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Research Article

The Structure and Ultra Structure of Anther Epidermis and Pollen in *Lagerstroemia indica* L. (Lythraceae) in Response to Air Pollution

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Abstract: The structure of the anthers and pollen of *Lagerstroemia indica* L. (crepe myrtles) (Lythraceae) in samples collected from clean and polluted areas was studied by OM, SEM, and TEM. The epidermal cells of the anthers enlarged during anther development. Their cuticle content increased and became thick and folded. The cytoplasm of epidermal cells was peripheral and degenerated in mature anthers. At this time, their major content was phenolic compounds. The epidermal cells in the anthers collected from polluted areas were shrunken, fragile, and burned at the tip, compared to those collected from non-polluted areas. Flavonoid stainability was greater in the anthers collected from polluted areas than in control samples. In addition, the cuticles were thinner, unfolded, and irregular. Pollen grains in anthers collected from polluted areas were irregular, shrunken, and smaller in comparison to the controls. Pollen cytoplasm in polluted samples was less dense and without cellular differentiation.

Key Words: Air pollution, anther epidermis, flavonoids, *Lagerstroemia indica*, pollen

Introduction

Lagerstroemia indica L. (Lythraceae) is a widespread garden plant appreciated for its long lasting summer flowers. The family is the most palynologically diverse of the order Myrtales. The most simple pollen types and the ones common to the largest number of genera are prolate-spheroidal to prolate, tricolporate without pseudocolpi, psilate, scabrate, or finely verrucated. Specialisations include oblate grains, development of pseudocolpi (3 or 6 in number), diversification of exine sculpturing, broadening of the colpal and pseudocolpal areas, and reduction in the conspicuousness of the colpi (Graham et al., 1990). The outermost cell layer of the anther is epidermis, which in most plants is slightly modified during development. This layer is a multifunctional tissue, playing important roles in water relations, defence, and pollinator attraction. In many plants, anther epidermis comprises simple and small cells, but studies on the anther of *Aristolochia* L. (Johri & Bhotnagar, 1955), *Calycanthus* L. (Mature, 1968), and *Chelone glabra* L. (Arekal, 1963) showed that epidermal cells are as epidermis hairs. Despite the global problem of air pollution in urban areas, little attention has been paid to the effect of air pollution on the development and structure of the anther and pollen. Studies by Pfahler (1981), Majd et al. (1996), and Emberlin (1998) showed that air pollutants affect pollen structure, viability, germination, and tube growth. These effects cause pollen sterility and reduced fertilisation. The purpose of this study was to investigate the structure of the anther epidermis, its contents, and pollen structure, and the effects of air pollution on these characters in *Lagerstroemia indica* L.

Materials and Methods

Preparation of Microscopic Slides

Samples were collected from *Lagerstroemia indica* plants grown in a control area (National Botanical Garden, Paykanshahr, 30 km from Tehran) and from plants grown in a polluted area surrounded by heavy traffic (the city centre). According to reports by the Air Quality Centre at the Environment Protection Agency, the mean air pollutant concentrations in the control and polluted study areas are shown in the Table. The flower buds and anthers were fixed with FAA (formalin:acetic acid:alcohol ethyl 96%, 2:1:17) at different stages of development. Then, they were dehydrated in a graded alcohol series

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and embedded in paraffin. Serial sections of 8-12 µm were prepared and examined by light microscopy (LM).

Some samples were fixed in 3% glutaraldehyde and 1% paraformaldehyde in 0.1 M phosphate buffer (pH 7.2) for 5 h at room temperature. After washing with rinsing buffer (0.2 M phosphate buffer, pH 7.2), the samples were postfixed in 1% osmium tetra oxide in 0.1 M phosphate buffer for 2 h at 4 °C. They were dehydrated in a graded alcohol series and embedded in Spurr's resin. For LM, sections 2 µm thick were prepared using glass knives on an Ultracut microtome (MT 5000, Sorvall Ultra Microtome), and then stained with toluidine blue O. Ultrathin sections were prepared with a diamond knife on the same microtome. The sections were observed by TEM (JEOL JEM. 1010) and the images were photographed.

