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The Effects of Air Pollution on Cellular Material Release, Allergenicity and Allergens Proteins of Three Ornamental Plants

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1. Introduction

The term allergy, coined by von Pirquet in 1906, is used to define the series of events which occurs when an antigen, which is not harmful in itself, causes an immune response, leading to symptoms and disease in genetically predisposed individuals. An antigen that induces the allergic response is called an allergen (Shaikh, 2001). The allergic response manifested on exposure to a harmless allergen is the result of a complex orchestrated interaction of various immune cells and immunoglobulins. Allergy and asthma are a spectrum of diseases based on various genetic and physiologic mechanisms (Shaikh, 2001). The changes in life style and environment have results in a sufficiently large increase in the number of patients with disease to constitute a social problem. Asthma is a complex syndrome with many clinical phenotypes. Common to all is chronic inflammation with reversible airway obstruction and airway hyperresponsiveness (AHR). The most prevalent form of asthma is atopic asthma which is initiated by the exposure to (inhaled) allergens and resultant allergen-specific immune responses (Shin et al., 2009). Asthma is characterized by inflammatory changes of the airways, in which inflammatory cells such as lymphocytes. Neutrophils and eosinophils and a number of cytokines including leukotrienes released from these cells participate in the late asthmatic reaction. Among inflammatory cells, activated lymphocytes and eosinophils play an important role in induction and persistence of the reaction (Walker et al., 1991). The results are consistent with the data showing enhanced production of LTC 4 by activated eosinophils during antigen challenge.

The transfer of pollen grains, the structures housing the male gametes of plants, from floral anther to stigma is the critical reproductive event among higher plants. Like any other plant cells, pollen grains contain many different types of proteins, which are located in major domains: in the cytoplasm and at the surface of exine and intine. These molecules are strategically sited to participate as the male partner in intercellular recognition reactions with the female stigma (Knox & Suphiohlu, 1996; Dickinson et al., 2000). Once pollen grains are placed on stigma or on artificial medium (or mucosal membrane), they release cellular material containing proteins and glycoprotein. These materials from pollen that contribute in interaction pollen-stigma can function as allergens, environment molecules interacting with the human immune system to elicit an allergic response in susceptible individual.

Pollen allergy is the most common form of seasonal respiratory allergic disease in most of countries.

Henry Hyde Salter was the first to report that air pollution was implicated in respiratory allergy. In his book "On asthma: its pathology and treatment" published in London in 1860, with great intuition he listed "Impure air, animal emanations, hay fever and foods" among the various causes of asthma (D'amato, 2002, as cited in Salter, 1869). He also described asthma as being "Paroxysmal dyspnea of a peculiar character, generally periodic with healthy respiration between attacks". Nearly 50 years latter, William Lioyd postulated that hay fever was a result of urbanization and agricultural changes (D'amato, 2002, as cited in Lloyd, 1907). Pollen allergy is one of the models most frequently used to study the interrelationship between air pollution and allergic respiratory allergy (D'Amato, 2002).

1.1 Allergies: Genetic and environment

The term 'allergy' refers to a grouping of symptoms that affects different body systems. The allergic patient may experience symptoms in only one, or several body systems. People may experience allergies that are due to a wide array of antigens (triggering substances), such as foods, pollen, environmental particles, animal dander, dust mites, cigarette smoke, ingredients in soaps, newsprint, latex and almost anything else that a person may come into contact with. Although many of the antigens that provoke allergic reactions are proteins, many non-protein components can illicit allergic reactions, such as toluene diisocyanate found in dry cleaning fluid. The following is a grouping of symptoms that are commonly associated with different body systems. Respiratory symptoms: stuffy/runny nose, sneezing, coughing, watery eyes, wheezing, recurring ear infections, tickling or sore throat, asthma. Integumentary symptoms: swelling of hands and feet, itchy, dry, red scaling skin patches, dark circles under the eyes, swollen tongue. Digestive symptoms: bloating, flatulence, diarrhea, constipation, weight loss or gain, burning anal and underarm rash, abdominal discomfort (Wayne et al., 2002).

Allergic reactions are initiated by the immune system's over response to substances it deems as distinct from itself. Although well tolerated by most people, in the allergic person contact with the offending substance (antigen), is the basis of the hypersensitivity reaction. Immunoglobulins are specific antibodies that have the responsibility of tracking down and destroying antigens that are recognized by the body. There are five different classes of antibodies, IgE, IgA, IgG, IgM, and IgD. Each of these specifically attacks different categories of antigens. Every antibody is 'antigen specific', and is programmed to react to a specific kind of invader. In the case of allergies, these antibodies become overzealous, and begin to recognize substances that are usually benign, as if they were dangerous invaders. Once an antibody recognizes a suspected antigen, it attaches to it and forms an 'antigen-antibody' complex. This complex initiates the release of histamines and other chemicals that lead to inflammation, the common theme to all hypersensitivity reactions (allergies). The inflammatory response causes the release of chemical mediators, vasodilation, increased vascular permeability, edema and tissue damage. Allergic reactions can range from a trivial annoyance such as an itchy rash and runny nose, to a fatal incident (Wayne et al., 2002). There are four different classes of allergic reactions: Type I (immediate hypersensitivity reactions), Type II (cytotoxic reactions), Type III (immune complex mediated reactions) and Type IV (cell dependent reactions). Type I reactions occur almost immediately after exposure to an antigen, and range from a temporary outbreak of hives, skin rash, headache,

