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Development of the leaf blades of *Acer platanoides* in industrially contaminated environment

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Abstract: Leaf blades of Norway maple (*Acer platanoides* L.), growing in heavily polluted industrial area have been studied for anatomical changes developed under the influence of the industrial contamination (with SO_2, N_xO_x, Pb, As). The aim of the examination was to reveal the dynamics in the development of leaf blades and to trace the impact of the contaminated air on the leaf structure of Norway maple.
The conducted study registered acceleration of the vegetative growth of the leaf blades that is manifested through approximately two weeks earlier appearance of leaves on the tree, faster linear growth and strengthened the xeromorphic traits in the leaf structure of the tree plants from the contaminated region. The observed changes are regarded as adaptation of the plant to the polluted environment, i.e. as tolerance.

Additional key words: Norway maple, leave blades, industrially contaminated air, sulphur dioxide

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Introduction

The increase of anthropogenic contamination in the environment is accompanied with many adverse ecological effects. Acidic deposition is comprised of sulfuric and nitric acids and ammonium derived from emissions of sulfur dioxide, nitrogen oxides, and ammonia. These compounds are largely emitted to the atmosphere by the burning of fossil fuels and other industrial activities. Once such compounds enter sensitive ecosystems, they can acidify soil and surface waters, bringing about a series of ecological changes. In acid-sensitive regions, acidic deposition alters soils and stresses forest vegetation (Driscoll et al. 2001). The main cause of forest decline is mostly the sulfur dioxide (Thiel 1985; Traca 1985; Hinrichsen 1983). The mitigation of stressful conditions of the industry environment can be achieved through creation of green forest bands from resistant to the toxicants tree plant species. Planting of tolerant plant trees are applied for localization point source gaseous contaminants such as sulfur dioxides, nitric acids and ammonium derived (Lorenz 1985).

The industrial polluted air affect directly on the assimilative organs of the plants (Thomas 1951; Ilkun 1978; Kummer 1983). Many studies show that under polluted conditions, plants develop different physiological, morphological and anatomical changes (Inamdar and Chaudahri 1984; Iqbal 1985; Karenlampi 1986; Gupta and Ghouse 1988; Bhatti and Iqbal 1988; Jahan and Iqbal 1992; Gravano et al. 2003; Novak et al. 2003; Veselkin 2004; Dineva 2004).

The aim of the investigation was to examinate the dynamics in the development of leaf blades and to record the influence of the contaminated air on the structure of the leaves of *Acer platanoides* as well as to assess the passive tolerance of the tree to the polluted conditions with sulfur dioxide and heavy metals.

Materials and methods

Characteristics of the regions

The study examined the leaf blades from *Acer platanoides* L. (Norway maple). The trees are developed in heavily polluted area of metallurgical factory "Kremikovtzi" (42°47'N; 23°30'E) and as a control National Park Vitosha, (42°30'N; 23°15'E). The region of a steel works "Kremikovtzi" is heavily polluted with SO_2, N_xO_x, Pb, As, Zn, Cu etc.

Major industrial processes in the region generate particles (dust). Particulates are not extremely damaging, but can inhibit or reduce photosynthesis by plugging stomates. Particles are usually washed from leaves by rain or irrigation, and are therefore more harmful during dry periods when other pollutants are not.

The main air pollutant is the sulfur dioxide. During the investigation period the amount of sulfur dioxide in the observed area was 0,5 mg/m^3. Table 1 gives data on the highest pollution concentration at ground surface level measured in the "Kremikovtzi" district (Tzekova, Delev & Tepavicharov 2004).

Description of the plant tree

Norway maple (*Acer platanoides*) grows best on moist, "adequately" drained, deep, fertile soils. It is intolerant of low soil nitrogen conditions and is rare on acidic (pH near 4) soils. Norway maple (*Acer platanoides*) makes "suboptimum" growth on sandy soils or soils high in lime or clay content, and does not tolerate high evaporation and transpiration or prolonged drought. Conflicting reports assert that it is rare on poorly drained soils, yet it reportedly can tolerate flooding for up to 4 months (Nowak and Rowntree 1990; Prentice and Helmisaari 1991).

