

Biomonitoring of pollen grains of a river bank suburban city, Konnagar, Calcutta, India, and its link and impact on local people

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Abstract

Introduction and objectives. Pollen grains released by plants are dispersed into the air and can become trapped in human nasal mucosa, causing immediate release of allergens triggering severe Type 1 hypersensitivity reactions in susceptible allergic patients. Recent epidemiologic data show that 11–12% of people suffer from this type of disorders in India. Hence, it is important to examine whether pollen grains have a role in dissipating respiratory problems, including allergy and asthma, in a subtropical suburban city.

Materials and methods. Meteorological data were collected for a period of two years, together with aerobiological sampling with a Burkard sampler. A pollen calendar was prepared for the city. A health survey and the hospitalization rate of local people for the above problems were documented following statistical analysis between pollen counts and the data from the two above-mentioned sources. Skin Prick Test and Indirect ELISA were performed for the identification of allergenic pollen grains.

Results. Bio-monitoring results showed that a total of 36 species of pollen grains were located in the air of the study area, where their presence is controlled by many important meteorological parameters proved from SPSS statistical analysis and by their blooming periods. Statistical analysis showed that there is a high positive correlation of monthly pollen counts with the data from the survey and hospital. Biochemical tests revealed the allergic nature of pollen grains of many local species found in the sampler.

Conclusions. Bio-monitoring, together with statistical and biochemical results, leave no doubt about the role of pollen as a bio-pollutant. General knowledge about pollen allergy and specific allergenic pollen grains of a particular locality could be a good step towards better health for the cosmopolitan suburban city.

Key words

Aerobiology, allergy, health survey, hospitalization, bio-pollution, ELISA, Statistical analysis

INTRODUCTION

Allergy is considered to be the most damaging factor for the cause of bronchial asthma [1, 2]. Pollen that causes allergy are quite variable in different eco-zones, which makes it very important to identify bronchial asthma causing species from every region [3]. Immunotherapy is the key treatment for allergy sufferers. The eastern sub-humid tropical zone near Kolkata city, India, was for the first time considered as the focus on airborne bio-pollutants and its impact on human health.

Objective. The aims of the study were: 1) to determine the peak concentration of dominating airborne pollen grains and their impact on human health; 2) to prepare a health survey report to note allergenic symptoms and their correlation with the report on two-years' bio-monitoring from the statistical aspect; 3) to check the hospitalization rate due to allergy and asthma.

MATERIALS AND METHOD

Study area. Konnagar city in the Hooghly district is located at 22.7°N 88.35°E. The 2011 census in the Indian state of West Bengal revealed that Konnagar had a population of 124,585, with a literacy rate of 88.89 [4]. Four distinct seasons: summer, monsoon, post-monsoon and winter are recognized in West Bengal. The temperature fluctuates between 24°–38°C during summer, and 12°–27°C in winter. The average rainfall is about 1,582 mm per annum (June–September).

Bio-monitoring. Two consecutive years (2010–2012) were continuously (24×7) monitored using a Burkard Volumetric Sampler (Burkard Manufacturing Co. Ltd., UK) during May 2010 – April 2012. It was installed at ground level on the sites in Konnagar where wild vegetation grows around the locality. The exposed tapes were mounted, and qualitative and quantitative analyses carried out using standard protocol [5] in association with the comparison of reference slides and consulting the published literatures [6]. The weekly data for the two years were estimated for average monthly data in order to produce a pollen calendar [7] for the study site.

Meteorological data. Meteorological data were collected from a website [8]. All the weekly average meteorological data, such as maximum and minimum temperature range, relative

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humidity, wind speed, precipitation rate for the consecutive two years, were taken into account to check their real effects on pollen dispersal, distribution and suspension [6].

