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## Pollen, Fungus and House Dust Mites Survey at the Residence of 90 Allergic Patients in Greater Silchar Area of Assam, India

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**Abstract:** In the present study, the quality and quantity of atmospheric pollen, fungus and house dust mites was evaluated at the residence of 90 allergic patients in Greater Silchar area of Assam, India. Atmospheric pollen and fungal survey was done using volumetric Burkard personal slide sampler and volumetric Andersen sampler, respectively. Dust mites survey was done by collecting 500 mg of house dust sample from bed and floor of 90 atopic allergic patient's residence. Atmospheric pollen concentration was found to be higher in outdoor environment as compared to the indoor air. The dominant pollen types recorded were Poaceae followed by *Allium* sp., *Cocos nucifera*, *Cassia* sp. etc. In contrast, the fungal concentration was found higher inside the patient room as compared to the outdoor air. The dominant fungal genus recorded were *Aspergillus* sp., followed by *Botrytis* sp., *Cladosporium* sp. etc. The dominant house dust mites recorded were *Blomia* sp., *Dermatophagoides* sp., *Chyletus* sp. and *Acharus* sp. No significant difference was found between the indoor and outdoor pollen and fungal types. Similar kind of result was recorded in the distribution of floor and bed mites' population. Patients showing higher indoor biopollutants were found to be suffering from severe respiratory allergic disorders which indicate that some of the indoor fungus, pollens and dust mites could be responsible for eliciting allergic response in respective patients.

**Key words:** Allergy, dust mites, fungus, indoor, pollen

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### INTRODUCTION

Except for specific chemical or toxic gas exposures such as carbon monoxide, building related illnesses are usually associated with exposure to bioaerosols (Macher, 1987; Burge, 1990). *Bioaerosols* are airborne particles that are living, or originated from living organisms. They include microorganisms, fragments, toxins and particulate waste products from a variety of sources (e.g., viruses; bacteria; fungi; plants; protozoa and animals such as arthropods, birds and mammals) (Fung and Hughson, 2003). Modern lifestyle and environmental conditions may be contributing to the production and concentration of indoor allergens and consequently have been associated with increase in the prevalence of several allergic diseases, including asthma (Jones, 1998; Platts-Mills, 2003).

Among the biopollutants responsible for causing allergic symptoms in susceptible individual, pollens are established as major aeroallergens. Fungal spores however are among the most abundant

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and least well known airborne allergens. Fungi grow on structural materials and furnitures where humidity is high or on food materials and stored products where there is condensation of water. Humidifier, air-conditioning system, carpeting and damp wall are the potent source of indoor fungal growth (Basel, 1987). Outdoor fungi such as *Alternaria*, *Aspergillus*, *Botrytis*, *Cladosporium*, *Curvularia*, *Epicoccum*, *Fusarium*, *Penicillium* etc. have been reported regularly from the damp indoors (Sneller and Roby, 1979; Sharma and Dutta, 2001). Several progresses have been done in fungal allergens during the last two decades and efforts have been made to develop new improved allergens of use in fungal vaccines (Bisht *et al.*, 2003). Although the number of studies on pollen concentrations inside and outside buildings is increasing, little is known about the efficiency of penetration of pollen from outdoor to indoor air (Hugg and Lehtimaki, 2007). A wide variety of pollens responsible for eliciting allergic reactions are reported from different parts of the world (Ordman, 1955; Singh and Kumar, 2003; Sharma *et al.*, 2004).

