

Monitoring of airborne fungal spore load in relation to meteorological factors, air pollutants and allergic symptoms in Farakka, an unexplored biozone of eastern India

Shipra Roy · Arindom Chakraborty · Saibal Maitra · Kashinath Bhattacharya 

Received: 3 February 2017 / Accepted: 30 May 2017
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Abstract Fungi are important components of atmosphere which play a major role in causing respiratory allergy upon inhalation. An airborne fungal spore survey was carried out in two outdoor environments in Farakka—an unexplored township covering the National Thermal Power Station, West Bengal, India for a period of 2 years (October 2013 to September 2015). A Burkard personal volumetric air sampler was used at 15 days interval to collect the total fungal spore load. A fungal spore calendar has been prepared depicting monthly average spore concentration in the air. The relationships between fungal spore concentration and different climatic factors were analysed statistically. Higher concentration levels of aerospora and pollutants were recorded during winter season. A detailed questionnaire was used to obtain medical history data of 523 local

patients visiting the outpatients department of Farakka NTPC (National Thermal Power Station) hospital. A significant positive correlation was found between fungal spore concentration, atmospheric pollutants and allergic manifestation. The dominant fungal spores were isolated, sub-cultured and tested for allergenic potential by skin prick tests (SPTs) among subjects with clinical history of respiratory allergy, which evoked >45.0% skin reactivity upon individuals.

Keywords Aeromycoflora · Burkard sampling · Meteorological factors · Air pollutants · Allergic symptoms · Farakka · West Bengal · India

Introduction

Fungal spores constitute important airborne allergens which may cause serious allergic problems in upper and lower respiratory tracts in susceptible allergic patients upon inhalation. More than 80 fungal genera have so far been found to be associated with respiratory tract disorders (Horner et al. 1995; Ormen et al. 2005). Fungal spore induced allergic diseases are estimated in the range of 20–30% in atopic individuals and up to 6% in the general population (Singh et al. 2003; Singh 2014). In India, fungi were reported as sole offenders in 2.3% of asthmatics and contributing offenders in another 34.6% of patients (Brist et al. 2003). Besides, fungi

Electronic supplementary material The online version of this article (doi:10.1007/s10661-017-6044-x) contains supplementary material, which is available to authorized users.

S. Roy · K. Bhattacharya (✉)
Department of Botany, Environmental Biology Laboratory,
Visva-Bharati, Santiniketan, West Bengal Pin-731235, India
e-mail: kashinathb23@rediffmail.com

A. Chakraborty
Department of Statistics, Visva-Bharati, Santiniketan, West
Bengal Pin-731235, India

S. Maitra
Allergy and Asthma Research Centre, 48/7 Purna Das Road,
Kolkata, West Bengal Pin- 700029, India

are also responsible for bio-deterioration, mycotoxin production and spread of plant, animal and human diseases (Miller et al. 1998). Aeromycoflora of a particular place depends upon various factors including geographical location, climate, air quality, vegetation and human activity (Kozak et al. 1979). In West Bengal, aerobiological monitoring have been performed by several workers (Raha and Bhattacharya 1992; Chakraborty et al. 2000; Majumdar and Bhattacharya 2000, Chakraborty et al. 2003; Das and Gupta-Bhattacharya 2008, 2011). However, detailed report of aeromycoflora in relation to meteorological factors and human health is scanty (Das and Gupta-Bhattacharya 2008; Chakrabarti et al. 2012). The manifestation of allergic diseases results from interaction between environmental factors and genetic predisposition (Jenerowicz et al. 2012).

The objectives of the present study were (1) to record the airborne fungal aerosol at two different outdoor environments in Farakka—an unexplored biozone of West Bengal by using Burkard personal volumetric sampler, (2) to prepare an airborne fungal spore calendar of the sampling site, (3) to explore the influence of different climatic factors on the occurrence of fungal spores and (4) to prepare a health survey report of allergic patients in the locality and their correlation with fungal spore concentration in the air.

Materials and methods

Aeromycological sampling

The present study was conducted for a continuous 2-year period, from October 2013 to September 2015 at Farakka (Fig. 1) (24° 47' 51" N, 87° 54' 51" E) in Murshidabad district of West Bengal, India, comprises of 144.78 km² with population density of 2100/km² and literacy rate of 59.75% as per Census of India (2011). The town, situated on the banks of river Ganga, gets importance for having the National Thermal Power Station (NTPC) in its vicinity. Two different outdoor environments at a distance of 9 km were selected as the sampling sites (Fig. 1). Site A is situated near the river Ganga comprises of rich vegetation, mainly dominated by mango (*Mangifera indica*) trees and rice fields (rice

is the staple food). Since it is located near Farakka barrage having 104 lock gates, controls water supply between India and Bangladesh, river Ganga remains filled with water almost throughout the year, making the atmosphere humid. Site B is adjacent to Kendua village, a dust prone area. A cement industry and the National Thermal Power Plant located within 2 km radius of site B (Fig. 1). Air sampling was done using a Burkard personal volumetric air sampler (Burkard Manufacturing Co. Ltd., Hertfordshire, UK). The sampler was operated at 15 days interval and was placed at a height of 2 m above the ground level. The sampler (air suction rate = 10 L/min) was used for 10 min for monitoring and assessment of both non-viable and viable airborne fungal spores. The exposed slides were mounted with DPX as a mountant and were scanned by a high resolution light microscope. The counts are then expressed as spores/m³ air. The fungal spores were identified following the standard literature (Gregory 1973; Ellis 1971; Barnett 1960) and counted according to the guidelines of The British Aerobiology Federation (1995). The fortnightly data for two consecutive years were expressed as average monthly data for constructing a fungal spore calendar.

