



Deposition of Calcium Oxalate Crystals and Tolerance of Deciduous Trees to Pollution

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Received on June 6, 2019; Accepted on June 19, 2019.

Abstract

Plants are frequently utilized as biological indicators for the extent of air pollution. They are organisms which are sensitive to pollutants in the air and many studies are focused on morphological, physiological, and histochemical effect of air pollutants on plants. Various experiments have been conducted to explain the interaction of plants and those pollutants. Deciduous tree plant species growing naturally in Bulgaria, such as *Acer campestre* L. (field maple), *A. tataricum* L. (tatarian maple), and four cultivated: *A. negundo* L. (box elder), *A. saccharinum* L. (silver sycamore), *Morus alba* L. (white mulberry), and *Platanus acerifolia* Willd. (London plane) have been investigated. There were found increasing the formation of Ca-oxalate crystals in the lamellae of *Morus alba* L. under polluted air conditions or scattering of them in different way in *Acer negundo* L. The most pronounced depositions of different types of calcium crystals were observed in the most resistant to pollution and dry environment species *Morus alba* L. Further investigations are needed to specify the relation between biomineralization and specific environmental conditions.

Keywords: Calcium oxalate crystals, deciduous trees, pollution.

Introduction

Calcium oxalate crystals occur in more than 215 higher plant including gymnosperm and angiosperm families (McNair, 1932; Franceschi and Horner, 1980; Lersten and Horner, 2006). The morphologies and precise locations of crystals are under strict genetic control (Franceschi and Nakata, 2005). Therefore, the shape and location of the crystals within a taxon are often very specific and may be represented as a taxonomic character (Genua and Hillson, 1985; Prychid and Rudall, 1999; Lersten and Horner, 2000). Nevertheless, it is found that their shape, size and formation greatly changes under specific environmental circumstances (Umemoto and Hozumi, 1972; Weiner and Dove, 2003; Nakata and McConn, 2007; Tomašević *et al.*, 2008; Gostin I., 2010; Khan and Siddiqi, 2014; Gostin I., 2016; Cuadra and Cambi, 2017).

Even though the CaOx crystals have intrigued plant scientists for a long time, their functional roles are still poorly understood (Tooulakou *et al.* 2016). In *Tradescantia pallida*, crystal formation is influenced by mercury stress that increased raphide and prismatic crystals in the treated plants (Khan and Siddiqi, 2014). Plants growing under Hg stress have high metabolic rate that increase oxalic acid formation and formation of crystals which may be due to effort of plants to maintain ionic equilibrium (Khan and Siddiqi, 2014). One of the possibilities is that their accumulation is an indication

that these crystals are doing effective role in sequestration of metal ions (Franceschi and Nakata, 2005).

There are many evidences that the environmental factors such as air pollution, metal ions, salinity and other, influence on the number, size and shape of formed crystals within the species (Yang *et al.*, 2000; Choi *et al.*, 2001; Gostin., 2010 and Gostin., 2016). The addition of the heavy metals to the nutrient medium decreased the number of calcium oxalate crystals of hydroponically grown plants *Phaseolus vulgaris* (Jáuregui-Zúñiga *et al.*, 2005). Mercuric chloride at high doses increased all types of crystals in *Tradescantia pallida* as compared with low doses (Khan and Siddiqi, 2014). Cd, Pb, Ni, Mn and Al are detoxified through formation of metal-organic acid or metal-amino acid complexes. Cd and Zn resistance of *Atriplex halimus* L. is due to precipitation of metals in oxalate crystals (Lutts *et al.*, 2004). In *Oryza sativa* synthesis of oxalate represents a significant response mechanism that enhances tolerance to Pb (Yang *et al.*, 2000). Crystals of tobacco seedlings are composed of Ca-oxalate, in which toxic Cd is embedded, and under Cd stress production of that crystals increase (Choi *et al.*, 2001).