Plant material and methods

The study examined the leaf blades from *Acer platanoides*. The plant material was collected monthly from April to October. Samples were taken randomly (30 leaf blades from each tree), from the south side of the crown at 160–200 cm of the trees (10–15 trees of species) from both regions. The trees were of a similar age (15 years), sun expose, uniform height and growth form. The middle parts of the leaf blades were cutting and fixed in FAA (90% ethanol – 90 cm^3, ice acetic acid – 5 cm^3 and formalin – 5 cm^3). Standard morphological, histological techniques and light microscopy were used to examine the anatomical characteristics of the leaf blades. The cross-sections of the leaf blades were prepared and observed under light microscopy measured, drawn and photographs were taken. The measurements were repeated 30 times per one parameter. Cell size and thickness of the layers were assessed statistically (Student t-test, $p<0.05$). The influence of the pollution and the time on the linear growth of the leaf blades, length and width, was evaluated with ANOVA (two way).

Results

The leaf blades of Norway maple are characterized with fast growing and development in the commencement stages of vegetative period. The leave and linear increasing of leaf blades size in trees from polluted region is accelerated significantly compare with that which is registered for the control. Nevertheless, the size of the surface of leaf blades from the polluted trees stayed smaller than that from non-polluted on mature leaves (Table 2).

The mature leaf blades of *Acer platanoides* vary from 17 to 19 cm in length and from 18 to 20 cm in width. The surface area of completely developed mature leaf was approximately from 200.67 cm^2 up to 210.07 cm^2. In the control (measurements September) the mean of leaf blade surface had value 200.67 cm^2 (σ = 6.5 cm^2), while that from the polluted tress was about 137.43 cm^2 (σ = 7.48 cm^2).

The obtained results of the conducted morphological and anatomical measurements for April are shown in Table 2.

In the contaminated area, the leaf blades were appeared earlier approximately two weeks before these from the control field. The length and the width of the leaves from the polluted trees were twice bigger than that from the non-polluted (Table 2). The common thickness of the leaf blades from the polluted site was significantly less. The upper and lower epidermis, in the leaves from the polluted region, was represented especially from small-cells compared with the size of these cells from the control (Fig. 1, 2 and Table 2). The manifested trend of smaller epidermal cells in the leaf blades from polluted trees was registered at the

Table 1. Concentration of pollution components in the industrial district "Kremikovtzi"

Year	SO_2 [mg]	NO_x [kg]	CO [kg]	Pb [kg]	Dust [mg]
1994	8203.8	4480525	67359862	15897341	70869
1995	8302.2	4523238	73042454	16570430	74668
1996	11897.5	5493854	66379819	12501979	56679
1997	11869.1	5493854	74094740	16090905	72371
1998	11905.6	5441284	63205819	13981770	62811

first measurements on April and those difference between the means of polluted and control samples sustained up to entire development of a mature leaf (Table 2). All measurements providing during April had significant difference of means between polluted and control with the exception of the thickness of upper cuticle and the thickness of the spongy mesophyll.

The acceleration of vegetative development manifested as earlier appearing of leaves and faster development and forming of leaf blades in the polluted field can be accepted as adaptation the plant to the industrial contamination, i.e. sign of tolerance.

On May the registered length and width of the leaves from the polluted field were smaller, than these from the uncontaminated region, and with no significant dissimilarity between the thicknesses of the leaf blades (Table 2). The noticed expanse of the common thickness of the leaves was mostly due to the enlargement of the mesophyll tissue, compared with the cross-sections made on April. The thickness of the upper and lower cuticle layers were without considerable distinction.

On May the leaf blades ended their linear growth and the surfaces were fully developed. Therefore, there was no dissimilarity in the results of the morphological measurements, obtained on July with those from May. The trend kept up the same – the length and the width of the leaves from the impure field were smaller, than these from the uncontaminated region without significant variation between the thicknesses of the leaf blades (Table 2).

The size of the cells from the upper and lower epidermis was significantly smaller from the polluted field, than those from the control. The differentiation of the palisade mesophyll between polluted and control was greater. On the cross-sections of the leaves from the impure site (Fig. 3), was easy to mention lacks of large air spaces typically for the leaf blades structure of *Acer platanoides* (Fig. 2, 4, 6).

