STRUCTURAL MODIFICATION INDUCED BY AIR POLLUTANTS IN *PLANTAGO LANCEOLATA* LEAVES

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Abstract. Some structural parameters of *Plantago lanceolata* leaves, which may be considered as biomarkers, were investigated, in order to establish what modifications occur under the pollutants action. The material was represented by leaves of different ages collected from sites with different pollution degrees of the Ceahlau Mountain.

External symptoms such as necrotic areas were observed on plants leaves exposed to air pollution. The leaf structure of the analyzed species show some dark deposits in the assimilatory cells, especially from palisade parenchyma. The necrotic areas shows hypertrophied assimilatory cells with thick walls and tannin deposits. Solid deposits are present on both on upper and lower epidermis.

Keywords: leaf, palisade parenchyma, pollution, polyphenolic compounds

INTRODUCTION

Human activities lead to increase concentrations of heavy metals like lead, zinc, cadmium, etc in soil, water, air and living organisms. One of the most important environmental impacts of anthropogenic activity in the urban ecosystem is the atmospheric pollution [11]. The interactions between different plant species and air pollutants were extensively investigated by different authors. Most studies on the influence of environmental pollution focus on physiological and ultrastructural aspects [10, 12, 17]. Studies concerning the anatomy of the vegetative organs under conditions of pollution have been also carried out [1, 2, 7, 8, 13, 14, 18].

It has been observed that plants particularly growing in the urban areas are affected greatly due to varieties of pollutants and their survival is correlated with structural and metabolic adaptations to the stressful environmental conditions.

Plantago lanceolata is a member of the *Plantaginaceae* family; it is widely distributed throughout the world and is a common roadside plant. It is relatively drought resistant and is able to grow on dry sites. It was chosen for this investigation because it is a plant adapted to different ecological conditions and a widely distributed in the analyzed area.

MATERIALS AND METHODS

The vegetal material consists in leaves of *Plantago lanceolata* 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 was fixed 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 biometrical software from Nikon (NIR-Demonstration). From each leaf one section was investigated; for each parameter 50 measurements was made. The following anatomical characteristics were measured: leaf thickness (LT), height of the palisade cells (under the upper and lower parenchyma) (HPCu, HPC l), the diameter of the spongy cells from the external and internal part of the lamina (DSCi, DSCe) stomata length (SL u, SL l) and stomatal index (SI u, SI l). Each set of parameters were 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 (Table 1).

Table 1. Pollutants concentrations in the investigated areas

Collection point \ Pollutant type	Dust suspensions (mg/m^3)	NO_2 (µg/m^3)	SO_2 (µg/m^3)	Pb	Cd	Zn	Cr	Cu	Co
Control (protected area) (M)	0.021	7.651	4.719	45.31	0.214	210.05	4.05	27.02	3.59
Tasca village (V1)	0.132	16.137	5.393	124.31	0.994	826.18	22.41	124.51	21.59
Caprpatcement carrying station (V2)	0.481	21.344	7.829	142.46	2.519	1476.78	82.49	181.64	44.61

RESULTS

The leaves of *Plantago lanceolata* have isobilateral structure (Fig. 1A & 1B). Epidermal cells are tangentially elongated, with relative thin walls (including the external one which is covered with a thin cuticle).

