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Air Pollution Effects on the Leaf Structure of some *Fabaceae* Species

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Abstract

Plants growing in the industrial areas and near the major roads absorb the pollutants at their foliar surface. In this paper, histological changes induced by air pollutants (from a cement factory combined with the pollution generated by human activities) in *Lotus corniculatus* L., *Trifolium montanum* L., *T. pratense* L. and *T. repens* L. leaves were followed. Some plant species have been identified to be able to absorb, detoxify and tolerate high levels of pollution. The tolerance degree is indirectly correlated with the intensity of injuries which occur in plant structure. Leaf thickness, height of palisade cells, diameter of the spongy cells, height and width of the upper epidermis cells, the thickness of the external wall of the upper epidermis cells, stomata length and stomatal index number of these species from highly polluted sites and lowly polluted sites were investigated by light microscopy. The stomata decrease in size and increase in density in leaves from high polluted sites. In the mesophyll cells (both in palisade and in spongy parenchyma) dark phenolic deposits could be observed.

Keywords: epidermis, leaf structure, phenolic compounds, pollutants, stomata

Introduction

Air pollution is a major problem in modern society. Even though air pollution is usually a greater problem in cities, pollutants contaminate air everywhere. These substances include various gases and tiny particles, or particles that can harm human health and damage the environment. The interactions between plants and different types of pollutants were investigated by many authors: most studies on the influence of environmental pollution focus on physiological and ultrastructural aspects (Heumann, 2002; Psaras and Christodoulakis, 1987; Velikova *et al.*, 2000). Studies concerning the anatomy of the vegetative organs under conditions of pollution have been also carried out (Alves *et al.*, 2008; Ahmad *et al.*, 2005; Silva *et al.*, 2005, 2006, Verma *et al.*, 2006). The reaction of different species to the altered environmental conditions is strongly correlated with their structural and functional features. Christodoulakis and Fasseas (1990) show no significant changes in *Laurus nobilis* (a resistant xerophyitc plant) leaf structure exposed to air pollutants in Athens. Studies show that under the action of pollutants, plants develop different morphological and anatomical changes.

Various authors investigated the effects of pollution on different species of *Fabaceae*. The strong correlation between the degree of contamination and concentrations in all plant leaves assessed displayed that the leaves of *Robinia pseudo-acacia* (*Fabaceae*) reflect the environmental changes accurately, and that they appear as an effective biomonitor of environmental quality (Celik *et al.*, 2005). Bidar *et al.* (2006) have considered the species *Trifolium repens* as resistant to the action of heavy metals, based on the activity of superoxide dismutase.

In comparison with gaseous air pollutants, many of which are readily recognized as being the cause of injury to various types of plant species, relatively little is known and limited studies have been carried out on the effects of cement dust pollution on the their growth and structure. Cement dust had a significant effect on the growth and structure of some plant species compared with non polluted plants. Toxic compounds such as fluoride, magnesium, lead, zinc, copper, sulphuric acid and hydrochloric acid were found to be emitted by cement manufacturing factories (Iqbal and Shafig, 2001).

In this paper the influence of the air pollution from a cement factory combined with the pollution generated by the human activities in the area (including motor vehicles) upon some *Fabaceae* species (*Lotus corniculatus* L., *Trifolium montanum* L., *T. pratense* L. and *T. repens* L.) anatomy was followed. These plants were chosen because they are common and can be found in different polluted sites. In this study the histo-anatomical structure of the leaves was investigated.

Materials and methods

The vegetal material consists in whole plants of *Lotus corniculatus, Trifolium montanum, T. pratense* and *T. repens* collected from Ceahlau National Park and the adjacent area. The control sample (M) was collected from the protected area of the park and the variants from rail station Tasca (V1) and near the main road of the area (V2).

For each site, 5 plants were collected. We considered only 4 leaves from the central part of each plant. For histo-anatomical analysis the material-leaves, were fixed in formalin/ethanol/acetic acid/water (FEA; 4:69:5:22 volume to volume) and conserved in 70% ethanol and conserved in ethylic alcohol 70%. Free hand sections were performed using a razor blade. The sections were observed without coloration in order to show the phenolic and tannin deposits from different tissues. The photos were taken with an Olympus E-330 photo camera, using an Olympus BX51 research microscope.

