Atmospheric pollen grains of a suburban area near India–Bangladesh border with reference to their allergenic potential and probable effect on asthma-related hospital admission

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To study the role of atmospheric pollen on respiratory allergy and asthma-related hospitalization (ARH), a pollen calendar was prepared for a suburban area (Habra) of West Bengal, near India–Bangladesh border on the basis of seven-year (2007–2013) aeropollen monitoring with Burkard slide-sampler. Among 40 pollen types, Poaceae/grass showed highest contribution (12.32%) followed by *Trema orientalis* (11.45%) and others. Among 30 allergenic pollen types, Poaceae/grass showed the highest sensitivity in skin-prick test (>50%) and IgE-ELISA. ARH of local population ($n = 9492$) showed significant positive correlation ($P < 0.05$) with airborne pollen of grass, *Bombax ceiba*, *Mangifera indica* and total aeropollen too.

Keywords: Airborne pollen calendar, allergenic pollen, asthma-related hospitalization, IgE-ELISA, skin-prick test.

AIRBORNE pollen grains can induce IgE-mediated respira tory allergy and asthma in susceptible individuals[1] with variable seasonal patterns according to time, geographical location and climate[2]. Thus, study of atmospheric pollen biodiversity is an essential prerequisite for assessment of allergenic pollen exposure and selection of proper antigen during desensitization treatment of respiratory allergy and allergic asthma.

Atmospheric pollen exposure often correlates with seasonal allergic rhinitis and emergency asthma attacks from different parts of the world[1,3,4], but the aspect has received relatively less attention in India, a country of rich and diverse vegetation with various geoclimatic regions[5]. So, diversity of airborne pollen grains was studied in Habra, West Bengal, for seven years (January 2007–December 2013) to observe their probable effect on respiratory allergy and asthma-related hospitalization (ARH) on susceptible local population.

Habra (22.83°N, 88.63°E) is a town in North 24 Parganas district of West Bengal, 40 km north from Kolkata city and 32 km away from India–Bangladesh border (Petrapole) over the Gangetic delta (Figure 1). It is an important hub for import–export between India and Bangladesh. Basically surrounded by rural areas with rich tropical and moist vegetation, this town experienced two remarkable population influxes in 1947 (partition of India, after independence) and 1971 (when Bangladesh became independent from Pakistan). This area has a population of 149,675 (2011 census) and the local health questionnaire survey showed that a number of people (9.82%) suffered from respiratory allergy and asthma.

Airborne pollen grains were monitored at approximate human height (1.5 m) using portable, battery-operated personal slide sampler (flow rate 10 l/min). The sampling frequency was five days/week for 10 min, thrice a day (11 : 00 am and 1 : 00 pm and 3 : 00 pm). The trapped pollen grains were microscopically studied[6] with acetolysed[7] reference pollen slides. Meteorological data were recorded from the Netaji Subhas Chandra International Airport, Dum Dum, North 24 Parganas.

After fresh pollen collection (>95% pure), soluble proteins were extracted in phosphate buffered saline (PBS)[8] and stored in sterile vials at –20°C.

Skin-prick tests (SPT) were carried out with the pollen extract according to the case history[9] (allergic rhinitis and/or bronchial asthma) of adult respiratory allergic patients of the study area attending Mediland Diagnostic Centre, Kolkata. Histamine diphosphate (1 mg/ml) and PBS were used as positive and negative controls respectively. The weal response was measured after 15 min and graded at +1 to +3 levels[10].

Sera were collected from pollen allergic subjects with +2/+3 level skin reactivity, not receiving immunotherapy. Control sera were collected from non-atopic healthy volunteers (confirmed by negative skin reaction) and having no history of allergic diseases. The consent of the patient was obtained prior to sera collection. The entire study was approved by the Ethics Committee of the clinic. Enzyme-linked immmunosorbent assay (ELISA) was performed to measure pollen-specific IgE levels with the whole pollen extract against patients' sera[8].