Recording of meteorological parameters and atmospheric pollutants

During the entire sampling period, monthly average meteorological data such as temperature (°C), relative humidity (%), precipitation (mm) and wind speed (mph) were collected from World weather online website (n.d.) (<http://www.worldweatheronline.com/farakka-weather-history/west-bengal/in.aspx>). Monthly average air quality data such as PM₁₀ (µg/m³), SO₂ (µg/m³) and NO₂ (µg/m³) were also collected from official website of West Bengal Pollution Control Board (n.d.) (emis.wbpcb.gov.in/airquality/citizenreport.do#) to show their effect on fungal spore concentration in the air.

Hospital survey

A health survey report of 523 patients (age between 4 and 84 years) with respiratory allergic symptoms was obtained by visiting the outpatients department of Farakka NTPC hospital. Number of allergic

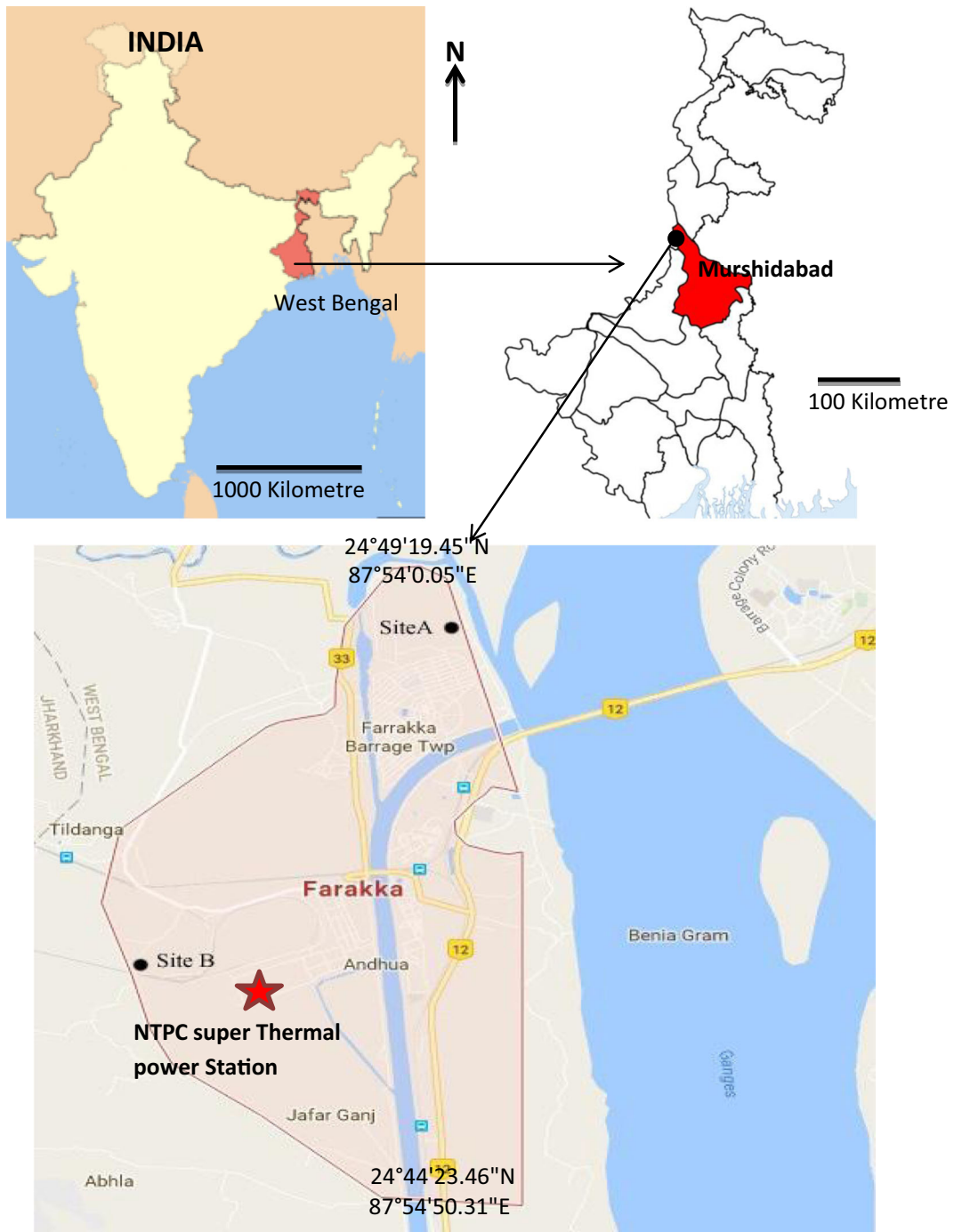


Fig. 1 Geographical location of Farakka, West Bengal, India showing the sampling sites

patients visiting the hospital in each month was noted down from the NTPC hospital record from October 2013 to September 2015. The data on demographic and medical history of the studied allergic patients were collected in the presence of

physicians using a standard questionnaire. The patients were clinically examined by the physician before collecting information from them. The questions concerning allergic symptoms were related to cough, breathlessness (wheezing, chest tightness or

pain), allergic rhinitis (running nose, itchiness of nose, sneezing, nose stuffiness), allergic conjunctivitis (irritation, redness and watering of eyes), allergy related skin disease and food allergy. Occurrence pattern of symptoms (seasonal, perennial, irregular), worst month and time of onset of symptoms were also noted. Pulmonary function tests (PFTs) by Spirometry was performed on 75 selected patients recommended by the physician using a portable, electronic spirometer (model Helios 701 Spirometer, Recorders and Medicare systems Pvt. Ltd. Haryana, India) suggested by the American Thoracic Society guidelines (1995) to determine the forced expiratory volume in 1 s (FEV1), forced vital capacity (FVC) and other parameters (data not shown). The study was performed with written consents from all the patients.

Statistical analysis

The relationship between monthly fungal spore concentration, different meteorological parameters (temperature, relative humidity, precipitation and wind speed) and air quality data (PM₁₀, SO₂ and NO₂) were analysed by Pearson's Product Moment method. The relationship between fungal spore concentration and hospitalization data was also analysed by correlation and regression analysis. All computations were done in R software (R- 3.2.2). Since the response, number of allergic patient, was count data, a generalized linear model (GLM) was used using log link.