The coefficient of palisadeness (K %) rose up to 53% for polluted leaves and kept the same in non-polluted 41%, as it was measured on April.

The enlargement of the palisade tissue corresponded to the escalating of the coefficient of palisadeness (K %), which is an index of the rate of gas exchange in the plant leaves:

$$K\ (\%) = [Rp/Rm] \times 100,$$

where R_p – is the length of the palisade mesophyll; Rm – is the length of the mesophyll tissue.

On July the thickness of the leaf blades from the contaminated region were 319.92 μm (σ = 5.9) and only 263 μm (σ = 28.16) for the sample from the control site. There was no difference in the measurements for the thickness of the upper and lower cuticle layers. The tendency of enlargement the palisade parenchyma continued for the mesophyll layer. On the represent cross-sections of the leaves from the polluted environment, the palisade tissue had value 112.84 μm (σ = 7.19), while from the pure area the value was about 69.44 μm (σ = 7.04). The coefficient of palisadeness resided approximately in the same precincts 41% for control as it was measured on April and June, and 50% for the tainted plants. On September the margins of leaf blades were scorch as well as the tops of the lobe parts of the leaves from the trees

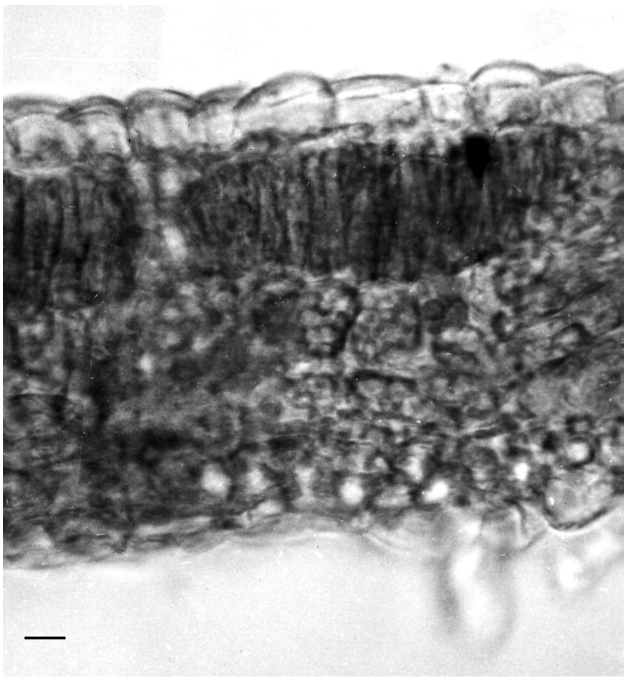

Fig. 1. *Acer platanoides* (bar = 10 μm) Àpril – polluted

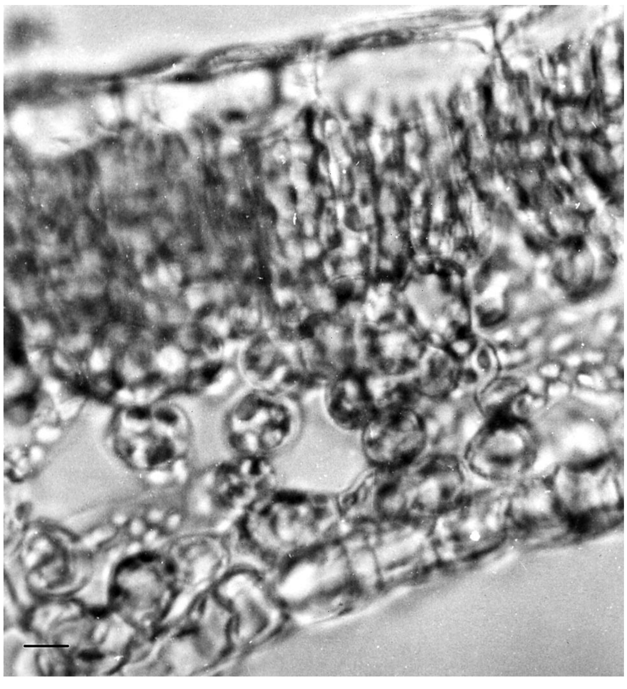

Fig. 2. *Acer platanoides* (bar = 10 μm) April – control

Table 2. Results of the linear growth of the leaf blades – ANOVA (two way)