Health survey. A survey of 2,369 people relating their health hazards in the seasonal changes [9, 10], together with their lifestyle, male-female ratios, smoking habit and fuels for cooking were taken into account, because other factors also enhance the chance of susceptibility to allergy-related symptoms. Data were noted down and placed onto excel sheets to clarify their link to susceptibility in allergy and related symptoms. A questionnaire was derived from the Department of Genomics and integrative Biology, Delhi University, India, for conducting the survey.

Survey in hospital. Data of the patients who were suffering from respiratory allergy and asthma-related illnesses were taken from a non-government, renowned hospital, Kamala Ray Hospital, Hind Motor Station, located near the study site, to check the rate of hospitalization due to the above illnesses [11]. Hospitalization rate data (HRD) was collected during the two consecutive years.

Statistical analysis. Because all the data were unevenly distributed, Spearman's correlations were utilised for statistical analysis in order to chart correlations between Pollen counts (PC) and monthly weather-related variables (mean, maximum and minimum temperature; relative humidity and precipitation, wind speed) [12]. Spearman's correlation was also performed to chart the correlations between PC with HSD and HRD separately [13]. Besides the effects of dust, mites and smoke, the intention was to verify and correlate the bio-monitoring with the data from the survey and the hospital. All the correlations were followed by multiple linear regression analysis for further confirmation.

All statistical tests were performed using SPSS 20.0 for Windows software package.

Patient population and skin prick tests. Sterile pollen allergen extracts were used for SPT on atopic patients and responses graded according to established literature [14].

Indirect Enzyme Linked Immuno-Sorbent Assay (ELISA). Diluted antigen extract of allergic pollen grains were coated on an ELISA plate and specific IgE titre estimated in selected patients following a standard method [15].

RESULTS

Aerobiological sampling. The results showed that a total of 36 species of pollen grains were present in the air during their blooming periods. Pollen grains of well-known allergen species, such as Poaceae (18.55%) [16], were the in highest number, followed by many species of Asteraceae (15.77%) [17], *Lantana camara* (6.2%) [18], *Trema orientalis* (2.54%), Chenopodiaceae-Amaranthaceae (4.78%), *Cocos nucifera* (4.31%) [19], *Areca catechu* (2.84%) [20], *Phoenix sylvestris* (5.15%) [21], *Borassus flabellifer* (1.95%) [22], *Petlophorum pterocarpum* (2.07%) [23], *Mangifera indica* (1.52%), *Carica papaya* (5.61%) [24], among others, as well as a number of herbs and shrubs, e.g. *Parthenium hysterophorus*

(2.99%), *Cyperus sp* (4.06%), *Justicia simplex* (1.71%), and *Catharanthus roseus* (3.07%) [25]. A pollen calendar (Fig.1) was prepared which showed the prevalence of different pollen grains in air throughout the year. A total of 36.

Correlations with meteorological parameters. Correlation analysis showed that together with maximum and minimum temperature, four independent factors revealed highly negative correlations with pollen counts in the air: 1) average minimum temperature; 2) maximum relative humidity; 3) minimum relative humidity and 4) maximum precipitation. Minimum humidity scored the highest *p* value (< 0.01) and *r* value (Tab.1).

Table 1. Results of bivariate correlation of meteorological factors as independent variables, with pollen counts as dependent variables for 2010–2011 and 2011–2012, following Spearman's Rho method

Meteorological factors/ pollen counts	Correlation coefficient (<i>r</i>) value		Significant (2 tailed) <i>p</i> value	
	2010–11	2011–12	2010–11	2011–12
Avg Max. Temp	-0.078	0.025	0.5977	0.868
Avg Min. temp	-0.578**	-0.548**	1.7 × 10 ⁻⁵	5.5 × 10 ⁻⁵
Max Wind speed	0.168	0.198	0.252	0.177
Min. Wind speed	-0.058	0.150	0.697	0.308
Min. Relative Humidity	-0.679**	-0.500**	1 × 10 ⁻⁶	3 × 10 ⁻⁴
Max. Relative humidity	-0.478**	-0.813**	5.8 × 10 ⁻⁴	1 × 10 ⁻⁶
Max. Precipitation	-0.498**	-0.527**	3.7 × 10 ⁻⁴	11.8 × 10 ⁻⁴
Min. Precipitation	-0.127	-0.320*	0.390	0.026