The data of ARH were recorded from Habra State General Hospital and Barasat District Hospital, two important government hospitals for local people. After baseline study, data from 2007 to 2013 were used for statistical analyses, including 9492 patients (age range of 18–75 years) with principal diagnosis of asthma[11]. All the statistical studies were performed using Spearman non-parametric correlation analyses (SPSS version 20.0).

Forty airborne pollen types were identified up to family/genus/species level. Pollen grains of Asteraceae, Chenopodiaceae–Amaranthaceae, Poaceae (grasses) and other stenopalynous members could be identified only up to family level. It was found that 55% of aeropollen were of arboreal origin, 33.84% came from herbs and 6.3% from shrub members.

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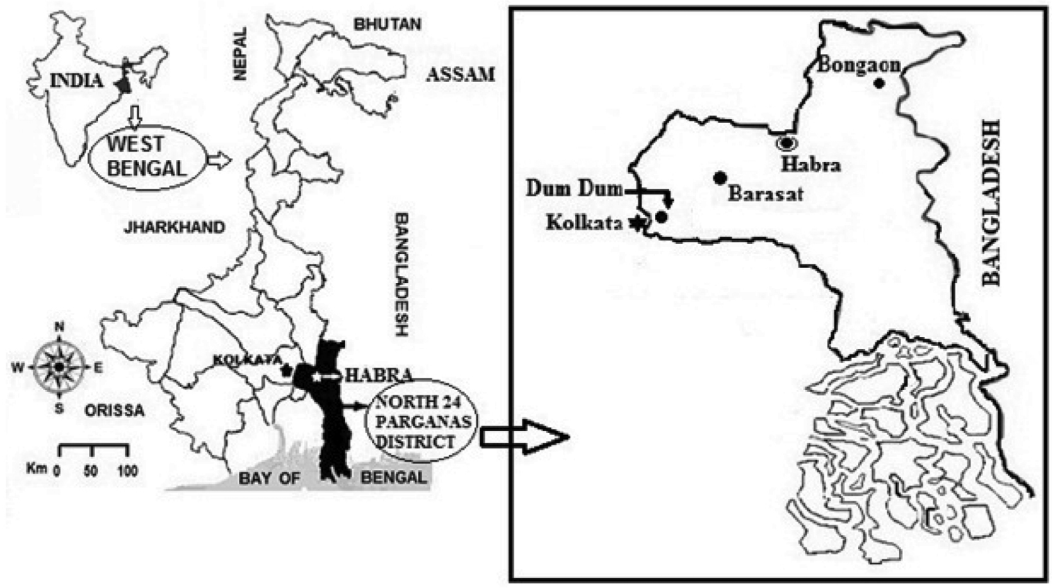

Figure 1. Location of aeropalynological sampling site at Habra town, local hospitals (Habra, Barasat and Madhyamgram) and meteorological station (Netaji Subhas International Airport, Dum Dum) in North 24 Parganas district of the state of West Bengal, India.