Material and methods

The material used for this study was collected from two regions: heavily polluted (42°47'N; 23°30'E) and relatively clear (42°30'N; 23°15'E). The trees from the both fields were sun exposure with uniform height and

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growth form. The investigated trees were 4 taxa of *Acer* genus (*Aceraceae* family), and 1 taxa of genus *Morus* (*Moraceae* family) and *Platanus* genus (*Platanaceae*). Of these, 2 species grow spontaneously in Bulgaria: *Acer campestre* L. (field maple), *A. tataricum* L. (tatarian maple), and 4 cultivated: *A. negundo* L. (box elder), *A. saccharinum* L. (silver sycamore), *Morus alba* L. (white mulberry), and *Platanus acerifolia* Willd. (London plane). Taxonomically, the *Acer* species are of 4 different sections: the Ginnala section (*A. tataricum*), the Negundo section (*A. negundo*), the Platanioidea section (*A. campestre*) and the Rubra section (*A. saccharinum*).

Plant tissue samples were taken randomly, from the middle parts of the leaf blades and fixed in FAA - 90% ethanol (90 cm³); iceacetic acid (5 cm³); and formalin (5 cm³). Standard histological techniques were used to examine the anatomy of the leaf blades.

Results and discussions

In the lamellae cross sections of observed species have been found numerous calcium biomineral formations in the leaf blades of plants from both regions (figure 1, 2, 3, 4). Among the various plant nutrients, calcium appears to occupy a unique position, acting as

an important regulator in many processes related to both growth and responses to environmental stresses (Lautner and Fromm, 2010). Oxalate is a common cellular constituent involved in Ca²⁺ regulation, ion balance, and metal detoxification (Franceschi and Nakata, 2005; Rahman and Kawamura, 2011). Ordinarily, plants exposed to stressful air conditions formed in the leaves numerous single or aggregated crystals (Tomašević *et al.*, 2008).

In laminas of *Morus alba* L. (figure1), formation of Ca oxalate druses and carbonate formations as cystoliths were found much more in leaf blades from polluted region (figure1, A) than those in control samples (figure1, B). The idioblasts of bigger size, cystolith-containing idioblasts were found on the adaxial epidermis of leaves (figure1, A), and in the mesophyll (figure1, B). According to Katsumata (1971), mulberry varieties can be classified based on the morphology of cystolith-containing idioblasts. In no other species the idioblasts have not been observed in their cross sections of leaf blades. Removal of heavy metal by accumulation in calcium crystals appears to be common mechanism among plants known to be tolerant to pollution.

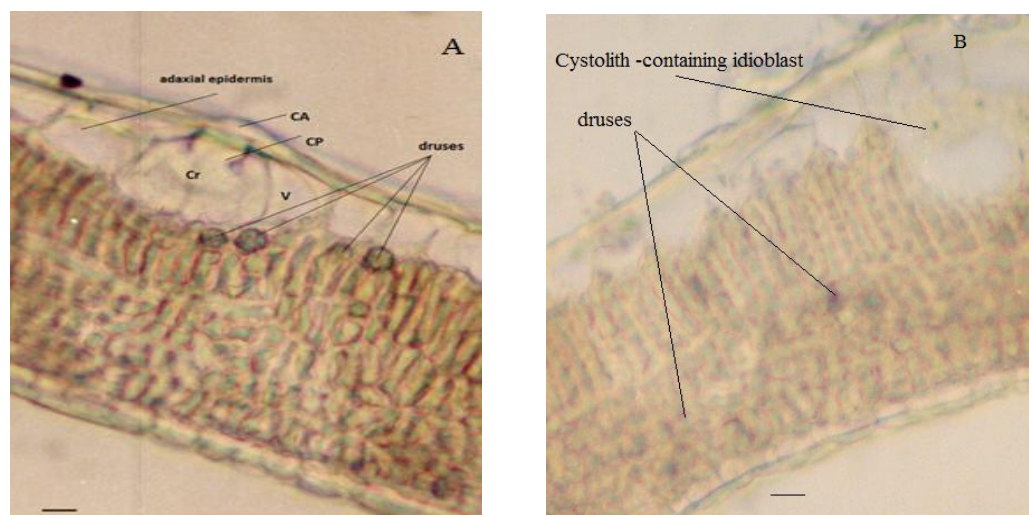


Figure 1. *Morus alba* L. – different types of Ca crystals.
(Cystolith -containing idioblast: CA – cap region; CP - cylindrical protuberance; Cr – crystal; V – vacuole)
(A) polluted area; (B) control.

