Effect of salt stress on the chemical composition of leaves of different tree species in urban environment

Article *in* Fresenius Environmental Bulletin · January 2013

EFFECT OF SALT STRESS ON THE CHEMICAL COMPOSITION OF LEAVES OF DIFFERENT TREE SPECIES IN URBAN ENVIRONMENT

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ABSTRACT

Urban soil salinity caused by deicing of streets and sidewalks with sodium chloride in the winter season is considered to be the main cause of the poor state of health and dying of street side trees. The purpose of the study was to determine the effect of soil salinity on changes in the contents of microelements (Zn, Cu, Fe, Pb) in leaves of 5 urban tree species (*Quercus rubra* L., *Gleditsia triacanthos* L., *Platanus* x *hispanica* Mill., *Robinia pseudoaccacia* 'Umbraculifera', and *Tilia* x 'Euchlora') that were significantly different in their resistance to salt stress and growing in diverse habitats: the first row away from the main streets in the city center, and in the central areas of downtown parks. The control trees were growing in the Botanical Garden of the Polish Academy of Sciences in Powsin, in a suburban setting far from roads and other emission sources. Considerable differentiation was found in the content of chlorine and sodium in the leaves, both between the studied species of trees and between their locations. The leaves of *Q. rubra* contained a minimal amount of chlorine in comparison with the other species of trees at all the studied sites. The leaves of *T.* 'Euchlora' with strong damage contained extremely high sodium contents. The study showed very little or no effect of salt stress on the content of microelements in the leaves of the trees. Salt stress had no effect on the occurrence in the leaves of deficient levels of Cu and Zn, or toxic levels. The study confirmed the usefulness of *Q. rubra*, *R. pseudoaccacia* 'Umbraculifera', *P.* x *hispanica* and *G. triacanthos* for street side planting in city centers, and the high sensitivity of *T.* 'Euchlora'.

KEYWORDS: Salt stress, deicing, trace elements, ion balance, urban trees

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1 INTRODUCTION

Conditions prevailing in the urban environment adversely affect the health status of trees, even causing their premature dying-off. Especially at risk are the street trees in the city center. Salinity of the soil, caused in Poland mainly by the application of NaCl to deice the winter streets and sidewalks, is considered to be the main cause of tree dying [1-9]. The largest losses concerned the following tree species: *Sorbus aucuparia* L. 94%, *Acer pseudoplatanus* L. 83%, *Tilia cordata* L. 65%, and *Tilia* 'Euchlora' 62%. *Tilia platyphyllos* Scop. had the smallest losses which were still as high as 44% [10]. In Liverpool [11], 39% of the city trees, planted there in the recent years, had died within 5 years. In Edmonton, Canada, more than 20,000 trees had died out due to drought and salinity [12].

The effect of salt stress on the trees is very extensive. The increased salinity had reduced net photosynthesis and transpiration rates, and stomatal conductance [13, 14]. This disturbs the phenological phases by shortening the period of vegetation activity and the phase of autumn foliage discoloration [15, 16]. It also causes a reduction in mycorrhiza [17, 18]. The increased chlorine content in the leaves significantly affects the reduction of Lime Aphid feeding on the leaves (*Eucallipterus tiliae* L.) [19].

The disturbance of ionic balance is considered to be the main reason for the worsening state of tree health, and for their death. Salinity upsets the nutritional balance of plants by one or more mechanisms, such as the osmotic effects of salts, the competitive interaction among ions in the substrate, and the effects on membrane selectivity [2, 20-26].

The relationship between salt stress and mineral nutrition of plants is complex because the salinity may increase, decrease, or have no effect on the concentration of microelements in the plants [27]. The observed differences may result from the impact of salinity on the intake of microelements, their availability, the competitiveness of the ions, or their transport within the plant [28, 29]. There are a number of reports about the lack of effects of salinity on the content of basic elements in the plants [30, 31] while, in other studies, its influence was confirmed [32, 33].

The salinity increases soil pH, which has a substantial effect on the solubility of metal salts and the possibility of their uptake by the roots of trees. High pH impedes the intake of microelements [3, 4, 34-37].

The purpose of the study was to determine the effect of soil salinity on changes in the contents of microelements (Zn, Fe, Cu, Pb) in the leaves of 5 species of urban trees that were significantly different in their resistance to salt stress.