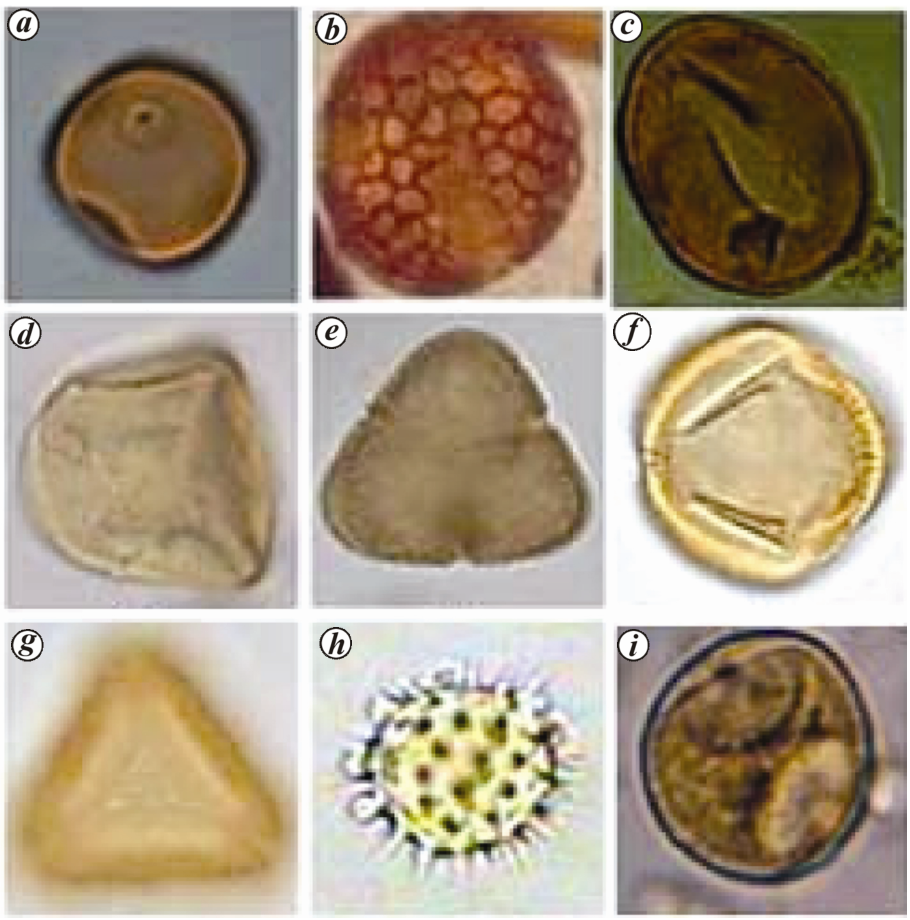

Figure 2. Light microscopy (under 100×) of some dominant and common pollen grains present in the atmosphere of study area. EV, Equatorial view; PV, Polar view. ***a***, *Saccharum officinarum* (Poaceae); ***b***, *Peltophorum pterocarpum* (EV); ***c***, *Cocos nucifera* (EV); ***d***, *Cyperus rotundus* (Cyperaceae); ***e***, *Bombax ceiba* (PV); ***f***, *Mangifera indica* (EV); ***g***, *Eucalyptus citriodora* (PV); ***h***, *Helianthus anuus* (Asteraceae) (EV); ***i***, *Trema orientalis* (EV).

Table 1. Pollen types identified in the atmosphere of study area along with their contribution to total aeropollen load

Pollen types	Mean annual contribution to total aeropollen load (%)	Pollen types	Mean annual contribution to total aeropollen load (%)
Trees			
Trema orientalis	11.45	*Tectona grandis*	0.65
Cocos nucifera	7.31	*Mangifera indica*	0.36
Peltophorum pterocarpum	4.48	*Aegle marmelos*	0.24
Phoenix sylvestris	4.31	Shrubs	
Fabaceae	3.76	*Ricinus communis*	3.69
Borassus flabellifer	3.29	*Lantana camara*	1.3
Eucalyptus citriodora	3.18	Malvaceae	1.3
Delonix regia	2.40	Herbs	
Polyad (Fabaceae)	2.25	Poaceae	12.32
Lagerstroemia speciosa	2.0	Cyperaceae	5.98
Carica papaya	1.90	Asteraceae	3.71
Polyalthia longifolia	1.52	Cheno-Amaranthaceae	2.88
Azadirachta indica	1.38	*Cassia* sp.	2.77
Areca catechu	1.35	*Catharanthus roseus*	1.96
Terminalia arjuna	1.07	*Argemone mexicana*	1.24
Zizyphus zuzuba	1.0	Lamiaceae	0.75
Bombax ceiba	0.93	*Croton* sp.	0.63
Tamarindus indicus	0.79	*Justicia* sp.	0.63
Mimusops elengi	0.76	*Clerodendrum* sp.	0.50
Dillenia indica	0.72	*Brassica* sp.	0.22
Alstonia scholaris	0.66	*Datura* sp.	0.20