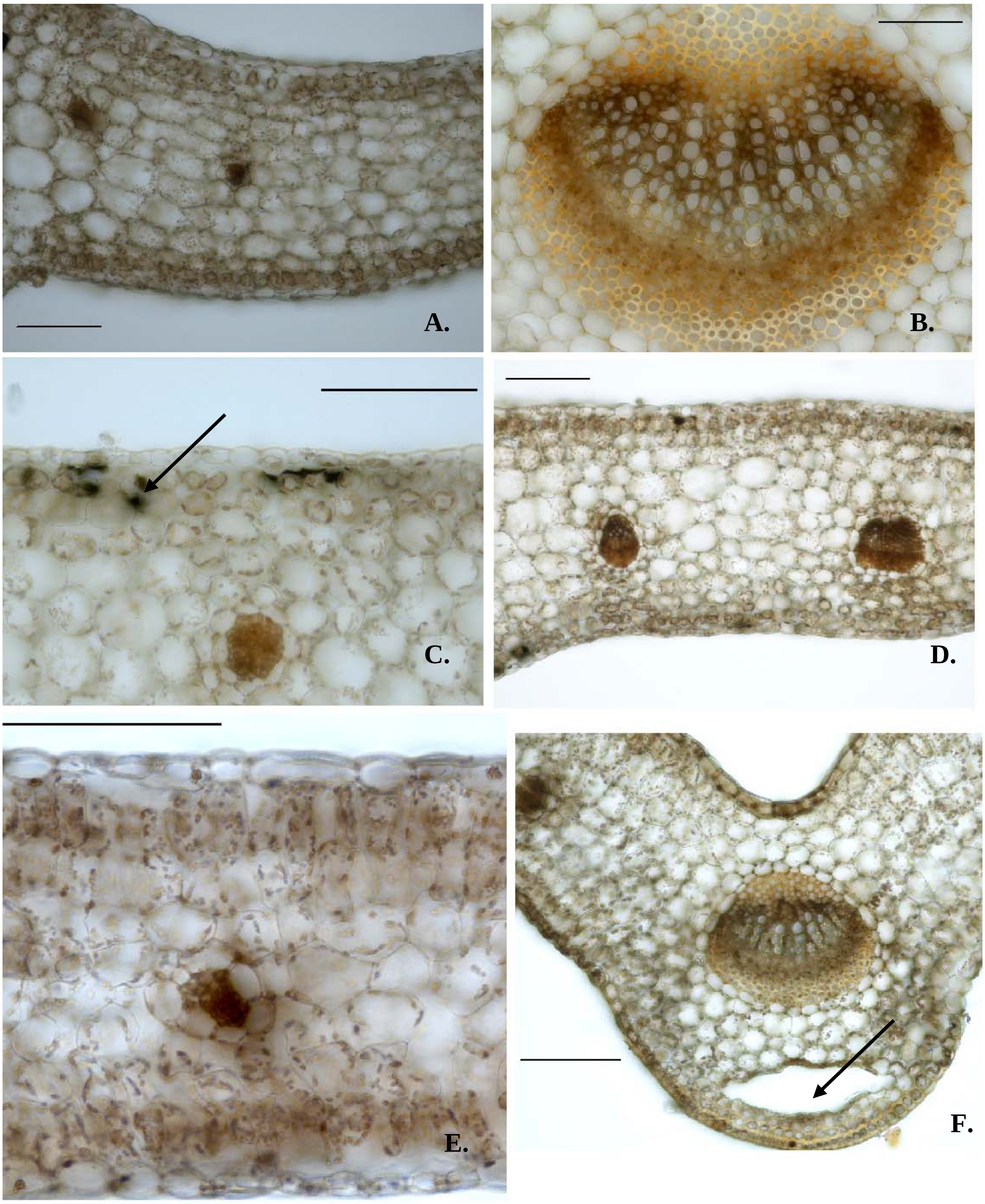

Figure 1. Crossections through the leaf: **A, B** – Mature leaf from control area; **C-D** – leaves from V1 sample; **C** – arrow indicate the polyphenolic compounds from the lamina; **F** – arrow indicate a lacuna from the midvein (original).

Figure 2. Crossections through the leaf: **A-B** – Mature leaf from V2 sample; **A** – arrow indicate the partial necrosis of the tissue from midvein lower part; **B** – arrow indicate the crushed lower epidermis and tannin deposits; **C** –tannin deposits in mesophyll cells) (original)

The diacytic stomata are present in both epidermises, so the lamina has amphystomatic structure. Tector hairs are rare, located especially in the lower epidermis. Glandular hairs are more abundant; they were shorts, multicellular and bi- or triseriate. They consisted in 1 basal cells, 2-3 peduncle cells and a secretory head with 10-12 cells. The mesophyll is differentiated into 1-2 layers of palisade parenchyma, both under upper and lower epidermis and a thick central zone with spongy parenchyma. The cells from spongy parenchyma and their diameter grow from the external to the internal part of the mesophyll.