Examination of Pollen Structure

Structure, agglomeration of airborne particulate material (APM), and cellular material release in pollen grains were examined by LM and SEM. Pollen regularity was assessed based on the percentage of well-regulated and normal pollen grains. At least 100 pollen grains were studied. The size of pollen grains from the control and polluted study areas was compared by measuring the diameter of 20 pollen grains on each slide. All experiments were repeated 4 times. The results were analysed using one-way ANOVA in SPSS to test the significance ($P < 0.05$) of the treatments (Wang et al., 2006).

Study of the Epidermal Compounds

Hand sections were prepared and stained with specific dyes for protein, starch, lipid, lignin, and phenolic material. The presence of phenolic compounds was determined using Fast Blue BB salt, which has the ability to react specifically with these compounds (O'Brian & McCully, 1981) and gives a characteristic reddish-brown reaction product.

Results

LM showed that epidermal cells extended and differentiated into the upper and lower epidermis in immature anthers (Figure 1a). During development, the length of epidermal cells increased. Consequently, they were observed as completely enlarged cells during the anther dehiscence stage (Figures 1b-f). At this stage of anther development, the upper and lower epidermis had a fully enlarged shape. The epidermal cells located at the margins (right and left) of the anthers were small and identical to the epidermis in most plants (Figures 1a-f). The outer surfaces of the upper and lower epidermis were covered by an intact cuticle-like film (Figure 1e). This layer was stained by Sudan Black B (Figure 1e). At the early stages of anther development it was smooth (Figure 2a) and with further development became thicker and folded (Figures 2b-c).

The various staining reactions of epidermal cells suggested that flavonoids were the main compounds (Figure 3). These compounds were visualised by reaction with Fast Blue BB salt, resulting in a reddish-brown reaction product (Figures 1e-f). The effects of air pollution on epidermal cells were observed as plasmolysis, shrinkage, fragility, and burning at the tip (Figure 4). An enhanced accumulation of flavonoids was observed in some samples collected from the polluted area (Figure 4a). In some other samples, degeneration and collapse of epidermal cells, as well as their compounds were observed (Figures 4b-c). The cuticle showed fragility and decreased folding (Figures 4b,d-f).

Pollen grains collected from the control area were regular (Figures 5-6), 4-colporate (Figure 5b, asterisks show 4-colporate pollen grains), usually 3-colporate (Figures 5a and 6a), sometimes had pseudopores (Figure 6b), and contained differentiated cellular organelles (Figure 7). Ornamentation was verrucated (Figure 6b). Pollen grains from the polluted area showed structural irregularities, such as shrinkage (Figure 8a) and precocious pollen germination from one or more apertures (Figures 8-10). The pollen walls were irregular as well (Figure 10b). In some pollen from the polluted area, cytoplasm had less content and differentiated cellular organelles were not seen (Figures 10c-d). There were some pollen grains that adhered to each other (Figure 10e). The mean pollen regularity and pollen size are shown in Figures 36 and 37, respectively. Statistical analysis of the regularity and diameter of pollen showed significant differences among samples collected from polluted and control sites (Figures 11 and 12).