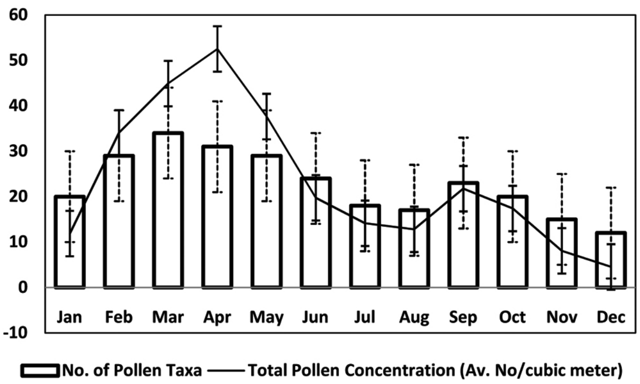

Figure 3. Monthly variation of mean value of total aeropollen load and number of pollen taxa in the atmosphere of Habra town during 2007–13. The bars indicate standard deviations.

Poaceae pollen type was the major single contributor (12.32%) to mean annual aeropollen concentration. *Trema orientalis* (11.45%), *Cocos nucifera* (7.31%), Cyperaceae (5.98%), and others (Figure 2, Table 1). Contribution of first 14 members was more than 70% (71.57%) altogether. There are two peak pollen seasons in a year: one in March–April and another in September–October (Figure 3). Maximum pollen catch was recorded in April (65.5 pollen/m^3 air in 2009). In July–August and December, lowest pollen count was recorded (4.5 pollen/m^3 air in December 2012). Every year, the maximum number of contributory pollen taxa was recorded in March, whereas the minimum number of taxa was recorded in December.

The pollen calendar of the study area shows the overall periodicity patterns of all recorded pollen taxa (Figure 4). Pollen grains of *Areca catechu*, Asteraceae, *Carica papaya*, Chenopodiaceae–Amaranthaceae, *Cocos nucifera*, Cyperaceae, Fabaceae, Poaceae, *Ricinus communis* and *Trema orientalis* were found in atmosphere round the year, whereas other types showed distinct seasonal patterns.

The aeropollen count showed positive correlation with average temperature ($r = 0.587$, $P < 0.05$ level) and wind speed ($r = 0.650$, $P < 0.05$ level) (Figure 5). There was no significant effect of average humidity and rainfall on atmospheric pollen count.

Thirty pollen types elicited positive response in SPT with pollen extracts in a population of adult respiratory allergic patients of the study area attending the allergy clinic. Strongest hypersensitivity was demonstrated to grass pollen members (*Saccharum officinarum*, 53.79% and *Imperata cylindrica*, 52%), followed by *Azadirachta indica* (neem, 50.80%), *Cocos nucifera* (coconut, 44.37%) and others (Table 2). *Croton bonplandianum*, *Amaranthus viridis*, *Trema orientalis*, etc. failed to exhibit +2/more level of skin reaction. During the study period, a total of 9492 asthma-related hospital admissions (mean age = 39 ± 1.4 years, male/female = 0.9) were registered. There were two peak periods (Figure 6) of ARH–March (up to 8.15/day in 2009) and September (up to 5.45/day in 2013) respectively. Lowest mean value of hospitalization was observed in November–December and May–June.

