

PHYSIOLOGICAL RESPONSE OF CHESTNUT (*AESCULUS HIPPOCASTANUM* L.) ROADSIDE TREES TO POLLUTION

Carmenica Doina JITĂREANU¹, Cristina SLABU¹, Doina Liana TOMA¹,
Alina Elena MARTA¹, Mirela RADU¹

¹ University of Agricultural Sciences and Veterinary Medicine of Iași

Abstract

Roadside trees are exposed to greater pollution caused both by exhaust emissions and road salt usage in winter months. To examine the pollution effects on *Aesculus hippocastanum* L. trees, leaves from damaged plants, located near the roadside were compared to leaves from healthy trees, located far away from the road. The plants from the roadside environment displayed marginal leaf necrosis accompanied by chlorosis.

The Na⁺ and Cl⁻ concentrations in the necrotic leaves were two- and 14-fold higher, respectively, compared to those of the control plants. This increase was correlated with a decrease in chlorophyll concentration in leaves. The latter may be explained as a result of high Cl⁻ concentration. This effect is further amplified by a simultaneously high Na⁺ concentration. The dehydration rate in leaves with toxicity symptoms was higher than in healthy ones, indicating an uncontrolled transpiration. Salt stress induced Na⁺ toxicity which caused an apparent K⁺ deficiency, primarily affecting stomatal closure. Furthermore, it caused high transpiration and loss of water. This may explain the appearance of marginal necrosis. In those trees located near the roadside high calcium, potassium, magnesium, copper, and iron concentrations were determined which are accompanied by a simultaneous deficiency of phosphorus and manganese.

Key words: *Aesculus hippocastanum*, pollution, salinity

Roadside trees are, to a greater extent, to pollution caused by both vehicle emissions and as a result of using the antiskid material during winter. Together with the antiskid, other salts are also distributed, most commonly sodium chlorides and calcium.

A great part of these salts, either after rainfalls or driven by the wheels of the vehicles, gets to the immediate proximity of the access ways, thus increasing the impact on the environment by contaminating the soil, the underground waters and producing physiological disorders to the trees used in street alignments. The sodium and especially the chlorine are mobile ions with a strong special impact on vegetation. For herbaceous plants, Munns (1993) has proposed a biphasic pattern of the reaction to saline stress. According to this pattern, at a *first phase* saltiness reduces the ability of plants to absorb water from the soil – a phenomenon known under the name of *physiologic drought* which causes the decrease in the growth rate. The initial breakage of the growth is due to the hormonal signals sent at the level of the roots (Munns, R., 1993). All along this first phase, within the bodies of the plant significant quantities of mineral ions bring together and they are meant to contribute to the increase of the plant's capability to lay in a stock of water from the soil. At a given point, the ions reach critical

concentrations and *the second phase* triggers, being caused by *the toxicity of the ions in excess*. During the second phase, one can observe differences among genotypes as far as resistance to saline stress is concerned. It may also be assumed that timber species react according to this biphasic pattern and that the searing of the alignment vegetation is the consequence of the storage, over the winter, of significant quantities of salts in the soil from the immediate proximity of the access ways.

The trees have characteristic aspects: *the chlorosis of the leaf unit*, as a result of the reduction in the concentration of chlorophyll pigments responsible for the photosynthesis process, followed by *necrosis* of the leaf edges which reduce significantly the assimilating surfaces of plants. Leaves sear and fall in the middle of the summer. Under special metabolic efforts, new leaves appear from buds but unfortunately they shall sear shortly, the phenomenon taking place a couple of times by the time autumn comes Bergmann, W. (1993), Marschner, H. (1995). The photosynthesis is perturbed, the plant can not feed itself, the existing reserves waste and, finally, the plant sears totally Fuhrer J. K. H. Erismann (1980), Kayama, M. et al (2003), Lutts S., et al (1996), Slabu Cristina et al (2009).

