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# Analyzing airborne fungal concentration in Kolkata, India: temporal distribution, the effect of atmospheric parameters and health impact

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**Abstract**
Airborne fungal spores constitute a significant fraction of atmospheric bioparticles, and most of them are responsible for causing the respiratory allergy. The present study deals with the evaluation of fungal aerospora by microscopy-based and culture-based methods in one outdoor and six indoor microenvironments in Kolkata, India, from May 2014 to April 2017. The association of environmental parameters with spore concentrations was explored by Spearman's rank correlation analysis and multiple regression analysis. The impact of spore concentrations on the local population was assessed through a questionnaire survey, linear regression analysis, and Skin Prick Test (SPT). The maximum spore concentration was found in the outdoor environment. Ascospores, *Cladosporium* spp., *Aspergillus*/*Penicillium* spp., and basidiospores were found as major taxa recorded by microscopy-based method, whereas in culture-based method, *Aspergillus* spp. were abundant. In the outdoor, particles with aerodynamic diameter < 10 μm ($PM_{10}$) and in indoors, temperature, relative humidity, wind speed, average sun hour, $PM_{10}$, and ambient nitrogen dioxide concentration ($NO_2$) were identified as significant predictors. The linear regression analysis showed several positive associations of major taxa with respiratory diseases in the local inhabitant. SPT with several fungi was able to induce allergic inflammation in a selected atopic patient cohort. Analysis of spore concentrations and their relation with environmental parameters will give an insight into the air quality in Kolkata. The association with respiratory diseases will shed a light on the increasing burden of airway diseases in the urban megacity. Observations from this study will be useful for assessing the potential health impact on residents.

**Keywords** Airborne fungi · Indoor environments · Air pollutants · Correlation · Multiple regression analysis · Respiratory diseases

## Introduction

Exposure to airborne bio-particulate matters, such as fungal spores and pollen grains, commonly termed as "bioaerosols" results in a variety of adverse health effects including allergic disorders like severe asthma, allergic rhinitis, anaphylaxis, allergic broncho-pulmonary diseases, and other respiratory diseases. Fungi, being ubiquitous, contribute approximately 4–11% of ambient bioaerosols in the urban and rural environment (Womiloju et al. 2003) and are known to cause major health risks by having a greater impact on human health as they are associated with a broad panel of diseases including IgE-mediated type I hypersensitivity, life-threatening primary and secondary infections in immunocompromised patients, allergic bronchopulmonary mycosis (ABPM), hypersensitivity pneumonitis, fungal sinusitis, and toxic pneumonia. Additionally, they can produce a variety of mycotoxins having neurotoxic, mutagenic, carcinogenic, and teratogenic effects and cause mycotoxicoses (Simon-Nobbe et al. 2008). They also produce proteases, enzymes (Kauffman et al. 2000), and volatile organic compounds (Fischer et al. 1999) which may affect airways (Simon-Nobbe et al. 2008). Moreover, they can colonize inside the human body. Approximately, 1–1.5 million fungal species exist across the world of which 80,000 have been identified so far (Teresa et al. 2015). Fungi are common in the ambient air, and their small spore size (average size: 2–10 μm; Simon-Nobbe et al. 2008) leads to the enhancement of their dispersion in the atmosphere and deposition in the human respiratory tract as

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well (Yamamoto et al. 2012). About 30% of the total allergic patients are sensitized by fungal aeroallergen–induced allergy, imposing a major public health concern (Kurup et al. 2000; Singh and Shahi 2008). Till now, 113 fungal allergens from 30 fungal genera have been officially recognized by the World Health Organization and International Union of Immunological Society (WHO/IUIS) Allergen Nomenclature Sub-committee (www.allergen.org). Approximately, 112 genera especially belonging to Ascomycota and Basidiomycota phylum are considered as the possible source of allergens to induce type I hypersensitivity reactions (Teresa et al. 2015).

The influence of climate change on the prevalence and temporal distribution of airborne fungi in various countries has been documented in recent studies (Fernández-Rodríguez et al. 2018). Atmospheric variables influence the fungal spectrum of a specific area. Various studies have demonstrated the effect of meteorological parameters and air pollutants on ambient fungal spore concentrations in different geographic regions (Kallawicha et al. 2017; Pyrri and Kapsanaki-Gotsi 2017). Both indoor and outdoor fungal aerospora can be able to induce sensitization in atopic patients (Green et al. 2006). Therefore, to evaluate the exposure of ambient spore load, it is important to explore the diversity of fungal spores and their seasonal variation in outdoor as well as indoor environments, as the outdoor environments directly influence indoor environments (D'Amato et al. 2015).

In the present study, three-year-long biomonitoring was conducted in outdoor and several indoor environments of the Kolkata megacity. The aim of this study was the quantification of the fungal aerospora by monitoring in the given environments to investigate their temporal distribution and association with environmental factors and to determine the impact of their allergenicity on the health of the local population regarding respiratory allergy. Previously, several studies have been performed only in the outdoor environment of an urban area (Dey et al. 2018) and several suburb areas in Kolkata (Chakrabarti et al. 2012; Chakraborty et al. 2003; Das and Gupta-Bhattacharya 2012). This study was the first attempt to evaluate fungal aerospora in the different indoor areas along with the outdoor atmosphere in the core of the urban area in Kolkata and to investigate the impact of atmospheric pollutants on the spore concentrations.

## Materials and methods

### Study area and sampling sites

The aerial concentration of fungal spores was monitored from May 2014 to April 2017 in Kolkata. Kolkata (22.57° N and 88.37° E), situated on the Indo-Gangetic plain of West Bengal, is one of the largest urban megacities of India. It is the seventh-most populous city in India (Census India 2011) with a population density of 24,252/km$^2$ and a male to female ratio of 1000:899. The study area is thickly populated and crowded with all civic amenities. Many institutional, commercial, and residential buildings along with the congested marketplace and garden are present in and around the area. The connecting roads have high traffic throughout the day. Several weeds, herbs, shrubs, and large trees including ornamental trees are present in the proximity of the sampling area. Seven microenvironments were selected as sampling sites in this study area. Among them, school, university, and library were chosen for the educational area, whereas a hospital, bank, and an institutional workshop were categorized under the occupational area. Along with these six indoor environments, a garden with an open field area was also selected as an outdoor environment. The geographic locations of selected sampling sites are presented in Fig. 1. The characteristic features of each site are described in Online Resource 1.

### Sampling and identification of airborne fungal spores

Biomonitoring of fungal aerospora in different indoor and outdoor environments was executed by using Burkard personal volumetric sampler (Burkard Manufacturing Co. Ltd. Hertfordshire, UK) as well as Andersen two-stage sampler (Thermo Andersen, Smyrna, USA). The sampling was carried out at a height of 1.5 m above the ground level between 12 noon and 3 pm once every week of a month. Monthly average values of spore concentrations recorded by both samplers were considered for analysis.

Total fungal spores were trapped onto vaseline-coated glass slides placed inside the Burkard personal volumetric sampler (suction rate: 10 l/min). The sampling time was standardized for 15 min. Exposed slides were mounted with a coverslip and Dibutylphthalate Polystyrene Xylene (DPX). Trapped spores were observed by using Nikon high-resolution light microscope (Labophot 2, Nikon Corp., Japan) at 400x magnification. The spores were identified up to the genus level by using standard manuals (Ellis 1971; Smith et al. 1981) and counted according to the guidelines of The British Aerobiology Federation (1995). Spores that were difficult to identify were considered as "Others". Finally, total spore counts were converted into a number per cubic meter of air (m$^3$) by multiplying with the conversion factor of 6.67.

Ambient fungal spores were captured by using Andersen two-stage sampler (suction rate: 28.3 l/min) through the orifice at the top. Spores were trapped on petri plates containing potato dextrose agar (PDA) medium supplemented with chloramphenicol (34 µg ml$^{-1}$) (SRL, India) for preventing bacterial growth. After 3 min of exposure, the petri plates

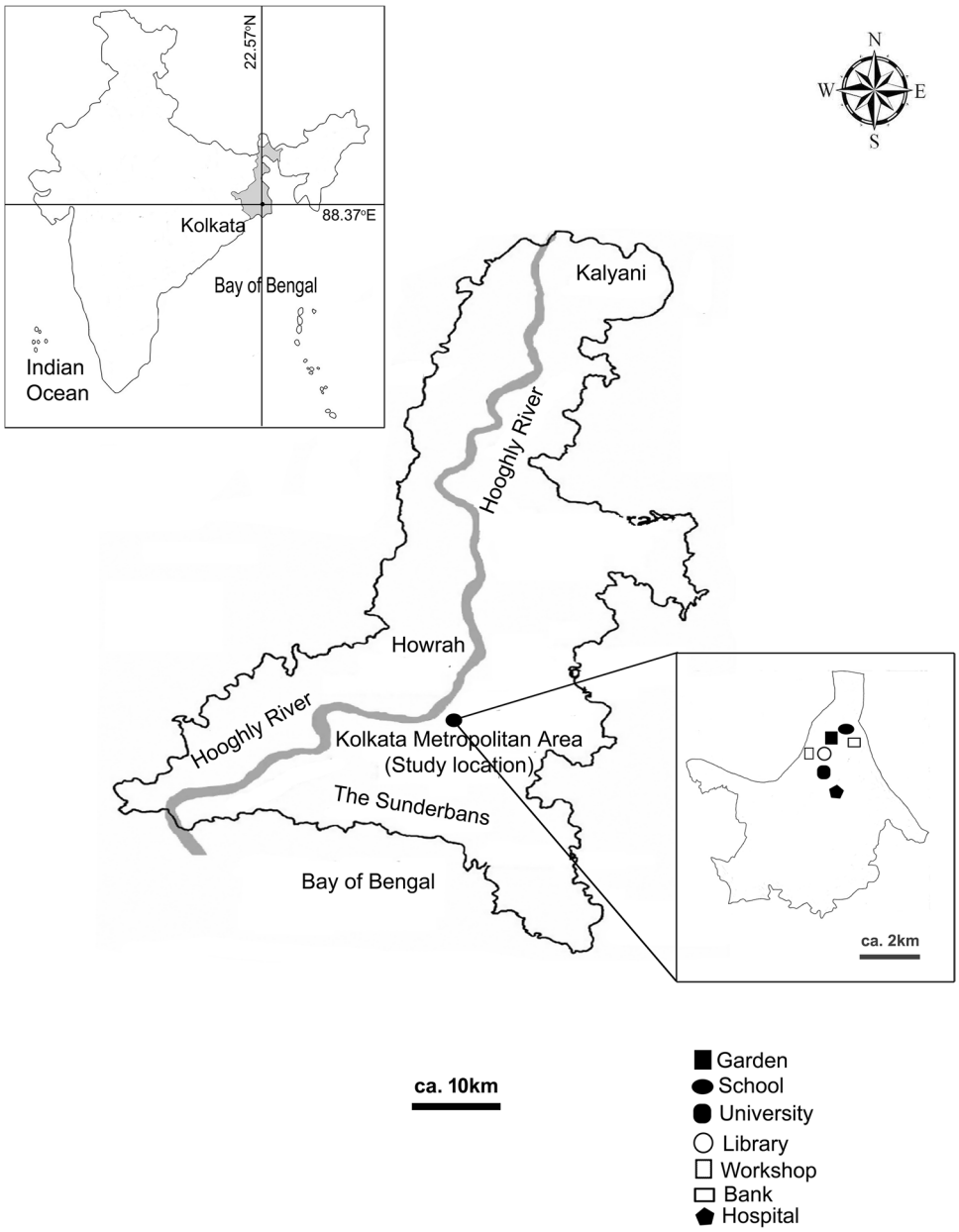


**Fig. 1** The geographical position of seven sampling sites

were incubated at 27 ± 2 °C for 3–7 days until sporulation. The pure isolates were obtained through periodic subculturing and observed under the Nikon high-resolution light microscope (Labophot 2, Nikon Corp., Japan) at 400x magnification by using lactophenol blue stain (Sigma–Aldrich, USA). The fungal isolates were identified up to the genus level by following standard reference manuals (Onions et al. 1981; Subramanian 1971). Non-sporulating colonies or sterile mycelia were grouped as "Others." Fungal colonies were counted and expressed as Colony Forming Units (CFU) per $m^3$ of air by multiplying with the conversion factor of 11.77.