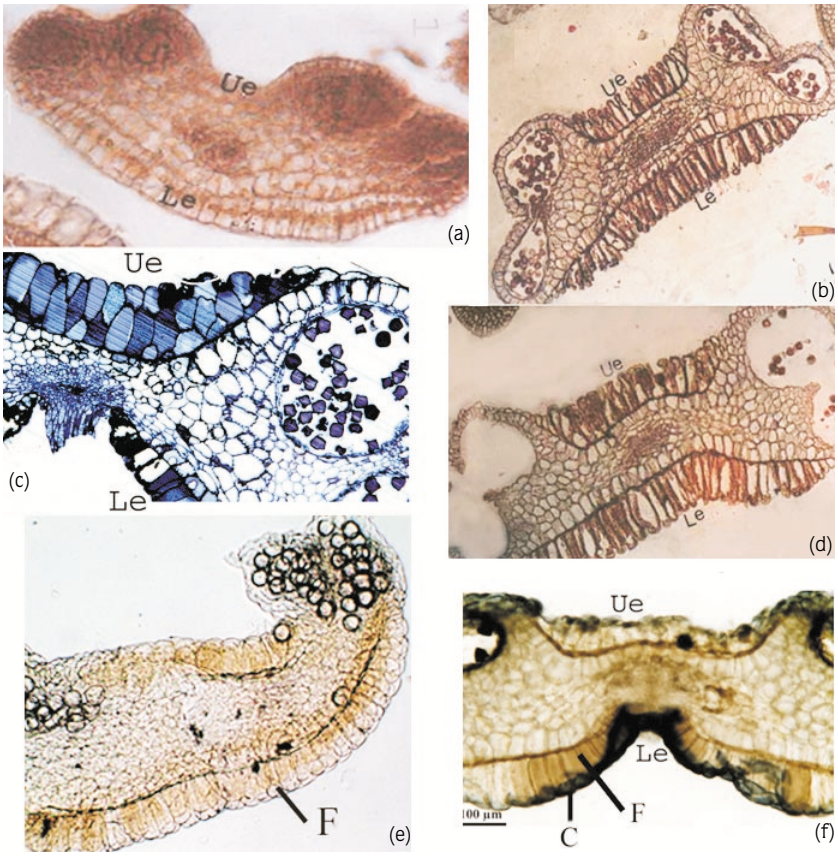


Figure 1. Light micrographs of the cross section of an anther under normal conditions (less polluted): (a). Epidermal cells in young anther. LM 400×, (b-c). Epidermal cells in mature anther before dehiscence. LM 100× and 150×. respectively, (d). Epidermal cells in mature anther after dehiscence. LM 100×, (e). Epidermal cells in young anther stained with fast blue BB, phenolic compounds stained with fast blue BB. LM 400, (f). Epidermis stained with fast blue BB and cuticle stained with Sudan black B. LM 150. Ue: upper epidermis ; Le: lower epidermis; F: flavonoid; C: cuticle.

Discussion

Lagerstroemia indica L. pollen grains have different forms. They are triporate, tetraporate, and pseudoporate. Studies by Graham et al. (1987) and Tutuncu et al. (2007) on the pollen morphology of this species report results similar to ours. Both studies reported that pollen grains are spheroidal and tricolporate, and that ornamentation is verrucated. Pacini & Bellani (1986) and Nepi et al. (2003) reported the presence of 3- and 4-porate pollen grains. They did not indicate the presence of colpi in the pollen of this species. The outermost cellular layer of the anther is epidermis. This layer protects inner tissues against various stresses. The anther epidermal cells in most plants are the same size and relatively small. They do not undergo obvious changes in comparison to other cellular layers during anther development; therefore they are slightly modified during development. Previous studies on the anther of *Aristolochia* L. (Johri & Bhotnagar, 1955), *Calycanthus* L. (Mature, 1968), and *Chelone glabra* L. (Arekal, 1963) showed that anther epidermis in these plants differentiate as epidermal hairs. These researchers did not indicate the compounds of this layer. In *Lagerstroemia indica* L., the elongations of anther epidermal cells was worthy of note. They differentiated at a very early stage of anther development, prior to the differentiation of the wall layers, as upper and lower epidermis. The compounds in epidermal cells are often flavonoids. There was no