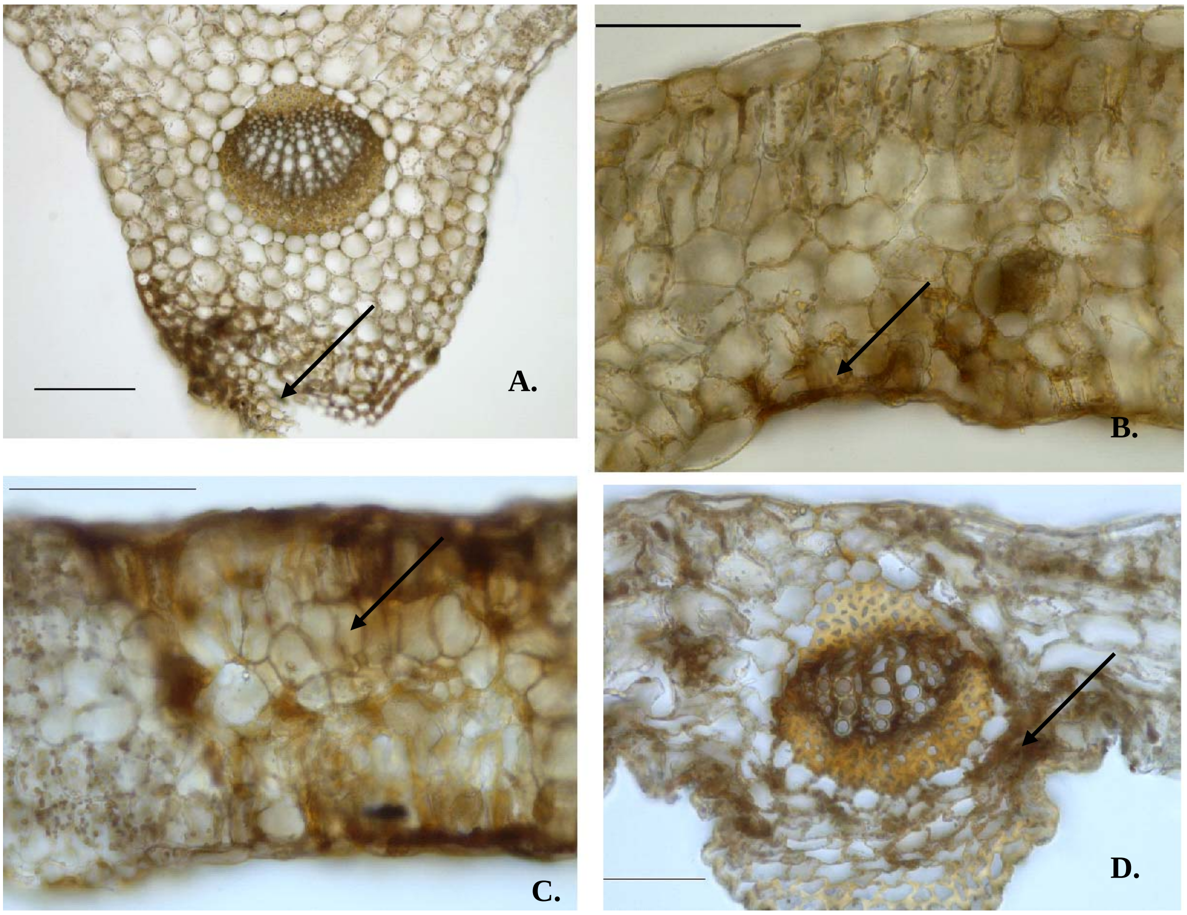

Table 2. Variations of the anatomical parameters in *Plantago lanceolata* leaves from control and pollutes areas Data represented as a mean ± standard error; y = Means within columns having different letters are significantly different according to the least significant difference (LSD) at 0.05 level of probability.