GI symptoms, rhinitis, and asthma-like symptoms, to fatal anaphylactic shock characterized by difficulty breathing, closing of the throat, decreased blood pressure, tachycardia, seizures and loss of consciousness. The symptoms can be local or systemic, depending on the port of entry of the antigen. IgE immunoglobulins are widely believed to be responsible for Type I reactions. However, recent studies report that IgG, previously thought to only be involved with delayed reactions, may also be involved in the initiation of Type I reactions. This evidence supports complementary health care practitioners, who have been regularly testing patients for both IgE and IgG mediated reactions for decades, although most conventional physicians still disregard IgG testing. Type II- Type IV reactions are referred to as delayed reactions, since they can occur 24- 72 hours after encountering the offending substance. They involve IgG, and to some degree IgM, although many allergic reactions do not involve any antigen-antibody complex, but are due to the direct release of histamines and other inflammatory compounds by mast cells and other white blood cells (Wayne et al., 2002). Pollinosis occurs in 10–20% of European inhabitants. Type I immunological reaction, dependent on the presence of specific IgE antibodies, mast cells and eosinophil cells, forms the basis of this disease (Lipiec et al., 2005, as cited in Bertel et al., 2001; Kowalski, 2000).
The ability of an individual to form an allergic response and its subsequent severity is dependent on a combination of intrinsic and extrinsic factors. Intrinsic traits such as age and genetic background have been shown to have a strong influence on the development and severity of allergic disease. In addition, a multitude of experimental and epidemiological studies have demonstrated that environmental factors such as infections, air pollution, and indoor allergens may affect the symptoms of established allergic disease. Less is known about specific environmental factors leading to primary allergic sensitization in humans, i.e., the initial formation of Ag-specific IgE following Ag exposure (Riedl et al, 2005). Cookson et al (1989) noted the linkage of a specific chromosome region to allergic phenotype the 11q13 gene locus. Cytokine gene cluster at 5q is a large genetic region spanning a gamut of DNA and contains numerous genes influencing the atopy phenotype. Researchers have analyzed a mouse chromosomal segment homologous to human chromosome 5 locus exhibiting a T cell and airway phenotype regulator (Shaikh, 2001). The interaction between genetic and environmental factors is generally accepted to cause individuals to be sensitized with environmental allergens and to suffer from allergic diseases. However, it is believed that recent changes in the environment have contributed to the increase more significantly than genetic factors, since it seems unlikely that genes would change over one or two generations. Thus, it is a central issue to reveal what environmental factor(s) cause such high prevalence and to find strategies to prevent their development. As environmental factors that influence the susceptibility to the development of asthma in predisposed individuals, the Global Strategy for Asthma Management and Prevention lists indoor and outdoor allergens, occupational sensitizers, air pollution, respiratory infection, parasitic infections, socioeconomic status, family size and obesity (Kawai et al., 2007). Human allergic responses to the pollen of certain plant species (hay fever, allergenic rhinitis, pollinosis) is a serious environmental health issue (Wayne et al., 2002).

1.1.1 Air pollution

Air pollution is a major and growing environmental health problem worldwide. The World Health Organization [WHO] considers that air pollution is damaging the resources that are needed for the long term sustainable development of the planet (Chan-Yeung, 2000; as cited

in WHO, 1999). This growth is largely attributable to increases in: a) the world population, b) economical activities, c) energy consumption, d) industrial activities, and e) motor vehicles (Bartra et al., 2007, as cited in Parnia & Frew, 2001). In most countries including Iran, the main source of air pollution due to particles in suspension is represented by motor vehicles particularly those that use diesel fuel. The sources of air pollution fall into three broad categories: 1- Mobile sources, which include combustion-engine vehicles such as gasoline powered cars, diesel-powered vehicles, motorcycles, and aircraft; 2- Stationary sources, which include rural sources such as agricultural production, mining, and quarrying; industrial sources such as manufacturing; and community sources such as the heating of homes and buildings, municipal waste, and incinerators; and 3- Indoor sources, which include combustion, tobacco smoking, and biological sources; and emissions from indoor materials or substances such as volatile organic compounds, asbestos, and radon (Chan-Yeung, 2000).

1.1.1.1 Suspended particulate matter and gaseous pollutants

Air pollutants are usually classified into suspended particulate matter (dusts, fumes, mists, and smokes), gaseous pollutants (gases and vapours), and odours. The most abundant components of air pollution in urban areas are nitrogen dioxide (NO2), Hydrocarbons (HC), sulphur dioxide (SO2), ozone (O3), carbon monoxide (CO) and airborne particulate matter (APM). Suspended particulate matter (PM) consists of finely divided small particulates with diameters of less than 10 μm (PM10). Suspended particulate matter comprises a wide variety of substances, which include inorganic and organic carbon (containing polycyclic aromatic hydrocarbons), acidic or neutral sulphates and nitrates, fine soil dust, residues of lead and other metals, asbestos, and other fibres. Most of these particulates are smaller than 1 μm and remain suspended for hours or days. Particles that are smaller than 2.5 μm in diameter (PM2.5) arise mainly from combustion processes, whereas larger particles are generated by grinding and other mechanical or agricultural processes (Chan-Yeung, 2000). Small particles efficiently penetrate indoors, where levels are typically 70% to 80% of outdoor levels in the absence of indoor sources (Chan-Yeung, 2000). Much research is now being carried out on diesel exhaust particles (DEPs) and their components (e.g., polycyclic aromatic hydrocarbons (PAH), since a large part of urban particulate matter originates from diesel engines. DEPs account for most airborne particulate matter (up to 90%) in the world's largest cities, and are composed of fine particles (0.1- 2.5-μm) and ultrafine (<0.1 μm) particles, although these primary DEPs can coalesce to form aggregates of varying sizes (D'Amato et al., 2010). Diesel fuel combustion results in the production of diesel exhaust particles (up to 100 times more particles than gasoline engines) and gaseous compounds such as NO2 and hydrocarbon precursors of ozone. Diesel engine use has increased in many parts of the world due to superior energy eficiency and durability. Thus, diesel exhaust particles (DEP) are a predominant and representative particulate pollutant widely used to study the effects of PM (Riedl, 2008).

1.1.2 Pollen grains

The word pollen is derived from the Greek word meaning 'fine flour'. Pollen development is an integrated process in which three elements take part: 1- The cells of the external part of the anther that are modified slightly during development. 2- The tapetum cells that differentiate during development, their content being wholly or partly reabsorbed and 3-

The microspore mother cells that under go meiosis and haploid mitosis. During development, various types of cells differentiate, with modifications in the case of tapetum and pollen that mostly involve the walls, division and differentiation (Pacini & Franchi, 1992). The transfer of pollen from floral anther to stigma is the critical reproductive event among higher plant. Like any other plant cells, pollen grains contain many different types of proteins, which are located in major domains: in the cytoplasm and at the surface of exine and intine. These molecules are strategically sited to participate as the male partner in intercellular recognition reactions with the female stigma (Rezanejad, 2009, as cited in Dickinson et al., 2000; Knox & Suphiohlu, 1996).