MATERIAL AND METHOD

In order to examine the effects of pollution on the *Aesculus hippocastanum* L species, it was used the vegetal material coming from trees situated in the immediate proximity of highways, which presented symptoms of metabolic disorders, represented by marginal necrosis of the leaf limbs accompanied by chlorosis. Healthy vegetal material was used as control.

The loss of water at leaf level was estimated based on the dehydration rhythm of the leaves by means of weighing to the precision balance respecting intervals of 1, 2, 3, 4 and 24 hours respectively (Toma Liana Doina et al., 1999).

The concentration of cations was determined by means of atomic absorption spectroscopy (AAS). The concentration of chlorophyll *Zörb C.* (2004) de ions was analyzed by Ag potentiometer titration. The concentration of the chlorophyll was determined spectrophotometrically in acetone extract (Toma Liana Doina et al., 1999).

RESULTS AND DISCUSSIONS

The evaluation of the temperature and precipitations during the active vegetation period (March – September) in 2009.

The metabolic disorders displayed through chlorosis followed by the mortification of the edges of the leaf limbs usually appear starting with the middle of July and are favored by reduced precipitations. Taking into account the climatic conditions of 2009, such disorders developed much earlier, down to the beginning of June. The analysis of the climatic factors of temperature and precipitations during the active vegetation period (March - September) reveals the fact that the year of 2009 was generally characterized by climatic stress conditions represented by dryness. In 2009 the drought was provoked less by the abnormalities of the thermal conditions which did not go too much over or under the multi-annual average values (*tab. 1*), but more by a permanent and constant deficit in precipitations which reached a total representing 55, 6% of the multi-annual average value (*tab. 2*).

Table 1

Dynamics of average monthly temperatures in 2009, during the growing season

	Month							Average
	III	IV	V	VI	VII	VIII	IX	
Average monthly temperature 1971-2000	3.5	10.3	16.1	19.5	20.8	20.0	15.6	15.11
Average monthly temperature	4.0	12.0	16.8	21.0	23.1	21.3	17.5	16.53
Deviation	+0.5	+1.7	+0.7	+0.5	+2.3	+1.3	+1.9	+1.42

Table 2

Dynamics of rainfall recorded in 2009 during the growing season

	Month							Total
	III	IV	V	VI	VII	VIII	IX	
Multi-annual average rainfall	30.7	50.7	62.7	97.6	81.8	58.0	56.4	437.9
Average monthly sum	30.6	1.1	30.8	57.1	30.1	36.8	7.7	194.2
Deviation	-0.1	-49.6	-31.9	-40.5	-51.7	-21.2	-48.7	-243.7

The dehydration rhythm of the leaves

As a result of the repeated weighing of the leaves, it was found that, no matter the moment of determination, water losses to leaves with marginal necrosis were significantly superior to the control leaves, which may stand proof that in this case the opening – closing mechanism of the stomata is damaged. This phenomenon usually takes place in the case of amassment in excess of the sodium

ions. The latter, at the level of the stomata cells, block the potassium channels, thus preventing further the stomata movements (Bhandal, I.S., Malik, C. P., 1988, Maathuis, F. J. M., Amtmann, A., 1999, Tester, M., Davenport, R., 2003). The phenomenon could be observed to *Vicia faba* L. (Slabu Cristina, et al., 2009, *Tilia cordata* Mill. (Slabu Cristina et al., 2009) plants exposed to the excess of NaCl.

