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DE-ICING SALT IMPACT ON LEAVES OF STREET TREES (*TILIA X VULGARIS* H.) IN RIGA, LATVIA

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In Riga (Latvia), common lime (*Tilia x vulgaris* H.) is a popular taxon used for street greenery. The foliar accumulation of de-icing salt (NaCl), spread on roadways during winter and translocated to leaves during subsequent vegetation seasons, has been shown to cause leaf necrosis and contribute to the decline of street trees. However, injury and tolerance mechanisms in response to salt accumulation in *Tilia* spp. are still poorly understood. Thereby the main objective in this study was to analyse the effects of de-icing salt (NaCl) accumulation in leaves of street trees on the concentration of nutrients and leaf structure.

Leaf samples of *Tilia x vulgaris* were collected at 7 uncontaminated and contaminated street sites in the centre of Riga and one control site in the National Botanical Garden (NBG) in September 2014 before leaf yellowing. After evaluating the percentage of necrotic leaf area, leaf samples were prepared with a view to chemical and microscopical analysis. The leaf concentration of N, P, K, Ca, Mg, S, Fe, Mn, Cu, Zn, Mo, B, Na, and Cl was determined. The structural injuries in the central and rim part of leaves were analysed by means of semi-thin resin-embedded sections observed in diascopic light microscopy.

The results revealed that in comparison to lime trees from the NBG, the asymptomatic leaves of healthy street trees contained higher concentration of Na, Cl, P, Ca, Mg, Fe, Mn, Zn, Cu and Mo and showed no nutrient deficiency. In symptomatic foliage, the amounts of K, Ca, Mg and Mn were significant lower to compare with the concentration of these elements in the asymptomatic foliage in the healthy street trees. In severely damaged leaves, the concentration of K and Mn was close to the deficiency limit. In street trees, the intensity of leaf necrosis was positively correlated to leaf concentration of Na, Cl, Zn, and Mo but negatively to that of K. Given the chemical composition of de-icing salt, positive correlations between Na and Cl on one hand and Zn, and Mo on the other hand could indicate the simultaneous uptake of salt and other environmental contaminants. Negative correlation between salt and Ca, B and Mg may relate to injury in leaf cells caused by NaCl accumulation. In response to salt accumulation, the mesophyll cells in symptomatic leaves showed an increased turgescence and the disruption of cell compartments in the case of the most contaminated samples.

Key words: NaCl, mineral nutrition, micromorphological changes, leaf damages.

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INTRODUCTION

Street trees and other urban vegetation provide city inhabitants with different sorts of ecosystem services, including the mitigation of air pollution, attenuation of climate extremes or improvement of biodiversity. In Riga (Latvia) within the boreo-nemoral zone along the Baltic Sea as well as in other European regions, common lime (*Tilia* x *vulgaris* H. known also as *T. x europea* L., *T. intermedia* DC., *T. x hollandica* K. Koch.; Bengtsson 2005) is a popular taxon used for street greenery because of its robustness and tolerance to various environmental constraints and also for cultural reasons. Since the end of last century however, the extensive decline and mortality of street *Tilia* has been observed and led to increased tree felling and replacement by young trees. The foliar accumulation of de-icing salt (NaCl), spread on roadways during winter and translocated to leaves during the subsequent vegetation seasons, has been shown to cause leaf necrosis and contribute to the decline of street trees, not only in the urban areas of Latvia (Cekstere et al. 2008, Cekstere & Osvalde 2013), but also in other countries (Dobson 1991, Chmielewski et al. 1998, Bryson & Barker 2002, Paludan-Müller et al. 2002, Kwasowki & Czyz 2011, Dmuchowski et al. 2014, Baczewska et al. 2014). Within leaf cells, the accumulation of Na and Cl ions can decrease chlorophyll concentration, inhibit membrane functions, impair several enzymatic activities, etc., and, as a consequence, significantly affects essential physiological activities such as e.g. leaf gas exchanges and photosynthesis (Munns 2002, Sieghardt et al. 2005). By lowering the osmotic potential of soil solution, the accumulation of de-icing salts can also reduce the water availability and cause a so-called 'physiological drought stress' (Dobson 1991). Moreover, the uptake of Na and Cl ion can interfere with that of other nutrients and affects the mineral nutrition of street trees (Marschner 1995). Throughout the northern hemisphere, a wide range of optimal and sub-optimal concentrations of nutrients in *Tilia* sp. leaves has been documented (Bergmann 1988, Kopinga & van den Burg 1995, Nollendorfs 2003, Hagen-Thorn et al. 2004, 2005, Dmuchowski et al. 2014). According to different investigations (Hagen-Thorn et al. 2004, Mertens et al. 2007) there are differences in plant nutrient accumulation between species growing in the same soils, as well as differences due to sampling time, pollution, regional variations, etc. Low levels of essential nutrients in foliage - sometimes below the deficiency limit despite a sufficient supply from the soil - raise the question of the stress, defence and tolerance reactions in foliage in response to the accumulation of de-icing salts (Günthardt-Goerg & Vollenweider 2007, Hermle et al. 2007, Vollenweider et al. 2011). Our previous work on horse-chestnut and lime in Riga showed a severe imbalance in the mineral nutrition of old urban trees and toxic effect of NaCl, as shown by leaf necrosis (Čekstere et al. 2005, Cekstere et al. 2008, Čekstere 2011). However, the injuries, as well as tolerance mechanisms in response to salt contamination in foliage of Tilia x vulgaris are still poorly understood. Therefore, the main objective in this study was to analyse the effects of de-icing salt (NaCl) accumulation in leaves of *Tilia x vulgaris* planted in streets of Riga, Latvia, on the concentration of nutrients and leaf structure.

