Research Article

Fungal bio-aerosol in multiple micro-environments from eastern India: source, distribution, and health hazards

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Abstract
Health hazards associated with bio-aerosol is gaining immense importance in recent time. However, studies linking the probable source characterization of bio-aerosol, and their potential effect as respiratory ailments on the local people are extremely rare over the Indian subcontinent as well as in global perspective. To fill our knowledge gap, we have conducted a 3-year study on fungal bio-aerosol characterization, possible source segregation, identification of health hazardous fungal species and their role in causing allergy from seven different micro-environments over a semi-rural site of lower Indo-Gangetic Plain, West Bengal, India. The result showed the highest fungal spore concentration as well as spore diversity in the crop field (4477 ± 1343 spores m^{-3}) and the lowest in the classroom (1994 ± 651 spores m^{-3}). Ascospore, basidiospore, *Cladosporium*, and Aspergilli group were the primary indoor spores. The marker species for the crop field were *Curvularia* and *Drechslera*, whereas *Ganoderma* was for the factory environment. Source profile of the fungal spore of different micro-environments indicates the domination of outdoor species, whereas enclosed micro-environments have been dominated with spores of indoor origin. *Aspergillus*, *Curvularia*, *Penicillium*, and *Rhizopus oryzae* showed maximum correlation with the local allergic rhinitis and asthmatic patients. In skin prick test, *Aspergillus* sp., contributing a maximum percentage of the total culturable fungi, was found to be an expected potent allergic candidate in both non-asthmatic and asthmatic patients, as well. This study will directly help the inhabitants to avoid the hazardous fungal sensitization in different micro-environments.

Keywords Health hazards · Bio-aerosol · Source segregation · Allergy · Skin prick test

1 Introduction

Bio-aerosol, often known as airborne particles and their derivatives such as allergens, endotoxin, etc. of different biological origin, is of the major cause of respiratory ailments. Among these bio-aerosols, fungal spores represent one of the most prevalent components [36]. They can originate from both natural (soil, vegetation, debris, etc.) and human-made (building materials, organic waste, etc.) sources. Through the process of aerosolization, they disperse into their surrounding environments from the sources [35]. Being respirable by size (2–10 µm), fungal spores can easily enter the human respiratory tract [67]. Fungus-induced allergic respiratory symptoms can occur from the indoor, occupational environment or both [3, 9]. Throughout the world, 20–30% of the allergic problems are attributable to fungal spores, which include 44% atopic and 80% among asthmatics [60]. In

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India, about 20–30% of the population suffer from allergic rhinitis, and 15% of them suffer from asthma alone [61].

Previous studies have shown that outdoor spore concentration is dominant over the indoor [20]. Outdoor spore level is mostly governed by different meteorological factors (temperature, relative humidity, wind speed, and rainfall), the host–fungus relationship, and source strength potential [19, 27, 33]. On the other hand, indoor spore diversity is a mixture of both indoor and outdoor species [34, 39]. Growth of indoor fungi primarily depends upon the indoor moisture content, temperature, and presence of suitable growing surfaces that may cause fluctuations in fungal level [44, 47, 68]. In rural areas of eastern India, *Alternaria*, *Aspergillus niger*, *Aspergillus flavus*, *Cladosporium cladosporioides*, are reported to be the major indoor culturable fungi while ascospores, basidiospores, *Alternaria*, *Nigrospora*, and Aspergilli group are predominantly outdoor fungi [5, 6].

Numerous studies have been carried out to identify the fungal spores, which could be potential sources of allergen released in the environment. So far, around 180 fungal allergens have been identified to induce IgE-mediated hypersensitivity in atopic patients [48, 60]. A study conducted in a rural area with tropical climate from eastern India has revealed that 95.1% of atopic patients were sensitized to *A. fumigatus* through skin prick test (SPT) [6]. Both the young children and the respiratory allergic patients have been reported to display potential health issues on fungal spores' exposure [16, 63]. However, very little is known about the spore concentration, seasonal distribution, and their relation with allergic sensitization among the local people in rural and semi-rural regions of eastern India. These regions have higher vegetation coverage and can contribute to a higher spore source than urban areas. Therefore, it is essential to conduct a study on both spore concentration and their probable sources over different environments of rural or semi-rural areas and then to determine their potential impact on the health of local people in terms of allergic sensitization. This could improve awareness among the local people by forecasting through an allergist for the up-gradation of health outcomes of the sensitized patients.

The present study aims to investigate the potential sources and spatiotemporal variation of fungal spores over different environments within the geographical location of Habra, a semi-rural site in eastern India. Further studies are done to check the seasonal variation of culturable fungi in different micro-environments and their role in causing allergic disease in people residing in those areas. The study also focuses on determining the probable allergenic impact of exposed dominant aero-mycoflora from different environments through extract-based SPT.

2 Materials and methods

2.1 Study design, location, and characteristics of sampling sites

This aero-mycological study was carried out in a semi-rural site (Habra, latitude: 22°49′48.00″ N and longitude: 88°37′48.00″ E) of lower Indo-Gangetic Plain (IGP), West Bengal, the eastern part of India (Fig. 1). Total seven different environments such as bank, hospital, factory, crop field, classroom, library, and institutional office room were selected in Habra. Each of these environments is considered as micro-environments, and their characteristics are described in Online Appendix A, Table. A.1. The selected area is surrounded by the lush greenery of tropical moist deciduous forest, horse-shoe shaped lake and agricultural field. According to provisional reports of Census India, 2011, the population of Habra was 147,221. The principal occupation of the people is agriculture, and paddy is the primary cultivar here. However, very recently, due to the construction of many housing projects and investment activities for commercial as well as industrial purposes, the area became populated. Thus, the study site perfectly executes the features of a semi-rural area over the eastern part of India.

2.2 Sampling, instrumentation and sample analysis

The total fungal spore as well as culturable fungal spore content of the air was measured in all the seven micro-environments of Habra from May 2014 to April 2017, using the Burkard Personal Slide sampler (Burkard Manufacturing Co.Ltd. Hertfordshire WD3 1PJ, UK) and Andersen Two-Stage sampler ([10]; manufactured by Thermo Andersen, Smyrna, 300082-5211, USA), respectively. The sampling was conducted at the height of 1.5 m above the floor or ground level. Samples were collected once in a month, in between 10.00 h and 15:30 h. After mounting of the fungal spores, trapped by Burkard Personal Slide sampler (10 ± 0.1 L min^{-1}), the scanning was carried out under 400× and 1000× magnification of a Nikon high-resolution light microscope (Labophot 2, Nikon Corp., Japan). Identification of fungal spores based on morphology was made up to the genus level using the guidelines of the British Aerobiological Federation (The British Aerobiology Federation 1995) and from the previous studies [18]. Finally, the spore concentration was expressed as the number of spores per meter cube of air (spores m^{-3}, Online Appendix B). Within total aero-spora count, we have listed a group, named "Aspergilli" ("Asp/Pen"). The conidia of this group

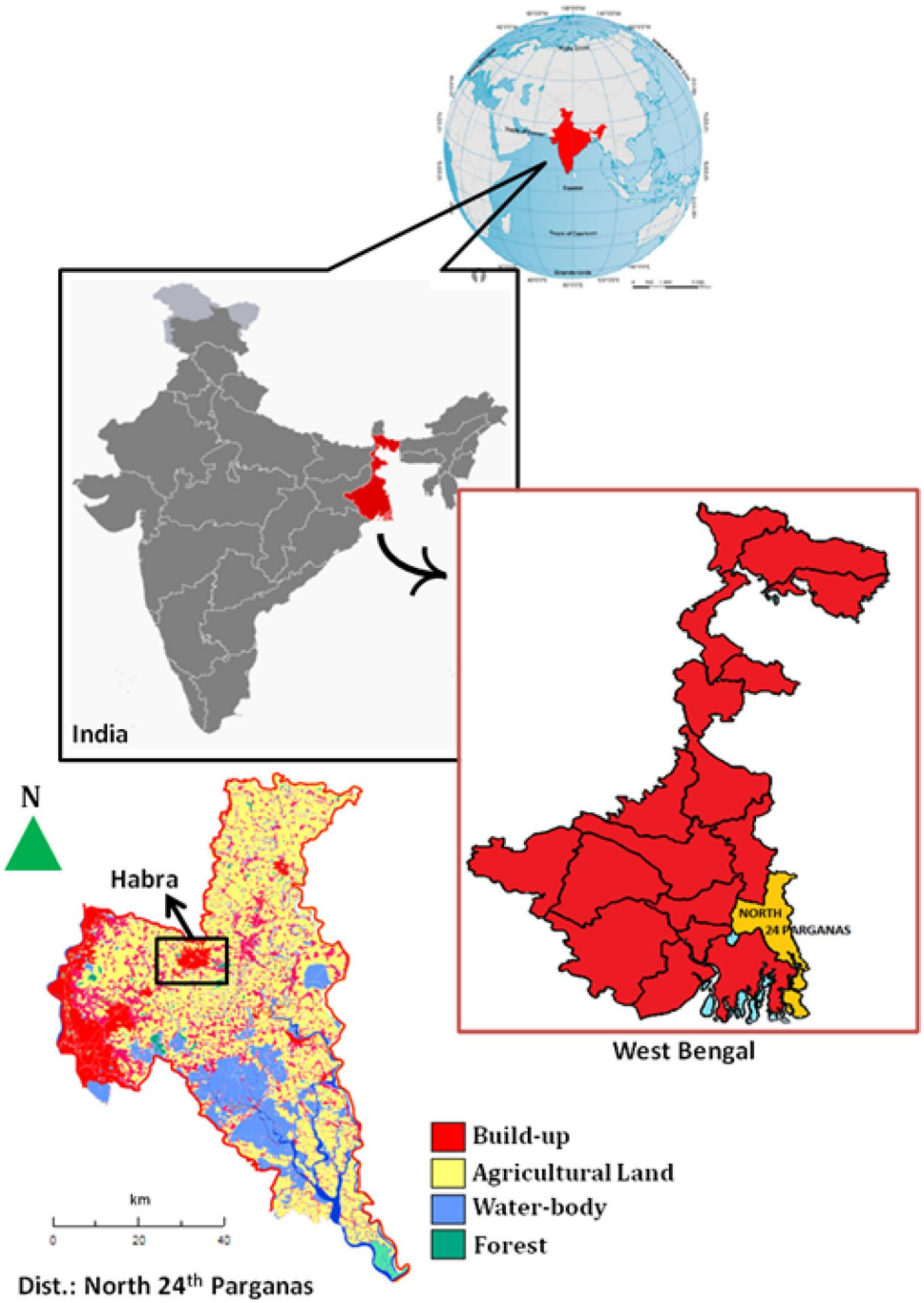

Fig. 1 Map showing the location of the sampling area

are indistinguishable, and they are identified only with the viable method [55].

Andersen two-stage sampler with total 200 holes, having upper and lower stages of 1.5 mm and 0.4 mm of holes' diameter, drew air (28.3 ± 0.3 L min^{-1}) through the orifice at its top. This instrument has a particle diameter cutoff size (d50) of 8.0 µm and 0.95 µm for the upper and lower stages, respectively. Above this aerodynamic diameter, the sampler meets the cutoff size requirements for most of the fungal spores. Collection of the fungal sample was performed onto two petri-plates containing potato dextrose agar media with chloramphenicol (25 mg mL^{-1}) added to cease the bacterial growth. Plates were then incubated at 27 ± 0.5 °C temperature for 2–7 days to get the optimum growth of fungal colonies as previously described by Sharma et al. [59]. For the identification, each and every fungal colony from the exposed petri-plate was isolated and subcultured for getting pure culture on PDA slant. Finally, the microscopic morphological identification was confirmed from Agharkar Research Institute,

Pune. However, culturable airborne fungal spore counts were represented as colony-forming units per meter cube of air (CFU m^{-3}, Online Appendix B). The growth of these spores was measured by dividing the concentration of culturable fungus with the total fungal spores' concentration, followed by the multiplication with a hundred.