2 MATERIALS AND METHODS

The subject of the study was to determine the level of accumulation of microelements in the leaves of urban trees. The study involved 5 species of trees: *Quercus rubra* L., *Gleditsia triacanthos* L., *Platanus* x *hispanica* Mill., *Robinia pseudoaccacia* 'Umbraculifera' and *Tilia* 'Euchlora', growing in Warsaw, in diverse habitats in the row adjacent to the main streets in the city center, and in the central regions of downtown parks. The control trees were growing in the Botanical Garden of the Polish Academy of Sciences in Powsin, in a suburban setting, far away from roads and other emission sources.

At each station, leaf samples were collected in mid-July from 8 trees of each species. The leaves were harvested from the outer belt of the crown, along its entire circumference at heights from 2 to 4 m.

The materials collected were placed in linen bags and dried at 70 °C for 6 days. The dried materials were ground to a powder in a stainless steel impact mill (Fritsch 14702) and stored in tightly sealed plastic containers until analysis. Leaves were washed for 1 min in distilled water before being dried and ground. The powdered samples were dry-mineralized in a muffle oven (Naberthern L40/11/P320) using the following time/temperature procedure: 120 ºC – 2 h, 200 ºC – 1 h, 300 ºC – 1 h, and 450 ºC – 5 h. The ashes were digested in 30% HCl (Merck, Suprapur) and filtered through a filter paper [38]. Chemical analyzes were done for each tree, and in the rankings, the average values were reported.

The analyses were performed by flame-AAS (Perkin Elmer, 1100A, connected to deuterium background correction, hollow cathode lamps (HCl) and acetylene burner). Analytical wavelengths were 328.8.8 nm for Cu, 217.0 nm for Pb, 589.0 nm for Na, 313.9 nm for Zn, and 248.3 nm for Fe [39]. Three replicate sub-samples of each sample were processed. Three blanks were run with each batch of samples; thus, each sample was blank-corrected.

Chlorine was marked as soluble chloride after extraction from leaf powder by potentiometric titration method (0.01N HNO_3) using ion-selective electrode and Orion Star Plus ion meter [40].

To provide quality control (QC), the elemental content in the plant samples was determined using certified reference materials from the NIST- USA - Apple leaves nr 1515. The obtained results were in good agreement with the certified values. The recovery range was from 90 to 96%.

For statistical analysis of the results, the following methods were used: one-way analysis of variance and multiple comparisons of means (based on LSD value), Pearson's linear correlations for evaluation of relationships between the contents of examined elements, and principal component analysis for multivariate evaluation of relationships and multivariate characteristics of examined species grown at various places. Analyses were performed using STATISTICA 8 software (StatSoft). The significance level for all analyses was set at 0.05 [41].

3 RESULTS AND DISCUSSION

The study involved tree species differing greatly in their sensitivity to the adverse conditions of the urban environment, especially to the salt stress. Previous studies have shown that *Q. rubra, G. triacanthos, P.* x *hispanica,* and *R. pseudoaccacia* 'Umbraculifera' were relatively resistant, but *T.* 'Euchlora' particularly sensitive [1, 8, 9, 42]. Observations of the health conditions of the trees studied showed no damage to the leaves of all species grown in parks and suburbs. Oaks, gleditsias, plantains, and black locust from the street side locations did not show any damage. However, among the Crimean linden trees there were distinguished groups with heavily damaged leaves and with relatively healthy ones, which were subjected to chemical analyses.

Table 1 and Figs. 1 and 2 show the average contents of Cl, Na, Cu, Fe, Zn and Pb in the leaves of the studied trees. The statistical analysis of the obtained results showed considerable variation in the content of the studied elements in the leaves, both between the studied tree species, and the habitat conditions (city, park, suburban area).

The Cl content in Crimean linden ranged for the street side plantings from 0.74% in relatively healthy leaves to 1.88% in heavily damaged leaves; it was 0.35% for the park trees, and 0.26% for the trees in the suburban areas. The differences between the street side trees, both the damaged and the relatively healthy ones, and the trees from the remaining locations were statistically significant. On the other hand, the differences between the park trees and the suburban ones were not significant. For the species of Crimean linden trees, the level of 0.60-1.0% of Cl in the leaves is considered to be toxic [1, 43, 44]. The leaves of the street side trees from the heavily damaged group had marginal necrosis, a characteristic of salt stress [2, 9, 15, 45-47].