MATERIAL AND METHODS

Study sites

The study was conducted in Riga, Latvia, in the boreo-nemoral climate zone (56.9489° N, 24.1064° E). With average annual precipitation of 708 mm, temperature in January of −3.0 ◦C and July of +17.7 ◦C, the climate in Riga is moderately warm and humid (Lizuma 2000). In Riga, parks, gardens, squares and other greenery represent 8 % of the percentage area in the city centre (Nikodemus et al. 2003). Soils in the central part of Riga could be characterized as artificial, sandy, highly heterogeneous and compacted. The average content of nutrients in the topsoil of the studied sites in Riga using 1 M HCl extract are: N 36; P 407; K 163; Ca 18776; Mg 6390; S 19; Fe 2243; Mn 137; Zn 138; Cu 48; Mo 0.07; B 0.29 mg/l, but pH/KCl – 6.94; in

NBG: N 39; P 354; K 177; Ca 6087; Mg 2220 mg/l, and pH/KCl – 6.74 (unpublished data by Cekstere, G). Trees in the street greenery are mainly not watered. Regularly increased air pollution level can be stated. In 2013, the average parameter values for air quality in a street (K Valdemara) with high intensive traffic in the city centre were: NO_2 50.6, NO 94, O_3 29.9, CO 400, PM10 41.7 $\mu g/m^3$ (Gaisa piesārņojuma …, 2014).

Sampling

In September 2014 before leaf yellowing started, foliage material was collected at 7 typical uncontaminated and contaminated street sites lined with lime trees in the centre of Riga and one control site in the National Botanical Garden (NBG) at Salaspils, 20 km from Riga. In each site, foliage samples were collected from three trees (total = 24 trees) with a view to leaf chemical and microscopical analysis.

After pruning one branch per tree, the percentage of necrotic leaf area (6 classes of injury severity: 0 %, 1-5 %, 6-15 %, 16-30 %, 31-50 %, 51-100%) was estimated and leaf material sampled with a view to analysis back to the laboratory. For the microscopical analysis of Na and Cl effects on the tissue and cell structure, disks 10 mm in diameter were excised from the asymptomatic leaf central part and next to necrosis in the leaf rim using a cork borer and the 2nd or 3rd leaf from the branch apex. They were immediately fixed by immersion in 2.5% LM grade glutaraldehyde, buffered at pH 7.0 with 0.067 M Soerensen phosphate buffer, within 1.5 ml Eppendorf tubes with screw caps (two disks per Eppendorf tube). Back in the laboratory, samples were fully evacuated prior to storage at 4 °C until further processing.

Laboratory analysis

The leaf concentration of Ca, Mg, Fe, Cu, Zn, Mn, N, P, Mo, B, K, Na, and Cl was determined at the Laboratory of Plant Mineral Nutrition of the Institute of Biology of the University of Latvia. The levels of Ca, Mg, Fe, Cu, Zn and Mn was estimated by AAS (*Perkin Elmer AAnalyst 700*), that of N, P, Mo, B by colorimetry, S by turbidimetry using a *JENWAY 6300* spectrophotometer, K and Na by flame photometer (*JENWAY PFPJ*) and Cl by $AgNO_3$ titration (Ринькис и др. 1987). Analytical replication was three times.

The microscopical analyses were performed at WSL in Switzerland. Leaf material was dehydrated with 2-methoxyethanol (3 changes), ethanol, *n*-propanol, *n*-butanol and embedded in Technovit 7100 resin. Semi thin sections (1.5 µm thick) were cut using a *Reichert* UltraCut S ultramicrotome, stained with toluidine blue and acid fuchsine, mounted in DPX and observed using a diascopic light microscope *Leica DMRB* microscope and 5x to 100x objectives. Micrographs were taken using the *Luminera INFINITY 2 ANALYZE* camera and software.