Skin prick tests (SPTs) and study of the allergic patients

Skin prick tests (SPTs) were performed on selected allergic patients recommended by the physician with fungal crude antigenic extract (1:10 w/v) following the standard method (Shivpuri 1982; Deborg and Few 1993; Hussain et al. 2013, 2014). Antigenic extracts were prepared from seven dominant fungal species. Histamine diphosphate (1 mg/ml) and PBS (0.01 M, pH 7.2) were used as positive and negative control, respectively. Tests were performed with 20 µl of antigenic solution placed on the ventral side of the arms and then pricked with a sterile lancet. The wheal response was measured after 20 min and graded according to Styttis et al. (1982). The study was conducted at nearby SPT

Centre of Dishari Hospital, Malda through Allergy and Asthma Research Centre, Kolkata. The study was approved by the Ethics Committee of the concerned Institute and prior written consent was obtained from all the patients.

Results

Aeromycological study

During the 2-year sampling period, a total of 32 fungal spore types at two sampling sites of Farakka were recorded (Table 1). The total monthly aerospora count near river Ganga (site A) fluctuated between 6270 and 12,870 spore/m³ in the first year and 7260–20,460 spore/m³ in the second year (Fig. 2). The spore concentration near Kendua village (site B) was 3894–12,573 spore/m³ in the first year and 6303–16,764 spore/m³ in the second year (Fig. 2). Among the 32 fungal spore types, 12 were found to be present with ≥1% mean annual contribution each to the total fungal spore load over the study area for two sampling years (Table 1). In both study sites, the highest percentage contribution was observed in *Cladosporium* sp. (20.10% at site A and 19.53% at site B), followed by ascospores (18.15% at site A and 19.18% at site B), *Aspergilli*/*Penicilli* (13.73% at site A and 12.67% at site B), *Alternaria* sp. (9.22% at site A and 8.19% at site B), *Nigrospora* sp. (7.82% at site A and 8.84% at site B), *Curvularia* sp. (6.64% at site A and 7.6% at site B), *Fusarium* sp. (6.61% at site A and 7.08% at site B) and *Drechslera* sp. (5.02% at site A and 4.51% at site B) (Table 1). The entire study period has been categorized into four seasons for better interpretation namely, post-monsoon (September to November), winter (December to February), summer (March to May) and monsoon (June to August). In both study sites, highest concentration of fungal spores was observed during winter season, which was followed by post-monsoon season (Fig. 3). Spore concentration decreased during monsoon. A fungal spore calendar of Farakka was made with monthly average spore concentration in the air for two consecutive sampling years (Fig. 4). It is found that some of the fungal spores were seasonal, and some were perennial in occurrence and no spore free month has been found.

Table 1 Percentage contribution of dominant fungal spores to total annual count in Farakka, West Bengal, India

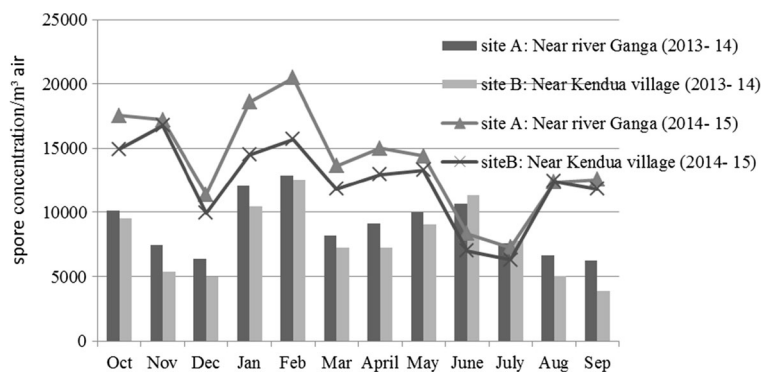
Sl. No.	Name of fungi	Percentage contribution					
		Near river Ganga (site A)			Near Kendua village (site B)		
		2013–14 (%)	2014–15 (%)	Average (%)	2013–14 (%)	2014–15 (%)	Average (%)
1	Ascospores	21.55	14.74	18.15	21.83	16.54	19.18
2	<i>Alternaria</i> sp.	14.05	4.40	9.22	12.78	3.60	8.19
3	Aspergilli/Penicilli	14.42	13.04	13.73	11.81	13.54	12.67
4	<i>Bispora</i> ↔sp.	0.80	1.00	0.90	0.56	1.01	0.78
5	<i>Bipolaris</i> ↔sp.	0.12	0.14	0.13	0.10	0.13	0.12
6	<i>Botryodiplodia</i> sp.	0.15	0.20	0.17	0.07	0.18	0.12
7	Basidiospores	2.06	2.74	2.40	1.61	2.39	2.00
8	<i>Beltrania</i> sp.	0.37	0.16	0.26	0.15	0.12	0.14
9	<i>Chaetomium</i> sp.	0.28	0.27	0.28	0.56	0.20	0.38
10	<i>Cercospora</i> sp.	0	0.13	0.07	0	0.04	0.02
11	<i>Cladosporium</i> sp.	16.85	23.36	20.10	17.74	21.32	19.53
12	<i>Corynespora</i> sp.	1.14	1.11	1.13	1.12	0.74	0.93
13	<i>Curvularia</i> sp.	3.54	9.74	6.64	4.65	10.56	7.60
14	<i>Drechslera</i> sp.	4.70	5.34	5.02	4.37	4.65	4.51
15	<i>Dendrophysalis</i> sp.	0.03	0.02	0.03	0.07	0.04	0.06
16	<i>Dwayabeeja</i> sp.	0.06	0.14	0.10	0.07	0.27	0.17
17	<i>Epicoccum</i> sp.	1.91	0.39	1.15	1.64	0.40	1.02
18	<i>Fusarium</i> sp.	4.67	8.54	6.61	5.27	8.88	7.08
19	<i>Ganoderma</i> sp.	0.49	0.04	0.27	0.66	0.02	0.34
20	<i>Nigrospora</i> sp.	5.44	10.21	7.82	6.74	10.94	8.84
21	<i>Pleospora</i> sp.	0.15	0.02	0.09	0.35	0.09	0.22
22	<i>Pithomyces</i> sp.	1.35	0.43	0.89	1.05	0.36	0.70
23	<i>Periconia</i> sp.	1.97	1.25	1.61	2.34	1.21	1.77
24	<i>Periconiella</i> sp.	0.43	0.18	0.30	0.42	0.31	0.37
25	<i>Phacotrichoconis</i> sp.	0.03	0.43	0.23	0.07	0.49	0.28
26	<i>Sporidesmium</i> sp.	0.28	0.31	0.29	0.38	0.27	0.33
27	<i>Stachybotrys</i> sp.	0.12	0.02	0.07	0	0.04	0.02
28	<i>Stemphylium</i> sp.	0.09	0.12	0.10	0.14	0.13	0.14
29	<i>Tetraploa</i> sp.	0.03	0.12	0.07	0.07	0.11	0.09
30	<i>Tetracoccosporium</i> sp.	0	0.13	0.07	0	0.09	0.04
31	<i>Trichoconis</i> sp.	0.43	0.27	0.35	0.87	0.58	0.73
32	<i>Torula</i> sp.	2.49	1.29	1.89	2.48	0.83	1.65