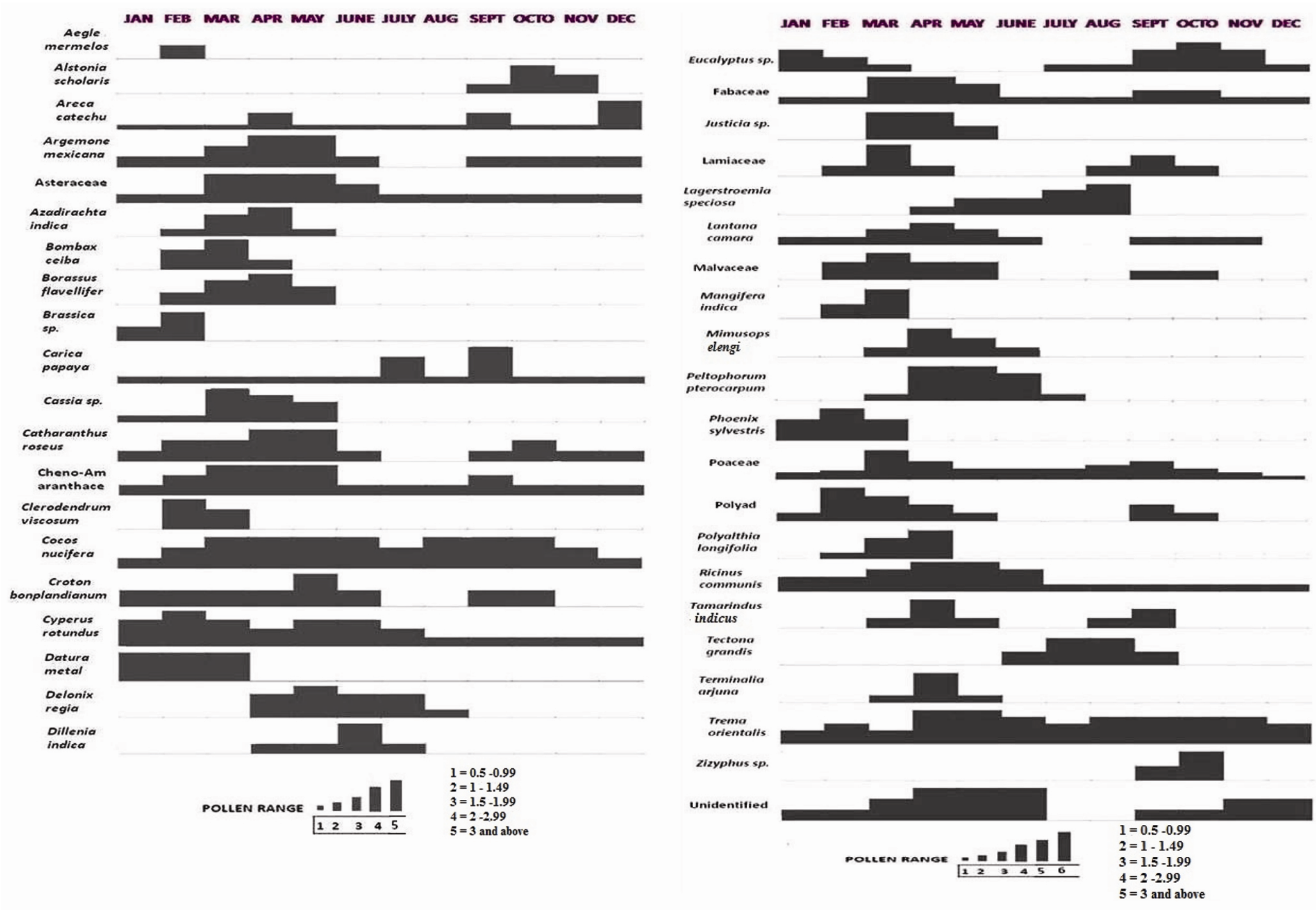

Figure 4. Pollen calendar of Habra town (2007–13). (Classes and values of pollen concentration in number/m^3 air represented in calendar graph.)

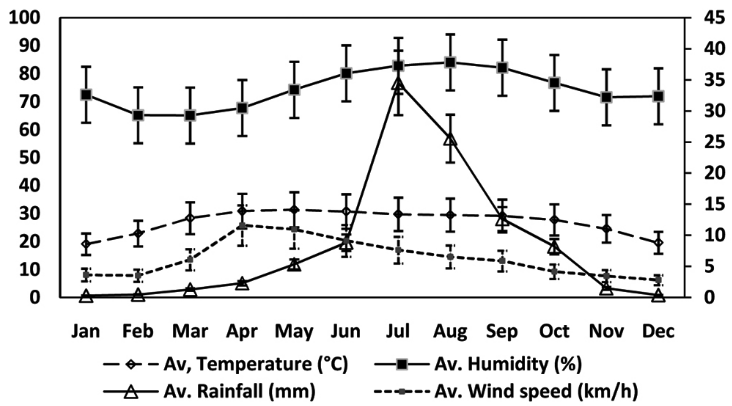

Figure 5. Monthly mean variation of meteorological parameters near sampling site during 2007–13. The bars indicate standard deviations.