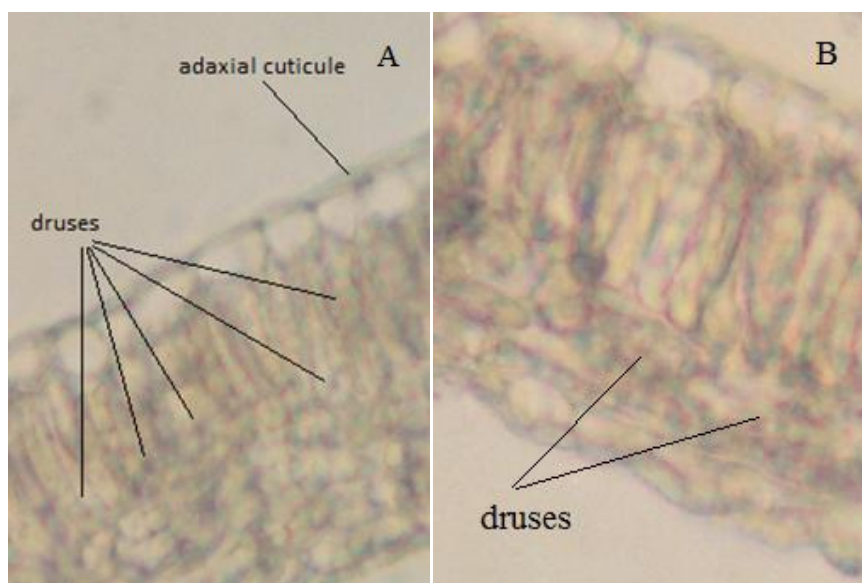


Figure 2. *Acer saccharinum* L.–Ca crystals (A) polluted area; (B) control.

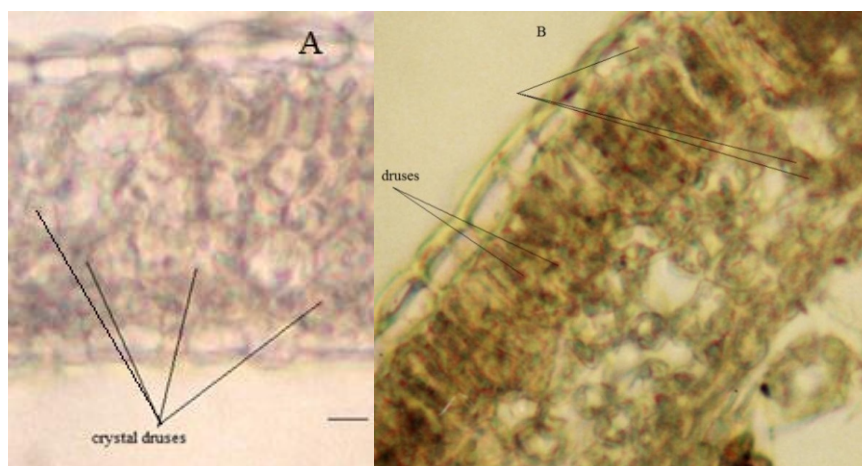


Figure 3. *Acer negundo* L.–Ca crystals formations polluted area; (B) control.

Ca-oxalate plays role in biologically controlled mineral deposition (Arnott 1983 and Webb 1999) and it is deposited in intravascular membrane chambers of specialize cells, idioblasts (Webb, 1999). For example, *Armeria maritima* accumulates Cu in idioblasts, by chelation with phenolic compounds (Lichtenberger and Neumann, 1997). Heavy metals ions are accumulated in calcium-containing crystal in idioblasts (Van Balen *et al.* 1980; Franceshi and Schueren, 1986; Van Stevenick and Fernando, 1995; Mazen and El Maghraby, 1997; Webb, 1999).

The calcium oxalate crystals have been observed on the cross sections of *Acer negundo* L. from both areas (figure3), but in polluted region the deposition in spongy parenchyma was much higher. The calcium

oxalate crystals were observed in *Acer negundo* L. from Toma, *et al.*, (2015) in the palisade mesophyll. In the clear environment the mineral formations were also mainly in palisade (figure 3,B).