The role of house dust mites in inducing allergy has been increasingly recognized by allergologist and aerobiologists. However, clinical investigations of house-dust mite allergy in tropics are few (Pepys *et al.*, 1968; Voorhorst *et al.*, 1969; Anand, 1981; Hurtado and Mava, 1987). The significant role of mites in the house dust responsible for health hazard such as respiratory allergy, nasobronchial, nasal and skin allergy in sensitive individuals is well documented. Group 1 allergens of the mites *Dermatophagoides farinae* (*Der f 1*) and *Dermatophagoides pteronyssinus* (*Der p1*) are the most significant allergens and 80 to 95% of patients allergic to dust mites have an elevated IgE response to *Dermatophagoides* allergen (Peng *et al.*, 1997). Shivpuri (1962, 1977) and Shivpuri and Dua (1974) were the first Indian acarologists to conduct extensive and intensive studies on mites and recorded that mites could grow well in house dust at 25°C temperature and 80% relative humidity.

In the present study, an attempt has been made to provide valuable information's on the prevalence of pollen, fungus and house dust mites at the residence of some selected atopic allergic patients in Greater Silchar area which lies between 24°5' N latitude and 92°48' E longitude and 26-27 m above mean sea (Fig. 1). Emphasize has also been given to correlate the indoor biocomponent concentrations in the selected patients residence with their severity of allergic disorders.

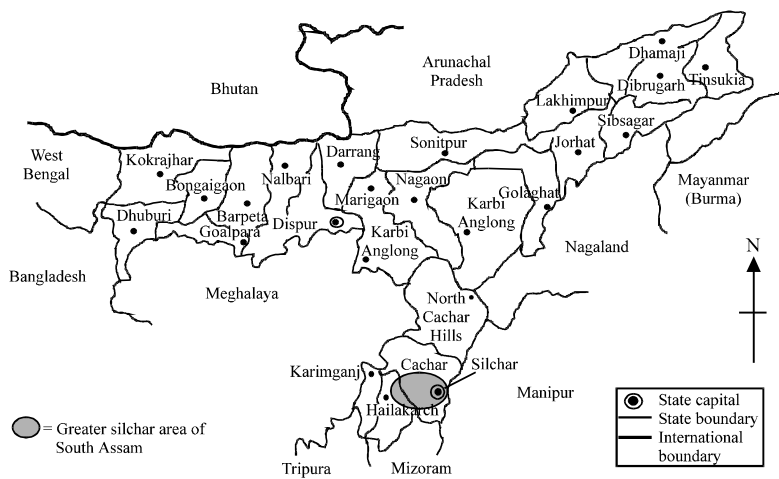


Fig. 1: Map of Assam presenting the location of Greater Silchar area

## MATERIALS AND METHODS

Aerobiological and dust mites survey was done at the residence of 90 atopic allergic patients in Greater Silchar area of Assam (Fig. 1). Patients of different economic status, age, sex were selected for the present study. About 47% of the patients were living in RCC type building, 39% were in Assam type building, 13% in Bamboo house and rest 7% in wooden house. Sex wise, 73% of the patients were male and twenty seven were female. Twenty percent patients were below the age of 30 years, 60% were between 30-50 years and rest 20% were above 50 years. All of these patients were tested to be atopic allergic patients either from All India Institute of Medical Science or from Christian Medical College, Vellore. Among the 90 allergic patients, 60.0% were suffering from asthma/respiratory allergy and 40.0% from skin allergy (including nasal, ocular and dermal). Survey was done both at indoor and outdoor of patients house during the peak period of their suffering (i.e., from September 2002 to February, 2003). Sampling was done six times in each patient house after an interval of one month and the mean of six sampling was considered as standard. Pollen survey was done using Burkard personal volumetric air sampler (Burkard Manufacturing Co, UK). Slide smeared with gelatine jelly (containing gelatine-50 g, glycerine-150 mL, phenol crystal-7 g and distilled water-175 mL) was inserted inside the sampler and the sampler was run for 10 min. Pollens collected over the jelly were identified with the help of reference slides and available literatures (Erdtman, 1952; Faegri and Iversen, 1964; Nair, 1970). Total pollen count was correlated to the nearest count with the help of correction factor and was expressed as number per m<sup>3</sup> of air. Fungal spores were collected using volumetric two stage Andersen sampler (Andersen, USA). Petriplates containing Rose Bengal agar media (containing glucose-1%, peptone-0.5%, K<sub>2</sub>HPO<sub>4</sub>-0.1%, MgSO<sub>4</sub>.7H<sub>2</sub>O-0.05%, Agar-1.5%, Rose Bengal-0.0035%, streptomycin-0.003%) was placed inside the sampler and the sampler was run for 10 min at human height. After sampling, petriplates were incubated at 27±2°C for 5 days. Identification was done with the help of reference slides and literatures available (Dumsch *et al.*, 1980; Gillman, 1975). Fungal count was correlated to its nearest count with the help of correction factor given by Andersen (1958). Fungal count was expression as colony forming unit (cfu) per m<sup>3</sup> of air.