The measurements of the epidermic cells, stomata and assimilating parenchyma were made using a biometrical software from Nikon (NIR-Demonstration). One section was investigated from each leaf; for each parameter 50 measurements were made. The following anatomical characteristics were measured: leaf thickness (LT), height of the palisade cells (HPC), the diameter of the spongy cells (DSC), height and width of the upper epidermis cells (in cross section) (HEP and WEP), the thickness of the external wall of the upper epidermis cells (TW), stomata length (SL) and stomatal index (SI) (method after Weyers and Meidner, 1990). Each set of parameters was analyzed using ANOVA and the Least Significant Difference (LSD) test at the 95% probability level (with SPSS 1.6 EV software).

The stationary conditions (regarding air pollutants present in the area) were obtained from Neamt Environmental Protection Agency (Tab. 1).

Results

In all analyzed species, the leaves have dorsiventral structure. The upper epidermis consists of large cells, with a thin cuticle. The mesophyll is differentiating in a palisade parenchyma (single- or bilayered, with short cells) and a spongy parenchyma (in multiple layers, with round cells). The lower epidermis is similar to the upper one (some differences could be noted in the shape of the epidermal cells). The stomata are present in both epidermis (amphystomatic leaves).

In all *Trifolium* species leaves, some differences between the plants from control and from polluted areas were observed. The thickness of the foliar lamina slightly decreases under the influence of the air pollutants (Tab 2., 3., 4.); while the height of the palisade cells decreases, as well.

The thickness of the external wall of the epidermal cells significantly increase in V1 and V2 samples comparative with the control in *Trifolium montanum, T. repens* and *Lotus corniculatus* leaves. The epidermic cells generally have a decreased size in the leaves exposed to the pollutants (especially in *T. pratense* – Tab. 4.).

The stomatal index increase significantly in *Trifolium montanum* and in *T. repens* lower epidermis. In *T. pratense* and *Lotus corniculatus* leaves, no significant differences between the control samples and the variants were noted.

Tab. 1. Pollutants concentrations in the investigated areas

Pollutant type / Collection point	Dust suspensions (mg/m^3)	NO_2 (µg/m^3)	SO_2 (µg/m^3)	Pb ppm	Cd ppm	Zn ppm	Cr ppm	Cu ppm	Co ppm
Control (protected area) (M)	0.021	7.651	4.719	45.31	0.214	210.05	4.05	27.02	3.59
Rail station Tasca (V1)	0.121	17.152	5.164	104.11	0.798	887.06	21.31	122.90	20.85
Main road (V2)	0.281	20.974	6.833	138.30	2.817	1673.72	78.84	193.07	41.91

Tab. 2. Anatomical parameters from *Trifolium montanum* leaves

Plant samples	Control	V1	V2
WEP (µm)	35.57±2.05a	31.62±2.66b	29.54±1.44c
HEP (µm)	38.04±1.96a	43.89±1.98a	31.85±1.64a
TW (µm)	4.84±0,20a	8.81±0.74b	7.47±0.27c
LT (µm)	270.83±4.95a	243.90±4.63b	209,58±1.70c
HPC (µm)	49.43±2.85a	39.76±2.12b	35.35±1.68b
DSC (µm)	19.56±0.74a	21,66±0.92a	19.23±0.89a
SL (µm)	23.11±0.93a	22.31±1.17a	18.94±1.23b
SI	12,57±0.45a	13.53±0.47a	15.67±0.63b

Note: Data represented as a mean ± standard error; y = Means within columns having different letters are significantly different according to the least significant difference (LSD) at 0.05 level of probability.

The thickness of the external wall of the epidermal cells significantly increases in V1 and V2 samples comparative with the control in *Trifolium montanum, T. repens* and Lotus corniculatus leaves. The epidermic cells generally have a decreased size in the leaves exposed to the pollutants (especially in *T. pratense* – Tab. 4.). The stomatal index increases significantly in *Trifolium montanum* and in *T. repens* lower epidermis. In *T. pratense* and *Lotus corniculatus* leaves, no significant differences between the control samples and the variants were noted.

