

Aerobiological Investigation and In Vitro Studies of Pollen Grains From 2 Dominant Avenue Trees in Kolkata, India

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■ Abstract

Background: *Peltophorum pterocarpum* and *Delonix regia* are dominant avenue trees in the city of Kolkata in India. They are well adapted to the humid tropical climate and also grow commonly in different parts of the country. Their pollen grains are reported to be airborne.

Objective: The aim of this study was to conduct an aerobiological survey in Kolkata to determine the concentration and seasonal periodicity of pollen grains from *P pterocarpum* and *D regia* and to analyze the meteorological factors responsible for their levels in the atmosphere. In addition, we analyzed the prevalence of sensitization due to these grains among patients with seasonal respiratory allergy.

Methods: An aerobiological survey was conducted with a volumetric Burkard sampler from 2004 to 2006. Correlations between meteorological parameters and pollen grain concentrations were assessed by Spearman correlation test. The protein profile of the pollen extracts was studied by sodium dodecyl sulfate polyacrylamide gel electrophoresis. Finally, the allergenic potential of the pollen extracts was evaluated in patients with respiratory allergy by skin prick test, immunoglobulin (Ig) E enzyme-linked immunosorbent assay, and IgE immunoblotting.

Results: *P pterocarpum* and *D regia* pollen grains occur from March to June and April to July, respectively. The pollen concentrations showed statistically significant positive correlations with maximum temperature and wind speed. Positive reactions to *P pterocarpum* and *D regia* were observed in 26% and 22% of the patients, respectively. Many protein bands were detected in the pollen extracts over a wide molecular weight range. A total of 5 (*P pterocarpum* pollen) and 8 (*D regia* pollen) protein fractions were detected by IgE immunoblotting.

Conclusion: *P pterocarpum* and *D regia* pollen grains are dominant in the atmosphere of south Kolkata and they are influenced by temperature. The pollen grains release proteins that may be responsible for immediate hypersensitivity reactions in sensitive patients.

Key words: Aerobiology. Meteorological parameters. Pollen grains. Respiratory allergy. Skin prick test.

■ Resumen

Antecedentes: El *Peltophorum pterocarpum* (Flamboyant dorado) y el *Delonix regia* (Ponciana Real) son las especies de árboles predominantes en las calles de la ciudad de Calcuta, en la India. Están muy bien adaptadas al clima tropical húmedo y crecen espontáneamente en diversas partes del país. Se ha revelado la presencia de los granos de polen de estos árboles en el aire.

Objetivo: el propósito de este estudio fue llevar a cabo un estudio aerobiológico en Calcuta para precisar la concentración y la regularidad estacional de los granos de polen de *P pterocarpum* y *D regia*, y analizar los factores meteorológicos implicados en la concentración de los mismos en la atmósfera. Además, se analizó la prevalencia de la sensibilización provocada por estos granos en pacientes con alergia respiratoria estacional.

Métodos: Se realizó un estudio aerobiológico con un captador volumétrico tipo Burkard de 2004 a 2006. Se midieron las correlaciones entre los parámetros meteorológicos y las concentraciones de los granos de polen mediante la prueba de correlación de Spearman. También se examinó el perfil proteico de los extractos de polen mediante electroforesis en gel de poliacrilamida con dodecil sulfato de sodio. Por último, se evaluó el potencial alergénico de los extractos de polen en pacientes con alergia respiratoria mediante la prueba cutánea, el enzimoimmunoanálisis de adsorción para la detección de inmunoglobulina (Ig) E y la inmunotransferencia de IgE.

Resultados: Los granos de polen de *P pterocarpum* y *D regia* están presentes de marzo a junio y de abril a julio, respectivamente. Las concentraciones de polen mostraron una correlación positiva estadísticamente significativa con una temperatura y una velocidad del viento máximas. Se observaron reacciones positivas al *P pterocarpum* y a la *D regia* en el 26 % y el 22 % de los pacientes, respectivamente. Se detectaron numerosas bandas proteicas en los extractos de polen de una gran variedad de pesos moleculares y un total de 5 (*P pterocarpum* polen) y 8 (*D regia* polen) fracciones proteicas mediante inmunotransferencia de IgE.

Conclusión: Los granos de polen de *P. pterocarpum* y *D. regia* predominan en la atmósfera del sur de Calcuta y están influidos por la temperatura. Estos granos de polen liberan proteínas que pueden provocar reacciones de hipersensibilidad inmediata en pacientes sensibles.

Palabras clave: Aerobiología. Parámetros meteorológicos. Granos de polen. Alergia respiratoria. Prueba cutánea.