Trait (mean and standard deviation)	Control area					Polluted area					ANOVA (p)		
	April	May	June	July	October	April	May	June	July	October	Pollution	Time	Interaction (P×T)
Length of leave (cm)	3.95	13.21	12.8			7.29	8.11	7.08			p < 0. 001	p < 0. 001	p < 0. 001
	(0.85)	(2.0)	(2.18)			(1.83)	(1.12)	(1.44)					
Width of leave (cm)	5.85	16.15	15.05			10.07	7.3	9.16			p < 0. 001	p < 0. 001	p < 0. 001
	(1.19)	(2.83)	(2.83)			(2.93)	(1.1)	(2.95)					
Leaf thickness (μm)	275.02	264.12	255.18	263	288.92	255.12	264.48	252.7	319.92	302.28	p < 0. 001	p < 0. 001	p < 0. 001
	(3.41)	(6.16)	(6.16)	(28.16)	(6.01)	(2.34)	(6.68)	(3.89)	(5.9)	(7.48)			
Upper cuticle thickness (μm)	6.82	8.37	7.75	17.98	22.94	7.56	10.23	14.57	17.98	22.94	p < 0. 001	p < 0. 001	p < 0. 001
	(1.3)	(2.54)	(2.54)	(3.78)	(2.97)	(2.1)	(2.54)	(4.15)	(2.41)	(7.75)			
Upper epidermis thickness (μm)	40.92	56.42	50.22	37.82	48.67	31.0	39.68	35.96	38.75	36.58	p < 0. 001	p < 0. 001	p < 0. 001
	(7.25)	(7.93)	(7.93)	(5.2)	(4.15)	(0.0)	(4.34)	(5.64)	(4.4)	(3.47)			
Palisade mesophyll thickness (μm)	93	79.32	65.72	69.44	74.09	76.88	96.72	90.52	112.84	100.44	NS	p < 0. 001	p < 0. 001
	(4.09)	(7.7)	(6.63)	(7.04)	(5.51)	(7.25)	(11.34)	(8.37)	(7.19)	(9.11)			
Spongy mesophyll thickness (μm)	96.1	89.28	92.38	97.03	104.78	106.64	111.6	77.5	112.84	106.02	p < 0. 001	p < 0. 001	p < 0. 001
	(16. 3)	(10.6)	(12.52)	(6.82)	(19.46)	(6.82)	(18.91)	(5.2)	(17.23)	(18.78)			
Lower epidermis thickness (μm)	31.93	30.69	32.86	32.55	29.76	28.83	27.59	27.9	28.83	27.59	p < 0. 001	p < 0. 001	p < 0. 001
	(2. 9)	(4.46)	(5.08)	(3.9)	(2.1)	(2.91)	(3.03)	(2.91)	(2.91)	(3.03)			
Lower cuticle thickness (μm)	6.25	6.25	6.25	8.18	8.68	4.21	6.25	6.25	8.68	8.68	p < 0. 001	NS	p < 0. 001
	(0.0)	(0.0)	(0.0)	(1.42)	(1.92)	(1.42)	(0.0)	(0.0)	(1.3)	(2.79)			
K – Coefficient of palisadness (%)	49%	47 %	41%	41%	41%	41%	46 %	53%	50%	48%	NS	p < 0. 001	p < 0. 001

*p<0.05
**p<0.001
***p<0.0001

growing in the polluted region. Dust particulates are damaging by plugging stomates. Hence, the plant excessively losing water through evaporation and irregular transpiration that is registered as "burn" of the margins of the leaves and decreasing of their surfaces (Ra 1980).