1.1.3 Influence of environmental pollution upon pollen allergenicity

Once pollen grains are placed on stigma or on artificial medium (or mucosal membrane), they release cellular material containing proteins and glycoprotein. These materials from pollen that contribute in interaction pollen-stigma can function as allergens, environment molecules interacting with the human immune system to elicit an allergic response in susceptible individual (Knox & Suphiohlu, 1996). Of all things that can cause an allergy, pollen is one of the most abundant and inescapable (Lokaj-Berisha et al., 2009, as cited in Shazo, 2000).

As for the health effects of air pollution in subjects living in polluted urban areas, it has been found, both in US (D'Amato, 2002; as cited in Dockery et al., 1759; Schenker, 1993) and in Europe (D'Amato, 2002; as cited in European Commission, Environmental Research Programme [UCERP], 1996) an association of daily concentrations of particulates and other components of urban air pollution (NO_2 and SO_2) and daily mortality. In this context Seaton et al. (D'Amato, 2002 as cited in Seaton et al., 1995) hypothesized that fine particulate of urban areas, penetrating deep into airways, is able to induce alveolar inflammation which is responsible for variation in blood coagulability and release of mediators which induce acute episodes of respiratory and cardiovascular diseases. Studies of Morgenstern et al. (2008) provided strong evidence for increased risk of atopic diseases and allergic sensitization when children are exposing to ambient particulate matter (Morgenstern et al., 2008). Asthma cases in China have risen an estimated 40% in the past 5 years concurrent with rapid increases in urban air pollution (Riedl, 2008, as cited in Watts, 2006).

Pollen allergy is one of the models most frequently used to study the interrelationship between air pollution and allergic respiratory allergy. Environmental pollution influences pollen allergenicity. Scientific evidence shows that pollen in heavily polluted zones expresses a larger amount of proteins described as being allergenic, compared with areas characterized by lesser pollution. The study of Cortegano et al. (2004) concluded that *Cupressus arizonica* in areas of heavy air pollution express a larger amount of Cup a 3 compared with pollen from areas with signifi cantly lower pollution levels, thus favoring sensitization of the exposed population or the appearance of respiratory allergic disease in sensitized individuals. Armentia et al. (2002) have confirmed that the protein content and biological allergenic activity of gramineous pollen specifically Lol p 5 is greater in areas of heavy air pollution. The pollen of Parietaria has also been considered in the investigation of the interaction between environmental pollution and allergenic expression. The emissions of gasoline engines without catalytic converters and of diesel engines increase the allergenic potential of Parietaria pollen compared with emissions from vehicles with catalytic

converters (Bartra et al., 2007, as cited in Armentia et al., 2002; Cortegano et al., 2004). Helander et al. (1997) and Majd et al. (2004) did not observe any significant difference between protein bands of pollen grains collected from polluted and control areas (Helanderet al., 1997; Majd et al., 2004). Jilek et al. (1993) observed an increase of the major birch pollen allergen, Bet v 1, in areas where nitrogen loads are high, while Parui et al. (1998) found a decrease in Bet v 1 concentration due to air pollution (Jilek et al., 1993; Parui et al., 1998). However, studies on pollen proteins collected from polluted and unpolluted areas have shown contradictory results.

Pollen particles contain pollinic allergens. High environmental humidity conditions can subject the pollen particles to osmotic shock, resulting in the release of microparticles or paucimicronic particles that may contain allergenic proteins. The presence of these paucimicronic particles would explain the discordance occasionally observed between the appearance of respiratory allergic symptoms in a pollinic patient and the absence of actual pollen particles in the atmosphere. In the same way, through physical contact with the pollen particles, DEPs can disrupt the former, leading to the release of paucimicronic particles and transporting them by air, thus facilitating their penetration of the human airways. In vitro studies have shown that Lol p 1 and Bet v 1, which are the prevalent or majority allergens of *Lolium* and birch, respectively, bind to DEPs thanks to the absorptive capacity resulting from their physicochemical characteristics. Such "affinity" has also been demonstrated for other allergens such as Der p 1, Fel d 1 and Can f 1. Other plant-derived allergenic particles are the so-called Ubish bodies. These are spheroid structures that develop with pollen exine and are found in the anthers of many plants. These structures, measuring only a few µm in diameter, may also contain allergenic proteins. Non-biological particles in suspension, such as DEPs, act as transporters for these structures, in the same way as for other pollen-derived paucimicronic particles (Bartra et al., 2007; D'Amato et al., 2010).

Inflammatory reactions generally involve a vast array of mediators and a variety of effector cells such as mast cells, macrophages, eosinophils, platelets, and neutropb.ils. It is well recognized that eosinophils are important cells in asthma. Eosinophils release basic proteins that are cytotoxic and lipid mediators such as cysteinyl leukotrienes that cause airway obstruction and bronchial epithelial damage. In contrast, the role of the neutrophil in asthma remains relatively obscure and neutrophils have been rather neglected in studies concerning allergic diseases, although an increase in neutrophil numbers and activity was found to be correlated to airway hyperresponsiveness in asthmatic patients (Bloemen et al., 2007). Nevertheless, recent evidence suggests that neutrophils not only contribute to acute asthma exacerbations, but also are present in high numbers in the airways of patients with chronic severe asthma. Production by neutrophils of lipid mediators, reactive oxygen intermediates (ROI) and proteases such as elastase, may contribute to airway obstruction, epithelial damage and remodeling (Bloemen et al., 2007 as cited in Sampson, 2000). Laboratory studies confirm epidemiologic evidence that air pollution adversely affects lung function in asthmatics. Damage to airway mucous membranes and impaired mucociliary clearance caused by air pollution may facilitate access of inhaled allergens to the cells of the immune system, thus promoting sensitization of the airway. Consequently, a more severe immunoglobulin (Ig) E–mediated response to aeroallergens and airway inflammation could account for increasing prevalence of allergic respiratory diseases in polluted urban areas.

(D'Amato et al., 2010). Miyabara et al. (1998) reported that the mechanisms underlying allergic asthma involve numerous factors, including antibody production, chemical mediators, cytokine expression, and activation of different cells. The airway inflammation of asthma is unique in that the airway wall is infiltrated by Th2 cells, eosinophils, and mast cells. Each of these cells is thought to contribute to the physiologic changes that characterize asthma. In particular, allergen-specific IgE is believed to play a central role in the hypersensitivity reactions via mast cells. Such IgE-mediated reactions are followed by chronic inflammation leading to increased airway hyperresponsiveness. (Miyabara et al., 1998).