In the investigated cross sections of *Acer tataricum* L. the mineral formation were found in both samples, polluted and control, scattered in palisade and spongy parenchyma (figure4, A, and B). In the tissue, they were densely distributed in the spongy tissue. According to Toma, *et al.*, (2015) in *Acer tataricum* L. the crystals as rhombic prisms are situated mainly in the spongy tissue.

In figure 5 it can be seen that in *Acer campestre* L. calcium crystals are situated in all mesophyll, and

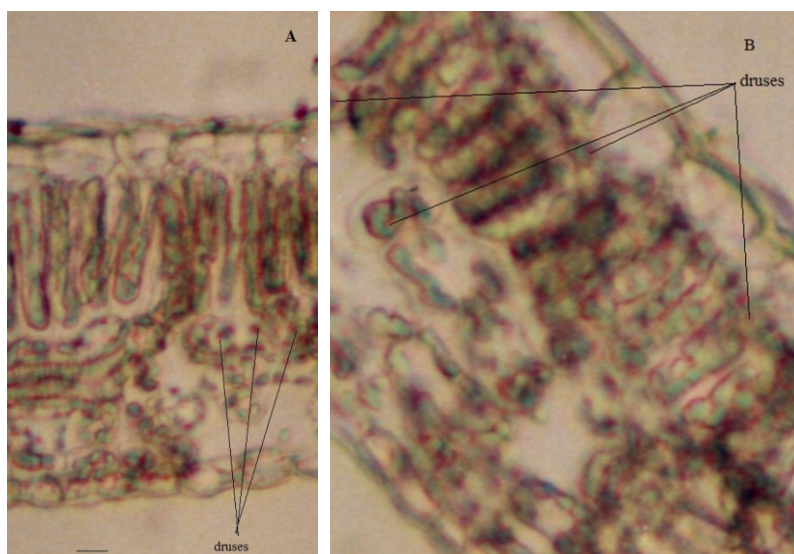


Figure 4. *Acer tataricum* L.– Ca crystals formations (A)polluted area; (B)control.

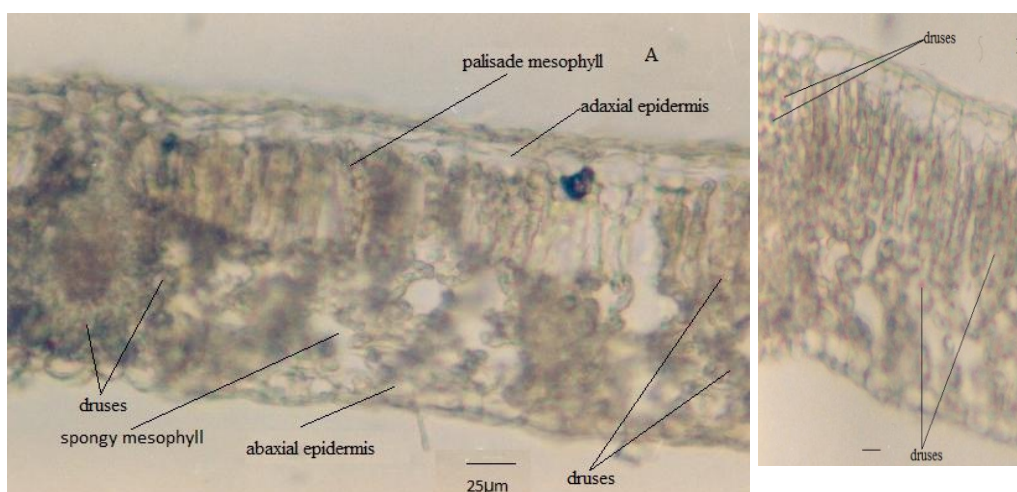


Figure 5. *Acer campestre* L.- Ca crystals formations (A)polluted area; (B)control.

around the vascular bundles (figure5, A and B). There were not pronounced differences in crystal deposition in the samples from both regions. In *Acer campestre* L. rhombic prism are found in the palisade (Toma, *et al.*, 2015). According to Toma *et al.*, (2015), the presences of the crystals are most probably influenced by ecological factors.

In the leaf of *Platanus acerifolia* Willd, druse crystals were observed under palisade in upper parenchyma tissue (Figure 6). About 9–10 crystals which arranged in one row of cells were observed along the margin between palisade and spongy mesophyll. However, only a few druse crystals were present in the palisade

tissue. Additionally, some of the druse crystals were in contact with the xylem or phloem vessels.