The entire sampling period was represented into four major seasons, including—summer (March–May), monsoon (June–September), post-monsoon (October–November) and winter (December–February).

2.3 Study population, hospitalization data, and questionnaire survey

We focused on pooling the patients' data, suffering from allergic rhinitis (including sneezing, runny nose, stuffy nose, coughing, and itchy eyes), and asthma with their age, sex, admission date and professions. In 3 years, a total of 179 children and 2,156 adults, age range between 6 and 60 years, visited Habra State General hospitals, Habra, with these symptoms. Children below the age of 6 years are difficult for the diagnosis of asthma [14]. The questionnaire was also done simultaneously with the selected patients. The format of questionnaire form (Online Appendix C) was prepared from Bose Institute, Kolkata, with a slight modification of the layout used by Singh et al. [62]. It was finally translated into the local language (Bengali).

2.4 Allergenic sensitivity of atopic patients against dominant culturable aero-mycoflora through SPT

Skin prick test (SPT) was performed according to the ethics of the hospital with our supplied fungal allergen extract, along with other standard allergens. The antigenic crude extract (1:10 w/v) was prepared from the individual dominant culturable fungi of the study area [4, 65]. Phosphate buffer saline (PBS-0.01M, pH 7.2) and histamine diphosphate (1 mg mL^{-1}) were taken as negative and positive controls, respectively. This test was performed on two hundred and sixty patients ($n = 260$) with prior permission from them. For individual atopic patients, 20 µL of each crude antigenic fungal extract was taken and pricked with a sterile lancet on the ventral side of the arms. After 20 min of pricking, the wheal response was measured based on gradation (1 +, 2 +, 3 +) [16].

2.5 Data analysis

Principal component analysis (PCA) using R-programming language (Version-3.4.4) was used to evaluate the possible sources of significant fungal spores over different micro-environments during the entire study period [52, 53, 69]. It is a mathematical tool that can combine information by correlating a large number of variables and transform them into a smaller set of statistically independent new variables retaining most of the data. The PCA-generated new components are linearly independent and can be interpreted physically by applying Kaiser's varimax rotation, which can aid the physical interpretation of the principal components. Components are classified according to the eigenvalue > 1. The PCA only indicates the relative influence of each identified source to each fungal species. Several studies have been used this receptor model for the source segregation of the fungal spores [23, 24]. In this study, the factors information from PCA, along with the correlation of total fungal spore with meteorological variables, was taken for the source apportionment. Moreover, the information of marker species with a single source of origin was incorporated.

3 Results and discussion

3.1 Inter-comparison of total fungal spore count in different micro-environments

Total fungal spore concentrations in different micro-environments over a semi-rural atmosphere are shown in Fig. 2a. The highest number of fungal spore concentration was observed in the crop field (average concentration 4477 ± 1343 spores m^{-3}), followed by the factory (average concentration 3891 ± 558 spores m^{-3}) environment. In the classroom, fungal spore concentration was found to be lowest with an average concentration of 1994 ± 651 spores m^{-3}. Higher outdoor spore concentration can be explained by the presence of vast vegetation and farmland in and around the sampling site which might act as a potential source for fungal spores [19].

The open field was marked by the highest fungal diversity (based on Shannon diversity index), whereas hospital showed the lowest spore diversity among the selected micro-environments. These spores diversity often depends on the sampling conditions and meteorological factors too [6, 33]. Other than unidentified ascospore and basidiospore, *Cladosporium* and Asp/Pen, consisting of 29–43% of the total spore load, were identified as the primary dominant species, in five indoor air (classroom, institutional office room, bank, library, and hospital). In the factory, *Ganoderma* and *Chaetomium* were found to be the dominant spore species, accounting for ~ 26% of total spore concentration. *Curvularia* and *Drechslera* were the two predominant fungal spores over the crop field, consisting of 32% of the total fungal spores. Unidentified ascospore and basidiospore significantly contribute 18–44% to the total fungal spore level for each micro-environment. This

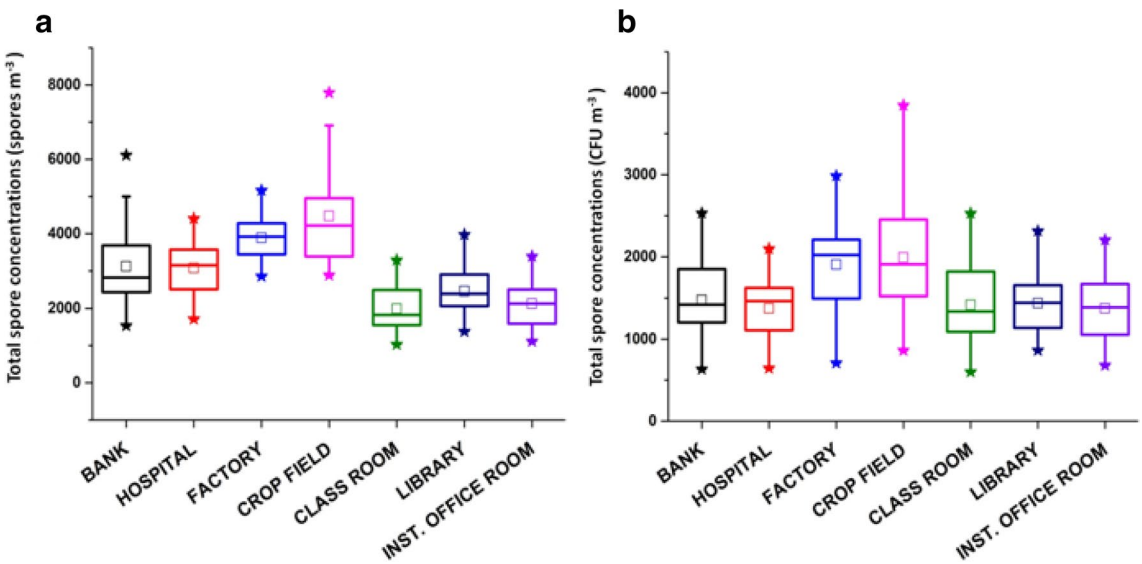

Fig. 2 Comparative study of fungal spore concentration. **a** Total fungal spore (Spores m^{-3}) and **b** culturable fungal concentrations (CFU m^{-3}) in seven different micro-environments from a semi-rural area of eastern India

result coincides with the previous study from eastern India [16, 21].

3.2 Seasonal variation of total fungal spores

Seasonality on the total fungal spore disclosed the highest and lowest spore levels during the post-monsoon and winter season in sampling area (Fig. 3a). A significantly higher spore concentration (performed one-way ANOVA test, $f_{value} < f_{critical\ value}$) during monsoon indicates that the fungal growth during this season exceeds the below-cloud scavenging of the fungal spore by precipitation.

The seasonal variation of *Cladosporium*, one of the indoor dominating species, showed the highest spore load in post-monsoon and the lowest during monsoon and winter seasons (Fig. 4). *Cladosporium* requires a combination of optimum meteorological factors for their growth [57] and release [56]. Low relative humidity (~ 60%), moderate wind speed (4–6 ms^{-1}), and occurrence of precipitation events during the post-monsoon season significantly increased the concentration over the sampling station. The other indoor dominating fungal group, Asp/Pen exhibited similar seasonal variation. The release mechanism of Asp/Pen spore can be both active and passive [42]. Elevated concentration of Asp/Pen in post-monsoon was observed along with higher temperature (30 °C) and lower relative humidity (58%). This indicates the domination of active spore release mechanism of Asp/Pen during sampling time. *Curvularia* was identified as the marker fungi in crop field with a very high frequency during summer and post-monsoon time as well. This genus is reported to be a potent plant pathogen of plants belonging to Poaceae family and is also reported in the rice field of eastern India [17, 41]. An increase in this spore count during the harvesting months of May and November is evident from the monthly fungal calendar (Online Appendix D, Fig. D.1–7). Within the factory aero-mycoflora, *Ganoderma*, the dominating fungus displayed higher spore consistency during post-monsoon and lower during winter season. Large number of rainy days (~ 22 rainy days in October–November) and availability of growing material, such as wood, elevate its concentration during the post-monsoon season [8].

3.3 Source segregation of airborne fungi

PCA has been used to determine the potential source profile of airborne fungus. The eigenvalues of each component for each micro-environment have been provided in Online Appendix A, Table. A.2. We have represented two principal components (PC1 and PC2) in Fig. 5 for all micro-environments.

3.4 Sources of fungal spore in bank micro-environment

Four major potential sources can be identified from the bank aero-spora through PCA analysis. Factor 1 consists of fungi such as rust spore, *Periconia*, and *Pithomyces*. Rust spore, found mostly in outdoor, was used as a marker fungus for this factor [37]. As these fungi showed a negative correlation with wind speed (Online Appendix A, Table. A.3, $r = -0.06$ to -0.18, p value < 0.01), it could be assumed that these fungi were transported from outdoor to the

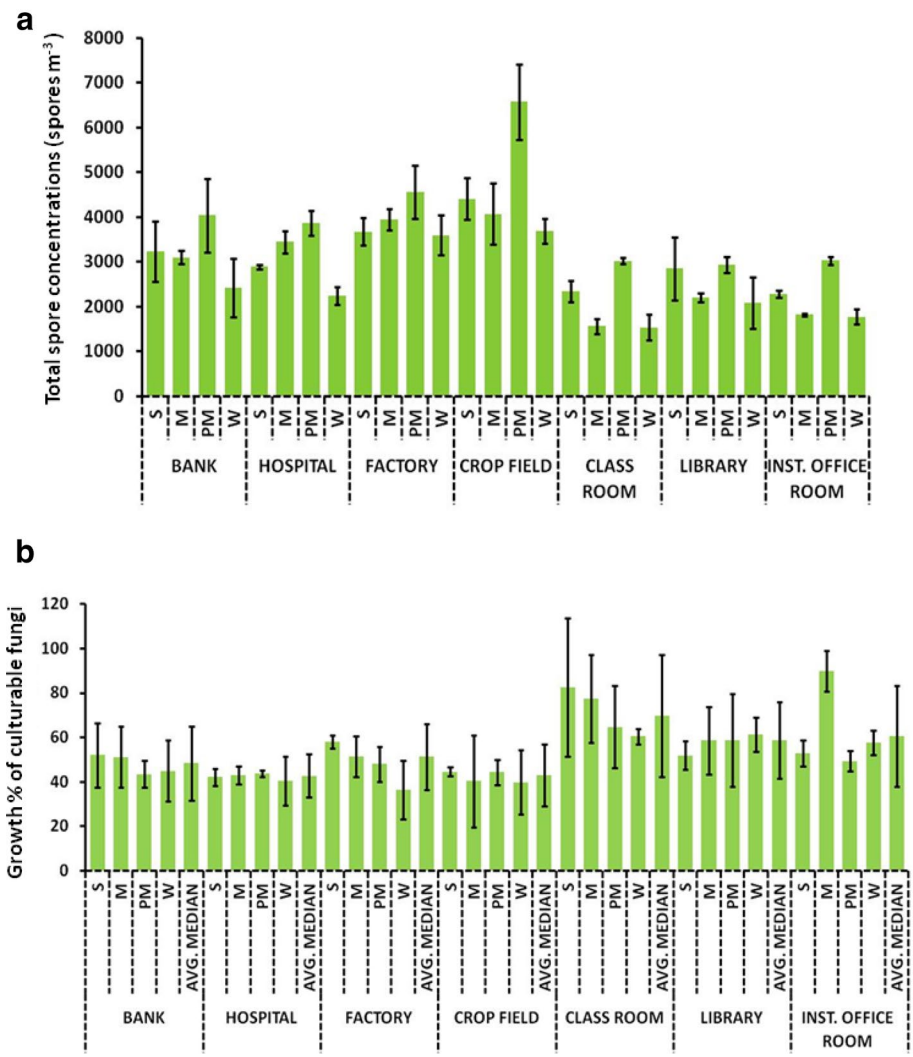

Fig. 3 Seasonal variation of **a** total fungal spore concentration and **b** growth % (median) of culturable fungi over different micro-environments of Habra. *S* summer, *M* monsoon, *PM* post-monsoon, *W* winter

indoor environment via human movement. For Factor 2, low and moderate correlation of the fungal spores, such as unidentified basidiospore, unidentified ascospore, and *Curvularia* with wind speed displays wind-borne as well as human-mediated transport inside the bank. *Curvularia* spores, used as a marker fungi for Factor 2, are majorly found in soil [7, 41]. The probable source for *Cladosporium*, Asp/Pen, and *Torula*, together correlated well with Factor 3, could be paper dust, and building material, too. From Factor 4, wood and /carpet materials would be the potential source for *Nigrospora*.