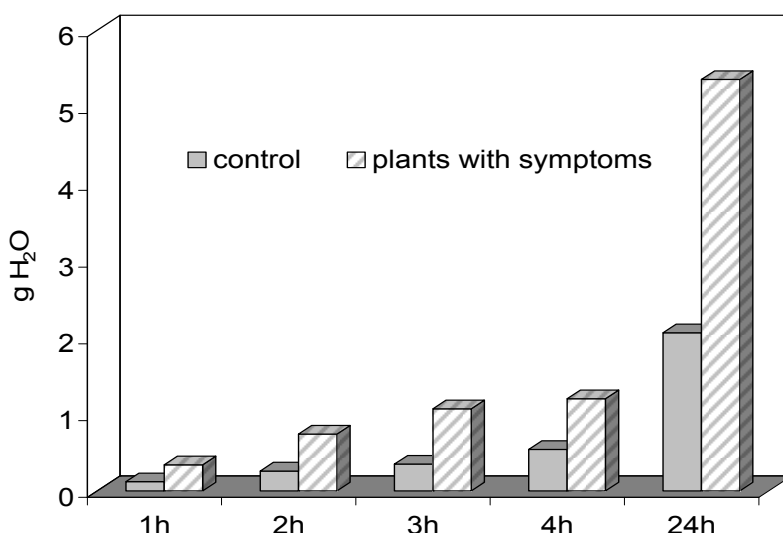


Figure 1 Pollution effect on dehydration rate of chestnut leaves

Concentration of the assimilatory pigments

For the determinations, the leaves of the control plants were entirely used and from the necrotic ones, only parts of the limb with no necrosis were taken. The analysis of the content of foliar pigments emphasized the decrease in the a-chlorophyll content of 663 nm and 431-432 nm, as well as the b-chlorophyll content 453-454 nm and an increase in the case of flavonoid pigments (fig 2). As far the content of the total chlorophyll is concerned, it was found a reduction of 23,3% to the plants with symptoms, compared to the control part: from 6,0 mg/g fresh substance to 4,5 mg/g fresh substance.

Consistent research have revealed the fact that plants exposed for long to salty stress have their chlorophyll concentration more reduced compared to control plants. Bergmann, W. (1993), Bergmann, W. (1993).

Concentration of sodium and chloride ions

To and in leaves of trees exposed to pollution, the concentration of the Na⁺ and Cl⁻ ions has exceeded 2 and respectively 14 times (fig 3 a,b), the concentration of the control plants, which being accumulated in excess at cell level get to chloroplasts where they produce the degradation of the chlorophyll molecules. The sodium ions, also found in excess, increase the toxicity of the chloride by means of a synergetic effect (Slabu Cristina et al., 2009). Fuhrer and Erismann (Fuhrer J. K. H. Erismann, 1980) have found to the horse chestnut tree the existence of a direct correlation between the chloride concentration at foliar level and the damage level of the leaves.