Indoor mite's survey was conducted at 90 patient's residence to record the quality and quantity of house dust mites in floor and bed of the patient's house. Five hundred milligrams (0.5 g) of the indoor house dust sample was collected both from the floor and the bed of the selected patient house. Since the vacuum cleaners are not used in majority of Indian homes, therefore, dust sample was collected manually. Patients were advised not to clean their floor and sleeping bed for two day before collecting the dust sample. Samples were collected in autoclaved plastic container. Large particles and fibrous materials in the dust were separated by sieving through 300 mesh special brass sieve of 6 diameter. Mites were isolated from the dust sample manually with the help of a painting brass (No. 6). Isolated mites were made clear by deeping in 50% lactic acid for 24 h. Then they were mounted in the centre of a glass slide with a drop of melted glycerine jelly. Identification of house dust mites were done with the help of reference slides and literatures available (Tilak *et al.*, 1994).

An attempt has been made to correlate the severity of the allergic/asthma attack with the pollen, fungus and house dust mite's population for each patients. On the basis of the allergic/asthma attack, patients were graded as follows:

**Group 1:** Occasional skin allergy attack

**Group 2:** Frequent skin allergy attack

**Group 3:** Occasional asthma attack

**Group 4:** Frequent asthma attack

Statistical analysis of data was done using the Minitab Software (Version 12.2).

## RESULTS

Survey was conducted at the residence of 90 atopic allergic patients in Greater Silchar area to isolate and identify the common indoor allergens (i.e, pollen, fungus and dust mites). A total of 21 pollens, 55 fungal and 20 dust mite types were identified during the survey period. Among the 90 houses surveyed, 53% showed higher indoor fungal concentration while 47% showed higher outdoor concentration. Twenty percent of the houses surveyed showed higher indoor pollen count while 80% showed higher outdoor pollen count whereas, 5% of the patients showed higher mites population in the bed dust sample and 95% in floor dust samples (Fig. 2). The dominant fungal genus recorded at the patients residence were *Aspergillus* (339.6 cfu m<sup>-3</sup>) followed by *Cladosporium* (313.0 cfu m<sup>-3</sup>), *Penicillium* (198.3 cfu m<sup>-3</sup>) and *Botrytis* (112.3 cfu m<sup>-3</sup>) (Fig 3b). Domination of *Aspergillus* is mainly represented by four species viz., *A. sydowi* (109.8 cfu m<sup>-3</sup>), *A. flavus* (38.4 cfu m<sup>-3</sup>), *A. fumigatus* (36.6 cfu m<sup>-3</sup>) and *A. humicola* (19.2 cfu m<sup>-3</sup>). Similarly, domination of *Cladosporium* is represented by *Cladosporium herbarum* (146.4 cfu m<sup>-3</sup>). The dominant pollen types recorded at the patient residence was Poaceae (201.6 m<sup>-3</sup>) followed by *Allium* sp. (50.4 m<sup>-3</sup>), *Cocos nucifera* (45.0 m<sup>-3</sup>), *Cassia* sp. (41.4 m<sup>-3</sup>) (Fig. 3a). A total of 90 patients houses were surveyed for indoor dust mite study. Although the house dust mites were found to be higher in the