Visual symptoms such as necrotic areas were observed on plants' leaves exposed to air pollution. In the leaf as-

Tab. 3. Anatomical parameters from *Trifolium repens* leaves

Plant samples	Control	V1	V2
WEP (μm)	32.57±1.66a	26.30±2.29b	27.75±0.84b
HEP (μm)	20.62±1.19a	19.34±0.64a	19.56±0.30a
TW (μm)	2,2±0,08a	3.24±0.10b	3.18±0.11b
LT (μm)	198.64±3.99a	187.39±1.29b	177.72±1.52c
HPC (μm)	29.97±1.81a	25,92±1.00b	23.21±0.44b
DSC (μm)	14.29±1.21	16.77±0.98	13.87±0.77
SL (μm)	31.09±0.79a	32.74±1.12a	26.97±0.89b
SI	18.43±0.76a	20.07±0.98b	23.22±0.98c

Note: Data represented as a mean ± standard error; y = Means within columns having different letters are significantly different according to the least significant difference (LSD) at 0.05 level of probability.

Tab. 5. Anatomical parameters from *Lotus corniculatus* leaves

Plant samples	Control	V1	V2
WEP (μm)	ʸ21,32±0.78a	20,35±1.02a	19,53±0.67a
HEP (μm)	16,76±0.88a	15,67±0.69b	15,89±1.14b
TW (μm)	2,08±0,13a	3,20±0,13b	2,86±0,09c
LT (μm)	169,62±4.20a	177,74±2.70a	168.83±3.40a
WPC (μm)	36,47±1.33a	26,36±1.03b	28,56±0.83b
DSC (μm)	17,62±1.32a	15,89±0.84b	16,34±0.93b
SL (μm)	36,96±0.97a	35,43±1.76a	28,87±1.54b
SI	11,90±0.67a	11,34±0.78a	12,04±0.82a

Note: Data represented as a mean ± standard error; y = Means within columns having different letters are significantly different according to the least significant difference (LSD) at 0.05 level of probability

similating tissues and epidermis of the plants from polluted sites variable deposits of polifenolic compounds are observed. In the midveins, there were black deposits along the walls of xylem and phloem vessels as compared to their respective controls (Fig. 1., 2., 3.).

In *T. montanum* leaves dark and smooth deposits could be observed under the external epidermis walls (Fig. 1. A); in some areas, the upper epidermis cells are collapsed (Fig. 1. C). In the mesophyll isolate dark spots (Fig. 1. D) or massive deposits (especially under the upper epidermis and related with a stomatal chamber – Fig. 1. F, 2 B) are visible. Similar types of modifications were detected in the other analyzed species (Fig. 2. B, F, C, 3 D).

In *T. pretense* leaves sometimes, the lower epidermis is detached from the mesophyll (Fig. 2. D). Completely collapsed epidermic cells are observed also in *T. repens* leaves (V1 and V2 samples) (Fig. 3. A, B, C). In *Lotus corniculctus* leaves (V1 sample), the same kind of phenolic deposits were observed inside the epidermis cells (Fig. 3. F) (in this case the external walls are intact). In some leaflets (from V2 samples) some taninic cells could be observed in the mesophyll (Fig. 3. E).

Tab. 4 .Anatomical parameters from *Trifolium pratense* leaves

Plant samples	Control	V1	V2
WEP (μm)	40.80±1.81a	31.81±2.10b	30.57±0.61b
HEP (μm)	31.47±2.54a	20.7±0.90b	24.45±1.76b
TW (μm)	4.76±0.20a	3.51±0.26b	4.89±0.39a
LT (μm)	358.70±3.84a	295.17±4.75b	290.99±3.96b
HPC (μm)	67.39±3.33a	61.89±0.83ab	56.22±2.88b
DSC (μm)	41.17±3.32a	34.34±1.80a	40.09±3.79a
SL (μm)	42.93±0.70a	42.25±0.85a	40.89±0.81a
SI	13.78±0.98a	14.12±0,79a	13.92±0.51a

Note: Data represented as a mean ± standard error; y = Means within columns having different letters are significantly different according to the least significant difference (LSD) at 0.05 level of probability.