The formation of biominerals occurs as a result of interactions between biological activity and exact environmental conditions (Weiner and Dove, 2003). The mechanisms controlling calcium oxalate formation and type of crystals in plants remain largely unknown, most certainly must be a combination of genetic, environmental conditions, and nutrient availability (Nakata and McConn, 2007). In gingko leaves grown in a clean atmosphere crystals were small isolated sparsely scattered throughout the mesophyll, while in leaves from high polluted areas were found in clusters larger

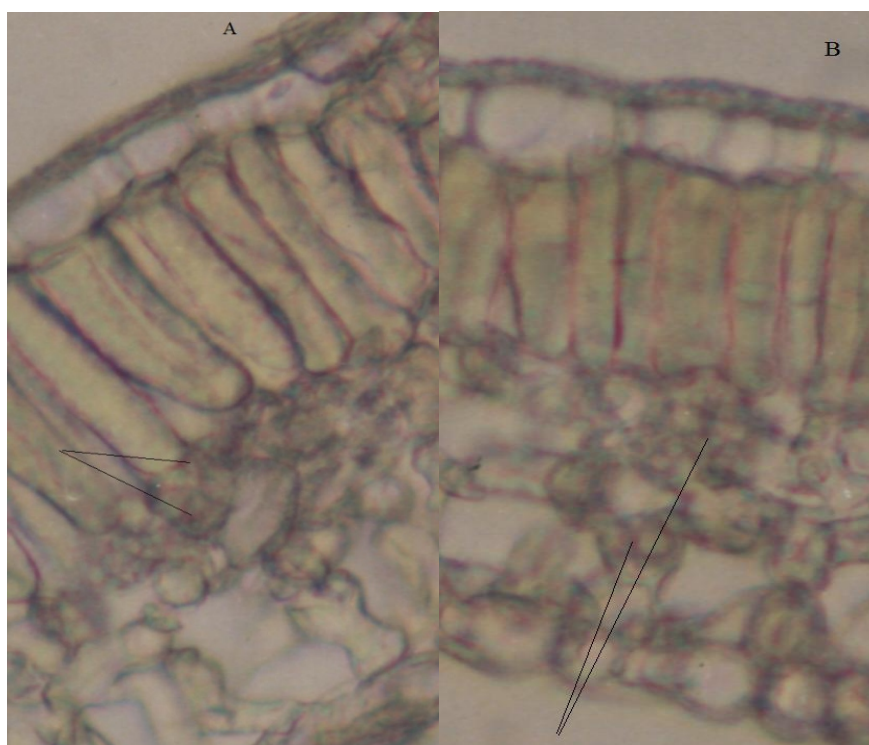


Figure 6. *Platamus acerifolia* Willd - Ca crystals formations (A) polluted area; (B) control.

and more irregular crystals lying along prominent veins (Umemoto and Hozumi, 1972). The alterations of size, type and distribution of calcium crystals were found among the plants, which are developed on soils with different salinity and amount of calcium (Cuadra and Cambi, 2017).

In *Abies alba* L. the needles from polluted region contain more calcium oxalate crystals observed as higher density compared to control (Gostin, 2010). Also it was found from Gostin (2016) that in the plants originating from extensively polluted area the frequency of the calcium oxalate crystals is increased. The similar results were found from Tomašević *et al.*, (2008), the leaves of *Aesculus hippocastanum* L. (Horse chestnut) and *Corylus colurna* L. (Turkish hazel) exposed to stressful air conditions were with numerous single or aggregated crystals.

Conclusions

In the polluted air environment the changes of leaf blade anatomy have been detected, as well as a deposition of biominerals. The observations showed that in the experiential tree species from the polluted region the formation of crystals in the leaf blades is increased in some of them, while in others have been changed the pattern of scattering. In the light of the

collected data and literature survey it can be concluded that there is no absolute and clear correlation between the presence of calcium oxalate crystals and tolerance of the plants to air pollution. Nevertheless, the biomineral depositions are strongly connected with the environmental conditions and can be used as biomarker for the certain environmental factors, but more data should be gathered.

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