Tilia spp. – Urban Trees for Future

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Abstract

Tilia spp. (lime trees) as ornamental trees have a privileged place in the European mythology and traditions and this is a reason of their constant presence in the urban landscape. Lime trees are in general very resistant to biotic and abiotic stress, although some sanitary problems may occur (e.g. aphids and other related organisms). Tilia spp. are considered a good bioindicator and accumulator of heavy metals. Seasonal accumulations of Pb, Ni, Cr, Mn, Cu and Fe in leaves of Tilia spp. in urban and periurban green areas of Western Romania, under different traffic conditions, were analyzed using atomic absorption spectrophotometry. The results confirmed the direct correlation between the traffic intensity and the accumulation of Pb in leaves. By mitigating the effects of air pollution, especially in terms of heavy metals, Tilia spp. should be further promoted as urban trees.

Keywords: bioaccumulator, bioindicator, climate change, heavy metals, seasonal accumulation

Introduction

Urban trees play an important role in the urban living space, by bringing social benefits, improving environmental quality (e.g. removing PM10 from urban air) and also contributing to local identity (McDonald et al., 2007; Nowak, 2007). One of the most common urban trees is the Lime tree (Tilia spp.), both native species and ornamental varieties. Out of 40 genera and over 350 species of the Tiliaceae family that are to be found especially in the tropical and subtropical regions, in Romania the lime trees are represented by a single genus (Tilia) with three species: Tilia cordata Mill. (the most common), T. tomentosa Moench. (the most drought tolerant), and T. platyphyllos Scop. (the most water demanding). Of all trees that tradition invested with special virtues, the lime tree holds a special place both in the Romanian (Eliade, 1995) and also the most frequently mentioned tree in the poems, songs and stories.

The potential use of Tilia spp. as ornamental urban trees and as medicinal plants is well known in all regions of Europe (Ivănescu et al., 1966; Șofletea and Curtu, 2007; Soukand et al., 2013). The air in urban and periurban areas has been polluted with numerous solid and liquid particles in suspension that vary in size, morphology and chemical composition. The Tilia spp. leaves have showed significant seasonal accumulation especially for Pb but also for Cr, Fe, Ni, Zn and Mn (Bargagli, 1998; Piczak et al., 2003; Tomašević et al., 2004b; Anicic et al., 2011). The aim of this

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Materials and methods

Firstly, a literature review has been performed on ornamental role, current health issues, potential use as pollution bioindicator and heavy metal bioaccumulation aspects.

Secondly, the seasonal accumulation of heavy metals in *Tilia* spp. leaves in Western Romania (Timişoara region) has been analyzed. In order to study heavy metal accumulation, samples (minimum 30 g of leaves) have been collected from individuals (11-14 years old) of *Tilia* genus, in street alignments, in locations with different pollution intensity levels: no traffic (Câvâran Forest - *T. tomentosa* and Borlova Forest - *T. platyphyllos*); low traffic (urban area in Timişoara, Albinder Street - *T. cordata*, *T. tomentosa*, *T. platyphyllos* and rural area Borlova - *T. platyphyllos*, *T. cordata*); medium traffic (urban area Timişoara - I. I. de la Brad Street - *T. cordata*); heavy traffic (road to Timişoara Airport - *T. tomentosa*).

Samples were encoded as follows: species (C - *T. cordata*, P - *T. platyphyllos*, T - *T. tomentosa*); locations (AL - Albinder Street, Timişoara; II - I. I. de la Brad Street, Timişoara; AR - Airport, Timişoara; M - Borlova Forest; B - Borlova village; C - Câvâran Forest); sampling data (M - May; A - August).

The representativeness in both the sampling design and the chemical analyses procedure was ensured. Thus, two sampling operations were performed in May (average size fresh leaves) and in August (average size mature leaves, before the autumn senescence). Samples were collected during the same week from 10 individuals/location, in three repetitions/individual (90 average size leaves). The leaves were collected from the south-facing upper part of the outer tree canopy and numbered (location, data, species, age). Leaves from the outer canopy accumulate more particles due to the higher transpiration rates (Banggah, 1998). Random chosen 30 g leaves/individual were washed for 25 seconds with deionized water (pH=5.8-5.9).

The samples were dried at 105 °C for 24 hours. 1.5 g of samples were mixed twice with 5 ml of nitric acid (65%) and the mixture was brought to 80 °C twice (about 2h) until completely dried. 5 ml of de-ionized water was added and the suspension was filtered through special paper filter (180 mm thick, with pores of 20-25 µm). The quantity of de-ionized water was increased up to 50 ml and heavy metal concentrations were measured during the atomic absorption with the spectrophotometer VARIAN 220 FAA. The recordings were performed after the calibration for each microelement, at the following wavelength: Cu 324.7 nm, Fe 259.9 nm, Cr 205.5 nm, Ni 231.6 nm, Pb 220.3 nm.