Mean aeropollen count showed significant positive correlation ($r = 0.658$, $P < 0.05$ level) with ARH (adjusted $R^2 = 0.334$) accounting for 33.4% variance in hospitalization (Table 3). Among all the 40 recorded pollen types, Poaceae/grass members were strongly correlated ($r = 0.834$, $P < 0.01$ level) with ARH (adjusted $R^2 = 0.848$), accounting for 84.8% variance in hospitalization. Except grass pollen, *Bombax ceiba* and *Mangifera indica* showed positive correlation with ARH ($P < 0.05$) accounting for 75.4% and 74.8% variance in hospitalization respectively (Table 3, Figure 6).

Similar to the present survey result, there are reports[12] from India, indicating tree pollen grains as major aeropollen contributor. On the contrary, some other reports[13,14] indicated the dominance of herbaceous taxa in the atmospheric pollen load. Seasonal periodicity pattern was clearly

Table 2. Results of skin-prick tests (SPT) using different pollen extracts on respiratory allergic patients of study area along with range of IgE-ELISA value for +2/more level sensitive patient sera

Pollen types	Total patients in SPT	Positive response	Percentage positivity in SPT	+2/more level SPT response	Percentage of +2/more level response	Range of *P*/*N* value* in IgE ELISA with +2/+3 level patient sera
Saccharum officinarum (Poaceae)	290	156	53.79	32	20.51	3.6–5.2
Imperata cylindrica (Poaceae)	250	130	52.00	30	23.07	3.5–4.9
Azadirachta indica	620	315	50.80	54	17.14	3.5–4.6
Cocos nucifera	800	355	44.37	80	22.53	3.5–4.6
Cyperus rotundus (Cyperaceae)	300	125	41.66	12	4	3.3–4.5
Phoenix sylvestris	620	220	35.48	32	14.54	3.7–4.7
Areca catechu	900	315	35.00	35	11.11	3.3–5
Carica papaya	626	210	33.54	24	11.43	3.4–4.7
Moringa oleifera	290	90	31.03	10	11.11	3.2–4.1
Borassus flabellifer	575	170	29.56	20	11.76	2.9–4
Parthenium hysterophorus (Asteraceae)	310	90	29.03	8	8.88	3–4.3
Catharanthus roseus	450	125	27.77	9	7.20	3.4–5
Chenopodium album (Cheno-Amaranthaceae)	260	65	25.00	4	6.15	2.4–3.8
Bombax ceiba	326	75	23.00	5	6.66	3.3–4.6
Eucalyptus citriodora	316	71	22.47	8	11.26	2.9–4.5
Peltophorum pterocarpum	255	57	22.35	6	10.52	3.2–5.0
Lagerstroemia speciosa	220	47	21.36	4	8.51	3.0–4.5
Lantana camara	300	42	21.00	4	9.52	2.2–3.5
Delonix regia	310	65	20.96	5	7.69	2.5–4.3
Mangifera indica	255	40	15.68	3	7.5	3.0–4.4
Oryza sativa (Poaceae)	265	48	18.11	8	16.66	2.4–3.6
Alstonia scholaris	400	70	17.5	6	8.57	3.8–5.2
Argemone mexicana	105	17	16.19	2	11.76	3.6–4.3
Datura metel	300	55	18.33	1	1.81	2.10
Croton bonplandianum	310	38	12.26	0	–	–
Ricinus communis	120	13	10.83	0	–	–
Amaranthus viridis (Cheno-Amaranthaceae)	300	32	10.67	0	–	–
Tamarindus indicus	210	22	10.47	0	–	–
Trema orientalis	250	23	9.20	0	–	–
Justicia gendurossa (Acanthaceae)	280	14	5.00	0	–	–

**P*/*N* value = ratio of optical density in IgE-ELISA of patient and normal sera (492 nm) for individual pollen extract.