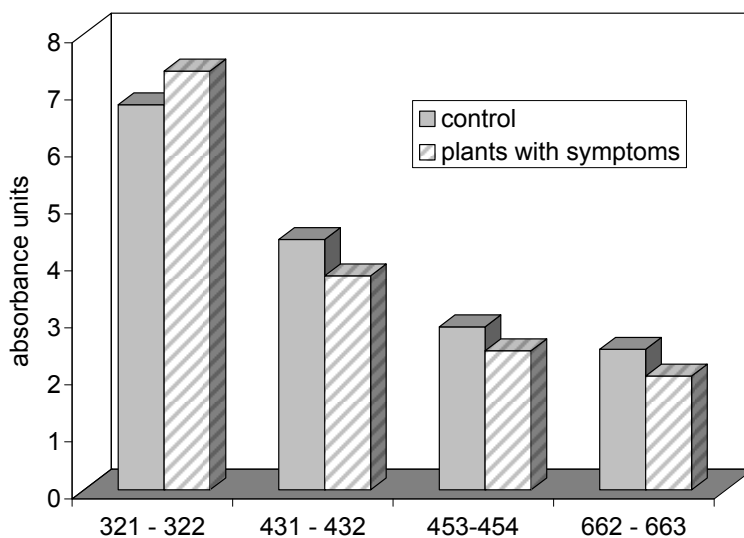


Figure 2 Pollution effect of assimilatory pigments

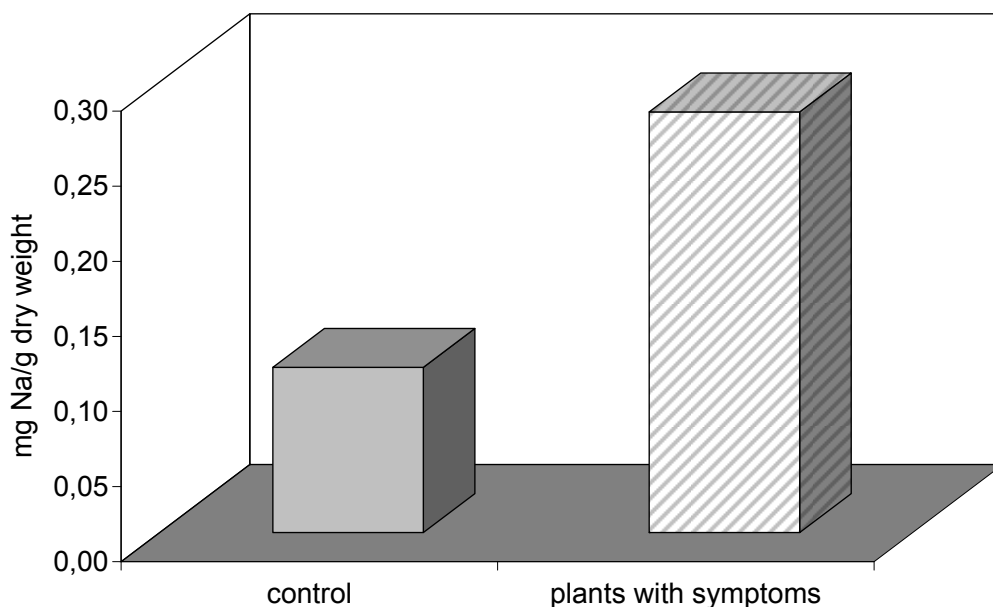


Figure 3a Pollution effect on sodium concentration in leaves

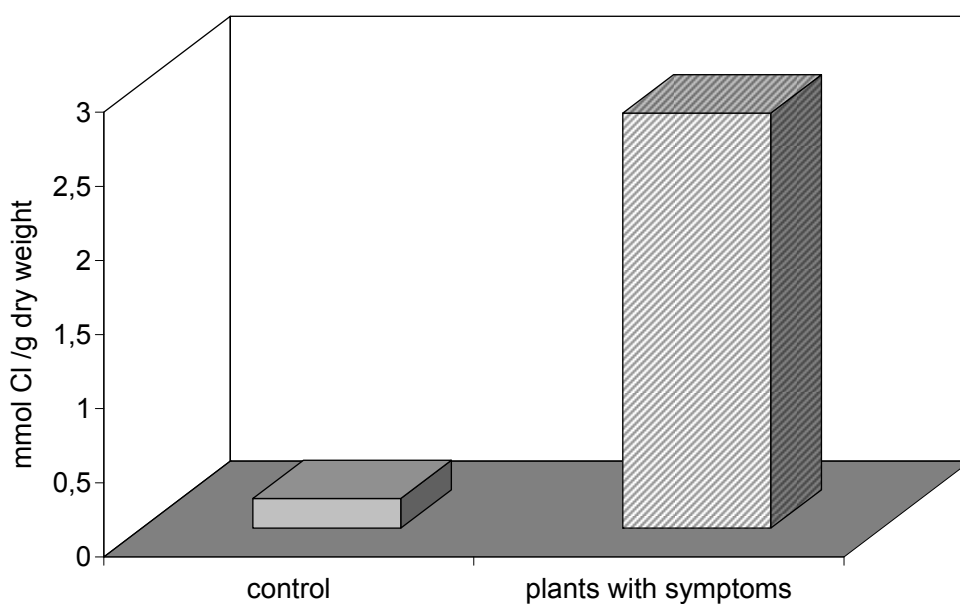


Figure 3b Pollution effect on chloride concentration in leaves

The nutritional state of plants

It can be appreciated by means of the analysis of the macro and micro-element concentration.

Among the macro-elements, there were analyzed: the potassium, calcium, magnesium and the phosphorus (*tab. 3*).