3.5 Sources of fungal spore in hospital micro-environment

From hospital micro-environment, three major possible sources of fungal spore have been identified. Factor 1 consists of Asp/Pen and could be interpreted as an indoor source. Previous studies have shown that wastes, such as fluid, cotton, puss, and tissues, are the good sources of Asp/Pen in hospital [51]. The positive correlation of relative humidity with Asp/Pen indicates higher growth of these fungal spores in a moist environment. The damp wall inside the hospital could be the probable source for the fungi (*Cladosporium*, Basidiospore, *Nigrospora*) of Factor 2. Fungal spores in Factor 2 also revealed a significant positive correlation with relative humidity ($r = 0.3–0.59$, p value < 0.1). Factor 3 (*Periconia*, ascospore) can be attributed to those spores which were transported inside through human activity.

3.6 Sources of fungal spore in factory micro-environment

Factor 1 from factory micro-environment is majorly loaded with *Ganoderma*, *Curvularia*, *Cladosporium*, and *Torula*. Wood is considered as a major probable source of these fungi. Spores of this factor were well correlated with relative humidity inside the factory [46]. Damp wall of the building can be interpreted as the potential sources of

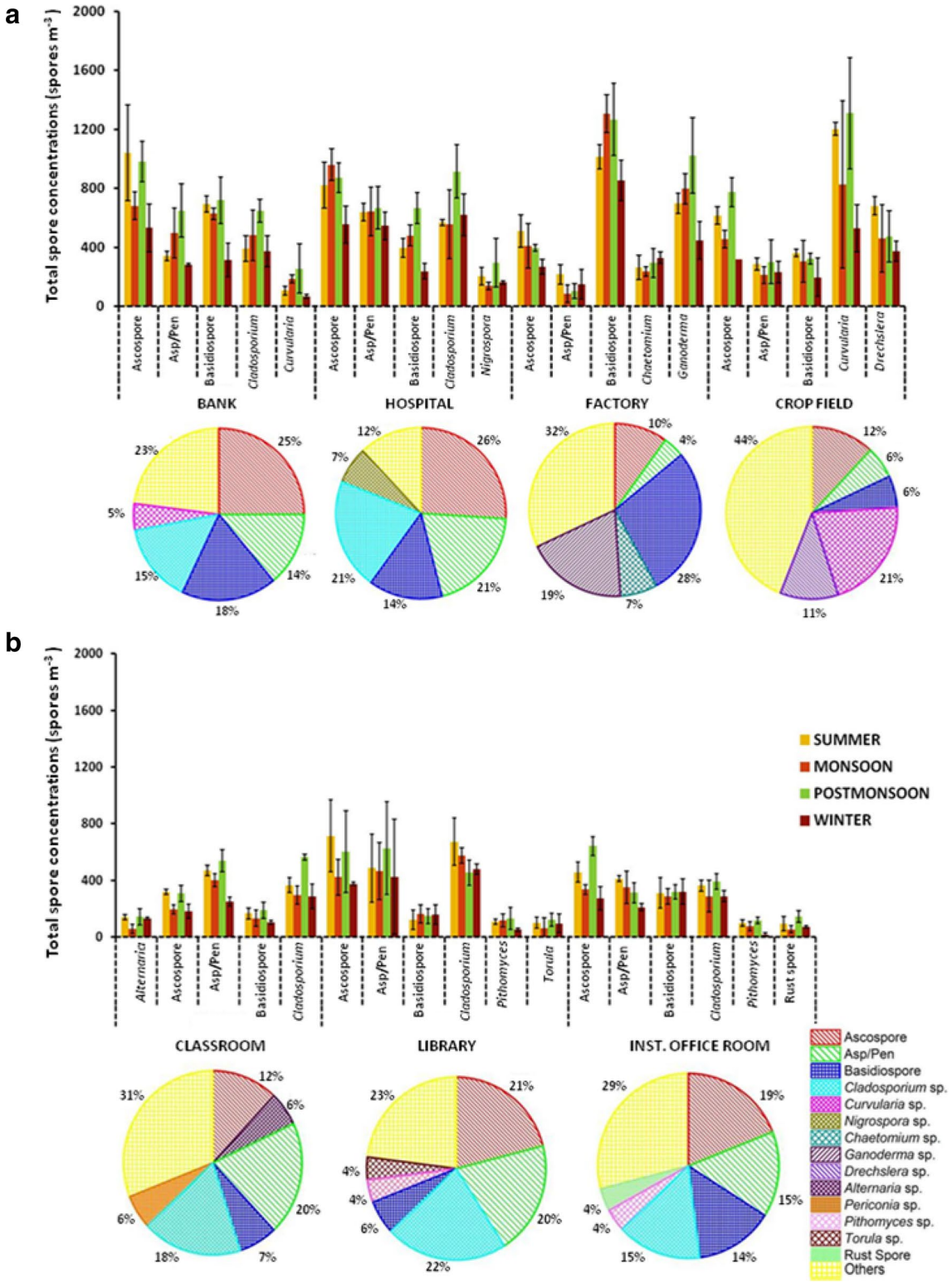

Fig. 4 Seasonal distribution of dominant fungal aero-spora with percent contribution in different micro-environments, **a** bank, hospital, factory, and crop field; **b** classroom, library, and inst. office room. *Inst*. institutional

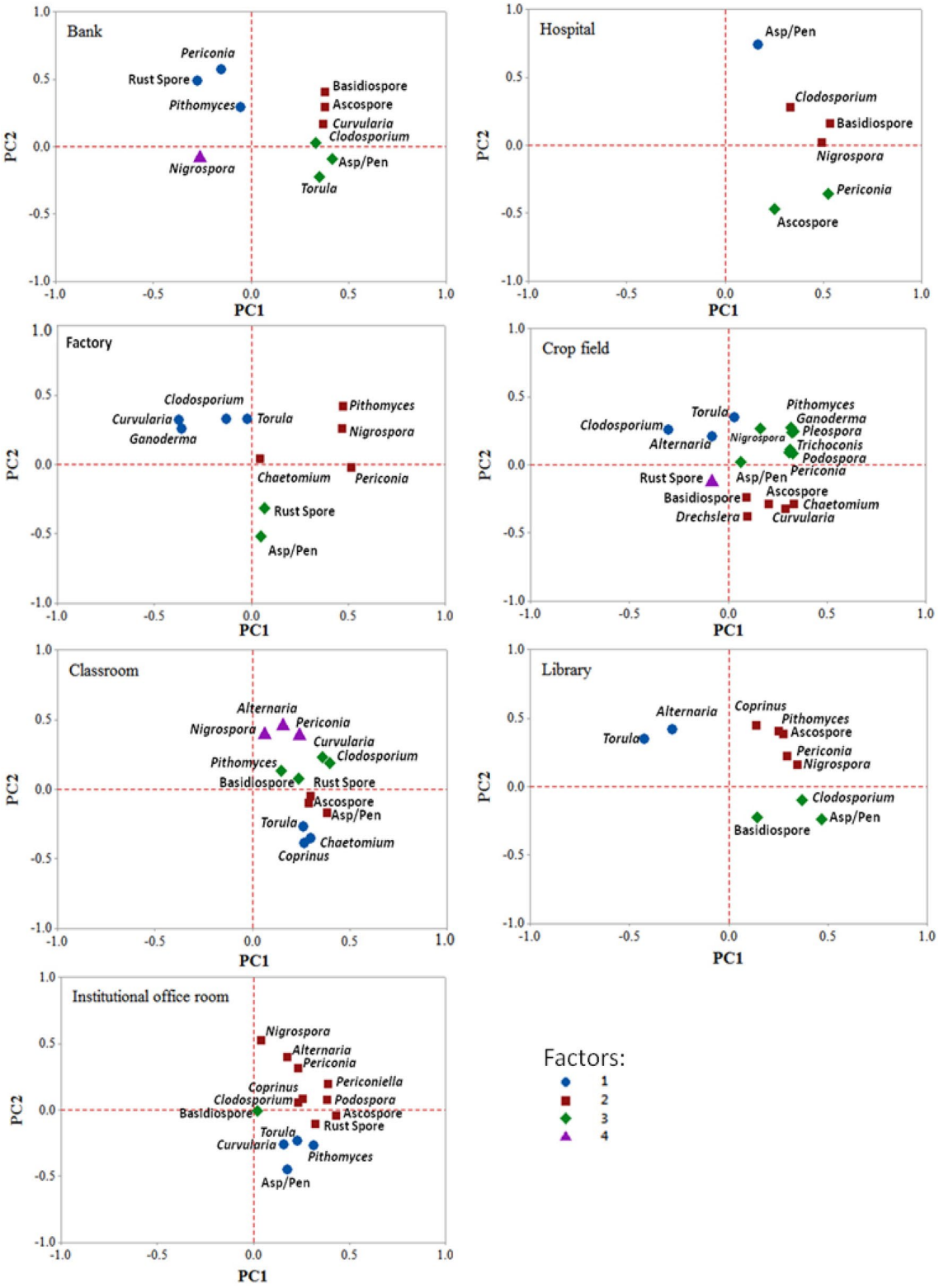

Fig. 5 Source segregation through PCA of fungal aerosol from seven different micro-environments

fungi (*Periconia*, *Chaetomium*, *Nigrospora*, and *Pithomyces*) from Factor 2. In Factor 3, positive correlation of the fungi with wind speed discloses wind-mediated ($r = 0.026–0.12$, p value < 0.1) transport from the outside.

3.7 Sources of fungal spore in crop field micro-environment

In the case of crop field, *Cladosporium*, *Alternaria*, and *Torula* in Factor 1 majorly arise due to post-harvesting activities. Dead leaf and plant debris during the post-harvesting period are considered to be a major source of these fungi [35, 53]. A negative correlation of wind speed ($r = -0.13$ to -0.2, p value < 0.1) with the fungus of Factor 1 reveals their dispersal from the nearby sources. Factor 2 could be explained by fungal spores, present during the rice-harvesting period. *Curvularia* and *Drechslera*, potential plant pathogens of Poaceae family, release in large number during the paddy harvesting time [38, 41]. Factor 3 entails fungi with mixed sources [35]. Factor 4 consists of rust spore, an outdoor fungus which infects plants of the Brassicaceae family during the winter season [37, 66].

