



Research Article

Assessing the Adaptability of *Acer saccharinum* L. to industrially contaminated environment according to its leaf blade structure

Snejana Boycheva Dineva

Trakia University - Stara Zagora; Faculty of Techniques and Technology, Bulgaria
E-mail: sbdineva@abv.bg

Assessing the adaptable capability of tree plants to air and soil pollution is an important task, because of nowadays increasing technogenic pollution and global warming trend of climate change. Plants are well known with their ability to absorb, filtrate, localized air contaminations, and mitigate the adverse effect of pollution. The goal of study is to estimate the adaptability of *Acer saccharinum* L. to industrial air pollution through observation of the leaf blade structure modifications. Under conditions of industrial air contamination has been registered reduction of lamina surfaces. The leaf blade structure of *Acer saccharinum* L. is characterized with high coefficient of palisadness naturally (65%). In conditions of industrial pollution silver maple developed thicker cutin ($p < 0.05$), smaller epidermal cells ($p < 0.05$) and expanded photosynthetic mesophyll tissue ($p < 0.0001$), which strengthen the capacity of plant to overcome the adverse conditions. The high coefficient of palisadness, the preserved thickness of cuticle, accompanied with the adapt alterations of mesophyll; provide a reason to classify silver maple as species with high pliability and as a tolerant to environmental stress. *Acer saccharinum* L. is a suitable for building green belts around areas with adverse emissions and for urban forestry.

Key words: leave blade structure, industrial pollution, polluted air, *Acer saccharinum* L. morphological and anatomical alterations

INTRODUCTION

Under environmental adverse conditions such a global warming trends, increasing of air and soil pollution, drought stress and other, plants expressed their genetic potential against the hostile environment developing visible morphological and anatomical modifications as a result of their struggle and adaptation (Jahan and Iqbal, 1992; Dineva, 2005 a, b; Azmat et al., 2006; Azmat et al., 2009; Stevovi and Sur, 2010; Pourkhabbaz et al., 2010; Kurteva and Dimitrova, 2014). The studies of plants plasticity and tolerance are very essential, because the unfavourable environmental conditions, which in the future are expected to enhance (El-Sharkawy, 2012).

Plants exposed to stress developed strategic defence mechanisms that vary between the species and the nature of stressing agent (Sharma and Dubey, 2005). Plants usually adapt to high pollutant concentrations by histological modifications observed in leaf blades that can be used as biological markers for air pollution presence (Gostin, 2009; Kurteva and Dimitrova, 2014; Rai, 2016). Any specific or predictable alteration in leaf blades due to contamination can serve as a diagnostic marker of exposure to various pollution types (Baker and Hunt, 1986). As an example the increase in heavy metal concentration in foliage of plants grown in contaminated

area caused changes in physiological and biochemical characteristics leading to reductions in morphological features (Azmat et al., 2009). However, to be accepted the certain trait as biomarker it should be clear recognized and predictable, as well as time cost valuable.

Studies on morpho-anatomical responses of leaf blade structure of plants to pollutants is an useful tool for establishing the sensitivity to pollutants and can provide crucial data to biomonitoring programs or retrieval of degraded areas (Sant et al., 2006). The deciduous trees are well recognized with the ability to absorb certain contaminants and to mitigate air pollution through construction of green belts or urban green spaces (Nowak et al., 2006; Giorgi and Zafiriades, 2006; Bealey et al., 2007; Jim and Chen, 2007; Escobedo et al., 2008; Simon et al., 2011; Kumar et al., 2013; Deepika and Haritash, 2016; Stratu et al., 2016). Therefore, the examination of tree potential to environmental weight in all points of view is a very actual and necessary part of work, before establishment of any forestry project.

The study aim is to assess *Acer saccharinum* L. adaptable capacity to industrial polluted conditions based on leaf blade structure and its modification in such conditions.

MATERIALS AND METHODS

Characteristics of *Acer saccharinum* L.

Acer saccharinum L. with the common names: river maple, silver maple, soft maple, creek maple, water maple, silverleaf maple, swamp maple, or white maple is native to Northern America, and is mostly used as an ornamental tree, this is one of the most common trees in the United States.