Burkard personal volumetric sampler trapped total fungal spores, i.e., both non-viable and viable fungi. In this method of sampling, we have identified and quantified the trapped fungal spores only by microscopy, due to being devoid of any growth medium. Therefore, in this study, this sampling method was considered as the microscopy-based method. On the other hand, Andersen two-stage sampler provided data on viable fungi cultured on the growth medium. Thus, this sampling method was denoted as the culture-based method.

## Environmental parameters

Meteorological parameters were obtained from the Indian Meteorological Department, Kolkata. The concentrations of air pollutants were collected from the public database of the official website of The West Bengal Pollution Control Board (www.wbpcb.gov.in) for Kolkata. The environmental

parameters used for this study were maximum, minimum, and average temperature ($T_{max}$, $T_{min}$, $T_{avg}$, respectively), relative humidity (r.h.), wind speed (W), total rainfall (R), average sun hour (Avg SH), suspended particulate matters ($PM_{10}$ and particles with aerodynamic diameter < 2.5 μm, $PM_{2.5}$), nitrogen dioxide ($NO_2$), sulfur dioxide ($SO_2$), carbon monoxide (CO), and ozone ($O_3$). For statistical analysis, monthly average values of the environmental parameters were considered. The entire sampling period was divided into four distinguished seasons such as summer (March–May), monsoon (June–September), post-monsoon (October–November), and winter (December–February).

### Collection of hospitalization data and questionnaire survey

Daily patient data were obtained by visiting the outpatient department of the nearest hospital for three consecutive years. 10,711 people (6574 males and 4137 females), from the local population, were taken into account for this survey. The total number of patients, with age groups ranging from 6–82 years, was divided into four categories, based on their diagnosed disease. Group A—"Asthma" ($n = 1955$); group B—"COPD" (chronic obstructive pulmonary disease) ($n = 1673$); group C—"PTB" (pulmonary tuberculosis) ($n = 2141$), and group D—"Others" ($n = 4942$), includes patients suffering from general respiratory and allergic symptoms like cough, cold, sneezing, wheezing, breathlessness, allergic rhinitis and some unspecific problems. The survey was executed with selected patients by using a set of questionnaires. The format was prepared by Bose Institute, Kolkata by following the format mentioned by Singh et al. (1999). The questionnaire consisted of questions on personal information of local people such as age, occupation, living environments, the impact of any respiratory symptoms and allergic disorders, and family history of atopic diseases.

### Allergenicity assessment of fungi by SPT

For investigating the allergenicity of several fungal species obtained by the culture-based method, the SPT was performed against atopic patients ($n = 145$) with their prior consent according to the ethics of the hospital. From the local population, asthmatic patients ($n = 52$) from group A and patients with allergic rhinitis ($n = 63$) and other respiratory discomforts ($n = 30$) from the "Others" group (group D) were considered for this experiment. All selected subjects visited the hospital more than four times per year due to frequent sensitization. Smoking, pregnancy, and patients with severe asthma and other severe systemic diseases were excluded from the study. SPT was carried out with our supplied 20 μl of crude antigenic extract (1:10 *w*/*v*), prepared by extracting the crude total protein from spore mycelia of the selected species. It was placed on the surface of the arms and pricked with a sterile lancet. After 20 min, the wheel reaction was measured and graded from + 1 to + 4 (Stytis et al. 1982). Histamine diphosphate (1 mg $ml^{-1}$) and phosphate buffer (0.01 M, pH 7.2) were used as the positive and negative control, respectively.

### Statistical analysis

The data were analyzed in GraphPad Prism 6.0 (San Diego, CA, USA) and IBM SPSS version 24.0 (IBM, Armonk, NY, USA). The normality of the dataset was checked. The consistency in spore concentrations during the monitoring period was analyzed through one-way ANOVA followed by Tukey's multiple comparison test. The temporal distribution of spore concentrations and environmental parameters across different seasons was analyzed through one-way ANOVA or Kruskal–Wallis test. The correlations of spore concentrations found by both sampling methods, with individual environmental parameters, were analyzed through Spearman's rank correlation coefficient (*r*) (two-tailed), for each sampling site. Further univariate analysis was performed between spore concentrations and potential predictor environmental variables. Variables with a *p*-value < 0.2 were included in multiple regression analysis with aerial concentrations of total spores and major fungal taxa, obtained through both sampling techniques, for every sampling site, considering a linear relation between them. The stepwise linear regression analysis was performed, and variables with *p*-value < 0.01 were included in the final regression models. The seasonal changes of fungal concentrations were also considered as a categorical variable in multiple regression analysis. The spore concentrations were transformed to logarithmic base 10 for regression analysis for an approximation to normality. The linear relation between concentrations of total spores as well as major taxa of both methods and recorded respiratory disease cases were analyzed through regression analysis. The incidence of respiratory disease between males and females for every disease category was analyzed through an unpaired *t*-test.

## Results

### Spore concentrations in different microenvironments

In this study, the concentration of total fungal spores, sampled with the microscopy-based method, ranged from 9561.8 to 22,727.5 spores $m^{-3}$ with mean ± SD: 13,176.11 ± 3795.618 spores $m^{-3}$, and the total spore concentrations sampled by the culture-based method varied from 7111.8 to 14,741.28 CFU $m^{-3}$ with mean ± SD:

10,748.89 ± 1698.957 CFU $m^{-3}$. Total spore concentration was maximum in the open field area, i.e., outdoor environment for both sampling methods, whereas minimum concentration was found in the indoors such as the occupational area in terms of the microscopy-based method and the educational area for the culture-based method (Online Resource 2).

In the microscopy-based method, among seven sampling sites, the garden exhibited the highest spore concentration (mean ± SD: 4018.239 ± 2472.521 spores $m^{-3}$), and the bank microenvironment showed the lowest concentration (mean ± SD: 607.4181 ± 250.7084 spores $m^{-3}$) (Fig. 2a). The frequency distribution of fungal aerospora and their concentrations are presented in Table 1. Among them, ascospores, *Cladosporium* spp., *Aspergillus/Penicillium* spp., and basidiospores contributed more than 66% with mean concentrations of 2593.1, 2490.9, 2116.9, and 1580.8 spores $m^{-3}$, respectively, hence considered as dominant taxa. However, the concentrations of major taxa varied in seven sampling sites (Fig. 2b). The distribution of fungi in seven microenvironments is also presented in Online Resource 3.

On the other hand, for fungal isolates grown by culture-based method, school exhibited maximum spore concentration with mean ± SD: 1829.091 ± 384.8068 CFU $m^{-3}$, whereas the lowest concentration was found in the bank (mean ± SD: 1014.451 ± 302.7418 CFU $m^{-3}$) (Fig. 2c). *Aspergillus* spp., *Penicillium* spp., *Rhizopus* spp., and *Curvularia* spp. were observed as dominant with mean concentrations of 5682.3, 1013.7, 668.2, and 605.2 CFU $m^{-3}$, respectively (Table 2). Being abundant in all studied microenvironments throughout the year, *Aspergillus* spp. was the most prevalent genus accounting for more than

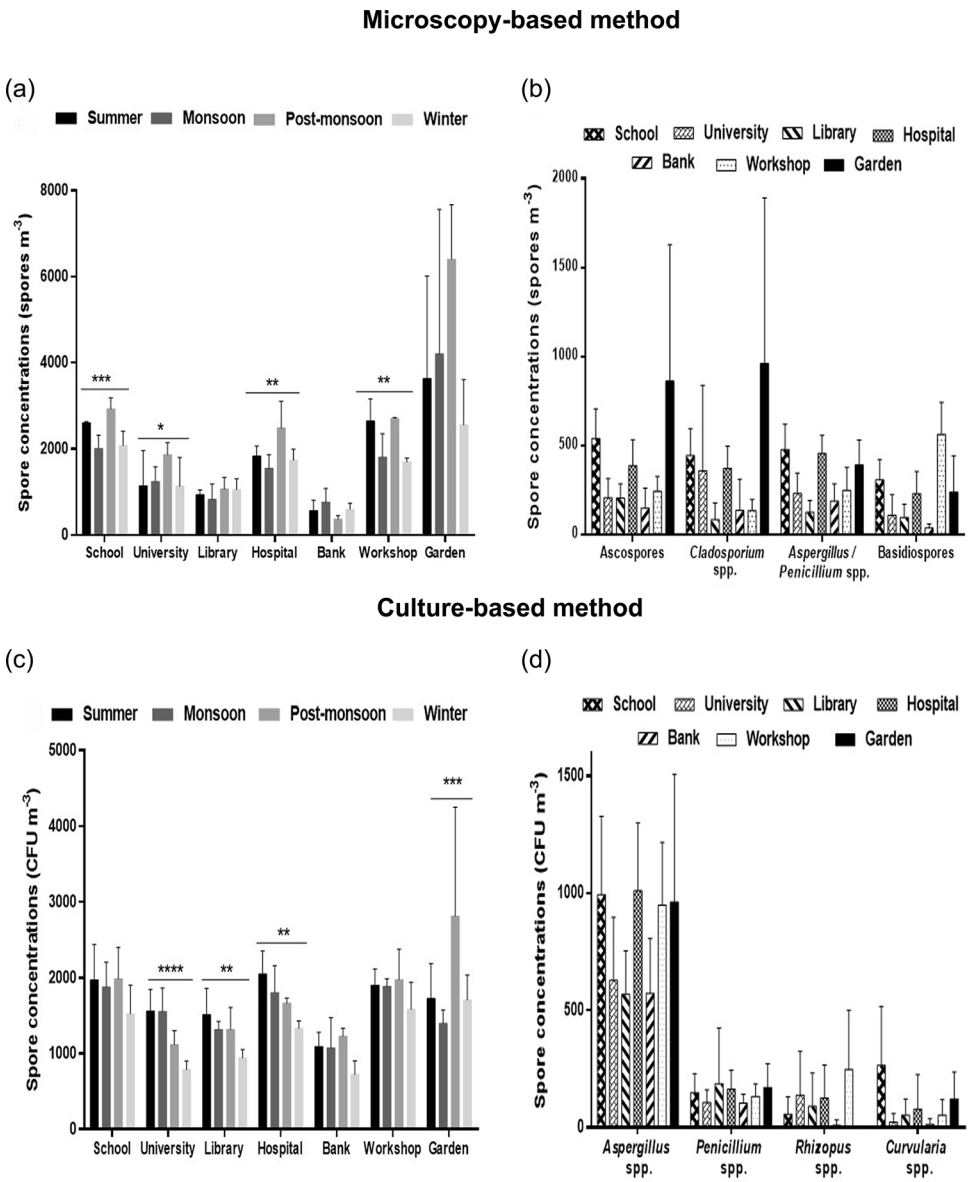


**Fig. 2** Temporal and spatial distributions of airborne fungi at all indoor and outdoor environments in Kolkata during May 2014–April 2017: **a** Total fungal spore counts from microscopy-based method with seasonal periodicity pattern. **b** Aerial concentrations of four major taxa obtained through the microscopy-based method. **c** Pattern of seasonal periodicity of total fungal spore concentrations of culture-based method. **d** Aerial concentrations of four major taxa found by using the culture-based method. Bars represent mean ± SD. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$ using One-way ANOVA or Kruskal–Wallis test