3.8 Sources of fungal spore in classroom micro-environment

Within classroom aero-spora, the factors are not very well separated due to a different mixture of sources. Factor 1 (*Torula*, *Chaetomium*, and *Coprinus*) could be interpreted as wind-borne outdoor spores as they showed a significant positive correlation with wind speed ($r = 0.06$ to 0.31, p value < 0.1). Factor 2 (rust spore, ascospore, Asp/Pen) could be explained as mixed transport due to showing low/moderate correlation with wind speed ($r = 0.01$ to 0.13, p value < 0.1). Fungal species, like unidentified basidiospore, *Pithomyces*, *Curvularia*, and *Cladosporium* from Factor 3, were negatively correlated with wind speed. Therefore, the transport of these fungi is suggested to be a human-mediated inside the classroom. Factor 4 is characterized by *Periconia*, *Nigrospora*, and *Alternaria* spores, and they can be identified as indoor fungus.

3.9 Sources of fungal spore in library micro-environment

In library air, Factor 1 is loaded with *Torula* and *Alternaria*, and their probable source could be interpreted as wooden materials, and damp wall of the library. The fungus from factor 2 exhibits human-mediated transport from the outdoor air. Factor 3 representing Asp/Pen, *Cladosporium* and Basidiospore could be explained as cellulose degrading fungus. Previous studies in similar micro-environment indicate these fungus feed on paper, glue, and other cellulosic materials found inside a book [64].

3.10 Sources of fungal spore in institutional office room micro-environment

The PCA result of the institutional office room indicates a mixed source profile for fungal aero-spora. Factor 1 (*Torula*, *Pithomyces*, Asp/Pen, *Curvularia*) is principally contributed by indoor sources. These fungal spores showed a high positive correlation with both temperature and relative humidity ($r = 0.26$ to 0.53, and $r = 0.11$ to 0.44, p value < 0.1). Factor 2 and factor 3 can be identified as fungal spores with an outdoor origin. Some fungal spores in factor 2 and 3 exhibited a positive correlation ($r = 0.25$, p value < 0.1) with wind speed, whereas other spores displayed a negative correlation ($r = -0.01$ to -0.5, p value < 0.1).

The air inside the classroom, institutional office room, and bank micro-environments have shown the predominance of outdoor spores, contributing 85%, 75% and 65% of outside sources. Shoes, shirts, and human body help in transporting the spores indoor [39]. Moreover, transportation of fungal spore by the wind is found to be another reason to increase the indoor spore level [1]. On the contrary, the indoor ambiance of hospital and library promotes the growth of the fungi, residing inside the room [2, 68]. In such cases, indoor temperature and relative humidity were two significant factors playing a role in the growth of indoor fungi under suitable conditions. Within the factory, the same indoor and outdoor ambiance might be the result for an equal percentage of outdoor and indoor spores' source, correlated with total variance of PC. For a crop field, the cutting of trees releases a high amount of spores in the neighboring atmosphere and the spore peak is totally independent on meteorological factors [25].

3.11 Comparative analysis on the growth and seasonality of culturable fungi

The Andersen two-stage sampler measured comparatively lower number of culturable fungal spores (1366–2073 CFU m^{-3}) than the Burkard Personal sampler (1994–4477 spores m^{-3}). The Andersen sampler collects only those spores, which can show their growth under certain conditions. This is consistent with the previous study from eastern India [6]. From our sampling, a total of ten fungal genera (*Aspergillus*, *Alternaria*, *Curvularia*, *Cladosporium*, *Penicillium*, *Pithomyces*, *Chaetomium*, *Candida*, *Rhizopus* and *Fusarium*) and twenty fungal species were identified. Considering all the micro-environments, the comparative study on the average total concentration of culturable fungi is shown in Fig. 2b. Like total fungal spore level, culturable fungal spore count was highest in the

crop field (avg. conc. 2073 ± 654 CFU m^{-3}) and lowest in institutional office room (avg. conc. 1366 ± 377 CFU m^{-3}). The seasonal variation in growth of culturable fungi is presented in Fig. 3b. The overall median value of growth was observed to be maximum for the classroom (69.9%), followed by library (58.8%) and institutional office room (60.7%). Presence of nearby sources of fungi along with adequate indoor temperature and moisture (Online Appendix A, Table A.4) favors the growth of culturable fungi over these three micro-environments [44]. The lowest median for growth rate was found in hospital (42.8%) followed by an outdoor crop field (43.1%) during the sampling period. Previous studies have reported low growth rate in outdoor sites due to harsh environmental condition [35].

Season-wise analysis reveals that the substantial seasonal variation with elevated culturable fungal growth was found during summer and monsoon season in the classroom and institutional office room aero-mycospora. For both of these two environments, the median percentage of growth was near 83–90%. For *Aspergillus* and *Penicillium*, the peak of growth rate was found highest during monsoon, followed by summer. During summer, low relative humidity leads to a higher spore buoyancy and facilitates the transport of spores from outdoors [22, 40]. But during monsoon season, heavy precipitation worsens the water leaking condition, which in turn increases the wall dampness and creates a perfect situation to elevate their growth [32]. In hospital and library, a seasonal variation on the growth level of culturable fungi was not prominent due to the high contribution of indoor sources (~ 60–70%). It is reported that in indoor, occupants frequently release fungi from their hair, skin, and fingernails. These are the most important source of indoor airborne fungi [68]. Also, the poor ventilation and homogenous environment over these two sites help to keep a continual growth. Similarly, the presence of a continuous indoor source of fungi, dynamics of year-long human movement and suitable growth conditions induces less variability within bank micro-environment [15, 39]. In the crop field, harvesting month of paddy plant had a direct effect on the release of *Curvularia* spores, and it directly increases the growth of this fungal genera. *Aspergillus*, the second most prevalent culturable fungi in the crop field air, registered a very low level of growth, and it comes from a mixed source. Due to unceasing fungal source, no seasonal fluctuation was noticed for *Aspergillus*. In the factory aero-spora, a sudden drop in growth of culturable fungi was seen during winter periods. Low relative humidity (50.6 ± 7.8%) and drought periods may hinder the development of many fungi during this season. *Rhizopus oryzae*, being a most prevailing culturable fungus of the factory, gained the maximum concentration in the post-monsoon season. During this time, both high temperature and moderate relative humidity facilitates the release of spore from the sources as well as the mixing of spores to the air. Within the same micro-environment, the second most dominating contributor was *Aspergillus* sp. This species was at its peak during the summer when high temperature and dry air facilitates their transport from the sources.

3.12 Relationship between culturable fungi with patients' data

To assess the intensiveness of the exposure of bio-aerosol, 2335 study subjects were divided into three categories: students—spending 6–7 h of a day in the classrooms; factory workers—staying in their work an average of 8–10 h day^{-1}, and farmers—usually spending 10–12 h in the open crop field. Lastly, regression analysis was performed with the major culturable fungal spore concentration of the corresponding micro-environments (classroom, crop field, and factory). The exacerbations of fungal allergy are correlated well with the concentrations of culturable allergic fungi rather than the total fungal concentration [5, 6, 16, 45].

During the summer season, the hospitalization records indicate the maximum number of allergic rhinitis patients exhibited a moderate to strong (strong: $r \geq 0.8$; moderate: $r \geq 0.6$–0.79; low: $r < 0.6$; [54] positive correlation with *A. fumigatus* ($r = 0.71$, p value < 0.05), *A. terreus* ($r = 0.79$, p value < 0.05) and *P. oxalicum* ($r = 0.83$, p value < 0.05) (Fig. 6a). PCA analysis reveals an outside source for Asp/Pen in the factory micro-environment. On the other hand, the maximum number of asthmatic workers visited hospital mostly during winter and monsoon periods. Several studies have reported significant associations between asthma hospitalization and colder seasons as respiratory viruses may reinforce the potential health effect during winter seasons [14]. However, very intense exposure to respirable fungal allergens may be associated with asthma in occupational environments [11, 43]. *R. oryzae* was found to be correlated low to moderate ($r = 0.36$, 0.68; p value < 0.05) with the factory workers' asthmatic data for both winter and monsoon seasons.

Among the farmers, allergic rhinitis was found to be more common than asthma. The frequency of hospital visits and patients' admission was maximum during summer and monsoon seasons for farmers. In these two seasons, farmers' hospital admission were found to be positively related (low to average) to *A. fumigatus* ($r = 0.52$, 0.28; p value < 0.05) (Fig. 6b). Insignificant correlation with *Curvularia lunata* and hospitalization records indicates that this major species of cropland does not contribute too much in respiratory problem among the farmers. During the twentieth century, studies on farmers have shown that

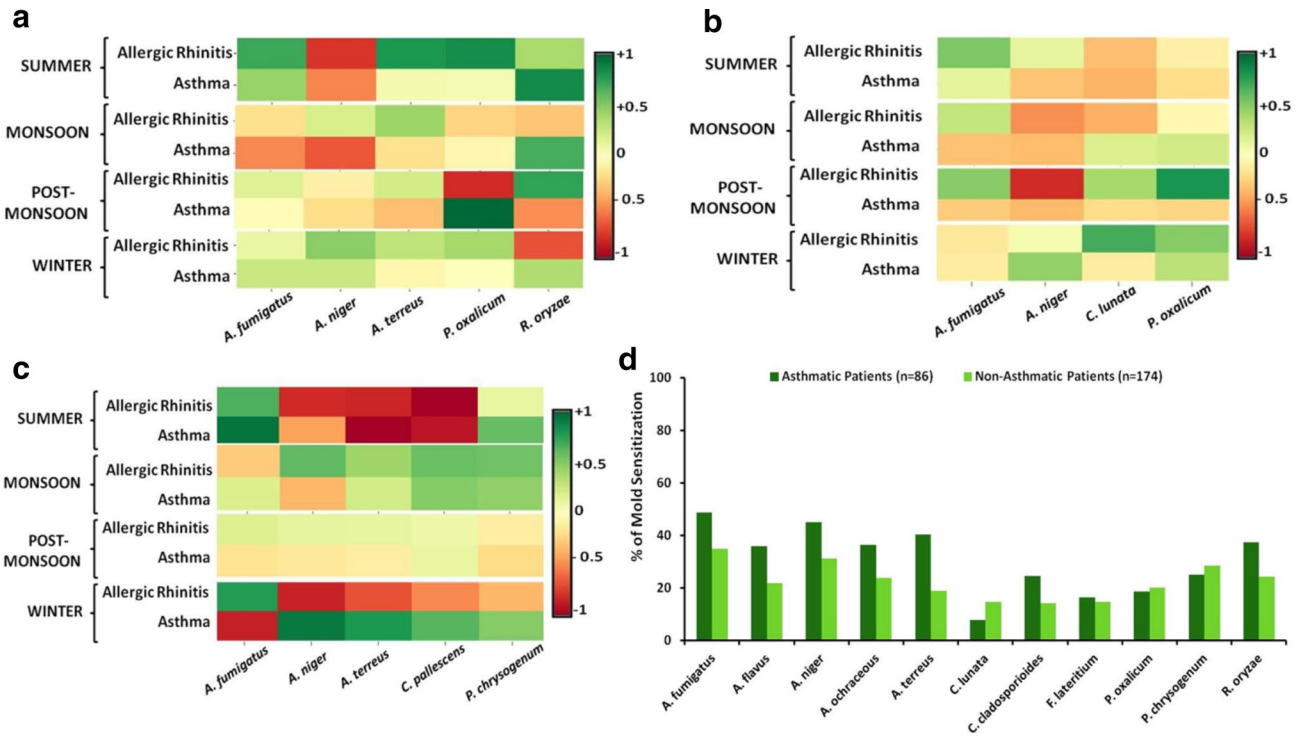

Fig.6 Heat-map showing correlation between patients' hospitalization data and concentration of culturable fungi (**a** for factory workers, **b** for farmers, and **c** for students), and **d** allergenicity assessment of individual dominant culturable fungi through SPT

agricultural exposures may result in higher rates of chronic pulmonary diseases [58].