1.2 Animal models for asthma and air pollutioon

However, the ability to completely assess the different disease phenotypes and inherent ethical issues are limiting factors in conducting many of the required clinical studies. As a result, animal models have been developed to study the pathogenesis of the disease, including genetic factors, to define the pathogenetic pathways and suggest new therapeutic approaches (Bice et al., 2000; Shin et al., 2009). Much of the researches on the adverse effects of air pollutants, both in vivo and in vitro, have been conducted in animals. Such experimental studies are reviewed critically and the findings are compared with those in human studies. Although a majority of studies of allergic airway disease are now carried out in the mouse, the guinea pig initially was utilized as an animal model of pulmonary hypersensitivity and AHR for many decades (Shin et al., 2009 as cited in Noelpp &Noelpp-Eschenhagen, 1952). Guinea pigs were used as a histamine-induced rhinitis model, since histamine induces nasal congestion, sneezing, and rhinorrhea well in guinea pigs among small experimental animals (Kobayashi, 2000). The guinea pigs demonstrated airway constrictive responses and production of hypersensitivity antibodies including IgG1 and IgE (Ricciardolo et al., 2008). The associated pulmonary inflammatory response is consistent with asthma, composed of both eosinophils and neutrophils. The guinea pig model identified the importance of airway inflammation in the development of altered airway function (Ricciardolo et al., 2008). However, there are several disadvantages in using guinea pigs as a model of asthma. The shortage of inbred strains prevents significant investigation of the genetic effects on susceptibility to sensitization and development of allergic airway disease. In addition, major problem to the guinea pig is the predominance of IgG1 rather than IgE as the major anaphylactic antibody (Ricciardolo et al., 2008).

In Iran, the main source of outdoor air pollution due to particles in suspension is represented by heavy traffic of motor vehicles such as gasoline powered cars, diesel-powered vehicles, motorcycles, mining, industrial sources such as manufacturing, community sources such as the heating of homes and buildings, municipal waste and combustion. This survey highlights cellular material release, pollen allergenicity and study of pollen allergens in three ornamental plants of *Spartium junceum* (spanish broom), *Tagetis patula* (French Marigold) and *Lagerstroemia indica* (crepe myrtles) that have distributed widely in parks and landscapes. Also, the importance of the exposure of pollen grains to air pollutants in the induction of allergic diseases is studied.

2. Experimental methods

Pollen grains were collected randomly from plants grown in a clean (nonpolluted) area (National Botanical Garden, Paykanshahr, Tehran, 30 km far from Tehran). After sifting and

drying of at room temperature, one half of pollen grains exposed to air pollutant in one area with heavy traffic (Shahid Mofateh Avenue, Tehran) during 10 days. In order to condition homogeneity, the control samples were covered with filter and placed in the vicinity of polluted ones at the same time. The type and mean of air pollutant concentrations in polluted area were reported as 0.068 ppm (SO2), 0.06 ppm (NO2), 9.7 ppm (CO), 3.1 ppm (HC) and 174 μgm^{-3} (APM). Pollen structure was studied by Scanning and Light Electron Microscopy. Pollen specimens were prepared for scanning electron microscopy observation by dusting them directly onto sticky-tape coated aluminum stubs and gold coating. Photographs were taken with a JEOL JSM 633 OF SEM. For light microscopy, Pollen grains were generally dusted onto a drop of water on a microscope slide. Specimens were viewed with an Olympus model Ah2 light microscopy connected to camera. The pollen extracts were prepared by incubating pollen grains in 0.1 M phosphate buffered saline, PBS, pH 7.4 in 15% ratio with stirring at 4–8°C for 4 h. Suspensions were centrifuged at 10 000 g for 20 minutes at 4°C and supernatants were removed. Total protein was measured by Bradford method (Bradford, 1967). Guinea pigs sensitized by injection of pollen extract on three days intervals through the intraperitoneal route with 75 µg protein, both in polluted extracts and non polluted ones. Male Hartley guinea pigs (weighing about 250- 300 g and 4 wk of age) were purchased from Pasteur institute (Karaj, Tehran). The animals were used for experiments at 5 weeks of age when they weighed about 300 to 350 g. The last injection was done subcutaneous to study skin prick test (SPT). Skin tests were read and results were recorded at 30 minutes. The definition of a positive skin test required a wheal diameter 3 mm or greater (≥3) than the saline control (Prakashkumar et al., 1998). At day 35, guinea pigs were sacrificed and the blood was collected by cardiac puncture. The sera of all animals were tested for IgE reaction using enzyme linked immunosorbent assay (ELISA). Proteins from samples and molecular markers were separated using SDS- PAGE (Laemmli, 1970). The unstained gels were electrotransferred to PVDF membrane for Western analysis. The membranes were incubated with the patients' sera. The IgE-binding proteins were revealed with an enzyme system using an anti-IgE peroxidase conjugate (Towbin et al., 1979). The results were analyzed using ANOVA in SPSS and Duncan's multiple range test to compare the significance ($p < 0.05$) of the treatments. Data are summarized and plotted as mean ± standard error of the mean. Five animals were used for each treatment.

3. Effect of air pollutants on pollen grains

3.1 Effect of air pollutants on structure and cellular material release in pollen grains

Pollen grains are triapertures (tricolpate) and echinate with small pores in base in *T. patula*, tricolpate with perforate sculpture in *S. junceum* and tricolporate or pseudocolpate with ornamentation of scabrate, striped-regulate, regulate –verucate and puctate depending on anther type (this species has large small anthers) and sculptures position (Figs. 1-3). Pollen sculpture and pollen coating especially in entomophilous pollen grains are resulted in adhering air pollutants to pollen surface when they are exposed to air pollutants. Studies of scanning electron microscopy showed that in pollen grains exposed to air pollutants, airborne particles accumulated on the surface of pollen and changed pollen wall structure (Figs. 1-3).