The silver maple is a large tree, can grow up to 35m tall with a trunk more than 100cm in diameter. Its light green leaves are 15 to 20cm long, with 5 or 7 lobes. The tree grows quickly that is the reason to be popular and commonly encountered tree in both urban and suburban landscapes. The silver maple is very similar to the red maple, both are temperate deciduous tree species (Jing-Yun and Ying, 2002), except that its leaves turn pale yellow or brown, not red, in the fall (McGarry, 2014). This plant species grows well in areas that have standing water for several weeks at a time and in acid soils that remains moist, but adapts to very dry and alkaline soils also. It should be saved for planting in wet areas or where nothing else will thrive (Gilman and Watson, 1993). Nevertheless, silver maple has very heat and high drought tolerance (Hightshoe, 1988; Sjöman et al., 2015). The tree has been successfully grown in urban areas where air pollution, poor drainage, compacted soil, and drought are common (Gilman and Watson, 1993).

Silver maple is given as SO₂ tolerant species (Davis and Gerhold, 1976; Appleton et al., 2014) and intermediate to fluorides (Kozłowski, 1986).

Characteristics of the regions

The plant material was collected from two regions – one heavily polluted, situated around metallurgical factory “Kremikovtzi” (42°47' N; 23°30' E); and another, as a control from National Park Vitosha (42°30' N; 23°15' E). Kremikovtzi factory was emitted significant quantities of dust, SO₂, NO_x, CO, CO₂, H₂S, HCN, heavy metal etc. Various industries, vehicles and power generators are the major sources of SO₂ (Gupta et al., 2015). SO₂ represents one of the main pollutants emitted by industrial combustion processes (Diab and Motha, 2007). Heat power stations and the “Kremikovtzi” metallurgic plant emitted 90% of the total pollution amount in the region. The field from where the plant material was collected stayed in distance of 2 km from the point source of main contamination.

Plant material and methods

The trees from the both fields were sun exposure with uniform height and growth form. Plant tissue samples were taken randomly, from the middle parts of the leaf blades and fixed in FAA - 90% ethanol (90 ml); ice acetic acid (5 ml); and formalin (5 ml). Standard histological techniques were used to examine the anatomy of the leaf blades. All the sections were embedded in emission and examined under a light microscope. The measurements were repeated 30 times per one parameter, assessed statistically with Student's t-test and photographed.

The coefficient of palisadness (K %) was estimated:

$$K(\%) = [M_p / M] \cdot 100,$$

where: M_p – is the length of palisade tissue;

M – is the length of mesophyll tissue.

RESULTS

In the field observation reduction of leaf surfaces in plants exposed to anthropogenic stress has been registered. The length of leaf surfaces was shorter with 4.39 cm, at significance $p < 0.0001$. For the silver maples growing in polluted region, was measured length with mean 10.23 cm ($s = 1.94$) and for the control plants the average was 14.62 cm ($s = 2.77$). The width of lamina also express alteration, for Kremikovtzi region the mean was 10.3 cm ($s = 2.48$), and for the control 12.57 cm ($s = 2.48$), with difference 2.27 cm, at $p < 0.001$. The surfaces of leaf laminae were normally with dry tops of units and necrotic spots around the veins, covered 2 - 3% of the total leaf area.

Table 1. Morphological measurements of *Acer saccharinum* L. leaf blades

Morphological measurements (cm)	Mean (cm)		Standard deviation	
	treated	control	treated	control
Length of lamina (cm)	10.23	14.62	±1.94	±2.77
Width of lamina (cm)	10.3	12.57	±2.48	±2.48

The leaf blades of *Acer saccharinum* L. have typical mesomorph structure (fig. 2, and 4). On the cross sections

of the middle part of laminas have been measured the upper cuticle layer, adaxial epidermis, mesophyll – palisade and spongy, abaxial epidermis and lower cuticle layer, the differences in that parameters were compared (table 2, and 3).

Table 2. Anatomical measurements of *Acer saccharinum* L. leaf blades

Anatomical measurements (μm)	Mean (μm)		Standard deviation (μm)	
	treated	control	treated	control
Thickness of adaxial cuticle (a)	2.75	2.57	± 0.45	± 0.17
Thickness of adaxial epidermis (b)	15.5	16.75	± 1.90	± 2.95
Thickness of palisade mesophyll (c)	49.75	41.58	± 3.10	± 7.7
Thickness of spongy mesophyll (d)	26.25	21.92	± 3.52	± 6.68
Thickness of abaxial epidermis (e)	10.17	10.33	± 1.73	± 1.82
Thickness of abaxial cuticle (f)	1.25	1.225	± 0	± 0.14

Table 3. Differences of means (μm), between polluted and control plants of *Acer saccharinum* L.