Statistical analysis

Statistical analyses were carried out with *RStudio*. Standard errors (SE) were calculated in order to reflect the mean results of chemical analysis. The Student's t-test was used to detect differences between the chemical characteristics in the leaf samples from healthy, damaged trees and the background level. Correlation coefficients (*Pearson*) between nutrients, salt contaminants and visible injury were calculated.

RESULTS AND DISCUSSION

The mean element concentrations and ranges of foliar nutrients and contaminants according to location and level of leaf injury are given in Tables 1 and 2. These results show that in comparison to lime trees from the NBG (background level), the asymptomatic foliage in healthy street trees contained higher concentration of Na, Cl, P, Ca, Mg, Fe, Mn, Zn, Cu and Mo. In comparison to reference values (K > 1.00 %, Mn > 22 mg/kg, Zn > 20 mg/kg; Bergmann 1988, Nollendorfs 2003, Čekstere 2011, Dmuckowski et al. 2013), the Zn, Mn and K level at the NBG could be characterized as in the deficiency due to, e.g., unfavourable soil conditions reducing element bio-availability or facilitating element antagonism. By contrast,

Table 1. Macronutrient content (%) in *Tilia x vulgaris* leaves in Riga in September 2014

Tree characterization	N	P	K	Ca	Mg	S
NBG, background level (n=3*)						
Mean	1.91 A	0.23 A	0.93 A	1.85 A	0.41 A	0.14 A
SE	0.08	0.00	0.07	0.08	0.04	0.00
Healthy street trees without leaf necrosis (n=3)						
Mean	2.00 Aa	0.28 Ba	1.11 Aa	2.04 Ba	0.67 Ba	0.14 Aa
SE	0.13	0.02	0.18	0.07	0.11	0.00
Tree leaves with necrosis (n=18)						
Mean	2.04 Aa	0.25 Aa	0.75 Bb	1.53 Bb	0.45 Ab	0.14 Aa
SE	0.05	0.01	0.10	0.07	0.04	0.00

*n – number of trees; SE – standard error; for each element, different letters indicate significant differences with amounts in the background (capital letters) and asymptomatic street (small letters) samples (t-test, $p<0.05$)

no plant nutrient deficiency was observed in asymptomatic foliage of healthy lime trees in the street greenery of Riga.

In symptomatic street foliage however, the concentration of K, Ca, Mg and Mn was significant lower to compare with the concentration of these elements in the asymptomatic foliage in the healthy street trees. In severely damaged leaves, the concentration of K and Mn was close to the deficiency limit, with levels of K being 3-4 times lower than those to be found in healthy street leaves. During the vegetation season, the K content in plant foliage usually decreases but that of Ca and Mn increases. In Riga the overall decrease of nutrient concentration in damaged tree leaves could be promoted by the abundance of antagonistic ions, especially Na^+, as well as by the insufficient level of foliar K in the context of elevated salt contamination. Indeed, low levels of K in symptomatic street foliage may cause various disturbances to the tree physiology, given the essential function of that element with regard to the osmoregulation, maintenance of electrochemical equilibria, protein conformation, regulation of enzyme activity etc. (Marschner 1995). According to previous investigations, the soils in Riga have elevated contents of Ca and Mg and show neutral or slightly alkaline soil reaction (Cekstere & Osvalde 2013). These soil conditions could also contribute to reduced Mn bio-availability.

The severity of leaf necrosis was positively correlated to the leaf concentration of Na, Cl, Zn, and Mo, but negatively to that of K (Table 3). Given the chemical composition of de-icing salt in Riga (NaCl), positive correlations between Na, Cl on one hand and Zn, and Mo on the other hand could indicate the simultaneous uptake of salt and other environmental contaminants by street trees. Alternatively in the case of Mo, they may also relate to plant resistance mechanisms to increased salinity: Mo-containing aldehyde oxidase is a key enzyme for catalyzing the final reactions of abscisic acid (ABA) biosynthesis (Huang et al. 2009) and ABA-mediated signalling plays a key role with regard to plant responses to salt stress (Zhu et al. 2005).

Negative correlation between salt and Ca and B concentration may relate to cell injury in mesophyll caused by NaCl accumulation, as these elements are involved in cell membrane formation (Marschner 1995). This finding could indicate to ion antagonism (Khan et al. 2003), similar to previous findings on street greenery in Riga (Čekstere 2011).