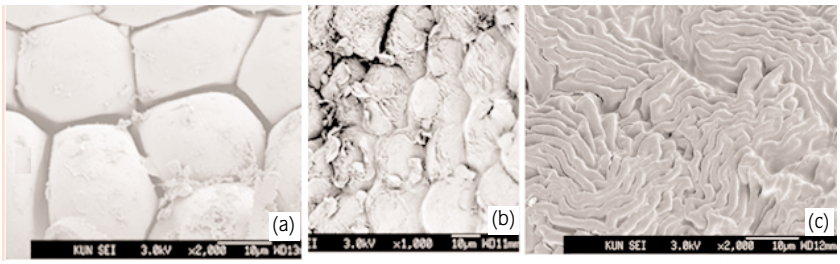


Figure 2. Electron micrographs of a cuticle: (a). Smooth cuticle, (b-c). Folded cuticle. Scale bars show the magnification.

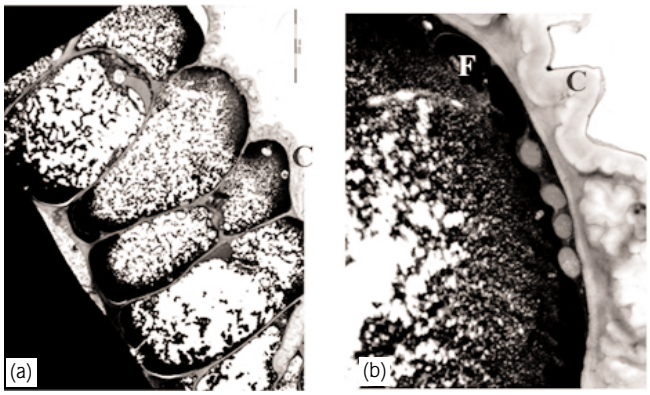


Figure 3. Electron micrographs of the anther epidermis and its cuticle. TEM 2000× and 14,000×, respectively (F: flavonoid; C: cuticle).

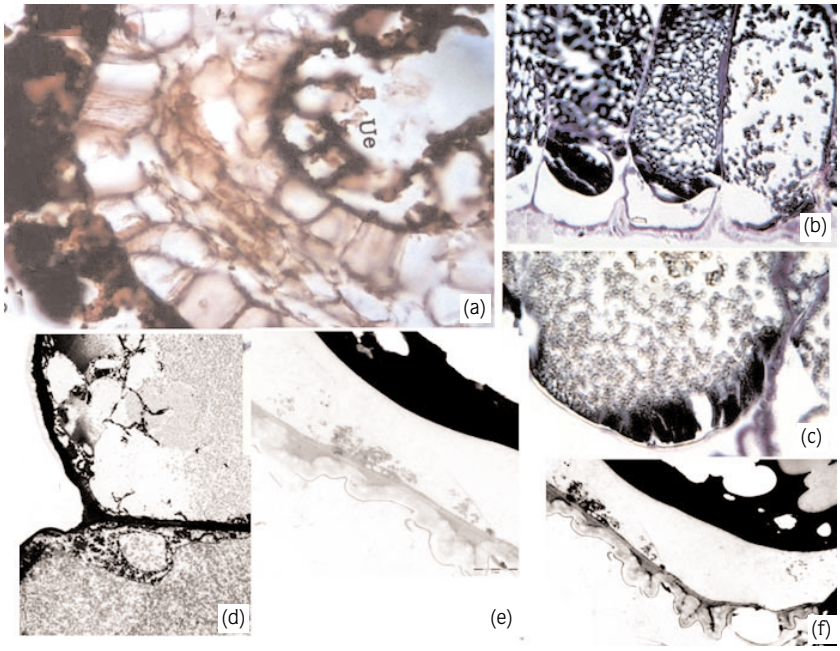


Figure 4. Light micrographs of the cross section of an anther and its epidermis under air polluted conditions: (a). Staining by Haematoxylin. LM 400×, (b-c). Staining by Aniline Blue. LM 1000× and 3000×, respectively, (d). Electron micrograph of the cross section of epidermis under air polluted conditions. TEM 4000×, (e-f). Electron micrographs of the cross section of a cuticle under air polluted conditions. TEM 14,000× and 8000×, respectively.