Correlation with meteorological parameters and air quality data

In the study area, monthly average temperature ranged from 31.25 °C (April) to 15.41 °C (January), average relative humidity ranged from 81.38(%) in September to 55.32(%) in March and optimum rainfall was noticed in the month of July (20.23 mm)

and lowest in March (0.26 mm). No rainfall was noticed in the month of November, December and January in both the sampling years. Average wind speed was higher in May (6.81 mph) and lower in the month of October (3.56 mph) (Fig. 5). Considering the pollutants level in the air, it shows that PM₁₀, SO₂ and NO₂ concentration were higher in the month of January (129.30, 5.61 and 25.26 µg/

Fig. 2 Monthly total spore concentration (No./m³) in two study sites of Farakka for two consecutive sampling years (2013–2015)



m³, respectively). Lowest PM₁₀ level was found in July (61.55 µg/m³), and concentration of SO₂ and NO₂ decreased in September (2.79 and 15.52 µg/m³, respectively) (Fig. 6). From the correlation matrix it is found that total fungal spore count/m³ of air showed no significant correlation with the meteorological parameters and air quality data at both study sites. But when individual fungal spore counts were taken into consideration, a significant correlation was found for some fungal spores (*p* value <0.05). The relationship between aerospora concentration and climatic factors is shown in Table 2. Analysis of Pearson product moment correlation showed that ascospores showed significant positive correlation with temperature, while *Cladosporium* sp. showed significant negative correlation with temperature. *Curvularia* sp., *Drechslera* sp. and *Nigrospora* sp. showed significant positive correlation with relative humidity. Basidiospores showed significant positive correlation with precipitation and negative correlation with PM₁₀ and SO₂ (Table 2).

Hospital survey report

The demographic profiles of the allergic patients have been presented in Table 3. Patients aged 41–60 years (41.3%) had visited the NTPC hospital more frequently with various allergic diseases in the studied period. Males (54.11%) were more susceptible to allergic symptoms than the females (45.88%), of which students and housewives were mostly affected (Table 3). Majority of the patients were from urban area (76.09%) than the rural area (23.90%) and most of them were residing in the locality for >10 years. 88.34% allergic patients consume non-vegetarian diet. Smoking habit is found in 98 male patients, while female smokers were not recorded. Inheritance of allergic diseases were reported in 34.03% patients, of which both father and mother were in the common relationship (Table 3). The most common allergic symptoms among the allergic patients of the study area were cough (75.71%), allergic rhinitis (68.45%),

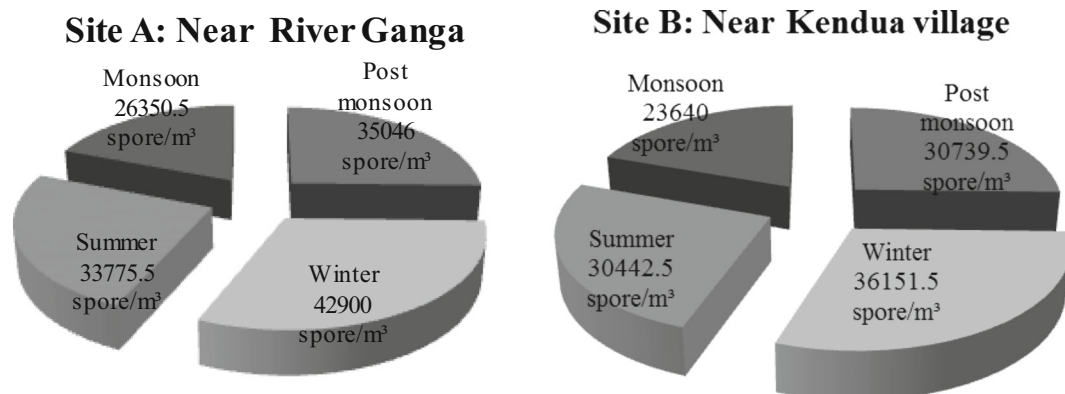


Fig. 3 Average seasonal variation pattern of aerospora in two study sites of Farakka for two consecutive sampling years (2013–2015)