Introduction

Allergenic plants display regional variations, depending on geography, climate, and vegetation [1]. It is essential that the major allergenic contributor be recognized in each area in order to identify atopic patients and prescribe appropriate treatment [2]. Pollen grains are well known to be common causative agents in respiratory allergic disorders [3-5]. In India, between 15% and 20% of individuals suffer from allergic manifestations such as allergic rhinitis and bronchial asthma, which impair the quality of life of the affected individual [6].

India is predicted to become the world's most populous nation by the year 2050. Industrialization and urban growth are now occurring at an unprecedented rate in this previously agrarian society [7]. Kolkata (formerly Calcutta), India's second largest city with a population of more than 13 million [8], is the fastest growing metropolis in the eastern part of India; as a result there has been an enormous change in the vegetation along with climatic conditions over the years. A previous study undertaken in Kolkata revealed a predominance of tree pollen grains comprising *Trema orientalis*, *Casuarina equisetifolia*, *Cocos nucifera*, *Azadirachta indica*, *Carica papaya*, *Areca catechu*, *Alstonia scholaris*, *Cassia* species, *Peltophorum pterocarpum*, and *Delonix regia* [9]

A number of tree pollen grains that are considered to be important constituents of the spectrum of allergenic agents are derived from the local vegetation [10]. Their atmospheric presence is strongly influenced by several meteorological parameters such as temperature, rainfall, relative humidity, wind speed, and sunshine hours [11,12]. Different eco-geographical regions in India support different allergenic pollen flora and many taxa need to be investigated for their allergenic properties in the local population. *Peltophorum pterocarpum* and *Delonix regia* are avenue trees with dominant pollen types that have been found to be potent inducers of respiratory allergy in susceptible individuals in Kolkata [13,14]. Previous studies of their atmospheric presence have been undertaken in the central part of India in Jabalpur [15] and the western part of the country in Bombay [16] and Pune city [17]. An aerobiological survey conducted in central Calcutta [13] and greater Calcutta [18] showed that they were prevalent in the atmosphere in those regions. Pollen grains from *P. pterocarpum* and *D. regia* have also been reported in previous studies to cause sensitization in 7% to 18% [13,14,16] and 14% to 23% [13,14] of atopic cases, respectively, as revealed by skin testing.

Peltophorum pterocarpum (DC) Backer ex K Heyne, family Fabaceae, commonly known as the Yellow Gulmohar, is native to tropical Asia [19] and is a dominant avenue tree in the southern part of Kolkata. Due to its spectacular display of

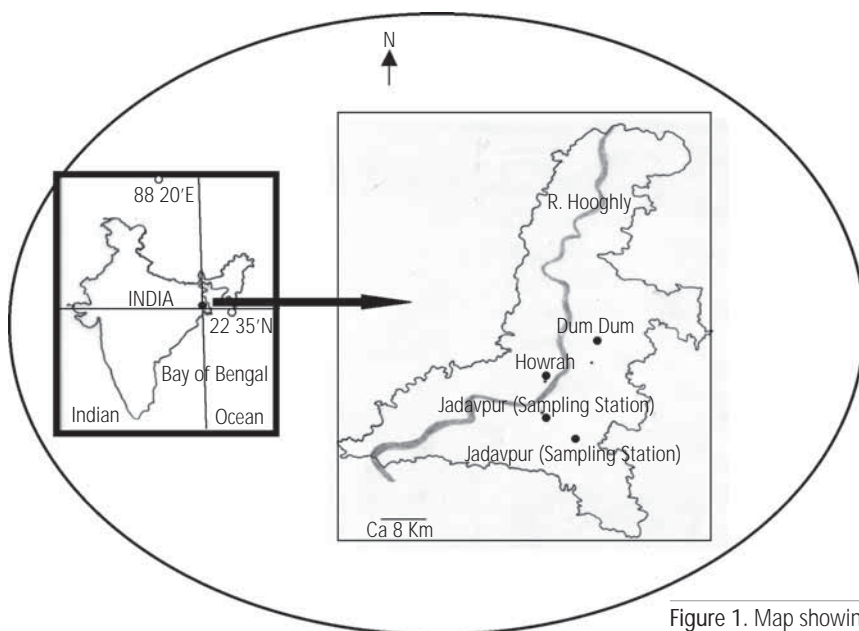


Figure 1. Map showing the location of Kolkata city and the sampling site.