Discussions

The relatively high concentrations of lead, zinc and cadmium from the investigated area were related to anthropogenic sources such as cement industry, agriculture activities and traffic emissions. Khashman and Shawabkeh (2006), in a study related to the distribution of the metals in soils around a cement factory, indicate that all of the metals are concentrated on the surface soil, and decreased in the lower part of the soil. This is due to reflect their mobility and physical properties of soil and its alkaline pH values. Foliar uptake through the stomata or leaf cuticle or both, may be the principle route for its accumulation. The metal entry into plants through the leaves is more significant for pollution elements because of aerosol deposits. A number of reports revealed that metal accumulation levels in plants are influenced by their distance from the source of pollution.

Pollution stress altered the structure of the leaves of the investigated species. Nevertheless, these species are quite resistant to air pollutant actions and despite the observed modifications they continue to grow and reach maturity (flowering stage). Various authors underlined the reduction of plant growth, as a consequence of pollution stress (Gupta and Iqba, 2005; Maruthi Sridhar *et al.*, 2005, 2007).

The modification of the frequency and sizes of stomata as a response to the environmental stress is an important manner of controlling the absorption of pollutants by plants. Stomatal size was decreased significantly in all analyzed species, while stomatal index was increased to about a maximum of 20% in *Trifolium repens* leaves. Reduction in stomatal closure under metal stress is not uncommon. The changes in the inner anticlinal walls of the guard cells have been shown to be induced by Pb stress (Ahmad *et al.*, 2005). The decrease of the stomatal size may be an avoidance mechanism against the inhibitory effect of a pollutant on physiological activities such as photosynthesis

