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EFFECT OF AIR POLLUTION ON SOLUBLE PROTEINS, STRUCTURE AND CELLULAR MATERIAL RELEASE IN POLLEN OF *LAGERSTROEMIA INDICA* L. (LYTRACEAE)

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Pollen of *Lagerstroemia indica* was collected from polluted (SO_2, NOx, CO, HC, airborne particulate matter) and less polluted areas of Tehran city, Iran. Some pollen from less polluted areas was exposed to polluted air for 10 and 20 days. To determine the effect of air pollution on proteins, pollen extracts were analyzed by the Bradford method and SDS-PAGE. Study of pollen structure by light and scanning electron microscopy showed that air pollution increased the number of shrunken and fragile pollen. Particle agglomeration and cellular material release were increased in polluted pollen. Total protein content and the staining intensity of protein bands by SDS-PAGE were decreased in polluted pollen.

Key words: *Lagerstroemia indica*, air pollution, pollen structure, protein release.

INTRODUCTION

Over the centuries, concurrent with industrialization and population growth, air pollution has increased from a local nuisance to a global problem. At the same time the incidence of allergies has been observed to increase in industrialized countries (Majd and Kiabi, 1994; Majd and Ghanati, 1995; Knox and Suphioglu, 1996; Behrendt et al., 1997; Helender et al., 1997; Emberline, 1998; Heo et al., 2001).

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 the exine and intine. These molecules are strategically sited to participate as the male partner in intercellular recognition reactions with the female stigma, but they also interact with the human immune system (Knox and Suphioglu, 1996). Air pollutants may effect pollen indirectly via stress on the growth of the plant, or directly by contaminating the anther on the plant or during the flight of pollen when it is dispersed. The combined effects on pollen can lead to the production of fewer, smaller pollen grains, and an increased number of deformed grains as compared with plants of the same species growing in less polluted areas. On its own this would result in a decreased allergen load in polluted areas (Emberline, 1998). However, studies on pollen allergens collected from polluted and unpolluted areas have shown contradictory results. Jilek et al. (1993) observed an increase of the major birch pollen allergen, Bet v 1, in areas where nitrogen loads are high, while Hjelmroos et al. (1994) and Parui et al. (1998) found a decrease in Bet v 1 concentration due to air pollution. Studies by Helender et al. (1997) did not show any significant difference.

Lagerstroemia indica is a species increasingly used for planting in parks and for landscaping. Our purpose was to characterize and compare its pollen structure, agglomeration and cytoplasmic material release in control and polluted areas.