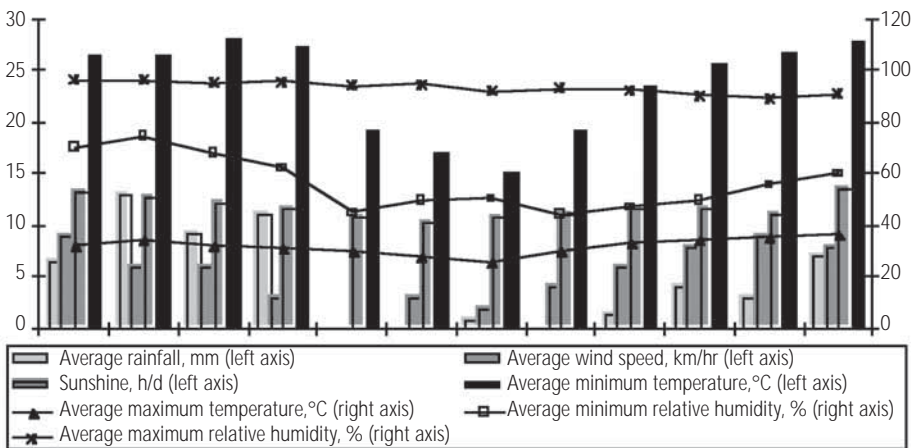


Figure 2. Meteorological parameters of Kolkata city from July 2004 to June 2005.

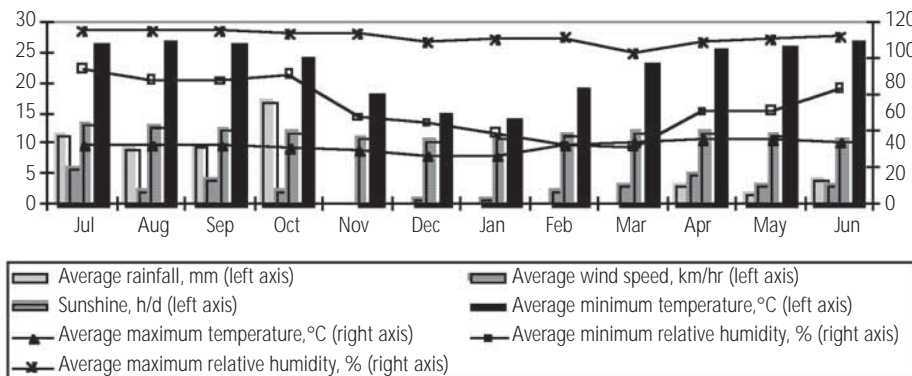


Figure 3. Meteorological parameters of Kolkata city from July 2005 to June 2006.

brilliant yellow blossoms, it is mostly grown as an ornamental tree on the roadsides. *Delonix regia* (Boj ex Hook) Raf, also a member of the Fabaceae family, is a native of Madagascar. Its common name is Royal Poinciana, also called the Gulmohar tree, and it has been described as the most colorful tree in the world. In addition to their ornamental value, they are usually planted as shade trees in tropical countries.

In this study, we conducted an aerobiological survey in Kolkata to determine the concentration and seasonal periodicity of pollen grains from *P pterocarpum* and *D regia*. The meteorological factors responsible for the frequency of the relevant pollen grains were analyzed. The prevalence of sensitization to these grains among seasonal respiratory allergic patients was also studied. Finally, we attempted to identify the proteins released by the pollen grains that may be responsible for immediate hypersensitivity reactions in sensitive patients.

Materials and Methods

Aerobiological Sampling

Twenty-four hour counts (12 noon to 12 noon) were carried out using a 7-day volumetric Burkard sampler (Burkard Manufacturing Co Ltd, Rickmansworth, Hertfordshire, United Kingdom). It was installed on the rooftop (about

40 feet above ground level) of the Solar Energy Building of The Indian Association for the Cultivation of Science, in Jadavpur in the southern part of Kolkata, India. The building is located in an open area, free from obstacles, allowing free airflow. Melinex tape coated with petroleum wax was fixed on the rotating drum of the sampler to trap airborne particles. Pollen grains trapped from the air were studied by microscopy. Grains were counted from the exposed tapes [20]. The resulting slides were counted along 24 traverses spaced at 2 mm (1 hour) intervals in order to obtain the mean hourly concentration. The 24 hourly counts were then averaged to obtain the daily mean concentration, which in turn gave the weekly and monthly mean concentrations. The daily pollen concentration was expressed as number of pollen grains per cubic meter of air.

Climate and Meteorological Data

Kolkata is located in the plains of eastern India at 22°82'N 88°20' E on the Ganges delta and is the capital city of the state of West Bengal (Figure 1). It spreads along the banks of the River Hooghly in a north-south direction. The city is at an elevation of 1.5 to 9 m above sea level. It has a tropical climate, with temperatures ranging from 32°C to 38°C in summer and from 10°C to 20°C in winter. May is the hottest month and January is the coldest month. The winter is short, from November until February. The average monsoon rainfall

is about 160 cm between June and September. The Alipore Meteorological Station in Calcutta, sited about 4 km away from the sampling site, provided the detailed meteorological data. The following data were recorded daily for the 24-month period of the study: maximum and minimum temperature expressed as °C, rainfall in mm, wind speed in km/h, relative humidity expressed as percent saturation, and sunshine in hours per day (Figures 2 and 3).