Anatomical measurements (μm)	Differences of means (μm)	Student's criterion
Thickness of adaxial cuticle (a)	0.18 *	2.04
Thickness of adaxial epidermis (b)	-1.25 *	1.95
Thickness of palisade mesophyll (c)	8.17 ***	5.39
Thickness of spongy mesophyll (d)	4.33 *	3.14
Thickness of abaxial epidermis (e)	-0.17	0.36
Thickness of abaxial cuticle (f)	0.025	1

*: $p < 0.05$; **: $p < 0.001$; ***: $p < 0.0001$.

DISCUSSIONS

Industrial pollution commonly caused low pH moisture as rainfalls, mist and fogs, because of dissolved sulfur dioxide, nitric acid and hydrochloric acid. In such environmental conditions, on the leaf blades of deciduous trees appears injuries as necrotic spots and chlorosis, which are covered different percentage of the total leaf area depending on the kind and level of pollution, and from the plant species (Temple et al., 1992; Silva et al., 2000; Orendovici et al., 2003). The necroses started from the adaxial epidermis, due to the direct expose to the pollutant, and plants accumulated phenolic compounds in necrotic areas (Sant et al., 2006), which made that spots black. On the leaf blades of all treated plants with simulated low pH acid rain (pH 3.0) have been observed necrotic spots and most of the injuries onset on the epidermis in all species studied (Sant et al., 2006).

Air pollution can lead to a high variability of the leaf area surfaces and specific leaf area, which is the result of the plant mechanism of survival (Azmat et al., 2006; Wuytack et al., 2011). The reduction of leaf blade area is classified as an adaptive response of plants under conditions of environmental stress and has been recorded from many authors (Jahan and Iqbal 1992; Dineva, 2004; Dineva, 2005 a, b; Seyyedneja et al., 2009; Azmat et al., 2009; Pourkhabbaz et al., 2010; Stevovi and Sur, 2010; Velickovi, 2010; Seyyednejad and Koochak, 2011;

Kurteva and Dimitrova, 2014). Decreasing of leaf area have been registered under high SO_2 concentration >50 mg/l (Pandey, 2005), and in environment with high levels of metal contamination. It has been found that Pb physically blocks the uptake of water and water stress appears, which led to reducing leaf area and that is the mechanism of plant survival (Azmat et al., 2006, 2009). The ambient air pollution caused adaptive modifications of specific leaf area, however that alterations are complicate species-dependent and related to the protective or adaptive mechanism of plants (Wuytack et al., 2011).

In laminas of deciduous trees, the cuticle consist of cutin that is a fatty substance and its thickness usually corresponds with the degree of xeromorphism of the leaf (Hall et al., 2013). The cuticle is an effective barrier to the penetration of gases into the leaf interior, and define its resistance to pollutant absorption (Unsworth et al, 1976; Bartiromo, 2013). The laminas of silver maple are coated with cuticle layers about $2.57 \mu\text{m}$ ($s = 0.17$) thick on the adaxial surface and $1.23 \mu\text{m}$ ($s = 0.14$) on the abaxial side. The adaxial epidermis is built from cells with height about $16.75 \mu\text{m}$, ($s = 2.95$) and under contaminated conditions diminish their size, with significance $p < 0.05$ (table 3). In regions with enhance air pollution in laminas very often is registered alterations of cuticle layers, increases or reductions, which depend of plant species and its genetic capacity of optimal adjustment to habitat condition (Margris and Mooney, 1981; Dineva, 2005a, b; Pourkhabbaz et al., 2010; Bartiromo, 2013). Any specific or predictable alteration in leaf cuticle due to pollution can serve as diagnostic marker of exposure to pollution type (Baker and Hunt, 1986). Nevertheless, Pb also induced changes in the leaf as more abundant wax coating and reduction in the cell size of epidermis (Weryszko-Chmielewska and Chwil, 2005). However, cuticle changes are still not use as specific markers of air pollution exposure, particularly to separate air pollution from other sources of stress (Berg, 1989). Usually, the anatomical changes of leaf laminas are not specific for a certain pollutant, as they result from the combine effects of different air contaminants that can be additive, synergistic or antagonistic (Kozlowski, 1986; Tomašević et al., 2008), but they result and can serve for evaluation of their adaptable capacity (Kovacic and Nikolic, 2005).