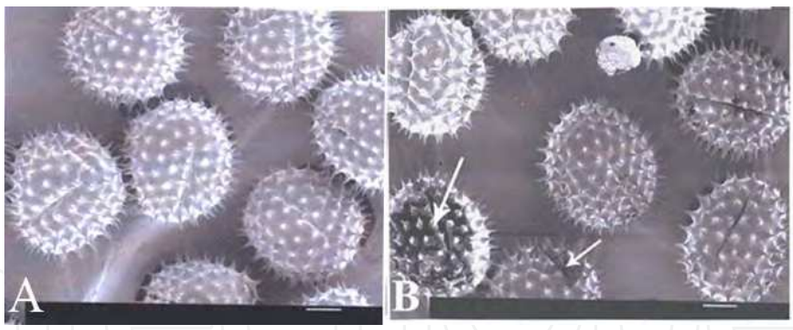


Fig. 1. A, B. Tricolpate pollen grains with echinate ornamentation in *T. patula*. A. Nonpolluted pollen, Agglomeration (arrows) of air pollutants (0.1 µm) is seen on pollen surface (B). Scale bars= respectively 50 & 10 µm.

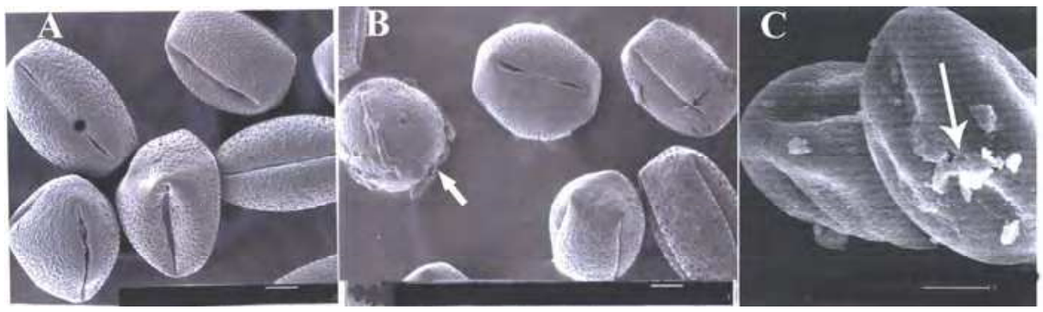


Fig. 2. A-C. Tricolpate pollen grains with perforate ornamentation in *S. junceum*. A. Nonpolluted pollen, arrows show agglomeration of air pollutants on pollen surface (B, C). Scale bars = 10 µm.

Precocious pollen germination is observed in *L. indica* as well (Fig. 3). Agglomeration of airborne particulate matter (0.1-10 µm) is resulted in thinness of pollen wall and induction of cellular material release when they are located in moist medium (Figs. 4-6). These release materials were agglomerated on the surface of pollen as well as on airborne particulate matter including DEP. Furthermore, other investigations of dust samples from highly polluted areas in Germany showed a significant degree of particle agglomeration on the surface of pollen grains (Morgenstern et al., 2008). Therefore, in vitro exposure of pollen grains to particles indicates morphological changes and increases pollen wall permeability and release of cellular matter including allergens from the pollen. In vitro exposure of pollen to gaseous pollutants (SO_2 and NO_2) under different conditions of humidity resulted in SO_2-induced, but not NO_2-induced reduction of allergen release from pollen (Behrendt et al. 1997). It is concluded that the bioavailability of pollen allergens may be modulated by air pollutants, supporting the concept of an interaction between pollen and pollutants in the atmosphere outside the organism which in turn may affect allergy-relevant phenomena. Air pollutants by attaching to the surface of pollen grains and of plant-derived paucimicronic particles, pollutants can modify the morphology of these antigen-carrying agents and alter their allergenic potential. In addition, by inducing airway inflammation, pollutants may overcome the mucosal barrier and so 'prime' allergen-induced responses. In other words airway mucosal damage and impaired mucociliary clearance induced by air pollution may facilitate the access of inhaled allergens to the cells of the immune system (D'Amato, 2002).

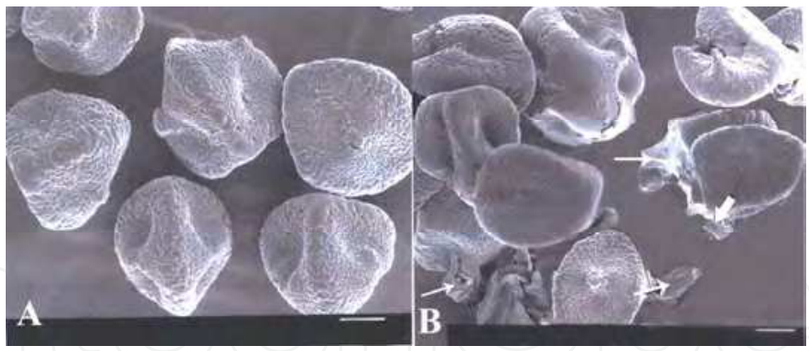


Fig. 3. A, B. tricolporate or pseudocolpate pollen grains with ornamentation of scabrate and regulate depending on anther type and pollen location in *L. indica*. Arrowhead shows agglomeration of air pollutants on pollen surface, arrow show precocious germination (B, due to air pollutants, pollen ornamentation is obscure), A. Nonpolluted pollen.

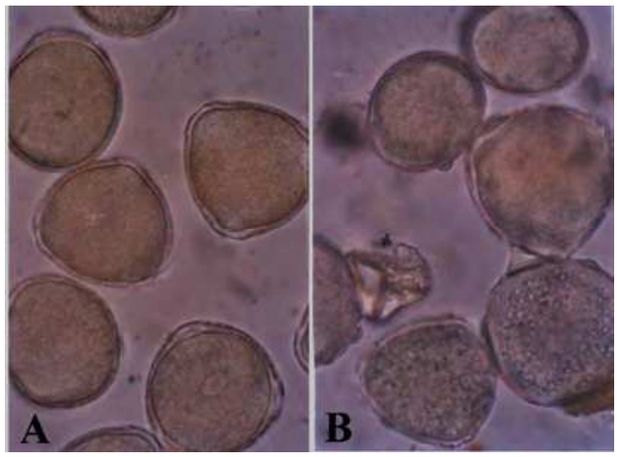


Fig. 4. A, B. Nonpolluted (A) and polluted pollen grains (B) in *S. junceum*. Thinness of exine and increase of cellular matter release in pollen exposed to air pollutants are observed.