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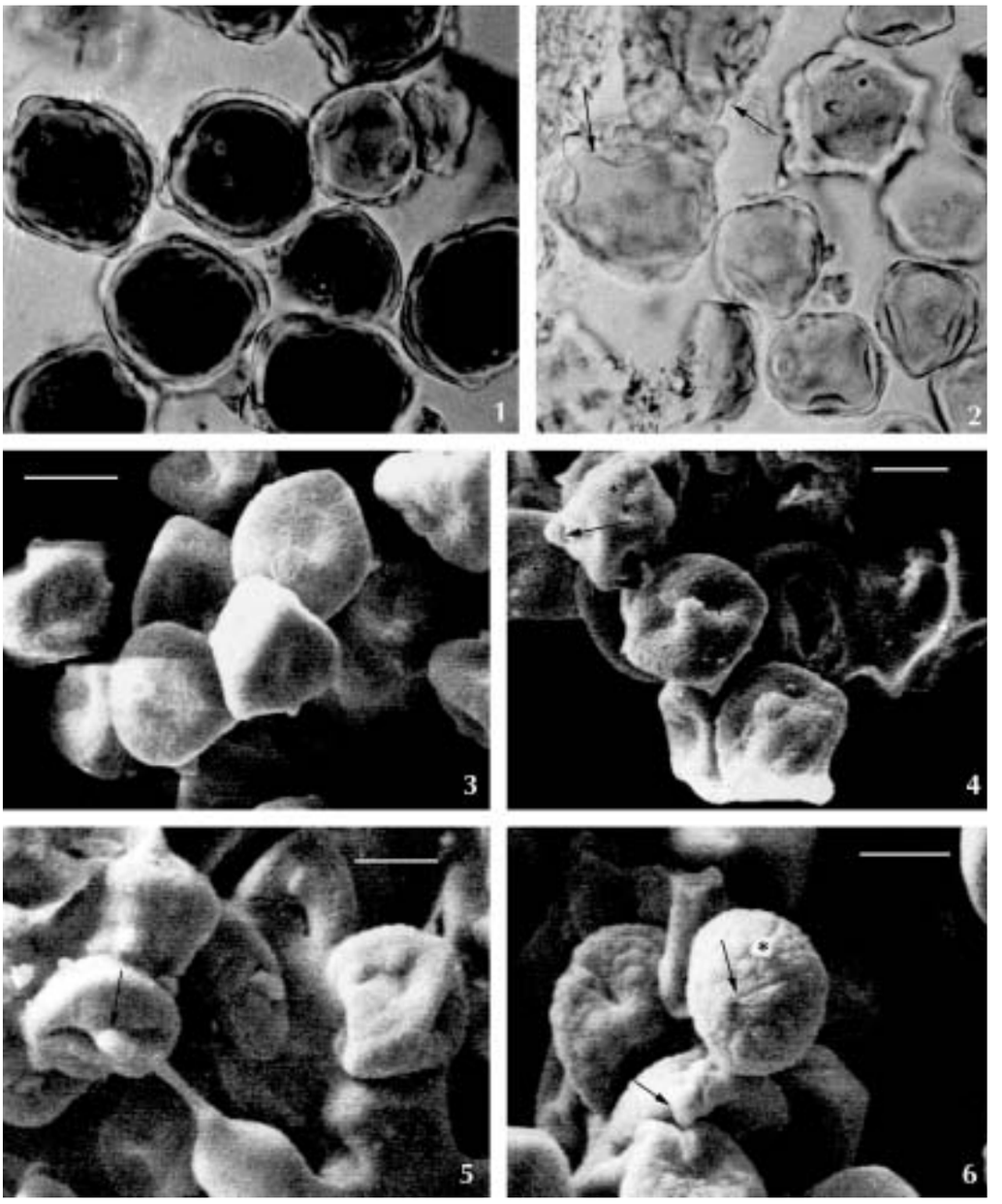
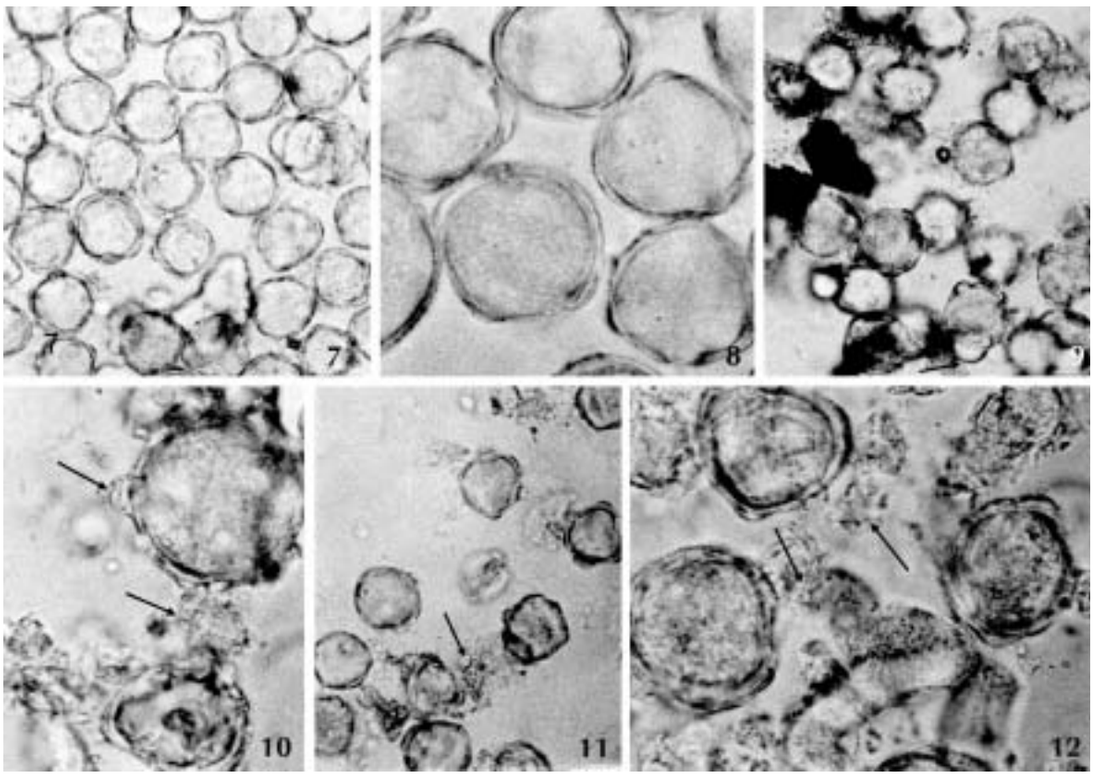


Figs. 1–2. Pollen obtained from control (**1**) and polluted (**2**) areas. × 1000. **Figs. 3–6.** Pollen grains of plants grown under control (**3**) and polluted (**4**) conditions, and pollen exposed 10 and 20 days to polluted air (**5** and **6,** respectively). SEM, Bars = 20 µm. Arrows show cellular material release; asterisks show APM agglomeration.