Statistical Analysis

The relationship between the meteorological parameters (temperature, relative humidity, average wind speed, average rainfall, and sunshine hours) and monthly pollen concentration was calculated using the Spearman nonparametric correlation coefficient. Comparisons of paired samples were performed by *t* test. Statistical analysis was undertaken with SPSS version 10.0. Values of *P* < .05 were considered statistically significant.

Collection of Pollen Samples

Pollen samples were collected from mature anthers of *P pterocarpum* and *D regia* flowers growing around the study area. The purity of the isolated pollen material was checked under the microscope. They contained not more than 1% nonpollen impurities.

Preparation of Pollen Extract

The pollen grains were defatted with diethyl ether and incubated with phosphate buffered saline (PBS; 0.1 mol/L sodium phosphate containing 0.15 mol/L NaCl, pH 7.2). Pollen was added at a 1:10 ratio (weight by volume [w/v]) and proteins were extracted by continuous stirring at 4°C for 16 hours. After centrifugation at 12 500 g for 40 minutes, the clear supernatant was dialyzed and passed through a 0.22 μm Millipore filter (Millipore Corp, Bedford, USA). The filtrate was then lyophilized and stored at -70°C in aliquots of known volume in sterile vials.

Determination of Soluble Protein Content

The total protein content of the crude pollen extracts was determined as described elsewhere [21], with bovine serum albumin (BSA) as the standard.

Patient Population and Skin Tests

Skin tests were performed with *P pterocarpum* and *D regia* pollen allergen extracts (1:50 w/v) on adult patients with respiratory allergies attending the Allergy Unit of the Institute of Child Health, Kolkata. Patients were selected because they had all displayed clinical manifestations of seasonal rhinoconjunctivitis or bronchial asthma. All patients reported that these avenue trees grew in their locality. The exclusion criteria were perennial or severe asthma, pregnancy or lactation, malignancy, or other severe systemic diseases during skin testing or serum collection. To avoid masking of possible symptoms, the use of corticosteroids and antihistamines was prohibited. The study was approved by the Ethics Committee of the hospital. The allergen extracts were prepared by extracting the pollen to 95%

purity with PBS [22,23]. The patients were also tested with a panel of allergens other than *P pterocarpum* and *D regia* pollen extract, including house dust mite, pollen grains of *Areca*, *Phoenix*, *Cocos*, *Borassus*, and different species of *Cassia*, *Catharanthus*, *Carica*, and *Eucalyptus*. Histamine diphosphate (1 mg/mL) and PBS (1 mg/mL) were used as positive and negative controls, respectively. The reaction was measured after 20 minutes. Tests were performed with 20 L of allergen solution placed on the ventral side of the forearm with a 26-gauge disposable hypodermic needle. According to international guidelines, positivity was defined as a mean wheal diameter of at least 3 mm compared with the negative control [24]. The reaction was graded from +1 to +3 according to Styttis et al [25]: +1, erythema, 20 mm in diameter; +2, erythema, >20 mm in diameter; +3, wheal and erythema. Control sera were collected from nonsensitized healthy volunteers (confirmed by negative skin reaction and enzyme-linked immunosorbent assay [ELISA] for specific immunoglobulin [Ig] E) with no personal or family history of allergic, systemic, or joint diseases. Serum was obtained from each patient and stored at -20°C for future use.

Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) of *P pterocarpum* and *D regia* crude pollen extract was carried out on 11% polyacrylamide gels using a discontinuous buffer system [26]. After completion of electrophoresis, the polyacrylamide gel was washed with water and stained at room temperature by incubating with staining solution (0.25% w/v Coomassie brilliant blue in a 9:2:2 volume by volume [v/v] solution of methanol, glacial acetic acid, and distilled water) for at least 1 hour. The gel was destained in a mixture of methanol, glacial acetic acid, and distilled water (9:2:9, v/v) until the background became clear and the bands were visible. The molecular mass of the fractions was calculated by calibrating with standard marker proteins (Genei, Bangalore, India).

IgE ELISA

ELISA was performed to analyze *P pterocarpum* and *D regia* pollen-specific IgE levels in individual patient sera using antihuman IgE horseradish peroxidase conjugate (Sigma Inc, St Louis, Missouri, USA; 1:1200 dilution) and o-phenylenediamine substrate [27]. The absorbance was measured with an ELISA reader (Labsystems Multiskan, Kolkata, India) at 492 nm. Individual sera in which the ratio of optical density of patient sera with respect to control was greater than 3.0 were used for immunoblotting.