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

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

The feasibility of using weekly PC and weekly meteorological data from 2010–2012 as dependent and predictive variables was tested by multiple regression analysis. The results were statistically significant, with an adjusted R² value of 0.50 (2010–2011) and 0.68 (2011–12), which accounted for approximately 50.2% and 68% of the variance in pollen counts in the two years (Tab. 2.) with the given probability value *p*<0.00. Moreover, the *t* value calculated for each variable indicated that all the associated coefficients were significant. The main negative variables for prevalence of pollen in air were minimum relative humidity, maximum relative humidity and maximum precipitation, where the average minimum temperature was a low positive variable. Their effects on pollen suspension in air are shown in Fig. 3.

Health report. The study revealed the following:

- 1) that the age ranges between 11–19 years of age showed the highest susceptibility rate. The ages of 20–40 were much more vulnerable to seasonal change and allergy-related symptoms, and the age range was in 2nd highest in position after the age range of 11–19 years.
- 2) Females (52.6%) were more susceptible to exposure of seasonal changes than males (47.4%).
- 3) Passive smokers (65%) sufferer more than active smokers (35%), respectively.
- 4) The majority of people in the study area use kerosene (39%), whereas gas, charcoal and gas+kerosene are equal in number (16%); kerosene +charcoal users are in the 2nd highest position (29%).



Figure 1. Pollen calendar for the study site – 2010–2012

Table 2. Multiple regression model of 4 dominating meteorological factors and pollen counts of successive 2years (2010–11 and 2011–12)

PC	R	(R2)	S	Standardized Coefficients (β)					T					Significance				
				V					V					V				
				C	AMT	MaRH	MiRH	MP	C	AMT	MaRH	MiRH	MP	C	AMT	MaRH	MiRH	MP
2010–11	0.709	.502	1 × 10 ⁻⁷	-	0.003	-0.201	-0.590	-0.029	2.913	0.017	-1.539	-3.065	-0.223	0.006	0.987	0.131	0.004	0.821
2011–12	0.825	.680	1 × 10 ⁻⁷	-	0.03	-0.134	-0.681	-0.140	2.878	0.232	-1.232	-4.566	-1.289	0.006	0.817	0.225	0.000	0.204

S – Significance of regression in ANOVA; C – Constant, PC=Pollen Counts; V – Variance; AMT – Average minimum temperature; MaRH – Maximum relative humidity; MiRH – Minimum relative humidity; MP – Maximum precipitation. Dependent variable: Pollen Counts. Predictors: C, AMT, MaRH, MiRH, MP.

- 5) The 500 people in the locality reported that they suffered more from frequent sneezing and coughing (17.01%), together with frequent respiratory problems/ allergy (12.45%). Additionally, there were tendencies of the symptom of eye itching and redness (10.97%) and running nose (10%) associated with them. Dry cough (7.4%) and frequent breathing problems (7.6%) were almost equally prevalent. Sore throat and sinus scored the same (3%), the lowest of all the scores (Fig. 2).
- 6) The relationship between the successive 2 years’ pollen counts and the data of the people’s hospitalization together showed much more parity (Fig. 2). On the graph, the blooming period of many species dominant in the different

seasons showed a similar curve to the increase in patients’ hospitalization. During the two-year study, data obtained from Kamala Ray Hospital revealed that 1,082 patients had been hospitalization due to respiratory allergy, lower track infection and asthma.

Correlations of pollen counts with hospitalization rate data and health survey data. Spearman’s correlation coefficients between total weekly pollen counts and data from the hospital (HRD) and survey (HSD) during 2010–11 and 2011–12, showed that both sets of data showed highly significant positive correlations with pollen counts in air where *p* value was < 0.01 in each case, with significant *r* value (Tab. 3).