**Table 1** Frequency distribution of fungi (spores $m^{-3}$) recorded by the microscopy-based method at different indoor and outdoor microenvironments in an urban area in Kolkata megacity, India, from May 2014 to April 2017

| Fungal categories | Freq (%)[a] | Mean | Median | SD[b] | Min[c] | Max[d] | IQR[e] |
|---|---|---|---|---|---|---|---|
| *Alternaria* | 58 | 225.3 | 205.1 | 110.9 | 0.0 | 461.3 | 162.0 |
| Ascopore | 67 | 2593.1 | 2526.2 | 1053.2 | 0.0 | 4440.4 | 815.7 |
| *Aspergillus/Penicillium* | 67 | 2116.9 | 2039.3 | 412.8 | 0.0 | 2808.3 | 748.1 |
| Basidiospore | 67 | 1580.8 | 1444.0 | 623.3 | 0.0 | 2633.4 | 641.9 |
| *Beltrania* | 8 | 2.2 | 0.0 | 7.7 | 0.0 | 26.7 | 0.0 |
| *Bipolaris* | 58 | 31.2 | 12.2 | 41.2 | 0.0 | 136.7 | 42.5 |
| *Bispora* | 58 | 25.9 | 23.3 | 14.2 | 0.0 | 46.7 | 24.2 |
| *Cercospora* | 58 | 35.5 | 23.3 | 46.7 | 0.0 | 163.4 | 46.1 |
| *Chaetomium* | 33 | 189.9 | 162.3 | 132.8 | 0.0 | 396.7 | 163.1 |
| *Choanephora* | 25 | 4.7 | 0.0 | 8.1 | 0.0 | 22.2 | 3.9 |
| *Cladosporium* | 67 | 2490.9 | 2174.5 | 1495.5 | 0.0 | 5754.7 | 1888.6 |
| *Curvularia* | 58 | 618.2 | 469.9 | 765.9 | 0.0 | 2974.7 | 256.9 |
| *Dendriphiopsis* | 8 | 1.3 | 0.0 | 3.8 | 0.0 | 13.3 | 0.0 |
| *Drechslera* | 50 | 62 | 51.7 | 63.8 | 0.0 | 207.9 | 80.0 |
| *Dwayabeeja* | 58 | 12.2 | 8.9 | 9.6 | 0.0 | 27.8 | 13.6 |
| *Fusarium* | 58 | 34.7 | 27.2 | 36.3 | 0.0 | 86.7 | 65.9 |
| *Ganoderma* | 50 | 670.2 | 610.3 | 220.4 | 0.0 | 1080.4 | 375.3 |
| *Lasiodiplodia* | 8 | 0.7 | 0.0 | 2.6 | 0.0 | 8.9 | 0.0 |
| *Nigrospora* | 50 | 329.6 | 306.8 | 207.8 | 0.0 | 684.6 | 219.9 |
| *Periconia* | 67 | 1005.6 | 436.3 | 1516.0 | 0.0 | 5401.2 | 587.2 |
| *Periconiella* | 58 | 24.1 | 19.4 | 22.1 | 0.0 | 76.7 | 26.7 |
| *Phaeotrichoconis* | 58 | 17.4 | 14.4 | 20.0 | 0.0 | 71.12 | 20.8 |
| *Pithomyces* | 50 | 119.5 | 119.5 | 88.0 | 0.0 | 241.1 | 148.6 |
| *Pringshemia* | 17 | 5.5 | 0.0 | 11.9 | 0.0 | 33.3 | 1.1 |
| Rust spore | 58 | 765.4 | 681.9 | 426.2 | 0.0 | 1679.4 | 661.7 |
| *Spegazzinia* | 58 | 23.9 | 13.3 | 37.4 | 0.0 | 133.4 | 28.9 |
| *Sporidesmium* | 17 | 4.8 | 0.0 | 10.4 | 0.0 | 33.4 | 1.5 |
| *Tetracoccosporium* | 8 | 0.9 | 0.0 | 0.6 | 0.0 | 2.2 | 0.0 |
| *Tetraploa* | 50 | 19.7 | 15.0 | 17.7 | 0.0 | 67.8 | 7.5 |
| *Torula* | 58 | 62.1 | 41.7 | 55.3 | 0.0 | 170.0 | 50.0 |
| *Trichoconis* | 50 | 32 | 27.8 | 30.6 | 0.0 | 95.6 | 48.6 |
| Others[f] | 58 | 47.9 | 45.0 | 23.8 | 0.0 | 93.4 | 19.2 |
| Total spores | 100 | 13,176.1 | 12,421.6 | 3795.6 | 9561.8 | 22,727.5 | 5872.8 |

[a]Frequency is the percentage of the individual fungal taxon present in the total sample ($n=252$) of the microscopy-based method; [b]standard deviation; [c]minimum concentration; [d]maximum concentration; [e]interquartile range, and [f]fungal spores that were difficult to identify, considered as "Others"

52% of total spores of the culture-based method (Fig. 2d; Table 2). The concentrations of major fungal isolates in the individual microenvironment are shown in Fig. 2d. Additionally, we have observed different fungal spectra for different culture techniques, and the spore concentration obtained through the microscopy-based method is higher than the culture-based method. However, for each sampling method, mean concentrations of the total fungal spores and major taxa between consecutive years remain statistically insignificant, except for *Aspergillus/Penicillium* spp. as shown in Online Resource 4.

## Association of ambient fungal spore concentrations with environmental parameters

The distribution of environmental parameters across different seasons varied significantly (Table 3). The seasonal changes showed a significant effect on ambient spore concentrations in indoor and outdoor microenvironments for both sampling techniques (Fig. 2a. c). Maximum spore concentrations were observed during the post-monsoon season with mean ± SD: 17,840.41 ± 2623.446 spores $m^{-3}$ (microscopy-based method) and 12,098.45 ± 2533.606 CFU $m^{-3}$ (culture-based method) and minimum during the

**Table 2** Percent contribution of fungi (CFU $m^{-3}$) recovered by the culture-based method at different indoor and outdoor microenvironments in an urban area in Kolkata megacity, India, from May 2014 to April 2017

| Fungal categories | Percent contribution (%)[a] | Mean | Median | SD[b] | Min[c] | Max[d] | IQR[e] |
|---|---|---|---|---|---|---|---|
| *Alternaria* | 0.52622 | 56.6 | 0 | 104.7 | 0.0 | 188.32 | 50 |
| *Aspergillus* | 52.86382 | 5682.3 | 5626.4 | 1487.9 | 117.7 | 2495.24 | 1249.9 |
| *Candida* | 2.325801 | 250 | 251.3 | 151.5 | 0.0 | 506.54 | 172.8 |
| *Chaetomium* | 0.1095 | 11.8 | 0 | 32.2 | 0.0 | 141.24 | 0 |
| *Curvularia* | 5.630307 | 605.2 | 406.3 | 475.1 | 0.0 | 1790.56 | 521.9 |
| *Fusarium* | 1.629176 | 175.1 | 31.4 | 252.7 | 0.0 | 565.44 | 274.3 |
| *Penicillium* | 9.430839 | 1013.7 | 964.5 | 286.2 | 0.0 | 1694.88 | 233.4 |
| *Rhizopus* | 6.216335 | 668.2 | 637.9 | 408.3 | 0.0 | 1507.84 | 720.8 |
| *Trichoderma* | 0.285916 | 30.7 | 33.3 | 18.3 | 0.0 | 70.62 | 26.5 |
| Others[f] | 20.98209 | 2255.3 | 2120.7 | 830.7 | 0.0 | 1600.72 | 1175.5 |
| Total spores | 100.0 | 10,748.9 | 10,859.1 | 1699 | 7771.1 | 14,741.28 | 1619.5 |

[a]Percent contribution is the percentage of individual fungal taxon among the total sample ($n = 252$) of the culture-based method; [b]standard deviation; [c]minimum concentration; [d]maximum concentration; [e]interquartile range, and [f]non-sporulating colonies or sterile mycelia were grouped as "Others"

**Table 3** Distribution of environmental parameters across different seasons in Kolkata from May 2014 to April 2017

| Environmental parameters[a]**** | | Summer (March–May) | Monsoon (June–Sep) | Post-monsoon (Oct–Nov) | Winter (Dec–Feb) |
|---|---|---|---|---|---|
| Meteorological parameters | $T_{Max}$ (°C) | 36.89 ± 2.713 | 34.25 ± 1.765 | 30.83 ± 1.169 | 28.67 ± 2.121 |
| | $T_{Avg}$ (°C) | 30.78 ± 2.587 | 30.25 ± 1.422 | 25.83 ± 1.722 | 21.44 ± 2.404 |
| | $T_{Min}$ (°C) | 26.00 ± 3.082 | 27.42 ± 1.240 | 21.67 ± 2.338 | 17.56 ± 1.878 |
| | r.h. (%) | 56.33 ± 5.568 | 73.58 ± 6.345 | 61.00 ± 9.381 | 44.44 ± 3.745 |
| | R (mm) | 15.38 ± 11.13 | 98.82 ± 43.88 | 15.52 ± 12.00 | 5.036 ± 8.404 |
| | W (Km/hr) | 13.63 ± 2.937 | 12.35 ± 2.903 | 7.417 ± 0.4997 | 8.433 ± 0.7969 |
| | Avg SH (hr) | 320.8 ± 38.81 | 269.3 ± 33.46 | 285.0 ± 5.441 | 223.1 ± 7.998 |
| Atmospheric pollutants | $PM_{2.5}$ (μg/$m^3$) | 51.11 ± 11.36 | 33.47 ± 7.120 | 83.01 ± 24.71 | 122.8 ± 26.69 |
| | $PM_{10}$ (μg/$m^3$) | 108.1 ± 20.41 | 70.08 ± 18.89 | 157.6 ± 46.82 | 234.0 ± 42.92 |
| | $NO_2$ (μg/$m^3$) | 40.40 ± 5.972 | 32.44 ± 4.454 | 55.37 ± 4.839 | 67.96 ± 6.733 |
| | $SO_2$ (μg/$m^3$) | 3.800 ± 0.6121 | 3.095 ± 0.7224 | 6.352 ± 1.095 | 7.464 ± 1.806 |
| | CO (μg/$m^3$) | 0.7589 ± 0.040 | 0.6967 ± 0.059 | 0.9133 ± 0.085 | 0.8567 ± 0.079 |
| | $O_3$ (μg/$m^3$) | 40.36 ± 13.46 | 33.92 ± 9.973 | 45.84 ± 11.01 | 39.45 ± 10.63 |

$T_{max}$, maximum temperature; $T_{avg}$, average temperature; $T_{min}$, minimum temperature; *r.h.*, relative humidity; *W*, wind speed; *R*, total rainfall; *Avg SH*, average sun hour; $PM_{2.5}$ and $PM_{10}$, suspended particulate matters with aerodynamic diameter < 2.5 μm ($PM_{2.5}$) and < 10 μm ($PM_{10}$); $NO_2$, nitrogen dioxide; $SO_2$, sulfur dioxide; *CO*, carbon monoxide; $O_3$, ozone

[a]Monthly average values of the environmental parameters were taken into consideration for the analysis

All meteorological parameters and atmospheric pollutants in the four seasons differed significantly

****$p < 0.0001$ using the one-way ANOVA or Kruskal–Wallis test ($n = 36$ for every parameter)

winter season (microscopy-based method: mean ± SD: 10,870.82 ± 2011.283 spores $m^{-3}$; culture-based method: mean ± SD: 8587.931 ± 803.483 CFU $m^{-3}$) (Fig. 3a, b).

For fungal spores recorded by microscopy-based method, the result revealed that *Cladosporium* spp. was the key contributor to the post-monsoon season showing the maximum peak. Ascospores were also identified as the major component of the subsidiary peak found in the summer season (Fig. 3a). Basidiospores were found to be higher in post-monsoon, whereas monthly concentrations of *Aspergillus/Penicillium* spp. revealed its abundance throughout the year and no such prominent seasonal dependency was displayed. Monthly concentrations of the major taxa of both microscopy-based and culture-based techniques were graphically represented in Online Resource 5 and Online Resource 6 respectively. In the outdoor area, only basidiospores showed significant seasonal variation, whereas it was prominent in total spore concentrations (Fig. 2a) and all major fungi in several indoor microenvironments (Online Resource 7).

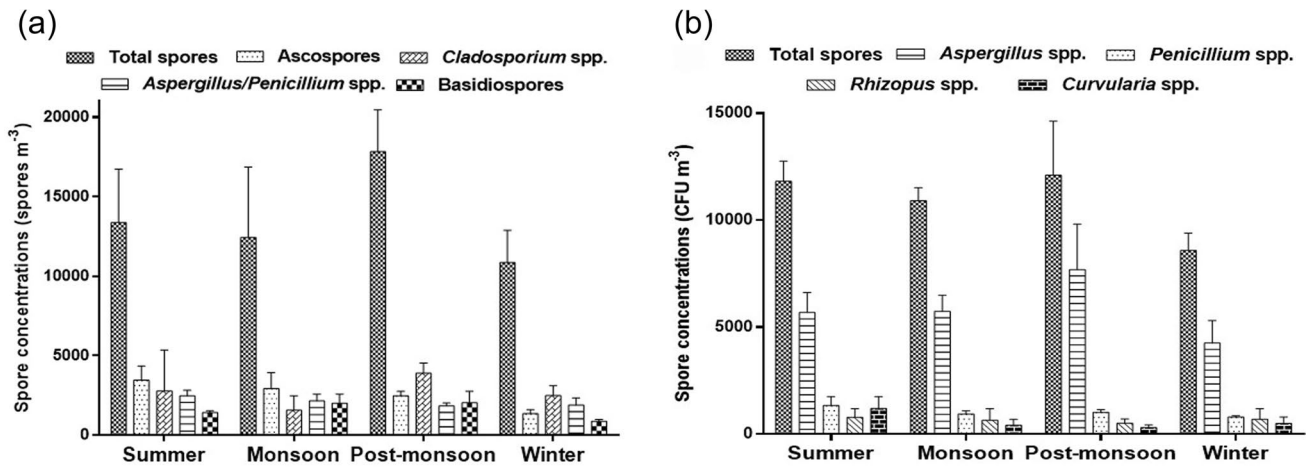


**Fig. 3** Seasonal variation of fungal aerospora in indoor and outdoor environments in Kolkata during May 2014–April 2017: monthly mean concentrations of total fungal spores and major taxa for **a** microscopy-based method and **b** culture-based method in the four distinguished seasons. Bars represent mean ± SD

In the culture-based method, *Aspergillus* spp. exhibited maximum concentration in the post-monsoon season, whereas, *Penicillium* spp., *Rhizopus* spp., and *Curvularia* spp. were found to be higher in the summer season (Fig. 3b). Concentrations of total spores (Fig. 2c), *Aspergillus* spp., *Penicillium* spp., and *Curvularia* spp. showed significant seasonal periodicity in the outdoor as well as in different indoor microenvironments (Online Resource 7). Contrarily, *Rhizopus* spp. did not show any significant seasonal variation.