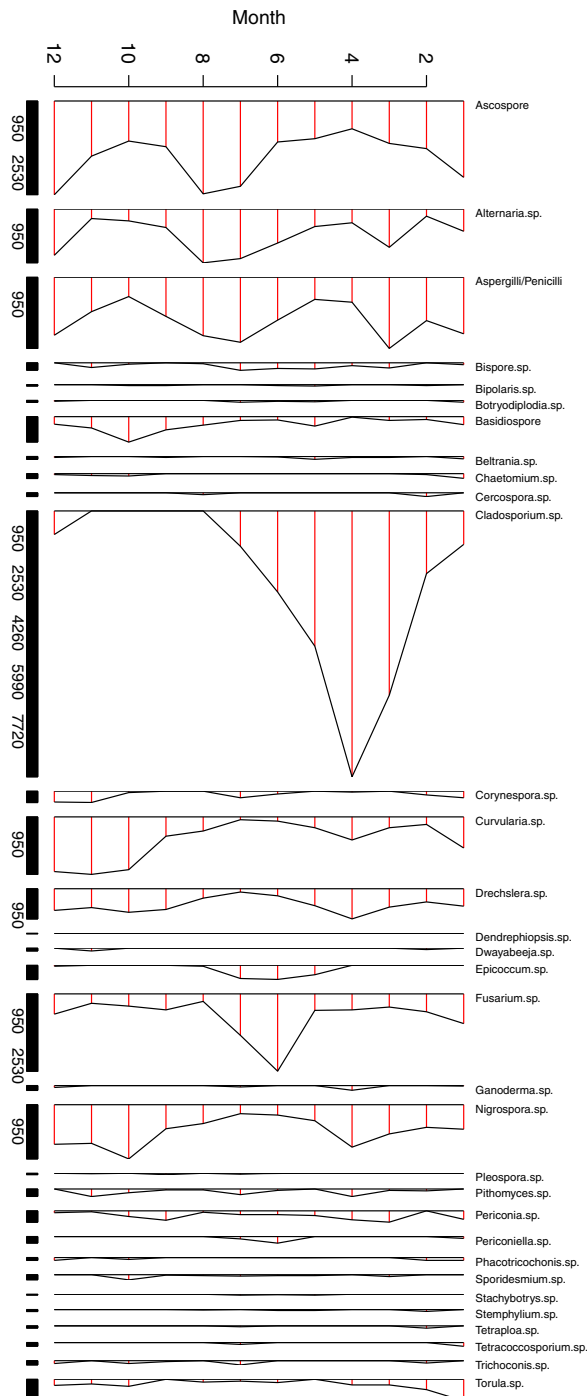


Fig. 4 Fungal spore calendar of Farakka representing 32 fungal spore types

breathlessness (62.52%), food allergy (47.41%), allergic conjunctivitis (36.52%) and allergy related skin diseases (35.18%) (Table 4 and Fig. 7). Most

of the allergic symptoms were much more vulnerable in 41–60 age group (Table 4). Males were more susceptible to cough, breathlessness, allergic rhinitis and allergy related skin diseases, whereas females were more susceptible to allergic conjunctivitis and food allergy (Fig. 8). Most of the allergic patients reported that they suffer more during winter and seasonal changes.

Correlation of fungal spore concentration and patients population

The relationship between the number of allergic patients who visited the NTPC hospital during each month of total survey period and the monthly fungal spore count data were shown in Fig. 9. The number of allergic patients showed significant positive correlation (p value <0.05) with monthly total fungal spore concentration in both the study sites along with atmospheric PM_{10} level in the air (Table 5). Using GLM with log-link, the regression equation is found to be

$$\text{Log (Total allergy patient)} = -31.45 + 0.0053 * \text{Total spore} + 0.5388 * PM_{10}$$

With adjusted R^2 value 0.7767 which means the above regression model can explain almost 78% of the variability. This also indicates the accuracy of the fitted model. From the above equation, we can predict the number of allergy patients if total spore count and PM_{10} data are available (Fig. 10).

Skin prick tests (SPTs)

Results showing the frequencies of positive reaction to antigenic extracts from seven dominant culturable fungi are presented in Table 6. SPT was performed on local patients with positive clinical history of asthma and/or allergic rhinitis. The highest skin sensitization was induced by *Aspergillus niger* (75.86%). The remainder fungal species also evoked remarkable skin reactivity ($>45.0\%$) in the symptomatic subjects. In general, females were shown to be more susceptible to the airborne fungal spores (Table 6).

Fig. 5 Average meteorological parameters of Farakka for two consecutive sampling years (2013–2015)

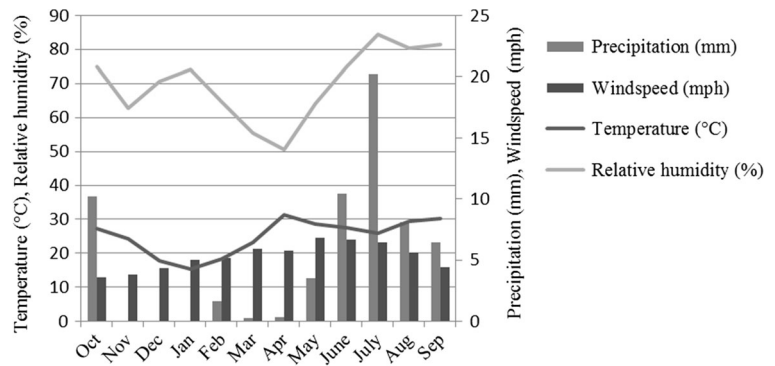
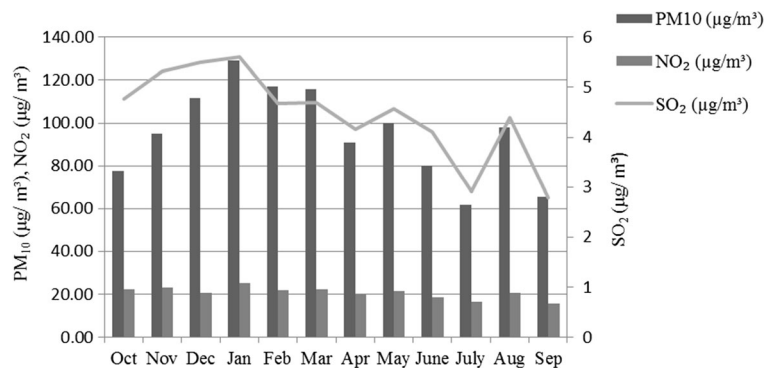


Fig. 6 Average air quality data of Farakka for two consecutive sampling years (2013–2015)



Discussion

Monitoring of two outdoor air of Farakka over a period of 2 years gives a clear picture of atmospheric fungal load both quantitatively and qualitatively.