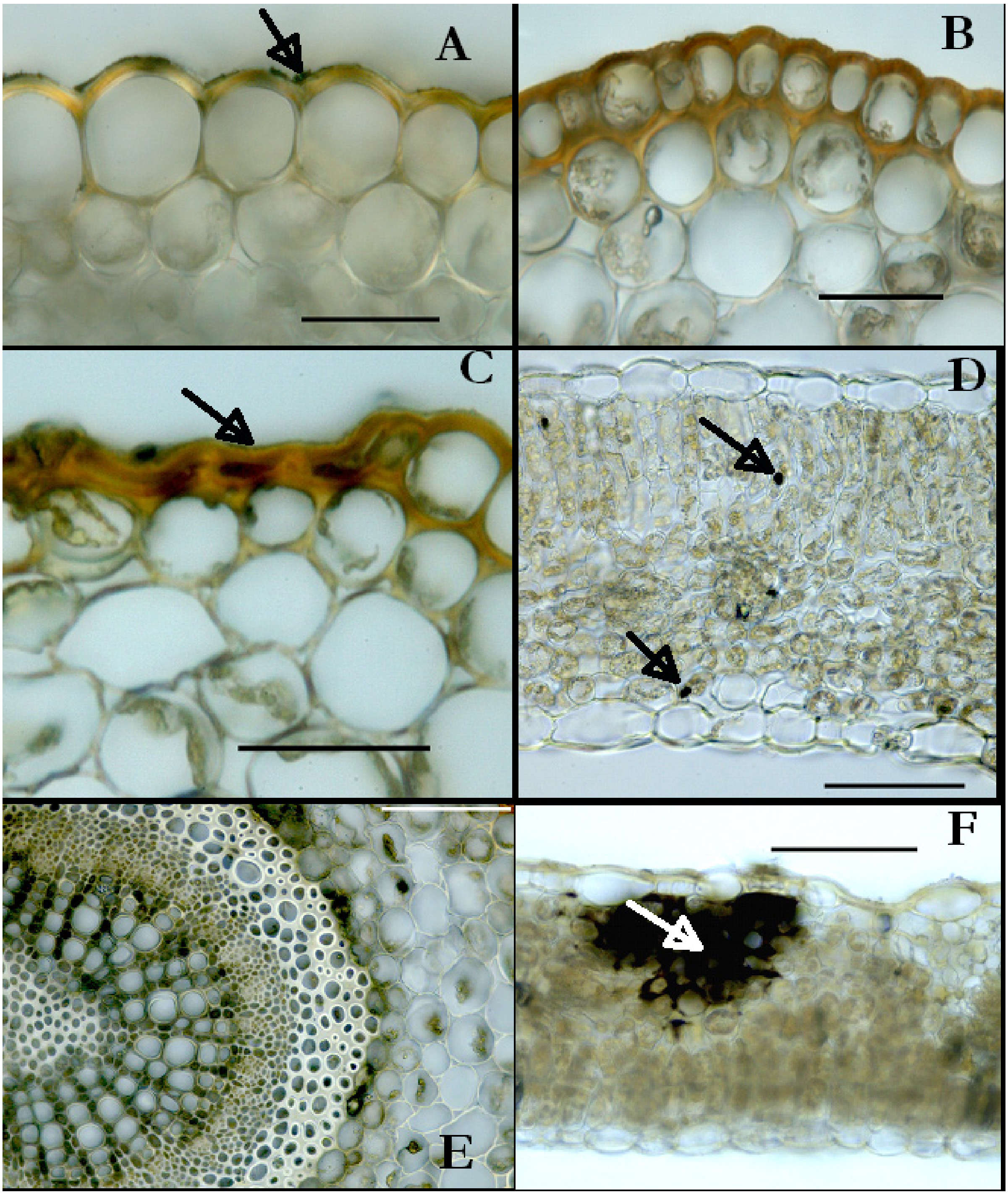

Fig. 1. Crossections through the leaf of Trifolium montanum: A - epidermis from the midvein (V1) (dark deposits under the external cell wall could be observed); B - epidermis from the midvein (C); C - epidermis from the midvein (V2) (crushed epidermis cells) (scale bar - 50 µm); D - crosssection through the leaf from V1 sample (small dark spots in the mesophyll); E - cross section through the midvein (V2); F - cross section through the leaf from V2 sample (large dark deposits in the mesophyll) (scale bar - 100 µm)

(Verma *et al.*, 2006). However, the increase of SI is not a common feature of plant species exposed to air pollution. Verma *et al.* (2006) find a significant decrease of stomatal density and stomatal index in *Ipomea pes-tigridis* grown under various degrees of environmental stresses (coal-smoke pollutants).

The thicker external walls of the epidermis cells also serve as a more efficient barrier against the pollutants penetrating into the mesophyll. Gaseous pollutants entering into the leaves via stomata may strongly interact with the surface of mesophyll cells, assuming that the larger proportion of intercellular space is positively correlated with a larger inner surface area of that tissue. Other authors have