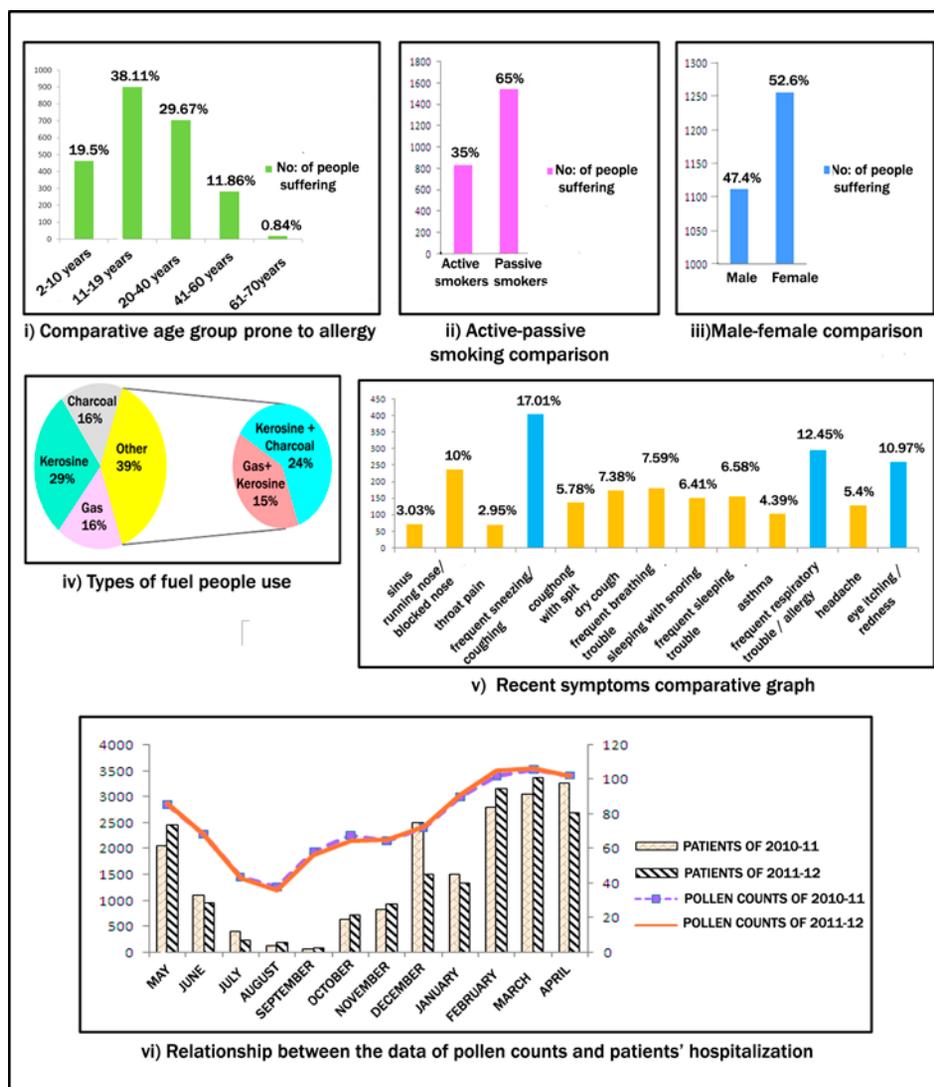


Figure 2. Comparative accounts derived from health survey, data of hospitalization and pollen counts

Table 3. Three-dimensional correlations with pollen counts, health survey and hospitalization following Spearman's rank method

PC	HRD				HSD			
	2010-11		2011-12		2010-11		2011-12	
	r value	P value						
2010-11	0.937**	7×10 ⁻⁶	NA	NA	0.979**	1×10 ⁻⁶	NA	NA
2011-12	NA	NA	0.958**	1×10 ⁻⁵	NA	NA	0.993**	1×10 ⁻⁶