Both the study sites were dominated by ascospores, *Cladosporium* sp., *Aspergilli*/Penicilli, *Alternaria* sp., *Nigrospora* sp., *Curvularia* sp., *Fusarium* sp. and *Drechslera* sp. Dominance of these fungal spores were also reported in similar outdoor

Table 2 Correlation with p- values for individual fungal spores of Farakka with different meteorological variables and air quality data

Sampling site	Variable 1	Variable 2	Correlation value	p value
Site A	Ascospores	Temperature	0.65	0.02
	Basidiospores	Precipitation	0.80	<0.001
	<i>Cladosporium</i> sp.	Temperature	−0.83	<0.001
	<i>Curvularia</i> sp.	Relative humidity	0.69	<0.001
	<i>Drechslera</i> sp.	Relative humidity	0.63	0.02
	<i>Nigrospora</i> sp.	Relative humidity	0.62	0.03
Site B	Basidiospores	Precipitation	0.78	<0.001
		PM ₁₀	−0.62	0.03
		SO ₂	−0.60	0.048
	<i>Cladosporium</i> sp.	Temperature	−0.83	<0.001
	<i>Curvularia</i> sp.	Relative humidity	0.69	0.01

Table 3 Demographic profile of respiratory allergic patients selected for the study conducted in Farakka ($n = 523$)

Demographic profile	Caregory	<i>n</i> (%)
Age (yrs)	<20 yrs	98 (18.73)
	21–40 yrs	167 (31.93)
	41–60 yrs	216 (41.30)
	>60 yrs	42 (8.03)
	Range	4–84 yrs
Sex	Male	283 (54.11)
	Female	240 (45.89)
Occupation	Students	124 (23.70)
	Housewife	166 (31.73)
	Agriculture	24 (4.58)
	Unskilled	97 (18.54)
	Service holder	63 (12.04)
	Business	49 (9.36)
Habitat/locality	Urban	398 (76.09)
	Rural	125 (23.90)
Period of stay in the locality	<10 yrs	180 (34.41)
	>10 yrs	343 (65.58)
Dietary habitats	Vegetarian	61 (11.66)
	Non-vegetarian	462 (88.34)
Smoking habit	Yes	98 (18.73)
	Current smoker:	
	Regular	49 (50)
	Irregular	18 (18.36)
	Chain smoker	6 (6.12)
	Ex-smoker	25 (25.51)
	No	425 (81.26)
Family history of allergy/asthma	Yes	178 (34.03)
	No	345 (65.96)
Relatives affected	Father	51 (28.65)
	Mother	75 (42.13)
	Siblings	17 (9.55)
	Children	13 (7.30)
	Grandfather/ grandmother	22 (12.35)

environment of West Bengal (Das and Gupta-Bhattacharya 2007, 2008; Majumdar and Bhattacharya 2000; Chakraborty et al. 2001; Adhikari et al. 2004) and also other parts of India (Sreeramulu and Ramalingam 1966; Singh et al. 1987; Deka and Devi 2012). No such difference is found in the prevalence of airborne fungal spores during the two sampling years. Two study sites were selected at Farakka only for aerobiological research.

Site A was adjacent to river Ganga and site B was 9 km away from river Ganga in Kendua village having super thermal power station (NTPC) and a cement industry. Aerobiological study was conducted concurrently on the two sites at fortnight interval to rule out and minimize any error during sampling. Because sampling in only one site may give incorrect result due to having different environmental factors. Hence, the prevalence of fungal spore in the air for all 32 fungal spore types remains almost same in the two study sites, though total spore count was slightly higher near river Ganga (site A) than the Kendua village (site B). Wet and humid environments and wind flow throughout the year near river Ganga (site A) may provide suitable growth condition of fungi, thus increasing the airborne spore load. On the other hand, dense vegetation, different human activities like throwing of garbage, prevalence of dead and decaying matter near river bed may help to increase fungal population. Spore concentration was found to be decreasing in monsoon due to heavy rainfall which washed out the spore mass from the air (Weber 2003). Whereas the atmospheric spore load was found to be increasing in winter months due to having favourable atmospheric conditions like moderate relative humidity and temperature, very low wind speed and minimal rainfall that help in the dispersion of dry spore mass. The constructed fungal spore calendar would be very much useful to predict the concentration of individual fungal spore load in different months, which would provide adequate information to the clinicians for diagnosis and treatment of allergy caused by fungal spores. Usually, the air is changed with fungal spores but their concentration or load would vary every month depending on local vegetation and human activities. Therefore, fungal spore-free month is ruled out. Spores of some fungal species are seasonal while some others are aseasonal, in the latter case, they may also be perennial. Sporulation of fungi depends upon various climatic factors like temperature, moisture content and precipitation. Dry air and high wind velocity enhance spore detachment and discharge, but such effect varied in different genera. Ascospore (a wet spore) and *Cladosporium* (a dry spore) showed significant association with temperature. Concentration of *Curvularia*, *Drechslera* and *Nigrospora* increased with increase in the moisture content of the

Table 4 Percentage of occurrence of different allergic symptoms in different age group of patients of Farakka