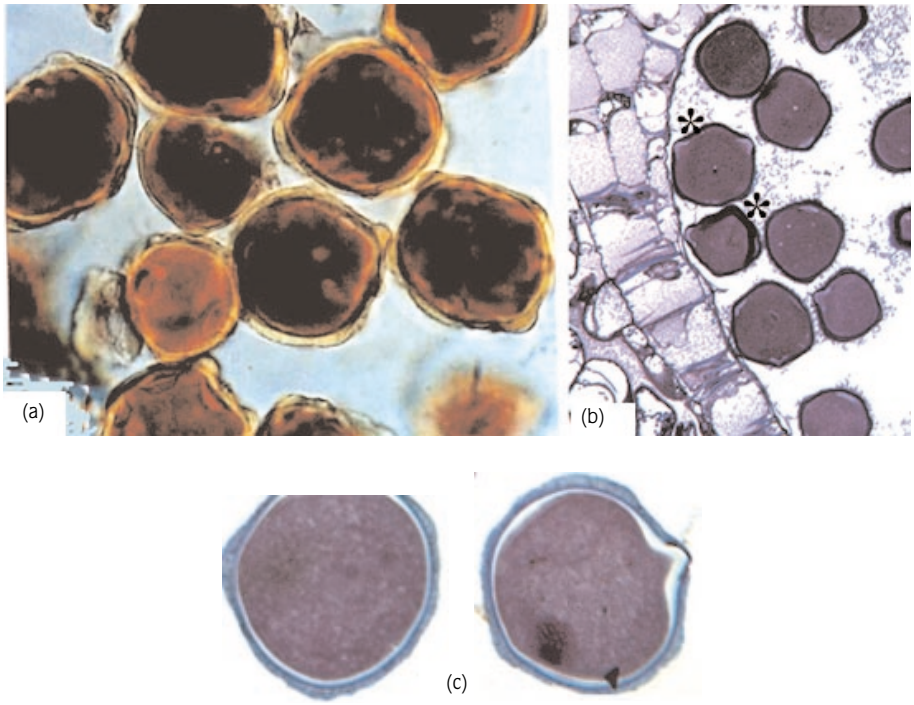


Figure 5. Light micrographs of pollen under normal conditions (asterisks in Figure 5b shows 4-colporate pollen).as LM 1000×, 400×, and 1500×, respectively.

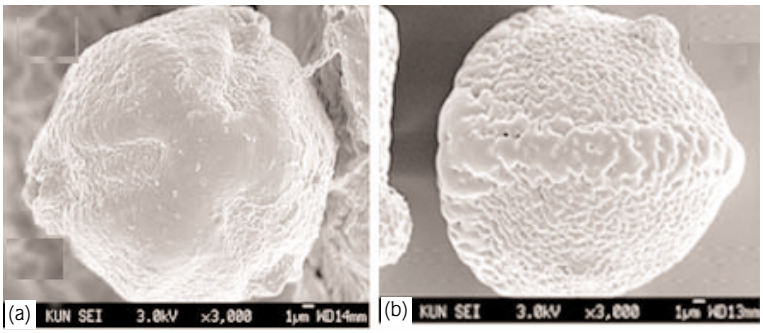


Figure 6. Electron micrographs of pollen under normal conditions (Figure 6a immature pollen before anther dehiscence and Figure 6b mature pollen), SEM 3000×.