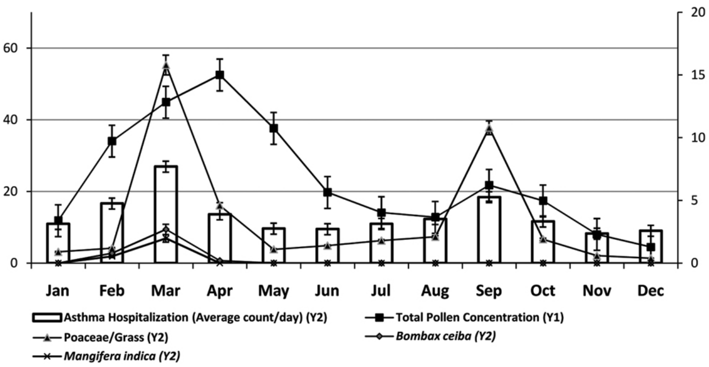

Figure 6. Monthly mean variation of asthma-related hospital admission in study area along with airborne concentration (mean count/day/m^3) of total pollen, pollen of *Bombax ceiba* and *Mangifera indica* during 2007–13. The bars indicate standard deviations.

reflected in the pollen calendar. Meteorological parameters also play an important role in phenology, pollen release and dispersal of grains in the atmosphere.

Among the allergenic pollen, type grass members, the most important aeropollen contributor, showed the highest positivity in most of the studies from India and abroad too[14,15]. Interestingly, the second dominant contributor *Trema orientalis* was found to be comparatively less reactive (9.20%) with almost no evidence of +2 level/more reaction as reported in earlier studies[14].

Severity and prevalence of allergic asthma is often related to airborne allergenic pollen exposure[16]. However, very few studies assessed the impact of atmospheric pollen level on ARH from India[17,18]. The total aeropollen

Table 3. Correlation and multiple regression with asthma-related hospitalization data and pollen counts (7-year average data)

Pollen count per m^3/ Asthma-related hospital admission	Correlation coefficient (*r* value)	Significance in *P* value (2-tailed)	(R^2) value in regression analysis	*t*-value
Total aeropollen count	0.658	0.019892*	0.334	2.238
Poaceae	0.834	0.000754**	0.848	7.471
Bombax ceiba	0.662	0.019008*	0.754	5.535
Mangifera indica	0.592	0.042413*	0.748	5.449

*Correlation is significant at the 0.05 level (2-tailed). **Correlation is significant at the 0.01 level (2-tailed).

load and some pollen members showed positive correlation with ARH of the study area. Such result confirms the synergistic effect of atmospheric pollen in triggering respiratory allergy and asthma[19]. In addition to total aeropollen, pollen grains of grasses, *Bombax ceib*a (kapok) and *Mangifera indica* (mango) were strongly correlated with ARH. In this context, there has been a previous report from nearby area, reporting positive correlation between *Areca catechu* pollen and ARH[17] during 2004–05. A similar finding from Australia[1], reported strong nonlinear association of the airborne pollen grains of Myrtaceae, Poaceae, Cyperaceae, Arecaceae and respiratory hospital admission. Formulation of pollen calendar of such an area characterized by rapid urbanization due to population influx will be helpful in obtaining an idea about aeroallergen exposure and subsequent planning of indoor/outdoor activities for susceptible allergic population.

More than 10% of the Indian population suffers from different allergic disorders[5]. It was found that among the Indian population aged ≥15 years (845 million), 2.04% (17.25 million) suffers from asthma[20]. Environmental factors such as global warming have certain effect on the timing of flowering and anthesis[21] with direct impact on aeropollen count. An updated aeropalynogical survey and formulation of pollen calendar will be helpful to combat respiratory allergy diseases and improve the quality of public life of that population.

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