The Cl content in the leaves of other street side tree species studied was statistically significant compared with the park and suburban area trees. There were no signs of damage observed in these species. *Quercus* leaves were characterized by particularly low contents of Cl: 0.04% for street side trees, and 0.02% for park and suburban trees. This special feature of accumulation in the leaves of small amounts of Cl in this species was previously described by Pracz [48] and Dmuchowski *et al.* [49]. For the toxic level of *Quercus,* Pracz [48] determined a Cl content of 0.15%. The leaves of *Gleditsia* growing under street side conditions had 0.27% Cl, 0.13% in park, and 0.09% in suburban areas. The Cl content in the leaves of *Robinia* was higher than in the case of *Gleditsia*, and for sidewalk trees, it was 0.65%, but 0.22% for park and 0.20% for suburban trees. Relatively large Cl contents were also found in the leaves of *Platanus*, respectively (1.33, 0.25 and 0.14%). No toxic level values were found in the literature for these species.

TABLE 1 - Mean values and standard deviations for examined elements.

	Cl (%)		Na (mg/kg)		Cu (mg/kg)		Fe (mg/kg)		Zn (mg/kg)		Pb (mg/kg)	
Species	mean ± SD		mean ± SD		mean ± SD		mean ± SD		mean ±S D		mean ± SD	
Street trees												
Gleditsia triacanthos L.	0.27±0.06	b*	203.9±32	a	11.48±2.29	bc	277.3±54.5	b	34.8±5.44	bc	2.14±0.4	a
Platanus x hispanica Mill.	1.33±0.25	d	330.2±99.1	a	12.70±2.55	cd	191.2±40.6	a	36.8±7.17	c	2.78±0.68	b
Quercus rubra L.	0.04±0.01	a	105.6±14.9	a	12.61±2.26	cd	332.6±112.2	b	45.8±13.07	d	3.71±1.11	c
Robinia pseudoacacia 'Umbraculifera'	0.65±0.15	c	127.7±9.5	a	8.25±0.76	a	200.4±17.6	a	55.2±10.62	e	2.07±0.2	a
Tilia 'Euchlora'- damaged	1.88±0.29	e	3291.2±1970.3	b	10.07±0.81	ab	284.0±43.5	b	25.6±3.51	a	1.68±0.29	a
Tilia 'Euchlora'- healthy	0.74±0.14	c	167.1±26.3	a	13.51±1.94	d	291.6±52.8	b	27.8±3.55	ab	1.81±0.43	a
Urban parks												
Gleditsiatria canthos L.	0.13±0.03	b	45.4±12.4	b	8.27±0.93	b	96.1±20.1	a	26.1±4.88	ab	1.71±0.32	ab
Platanus xhispanica Mill.	0.25±0.05	c	27.1±7.4	a	10.60±1.59	d	114.0±18.4	a	24.3±5.55	a	2.01±0.59	ab
Quercus rubra L.	0.02±0.01	a	43.9±6.5	b	9.09±1.03	bc	132.8±16.3	b	30.9±5.1	bc	2.26±1.92	b
Robinia pseudoacacia 'Umbraculifera'	0.22±0.05	c	66.7±7.4	c	7.02±0.92	a	146.3±12.8	b	41.2±6.55	d	1.86±0.18	ab
Tilia 'Euchlora'	0.35±0.07	d	86.4±13.9	d	10.10±1.08	cd	148.9±22.8	b	32.2±4.59	c	1.27±0.34	a
Suburban area (Botanical Garden PAS)												
Gleditsia triacanthos L.	0.09±0.01	b	21.2±5.1	a	5.05±0.67	a	67.0±9.6	a	16.9±2.03	a	1.28±0.24	bc
Platanus xhispanica Mill.	0.14±0.03	c	19.4±7.5	a	7.66±1.53	c	87.0±19	b	18.6±6.91	a	1.35±0.26	c
Quercus rubra L.	0.02±0.01	a	35.9±2.4	b	6.13±0.72	b	94.1±12.7	b	26.5±5.16	b	1.04±0.15	a
Robinia pseudoacacia 'Umbraculifera'	0.20±0.03	d	63.1±4.4	c	6.31±0.89	b	79.6±7.6	ab	27.6±5.32	b	1.33±0.13	c
Tilia 'Euchlora'	0.26±0.06	e	65.9±21.2	c	8.40±1.11	c	114.5±19.1	c	24.3±3.71	b	1.09±0.26	ab