To assess elaborately, the correlations between environmental parameters and the ambient fungal spore concentrations sampled by both methods, in different microenvironments, were determined through Spearman's rank correlation coefficient (r) (Table 4). Furthermore, the stepwise multiple regression analysis identified the key atmospheric predictors of the total spores and dominant taxa in different microenvironments (Table 5).

### Correlation of fungal spore concentrations with meteorological parameters

In this study, the ambient temperature showed a significant positive association with all major fungal taxa found by microscopy-based as well as culture-based methods in both outdoor and indoor environments except *Cladosporium* spp. (Table 4). A significant negative association between temperature and *Cladosporium* spp. was found in the garden. Besides, in the university, the average and minimum temperature were found to be significant predictors for *Aspergillus/Penicillium* spp. and *Aspergillus* spp., respectively, by multiple regression analysis (Table 5). The regression model also showed the average temperature as a significant predictor of total spores of the culture-based method in the hospital environment (Table 5).

A significant positive association between average sun hour and ascospores in outdoor as well as indoor environments (school and workshop) was observed. In several indoor areas, it also had a significant positive impact on *Aspergillus/Penicillium* spp., basidiospores, total spores of the culture-based method, *Aspergillus* spp., and *Penicillium* spp. (Table 4). Moreover, it was attributed as an important predictor of ascospores in the school along with total spores of the culture-based method in the university and the library through the multiple regression analysis (Table 5).

Rainfall presented negative correlations with *Cladosporium* spp. in outdoor and several indoor microenvironments (Table 4), whereas ascospores showed a significant positive relationship with it in the garden.

Relative humidity was positively associated with ascospores and basidiospores in the outdoor environment (Table 4). It showed a positive association with basidiospores in all indoor microenvironments and was identified as a significant predictor in the university and the library through the regression analysis (Table 5). Conversely, in the library, it was negatively correlated with *Cladosporium* spp. and detected as a key predictor as well (Table 5).

Wind speed showed a significant positive association with ascospores and basidiospores in the garden. A significant negative impact of wind speed was also observed on *Cladosporium* spp., *Curvularia* spp., and total spore concentrations of the culture-based method in the outdoor environment (Table 4). Among several positive associations observed indoors, it was identified as a key predictor for ascospores in the university, whereas in the library, it was associated negatively with total spore concentrations of the

**Table 4** Two-tailed Spearman's correlation coefficients (r) of ambient fungal spore concentrations obtained through microscopy-based as well as culture-based method at outdoor and different indoor microenvironments in Kolkata from May 2014 to April 2017

| Fungal taxon | Environmental parameters | Outdoor | Indoor | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Open field area | Educational area | | | Occupational area | | |
| | | Garden | School | University | Library | Hospital | Bank | Workshop |
| Microscopy-based method | | | | | | | | |
| Total spores | $T_{Max}$ | 0.152 | 0.148 | −0.019 | −0.141 | −0.117 | 0.107 | 0.209 |
| | $T_{Avg}$ | 0.095 | 0.107 | −0.179 | −0.267 | −0.147 | 0.019 | 0.159 |
| | $T_{Min}$ | 0.064 | 0.004 | −0.135 | −0.299 | −0.154 | 0.114 | 0.047 |
| | r.h | 0.070 | −0.131 | −0.051 | −0.281 | −0.077 | 0.143 | 0.045 |
| | R | −0.166 | −0.169 | −0.381* | −0.215 | −0.270 | 0.105 | −0.002 |
| | W | 0.003 | −0.297 | 0.026 | −0.395* | −0.277 | 0.258 | −0.063 |
| | Avg SH | 0.524** | 0.466** | 0.165 | −0.042 | 0.092 | −0.147 | 0.353* |
| | $PM_{2.5}$ | 0.052 | 0.245 | 0.203 | 0.294 | 0.268 | −0.181 | 0.059 |
| | $PM_{10}$ | 0.031 | 0.244 | 0.192 | 0.271 | 0.228 | −0.153 | 0.004 |
| | $SO_2$ | 0.066 | 0.289 | 0.208 | 0.280 | 0.388 | −0.229 | 0.075 |
| | $NO_2$ | −0.017 | 0.194 | 0.110 | 0.319 | 0.235 | −0.279 | −0.002 |
| | CO | 0.085 | 0.308 | 0.136 | 0.199 | 0.406 | −0.406* | 0.184 |
| | $O_3$ | 0.186 | −0.028 | −0.032 | −0.098 | −0.196 | −0.407 | −0.197 |
| Ascospores | $T_{Max}$ | 0.700** | 0.365* | 0.305 | 0.412* | 0.239 | 0.322 | −0.066 |
| | $T_{Avg}$ | 0.759** | 0.398* | 0.250 | 0.227 | 0.169 | 0.255 | −0.044 |
| | $T_{Min}$ | 0.722** | 0.367* | 0.428** | 0.252 | 0.133 | 0.334* | −0.085 |
| | r.h | 0.485** | 0.308 | 0.614** | 0.018 | 0.013 | 0.195 | 0.055 |
| | R | 0.486** | 0.420* | 0.099 | 0.323 | 0.084 | 0.205 | −0.221 |
| | W | 0.388* | 0.247 | 0.685** | −0.035 | −0.149 | 0.280 | −0.016 |
| | Avg SH | 0.741** | 0.351* | 0.016 | 0.324 | 0.361* | 0.171 | 0.297 |
| | $PM_{2.5}$ | −0.496** | −0.310 | −0.496** | −0.071 | 0.057 | −0.208 | 0.142 |
| | $PM_{10}$ | −0.483** | −0.306 | −0.480** | −0.086 | 0.038 | −0.212 | 0.101 |
| | $SO_2$ | −0.396* | −0.261 | −0.387* | −0.086 | 0.071 | −0.133 | 0.143 |
| | $NO_2$ | −0.533** | −0.312 | −0.547** | −0.148 | 0.065 | 0.334* | 0.101 |
| | CO | −0.273 | −0.178 | −0.443** | −0.065 | 0.209 | −0.351* | 0.274 |
| | $O_3$ | −0.116 | −0.356* | −0.485** | −0.249 | 0.035 | −0.336* | −0.031 |
| *Cladosporium* spp. | $T_{Max}$ | −0.228** | 0.036 | −0.126 | −0.219 | −0.252 | −0.090 | −0.110 |
| | $T_{Avg}$ | −0.322** | 0.029 | −0.320 | −0.260 | −0.298 | −0.176 | −0.139 |
| | $T_{Min}$ | −0.294** | 0.000 | −0.368* | −0.440** | −0.206 | −0.265 | −0.115 |
| | r.h | −0.466 | −0.027 | −0.288 | −0.501** | −0.133 | −0.332* | −0.128 |
| | R | −0.378* | −0.137 | −0.420* | 0.016 | −0.361* | 0.026 | −0.226 |
| | W | −0.343** | −0.094 | −0.251 | −0.488** | −0.098 | −0.157 | −0.147 |
| | Avg SH | 0.026 | 0.045 | −0.027 | −0.195 | 0.174 | −0.188 | −0.032 |
| | $PM_{2.5}$ | 0.535** | 0.096 | 0.228 | 0.354* | 0.271 | 0.175 | 0.309 |
| | $PM_{10}$ | 0.537** | 0.170 | −0.172 | 0.343* | 0.282 | 0.196 | 0.260 |
| | $SO_2$ | −0.228** | 0.058 | 0.362* | 0.203 | 0.301 | 0.104 | 0.201 |
| | $NO_2$ | −0.322** | 0.041 | 0.358* | 0.377* | 0.318 | 0.050 | 0.310 |
| | CO | −0.294** | 0.128 | 0.326 | 0.146 | 0.466** | −0.101 | 0.294 |
| | $O_3$ | −0.466 | 0.091 | 0.267 | 0.244 | 0.096 | −0.152 | −0.086 |

**Table 4** (continued)

| Fungal taxon | Environmental parameters | Outdoor | Indoor | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Open field area | Educational area | | | Occupational area | | |
| | | Garden | School | University | Library | Hospital | Bank | Workshop |
| *Aspergillus/Penicillium* spp. | $T_{Max}$ | −0.006 | 0.078 | 0.552** | 0.265 | 0.129 | 0.137 | 0.316 |
| | $T_{Avg}$ | 0.036 | 0.037 | 0.540** | 0.395* | 0.070 | 0.149 | 0.228 |
| | $T_{Min}$ | 0.067 | −0.070 | 0.521** | 0.345* | −0.030 | 0.190 | 0.130 |
| | r.h | 0.202 | −0.174 | 0.230 | 0.315 | −0.215 | 0.302 | 0.077 |
| | R | −0.218 | 0.168 | 0.324 | 0.269 | 0.139 | 0.128 | 0.172 |
| | W | 0.110 | −0.264 | 0.219 | 0.143 | −0.223 | 0.225 | 0.005 |
| | Avg SH | 0.017 | 0.021 | 0.346* | −0.020 | 0.125 | 0.023 | 0.185 |
| | $PM_{2.5}$ | 0.003 | 0.021 | −0.258 | −0.333* | 0.149 | −0.190 | −0.118 |
| | $PM_{10}$ | −0.022 | 0.040 | −0.247 | −0.343* | 0.141 | −0.201 | −0.133 |
| | $SO_2$ | 0.124 | 0.068 | −0.148 | −0.174 | 0.287 | −0.122 | 0.003 |
| | $NO_2$ | 0.060 | 0.080 | −0.417* | −0.273 | 0.139 | −0.247 | −0.064 |
| | CO | 0.221 | 0.027 | −0.385* | −0.315 | 0.123 | −0.281 | −0.063 |
| | $O_3$ | 0.130 | −0.257 | −0.331* | −0.221 | −0.406* | −0.260 | −0.335* |
| Basidiospores | $T_{Max}$ | 0.346* | 0.032 | 0.353* | 0.326 | 0.267 | 0.247 | 0.268 |
| | $T_{Avg}$ | 0.413* | 0.107 | 0.429** | 0.508** | 0.414* | 0.351* | 0.249 |
| | $T_{Min}$ | 0.474** | 0.104 | 0.547** | 0.581** | 0.390* | 0.371* | 0.216 |
| | r.h | 0.687** | 0.335* | 0.719** | 0.553** | 0.461** | 0.533** | 0.257 |
| | R | 0.171 | −0.109 | 0.260 | 0.311 | 0.120 | 0.081 | 0.033 |
| | W | 0.664** | 0.134 | 0.749** | 0.511* | 0.217 | 0.374* | 0.255 |
| | Avg SH | 0.095 | 0.254 | 0.000 | 0.239 | 0.375* | 0.005 | 0.284 |
| | $PM_{2.5}$ | −0.553** | −0.143 | −0.664** | −0.556** | −0.333* | −0.493** | −0.164 |
| | $PM_{10}$ | −0.589** | −0.200 | −0.665** | −0.567** | −0.395* | −0.543** | −0.213 |
| | $SO_2$ | −0.437** | −0.132 | −0.563** | −0.389* | −0.244 | −0.352* | −0.116 |
| | $NO_2$ | −0.528** | −0.188 | −0.618** | −0.542** | −0.320 | −0.433** | −0.168 |
| | CO | −0.405* | −0.017 | −0.544** | −0.465** | −0.063 | −0.339* | −0.049 |
| | $O_3$ | −0.153 | 0.281 | −0.198 | −0.185 | 0.160 | 0.170 | −0.139 |
| Culture-based method | | | | | | | | |
| Total spores | $T_{Max}$ | −0.324 | 0.224 | 0.765** | 0.552** | 0.493 | 0.235 | 0.155 |
| | $T_{Avg}$ | −0.275 | 0.180 | 0.664** | 0.498** | 0.535** | 0.180 | −0.275 |
| | $T_{Min}$ | −0.343* | 0.089 | 0.710** | 0.445** | 0.462** | 0.124 | 0.155 |
| | r.h | −0.423* | −0.040 | 0.598** | 0.296 | 0.282 | 0.234 | 0.071 |
| | R | −0.337* | 0.109 | 0.519** | 0.415* | 0.488** | −0.218 | 0.087 |
| | W | −0.570** | −0.050 | 0.560** | 0.305 | 0.320 | 0.106 | 0.035 |
| | Avg SH | 0.219 | 0.309 | 0.616** | 0.612** | 0.429** | 0.375* | 0.399* |
| | $PM_{2.5}$ | 0.479** | 0.026 | −0.732** | −0.417* | −0.275 | −0.191 | −0.069 |
| | $PM_{10}$ | 0.466** | 0.050 | −0.733** | −0.394* | −0.232 | −0.226 | −0.051 |
| | $SO_2$ | 0.500** | −0.127 | −0.675** | −0.511** | −0.397* | −0.140 | −0.165 |
| | $NO_2$ | 0.553** | −0.103 | −0.685** | −0.431** | −0.243 | −0.161 | −0.122 |
| | CO | 0.517** | −0.092 | −0.521** | −0.369* | −0.324 | −0.024 | −0.054 |
| | $O_3$ | 0.343* | −0.074 | −0.141 | −0.201 | −0.397* | 0.015 | −0.026 |