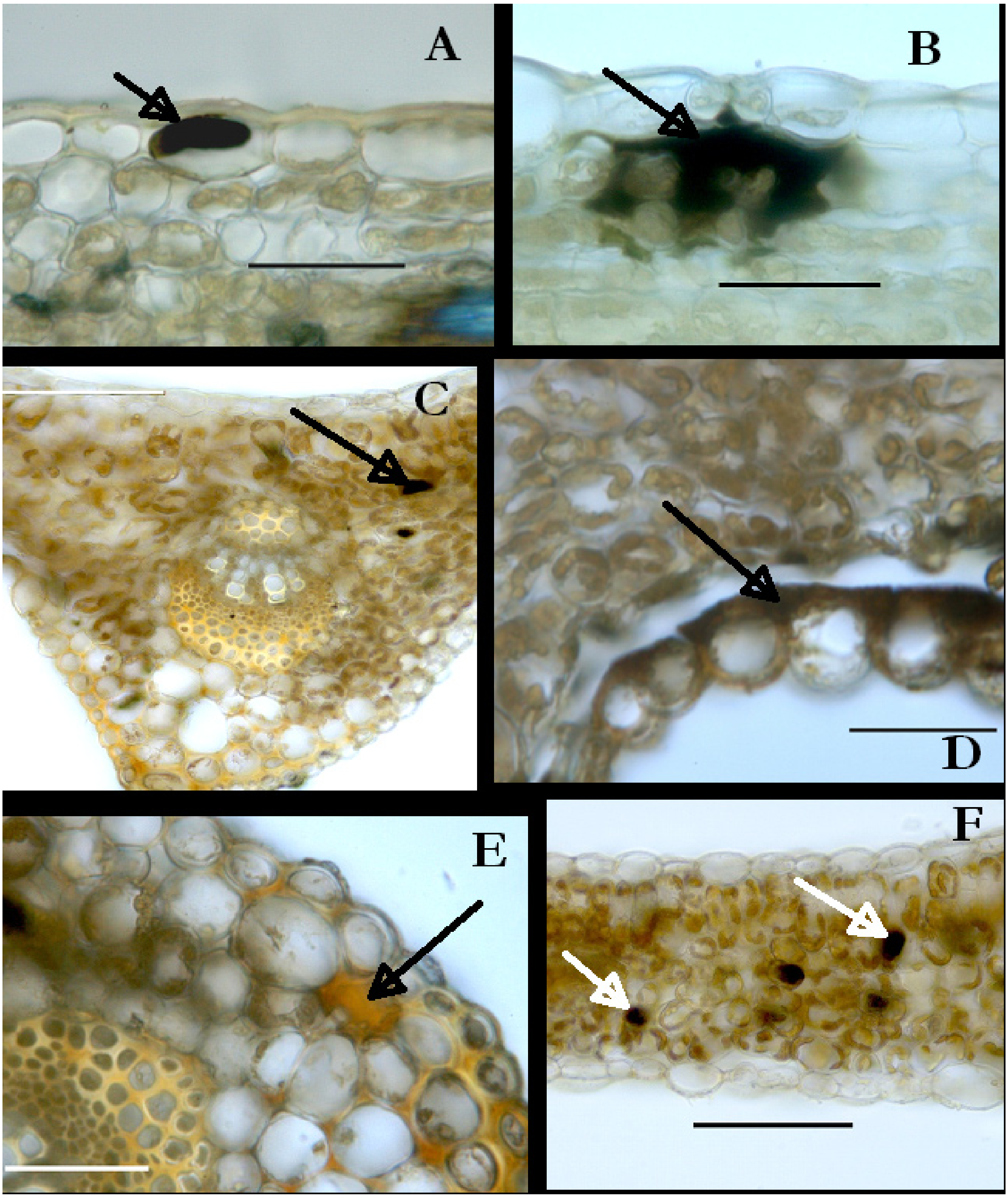

Fig. 2. Crossections through the leaf of T*rifolium montanum* and *T. pretense*: A - *T. montanum* - upper epidermis from V1 leaf (dark deposit into a epidermic cell); B - lower epidermis from V2 leaf (dark deposit into a substomatal chamber) (scale bar - 50 µm); C - T. pretense - cross section through the midvein of V1 leaf (scale bar - 100 µm); D - cross section through the midvein of the V2 leaf (detachment of lower epidermis could be observed); E - cross section through the midvein of V2 leaf (cell with tannin) (scale bar - 50 µm); F - cross section through the lamina of V1 leaf (dark deposits in the mesophyll) (scale bar - 100 µm)

suggested changes in these parameters in connection with atmospheric pollution or other stress factors. The external walls of the cells from upper epidermis show significant increases in almost all investigated species and variants (with a maximum in *T. montanum* - V1 - leaves).

Leaf anatomy of the above mentioned species also showed reduction in epidermis, palisade parenchyma and