* - letters denote homogenous groups (means which do not differ significantly) of species at each place, based on multiple comparisons using $LSD_{0.05}$ (Number of observations for each species in individual location was equal)

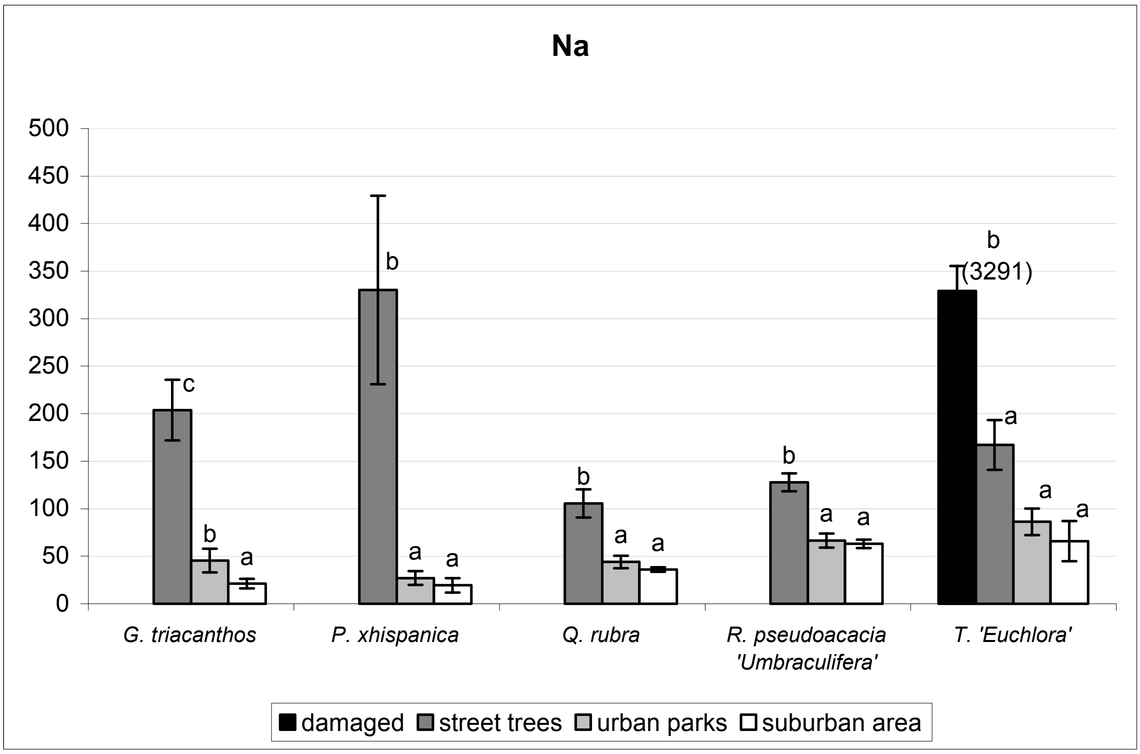

FIGURE 1 - Content of sodium in leaves (mg/kg) of trees grown under different conditions (different letters denote significant differences based on multiple comparisons between examined locations for each of the species).