Plant samples	Control	V1	V2
LT (µm)	215.63 ± 3.75a	225.83 ± 4.63a	298.58 ± 1.44b
HPC u (µm)	27.43 ± 0.85b	18.54 ± 1.14a	34 ± 0.96c
HPC l (µm)	24.45 ± 1.87a	16.76 ± 2.12b	19.35 ± 1.68b
DSCi (µm)	21.36 ± 0.74a	28,66 ± 0.92b	24.23 ± 0.89c
DSCe(µm)	33.13 ± 1.65a	36.21 ± 1.43b	40.21 ± 1.19c
DMxV	12 ± 0.96	11.53 ± 0.54	11.12 ± 0.67
DPxV	8.97 ± 0.32	8.54 ± 0.45	6.57 ± 0.82
SLu (µm)	22.64 ± 0.82a	23.27 ± 0.92a	24.34 ± 1.04b
SIu	15.52 ± 0.67a	15.12 ± 0.68a	14.95 ± 0.87a
SLl(µm)	20.21 ± 0.92a	21.51 ± 1.12a	24.34 ±1 .18b
SIl	16.30 ± 0.45a	16.13 ± 0.47a	15.77 ± 0.63a

Regarding the histo-anatomical parameters, some differences between the plants from control and from polluted areas were observed (Table 2). The thickness of the foliar lamina has increase significantly in V2 sample (298.58 µm) comparative with the control and V1 sample. These increasing are related with the increase of the cells of spongy parenchyma (especially those from the central part of the leaves). The height of the palisade parenchyma (under the upper epidermis) is larger in the V2 sample. In V1 sample a slightly decrease of the height of the palisade parenchyma could be observed. The diameters of the spongy parenchyma cells generally increase in polluted leaves comparative with the control.

The stomatal apparatus is no affected by the influence of the air pollutants presents in the areas. Both the length of the stomata and the stomatal index (from upper and lower epidermis) show no significant variations reported to the control samples.

Visual symptoms such as necrotic areas were observed on plants leaves exposed to air pollution (V1 and especially V2 samples). In the leaf assimilating tissues and epidermis of the plants from polluted sites variable deposits of tannin and polyphenolic compounds could be observed (Fig. 1C & 2A-D). The polyphenolic compounds (dark deposits) are localized in palisade parenchyma cells (rarely in the spongy parenchyma ones) and in substomatal chambers. The anatomical structure of the necrotic areas shows hypertrophied assimilatory cells with thick walls and tannin deposits. But the hypertrophy of these cells is not related with an increase of the leaf thickness (Fig. 1D & 1E); on contrary, this parameter decrease in the necrotic areas (probably because some cells are already dead and collapsed). In other cases, the necrosis is visible only at the epidermal level (and the epidermis cells are totally crushed in these areas) (Fig. 2B). In the leaves from V1 samples some cavities appears into the parenchyma from the mid-vein areas; they are formed as results of the disintegration of some subepidermal layers (Fig. 1F).

DISCUSSIONS

Plant adaptation to changing environmental factors involves both short-term physiological responses and long-term physiological, structural, and morphological modifications [9]. These changes help plants minimize stress and maximize use of internal and external resources. The structure of the leaves has an important role in determines the response of plants to the air contamination.

The pollution stress altered the structure of the leave of *Plantago lanceolata*. Nevertheless, this specie is quite resistant to the air pollutant actions and despite the observed modifications they continue to grow and reach maturity (flowering stage).

The increase of the leaf thickness in polluted plants samples indicates a high resistance of these species to this kind of stress. We must underline that the investigations was made by specimens whom grow naturally in the polluted areas and the adaptations was acquired in many years by local populations.

The width of palisade mesophyll and the greatest number of palisade coefficient are the main properties that distinguish the tolerant and resistant plant species from the sensitive ones to atmospheric pollution [6, 4]. *P. lanceolata* accumulated heavy metals mainly in roots [15].

In an experiment related to the influence of the UV radiations upon *P. lanceolata* grown in mediums with low and height nutrient supplies Tosserams et al. (2001) [16] concluded that the increased accumulation of carbon in nutrient-stressed plants, may lead to a reduction of UV-B induced damage because of increased foliar UV-B absorbance by enhanced accumulation of phenolic compounds and leaf thickening. In *P. lanceolata* leaves from pollutes sites we also observed 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 [19]. Plants contain a great diversity and quantity of secondary metabolites, some of which are toxic and deterrent to herbivores and pathogens. Observations from natural populations suggest that ontogenetic patterns in plant secondary chemistry are likely to have important consequences for plant fitness [3].

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