IgE Immunoblotting

Crude pollen extract was resolved by SDS-PAGE (11% T, 5% C) in the presence of β-mercaptoethanol. Following electrophoresis, the resolved bands (without staining) were transferred to a polyvinyl difluoride (PVDF) membrane, by semi-dry blotting for 1 hour at 0.8 mA/cm². The membrane was pretreated with methanol, water, and anode buffer II, according to the manufacturer's instructions (NEN Life Science Products, Boston, USA). After blotting, the PVDF

membrane was incubated in 0.1 mol/L Tris buffered saline (TBS) pH 7.5, containing 0.05% (v/v) Tween 20 (Sigma Inc, St Louis, Missouri, USA) (TBST) for 1 hour as blocking solution. For protein staining, a piece of the membrane was incubated with staining solution. For detection of IgE-reactive proteins, the membrane was cut into 0.5 mm strips that were separately incubated overnight with serum samples collected from individual patients showing positive skin reaction to *P pterocarpum* pollen extract. Serum samples were diluted 1:20 in TBST. Membrane strips were then washed in TBST and incubated in secondary antibody for 3 hours. Alkaline phosphatase-conjugated monoclonal antihuman IgE (1:1000) (Sigma Inc) was used as secondary antibody. Binding patterns were visualized with substrate solution containing 0.033% w/v nitroblue tetrazolium chloride and 0.017% w/v 5-bromo,4-chloro,3-indolyl phosphate potassium salt (Genei, Bangalore, India) in 0.1 mol/L TBS pH 9.5.

Results and Discussion

Aerobiological Studies

Seasonal behavior. A 2 year period of aerobiological monitoring of the southern part of Kolkata city was initiated in July 2004 and continued nonstop until July 2006. *P pterocarpum* pollen grains were found to be present in the air from March to June and sporadic occurrence was also seen during the months of August and September (Figure 4). Although the occurrence of pollen was seasonal, during the month of maximum concentration (May in 2005 and 2006), *P pterocarpum* pollen represented a relatively high percentage of the total airborne pollen load (6.56% and 8.92% in 2005 and 2006, respectively). Microscopy revealed that the *P pterocarpum* pollen grains were 55 to 57 μ m in size, had tricolporate aperture, with reticulate ornamentation, and were oblate to spheroidal in shape. In earlier studies in Kolkata, *P pterocarpum* pollen grains

were found only during the summer months [13,14]. This is the first report of 2 pollen seasons in the city. Similar results were obtained in an aerobiological survey of Pune city, in western India [17]. Such a high concentration of *P pterocarpum* pollen grains has not been found in previous studies from Kolkata [13,18]. The change is probably due to the extensive introduction of *P pterocarpum* in recent years as ornamental trees. Therefore, periodic monitoring is required to gain a better understanding of the dynamic nature of the atmospheric load in a particular area.

The aerobiological survey revealed the seasonal presence of *D regia* pollen in the atmosphere of south Kolkata from April to July. During the peak month of May in 2005 and 2006 it contributed to about 4.8% and 6.3%, respectively, of the total airborne pollen load (Figure 5). Microscopy showed that the pollen grain was 59 to 60 μ m in size, had tricolporate aperture with reticulate ornamentation, and was prolate to spheroidal in shape. Mixed pollination mechanisms (anemophily and entomophily) are employed in both cases.

Statistical analysis. Meteorological parameters like temperature, rainfall, relative humidity, and wind direction and velocity are responsible for fluctuations in pollen concentration [28,29]. The results of the Spearman correlation analysis between the meteorological parameters and the average monthly pollen concentrations revealed significant correlations for the whole study period. When data were considered separately for each year, the variation of the total *P pterocarpum* pollen load showed a statistically significant positive correlation with mean maximum temperature ($P=.001$) and average wind speed ($P=.017$) in both years. There was a statistically significant positive correlation between the total *D regia* pollen load and mean maximum temperature ($P=.002$ in 2004-05 and $P=.021$ in 2005-06) and average wind speed ($P=.002$ in 2004-05 and $P=.017$ in 2005-06) (Table 1).