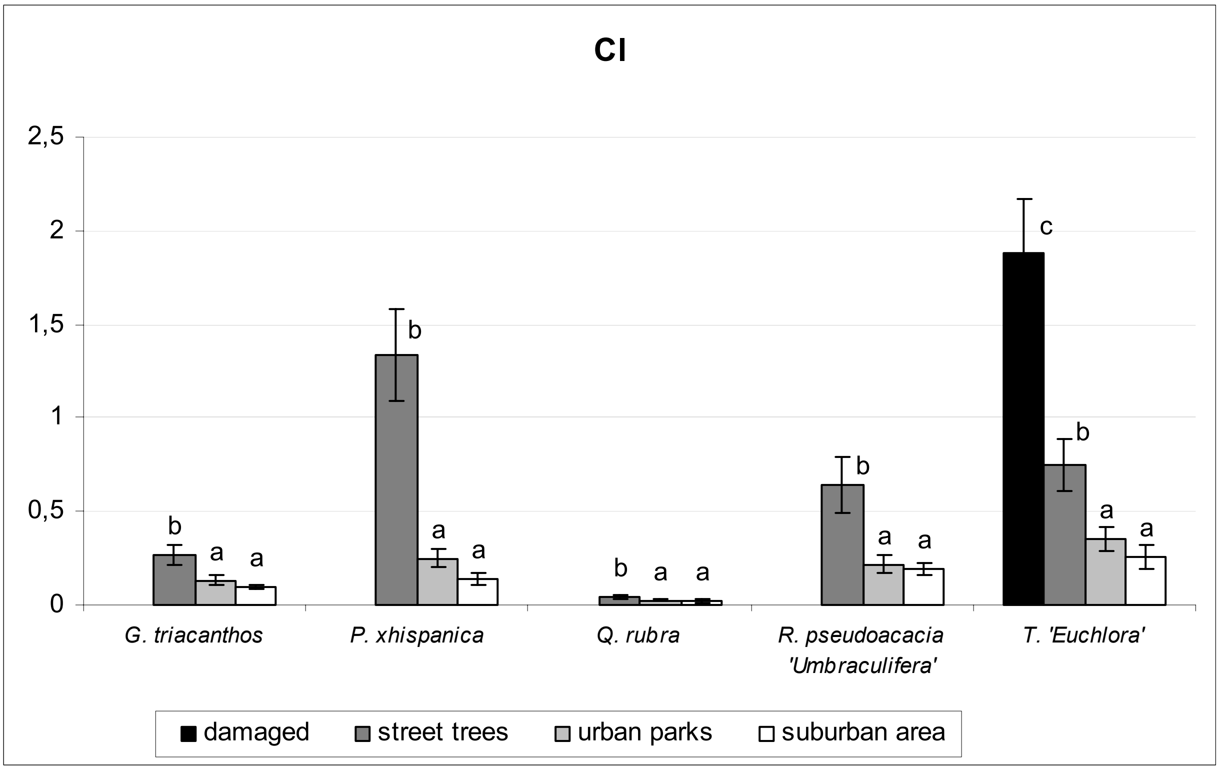

FIGURE 2 - Content of chloride in leaves (%) of trees grown under different conditions (different letters denote significant differences based on multiple comparisons between examined locations for each of the species).

Similar dependencies as in the case of Cl were found for the Na contents in the leaves. Extremely high Na content was determined in the leaves of Crimean linden trees (3291 mg/kg) with strong damage, while relatively healthy trees contained only 167 mg/kg. The oak leaves contained the least Na but, when compared with other species, these differences were not as high. The interpretation of the results for Na levels in the leaves of trees is made difficult because there is no information about the limit levels of toxicity. Na is characterized by a high lability in soils as well as in plants, and its excess results primarily in a disturbance of the ionic balance, and not in a simple toxic action. Na can be deposited on the stems and other parts of the trees, and, after exceeding a certain undefined threshold value, can be transmitted to the leaves. Possible is also a transmission of Na from leaves to roots through phloem, and from roots to culture medium [50, 51]. A very high value of SD can also indicate a high mobility of Na in the plant; with the average content of Na in the leaves of the Crimean linden at 3291% of the SD resulting in 1970.

The leaves of Crimean linden contained the highest levels of Cl and Na among all the trees surveyed. This was applied to all the stations studied. In studies of Paludan-Müller [45], linden *T. cordata* also accumulated much more Cl and Na than *Acer pseudoplatanus, Aesculus hippocastanum* and *Fagus silvatica*. At the same time, the linden trees are characterized by a relatively poor state of health in Warsaw street plantings, and are among the species most subjected to dying.

The contents of microelements Cu, Fe, Zn and Pb were determined in the leaves of the studied trees, and were all found to be below the toxic level to plants (for Pb > 30 mg/kg for plants in general [52, 53], for *Picea sitchensis* > 19 mg/kg [54], for Zn - deciduous trees > 250 mg/kg [55], and Cu - deciduous trees > 30 mg/kg [56]). No toxic levels were found in the literature for Fe. The content of microelements in the leaves of the street side trees was higher than for the park trees, and the least content was present in the leaves of trees from the suburban areas. However, the differences were much smaller than in the case of Cl and Na contents in leaves. The differences between the studied species were also small. No significant differences were found in microelement contents between the leaves of heavily damaged Crimean linden trees and the relatively healthy ones.