Acer saccharinum L. has a great coefficient of palisadness (65%), for the trees from the both places polluted and control (table 2). The leaf blades of *Acer saccharinum* L. have a typical mesomorph structure and are characterized with well-developed mesophyll with laterally contiguous spongy tissue and noncontiguous vertical palisade (fig. 3 and 4). The size of palisade parenchyma has a value $41.58 \mu\text{m}$ ($s = 7.7$) and the mean of spongy parenchyma $21.92 \mu\text{m}$ ($s = 6.68$).

Under the influence of industrial pollution, it has been registered significant expansion of palisade mesophyll and

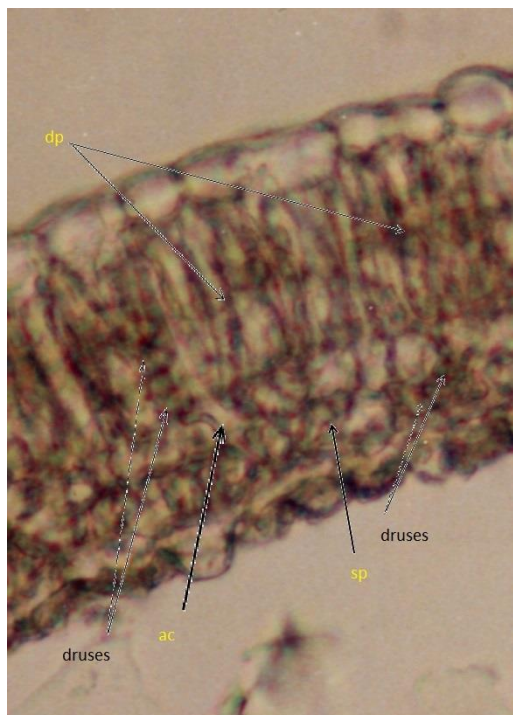


Figure 1. *Acer saccharinum* L cross section of leaf blade from Kremikovtzi region;
Legend: dp – double stratified palisade mesophyll; ac – air cavity; sp – spongy parenchyma; calcium oxalate druses

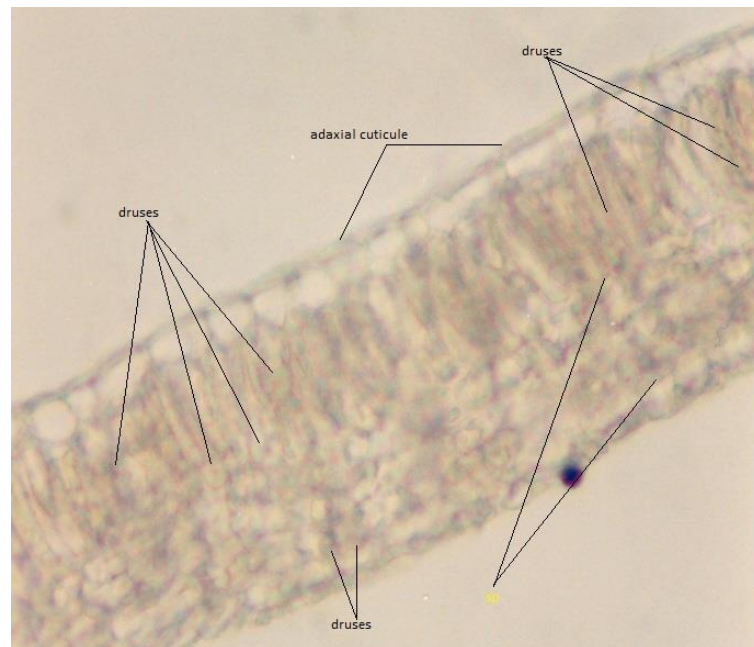


Figure 2. *Acer saccharinum* L cross section of leaf blade from Kremikovtzi region;
Legend: calcium oxalate druses sp – spongy parenchyma



Figure 3. *Acer saccharinum* L cross section of leaf blade from Vitosha region;
Legend: p – palisade mesophyll; ac – air cavity; sp – spongy parenchyma; calcium oxalate druses