**Table 4** (continued)

| Fungal taxon | Environmental parameters | Outdoor | Indoor | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Open field area | Educational area | | | Occupational area | | |
| | | Garden | School | University | Library | Hospital | Bank | Workshop |
| *Aspergillus* spp. | $T_{Max}$ | −0.141 | 0.195 | 0.472** | 0.167 | 0.346* | −0.249 | −0.101 |
| | $T_{Avg}$ | 0.062 | 0.138 | 0.649** | 0.341* | 0.370* | 0.223 | −0.048 |
| | $T_{Min}$ | 0.090 | 0.116 | 0.702** | 0.278 | 0.284 | 0.147 | −0.015 |
| | r.h | −0.016 | 0.102 | 0.617** | 0.036 | 0.092 | 0.253 | 0.124 |
| | R | −0.164 | 0.026 | 0.447** | 0.261 | 0.451** | −0.023 | −0.162 |
| | W | −0.146 | 0.079 | 0.448** | −0.075 | 0.122 | 0.116 | −0.038 |
| | Avg SH | 0.261 | 0.404* | 0.437** | 0.328 | 0.442** | 0.397* | 0.161 |
| | $PM_{2.5}$ | 0.131 | −0.055 | −0.637** | −0.055 | −0.089 | −0.229 | 0.038 |
| | $PM_{10}$ | 0.143 | 0.000 | −0.628** | −0.023 | −0.066 | −0.241 | 0.031 |
| | $SO_2$ | 0.301 | −0.116 | −0.403* | 0.014 | −0.092 | −0.252 | −0.058 |
| | $NO_2$ | 0.089 | −0.143 | −0.623** | −0.219 | −0.197 | −0.253 | −0.132 |
| | CO | 0.209 | −0.060 | −0.473** | −0.134 | −0.133 | −0.198 | 0.029 |
| | $O_3$ | 0.248 | 0.049 | −0.077 | −0.057 | −0.179 | −0.106 | 0.252 |
| *Penicillium* spp. | $T_{Max}$ | −0.103 | −0.004 | 0.304 | 0.544** | −0.181 | 0.238 | 0.149 |
| | $T_{Avg}$ | 0.078 | −0.083 | 0.299 | 0.431** | −0.105 | 0.346* | 0.035 |
| | $T_{Min}$ | −0.007 | −0.135 | 0.253 | 0.288 | −0.121 | 0.291 | 0.082 |
| | r.h | 0.038 | −0.068 | 0.008 | −0.002 | 0.151 | 0.314 | 0.242 |
| | R | −0.090 | −0.067 | 0.176 | 0.365* | −0.081 | 0.035 | −0.027 |
| | W | −0.069 | −0.090 | −0.026 | −0.066 | 0.119 | 0.166 | 0.210 |
| | Avg SH | 0.122 | −0.227 | 0.299 | 0.430** | −0.178 | 0.240 | 0.086 |
| | $PM_{2.5}$ | 0.081 | 0.080 | −0.142 | −0.201 | 0.021 | −0.285 | −0.052 |
| | $PM_{10}$ | 0.044 | 0.073 | −0.102 | −0.194 | 0.025 | −0.324 | −0.070 |
| | $SO_2$ | 0.285 | −0.034 | −0.114 | −0.255 | −0.013 | −0.098 | −0.066 |
| | $NO_2$ | 0.068 | −0.010 | −0.204 | −0.249 | −0.040 | −0.235 | −0.155 |
| | CO | 0.165 | −0.151 | −0.244 | −0.236 | 0.046 | −0.032 | 0.008 |
| | $O_3$ | −0.070 | −0.210 | −0.160 | −0.116 | −0.210 | −0.096 | −0.226 |
| *Rhizopus* spp. | $T_{Max}$ | 0.255 | −0.164 | 0.417* | 0.166 | −0.043 | 0.306 | 0.045 |
| | $T_{Avg}$ | 0.266 | −0.059 | 0.253 | 0.192 | 0.005 | 0.353* | 0.060 |
| | $T_{Min}$ | 0.267 | −0.001 | 0.303 | 0.249 | 0.158 | 0.440** | −0.024 |
| | r.h | 0.154 | −0.036 | 0.069 | 0.171 | 0.209 | 0.255 | −0.333* |
| | R | 0.127 | 0.047 | 0.229 | 0.301 | −0.133 | 0.294 | 0.289 |
| | W | 0.170 | 0.032 | 0.235 | 0.167 | 0.221 | 0.370* | −0.121 |
| | Avg SH | 0.078 | −0.158 | 0.280 | −0.051 | −0.247 | 0.285 | −0.090 |
| | $PM_{2.5}$ | −0.136 | 0.020 | −0.084 | −0.240 | −0.206 | −0.260 | 0.036 |
| | $PM_{10}$ | −0.113 | 0.066 | −0.061 | −0.227 | −0.176 | −0.241 | 0.080 |
| | $SO_2$ | −0.240 | −0.034 | −0.195 | −0.247 | −0.059 | −0.223 | −0.059 |
| | $NO_2$ | −0.175 | −0.019 | −0.208 | −0.292 | −0.168 | −0.286 | 0.048 |
| | CO | −0.142 | −0.217 | −0.095 | −0.325 | −0.137 | −0.341* | −0.173 |
| | $O_3$ | 0.133 | 0.402* | −0.148 | −0.164 | −0.214 | 0.069 | 0.045 |

**Table 4** (continued)

| Fungal taxon | Environmental parameters | Outdoor | Indoor | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Open field area | Educational area | | | Occupational area | | |
| | | Garden | School | University | Library | Hospital | Bank | Workshop |
| *Curvularia* spp. | $T_{Max}$ | 0.024 | 0.307 | 0.232 | 0.232 | 0.383* | −0.069 | 0.184 |
| | $T_{Avg}$ | −0.076 | 0.241 | 0.135 | 0.135 | 0.369* | 0.001 | 0.239 |
| | $T_{Min}$ | −0.164 | 0.199 | −0.040 | −0.040 | 0.314 | 0.011 | 0.326 |
| | r.h | −0.464** | −0.117 | −0.145 | −0.145 | 0.169 | −0.098 | 0.482** |
| | R | 0.052 | 0.243 | 0.104 | 0.104 | 0.286 | −0.080 | 0.141 |
| | W | −0.423* | −0.032 | −0.211 | −0.211 | 0.263 | −0.164 | 0.460** |
| | Avg SH | 0.144 | 0.320 | 0.302 | 0.302 | 0.134 | 0.140 | −0.017 |
| | $PM_{2.5}$ | 0.352* | 0.066 | 0.077 | 0.077 | −0.247 | 0.183 | −0.374* |
| | $PM_{10}$ | 0.333* | 0.073 | 0.053 | 0.053 | −0.208 | 0.158 | −0.372* |
| | $SO_2$ | 0.252 | −0.053 | 0.074 | 0.039 | −0.280 | 0.205 | −0.426** |
| | $NO_2$ | 0.275 | −0.042 | 0.039 | 0.074 | −0.314 | 0.319 | −0.513** |
| | CO | 0.237 | 0.041 | 0.118 | 0.118 | −0.312 | 0.357* | −0.376* |
| | $O_3$ | −0.168 | −0.061 | 0.043 | 0.043 | −0.087 | 0.147 | −0.226 |

Monthly average of meteorological parameters ($T_{max}$, $T_{min}$, $T_{avg}$, r.h., W, R, Avg SH), atmospheric pollutants ($PM_{2.5}$, $PM_{10}$, $NO_2$, $SO_2$, CO, $O_3$), and ambient concentrations of total fungal spores and major taxa sampled by both methods were considered for the analysis

*$p < 0.05$

**$p < 0.01$

$n = 252$

microscopy-based method and also detected as a significant predictor (Table 5).

### Correlation of fungal spore concentrations with atmospheric pollutants

The concentrations of both $PM_{2.5}$ and $PM_{10}$ in the outdoor area have a significant positive correlation with *Cladosporium* spp., total spores of the culture-based method, and *Curvularia* spp. (Table 4). In the outdoor environment, $PM_{10}$ was identified as the key predictor regulating aerial concentrations of *Cladosporium* spp. by the regression model (Table 5). Conversely, ascospores and basidiospores were negatively associated with $PM_{2.5}$ and $PM_{10}$ in the outdoor area (Table 4). On the other hand, in indoor areas, both PMs showed a negative impact on ascospores, *Aspergillus/Penicillium* spp., and basidiospores. The multiple regression analysis also detected $PM_{10}$ as an important predictor variable having a negative effect on basidiospores in the bank as well as total spores of the culture-based method in the university (Table 5).

All gaseous pollutants ($SO_2$, $NO_2$, CO, and $O_3$) mostly affect different major taxa negatively in both environments (Table 4). $SO_2$ and $NO_2$ were negatively associated with ascospores and basidiospores in outdoor and indoor areas. *Cladosporium* spp. also showed negative correlations with them in outdoor. Multiple regression analysis also revealed that $NO_2$ could be a key predictor of *Curvularia* spp. by affecting negatively in the workshop (Table 5). Although $SO_2$ and $NO_2$ exhibited few significant positive associations with total spores of the culture-based method in the outdoor as well as with *Cladosporium* spp. in the university. $NO_2$ also had negative impact on ascospores and *Cladosporium* spp. in the bank and library respectively.

CO showed several significant negative associations in indoor microenvironments, whereas in outdoor, *Cladosporium* spp. and basidiospores were negatively correlated with CO (Table 4). A significant positive impact of CO was only observed with total spores of the culture-based method in the garden, *Cladosporium* spp. in the hospital, and *Curvularia* spp. in the bank microenvironment. A negative association between $O_3$ and *Aspergillus/Penicillium* spp. was also observed in the hospital microenvironment. Similarly, ascospores and total spores of the culture-based method exerted a negative association with it, whereas a positive correlation was also noticed with *Rhizopus* spp. (Table 4).