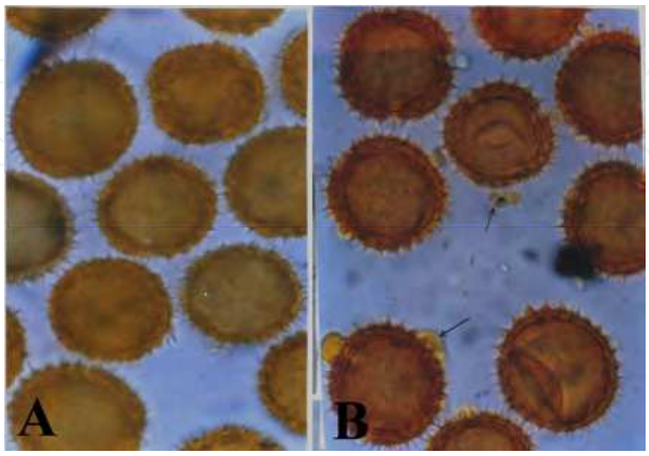


Fig. 5. A, B. Nonpolluted (A) and polluted pollen grains (B) in *T. Patula*. Increase of cellular matter release in pollen grains exposed to air pollutants is observed.

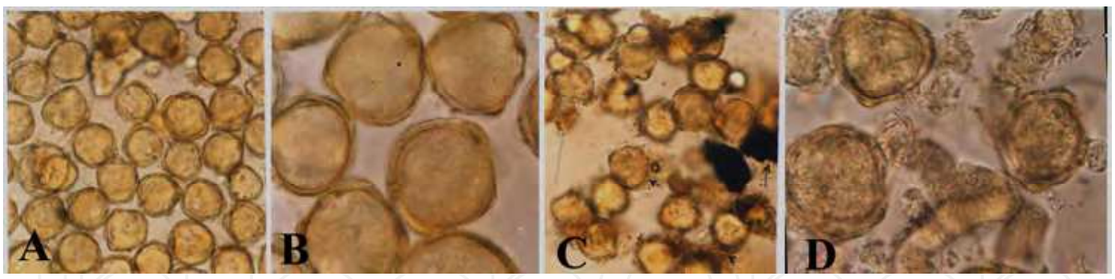


Fig. 6. A-D. Nonpolluted (A, B) and polluted pollen grains (C, D) in *L. indica*. Cellular matter release in pollen exposed to air pollutants is seen. Magnification: X100 (A, C) & X400 (B, D).

3.2 The effect of air pollution on clinical and serological tests

The results of skin tests in guinea pigs showed which pollen extracts result in a positive skin test response compared to control (buffer) ones; also the response of polluted extracts was more than nonpolluted ones (Fig. 7). Injection of polluted extracts especially in S. junceum caused the formation of wound in the place of injection so that its scar exists up to 72 hours.

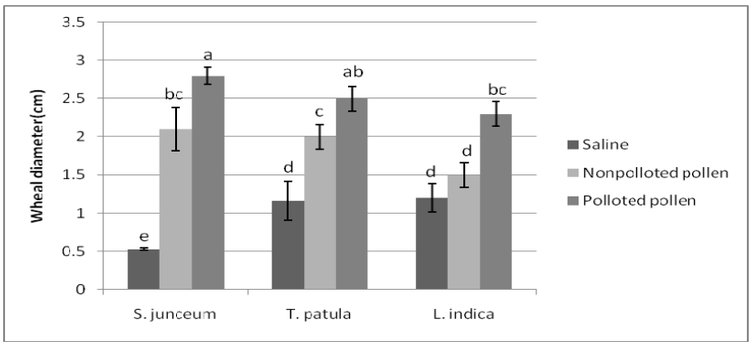


Fig. 7. Prick skin test reactivity in guinea pigs immunized with pollen extracts.

The level of IgE was higher in animals immunized and challenged with pollen extracts than control (buffer) ones. The higher level of IgE was observed in serum of animals injected with polluted pollen extract compared with nonpolluted (less polluted) ones. The induction of antibody production after DEP administration has been reported previously. For example, intranasal instillation of DEP and antigen resulted in increased levels of antigen-specific IgE in murine and human sera. Furthermore, DEP and the polyaromatic hydrocarbon fraction derived from DEP enhanced IgE production from purified human B cells in vitro (Miyabara et al., 1998, as cited in Diaz-Sanchez et al., 1994; Takafuji et al., 1987; Takenaka et al., 1995; Tsien et al., 1997). Diaz-Sanchez (1997) studied the effect of DEP on allergenecity in ragweed. He reported that DEPs can interact with aeroallergens to enhance antigen-induced responses, with the result that allergen-specific IgE levels are up to 50-fold greater in allergic patients stimulated with DEPs and allergens than in patients treated with allergen alone. Combined challenge with DEPs and ragweed allergen markedly increases the expression of human nasal ragweed-specific IgE in vivo and skews cytokine production to a type 2 helper T-cell pattern (D'Amato et al., 2010 as cited in Diaz Sanchez et al., 1997). Similarly, Kobayashi (2000) reported that the administration of DEP or exposure to DEP enhances IgE antibody production in mice and in humans (Kobayashi, 2000, as cited in Fujimaki et al., 1997 and Diaz-Sanchez et al., 1994).

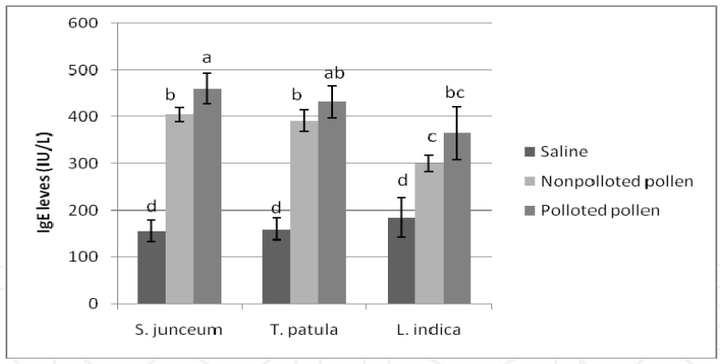


Fig. 8. ELISA reactivity of sera from guinea pigs immunized to pollen extracts.