Potassium. The concentration of the potassium ions reached values between 11,0 mg/g dw to the control plants and 13,0 mg/g dw to marginal necrotic ones. As far the potassium supply of the plants is concerned, there is no data referring to chestnut trees in the specialty

literature. Comparing the values presented by Bergmann (1999) as normal for other arboreal species (0,6-1,8%), we may state that, in the case of the vegetal material taken under study, the plants do not present any shortages in potassium provisioning.

Calcium. Macro-element representing 0,2 and 1,5% of dw of the leaves of timber plants, it registered values between 15,0 and 19,5 mg/g dw to the plants taken under study, which proves an excess in calcium in the case of the plants with symptoms, the probable cause being the replacement of the sodium chloride from the

antiskid material with the calcium chloride. The direct effects of calcium ion excess are not known but they can indemnify the toxicity of the chlorine or sulfate ions.

Magnesium. The values of 3,5 - 4,3 mg/g dw, found in the leaves of plants under research prove an optimum provisioning with such element. However, the chlorophyll concentration in the leaves with symptoms was with 23,3% more reduces than in the control plants, fact which proves that the magnesium is stored in leaves with marginal necrosis in greater quantities outside the

chloroplasts, to assume in the vacuole, in order to increase the osmotic potential of the vacuolar sap.

Phosphorus. It recorded values between 3,0 – 1,5 mg/g dw, which proves an optimum provision, normal values, according to Bergmann (1993) Bergmann, W. ,1993 being of 1,2 - 3,0% from dw However, we may state that reduced phosphorus content in the leaves of the plants with symptoms proves the decrease of the energetic status of plants with repercussions upon the cell metabolism.

Table 3

Pollution effect of on the of potassium-, calcium-, magnesium- and phosphorus-ions concentration in leaves of chestnut trees

	CHEMICAL ELEMENT			
	Potassium (mg g/dw)	Calcium (mg g /dw)	Magnesium (mg g /dw)	Phosphorus (mg g /dw)
control	11.0	15.0	3.5	3.6
plants with symptoms	13.0	19.5	4.3	1.5

The contents of micro-elements. Compared to the data offered by the literature, the values resulted from the analysis of the concentrations of the copper, iron manganese and zinc (tab.4) prove an optimum provisioning. The plants with

symptoms, compared to the control plants, have shown higher values in the case of copper and iron ions and lower values in the case of manganese and zinc ions.

Table 4

Pollution effect on the copper-, iron-, manganese- and zinc- ions concentration in leaves of chestnut trees

	CHEMICAL ELEMENT			
	Copper (ppm)	Iron (ppm)	Manganese (ppm)	Zinc (ppm)
control	17.3	150.2	19.6	26.5
plants with symptoms	18.2	271.7	5.7	23.5
normal values (from literature)	2 - 20	50 - 1000	20 - 200	10 - 100

CONCLUSIONS

During summer the alignment trees present symptoms characteristic to some metabolic disorders: the chlorosis of the leaf unit accompanied by necrosis of the limb edges.

The necrosis are a consequence of the excess in sodium which prevent the closing of the stomata, leading to the uncontrolled loss of water while the dehydration rhythm of the leaves presenting toxicity symptoms is always higher than in the case of the control plants.

The chlorosis, occurred as a result of the decrease in the chlorophyll concentration, are a consequence of the chlorine ions stored in excess, amplified by the presence of the sodium.

The high concentrations in calcium, magnesium, potassium, copper, iron and the deficit in phosphor and manganese ions, determined in the

alignment tree leaves compared to the control plants, prove the existence of some nutritional disorders although there are no characteristic symptoms.

The nutritional disorders manifested by an excess in calcium, magnesium, potassium, copper and a deficit in phosphor and manganese ions.