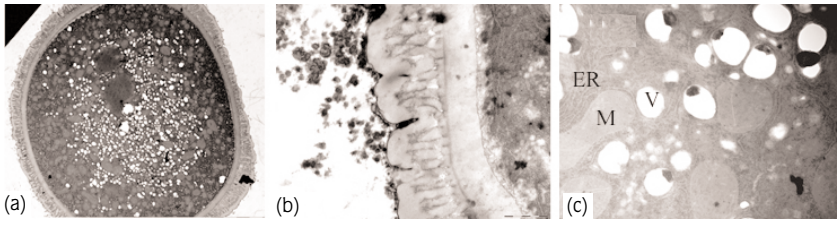


Figure 7. Electron micrographs of pollen under normal conditions. TEM 3000×, TEM 45,000×, and TEM 45,000×, respectively.

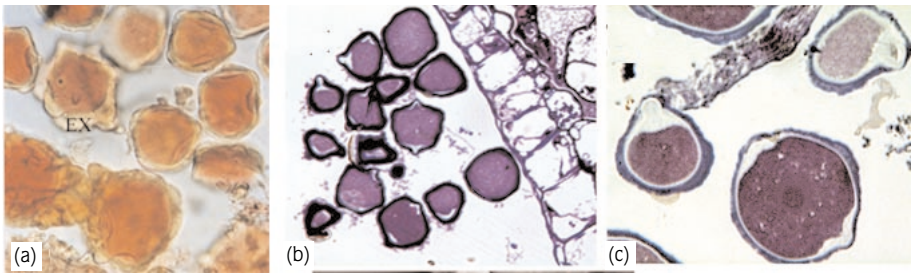


Figure 8. Light micrographs of pollen under polluted conditions. LM 1000×, 400×, and 1500×, respectively.

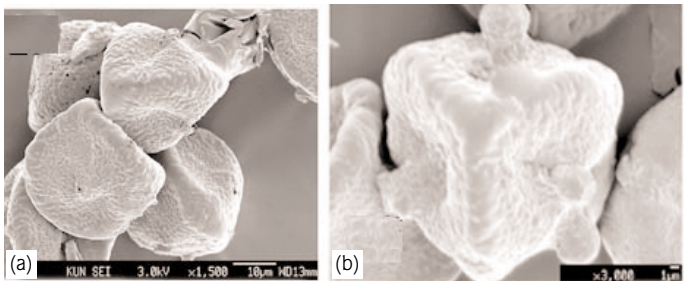
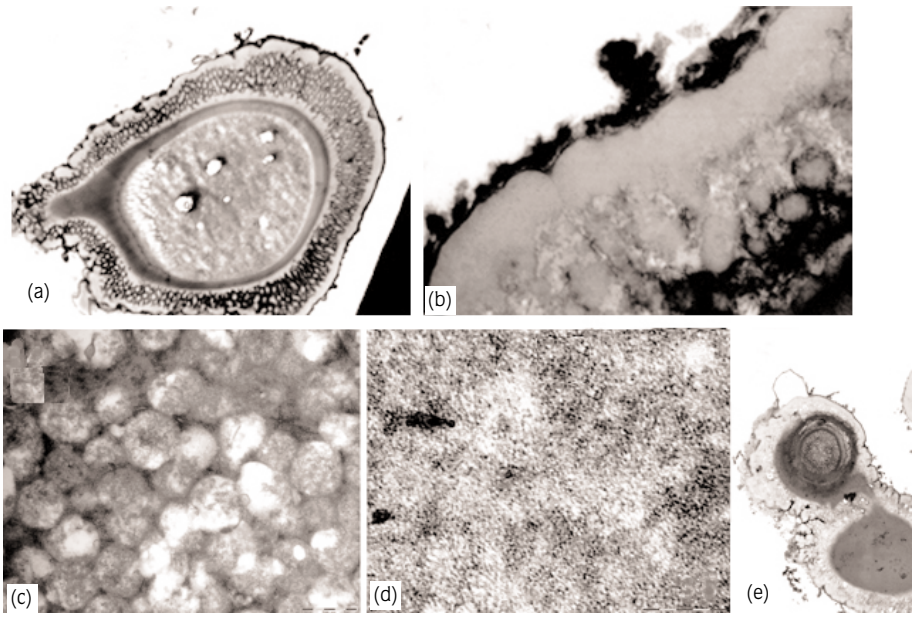


Figure 9. Electron micrographs of pollen under polluted conditions. Scale bars show the magnification. SEM 1500× and 3000×, respectively.