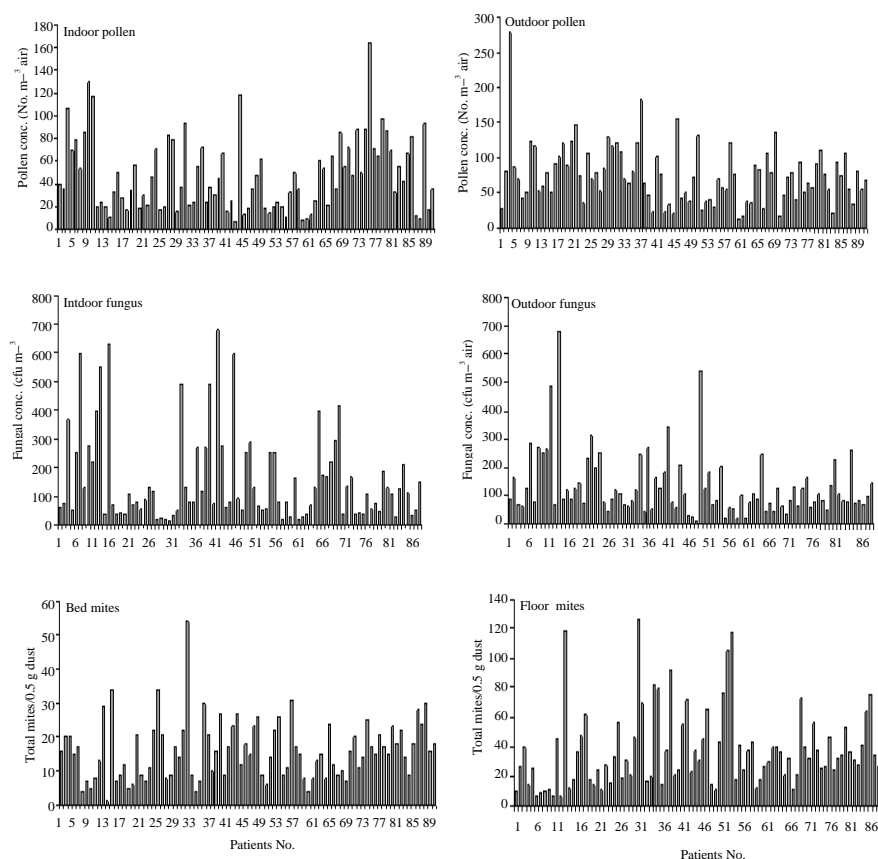


Fig. 2: Total indoor bio-components collected and identified from the residence of selected patients in Greater Silchar area. (a) Indoor and outdoor pollen (b) Indoor and outdoor fungal spores (c) Bed and floor dust mites