Different symptoms	Total number of patients (%)	Age group			
		<20 yrs. (%)	21–40 yrs. (%)	41–60 yrs. (%)	>60 yrs. (%)
Cough	396 (75.71)	59 (14.89)	120 (30.3)	176 (44.44)	41 (10.35)
Breathlessness	327 (62.52)	35 (10.7)	127 (38.83)	131 (40.06)	34 (10.39)
Allergic rhinitis	358 (68.45)	24 (6.7)	152 (42.45)	174 (48.6)	8 (2.23)
Allergic conjunctivitis	191 (36.52)	8 (4.18)	96 (50.26)	84 (43.37)	3 (1.57)
Allergy-related skin disease	184 (35.18)	17 (9.23)	62 (33.39)	93 (50.54)	12 (6.52)
Food allergy	248 (47.41)	24 (9.67)	137 (55.41)	62 (25)	25 (10.08)

atmosphere. This observation also supports the previous report of Chakrabarti et al. (2012), where they found that dry spores like *Cladosporium* had significant negative correlation with temperature and wet spores like *Curvularia*, *Drechslera* and *Nigrospora* had significant positive correlation with relative humidity. Precipitation is an important factor for discharge of basidiospores (Gregory 1973). In our study, basidiospores have significant positive correlation with rainfall. The total spore count in the air did not show any significant association with any meteorological factors, and air pollutants may be due to combined effect of different environmental factors like local vegetation, harvesting period, sunlight intensity, air pressure and wind direction.

From the hospital survey report, it is found that a significant part of the local population is suffering from various allergic diseases. The prevalence of allergic diseases increases with increasing age which may be due to increased exposure to outdoor environments. Moreover, monthly hospital visit record of allergic patients showed significant positive correlation with the monthly total airborne spore concentration and PM₁₀ in the air. It is an established fact that PM₁₀ concentration can enhance nasal irritation and wheeze in asthmatic patients. Patients were suffering mostly during winter season when the fungal spore load and atmospheric pollutants level in the air were very high. This observation was also proved by the clinical investigation

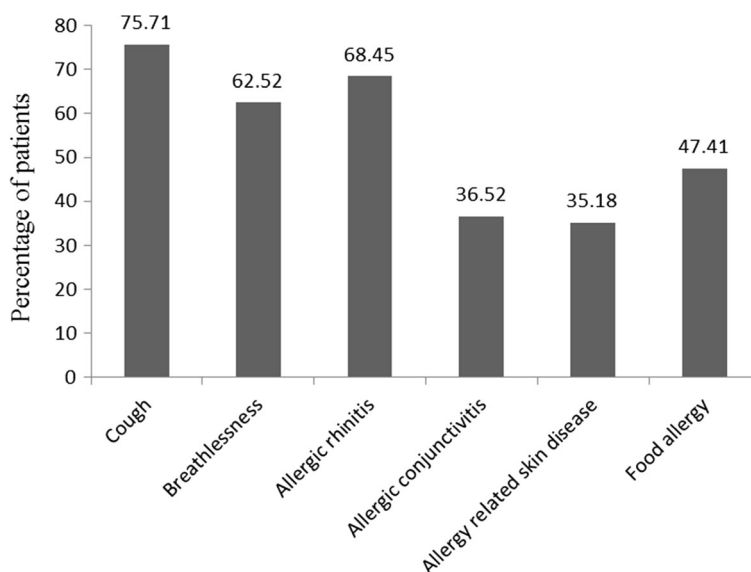
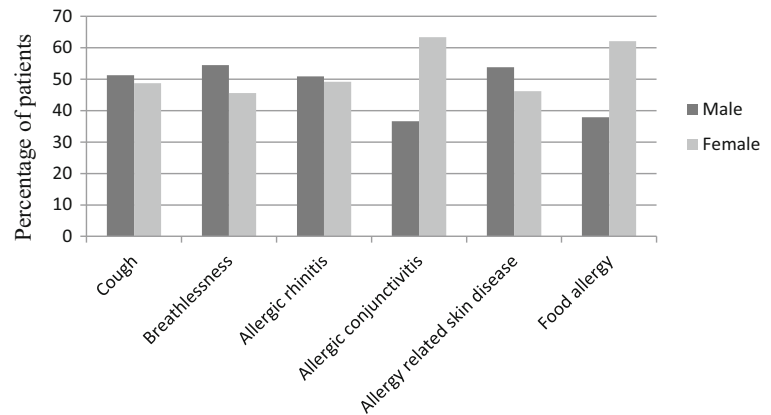
Fig. 7 Percentage of patients of Farakka showing different allergic symptoms

Fig. 8 Sex distribution of patients of Farakka showing different allergic symptoms



(SPTs) where a significant part of the allergic patients showed high positive sensitivity to dominant fungal allergens. The mould which gave the highest positive reaction was *Aspergillus niger* (75.86%) followed in the degree of prevalence by *Fusarium incarnatum* (65.38%), *Cladosporium oxysporum* (63.39%), *Aspergillus fumigatus* (56.69%), *Cladosporium herbarum* (51.42%), *Aspergillus japonicus* (47.95%) and *Curvularia lunata* (45.09%). Several authors in West Bengal (Bhattacharya et al. 2001; Majumdar and Bhattacharya 2004; Adhikari et al. 2000; Das and Gupta-Bhattacharya 2008; Chakrabarti et al. 2012; Chakraborty et al. 2001) and in other parts of India (Verma et al. 2011) reported such fungi except two as important aeroallergens by SPTs. Adhikari et al. (2000) recorded *Cladosporium oxysporum* in a rural bakery of West Bengal, India, using Andersen two-stage viable sampler during months of January to March. But allergenicity test of this fungus was not

conducted earlier. The prevalence of *Fusarium incarnatum* in the air was reported firstly in West Bengal from our study. The culture of *Fusarium incarnatum* was sent to “Agharkar Research Institute”, the National Fungal Culture Collection of India at Pune for its proper identification and this was confirmed. Till date, no research work has so far been conducted regarding the allergenic significance of *Fusarium incarnatum*. Thus, the allergenicity of *Fusarium incarnatum* and *Cladosporium oxysporum* is the first and new report from India. Higher percentage of positive response among the local patients might be due to higher prevalence of such fungal forms in the surroundings, which suggests that higher exposure to these fungal forms in the surroundings can increase the risk factor of allergic diseases among the local people.