** Correlation significant at the 0.01 level (2-tailed).
P C – Pollen Counts; HRD – Hospitalization Rate Data; HSD – Health Survey Data

The feasibility of using monthly (1×4 weeks) PC and monthly patients' data from the hospital and health survey from 2010–2012 as predictive and dependent variables (HRD and HSD) was tested by multiple regression analysis. The results for the PC and HRD were statistically significant with an adjusted R² value of 0.798 (2010–11) and 0.867 (2011–12), with a given probability value p<0.00 (Tab. 4). The results for the PC and HSD were statistically significant with an adjusted R² value of 0.963(2010–11) and 0.962 (2011–12), with a given probability value p<0.00 (Tab. 5). Moreover,

Table 4. Multiple regression model with hospitalization rate data (HRD) and pollen counts (PC) for 2010–2011 and 2011–2012

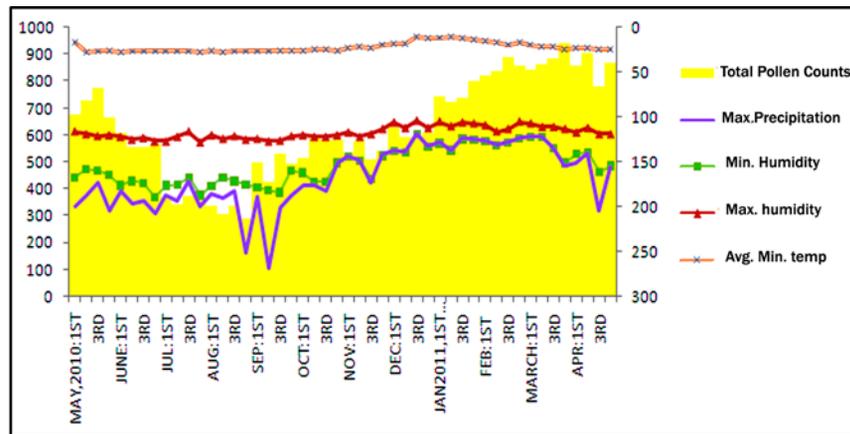
Patients' data	R	(R ²)	S	β		t		Significance	
				V		V		V	
				C	PC	C	PC	C	PC
2010-11	0.893	0.798	0.91×10 ⁻⁵	-0.893	-3.378	6.287	0.007	0.91×10 ⁻⁵	
2011-12	.931	.867	1.1×10 ⁻⁵	-0.931	-4.420	8.064	0.001	1.1×10 ⁻⁵	

S – Significance in ANOVA; β – Standardized coefficient; C – Constant; PC – Pollen Counts; V – Variance; Dependent variable – HRD; Predictors – C, PC

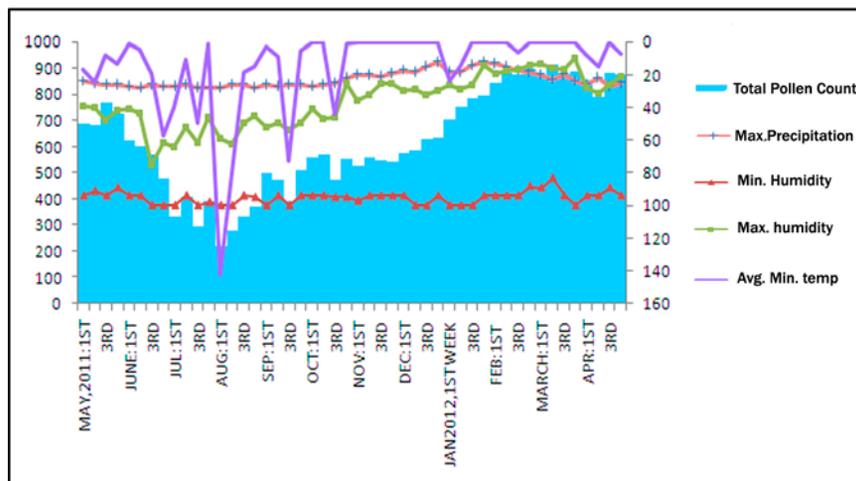
Table 5. Multiple regression analysis with health survey data (HSD) regarding symptoms suffered during seasonal change and pollen counts (PC) for 2010–2011 and 2011–2012