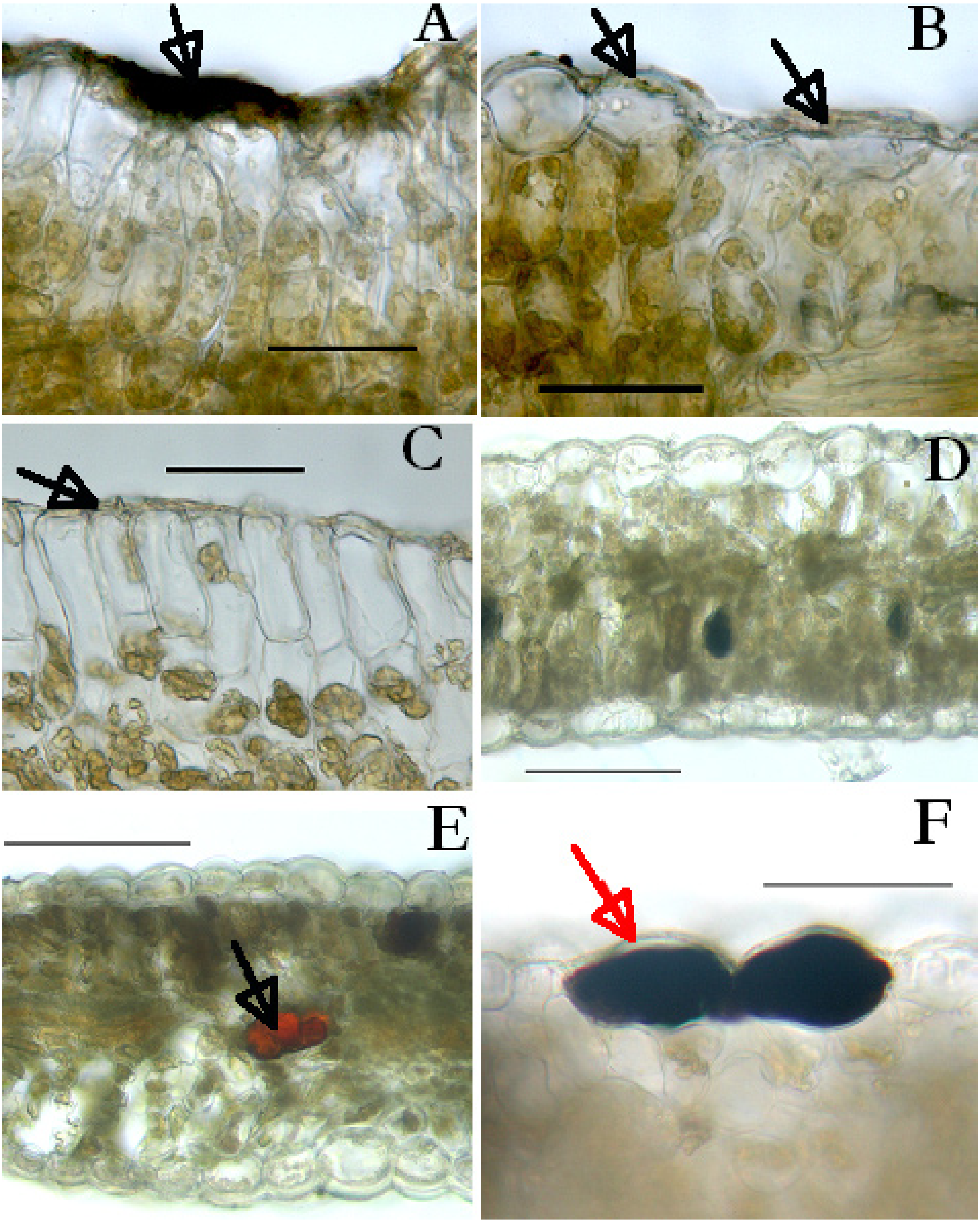

Fig. 3 - Crossections through the leaf of *Trifolium repens* and *Lotus corniculatus*: A - *Trifolium repens* - cross section through the leaf of V1 sample (completely crushed epidermis with dark depositions); B, C - cross section through the leaf of V2 samples (B - normal epidermis cells near crushed epidermis cells, C – destroyed upper epidermis); (scale bar - 50 µm); D - Lotus corniculatus - cross section through the V1 leaf; E - cross section through the V2 leaf (cell with tannin) (scale bar - 1000 µm); F - upper epidermis from V1 leaf (dark deposit into an epidermic cell) (scale bar - 50 µm)

spongy parenchyma cells in polluted leaves as compared to leaves collected from non polluted plants. Significant results were particularly observed in spongy parenchyma in *T. montanum* and *T. pretense* leaves. Mesophyll cells have thinner walls and are in direct contact with the environment through stomata. Sometimes, the substomatal chamber from *Lotus corniculatus* leaves (V2 area) was filled with dark deposits.

In this study, the spongy parenchyma cells show no significant decrease in the polluted areas. Iqbal (1985) has shown significant reduction in palisade and spongy parenchyma in leaves of white clover of a polluted population.

Conclusions

The presence of the phenolic compounds (dark deposits from the epidermis, assimilatory and vascular tissues) indicate that long-term exposure to air pollutants leads to enhanced accumulation of these compounds. The enhanced accumulation of phenolics and lignin is considered to be one of the most common reactions of plants to stress (Wild and Schmitt, 1995).

The structural characteristics of all investigated species indicated a significant potential for resistance to air pollutants. The histological modifications which occurred may potentially be used as biological markers for air pollution presence.

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