**Table 5** Multiple regression models for total fungal spores and major taxa for both microscopy-based and culture-based methods at outdoor and different indoor microenvironments in Kolkata from May 2014 to April 2017

| Site | Fungal taxon | Predictors | $\beta$ coefficient | SE[a] | $p$-value | $R^2$ |
|---|---|---|---|---|---|---|
| Microscopy-based method | | | | | | |
| Garden | *Cladosporium* spp. | Intercept | 1.898 | 0.231 | 0.000 | 0.218 |
| | | $PM_{10}$ | 0.005 | 0.002 | 0.004 | |
| School | Ascospores | Intercept | 2.100 | 0.208 | 0.000 | 0.191 |
| | | Avg SH | 0.002 | 0.001 | 0.008 | |
| University | Ascospores | Intercept | 2.038 | 0.074 | 0.000 | 0.251 |
| | | W | 0.004 | 0.001 | 0.002 | |
| | Basidiospores | Intercept | 0.017 | 0.284 | 0.951 | 0.542 |
| | | r.h | 0.029 | 0.005 | 0.000 | |
| | *Aspergillus/Penicillium* spp. | Intercept | 1.360 | 0.306 | 0.000 | 0.215 |
| | | $T_{Avg}$ | 0.034 | 0.011 | 0.004 | |
| Library | Total spores | Intercept | 3.014 | 0.031 | 0.000 | 0.229 |
| | | W | −0.002 | 0.000 | 0.003 | |
| | Basidiospores | Intercept | 1.562 | 0.247 | 0.000 | 0.197 |
| | | r.h | 0.012 | 0.004 | 0.007 | |
| | *Cladosporium* spp. | Intercept | 3.358 | 0.629 | 0.629 | 0.252 |
| | | r.h | −0.035 | 0.010 | 0.010 | |
| Bank | Basidiospores | Intercept | 1.826 | 0.083 | 0.000 | 0.320 |
| | | $PM_{10}$ | −0.002 | 0.001 | 0.000 | |
| Culture-based method | | | | | | |
| University | Total spores | Intercept | 2.976 | 0.104 | 0.000 | 0.744 |
| | | $PM_{10}$ | −0.001 | 0.000 | 0.000 | |
| | | Avg SH | 0.001 | 0.000 | 0.002 | |
| | *Aspergillus* spp. | Intercept | 2.029 | 0.160 | 0.000 | 0.380 |
| | | $T_{Min}$ | 0.030 | 0.007 | 0.000 | |
| Library | Total spores | Intercept | 2.765 | 0.100 | 0.000 | 0.244 |
| | | Avg SH | 0.001 | 0.000 | 0.002 | |
| Hospital | Total spores | Intercept | 2.850 | 0.112 | 0.000 | 0.251 |
| | | $T_{Avg}$ | 0.014 | 0.004 | 0.002 | |
| Workshop | *Curvularia* spp. | Intercept | 2.210 | 0.233 | 0.000 | 0.263 |
| | | $NO_2$ | −0.016 | 0.005 | 0.001 | |

For the final regression model to identify the significant predictor, the monthly average of total 13 candidate variables (maximum, minimum, and average temperature ($T_{max}$, $T_{min}$, $T_{avg}$, respectively), relative humidity (r.h.), wind speed (W), total rainfall (R), average sun hour (Avg SH), $PM_{2.5}$, $PM_{10}$, $NO_2$, $SO_2$, CO, and $O_3$) was used. $p < 0.01$; $n = 252$

[a]Standard error

## Assessment of the effect of ambient spore concentrations on respiratory health

To evaluate the effect of fungal spore concentrations on the local people, patients' data were collected during the entire monitoring period. It revealed that among four disease categories, the "Others" group which includes cough, cold, sneezing, wheezing, breathlessness, allergic rhinitis, etc. was the most frequent, reported in a maximum number of patients (46.14%), followed by "PTB" (19.99%), "Asthma" (18.25%), and "COPD" (15.62%). It was also observed that patients visited the hospital most in the winter season and minimum in the post-monsoon season, contradicting our findings from the monitoring. However, upon analyzing the monthly patients' visits to the hospital and monthly concentrations of total spores and major taxa of seven microenvironments (Fig. 4), as well as total spores of the outdoor environment and indoor environments (Online Resource 8) for both sampling methods, it seems that the exposure to fungal spores might have an important role in promoting sensitization to the patients with underlying lung conditions.

For further analysis, the relationship between diseases and total spore concentrations of both sampling methods along with the major taxa of both environments was analyzed by linear regression analysis. It showed several direct linear relationships, i.e., positive association. It reveals that the total number of hospitalized patients had a direct linear relationship with total spores, *Aspergillus/Penicillium* spp., and *Cladosporium* spp. found through the microscopy-based method as well as total spores and *Penicillium* spp. from the culture-based method (Online Resource 9).

Among four categories of diseases, only *Aspergillus/Penicillium* spp. showed a positive linear relationship with the number of hospitalized patients for asthma exacerbation

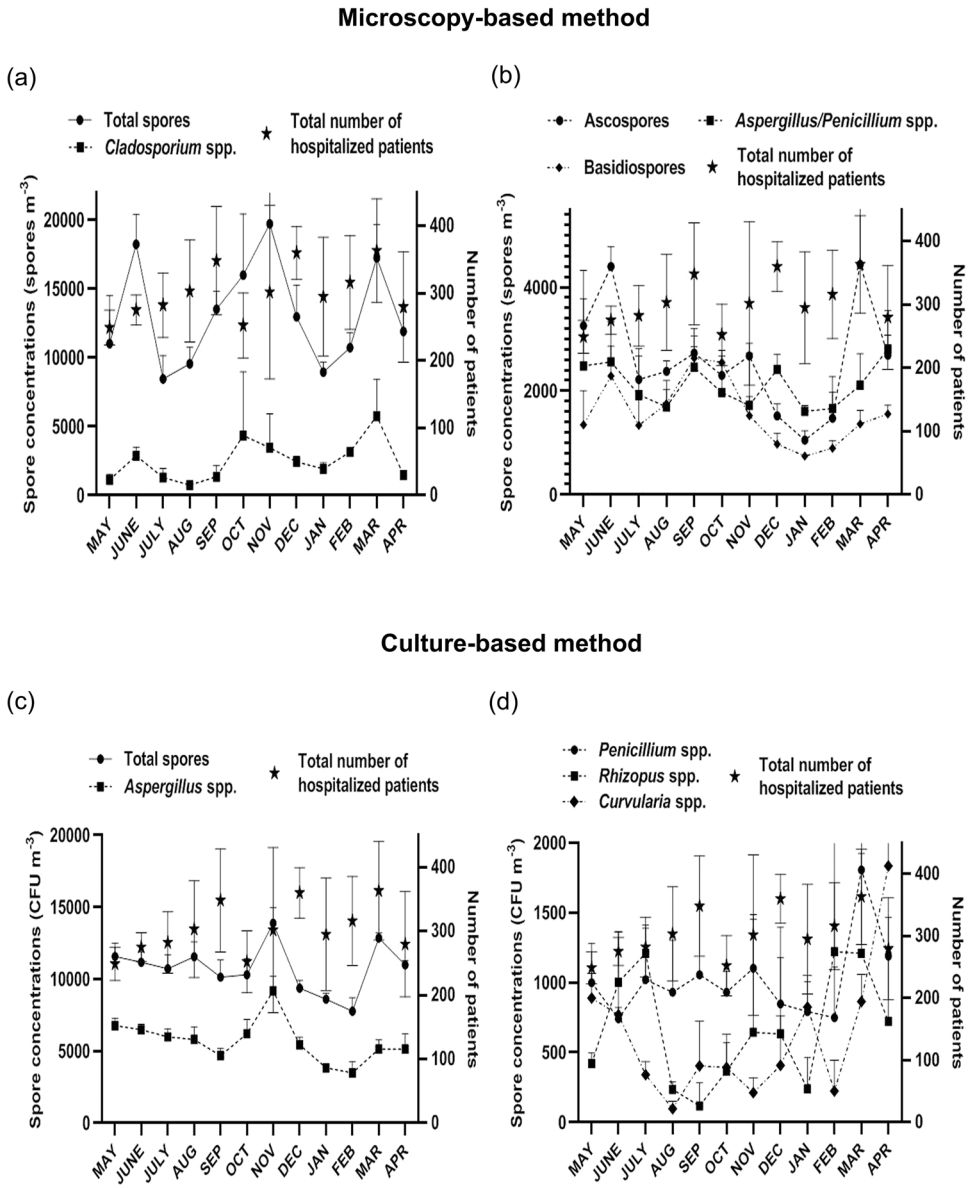


**Fig. 4** Relationship between monthly respiratory disease cases and concentrations of total spores as well as major taxa at both indoor and outdoor environments in Kolkata from May 2014 to April 2017: monthly patients' visits with **a** total spores and *Cladosporium* spp.; **b** ascospores, *Aspergillus/Penicillium* spp., and basidiospores, recorded by the microscopy-based method; **c** total spores and *Aspergillus* spp.; **d** *Penicillium* spp., *Rhizopus* spp., and *Curvularia* spp., obtained by the culture-based method. Symbols represent mean ± SD

(Fig. 5a). COPD patients were positively associated with *Cladosporium* spp. (Fig. 5b), *Penicillium* spp. (Fig. 5c), and total spores of culture-based method (Online Resource 9). "PTB" also showed direct linear associations with *Penicillium* spp. (Fig. 5d), *Aspergillus/Penicillium* spp. (Fig. 5e), and total spores of the culture-based method (Online Resource 9). Besides, the "Others" group of patients had positive associations with total spores of both microscopy-based and culture-based methods (Online Resource 9), *Penicillium* spp. (Fig. 5f), *Aspergillus/Penicillium* spp. (Fig. 5g), and *Cladosporium* spp. (Fig. 5h). The rest of the major taxa showed an inverse linear relationship with diseases. In addition, several positive associations of total spore concentrations in indoor environments were also observed with the total number of hospitalized patients, "PTB" and "Others" groups by both sampling methods, whereas patients from the "COPD" group showed a positive association only with total fungal spore concentration observed through the culture-based method.

For every disease category, males were more susceptible than females. The unpaired *t*-test revealed significant variations in the male to female ratio of all four categories and the total number of hospitalized patients (Fig. 6a). Patients aged 15–45 years suffered the most and visited the hospital more. Family history of allergenic respiratory symptoms was reported in 56.2% of the total number of hospitalized patients.

Finally, to assess the allergenic potential of the fungi recovered by culture-based sampling in the studied area, the skin prick test was performed. Thirteen antigenic extracts were prepared from thirteen different individual species based on their dominance and availability.