Survey data	R	R Square (R ²)	S	B		t		Significance	
				V		V		V	
				C	PC	C	PC	C	PC
2010-11	0.963	0.926	1×10 ⁻⁵	-0.963	-0.440	11.225	0.669	1×10 ⁻⁵	
2011-12	0.962	0.926	1×10 ⁻⁵	-0.962	-1.749	11.211	0.111	0.1×10 ⁻⁵	

S= Significance in ANOVA, β= Standardized coefficient, C= Constant, PC=Pollen Counts, V= Variance; Dependent variable: HSD, Predictors: C, PC



a) The four main meteorological factors controlling pollen counts in air of 2010-11



b) The four main meteorological factors controlling pollen counts in air of 2011-12

Figure 3. Effects of four most significant meteorological parameters on prevalence of pollen counts in air of 2010-12

the *t* value calculated for each variable indicated that all the associated coefficients were significant in each analysis (Tab. 4, 5).

Analysis of Spearman's correlation and multiple regressions showed that there was a high correlation between the people's health survey data and hospitalization rate data with pollen counts in the air.

Results of SPT and indirect ELISA. The study represents the diagnostic and immunological research into the allergenicity of different pollen grains. Positive cutaneous responses were elicited in 96 (64%) of the 150 patients tested. Among those with positive cutaneous response, 95% had previous reports of bronchial asthma, together with or without allergic rhinitis; the remainder reported allergic rhinitis only. +2 grade of positivity was found to be predominant followed by +3 and +1. Significantly, no positive skin reactions were observed in healthy volunteers whose sera were used as the negative control. In specific IgE ELISA, significantly elevated IgE levels were observed in 15 of those patients, compared to normal controls ($P/N > 2.5$) (Tab. 6), and positive patient sera (+2/+3) showed high antibody titer and a more intense colour development in the titer plate. Among all the aeroallergens in the SPT and ELISA tests, all the dominating pollen grains of air showed positive +2/+3 in SPT and high antibody titer

in ELISA. This result was significant enough to infer the presence of strong IgE binding proteins in the aero allergens dominating pollen grains found from the bio-monitoring data.

DISCUSSION

The results of bio-monitoring over 2 years provided information and a clear picture of the air at the site In Konnagar, an industrial suburban city with cosmopolitan people, and with sub-humid subtropical vegetation. There is a complete blending of the two different developments where rural vegetation in a few areas is associated with the civil urban development, including multi-storied buildings and markets. On one side In the city, vegetation is chopped down to make way for urbanization, and on the other side, wild vegetation grows around the localities. From statistical analysis, the four main meteorological parameters have been identified which play important roles in pollen dispersion and prevalence in air. Regular noting down and recording of pollen grains which is very informative and useful for the future study of bio-monitoring of a subtropical suburban city. Many of the pollen grains found in the sampler were allergenic, with mild-severe sensitivity, data supported by the subsequent SPT and ELISA tests.