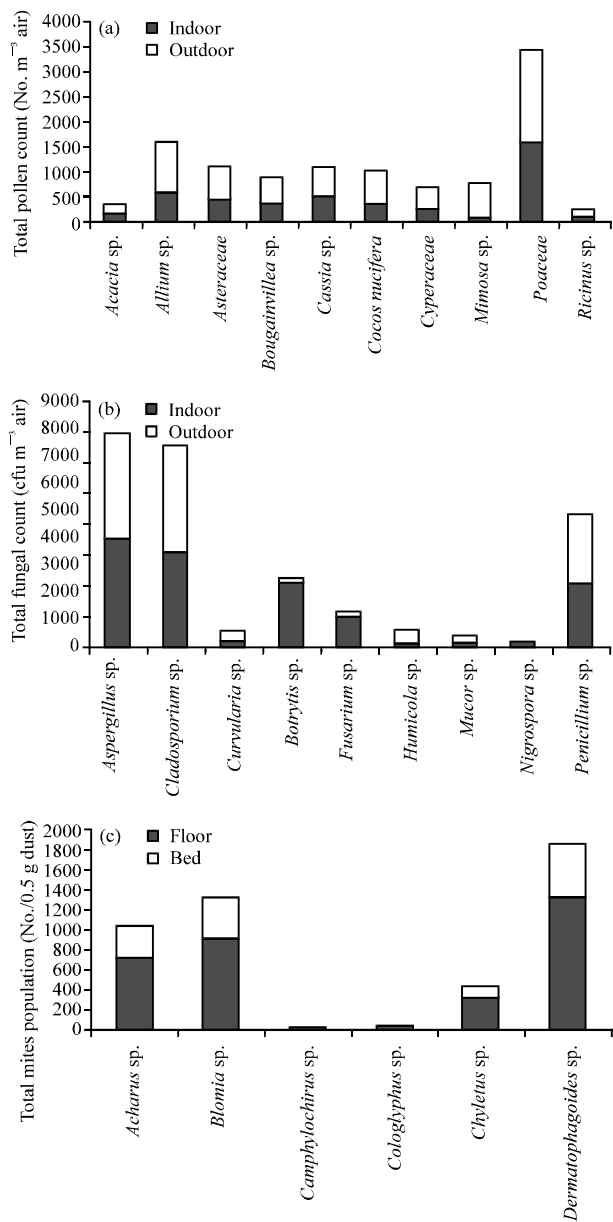


Fig. 3: Dominant genus of pollen, fungus and house dust mites collected from indoor environment in Greater Silchar area (a) Pollen grains (b) Fungal spores (c) House dust mites

samples collected from the floor but in certain cases, the number and variety of dust mites collected and identified from the bed of allergic patients house were found to be fairly high (Fig. 2). Total numbers of the dominant dust mites recorded from the patient's house were *Blomia* sp. (912 g<sup>-1</sup>), *Dermatophagoides* sp. (546 g<sup>-1</sup>), *Chyletus* sp. (354 g<sup>-1</sup>) and *Acharus* sp. (372 g<sup>-1</sup>) (Fig. 3c). Among *Dermatophagoides*, *D. pteronyssinus* (198 g<sup>-1</sup>) was found to be the dominant species. Number of male mites was found to be higher as compared to female mites in most of the genus except for *Dermatophagoides* where female dominate the male mites (33.7% female and 16% male).

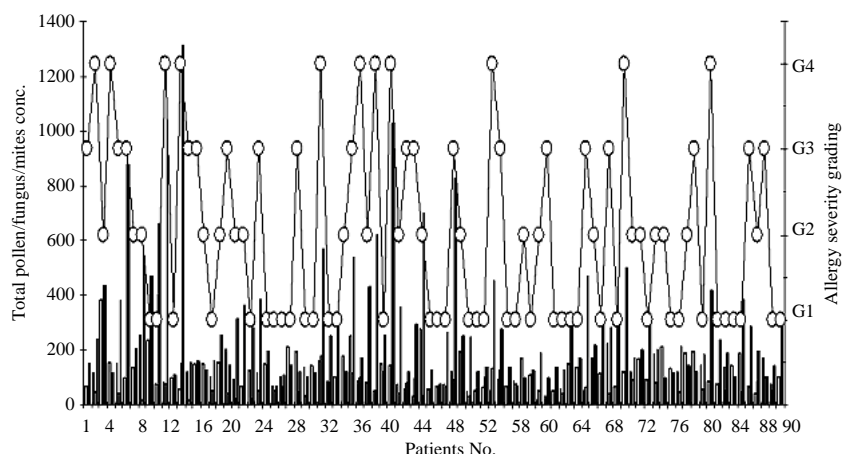


Fig. 4: Severity of allergic disorders in patients, showing relationship with indoor pollen, fungus and dust mites concentration. Symbols: ■ fungal count (cfu m<sup>-3</sup> air), □ pollen count (No. m<sup>-3</sup> air), ▨ total dust mites population (No. 0.5 g<sup>-1</sup> dust) and ○ severity of allergic diseases