In classroom micro-environment, a significant correlation was observed for *A. fumigatus* between asthmatic and allergic rhinitis patients during summer. During monsoon and winter, *A. niger*, *A. terreus*, and *Curvularia* showed a strong to a medium positive correlation with asthma and allergic rhinitis ($r = -0.36$ to 0.92, and $r = -0.85$ to 0.61; p value < 0.05) (Fig. 6c). The occurrence of these two diseases was observed to be much high during monsoon and winter, compared to other seasons. The origin of these fungi, as concluded from PCA, is majorly outdoor, and the high growth of these fungi within the classroom might help to build up disease-causing environment.

There are some limitations of the study as our findings are mainly confined on the fungal spore, while aeroallergens also include pollen grains. But fungal spores have a higher and consistent allergenicity effect than pollen grains over the general population. It is mainly because of two reasons: comparatively higher fungal spore concentration and being easily respirable spore size than pollen [26, 61]. Though many reports have shown positive associations of fungal concentration with multiple allergic and respiratory effects [6, 11, 13, 16], some studies do not support quantitative relationships between fungal exposure and allergy [12, 50].

3.13 Allergenicity assessment of dominant culturable fungus

A precise diagnosis of the fungal sensitization is essential in both atopic and asthmatic patients. Dominant culturable fungal species (Online Appendix A, Table A.5) were selected for the allergenicity assessment through SPT upon asthmatic as well as non-asthmatic patients, suspected to have a fungal allergy. Germination of spores might be the main cause to increase allergen release from some allergic fungi [31, 65]. Hence, the exposure to allergens might be the opportunity for spores to germinate after inhalation.

In the selected sampling area, 60% of a total of asthmatic patients ($n = 86$) and 49% of non-asthmatic patients ($n = 174$) were found to have sensitivity against one of those tested fungal species. Figure 6d represents that the percentage of atopicity to individual fungal species was higher among the asthmatic patients as compared to the non-asthmatic ones. The extract of *A. fumigatus* had shown the strongest fungal sensitization, with 48% in asthmatics and 35% in non-asthmatic patients. The Asian population with severe asthma has noted maximum allergenic reactivity in *Aspergillus* sp. through SPT [29, 30]. The highest degree of sensitization (3 +) was also found for *A. fumigatus* (Online Appendix A, Table A.6). A low percentage of

sensitivity was noticed to the extract of *C. lunata* (7.8% in asthmatics) and *C. cladosporioides* (14.3% in non-asthmatics). Around Ambala city of Haryana, northern India, *A. flavus*, *A. fumigatus*, and *Penicillium* sp. are reported to be as the major allergens through SPT among the patients with asthma, and allergic rhinitis [49]. But our findings unearthed *Penicillium* sp. to be as a most sensitive fungus in non-asthmatic patients. In previous studies, the risk of hypersensitivity to *Penicillium* is reported among asthmatic children than among adults [28].

Therefore, in this present study, the potential adverse health effect due to mold exposure and mold allergenicity was determined for asthmatic and non-asthmatic patients too. *Aspergillus* species, contributing up to 60% of total culturable fungi, were found to be the most likely active causative candidate for atopic as well as asthmatic patients.

4 Conclusions

In a semi-rural area of eastern India, studies to identify the possible sources of fungi, their overall contribution pattern in relation to meteorological parameters, patients-specific exposure and sensitization profile have never been attempted before. From this study, we can conclude that (1) fungal spores can have different potential sources; however, maximum vegetation coverage was the main reason for flourishing the highest spore concentration in a semi-rural area, especially for outdoor environments. On the contrary, the indoor environment had the mixture of possible spore sources, (2) the seasonal fluctuation on the total spore concentration was noticeable. In all the sampling micro-environments, maximum spore load during post-monsoon season might be due to the effect of meteorological factors, and crop harvesting. (3) In indoor, maximum median growth level for culturable fungi was due to the presence of suitable growth conditions. In this case, the seasonality did not affect them. (4) *Aspergillus sp*. and *Penicillium sp*. showed maximum correlation with allergic rhinitis and asthmatic patients among the factory workers, farmers, and students, and (5) Culturable fungi could be associated with allergic reactions among both asthmatic and non-asthmatic patients, respectively. *Aspergillus* species, contributing a maximum percentage of the total culturable fungi, were found to be the most likely potential allergenic candidate in both asthmatic and non-asthmatic patients. However, this study gives useful insight for the local people to plan their daily activities for lowering the aeroallergen exposure. In the future, the identification of fungal species using gene sequencing method would help to reveal more fungal species composition and richness as well from a semi-rural area of India. Still, the quantification of fungal spores may not reveal allergy development in all subjects. Hence, the serological detection and identification of major allergens of the culturable fungi would help to reduce the health risk through proper medication.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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Human and animals rights Yes.

Informed consent Yes.

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Fungal bio-aerosol in multiple micro-environments from eastern India: source, distribution, and health hazards.

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The following are the supplementary data:

Appendix A. Supplementary tables

A. 1: Characteristic features of the selected micro-environments.

Micro-environments	Features
Bank	It was located on the ground floor of a roadside building. Windows and doors remained open mainly during official hours.
Hospital	Sampling was done within the overcrowded outdoor-general ward of Habra State General Hospital, where a large number of patients came across from the local area and remote villages daily.
Factory	The sawmill environment were distinguished by the presence of sawdust, waste wood chips, and defoliated barks that were kept for prolonged periods in the open air. The normal seasoning and processing of wood logs was taking place here.
Crop field	Large open crop fields on both sides of Jessore Road, a national highway, connecting India to Bangladesh, was marked by the plantation of seasonal crops and the presence of the large trees on the edges of the crop field and roadside. Here, mainly two types of rice crops were cultivated: Autumn (Aus) rice crop and Summer (Rabi/Boro) rice crop, respectively, followed by the cultivation of winter crops, likely, mustards, onions, peas, cabbage, cauliflower, and beans.
Classroom	The classrooms of school as well as college of Habra, surrounded by grassland, ponds, and large trees were chosen. Most of the school and college-going students from Habra and near-by villages of the age range between 6-20 years came here for their education. The participating classrooms were naturally ventilated, having large open windows and doors. However, sampling was done during school and college hours.
Library	The stack room within the library of the selected college of Habra was chosen for sampling due to deficiency of cleanliness, dust, poor ventilation, non-air conditioning systems. The most striking features of the stack room were the presence of stored untouched dusty old books, journals; wooden racks, and cardboard.
Institutional office room	The sampling was done during regular office hours. This micro-environment was characterized by the presence of poster and paintings attached to the walls, files, furniture and continuous entry of people for official purposes. The surroundings were marked by the presence of an open lawn, with shrubs, and trees.

Table A. 1: Characteristic features of seven different micro-environments from a semi-rural area of eastern India.

A.2: Eigen Values from PCA Analysis

Micro-environments	Eigen Value of PC1	Eigen Value of PC2	Eigen Value of PC3	Eigen Value of PC4
Bank	2.79	1.89	1.46	1.23
Hospital	2.31	1.19	1.01	-
Factory	2.31	1.74	1.37	-
Crop field	4.66	2.87	1.89	1.31
Classroom	3.69	2.04	1.80	1.30
Library	2.25	1.97	1.34	-
Inst. office room	3.77	2.14	1.72	-

Table A. 2: The eigen values of each component for each micro-environment. (Inst. - Institutional)

A.3: Correlation Matrix of Total Fungal Spore

Spores	Bank	Hospital	Factory	Crop field	Classroom	Library	Inst. office room
Alternaria	-0.23	0.27	0.14	-0.13	0.01	0.02	-0.5
Ascospore	0.04	0.01	0.23	0.07	0.01	0.05	-0.07
Aspergilli group	-0.04	-0.07	0.026	0.07	0.13	-0.2	0.5
Basidiospore	0.18	-0.11	0.16	0.08	-0.05	-0.2	0.25
Chaetomium	-0.19	NA	-0.13	0.03	0.31	0.31	-0.3
Cladosporium	-0.33	-0.4	-0.31	-0.2	-0.12	0.12	-0.06
Coprinus	NA	NA	-0.26	-0.22	0.21	0.04	-0.25
Curvularia	-0.01	0.11	-0.14	0.01	-0.31	-0.29	0.01
Drechslera	-0.23	0.19	-0.41	0.04	NA	NA	0.12
Ganoderma	-0.21	0.25	-0.08	-0.36	-0.12	-0.2	0.32
Nigrospora	-0.54	-0.2	-0.5	-0.53	-0.34	-0.06	-0.5
Periconia	-0.06	-0.25	0.1	0.05	-0.1	-0.21	-0.06
Periconiella	-0.14	0.42	0.23	0.04	0.02	0.18	-0.19
Pithomyces	-0.3	0.19	-0.24	-0.5	-0.17	0.07	-0.02
Pleospora	NA	NA	NA	-0.3	NA	NA	NA
Podospora	-0.25	0.01	-0.22	-0.21	0.18	0.01	-0.14
Rust spore	-0.18	0.05	0.12	0.11	0.09	0.07	-0.01
Torula	-0.01	0.37	-0.05	-0.16	0.06	0.15	0.16
Trichoconis	NA	NA	-0.4	-0.34	-0.1	-0.07	-0.32

Table A. 3: Correlation matrix of total fungal spore (spores' selection* based on PCA) with wind speed over different micro-environments (Inst. - Institutional) [*Selected spores contributed above 90% to the total spore concentration].

A.4: List of Meteorological Parameters

		Bank	Hospital	Factory	Crop field	Classroom	Library	Inst. Office room
Summer	M. T.	28	27.3	39	40.5	27.5	27	28
	SD.	2	1.5	2	2.1	1.2	1	1.6
	M. R. H.	42	41	32	34	42	45	41
	SD.	4.5	3.7	6.6	8.5	3.5	2	2.3
	M. T.	27.5	28	35.2	36	27	26.5	27.5

	SD.	1.5	1.6	1.5	2.5	1.3	1.5	1.3
Monsoon	M. R. H.	48.3	49	68	70	48	47	48
	SD.	3.4	3.5	5	4.7	2.3	1.6	1.8
	M. T.	27.5	27	38	39	28	27	27.6
Post-Monsoon	SD.	2.1	2	2.4	3.2	2.2	2	1.8
	M. R. H.	43	44	41	51	40	39	40
	SD.	3	2.1	5.8	10.2	2.5	1.5	1.7
	M. T.	23.4	23	29	31	22.3	21	22
	SD.	1.6	1.9	2.1	3.2	2	2.5	2
Winter	M. R. H.	25	24.5	32	36	26	25	24.8
	SD.	3.2	3.5	3.2	2.9	4.7	3.4	3.8

Table A. 4: List of mean temperature (M.T.) and mean relative humidity (M.R.H.) with standard deviation (S.D.) in seven micro-environments. [Meteorological parameters (such as temperature and relative humidity) were recorded using a portable Wet and Dry Bulb Hygrometer (G.H. Zeal, London, UK). Other factors, like precipitation and wind speed, were collected from online website (https://www.worldweatheronline.com/habra-weather-history/west-bengal/in.aspx)].