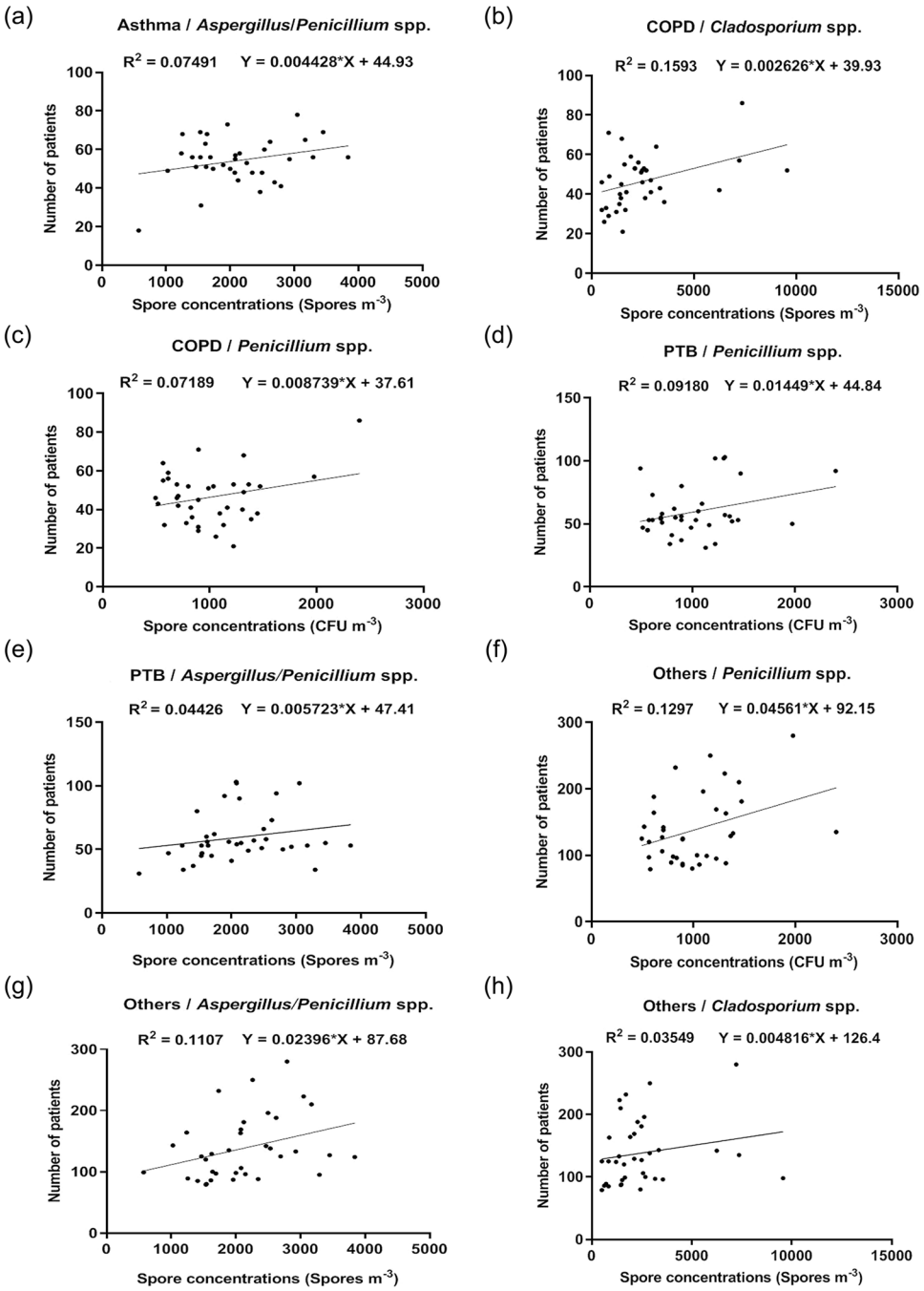


**Fig. 5** Linear regression analysis of four disease categories and their corresponding associated major taxa of both indoor and outdoor environments in Kolkata from May 2014 to April 2017 showing direct linear association: **a** asthma and *Aspergillus/Penicillium* spp. **b** COPD and *Cladosporium* spp. **c** COPD and *Penicillium* spp. **d** PTB and *Penicillium* spp. **e** PTB and *Aspergillus/Penicillium* spp. **f** Others and *Penicillium* spp. **g** Others and *Aspergillus/Penicillium* spp. **h** Others and *Cladosporium* spp.; *Aspergillus/Penicillium* spp., and *Cladosporium* spp. were found by the microscopy-based method, while *Penicillium* spp. was found by the culture-based method

The results of SPT demonstrated positive responses in 75.86% of the total subjects evaluated. The highest reactivity was observed from asthmatic patients (80.76%), whereas 77.77% of allergic rhinitis patients and 63.33% of patients with other respiratory discomforts showed allergenic sensitization. Among thirteen individual fungal extracts, *Aspergillus fumigatus* showed the maximum reactivity (50.9%), followed by *Aspergillus flavus* (40.0%), *Aspergillus oryzae* (37.27%), *Aspergillus niger* (36.36%), *Aspergillus ochraceus* (33.64%), and *Aspergillus terreus* (30.0%) (Fig. 6b). Extracts of these six species also showed + 1 to + 4 ranges of intensities of allergenic reaction. Apart from these species, *Penicillium oxalicum* (28.18%), *Curvularia pallescens* (22.72%), and *Rhizopus oryzae* (21.82%) also elicited higher sensitivity showing + 1 to + 3 intensity, whereas the other four species, *Fusarium lateritium* (14.55%), *Candida albicans* (13.64%), *Trichoderma harzianum* (11.82%), and *Alternaria alternata* (9.1%), reported < + 3 gradation.

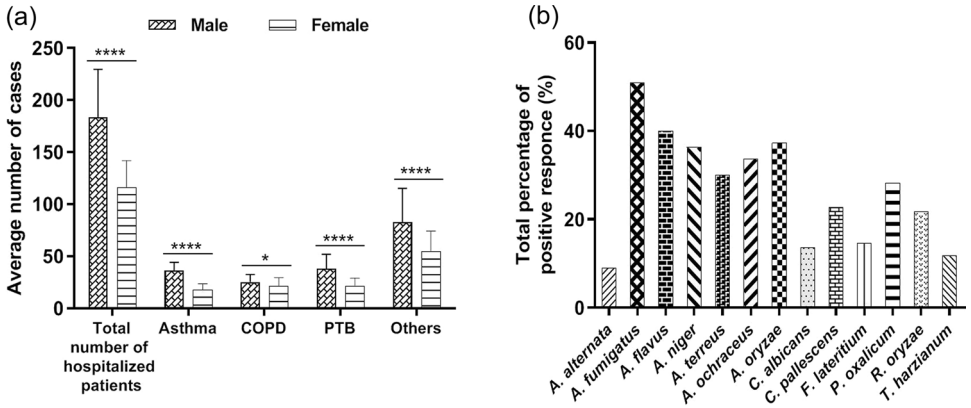


**Fig. 6** Determination of the male–female ratio of patients and assessment of the allergenic potential of fungal species obtained through the culture-based method: **a** Significant variations of male–female ratio of the total number of hospitalized patients and the patients of four groups of diseases such as asthma, COPD, PTB, and Others. $^{*}p < 0.05$; $^{****}p < 0.0001$ using unpaired *t*-test; **b** results of Skin Prick Test performed by antigenic extracts of thirteen species of fungi grown by the culture-based method revealing the total percentage of positive responses

## Discussions

Spore concentration varies largely across the world depending on the geographical locations. In the air of an external space of the University of Costa Rica, a tropical country like India, the mean total spore concentration was $443.86 \pm 201.68$ spores $m^{-3}$ (Brizuela-Hernández et al. 2019). However, in the rural agricultural areas of West Bengal, India, spore counts ranged from 82 to 2635 spores $m^{-3}$ for total fungal spore concentration and 72–1769 CFU $m^{-3}$ for viable fungi (Adhikari et al. 2004). It implies that the spore counts recorded in the present study were higher than the spore counts documented in the previous studies which might indicate the increase of risk factors of allergenic disorders for the local people of the studied area.

In this study, maximum spore concentration was observed in the open field area, an outdoor environment, rather than in the educational or occupational area for both sampling methods. A similar observation was also reported in earlier studies (Oberle et al. 2015; Sautour et al. 2009). The presence of the sources of fungal growth, like trees, weeds, grasses, and organic matter in the soil, and the effects of atmospheric parameters might be responsible for the prevalence of high airborne fungal spore load in the garden. This is in agreement with previously published findings. Ju et al. (2003) demonstrated that the abundance of multiple trees, shrubs, and herbaceous plants in the green area was responsible for higher fungal spore concentrations. The growth of several saprophytic and parasitic fungi on phylloplanes (leaf surface), reported by Picco and Rodolfi (2000), also enhanced the diversity and concentrations of fungi (Fang et al. 2019; Nageen et al. 2021). Fang et al. (2005) reported more diverse and significantly higher concentrations of culturable fungi in greener areas than in densely populated and polluted areas in Beijing. The strong influence of vegetation coverage on aerial concentrations of airborne fungi in outdoor environments was also observed in Hangzhou, China (Fang et al. 2019).

Among seven microenvironments, fungal spore concentration was also found to be maximum in the garden sampled with the microscopy-based method. On the contrary, for the culture-based method, indoor microenvironments were observed to be highly concentrated. These contrasting results might be reflections of the work purpose and infrastructures of studied indoor areas which provided favorable conditions for the growth of indoor fungi such as *Aspergillus* spp. and *Penicillium* spp. Large doors and windows in the airy classrooms of the school and the hospital might facilitate the influx of outdoor particles through air movements in indoor environments. Passive transport of spores by students, patients, and workers might be an important source of fungal growth inside almost all studied indoor microenvironments which was supported by the previous studies conducted in school (Oliveira et al. 2009) and hospital (Ekhaise et al. 2008). Additionally, the presence of cellulosic substrates like books, papers, and journals in the library might have served as a good source of cellulose-degrading fungi. Moreover, the moisture content of the wall, dampness, building materials, dust particles, and poor ventilation also influenced fungal colonization inside the hospital building, workshop, university, and library. Such kind of contamination of the indoor air was reported earlier (Górny 2004). Contrarily, the lowest spore concentration was observed in the bank microenvironment. The presence of air-conditioning, proper ventilation system, and thorough maintenance of human activity might be the reason for the less contamination of the air inside the bank which was reflected in the fungal spore concentrations.

During the monitoring period, both outdoor and indoor microenvironments showed a wide spectrum of various species of fungi. Most of the species belonged to phylum Ascomycota and Basidiomycota. According to Harley et al. (2009), early childhood wheezing was associated with high concentrations of ascospores and basidiospores. Chen et al. (2014) registered that approximately 1500 spores $m^{-3}$ were the threshold concentration of airborne fungi for the reduction of lung function. In the present study, the mean concentrations of all major taxa recorded by the microscopy-based method such as ascospores, *Cladosporium* spp., *Aspergillus/Penicillium* spp., and basidiospores were found to be greater than the reported threshold value (Table 1). Apart from these taxa, an elevated concentration of *Periconia* spp. was observed in the outdoor as well as the indoor area (university and library). Besides, *Ganoderma* spp. and Rust spores from Basidiomycota phylum showed remarkably higher concentrations in workshop and library microenvironments, respectively (Online Resource 3). The constant exposure of these taxa to the local people might induce allergenic or respiratory disorders in sensitive patients. However, detailed epidemiological studies are needed to determine the threshold concentration of fungal spores to induce an allergic response to the susceptible population of the studied environments. In our investigation, *Cladosporium* spp. was found as one of the predominant fungi by the microscopy-based method. But, surprisingly, we did not recover any colonies of *Cladosporium* spp. through the culture-based method. It might be due to the high incubation temperature and short incubation period set in our study that did not favor the germination of the spores. Another possible reason might be the antagonistic activities of other fungal colonies, grown in the exposed petri plates, which could inhibit the growth of *Cladosporium* spp. (Barbosa et al. 2001).

In the present study, maximum spore load was observed during the post-monsoon season, whereas minimum during the winter season for both sampling methods. A similar seasonal periodicity pattern has also been observed in the semi-rural and industrial townships in West Bengal (Karmakar et al. 2020; Roy and Gupta Bhattacharya 2020). Fang et al. (2019) also reported the lowest concentrations of culturable fungi during the winter at Hangzhou in southeastern China. However, our finding was different from some previous studies conducted in urban and suburban areas in Kolkata (Chakrabarti et al. 2012; Das and Gupta-Bhattacharya 2012; Dey et al. 2018) where they documented that monsoon season showed the highest spore concentrations. These variations might be occurred due to the yearly fluctuation of environmental variables, the nature of the sampling locations, and the selection of the sampler used for monitoring.

Spearman's rank correlation between environmental parameters and fungal spore concentrations has revealed significant effects of meteorological parameters as well as air pollutants on fungal aerospora. Additionally, the stepwise multiple regression analysis has pointed out that these parameters were significant predictors for spore concentrations, especially in indoor environments.

In the present study, the ambient temperature was observed as one of the most important meteorological variables. We observed a significant negative association of temperature with *Cladosporium* spp. at outdoor, triggering an elevated concentration in post-monsoon, winter, and early summer (Fig. 3a). Previous studies have also reported the abundance of *Cladosporium* spp. in a cooler climate (Fang et al. 2019; Oliveira et al. 2010; Radon et al. 2002). A positive effect of temperature was also observed on ascospores and basidiospores in the open field area. High temperature (> 30 °C) along with low relative humidity (< 70%) is important for the "active release" of ascospores (Kwon-Chung and Sugui 2013) which could be a possible reason for the observed higher concentration during summer (Fig. 3a). Further analysis with the regression model also showed ambient temperature as an important predictor in different indoor environments.

Average sun hour was also detected as another important meteorological variable in our investigation. Kallawicha et al. (2017) stated that sunlight has influenced fungi by increasing air temperature and activating the growth and sporulation of some fungal taxa. They reported a weak and insignificant positive association of sunlight with ascospores. Contrarily, in the present study, we observed a significant positive association between average sun hour and ascospores. Moreover, it was found an important predictor of ascospores and total spores of the culture-based method in indoors.

Excessive rain usually washes the spores out of the ambient environment and tends to decrease spore concentrations (Pakpour et al. 2017). This kind of negative association of rainfall with *Cladosporium* spp. was demonstrated earlier that we found too (Oliveira et al. 2010). However, rainfall increases atmospheric moisture content which is required for spore release, especially ascospores (Magyar et al. 2009). Similarly, we observed a significant positive correlation with ascospores in the garden indicating the reason for the abundance of ascospores in the monsoon season (Fig. 3a).