The increase in blood eosinophils was observed in Guinae pigs sensitized with pollen extracts compared with buffer alone although in *L. indica* was not significant. This increment was higher in the extracts of pollen grains exposed to air pollutants than nonpolluted pollen extracts (Fig. 9). Any significant increase in neutrophils value was seen in animals treated with nonpolluted pollen extracts compared with buffer while their percent increase in polluted pollen extract (Fig. 10). Thus, the combination of pollen plus air pollutant is resulted in a significant increase eosinophils and neutrophils. Inflammatory reactions generally involve a vast array of mediators and a variety of effector cells such as mast cells, macrophages, eosinophils, platelets, and neutropb.ils. It is well recognized that eosinophils are important cells in asthma. Eosinophils release basic proteins that are cytotoxic and lipid mediators such as cysteinyl leukotrienes that cause airway obstruction and bronchial epithelial damage (Bloemen et al., 2007, as cited in Sampson, 2000). Kämpe et al. (2007) reported that there is an eosinophil inflammation in both the nasal and bronchial mucosa and systemically in both allergic asthma and allergic rhinitis during the pollen season. Blood eosinophilia is a common feature in patients with bronchial asthma. The role of neutrophils in mild asthma and allergic rhinitis is uncertain, whereas in severe asthma neutrophils and the neutrophil chemoattractant CXCL8 play an important role in the pathogenesis of airway inflammation. Boulay et al. (2002) also demonstrated an increase in eosinophils in induced sputum after repeated very-low dose allergen challenge in allergic rhinitis (Bloemen et al., 2007, Kämpe et al., 2007). The studies have shown that air pollutants especially Diesel exhaust particles to be able to increased total and specific IgE production, the production of cytokines inherent to Th2 cell response, eosinophilic inflammatory response, the number of peripheral blood neutrophils and platelets and histamine levels (Bartra et al., 2007). Kinhult (2003) reported migration of neutrophils from the blood into the nasal mucosa increases during the grass pollen season (Månsson et al., 2010, as cited in Kinhult, 2003). It has been often observed by many investigators that number of eosinophils and neutrophils increases in brochial alveolar lavage (BAL) fluid after challenge with allergen. Thus, it may be that lower amount of blood neutrophils in animals sensitized with nonpolluted pollen extracts is correlated to their higher value in BAL fluid, nasal alveolar lavage (NAL) fluid and Sputum. Månsson et al. (2010) reported that migration of neutrophils from the blood into the nasal mucosa increases during the grass pollen season (Månsson et al., 2010, as cited in Kinhult, 2003). This migration may be the cause of blood neutrophils decrease. The response of guinea pigs to pollen extracts of *L. indica* was low in all of reactions related to allergenecity but this response was less in nonpolluted pollen extracts. It seems this speices in not allergenetic or its allergenecity potential is low.

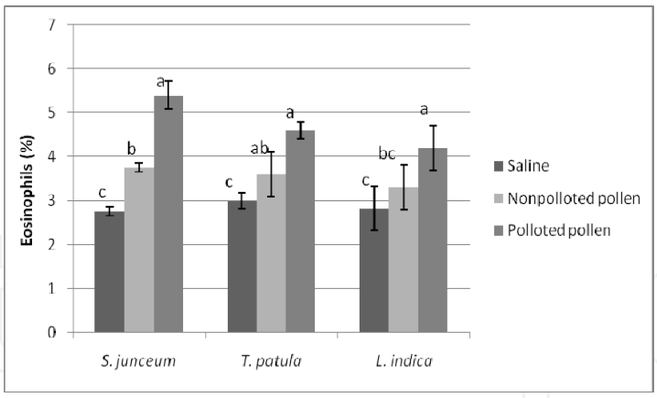


Fig. 9. Blood eosinophils in Guinae pigs immunized with nonpolluted and polluted pollen extracts.

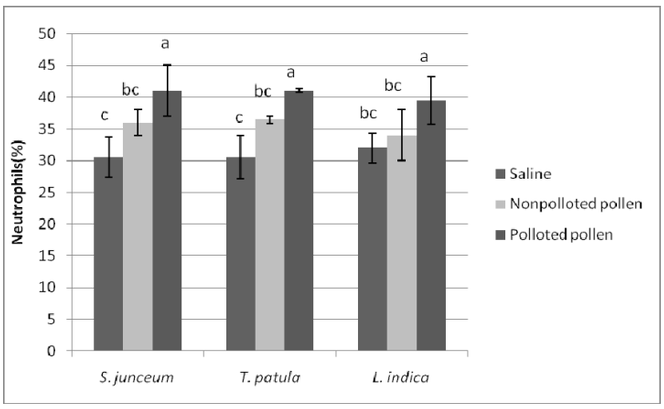


Fig. 10. Blood neutrophils in Guinae pigs immunized with nonpolluted and polluted pollen extracts.

3.3 Effect of air pollutants on total pollen protein and IgE-specific Imunoblotes

Exposure to air pollution showed no obvious difference between soluble proteins of polluted and control extracts in *S. junceum* and *T. patula*. A significant decrease of protein content was observed in pollen extracts under air pollution compared with nonpolluted ones in *T. patula* (Fig. 11).

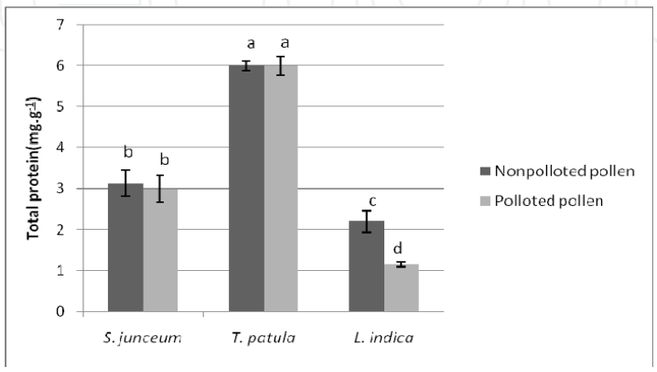


Fig. 11. Total protein content of nonpolluted and polluted pollen extracts.