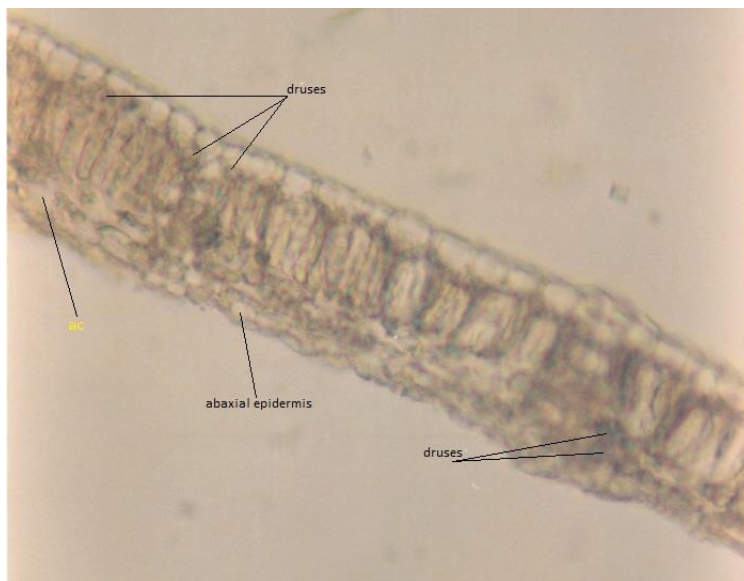


Figure 4. *Acer saccharinum* L cross section of leaf blade from Vitosha region;
Legend: ac – air cavity; sp – spongy parenchyma; calcium oxalate druses

spongy parenchyma, without alteration of their proportion (K%). The augmented palisade mesophyll can appear as bistratified (fig.1). The spongy mesophyll cells altered also under conditions of air pollution that can be defined as shortened cells with high compactness, densely

contiguous without air spaces (fig. 2). The small air cavities reduce the toxicant uptake and its speed of movement in the interface. The enhanced mesophyll is considered as adaptation to industrial contamination and has been registered from many researchers (Iqbal, 1985; Gostin,

2009; Noman et al., 2012). Plant adaptation to conditions of drought or large amounts of sunlight appears as increased palisade mesophyll also (Sefton et al., 2002).

Modifications of leaf anatomy and morphology in plants reflected on their adaptability to cope with the environmental stress and that allows this species to maintain good photosystem efficiency during the adverse conditions (Azmat et al., 2009). Thick palisade parenchyma is of superlative importance in plants growing under hampered conditions (Guerfel et al., 2009) and can be interpret as a potential for plasticity and adaptation in diverse conditions. Under low SO₂ dosages (1-4 µg/l) plants sinks pollutants and are not harmfully affected, but even can gain benefit from contamination (Kozłowski, 1986). Increased leaf palisade and spongy cell area under industrial polluted conditions is considered as healthier adaptability sign (Iqbal, 1985; Gostin, 2009).

In the cross-sections of *Acer saccharinum* L. (figure 2, and 4) are visible Ca oxalate crystal formations, but clear tendency between deposition of calcium druses and the ecological conditions were not found. Earlier in study of *Morus alba* L. laminas from polluted region have been observed much more Ca oxalate druses than those in control samples (Dineva, 2017). The calcium oxalate druses in silver maple lamina have been observed from Toma et al. (2015), found in the cellulosic parenchyma and collenchyma, in the phloem of the vascular fascicle of the midvein; but in the mesophyll the idioblasts of bigger size have not been observed. According to Toma et al. (2015), the appearances of crystals are most probably influenced by ecological factors. Tomašević et al. (2008) considered that plants exposed to stressful air conditions formed in the leaves numerous single or aggregated crystals.

CONCLUSIONS

Under conditions of industrially contaminated environment *Acer saccharinum* L. diminish the lamina surfaces, commonly accepted as a sign of adjustment to the unfavourable conditions. The leaf blades structure expressed some modifications regarding as healthier alterations and convey for high pliability. Under polluted circumstances *Acer saccharinum* L. well-maintained cuticle thickness and significantly decrease the size of adaxial epidermal cells that prevent from direct toxicant absorption and injury. The high coefficient of palisadness (65%), with the adapt amendments of mesophyll, stated as expanded and as bistratified palisade, with less and smaller air cavities in spongy parenchyma, afford a reason to classify *Acer saccharinum* L. as a tolerant species to industrial contamination and suitable for planting in such conditions.

Acer saccharinum L. showed good adaptable capacity and can be appropriate for constructing green belts around adverse point source emissions and for urban forestry.

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