Analysis of the relationship between the meteorological

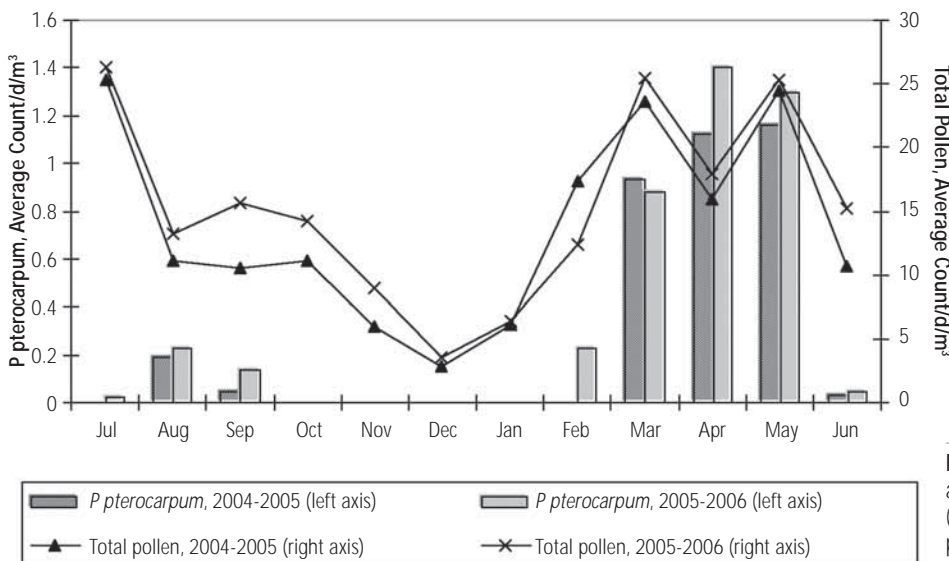


Figure 4. Seasonal mean periodicity of airborne *Pterocarpum pterocarpum* pollen (2004-2006) with respect to total airborne pollen load.

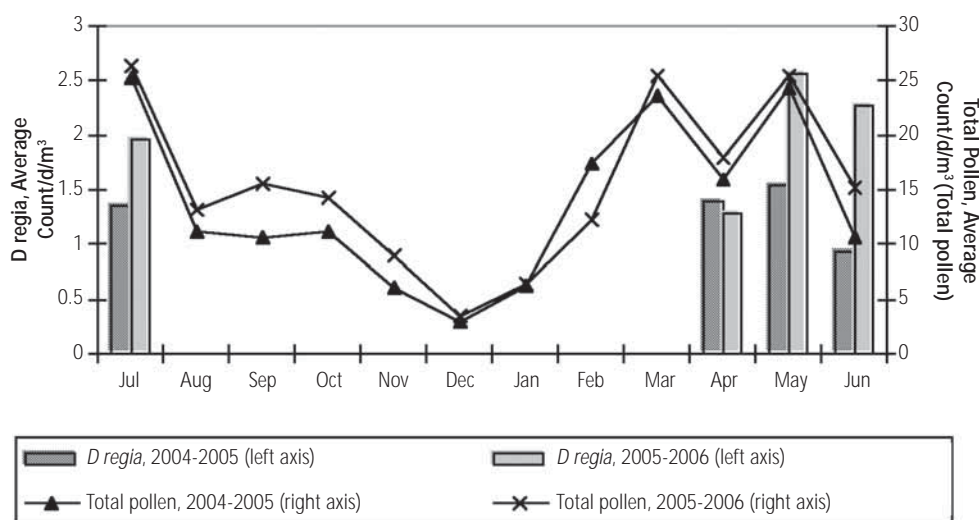


Figure 5. Seasonal mean periodicity of airborne *Delonix regia* pollen (2004-2006) with respect to total airborne pollen load.

Table 1. Spearman Correlation Coefficients Between Meteorological Parameters (Monthly Average) and Pollen Types

	July 2004-June 2005		July 2005-June 2006		July 2004-June 2006	
	PP	DR	PP	DR	PP	DR
Mean maximum temperature, °C	0.784 ^a	0.699 ^a	0.950 ^a	0.578 ^b	0.633 ^b	0.615 ^b
Mean minimum temperature, °C	0.381	0.366	0.436	0.570	0.322	0.522
Average rainfall, mm	0.333	0.158	-0.056	0.280	-0.229	0.060
Mean maximum relative humidity, %	-0.455	-0.512	-0.304	-0.1	-0.710 ^a	-0.228
Mean minimum relative humidity, %	0.160	0.121	-0.121	0.345	-0.274	0.267
Average wind speed, km/h	0.633 ^b	0.826 ^a	0.599 ^b	0.605 ^b	0.496	0.761 ^a
Sunshine hours, h/d	0.377	0.345	0.350	-0.021	0.001	0.307

Abbreviations: PP, *Peltophorum pterocarpum*; DR, *Delonix regia*.

^a $P < .01$

^b $P < .05$

parameters and *P pterocarpum* pollen load over the whole study period revealed a statistically significant positive correlation with mean maximum temperature ($P = .02$) and a statistically significant negative correlation with mean maximum relative humidity ($P = .007$). In the case of *D regia* pollen grains, a statistically significant positive correlation was seen with mean maximum temperature ($P = .013$) and with average wind speed ($P = .002$). Most studies report that Spearman coefficients indicate temperature and wind speed to be the meteorological parameters that best explain atmospheric pollen concentration variations, highlighting their fundamental importance in the dispersion and transport of pollen grains [30]. Although our results show that rainfall did not have a statistically significant effect on pollen counts, less pollen was trapped during heavy rainfall, indicating washing of pollen grains from the atmosphere, thus supporting the reports of Hjelmroos [31].