The leaf injury threshold (injury severity of 1-5 %) was exceeded when the Na and Cl leaf concentration ranged between 660-3100 mg/kg and 3000-7570 mg/kg, mainly similar or lower to previous findings with regard to Na (1800-2600 mg/kg) and Cl (6200-6600 mg/kg) (Čekstere 2011). According to Leh (1973), the toxicity limit for Cl in leaves of *T. x vulgaris* ranges between

Table 2. Micronutrient, Na and Cl content (mg/kg) in *Tilia x vulgaris* leaves in Riga, September 2014

Tree characterization	Fe	Mn	Zn	Cu	Mo	B	Na	Cl
NBG, a background level (n=3*)								
Mean	122.00 A	17.53 A	15.73 A	6.20 A	0.37 A	23.00 A	86.00 A	760.00 A
SE	9.87	2.27	0.81	0.12	0.04	2.89	7.57	120.97
Healthy street trees without leaf necrosis (n=3)								
Mean	493.33 Ba	29.33 Ba	21.33 Ba	15.40 Ba	0.84 Ba	24.33 Aa	116.67 Ba	4160.00 Ba
SE	40.55	2.91	0.67	1.47	0.03	3.18	9.40	780.77
Tree leaves with necrosis (n=18)								
Mean	435.56 Ba	22.12 Bb	25.33 Bb	18.19 Ba	1.32 Bb	22.89 Aa	5780.00 Bb	6669.44 Bb
SE	25.54	1.35	0.93	3.06	0.15	1.03	727.47	1014.94

*n – number of trees; SE – standard error; for each element, different letters indicate significant differences with amounts in the background (capital letters) and asymptomatic street (small letters) samples (t-test, $p<0.05$)

Table 3. Pearson's correlation coefficients between leaf necrosis and chemical element concentrations in *T. x vulgaris* leaves

Parameter	Na	Cl	N	P	K	Ca	Mg	S	Fe	Mn	Zn	Cu	Mo	B
Necrosis	**0.66**	**0.49**	-0.01	0.00	**-0.66**	-0.15	0.33	-0.33	0.14	0.03	**0.40**	-0.29	**0.40**	-0.07
Na	1.00	**0.60**	-0.04	-0.17	-0.31	**-0.67**	-0.25	-0.37	0.38	0.22	**0.68**	-0.16	**0.47**	**-0.44**
Cl	**0.60**	1.00	-0.28	-0.14	-0.06	-0.31	0.07	-0.19	0.37	0.12	**0.47**	0.07	0.23	-0.35

Values in bold: significant, *$p<0.05$*

18900–24300 mg/kg whereas other authors working on *Tilia* sp. proposed a threshold ≥ 9000 mg/kg (Kopinga and van den Burg 1995), ranging between 6000-10000 mg/kg (Dmuchowski et al. 2013) or above 3291 mg/kg (Czerniawska–Kusza et al. 2004, Dmuchowski et al. 2013). Irrespective the precise threshold value, these reports consistently indicate that leaf necrosis observed in *T. x vulgaris* in Riga during September 2014 should be attributed to the leaf accumulation of both Na and Cl up to toxic levels. As a consequence, the leaf content of both elements should be always determined prior to making any conclusion regarding the impact of de-icing salt on tree foliage and mechanistic relationship with leaf necrosis.

Observations in light microscopy revealed several structural changes in the leaf samples from salt-polluted *versus* unpolluted sites. At leaf level, these alterations increased in severity along the injury gradient between the leaf centre and leaf rim. They included a slight increase in the cell size, a more turgescent cell shape, an increase in the vacuolar volume and the disruption of cell content in the most extreme cases. In other studies, a reduction in the cell size and increase in the intercellular space as well as a higher stomatal density (Curtis & Läuchli 1987, Valenti et al. 1991, Iyer & Barnabas 1993) or different changes in the cell structure including plasmolysis, vesiculation of cytoplasm, distension of ER membranes, swelling of plastids (Pareek et al. 1997, Fink 1999) and the decrease in chlorophyll content or degradation of chloroplasts (Mitsuya et al. 2000, Yamane et al. 2012) have been reported. In coffee leaves, salt stress caused alterations in cell wall polysaccharides, increased monolignol content and structural damage to the mesophyll cells (Lima et al. 2014), but in pomegranate leaves - an increase in the cuticle thickness (Zarinkamar & Asfa 2005).

Hence our structural findings on *T. x vulgaris* appear to be preliminary and supplementary findings, at least partly matching previous reports, are expected from complementary assessments including cell size measurements and observations in transmitted electron microscopy. All findings will be compared to the microlocalisation of salt contaminants within tissue and cell compartments.

CONCLUSIONS

In lime trees lining the streets of Riga, mostly anthropogeneous soil can still provide trees with an adequate supply of nutrients. However, primarily the accumulation of NaCl from road de-icing salt and secondarily that of other environmental contaminants were found to cause severe macro- and micronutrient imbalance in foliage with especially the leaf content of K being negatively affected. Thereby, not only the toxic accumulation of salt but also nutrient imbalances appear to be responsible for the injury in foliage and tree decline observed in Riga's greenery. The toxicity mechanisms should be clarified by analysising the microlocalisation of contaminants and the structural effects of salt accumulation within leaf cells.

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