A.5: Dominant Culturable Fungal Contribution

Dominant culturable fungal species	% Contribution of dominant culturable fungi		
	Factory	Crop field	Classroom
Aspergillus fumigatus	10.9	13.4	26.2
Aspergillus flavus	3.4	1.8	4.3
Aspergillus niger	17.6	7.4	20.66
Aspergillus ochraceous	0.46	0.83	1.9
Aspergillus terreus	4.0	4.2	8.2
Curvularia lunata	2.8	45.4	8.6
Cladosporium cladosporioides	0	0.5	0.7
Fusarium lateritium	3.5	0.8	0.7
Penicillium oxalicum	7.3	18.8	2.4
Penicillium chrysogenum	0	0	6
Rhizopus oryzae	41.3	2	5.8

Table A. 5: Percentage (%) contribution of dominant culturable fungi in three micro-environments.

A. 6: Skin Prick Test Result

	Asthmatic Patients (n=86)				Non-asthmatic Patients (n=174)			
Fungal species	% of positive response	1+ (%)	2+ (%)	3+ (%)	% of positive response	1+ (%)	2+ (%)	3+ (%)
A. fumigatus	48.7	19.7	15.5	13.5	34.9	15.7	11.3	7.9
A. flavus	35.9	15.3	14.3	6.3	21.9	14.8	7.1	0
A. niger	45.1	19.1	14.8	11.2	31.2	16.6	9.8	4.8
A. ochraceous	36.4	18.5	15.8	2.1	23.9	14.5	7.4	2
A. terreus	40.4	18.6	13.6	8.2	19	11.6	6.2	1.2
C. lunata	7.8	5.8	2	0	14.7	11.2	2.6	0.9
C. cladosporioides	24.5	13.7	7.8	3	14.3	10.6	3.7	0

F. lateritium	16.6	9.7	4.8	2.1	14.7	11.4	3.3	0
P. oxalicum	18.8	11.7	7.1	0	20.2	12.2	6.9	1.1
P. chrysogenum	25.2	13.7	9.1	2.4	28.6	16.3	11	1.3
R. oryzae	37.4	24.1	11.2	2.1	24.4	12.2	10.1	2.1

Table A. 6: Skin Prick Test result showing allergic sensitivity to each fungal species among asthmatic and non-asthmatic patients. [Phosphate buffer saline (0.01 M, pH 7.2) and histamine di-phosphate (1mg ml^{-1}) were taken as negative and positive controls, respectively].

Appendix B. Calculation for spore count

<u>Calculation for total fungal spores sampled by Burkard personal slide sampler:</u>

The suction rate of the sampler = 10 L min^{-1}
Duration of the sampling = A min (eg. 5 minutes)
Amount of air sampled = 10 X A (10L/min X 5 min)
= Y Lit. (50 L)
Let the number of spores recorded = Z
Thus the numbers of spores recorded / m^3 of air = Z / (Y X 10^{-3}) ($1m^3$ = 1000 L)
=1000 Z /Y (50) ($1m^3$ = 1000 L)
= 20 X Z m^{-3} of air

Hence, the conversion factor of 5-min sampling is -- 20 X number of spores

<u>Calculation for culturable fungi sampled by Andersen two-stage sampler:</u>

Suction rate of the sampler = 28.3 L min^{-1} ($1m^3$ = 1000 L).
If operated for 10 minutes, air is taken = 0.283 m^3 [(28.3 X 10)/1000 m^3]

If 0.283 m^3 of air forms 1 colony
Then 1 m^3 of air forms 1/0.283 = 3.53 colonies
So, the conversion factor for 10-minute operation is -- 3.53
The spore count is represented as = CFU (colony forming unit) m^{-3}

Appendix C. Questionnaire Form

QUESTIONNAIRE FOR SURVEY OF OCCUPATIONAL AND NONOCCUPATIONAL RESPIRATORY DISORDERS DUE TO ENVIRONMENTAL HAZARDS (FUNGAL SPORES AND OTHER FACTORS)

Bose Institute, 93/1 Acharya P.C. Road, Kolkata-700 009, West Bengal, (India)

STUDY QUESTIONNAIRE
(পাঠ্য প্রশ্নমালা)

IDENTIFICATION AND DEMOGRAPHIC DATA (সনাক্তকরণ এবং পরিসংখ্যান তথ্যাবলী)

1. Centre Code (কেন্দ্রের নাম) ☐☐
2. Date of Survey (পর্যবেক্ষণের তারিখ) ☐☐ / ☐☐ / ☐☐☐☐
3. Current Residence (বর্ত্তমান বাসস্থান) Rural / Urban গ্রাম / পৌরসভা ☐
4. Address and phone (ঠিকানা এবং ফোন নম্বর)
5. Name (নাম)
6. Date of Birth (জন্ম তারিখ) ☐☐ / ☐☐ / ☐☐☐☐
7. Age (in years) বয়স (বৎসর অনুযায়ী) ☐☐
8. Sex (লিঙ্গ) Male / Female পুরুষ / মহিলা ☐
9. What has been your usual residence, where you have lived >75% of your life ? Rural / Urban / Mixed গ্রাম / শহর / দুই-ই ☐

   (আপনার নিয়মিত বাসস্থান যেখানে আপনি পঁচাত্তর ভাগ জীবন অতিবাহিত করিয়াছেন?)
10. For how may years have you received education? ☐☐

    (কত বৎসর যাবৎ আপনি লেখাপড়া করেছেন?)
11. **What is the occupation of the head of household ?** ☐

    (পরিবারের গৃহকর্তার জীবিকা কি?)

    (1) Unemployed or retired (কর্ম্মহীন অথবা অবসর প্রাপ্ত)

    (2) Housewife (গৃহবধূ)

    (3) Unskilled labourer (অদক্ষ শ্রমিক)

    (4) Skilled labourer (দক্ষ শ্রমিক)

(5) Business / Self employed professional (ব্যবসা /স্বনিযুক্ত পেশাদার)

(6) Agriculturist (কৃষিজীবি)

(7) Worker in a government or private service (সরকারী অথবা বেসরকারী কর্ম্মে নিযুক্ত কর্মচারী।)

(8) Supervisor in a government or private service (সরকারী অথবা বেসরকারী কাজ দেখাশুনা করেন)

(9) Officer in a gevernment or private service (সরকারী অথবা বেসরকারী কর্ম্মে নিযুক্ত উচ্চপদস্থ কর্ম্মচারী)

12. **What is your occupation ? (আপনার জীবিকা কি?)** ☐

(1) Unemployed or retired (কর্ম্মহীন অথবা অবসর প্রাপ্ত)

(2) Housewife (গৃহবধূ)

(3) Unskilled labourer (অদক্ষ শ্রমিক)

(4) Skilled labourer (দক্ষ শ্রমিক)

(5) Business / Self employed professional (ব্যবসা / স্বনিযুক্ত পেশাদার)

(6) Agriculturist (কৃষিজীবি)

(7) Worker in a government or private service (সরকারী অথবা বেসরকারী কর্ম্মে নিযুক্ত কর্মচারী।)

(8) Supervisor in a government or private service (সরকারী অথবা বেসরকারী কাজ দেখাশুনা করেন)

(9) Officer in a gevernment or private service (সরকারী অথবা বেসরকারী কর্ম্মে নিযুক্ত উচ্চপদস্থ কর্ম্মচারী)

RESPIRATORY SYMPTOMS (শ্বাস - প্রশ্বাস জনিত কষ্টের লক্ষণ)

Please answer Yes or No. If Yes, specify duration of symptoms (years)

(উত্তর দিতে হবে হ্যাঁ অথবা না। যদি হ্যাঁ হয় তবে নির্দিষ্ট করিয়া উপসর্গগুলির স্থায়ীত্ব উল্লেখ করতে হবে) (কত বছর)

Years

WHEEZING AND TIGHTNESS IN THE CHEST (বুকে সাঁই সাঁই শব্দ ও দম আটকানো ভাব)

13.	Have you ever had wheezing or whistling sound from your chest during the last 12 months ?	Yes / No	☐	☐☐
	গত বারো মাসের মধ্যে বুকে সাঁই-সাঁই বা বাঁশির মত শব্দ হয় কিনা?	হ্যাঁ / না		
14.	Have you ever woken up in the morning with a feeling of tightness in the chest or of breathlessness ?	Yes / No.	☐	☐☐
	বুকে চাপ ভাব নিয়ে ঘুম ভাঙ্গে কিনা?	হ্যাঁ / না		

SHORTNESS OF BREATH (শ্বাস প্রশ্বাসে ঘাটতি)।

15.	Have you ever felt shortness of breath after finishing exercises, sports or other heavy exertion during the last 12 months ?	Yes / No.	☐	☐☐
	কোনো ব্যায়াম, খেলাধূলা অথবা শ্রমসাধ্য কাজ করার পর শ্বাসকষ্ট হয়েছে কিনা গত বারো মাসের মধ্যে ?	হ্যাঁ / না		

16. Have you ever felt shortness of breath when you were not doing some strenuous work during the last 12 months ? Yes / No ☐ ☐☐

(কোন পরিশ্রম না করা অবস্থাতেও শ্বাসকষ্ট হয়েছে কিনা গত বারো মাসের মধ্যে) হ্যাঁ / না

17. Have you ever had to get up at night because of breathlessness during the last 12 months ? Yes / No ☐ ☐☐

(বিগত বার মাসে রাত্রিতে শ্বাস কষ্টের জন্য কখনও উঠে বসতে হয়েছে কিনা?) হ্যাঁ / না

COUGH AND PHLEGM (কাশি ও কফ্)

18. Have you ever had to get up at night because of cough during the last 12 Months ? Yes / No ☐ ☐☐

(বিগত বারো মাসে আপনি কি কাশির জন্য রাত্রে উঠে পরেছেন?) হ্যাঁ / না

19. Do you usually cough first thing in the morning ? Yes / No ☐ ☐☐

(ভোরে ঘুম ভাঙার পরেই কাশি হয় কিনা?) হ্যাঁ / না

20. Do you usually bring out phlegm from your chest first thing in the morning ? Yes / No ☐ ☐☐

(ভোরে ঘুম থেকে ওঠার পর কফ উঠতে থাকে কি?) হ্যাঁ / না

21. Do you usually bring up phlegm from your chest most of the morning for at least 3 consecutive months during the year ? Yes / No ☐ ☐☐

(বছরে অন্তত লাগাতার তিন মাস ধরে আপনার বেশির ভাগ সকালেই কফ উঠে কি?) হ্যাঁ / না

BREATHING (শ্বাস - প্রশ্বাস)

22. Select the most appropriate out of the following ☐ ☐☐

(নিম্নলিখিত বিররণ থেকে সঠিক নির্ণয় করুন)

a) I hardly experience shortness of breath.