MATERIALS AND METHODS

Mature anthers of opening flowers of *Lagerstroemia indica* were collected between 9:00 and 11:00 a.m. from control areas (National Botanic Garden, Tehran, Iran) and areas polluted with heavy traffic (Tehran city center). After fixation in FAA (formalin : acetic acid : ethanol, 2:1:17), anthers were dehydrated in a graded alcohol series and embedded in paraffin. For light microscopy, 10–12 µm sections were stained with hematoxylin-eosine. Mature pollen of both control and polluted areas were desiccated at room temperature and sifted. Some of the control pollen were exposed to the air pollutants of



Figs. 7–12. Pollen from plants grown in control (**7,8**) and polluted (**9,10**) areas, and pollen exposed to polluted air for 20 days (**11,12**), in PBS. Note pollen structure and cellular material release (arrows). Figs. 7, 9, 11 × 400; Figs. 8, 10, 12 × 1000.

heavy-traffic areas of Tehran city for 10 and 20 days. Pollen structure and deposition of polluted particles on their surface were studied by light and scanning electron microscopy. Cellular material release was examined in phosphate-buffered saline (PBS) by light microscopy. Pollen extracts were prepared by incubating in PBS in 15% (w/v) with stirring at 4–8˚C for 8 h. The protein content of the different extracts was estimated by Bradford's (1976) method. Proteins from the samples were separated using SDS-PAGE (Laemmli, 1970).

RESULTS

Mature anthers collected from control areas contained polycolpate and normal pollen with a relatively thick exine (Fig. 1). Those collected from polluted area contained shrunken, destroyed, defective and fragile pollen, and degradation (thinning) of the exine surface also was observed (Fig. 2).

SEM observations indicated that pollen from the polluted area and those exposed to air pollution for 10 and 20 days had more airborne particulate matter (APM) on their surface than the control samples (Figs. 3–6). Abnormality and shrinkage were high in pollen stressed by pollutants, and exocytosis was observed (Figs. 3–6).

In vitro experiments with polluted and control pollen in PBS showed induction of cellular material release and pollen degeneration in the polluted pollen (Figs. 7–12). The total concentration of soluble proteins per gram of pollen was significantly less in polluted pollen than in the control samples (Tab. 1). In electrophoresis, the proteins of the extracts of mature pollen from polluted areas and from mature pollen exposed to air pollution had lower staining intensity than those of the control samples (Fig. 13).

TABLE 1. Protein content of pollen extracts (mgg^{-1}).

Air condition	Control air	Polluted air	10 days exposed to polluted air	20 days exposed to polluted air
Protein	2	0.58*	0.72*	0.8*

* Significant difference

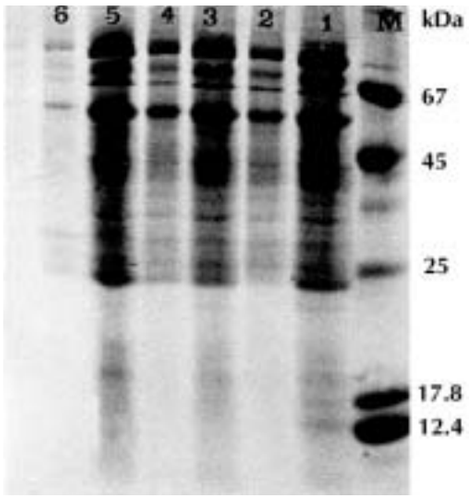


Fig. 13. SDS-PAGE of pollen extracts. Columns: M – protein standard (molecular weight markers); 1 – control pollen; 2 – polluted pollen; 3 – exposed 10 days to clean air (control); 4 – exposed 10 days to polluted air; 5 – exposed 20 days to clean air (control); 6 – exposed 20 days to polluted air.

DISCUSSION

Studies of the effect of air pollution on pollen showed changes such as shrinkage, thinning and fragility. Airborne pollen grains can be affected directly by air pollutants. Air pollution can affect pollen grains indirectly via the soil. If a plant grows in polluted soil, its physiological functions may change and affect the properties of the developing pollen grains (Helender et al., 1997). Also, injury to floral organs, especially the anther, can cause pollen abnormalities and sterility, and consequently malfunction of fertilization.

Increased particle agglomeration and cellular material release under conditions of contact with moisture have been observed in polluted pollen. Studies by Behrendt et al. (1992, 1997) showed that particle agglomeration on the pollen surface caused preactivation of pollen. It can be inferred that this function can induce discharge of pollen wall proteins (allergens) or cellular material when in contact with moisture (e.g., rainfall), on the sticky surface of the stigma, or on the mucous membrane of the nose and the eye, or on moist particles in the air. Two actions of the proteins, that is, participation in fertilization and interaction with the human immune system (Knox and Suphioglu, 1996), are affected by air pollution.

Total protein content and the staining intensity of the protein bands in the SDS-PAGE profile were reduced in pollen exposed to polluted air. These results are similar to those of other researchers (e.g., Behrendt et al., 1992, 1997; Parui et al., 1998; Hjelmroos et al., 1994), but Jilek et al. (1993) found increased Bet v 1, and Helender et al. (1997) showed the allergens not to be affected by air pollution. The differences in findings may be due to species differences, thinning of exine, or a false increase of pollen weight caused by deposition of pollutants on the pollen surface.

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