Table 1: Correlation in the distribution of indoor and outdoor biocomponent at the residence of selected allergic patients

Factors	Correlation (r)	p-value
Indoor vs outdoor fungus	0.476	<0.001
Indoor vs outdoor pollen	0.608	<0.001
Floor vs bed mites	0.488	<0.001

Table 2: Correlation of the severity of allergic diseases with the prevalence of bio-components

Factors	Correlation (r)	p-value
Pollen concentration	0.030	NS*
Fungal concentration	0.305	<0.001
Dust mites concentration	0.456	<0.001

\*NS = Not Significant

Statistical analysis shows significant correlation between the indoor and outdoor pollen and fungal count at 1% level (p<0.001). Dust mites concentration between floor and bed sample also showed significant correlation at 1% level of significance (Table 1).

Allergen load of pollen, fungus and dust mite in the samples collected from the patient's house was found to be proportional to the severity of allergic attack in some of the typical cases. Patient's number 13 revealed higher concentrations of both fungus and dust mites. The ratio of indoor vs outdoor fungal and bed vs floor mites' count was 5:1 and 1:3, respectively. Interestingly, the patient was found to be suffering from atopic bronchial asthma for last 12 years. Similarly patient number 40, suffering from bronchial asthma, showed higher fungal concentration both inside and outside the house (218.6 and 170.8 cfu m<sup>-3</sup>, respectively). Maximum pollen count (384 m<sup>-3</sup> air) was recorded from the residence of patient number 3 which was reported to be suffering from severe allergic attack. The dominant fungal genus recorded at the residence of patient number 3, 13 and 40 were *Aspergillus* followed by *Botrytis*, *Cladosporium*, *Fusarium*, *Humicola*, *Penicillium* while the dominant pollen types encountered were member of Asteraceae followed by *Cocos* and *Cyperaceae* (Fig. 4). Similarly, the dominant house dust mites identified were *Acharus*, *Blomia* and *Dermatophagoides*.

Statistical analysis showed significant correlation between the severity of allergic diseases and the indoor concentration of fungus and dust mites (p<0.001). However, indoor pollen concentrations have shown insignificant correlation with the severity of allergic disorders (Table 2).

## DISCUSSION

Domination of the genus *Aspergillus* and *Cladosporium* in the allergic patient's house studied above was also reported by Majumdar and Bhattacharya (2004). Comparison study carried out on indoor fungal spore levels before and after professional home remediation by Charles *et al.* (2007) reported that remediation can significantly reduce spore counts. They found that indoor collections in pre-remediation buildings are generally much higher than outdoor counts for critical spore types, including *Aspergillus/Penicillium* and *Stachybotrys*. Remediation provides indoor spore levels substantially lower than outdoor counts. Domination of *Aspergillus*, *Penicillium*, *Fusarium*, *Humicola* among the fungus, Asteraceae, *Cocos*, Cyperaceae, Poaceae among the pollens and *Acharus*, *Blomia*, *Dermatophagoides* among the dust mites in house number 3, 11, 13, 31, 40 and 44 etc. indicate that some of these antigens could be responsible for allergic reactions in susceptible patient. But the presence of fungus, pollen and dust mites, even in large quantity is unimportant unless their extracts are capable of producing immunological reaction (Gravesen, 1979; Vijay *et al.*, 1989). Similar findings were also reported earlier by Sneller and Roby (1979), Singh *et al.* (1998) and Sharma and Dutta (2001). An individual may prove to be highly susceptible to even a mild dose of allergens, irrespective of the concentration of spores. The higher percentage of positivity against some selected antigens might be due to a greater level of exposure to that particular kind of antigen (Majumdar and Bhattacharya, 2004). Threshold value could be one parameter which is needed to solve the cause and effect equation. The genuine magnitude of exposure of each of allergic patients is another obstacle in the search for a solution. High responses of the patient to high total indoor allergens may express the result of additive or combined effect rather than a direct response to on individual allergens (Waisel *et al.*, 2004).