In addition, relative humidity and wind speed also showed immense influence on fungal aerospora and were also detected as key predictors in several indoor microenvironments. Relative humidity can be positively associated with aeromycoflora by increasing moisture content that drives the production and release of spores, especially ascospores and basidiospores (Grinn-Gofroń and Bosiacka 2015). A similar result was also observed for ascospores and basidiospores in the present study. Besides, it showed a significant negative association with *Cladosporium* spp. as well. Increased humidity causes a reduction in the bouncy of suspended

spores in the air due to the high spore sedimentation rate, which might be a possible reason for this inverse relation (Pyrri and Kapsanaki-Gotsi 2017).

Spore release and dispersal are enhanced by wind speed. Higher wind speed is responsible for the "passive release" of ascospores (Kwon-Chung and Sugui 2013). Such a positive effect with ascospores and basidiospores was observed in this study. In contrast, high wind speed may dilute the spore load in the air and affect it negatively. The negative impact of wind speed on total spores has also been reported by Almaguer et al. (2014). A similar association was also noticed in both environments in the present study (Table 4).

The increasing concentration of air pollutants itself imposes a serious effect on respiratory health. The high concentrations of atmospheric pollutants due to vehicular emissions and uncontrolled biomass burning have registered Kolkata as one of the most polluted cities in India. Various studies have confirmed the association between atmospheric pollutants and fungal spore concentrations (Kallawicha et al. 2017; Pyrri and Kapsanaki-Gotsi 2017). Cakmak et al. (2012) have reported that the effect of aeroallergens on asthma can be modified by air pollution. Gaseous pollutants like $O_3$ and $NO_2$ can chemically alter aeroallergens by producing reactive oxygen species (ROS) and reactive nitrogen species (RNS) (Reinmuth-Selzle et al. 2017). Moreover, $O_3$ can negatively affect the viability of culturable fungi (Wu et al. 2007). Sommer et al. (1981) have stated the effect of CO on fungi by suppressing growth. Particulate matters (PM) could change the biological and morphological characteristics of microorganisms by binding with them (Alghamdi et al. 2014).

In our investigation, the fungal aerosol in different microenvironments also showed significant associations with atmospheric pollutant concentrations. Moreover, $PM_{10}$ was identified as the only predictor in the outdoor environment by the multiple regression analysis. Furthermore, $PM_{10}$ and $NO_2$ were also detected as significant key predictors in several indoor microenvironments. The concentrations of $PM_{2.5}$ and $PM_{10}$ had both positive as well as negative effects on fungal spore concentrations. Both $PM_{2.5}$ and $PM_{10}$ exhibited a significant positive association with *Cladosporium* spp. in the open field area. Some previous studies observed a similar association with *Cladosporium* spp. in the Taipei metropolis (Kallawicha et al. 2017) and Taiwan (Ho et al. 2005). Besides, Roy et al. (2017) reported a negative correlation between $PM_{10}$ and basidiospores which we also found in the outdoor area. Fan et al. (2021) observed negative correlation of residential culturable fungi with $PM_{2.5}$ and $PM_{10}$ at indoor rooms in China. In our study, $PM_{10}$ was also identified as a key predictor with a negative impact on total spores of the culture-based method in the university microenvironment.

Among gaseous pollutants, the negative association of $NO_2$ and basidiospores was also observed earlier (Roy et al. 2017). Kallawicha et al. (2015, 2017) reported the negative effect of CO on basidiospores by inhibiting the growth of mushrooms. Similarly in our study, CO showed several significant negative associations in indoor environments. Conversely, in outdoor, we observed the positive impact of CO and $NO_2$ on total spores of the culture-based method. A similar correlation with residential culturable fungi was also detected by Fan et al. (2021) but in indoors. Although Adhikari et al. (2006) found a positive association between $O_3$ and *Aspergillus/Penicillium* spp. as a function of temperature, in our study, we observed a negative association in the hospital microenvironment.

The $R^2$ values, indicating lower goodness of fit of the regression models, ranged from 0.191 to 0.744 (Table 5). The highest value ($R^2 = 0.744$, $p < 0.01$) was found to be greater than the optimal value of 0.7, observed for the total spores of the culture-based method in the university microenvironment, indicating that the aerial concentration was highly regulated by environmental parameters. The $R^2$ values of other fungal taxon models were comparable with previous studies (Kallawicha et al. 2017; Ponce-Caballero et al. 2013; Sousa et al. 2008). Among major taxa found by both microscopy-based and culture-based methods, basidiospores ($R^2 = 0.542$) and *Aspergillus* spp. ($R^2 = 0.380$), respectively, were mostly influenced by the environmental parameters in the university. Among all microenvironments, fungal aerospora in the university were immensely controlled by environmental parameters. Moreover, these regression models can predict the fungal spore concentrations by using these atmospheric variables which can be used for further investigation regarding health effects on local people, workers, and students and can help to minimize the exposure to fungal spores.

In the present study, biomonitoring clearly indicated the prevalence of allergenic fungi in the atmosphere in Kolkata. Thus, to evaluate the impact of these aeroallergens on the human health of the local population, a health survey was executed during the entire monitoring period. Our result showed that exposure to fungal spores might be one of the possible reasons to induce a delayed effect of the fungal aeroallergens on allergic respiratory symptoms. Besides, the exacerbation of asthma, COPD, or other allergenic diseases is not only dependent on one variable but also the combined interaction between aerial concentrations of fungal spores, air pollutants, meteorological factors, other bioaerosols, viral and bacterial infections, etc. Both PM and gaseous pollutants are harmful to human health. Table 3 also shows the elevated concentrations of air pollutants in the winter season. This fact might be responsible for increasing the rate of respiratory and allergenic disorders in the winter which was reflected in our findings. Moreover, during the survey, we noticed that there was a drifting of patients' numbers in the October and November months, i.e., in the post-monsoon

season. The unavailability of patients at the early stages of symptom development and air pollution due to the use of firecrackers in the festive seasons in October and November might contribute to an additional surge in patient numbers in December. However, exposure to ambient fungal concentration might be one of the key factors for promoting or worsening existing airway diseases.

The linear regression analysis revealed that four categories of diseases had direct linear relationships with several major taxa and total fungal spores obtained by both methods with a positive population regression coefficient ($\beta_1$ or slop), indicating a positive association with them. Among the fungi from the microscopy-based method, total spores, *Aspergillus/Penicillium* spp., and *Cladosporium* spp. exhibited a direct linear relationship with symptoms. On the other hand, among the fungal isolates recovered by the culture-based method, total spores and *Penicillium* spp. were found to have a positive association with patients' visits to the hospital. Our findings indicated that the number of hospitalized patients for asthma exacerbation was positively associated with only *Aspergillus/Penicillium* spp. There is vast evidence of the association between fungal exposure and asthma (Denning et al. 2014; Jones et al. 2011). Thus, further analysis of the monthly spore concentrations of *Aspergillus/Penicillium* spp., *Aspergillus* spp., and *Penicillium* spp. with asthmatic patients revealed that it might be due to the possibility of the delayed effect of *Aspergillus* spp. and *Penicillium* spp. (Online Resource 10). However, *Aspergillus* spp. did not exhibit a linear association with any diseases probably for this delayed effect.

For "COPD" patients, the coefficient of determination ($R^2$ value), indicating the closeness of fit of the regression line, was found to be maximum for *Cladosporium* spp. followed by *Penicillium* spp. and total spores of the culture-based method. It indicated that among these positive associations, *Cladosporium* spp. was able to induce maximum sensitization in "COPD" patients. Co-infection of *Aspergillus* spp. with *Mycobacterium tuberculosis* in Pulmonary Tuberculosis (PTB) patients is well-established (Hosseini et al. 2020; Xerinda et al. 2014). In our study, we observed that *Penicillium* spp. was more positively associated with PTB than *Aspergillus/Penicillium* spp. and total spores of the culture-based method. Besides, the maximum number of major taxa and total fungal spores found by both techniques can be able to stimulate allergenicity and respiratory diseases by showing direct linear relationships with the "Others" group of patients. Among them, the highest sensitization was elicited by *Penicillium* spp. having maximum $R^2$ value (Fig. 5f). In addition, it was also found that in the indoor environments, total spores of the microscopy-based method showed the maximum association with the "Others" group of patients, whereas total spores of the culture-based method showed a direct linear association with PTB (Online Resource 8).

The male to female ratio also showed the higher susceptibility of males to diseases, probably due to their nature of the occupation, regular transport in different outdoor and indoor areas, etc., leading to an increased level of fungal exposure. Maximum suffering of the patients of 15–45 years age group is also interpreted as the indication of the fungal exposure of students and workers belonging to the young and middle age population. Although studies have demonstrated that exposure to indoor fungal spores can worsen allergic responses (Adhikari et al. 2004; Levetin et al. 1995; Ye et al. 2021), outdoor exposure is more relevant for sensitization and disease expression. Generally, an average person spends most of the time his/her entire day in indoor environments; hence, both indoor and outdoor fungal concentrations were taken into account for analysis. However, the factors that may influence the spore concentration and species diversity in indoor environments should be taken into account. Besides, only one outdoor environment was selected for monitoring in our investigation. To overcome this limitation, more outdoor sampling sites should be considered for a better understanding of the fungal spectrum in the said environment.

The results of SPT exhibited the allergenic potentials of all the selected fungal species. Concerning inducing positive reactions and intensity of sensitization in the atopic patient cohort, *Aspergillus fumigatus* was found as the most reactive species. Similar findings were also reported in the previous studies (Chakrabarti et al. 2012; Dey et al. 2018). Besides, other dominant species of major taxa grown by the culture-based methods tested such as *Penicillium oxalicum, Curvularia pallescens*, and *Rhizopus oryzae* showed higher sensitization potential. Moreover, positive responses were also noticed to the extract of other fungal species as well. In our sampling area, we observed an abundance of these fungal species that are already reported as a potential source of aeroallergens, thus inferring that aeroallergens from these fungi can promote the worsening of respiratory health to the atopic patients in the population.

Therefore, the prevalence of aeroallergens in the air in Kolkata might fuel the burden of respiratory diseases and be a potential risk factor for human health. Thus, the regression equations, obtained from the regression analysis, can predict the value of the incidence of the diseases. Furthermore, the identification and characterization of allergens from the dominant fungi can facilitate a scope for clinicians for improved diagnostics in fungal allergy and can also design a sustainable model of allergen-specific immunotherapy for atopic patients.

## Conclusions

In the present study, aerobiological monitoring has described the diversity of fungal aerospora in different indoor and outdoor environments in Kolkata, their association with

environmental factors, and their impact on the local inhabitant. The outdoor environment showed a higher spore load than indoor environments, and the highest spore concentration was observed in the post-monsoon season. In the outdoor area, only the air pollutant $PM_{10}$ was identified as a significant predictor through multiple regression analysis. In different indoor microenvironments, $PM_{10}$, $NO_{2,}$ and all meteorological parameters except rainfall have a significant effect on aerial concentrations of fungi and can act as key predictor variables. These findings can help to understand the fungal spore distribution in different microenvironments in Kolkata. The regression models can be used as preliminary information for developing daily forecast models. The health survey revealed that the sensitization to these fungal allergens might promote a delayed response of allergic sensitization in patients with respiratory conditions. SPT also confirmed their allergenicity in the sensitized patients indicating the adverse effect of aeroallergens on human health. The entire study indicated that the prevalence of fungal aeroallergens in the atmosphere is significant enough to achieve as a risk factor for the local population of the studied area. The information obtained from the study will also be useful for clinicians in taking necessary safety measures and making awareness among the inhabitant in Kolkata of fungal spore exposure.

**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1007/s11869-023-01316-1.

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**Author contribution** Conceptualization and supervision: Swati Gupta Bhattacharya; investigation: Koyel SenGupta and Bijoya Karmakar; formal analysis: Koyel SenGupta and Sangeeta Roy; writing—original draft preparation: Koyel SenGupta; writing—reviewing and editing: Sangeeta Roy and Amarjeet Kaur.

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**Data availability** The authors declare that all data generated or analysed during this study are available within the article and its supplementary information files.

**Code availability** Not applicable.

## Declarations

**Ethical approval** All procedures performed in this study involving human participants were in accordance with the ethical standards of the hospital.

**Consent to participate** Consents were collected from the human participants according to the ethics of the hospital.

**Consent for publication** Not applicable.

**Conflict of interest** The authors declare no competing interests.

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