Figures 10. Electron micrographs of pollen under polluted conditions: (a). Pollen structure. TEM × 3000, (b). Pollen wall. TEM × 45,000, (c-d). Homogenous materials and less contents are seen in pollen. TEM 45,000×, (e). Pollen grains without separation. TEM × 1500.

information in the literature on the function of these compounds in epidermal cells. It appears that the increased size and the kind of compounds in these cells result in the seduction and deception of insects, and highly efficient pollination. In addition, they protect internal tissues against various stresses. The folding and thickening of the cuticle support the protective role for these cells. Despite the elongation and accumulation of

Table. Mean air pollutant concentrations in the control and polluted study areas.

	SO_2, ppm	NO_2, ppm	CO, ppm	HC, ppm	APM, µg m^{-3}
Polluted area	0.07	0.1	8.4	2.7	154
Control area	0.003	0.01	0.65	0.12	60

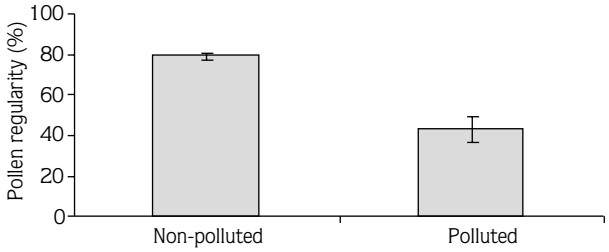


Figure 11. The mean pollen regularity (%) in the control (non-polluted) and polluted areas (mean ± SE).

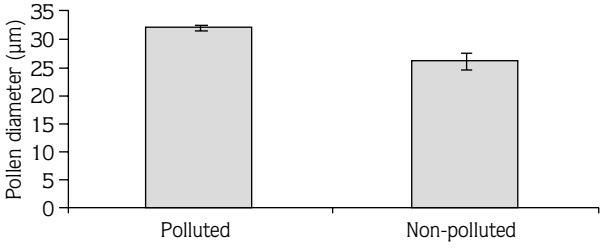


Figure 12. The mean pollen diameter (µm) in the control (non-polluted) and polluted areas (mean ± SE).

phenolic compounds in epidermal cells, due to a high concentration of air pollutants, especially particulate matter that in most days is 3-4 times greater than the standard dose, the epidermal cells and pollen suffer serious damage. The epidermal cells showed plasmolysis, shrinkage, fragility, and burning at the tip. Although we considered the cause as enhanced accumulation of phenolic compounds in some samples collected from the polluted region, quantitative study is necessary. A study on the needles of *Picea abies* (L.) H.Karst. by Soukupova (2001) considers the enhanced accumulation of phenolic materials to be one of the most common reactions of plants to stresses. Studies show contradictory results, i.e. enhanced accumulation and the opposite results under stress have also been reported in the literature. Therefore, it appears that the type of plant species and the type of stress are important. Pollen grains grown in the air polluted environment showed structural abnormality, decreased size, and fragility. Some studies have reported that airborne particle materials adhere to the pollen surface and cause the collapse and degradation of the exine surface, and shrinkage and abnormality of pollen (Majd & Mohamadi, 1992; Emberlin, 1998, 2000; Parui et al., 1998; Pelter, 1998). Pfahler (1981) showed that a high level of pollution could induce mutagenicity and physiologic changes; therefore, a high concentration of air pollutants, especially APM, causes abnormality in anther layers, microspore mother cells, and developing pollen. Because of the nutritional role of the tapetal layer, abnormality of the tapetum induces pollen sterility.

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