It has been reported earlier that microorganisms may enter buildings from outside but the most important sources are usually within the building (Vittal, 2003). If respiratory allergies are to be treated effectively, knowledge of indoor airborne fungi is perhaps even more important than that of the concerning propagules outdoor. Generally, the mycoflora of indoor air is qualitatively similar and quantitatively lower than outdoor air, but this is not always true if there are indoor sources (as observed in the present study). However, even in the absence of viable spores, allergenic proteins may still be present in dead spores or in spore fractions (Fluckiger and Morn, 1999). Many epidemiological studies have noted that residential exposure to molds and/or chronic dampness can increase asthma/wheezing incidence or morbidity in both children and adults (Williamson *et al.*, 1997; Jedrychowski and Flak, 1998; Ostro *et al.*, 2001; Gent *et al.*, 2002; Lee *et al.*, 2003). Studies with infants have reported that higher fungal exposures are associated with more wheezing, coughing and respiratory illness (Belanger *et al.*, 2003; Stark *et al.*, 2003). Kilburn (2003) have reported that patients exposed to high indoor fungal levels had significantly lower lung function than unexposed controls.

In the present study, lower pollen concentration was recorded in the indoor environment per m<sup>3</sup> of air as compared to outdoor air. The observation that pollen grains were found in higher concentrations outside than inside is in consistent with previous studies (D'Amato *et al.*, 1996; Carinanos *et al.*, 2004). The low indoor-to-outdoor ratios observed are also in accordance with previous findings (Lee *et al.*, 2006). Hugg and Lehtimaki (2007) in their study on *Betula* pollen also reported significantly decreased in pollen count from outdoors to indoors environment. They have also reported higher indoor pollen concentration in less frequently cleaned residence than that in more frequently cleaned. Pollen grains act as carriers of allergenic material and pollen counts may include pollen grains devoid of allergens and thus may not represent a true allergen load. Good agreement between numbers of pollen grains and the allergen concentration in outdoor air has been observed previously by Schappi *et al.* (1997). In particular, factors such as outdoor temperature, air-exchange rate, ventilation system, cleaning methods and activity of the occupants are important determinants of the indoor



concentration of pollen grains and pollen allergens (Sterling and Lewis 1998; Fahlbusch *et al.*, 2001). Member of Asteraceae, Poaceae, Cyperaceae and *Cocos* could be among the probable allergic pollens recorded at the residence.

Many of the dust mites reported above are known to be allergenic to human health (Voorhorst *et al.*, 1964, 1969; Martin *et al.*, 1987). Modak *et al.* (1991) reported the difference in the mite population in the house dust and variation in the mite fauna in the three study areas of West Bengal. They pointed out that in general it is due to the difference in the structure and materials of the buildings, socio-economic status of the individuals, type of mattresses used, standard of hygiene maintained and difference in the microclimatic conditions that contributes to the higher accumulation of mites in the house dust. The present findings conform to these observations. It has been suggested that the change in dwelling accompanied by treatment give the patient better relief.

A careful medical and environmental history is an essential first step in evaluating a patient for allergy related health problems (Marshall *et al.*, 2002; Eggleston, 2003). Particular attention should be paid to any history of exposure to visible allergens at the home or workplace. Environmental sampling in the air and dust can provide important exposure information. It has been seen that the patients living in low cost building having several ventilation/openings for free exchange of outdoor air showed maximum indoor pollen and fungal count. RCC building constructed in improper way leading to insufficient light entry, water seepage, damp indoor etc. record higher indoor fungal and dust mites concentration. Several studies have linked hospital construction work to increased rates of invasive aspergillosis (Panackal *et al.*, 2003; Loo *et al.*, 1996). Health care professionals, building managers, homeowners and the general public need to be much more aware of the potential adverse health effects of high indoor fungal exposures and the need for proper building construction, maintenance and remediation of dampness to prevent such effects.

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