In the present investigation, the two study sites are in 9 km aerial distance and are in the same climatic zone. Therefore, prevalence of fungal spore

Fig. 9 Relationship between monthly total spore count, PM₁₀ and hospitalization of allergic patients of Farakka

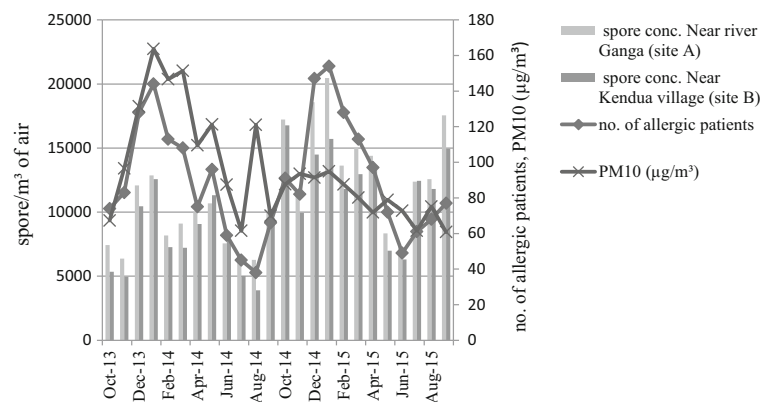
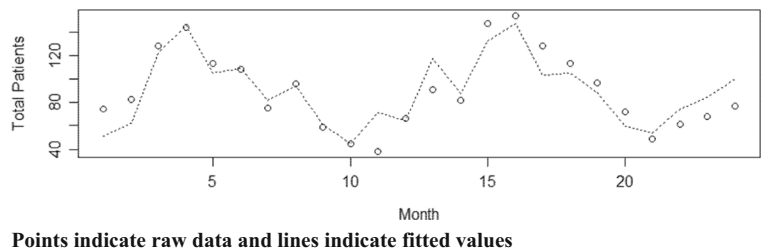


Table 5 Correlation with *p*-values for monthly hospital visit of allergic patients of Farakka with total spore concentration and pollutants in the air

Variable 1	Variable 2	Correlation value	<i>p</i> value
Number of allergic patients	Spore/m ³ air at site A	0.6453241	<i>0.0006612</i>
	Spore/m ³ air at site B	0.5740719	<i>0.0006612</i>
	PM ₁₀	0.4751452	<i>0.01896</i>
	SO ₂	0.1730509	0.4187
	NO ₂	0.2069456	0.3319

Italic values indicate significance

Fig. 10 Regression model for monthly hospital visit of allergic patients of Farakka with total spore count in the air. Points indicate raw data and lines indicate fitted values

and their concentration were found almost same in the two study sites. It can be taken as fungal spore load of Farakka town and adjacent rural area. To find out the relationship between monthly fungal spore concentrations in air with allergic disease manifestation in local inhabitants, people suffer from various allergic diseases in Farakka town and nearby rural area were included by collecting their demographic profile and medical history. We noticed significant positive correlation between monthly hospital visit of allergic patients with total spore

count and PM₁₀ level in air at both sites A and B using statistical analysis (Tables 5). A control is a prerequisite for interpreting the correlation between demographic profile and allergic symptoms. As the study was based on questionnaire survey, so no control was taken into consideration in our study. Hence, we could not able to interpret such relationship. However, we have performed chi-square test to check the association between patients' demographic profile, medical history and sensitization to fungal antigen. It was observed that period of stay in the

Table 6 Result of skin prick test with antigenic extracts of seven dominant airborne culturable fungi and the sensitive patients

Fungal allergen extract	Total number of subjects tested	Total number of positive response (%)	(+) Response (%)	(++) Response or above (%)	Male/female	Common aeroallergen sensitization
<i>Aspergillus niger</i>	87	66 (75.86)	36 (54.54)	30 (45.45)	1.8	Pollen: <i>Azadirachta indica</i> , <i>Brassica campestris</i> , <i>Carica papaya</i> , <i>Cocos nucifera</i> , <i>Cynodon dactylon</i> , <i>Eucalyptus terri-tecornis</i> , <i>Parthenium hysterophorus</i> ;
<i>Aspergillus fumigatus</i>	127	72 (56.69)	37 (51.38)	35 (48.61)	0.8	
<i>Aspergillus japonicus</i>	98	47 (47.95)	20 (42.55)	27 (57.44)	0.88	
<i>Cladosporium herbarum</i>	105	54 (51.42)	39 (72.22)	15 (27.77)	0.68	
<i>Cladosporium oxysporum</i>	112	71 (63.39)	51 (71.83)	20 (28.16)	0.91	Egg, Milk, Prawn, Lentil, House dust, Dust mite (<i>D. farinae</i>)
<i>Curvularia lunata</i>	51	23 (45.09)	12 (52.17)	11 (47.82)	1.55	
<i>Fusarium incarnatum</i>	130	85 (65.38)	39 (45.88)	46 (54.11)	0.7	

locality, smoking habit, family history of allergy/asthma, cough, breathlessness, allergic rhinitis, allergic conjunctivitis and skin disease were significantly associated with fungal antigen sensitization (Supplementary Tables 1, 2, 3). These can be treated as possible risk factor for development of allergy to fungi.

Conclusion

It is noteworthy that fungal spores play a major role in causing different respiratory allergic disease such as allergic rhinitis, allergic asthma, fungal sinusitis, allergic bronchopulmonary mycosis and atopic dermatitis. The present survey is a kind of awareness to the local people about the fungal spore load in the area which can cause adverse effect to their health. It may help the physicians in diagnosis and therapy of patients suffering from mould allergy in this unexplored biozone of eastern India where a significant part of the local people frequently suffer from various respiratory disorders. Furthermore, the clinician can also use the fungal spore calendar to make people aware of specific fungal spore in a particular season while dealing with seasonal allergic diseases.

Acknowledgements We thankfully acknowledge financial support to the first author (SR) from UGC-BSR. Sincere thanks are also to Dr. R. K. Mishra (NTPC hospital) for providing clinical data of patients visiting NTPC hospital.

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