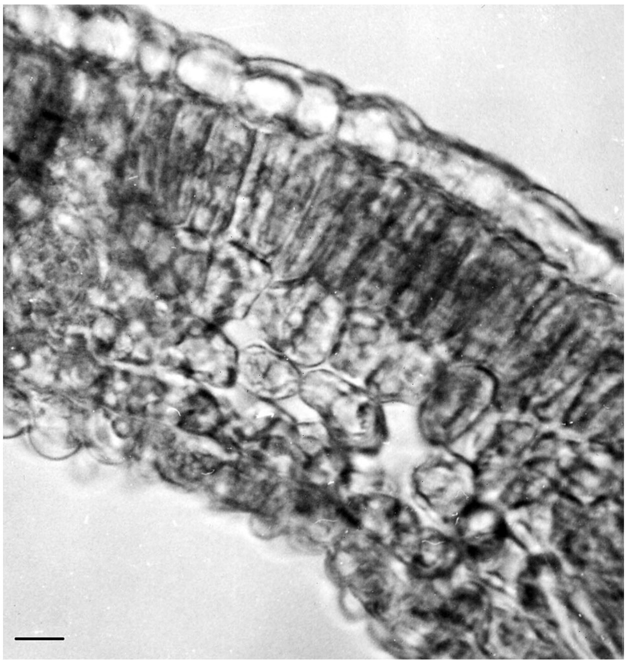

Fig. 3. *Acer Platanoides* (bar = 10 μm) June – polluted

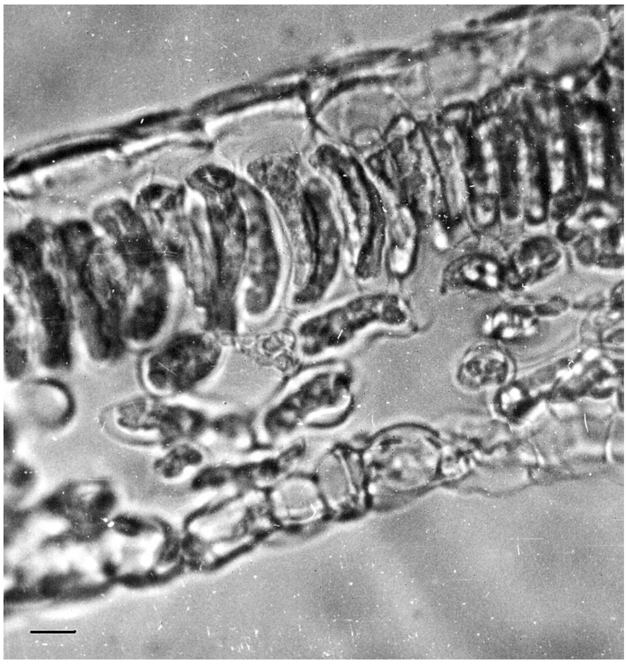

Fig. 4. *Acer platanoides* (bar = 10 μm) July – control

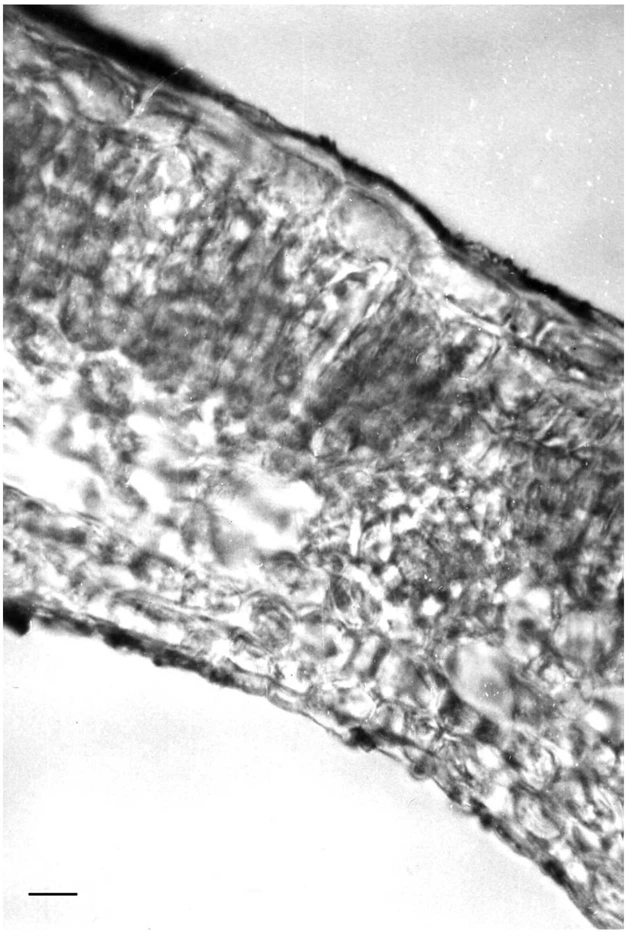

Fig. 5. *Acer platanoides* (bar = 10 μm) October – polluted

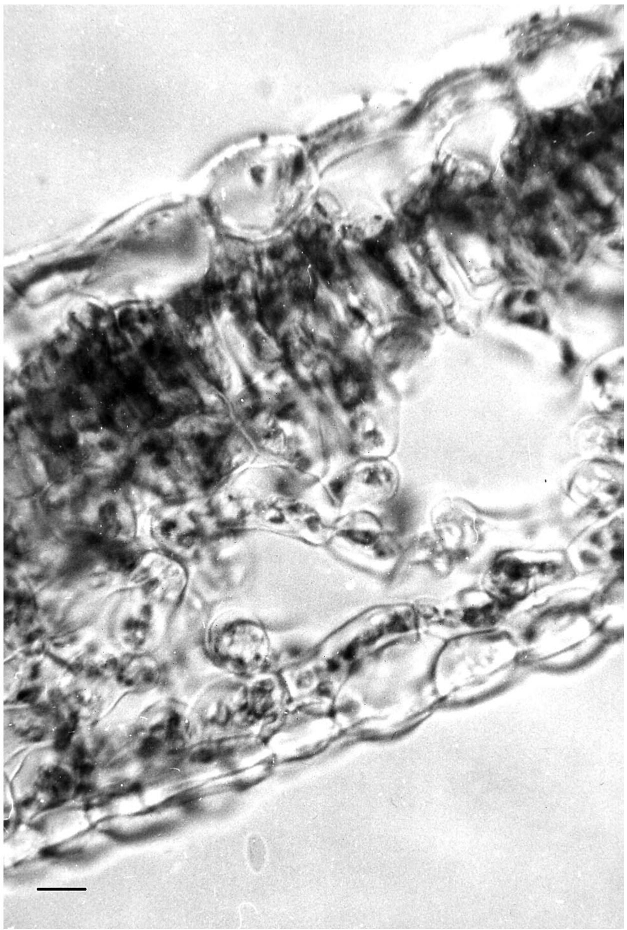

Fig. 6. *Acer platanoides* (bar = 10 μm) October – control

On October, the leaves from the both regions had insignificant structural differences. The main anatomical dissimilarities in the mature leaves sustained – higher thickness of the palisade mesophyll and smaller size of the epidermal cells from the upper epidermis were measured for the leaves from the contaminated area, compared with those from the pure one (Table 2). No distinctions were discovered between the upper and lower cuticle of the leaf blades from the both regions (Figs 5, 6 and Table 2).

The coefficient of palisadeness stayed on the same value for the leaves of control tree plants 41% (as it was measured during all vegetation period from April to October), and much higher for the leaves from polluted tree plants, about 48%. On the represented cross-sections from October, impressed the divergence in the structure of spongy mesophyll (Figs 5, 6). The Norway maple had typical mesomorphic leaf blade structure common for the plants that grew in average moisture (Fig. 4). The mesophyll structure of mesomorphic leaves is characterized with pallisade mesophyll situated in the upper part of the leaf, build up from columnar cells, very compact, with high chloroplast density and most PS carried out in these cells. The spongy mesophyll is located on the lower part of the leaf with large air spaces and fewer chloroplasts per cell. The lower epidermis contained bigger number of stomata that causes high rate of gas exchange.

Under the influence of industrially contaminated environment, the Norway maple changed some traits of its leaf blade structure (Figs 5, 6). The tree plant strengthened the xerophytic characteristics of the mesophyll tissue in the leaves. These adaptations help the tree plant to reduce water loss and to survive under the stress of pollution.