ক) আমার কখনই শ্বাসকষ্ট হয় না।

(b) I usually get short of breath but always get well

খ) আমার শ্বাস কষ্ট হয়, তবে কমেও যায়।

(c) My breathing is never completely satisfactory

গ) আমার শ্বাস-প্রশ্বাস কখনোই সম্পূর্ণ স্বাভাবিক নয়

DUST, FEATHERS AND PETS (ধূলো, পাখির পালক এবং পোষ্য প্রানী)

23. When you are exposed to dusty area, or pets like dog, cat or horse,

or feathers or quilts or pillows, etc., do you

(যদি আপনি ধূলো বালি অথবা পোষা কুকুর, বিড়াল, ঘোড়া, পাখীর পালক কিংবা লেপ বালিশ ইত্যাদির সংস্পর্শে আসেন তবে আপনার -

	(a) Feel tightness in chest ?	Yes / No	☐	☐☐
	ক) বুকের মধ্যে চাপ চাপ লাগে কি?	হ্যাঁ / না		
	(b) Feel shortness of breath ?	Yes / No	☐	☐☐
	খ) শ্বাস কষ্ট হয় কি?	হ্যাঁ / না		

ASTHMA (হাঁপানি)

24.	Have you ever suffered from asthma ?	Yes / No	☐	☐☐
	আপনার কখনও হাঁপানি হয়েছে কি?	হ্যাঁ / না		
25.	Have you ever had an attack of asthma the last 12 months ?	Yes / No	☐	☐☐
	গত বারো মাসের মধ্যে আপনার কখনও হাঁপানির টান হয়েছে কি?	হ্যাঁ / না		
26.	Are you taking any inhaler, pump, rotahaler or nebulizer or other medicines for treatment of breathlessness ?	Yes / No	☐	☐☐
	(আপনি কি শ্বাসকষ্টের জন্য ইন্‌হেলার, পাম্প, রোটাহালার, নেবুলাইজার অথবা কোন ওষুধ ব্যবহার করেন?)	হ্যাঁ / না		

<u>ATOPY AND FAMILY HISTORY</u> (পারিবারিক ইতিহাস)

27.	Do you often develop skin rash (such as urticaria or eczema) which come and go off and on ?	Yes / No	☐	☐☐
	(আপনার চর্মরোগ (র‍্যাশ, একজিমা, আমবাত) মাঝে মাঝে হয় কি?)	হ্যাঁ / না		
28.	Do you often develop sneezing or running nose ?	Yes / No	☐	☐☐
	(আপনি কি প্রায়ই হাঁচি অথবা নাক দিয়ে জল পড়ায় ভোগেন ?)	হ্যাঁ / না		
29.	Do you often develop itchiness in eyes ?	Yes / No	☐	☐☐
	(আপনার চোখে কি প্রায়ই চুলকানি হয়?)	হ্যাঁ / না		
30.	Do any of your family members have any of the above three symtoms ?	Yes / No		
	(আপনার পরিবারের কোনো সদস্যের ওপরের তিনটি উপসর্গের কোনোটি হয় কি?)	হ্যাঁ / না		
31.	If yes, who ? (যদি হয় তবে তিনি কে?)			
	Grandparents / parents / brother / sister / children / others			
	(দাদু-ঠাকুরমা / পিতা-মাতা / ভাই / বোন / বাচ্চারা / অন্যান্য)			
32.	Do any of your family members suffer from asthma ?	Yes / No	☐	☐☐
	(আপনার পরিবারের কারোর হাঁপানি আছে কি?)	হ্যাঁ / না		

33. If yes, who ? (যদি হয় তবে তিনি কে?) ☐
Grandparents / parents / brother / sister / children / others
(দাদু-ঠাকুরমা / পিতা-মাতা / ভাই / বোন / বাচ্চারা / অন্যান্য)

TOBACCO SMOKING AND CONSUMPTION (ধূমপান এবং তামাকের ব্যবহার)

34. What form of tobacco do/did you predominantly smoke ? ☐
(কোন ধরণের ধূমপানে আপনি প্রাধান্য দেন বা দিয়েছেন ?)
(1) Cigarette (2) Bidi (3) Hookah (4) Cigar (5) Pipe (6) Others
১) সিগারেট ২) বিড়ি ৩) হুক্কা ৪) সিগার (চুরুট) ৫) পাইপ ৬) অন্যান্য

35. How many cigarettes, bidis, cigar, pipe, hookah or chillum do you/did you usually smoke in 24 hours ? ☐☐
(আপনি ২৪ ঘন্টায় কতগুলি সিগারেট, বিড়ি, সিগার, পাইপ, হুক্কা ঐ ধরণের ধূমপান করেন?)

36. At what age did you start smoking ? ☐☐
(কোন বয়স থেকে আপনি ধূমপান সুরু করেছেন?)

37. Specify the number of years for which left smoking ☐☐
(ঠিক কত বছর হলো ধূমপান ছেড়েছেন?)

ENVIRONMENTAL TOBACCO SMOKE EXPOSURE (পরোক্ষ ধূমপান)

38. Do/did any of your family members (i.e. people residing in the same household) regularly smoke in your presence ? Yes / No ☐
(আপনার উপস্থিতিতে পরিবারের কেউ ধূমপান করেন কিনা?) হ্যাঁ / না

COOKING FUEL (রান্নার জ্বালানী)

39. At what age did you start cooking regularly ☐☐
(কোন বয়স থেকে আপনি নিয়মিত রান্না করা আরম্ভ করেছেন)

40. Does your house have a separate kitchen ? Yes / No ☐
(আপনার বাড়ীতে কি আলাদা রান্নাঘর আছে?) হ্যাঁ / না

41. Does the kitchen have windows/openings ? Yes / No ☐
রান্নাঘরে জানালা / ঘুলঘুলি আছে কি? হ্যাঁ / না

42. How many hours do you daily spend in the kitchen ? ☐☐
দিনে কত ঘন্টা আপনি প্রতিদিন রান্নাঘরে কাটান?

43. What cooking fuel do you predominantly use for cooking ?

রান্নার জন্য আপনি কি জ্বালানী ব্যবহার করেন?

a) Electricity (বিদ্যুৎ)
b) LPG (গ্যাস)
c) Kerosene (কেরোসিন)
d) Coal (কয়লা)
e) Wood (কাঠ)
f) Dung cake (ঘুঁটে)
g) Crop residue (তুষ)
h) Others (অন্যান্য)

TO BE FILLED BY THE INTERVIEWER
সাক্ষাৎকার গ্রহণকারী দ্বারা পূরন করিতে হইবে।

44. What, in your perception, is the overall socia-economic status of the individual ?

1. Low

2. Middle

3. High

আপনার কি উপলব্ধি? ইনি

(১) নিম্নবিত্ত

(২) মধ্যবিত্ত

(৩) উচ্চবিত্ত

মোটামুটি কোন আর্থসামাজিক ভুক্ত?

Form filled by (ফরম পূরণ কারী) _____

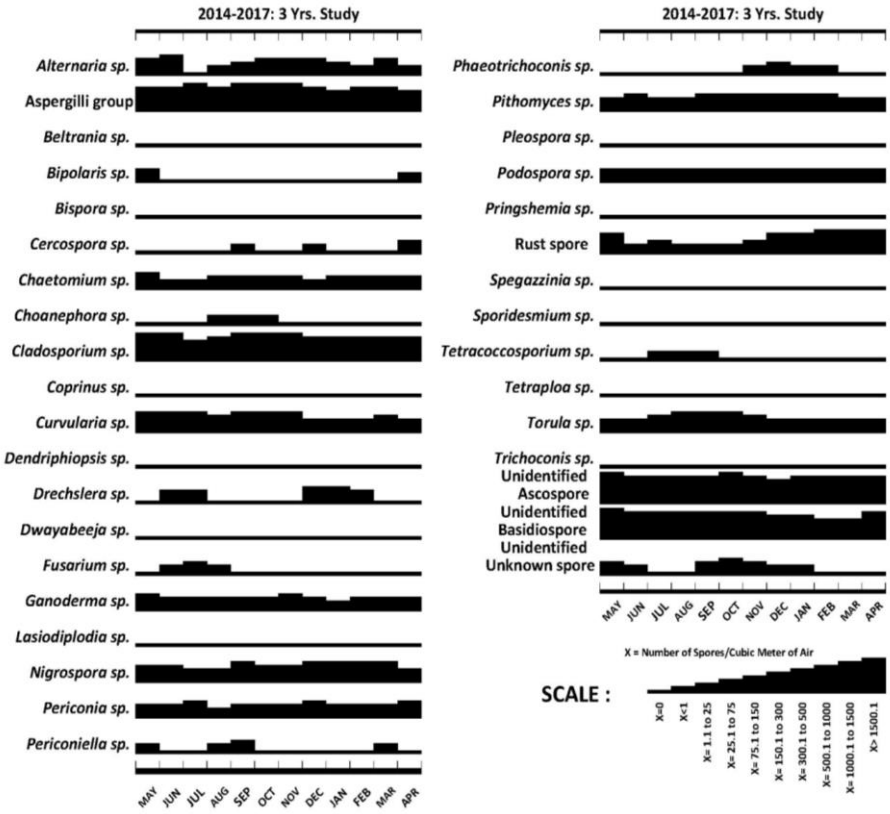
Data verified by (তথ্যাবলী যাচাইকারী) _____

Data entered into computer by
(তথ্যাবলী কম্পুটারে লিপিবদ্ধকারী) _____

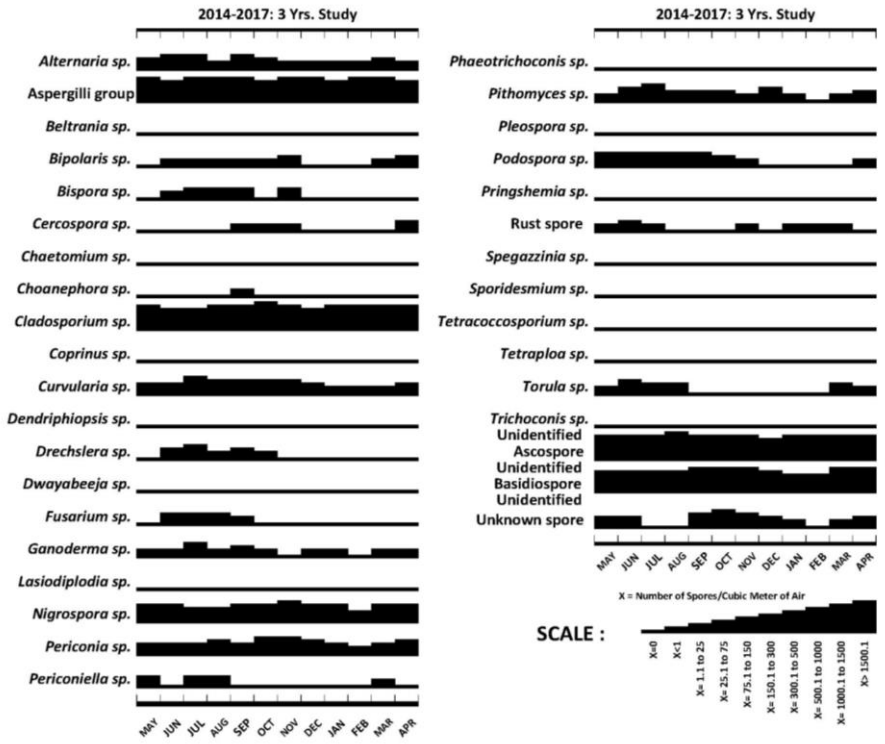
Appendix D. Supplementary Figures

Calendars:

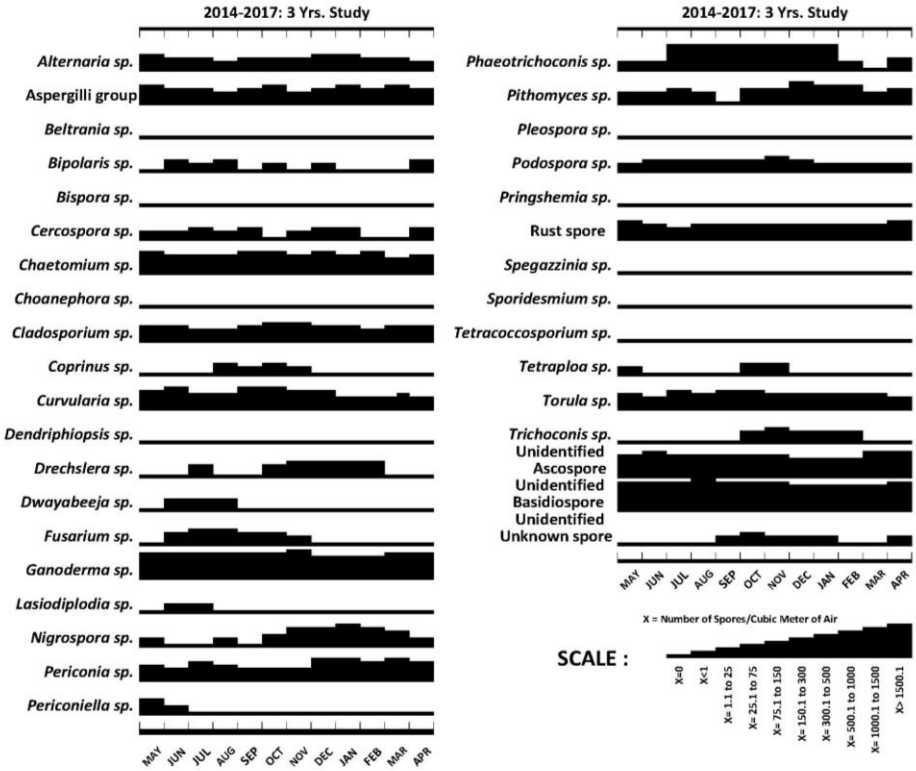
D.1: BANK



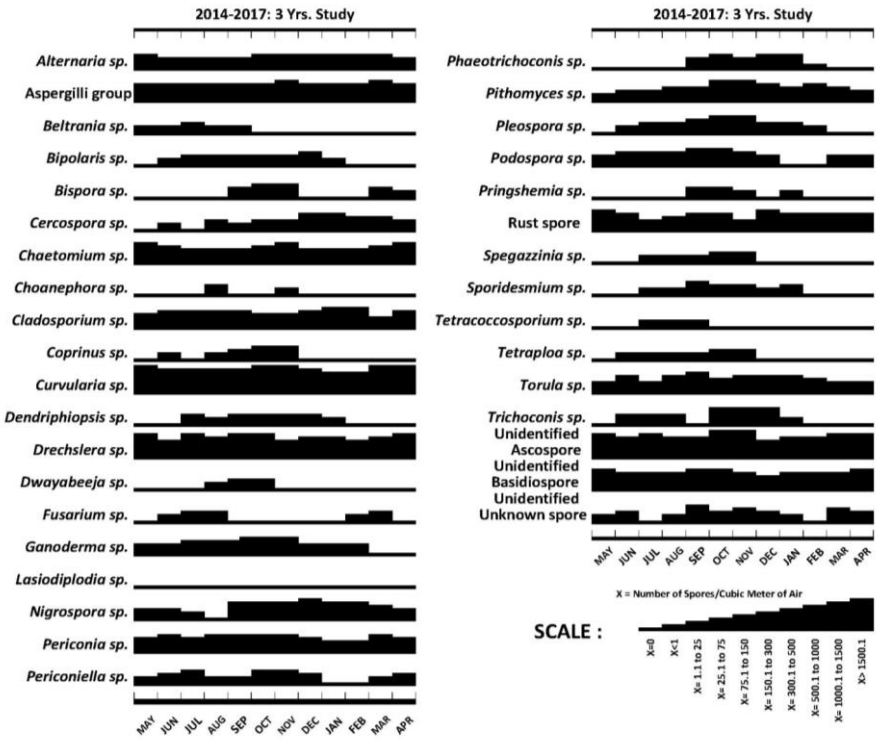
D.2: HOSPITAL



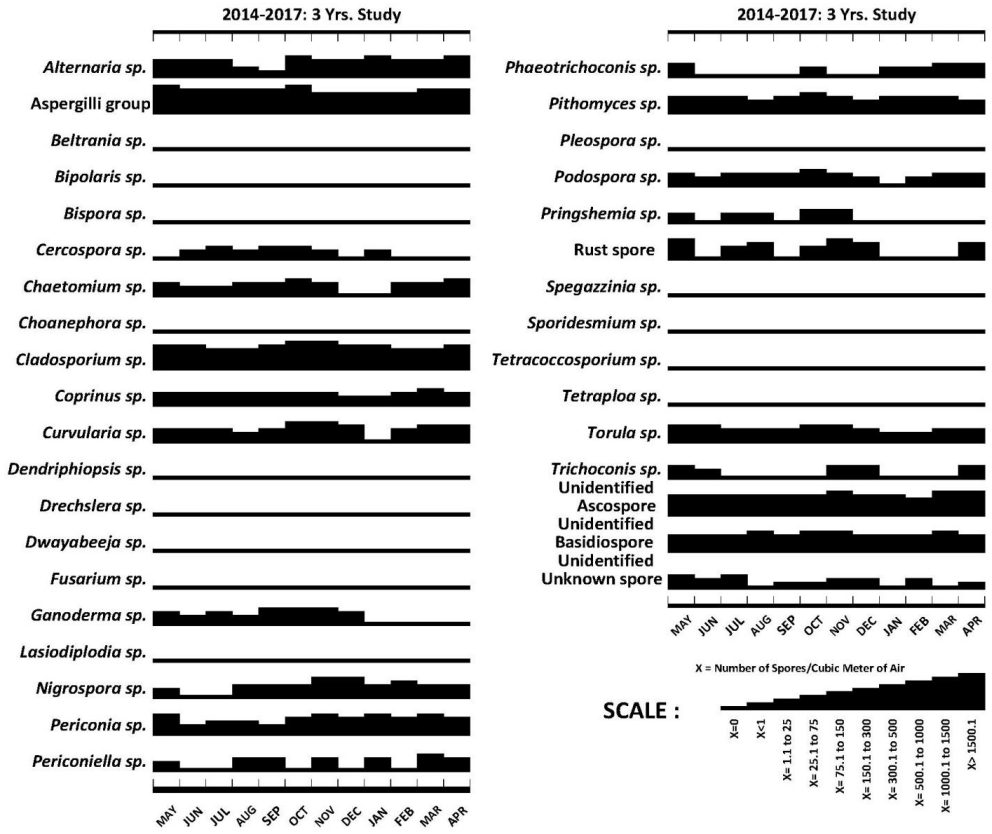
D.3: FACTORY



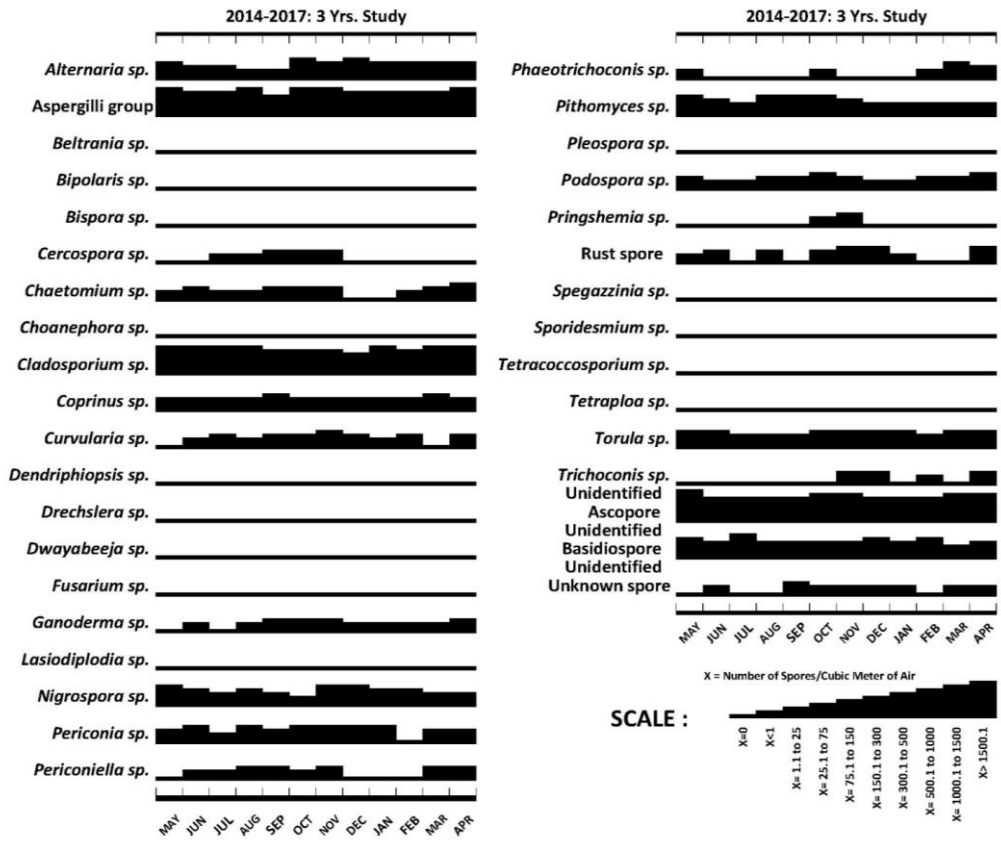
D.4: CROP FIELD



D.5: CLASSROOM



D.6: LIBRARY



D.7: INSTITUTIONAL OFFICE ROOM

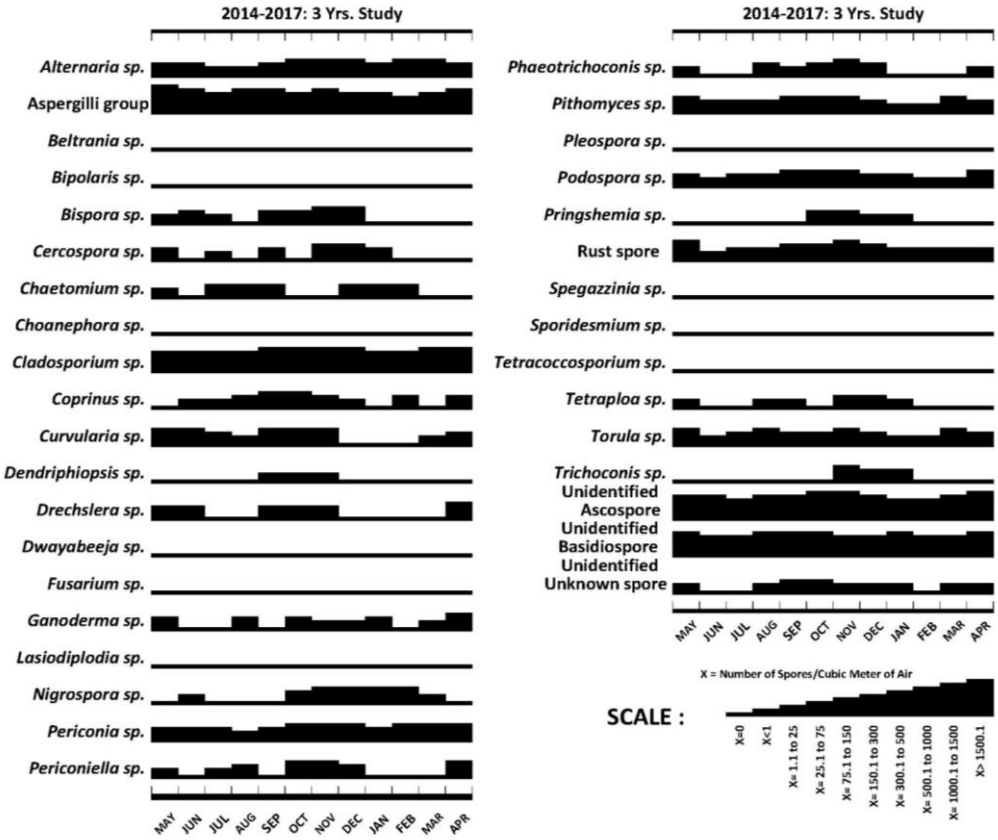


Fig. D.1-7: Calendars from different micro-environments of Habra, a semi-rural area of eastern India.