Immunoblot of IgE binding to *S. junceum* pollen proteins showed that in both polluted and non polluted extracts were revealed two bands in the ranges 46-55 kDa and 35 kDa (Fig. 12). No obvious difference was observed between polluted and non polluted extracts. The Immunoblot profile of *L. indica* did not reveal any clear IgE-binding protein band, although in a few of these blots a weak band in was observed. An IgE-binding band (52-60 kDa) was observed in the immlunoblots of *T. patula* (Fig. 13). Thus, our results did not show any difference in IgE-binding bands (proteins) between polluted and non polluted pollen extracts. Also, pollen extracts of *L. indica* showed no obvious allergen. It is evident that allergenecity potential of plants is a species character and depends on genotype. Studies of effect of air pollutants on pollen proteins show contradictory results. Our results are consistent with the results of Helender et al. (1997) who observed no significant difference between the protein bands of polluted and control areas. However, studies of Behrendt et al. (1997) showed a dose-dependent shift in the intensity of IgE binding reactivity to lower molecular weight bands. In addition, Parui et al (1998) found a decrease in Bet v 1 concentration under air pollution. It is possible that the type of plant species could easily be the cause for these differences, and therefore, this matter may need to be studied further.

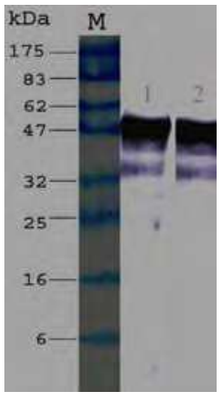


Fig. 12. Immunoblot profile of the pollen extracts in *S. junceum*. M: Marker; 1: Nonpolluted pollen extract; 2: Polluted pollen extract.

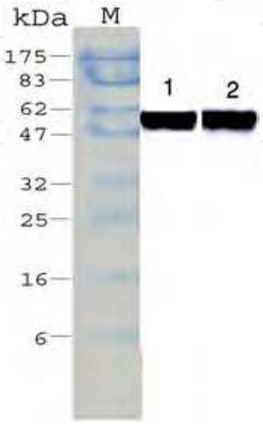


Fig. 13. Immunoblot profile of the pollen extracts in *T. patula*. M: Marker; 1: Nonpolluted pollen extract; 2: Polluted pollen extract.

4. Conclusion

There is growing evidence that air pollutants especially AMPs act as adjuvant in the immune system and lead to enhancement of allergic inflammation in predisposed animals and humans. Air pollutants may affect human B-cells and enhance IgE production by several mechanisms. Another possible mechanism of action of air pollutants on allergic responses is to act as a carrier of particles released from pollen (allergens) or derived from part of the plant such as leaf, stem and flower (for example, anther ubish bodies) allowing enhanced deposition of allergens especially pollen allergens in the lower airway. Also, allergens bound to AMP may trigger asthma attacks and AMP-binding may facilitate penetration of allergen through the airway mucosa. By attaching combination of AMP and allergen to the surface of mucosa and epithelial damage enhances epithelial permeability, stimulation of sensory nerve endings, and the release of chemical mediators. Therefore, AMP may affect both pollen wall and mucosa by increasing their permeability. Increasing pollen permeability induces cellular matter release such as allergens that in turn adheres to AMP. Induction of mucosa permeability may facilitate the access of inhaled allergens and AMP to the cells of the immune system. Furthermore, as cited by D'Amato (2002), air pollutants can modify the morphology of these antigen-carrying agents and alter their allergenic potential.

Taken together with the findings from the other researchers, living on cities with heavy traffic and industrial activities is associated with a higher risk for sensitization to pollen and other allergens. For this reason, it is suggested that government authorities could consider more carefully which plant species are used in populated areas. Species selection is also important to avoid aeroallergen from climate change mitigation tree planting and urban reforestation. Also, using of the fruits and vegetables containing polyphenols reduce damages of allergic disorders. Kishi et al. (2005) reported that a red perilla extract (luteolin being the main ingredient) and rubus suavissinmus extract (ellagitannin being the main ingredient) alleviated the symptoms of cedar pollinosis through antiallergic action (Kishi et al., 2005). Apple major polyphenol (Ap) is condensed tannin (ACT) and it is also contains such monomeric polyphenols as catechin, epicatechin, phlorizin, and chlorogenic acid. Studies on the antiallergic action of Ap have found that it suppressed the histamine release from mast cells and basophils (BRL-2H3) in rats, inhibited hyaluronidase and suppressed auricular swelling in mice. It has been reported from a clinical study that Ap alleviated the itching in atopic dermatitis patients. This phenomenon was attributed to the suppression of histamine release by ACT, the major component of apple (Kishi et al., 2005). Through analysis of the clinical evaluation of one kind of traditional remedy in patients with atopic dermatitis, flavonoids were shown to possess significant anti-allergic activity. Among the twenty kinds of flavonoids examined, fisetin, luteolin, apigenin, quercetin and kaempferol inhibited not only the release of chemical mediators but also the production of T-helper (Th)-2 type cytokines (Interleukin (IL)-4, IL-5 and IL-13) by basophils. These flavonoids inhibit expression of these cytokines through their inhibitory effect on the activation of several calcium-calmodulin dependent kinases (tanaka et al., 2003). Furthermore, air pollution control is an important character in the health of all of living organisms.

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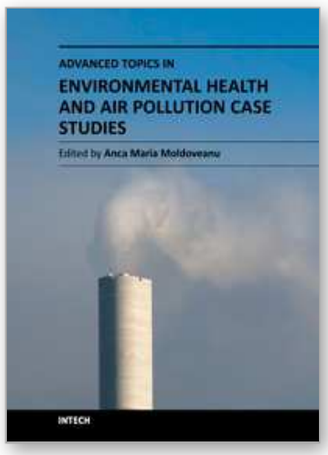
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The book describes the effects of air pollutants, from the indoor and outdoor spaces, on the human physiology. Air pollutants can influence inflammation biomarkers, can influence the pathogenesis of chronic cough, can influence reactive oxygen species (ROS) and can induce autonomic nervous system interactions that modulate cardiac oxidative stress and cardiac electrophysiological changes, can participate in the onset and exacerbation of upper respiratory and cardio-vascular diseases, can lead to the exacerbation of asthma and allergic diseases. The book also presents how the urban environment can influence and modify the impact of various pollutants on human health.

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