Excessive accumulation of Na and Cl ions leads to ionic imbalance, expressed especially in the contents of macroelements in the leaves [57, 58]. There is conflicting information on the topic of the effects of salt stress on microelements, see Introduction. On the basis of principal component analysis (Fig. 3) and correlation coefficients (Table 2), it is possible to evaluate the relationships between the examined elements as well as to characterize the examined species of trees grown under different conditions. Because the two principal components (PC1 and PC2) explain most of the total multivariate variability (about 80%, i.e. PC1 51.7% and PC2 28.4%), the results presented in Fig. 3 are useful for the multivariate evaluation of species, and for the correlation between examined elements. Quite strong correlations are observed between Na and Cl, as well as between Fe and Cu, or between Pb and

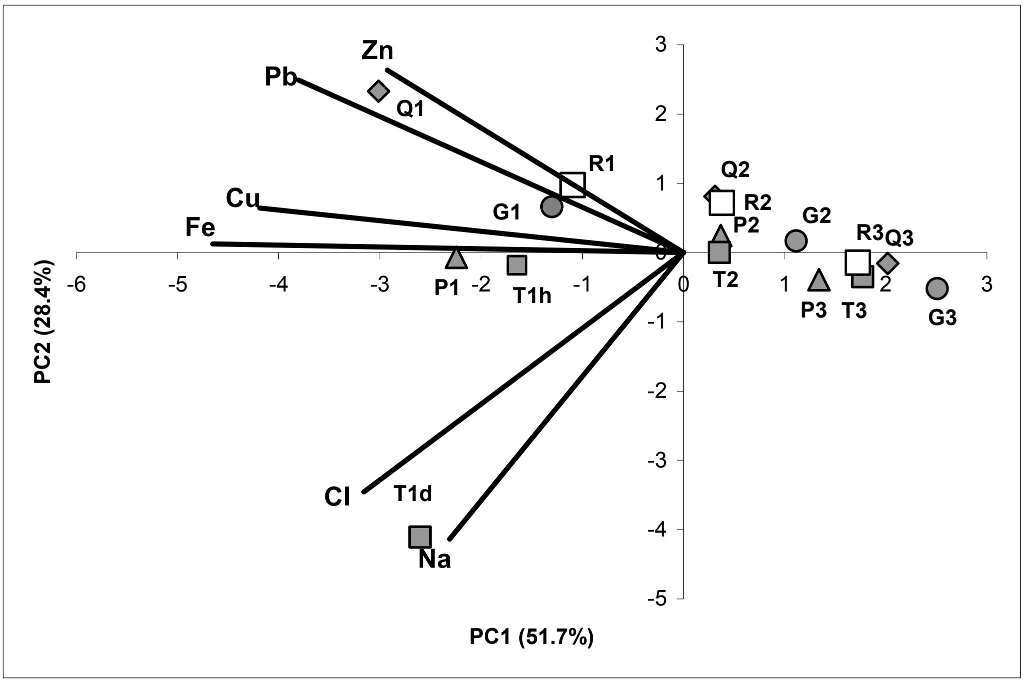

FIGURE 3 - Biplot of examined elements and examined species of trees and different places based on results of principal component analysis (Symbols: G- *Gleditsia triacanthos* L.; P- *Platanus xhispanica* Mill., Q - *Quercus rubra* L.; R - *Robinia pseudoacacia* 'Umbraculifera'; T - *Tilia* 'Euchlora'; 1 – street trees; 2 – urban parks; 3 – suburban area; d - damaged trees).