The xeromorphic features in pallisade mesophyll that present in the leaf structure of *Acer platanoides* from contaminated area were appeared as densely packed cells and correspondingly smaller thickness of the spongy mesophyll with few, little air chambers. The other typical xeromorphic trait that emerged in the leaf blade structure in the tree plants from polluted site was the hypodermis on the lower surface of the leaf (Fig. 5).

Discussion

The industrial polluted air causes pressure over the all plant and particularly on its assimilative organs. The study revealed that under contaminated conditions the tree plants of Norway maple developed leaf blades with smaller surfaces compared with these from the control plants. In our previous investigations with other deciduous tree plants the same trend was observed (Dineva 2004). Many authors reported similar results of decreasing leaf blade surfaces in tree plants under the influence of different type anthropogenic contamination (Iqbal 1985; Sodnik et al. 1987; Gupta and Ghouse 1988).

In polluted region was detected earlier appearing of leaves and acceleration of the vegetative development manifested as faster linear growth and forming of leaf blades. The appearance of leaves and the linear enlargement of their size in the commencement stages of the vegetative development in the trees from polluted region were speeded up significantly compared with that registered for the control. Nevertheless, the size of the surfaces of mature leaf blades from the polluted trees stayed smaller than that from non-polluted. The observed changes were regarded as adaptation of the plant to the polluted environment, i.e. as tolerance. In general, developing young leaves are more affected by acid rain than older leaves (Evans et al. 1978; Evans and Curry 1979; Swiecki et al. 1982; Paparozzi and Tukey 1983; Adams et al. 1984; Evans 1984; Crang and McQuattie 1986; Rinallo et al. 1986). Perhaps, with the increasing linear growth of the leaf blades in the commencement stages of developing the tree plants escape the adverse environmental conditions and met the highest peak of acidic rainfalls with fully expanded leaves that are less sensitive to acidic precipitations (Evans et al. 1978; Crang and McQuattie 1986; Rinallo et al. 1986).

Norway maple showed no significant changes of thickness of the cuticle layer between polluted and control trees with the exception of the sample measurements made on June that is coincided with the summer rainfall peak. The cells of epidermal layer diminished their size. The upper and lower epidermis was represented especially from small size cells compared with these from the control. The manifested trend (smaller epidermal cells in the leaf blades of polluted trees) was registered at the first measurements on April and sustained during the complete development up to mature leaf. The similar results reduction in size of epidermal cells at polluted sites as compared to that at reference site is received in studies of the foliar epidermal traits from other authors (Ferenbaugh 1976; Aggarwal 2000; Pal et al. 2000).

During the investigated vegetative period had been observed that *Acer platanoides* changed its leaf blade structure mainly by increasing the palisade mesophyll that is accompanied with the growth of the coefficient of palisadeness. On the cross-sections of the leaf blades from the impure site were detected lacks of big air spaces. The coefficient of palisadeness rose up to 53% for polluted leaves and stayed the same in non-polluted 41%, as it was measured on April. Trees remove gaseous air pollution primarily by uptake via leaf stomata. Gaseous pollutants, such as sulfur dioxide, enter plants usually through stomates. Passing through the stomates of the lower epidermis, gas meets spongy mesophyll; wide intercellular spaces provide the faster penetration of the toxicant toward the palisade cells. Once inside the leaf, gases diffuse into intercellular spaces and may be absorbed by water films to form acids or react with inner-leaf surfaces (Smith 1990). Therefore, many authors consider that one of the main criteria of resistance to air pollution is the higher coefficient of palisadeness (Nikolaevski 1963; Ilkun 1971; Ilkun 1978). The thickness of the upper cuticle, 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 (Nikolaevski 1963; Kulagin 1968; Ninova 1970; Bennett et al. 1992; Ferdinand et al. 2000).

Conclusion

The conducted investigation registered earlier appearing of leaves and acceleration the commencement stages of the vegetative development manifested as faster linear growth and forming of leaf blades in the polluted field, as well as strengthened the xeromorphic traits in the leaf structure of the *Acer platanoides* in the contaminated region. The changes were regarded as adaptive response of the plant to the grimy environment. All measurements of the structure of leaf blades suggested that Norway maple is tolerant plant to industrial contamination and can be used as tree plant for construction of forest belts around the point sources of air contamination.

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