Table 6. Results of SPT and Indirect ELISA

Patient No:	Age/Gender	Symptoms/Clinical history	Reaction in SPT	Specific IgE-ELISA P/N value	Total IgE (IU/ml)	Aeroallergen Sensitization
1	18/F	AR	++	3.32	675	CN, LC
2	25/M	AR	++	3.65	710	LC, Honey
3	30/M	AR+BA	+++	3.66	725	PP, LC
4	21/F	AR+BA	++	3.99	636	AF, LC, AI
5	17/F	AR	++	4.66	660	AC, CN, LC
6	15/F	AR+BA	+++	4.12	576	LC, House dust, AF
7	35/M	AR	+++	3.06	815	CR, LC, DR
8	37/F	AR+BA	++	3.77	678	DR, Egg, LC, House dust
9	28/M	AR+BA	++	4.02	529	LC, House dust, AI
10	19/M	AR	++	4.08	557	AF, DR, LC
11	22/F	AR	++	3.72	682	HOUSE DUST, AF, LC
12	32/F	AR	+++	4.1	723	LC, PP
13	14/M	AR+BA	++	4.73	886	AC, AF, CP, LC
14	16/F	AR+BA	++	3.65	773	AF, Honey, House dust, AI
15	28/F	AR	++	3.75	689	LC, CP

Control

1	25/F	AR+BA	-	0.847	150	AI, AF
2	15/F	AR	-	0.912	176	CP, CN
3	23/M	AR+BA	-	1.105	204	House Dust, Honey
4	30/F	AR	-	1.131	213	Dr, cp
5	28/M	AR+BA	-	0.984	198	TH, RN

Symptoms: AR allergic rhinitis, BA bronchial asthma.

P/N: ratio of optical density of patient serum with respect to control

Reactions in SPT: +no wheal and erythema of 20 mm in diameter; ++ wheal and erythema of 20 mm in diameter; +++wheal and Erythema >30 mm; - no wheal and no erythema.

Aeroallergens: AA - *Alternaria alternata*; AC - *Areca catechu*; AF - *Aspergillus flavus*; AI - *Azadirachta indica*; CN - *Cocos nucifera*; CP - *Carica papaya*; CR - *Catharanthus roseus*; CS - *Cassia sophera*; DR - *Delonix regia*; EC - *Eucalyptus citridora*; HD - House dust; LC - *Lantana camara*; PP - *Peltophorum pterocarpum*; PS - *Phoenix sylvestris*; RN - *Rhizopus nigricans*; SO - *Saccharum officinarum*; TH - *Trichoderma harzianum*.

Control group had P/N value <1.5

The local people helped a great deal with the study in order to better understand the bio-monitoring associated with the updated meteorological data. Moreover, the analysis shed light on the fact that there is a highly positive significant correlation between data from the local hospital with the seasonal pollen counts and yearly health survey report [19, 20]. Lack of knowledge about sanitation, as well as the type of fuel - charcoal and kerosene - for cooking, also act as a promoting factor for the allergy-related problems faced by the inhabitants. They suffered during the seasonal changes when different pollen grains are loaded in huge amounts during their blooming season. The annual survey report was confirmed by statistical analysis of all three sets of data by correlation and linear regression analysis. The period when people tend towards allergy almost resembles the blooming period of many known allergenic plant species, such as *Cocos nucifera* [19], *Areca catechu* [20], *Phoenix sylvestris* [21], *Lantana camara* [18], *Azadirachta indica*, and many other species known to be allergenic in earlier research articles by different research workers. Pollen grains, therefore negatively

affect human health, causing respiratory allergy, which was proved by the bio-monitoring for their presence, and confirmed by its biochemical tests.

CONCLUSION

This detailed study has attempted to heighten awareness of the fact that some pollen grains play an adverse role with respect to human health. The tendencies of people to allergy almost resemble the blooming period of many known allergenic plant species. Moreover, continuous recording of the occurrence of pollen data on a weekly basis over a period of two years elucidated the potential output, the scientific exploitation of which should pave the way for future clinical endeavours. It is also noteworthy that pollen grains also play a major role in causing mild-to-severe levels of respiratory allergy, such as rhinitis and bronchial asthma. Therefore, there should be an awareness programme, as recommended by clinical societies, to take preventive measures to avoid such health hazards. There should be a mandatory clinical test of SPT (Skin Prick Test) to make people aware of the specific aeroallergen(s) to which they are susceptible, for betterment of their health. This should be a step towards a healthier society.

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