TABLE 2 - Correlation coefficients between contents of Na and Cl and microelements.

	street trees		urban parks		suburban area	
	Gleditsia triacanthos L.					
	Cl	Na	Cl	Na	Cl	Na
Cu	0.26	0.23	0.68	0.21	0.04	0.25
Fe	0.63	0.59	0.2	0.64	0.30	0.42
Zn	0.26	0.18	0.25	0.55	0.66	0.70
Pb	0.05	-0.20	0.16	0.38	-0.07	-0.01
	Platanus xhispanica Mill.					
Cu	-0.22	-0.30	-0.62	-0.37	0.24	-0.69
Fe	0.67	0.41	-0.05	0.19	0.45	0.2
Zn	0.07	-0.14	-0.12	0.02	-0.42	0.39
Pb	0.01	-0.35	-0.51	0.13	-0.28	-0.66
	Quercus rubra L.					
Cu	-0.11	**0.79**	-0.55	0.18	0.08	-0.29
Fe	0.16	**0.86**	-0.33	0.23	0.11	0.01
Zn	0.34	0.09	-0.42	0.18	-0.08	-0.25
Pb	0.54	0.10	-0.46	0.19	0.14	-0.48
	Robinia pseudoacacia 'Umbraculifera'					
Cu	0.49	0.03	-0.01	0.16	0.33	**-0.75**
Fe	-0.33	-0.58	-0.33	0.16	-0.07	0.56
Zn	0.21	0.52	0.50	-0.57	-0.56	-0.41
Pb	0.44	0.23	-0.03	-0.26	-0.18	0.25
	Tilia 'Euchlora'					
Cu	-0.10 (0.25)	0.72 (-0.46)	-0.20	-0.01	-0.50	0.33
Fe	0.30 (-0.43)	0.69 (-0.32)	0.09	-0.35	0.23	0.19
Zn	0.28 (0.21)	0.16 (-0.48)	0.32	0.21	-0.15	0.21
Pb	-0.36 (0.44)	-0.37 (-0.48)	-0.23	0.26	-0.49	0.19

Significant correlations ($P<0.05$) are in bold; in brackets are values for damaged trees of *Tilia*.

Zn. The contents of Na and Cl, the elements responsible for salinity, were not correlated with the microelements.

Almost all correlations were weak. The only significant positive correlations were observed for Na with Cu and Fe for red oaks grown at street sides. Significant negative correlation was observed between Na and Cu for *R. pseudoaccacia* 'Umbraculifera' grown in suburban areas. Other correlations were not statistically significant. It means that, for all locations, the content of examined microelements, such as Cu, Fe, Zn and Pb, did not depend strongly on the content of Cl and Na in the leaves. The correlations presented in Table 2 were calculated for each location, and the resulting variability of the content of microelements was rather random, and only, to a small degree, determined by the content of Cl and Na in the leaves. Similar tendencies of a very small effect of soil salinity on the content of trace elements were demonstrated by Goodrich and Jacobi [59], but in their case, the salt was in the form of NaCl, and not $MgCl_2$.

4 CONCLUSIONS

Considerable differentiation was found in the content of Cl and Na in the leaves, both between the studied species of trees and their locations (city, park, suburban areas). The leaves of *Q. rubra* contained a minimal amount of Cl in comparison with the other species of trees, at all the studied sites. The leaves of *T.* 'Euchlora' with strong damage contained extremely high Na content.

The study showed very little or no effect of salt stress on the contents of Cu, Zn, Fe and Pb in the leaves of the trees. The only significant positive correlations were observed for Na with Cu and Fe for street side-grown *Q. rubra.* Significant negative correlation was observed between Na and Cu for *R. pseudoaccacia* 'Umbraculifera' grown in suburban areas. Other correlations were not statistically significant. It means that the content of the examined microelements in the leaves is, only to a small degree, determined by Cl and Na, and probably depends more on other factors which were not examined in the survey. Positive correlations were observed for each of the microelements (especially between Zn and Pb, and between Cu and Fe) but these microelements could not be indicative of salt stress. Salt stress had no effect on the occurrence in the leaves of deficient levels of Cu and Zn, or toxic levels.

The study confirmed the usefulness of *Q. rubra*, *P.* x *hispanica*, *R. pseudoaccaia* 'Umbraculifera', and *G. triacanthos* for street side planting in city centers, as well as the high sensitivity of *T.* 'Euchlora'.

ACKNOWLEDGEMENTS

The research was supported by the grant of Polish Ministry of Science and Higher Education; number of project: N N305 368938.

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Received: June 22, 2012
Revised: September 18, 2012
Accepted: November 12, 2012

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