Clinical Study

Out of the 600 patients who visited the Allergy Unit of the

Institute of Child Health, 528 (88%) had a history of pollen allergy to 1 or more pollen grains (Table 2). Most of those patients had symptoms of cough (97.16%), breathlessness (86.17%), wheezing (73.1%), sneezing (71.02%), nasal blockage (68%), and skin rash (34.1%). While 23% and 5.1% suffered only from asthma and rhinitis, respectively, 69.8% suffered from both (Table 3). The diagnosis of rhinitis and asthma was made on the basis of patient history, symptom score, skin reaction, and pulmonary function tests. From the skin prick test (SPT), with the whole pollen grain extract, maximum allergenic sensitivity was noted with the *P pterocarpum* pollen grain. Out of the 510 patients (mean age, 36.2 years; 260 men and 250 women) tested with *P pterocarpum* allergen extract, 135 (26.47%) showed +1 and 31 (6.07%) showed +2/+3 reactions in SPT. Forty-two patients (mean age 38.3 years; 23 men and 19 women) were selected for serum collection on the basis of stronger reactions in SPT and high titre of *P pterocarpum* pollen-specific IgE (data not shown).

Table 2. Distribution of Patients According to Symptoms and History of Pollen Allergy^a

Symptoms	Total Patients (n=600)	Patients With History of Pollen Allergy (n=528)
Bronchial asthma	138 (23)	92 (17.4)
Allergic rhinitis	31 (5.1)	27 (5.11)
Asthma with rhinitis	419 (69.8)	402 (76.14)
Asthma with skin allergies	17 (2)	7 (1.32)

^aData are shown as number (%).

Table 3. Result of Skin Prick Tests With Pollen From Avenue Trees in Patients With Respiratory Allergy

Pollen Allergen Extract	Patients Tested, No.	% Response, Total	% Response, $\geq +2$
<i>Peltophorum pterocarpum</i>	510	26.47	6.07
<i>Delonix regia</i>	500	22	4.8

Of the 500 patients (mean age, 34.7 years; 260 men and 240 women) tested with *D regia* allergen extract, 110 (22%) showed +1 and 24 (4.8%) showed +2/+3 reactions in SPT (Table 3). Among them, 33 patients were selected for serum collection on the basis of stronger reactions in SPT and high titre of *P pterocarpum* pollen-specific IgE (data not shown). These patients were sensitive to other pollen types, such as *A indica*, *C nucifera*, *C papaya*, and *Eucalyptus citriodora*. Most of these patients (65%) were from south Kolkata and the rest from other parts of Kolkata and the suburbs.

In our study, stronger skin reactions in SPT were seen with the allergen extracts of the pollen grains studied than in previous reports [13,14,16]. The city has undergone rapid urbanization, and as a result, the existing heterogeneous plant community has undergone large-scale destruction and new taxa have been introduced without considering their allergenic potential. Therefore it is likely that a large number of patients could have become sensitized to these pollen grains because of continuous exposure in these areas.

Biochemical Characterization of the Pollen Extracts

On reducing SDS-PAGE (11%), the crude extract of *P pterocarpum* resolved into more than 17 distinct bands between 15 kd and 98 kd (Figure 6). Protein fractions of 76, 66, 49, 41, 38, and 20 kd were most prominent and stained strongly. Bands of 56, 28, 25, 22, 16, and 15 kd were less distinct but sharp. Other bands were weak and less stained but were clearly visible and distinguishable on the gel. In another study of *P pterocarpum* pollen grains, 22 bands were resolved in their allergen extract, in the range of 10-116 kd [16].

The crude extract of *D regia* pollen grain resolved into more than 25 distinct bands between 16 and 109 kd (Figure 7). Protein fractions of 109, 94, 79, 67, 62, 54, 52, 49, 46, 40, 38, 32, 27, 25, and 21 kd were most prominent and stained strongly.

Immunological Studies

Immunoblots for specific IgE in individual patient sera revealed several reactivity profiles in the case of *P pterocarpum*

pollen extract (Figure 8). Of the different protein bands that resolved on SDS-PAGE, 5 of them were identified as specific IgE-binding fractions. Among the higher molecular weight proteins, a 76 kd band showed IgE reactivity with 6 individual patient sera. Protein bands of 56 kd and 49 kd were recognized by specific IgE antibodies from 3 and 9 individual patient sera, respectively. Among the lower molecular weight proteins, bands at 28 and 22 kd were recognized by as many as 6 and 5 individual patient sera, respectively. In a previous report, bands of 76, 55, 45, 26, and 23 kd showed IgE reactivity with sera from SPT-positive patients [16].