BIBLIOGRAPHY

- Bergmann, W. ,1993** - *Ernährungsstörungen bei Kulturpflanzen. 3rd Edition, Gustav-FischerVerlag, Jena, Stuttgart.* p. 127–137.
- Bhandal, I. S., Malik, C. P., 1988** - *Potassium estimation, uptake, and its role in the physiology and metabolism of flowering plants,* Int. Rev. Cytol. 110, p. 205–254.
- Blomqvist, G. & Folkesson, L. 2001** - *Indicators for monitoring the system of de-icing salt use and its impacts on groundwater, vegetation and societal assets,* Ph.D. Thesis, Royal Institute of Technology, Stockholm. <http://www.ectri.org/YRS05/Papiers/Session-1ter/blomqvist.pdf>.

- Fuhrer, J.K., Erismann, H., 1980** - *Tolerance of Aesculus hippocastanum L. to foliar accumulation of chloride affected by air pollution, Eironmemat Pollution (Srite A)* 21. p. 249-254.
- Kayama, M., Quoreshi, A. M., Kitaoka, S.; Kitahashi, Y., Sakamoto, Y., Maruyama, Y.; Kitao, M., Koike, T., 2003** - *Effects of de-icing salt on the vitality and health of two spruce species, Picea abies Karst., and Picea glehnii Masters planted along roadsides in northern Japan*, Environ. Pollut., 124, p. 127–137.
- Lutts, S., Kinet, J.M., Bouharmont, J., 1996** - *NaCl-induced senescence in leaves of rice (Oryza sativa L.) cultivars differing in salinity resistance*. Ann. Bot. 78, p. 389–398.
- Marschner, H., 1995** - *Mineral Nutrition of Higher Plants*, 2. Aufl., Academic Press, London.
- Maathuis, F.J.M., Amtmann, A., 1999** - *K⁺ nutrition and Na⁺ toxicity: the basis of cellular K⁺/Na⁺ ratios*, Ann. Bot. 84, p. 123–133.
- Munns, R., 1993** - *Physiological processes limiting plant growth in saline soils: Some dogmas and hypotheses*, Plant Cell Environ. 16, p.15-24.
- Pranas, B., Kazlauskienė, Agnė, Zaveckytė, J., 2006** - *Experimental investigation into toxic impact of road maintenance salt on grass vegetation*, J. Environ. Eng. and Landsc Manag, 14, 83–88.
- Slabu, Cristina, 2008** - *Effect of sodium and chlorine excess on chlorophyll pigments in leaves of Vicia faba L. plants*, Lucr. șt., seria Horticultura, vol. 49, U.Ș.A.M.V. Iași, p. 30-36.
- Slabu, Cristina., Zörb, C., Steffens, D., S. Schubert, 2009** - *Is salt stress fo faba bean (Vicia faba L.) caused by Na⁺ or Cl⁻ toxicity?* J. Plant Nutr. Soil Sci. 172, p. 644-650.
- Slabu, Cristina, Jitareanu, Carmen-Doina, Toma, Liana-Doina Robu, T., Marta, Alina-Elena, Radu, Mirela, 2009** - *Ecological impact of de-icing salt on Tilia cordata Mill. plants from roadside environment*, Lucr. șt., seria Agronomie, vol. 51, U.Ș.A.M.V. Iași, p. 40-44.
- Tester, M., Davenport, R., 2003** - *Na⁺ tolerance and Na⁺ transport in higher plants*, Ann. Bot. 91, p. 503-527.
- Toma, Liana-Doina, Milica, C., Robu, T., Jitareanu, Carmen-Doina, 1999** - *Fiziologie vegetală – Indrumator de laborator*, Edit. Ion Ionescu de la Brad, Iasi, p.43 - 44.
- Yokoi, S., Brestan, R. A., Hasegawa, P. M., 2002** - *Salt Stress Tolerance of Plants*, In: JIRCAS Working Report, p. 25–33.
- Zhu, J.K., 2001** - *Plant salt tolerance*, Trends Plant Sci., 6, 66–71.
- Zörb, C., Wiese, J., Wiese, H., Krämer, C., Yan, F., Mühling, K.H., Schubert, S., 2004** - *Biochemische Praktikumsversuche*. Verlag Grauer, Beuern-Stuttgart.