In the case of *D regia* pollen grains, IgE-specific immunoblotting with individual patient sera revealed 8 IgE-binding fractions (Figure 9). Among them, 6 bands were the most important, as they showed maximum binding with individual patient sera. Protein bands of 43 and 32 kd were recognized by 5 and 6 individual patient sera, respectively. Other bands at 96, 28, 25, 23 kd were identified in a lesser number of patients.

Allergens have also been characterized from pollen grains of other members of the Fabaceae family, such as *Cassia siamea* [32], *Prosopis julifera* [33], and *Acacia* [34]. Identification, isolation, and characterization of allergens are essential to improve the diagnosis and treatment of patients suffering from type-1 allergic disorders such as allergic rhinitis and asthma. Unfortunately relatively small numbers of pollen allergens have been biochemically characterized. Our study is the first to analyze *P pterocarpum* and *D regia* pollen grains in terms of their aerobiology and allergenicity in Kolkata, along with identification of the major IgE-binding proteins. These results will provide a platform for further isolation and molecular characterization of the major allergens in these pollens, which is a prerequisite for the treatment of allergic patients sensitive to *P pterocarpum* and *D regia* tree pollen grains.

Acknowledgments

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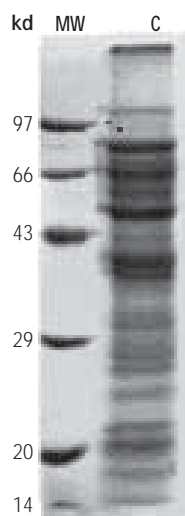


Figure 6. Sodium dodecyl sulfate polyacrylamide gel electrophoresis of *Peltophorum pterocarpum* crude pollen extract on an 11% discontinuous gel. The lane marked MW shows molecular weight markers indicated in kd to the left. The lane marked C shows protein bands from the crude pollen extract stained with Coomassie brilliant blue.

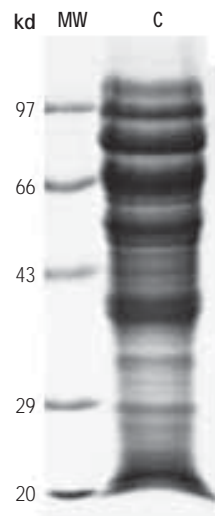


Figure 7. Sodium dodecyl sulfate polyacrylamide gel electrophoresis of *Delonix regia* crude pollen extract on an 11% discontinuous gel. The lane marked MW shows molecular weight markers indicated in kd to the left. The lane marked C shows protein bands from the crude pollen extract stained with Coomassie brilliant blue.

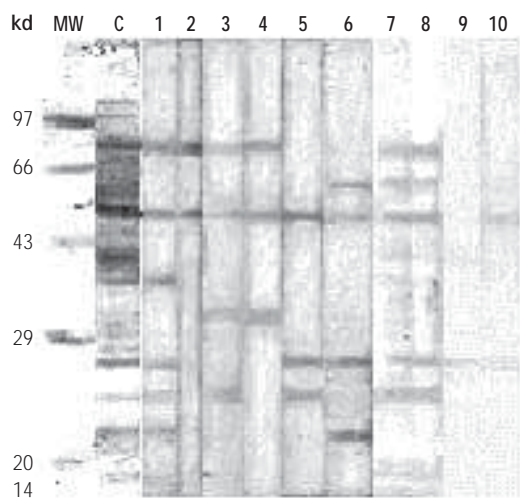


Figure 8. Immunoglobulin E immunoblotting with *Peltophorum pterocarpum* pollen extract. The lane marked MW shows molecular weight markers indicated in kd to the left. The lane marked C shows protein bands from the crude pollen extract. Lanes 1 to 10 show immunoblots using serum from individual sensitized patients.

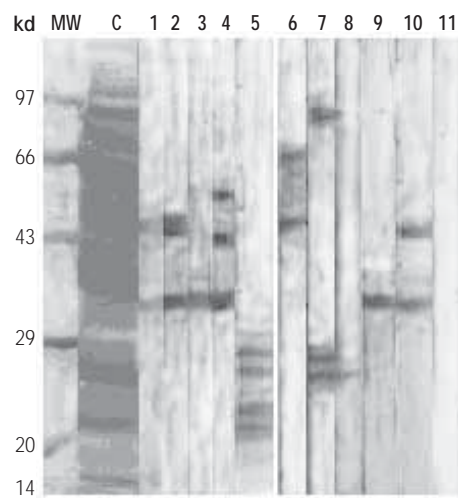


Figure 9. Immunoglobulin E immunoblotting with *Delonix regia* pollen extract. The lane marked MW shows molecular weight markers indicated in kd to the left. The lane marked C shows protein bands from the crude pollen extract. Lanes 1 to 10 show immunoblots using serum from individual sensitized patients.

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