The variance analysis and the post-hoc Duncan test of multiple comparisons were performed in order to compare the Pb concentrations recorded in the samples for each species, location and time of sampling. All calculations were done using Statistica 10 software.

Results and discussions

*Tilia* spp. as ornamental trees

Due to their special shape, *Tilia* spp. are used as main elements in designing grandiose and inspiring green areas. The beauty of these species all the year round is underlined by associating *Tilia* spp. with different ornamental shrubs in order to get perspectives focused upon *Tilia* spp. on coniferous background. Combinations in green areas with different species and varieties of *Tilia* spp. which blossom successively, are highly appreciated because of a longer period with flowers and fragrance. Some authors described hybrids (Ivănescu et al., 1966; Fricke et al., 1980), other authors reported new species (Zare et al., 2012) and others considered that genuine inter-specific hybrids are extremely rare (Neville-Jones, 1968). However, molecular markers can be applied to characterize hybridization and species differentiation in *Tilia* genus (Fineschi et al., 2003).

*Tilia* spp. produce a large number of sprouts with larger diameters and heights (Matula et al., 2012) and show no decline in sprouting vigour with age (Pigott, 1991). *T. cordata* leaves may represent a reliable indicator for evaluation studies on developmental stability based on the ability of its leaf morphometric characters to buffer their development under contrasting environmental conditions (Velikovic, 2010). The difference in the morphology of the crown as well as in the leaf structure seems to be an advantage for *Tilia* spp. when compared with other popular urban trees in Central Europe, considering the influence on air flow, deposition of air-borne particles, abatement of wind speed, reducing building energy use by lowering temperatures and shading buildings during the summer (Nowak, 2006). Sjöman and Oprea (2010) reported that *T. tomentosa*, *T. cordata* and *T. vulgaris* show long-lasting health and hardiness in street environments of Northern Europe.

In the system of green spaces in Timişoara, the global valuation method of the trees was applied by Ciupa et al. (2005), by using three components: ecological value, landscape value (general physiognomy of the species shape, individual size, subjectivity of the observer) and vitality; the results indicated *Tilia* spp. as the most valuable trees in this context. Different authors mentioned the high landscape value of *Tilia* spp. (Ivănescu et al., 1966; Neville-Jones, 1968; Jensen, 2003; Sjöman et al., 2012; Halajová and Halaj P, 2013). Remarkably old trees as well as the young ones are very common in parks and street alignments in Romania, both in groups and as isolated individuals, mainly in residential areas where citizens decide what species to be planted.

*Tilia* spp. resistance to biotic and abiotic factors

Climate change seems to increase the frequency of extreme events. In Romania, severe drought (1999-2000, 2003, 2011-2012) alternated with flooding (2005, 2010) and severe frost (2012) with milder winters (2013-2014). *Tilia* spp. seems to be more resistant to drought compared to other native tree species (Simionescu et al., 2012). *T. cordata* and *T. platyphyllos* showed sensitivity to backwater (3 months) and to very poor land drainage conditions (Dănescu et al., 2011).

Aphids and other related organisms, such as plant lice and mites, are strongly related to *Tilia* spp. in green areas but they only cause minor problems to the lime (Zuparko and Dahlsten 1994).

In flooded areas (2005, 2010, 2014), lime tree collar and roots were relatively fast affected by rot and dieback. *Phytophthora* spp. were among the first organisms to damage the
fine roots (Dănescu et al., 2011) and could lead to the death of the stressed trees. *Armillaria* spp. proved to be aggressive opportunists only on weakened lime-trees in forests, being followed by *Ganoderma lucidum* and other root destroying fungi. On upper roots and stem of trees in urban areas, the wounded bark of old trees was frequently colonized by *Schizopyllum commune* (sapwood parasite), followed by xylofagus fungi: *Stereum* spp., *Trametes* spp. and *Laetiporus sulphureus* or other Polyporaceae which rot the stem and favour the wind breaks (Chira et al., 2000). Lime-trees were the most resistant tree species to the biotic and abiotic factors in the decade 2001-2010 in Romanian forests (Simionescu et al., 2012), presenting only minor problems (e.g. only 120 acres defoliated by *Calderia annulifera* out of a total of infested 742,74 ha).

**Tilia spp. as a biomonitor and bioaccumulator**

Trees play a special role in reducing the level of fine, “high risk” breathable particles with potential side effects on the environment and on human health (Beckett et al., 2000 from Aničić et al., 2011). Tree leaves have been widely used as an indicator of atmospheric pollution and they are an effective alternative to the usual biomonitoring methods (Braun et al., 2007). *Tilia* spp. is one of the best indicators of pollution (especially with chlorine), displaying clear spring (*chlorosis*) and summer (*necrosis*) symptoms, in street alignments (Brașov, Romania) and industrial polluted areas (Dej, Romania) (Bolea and Chira, 2005).

Having an adequate morphology, canopy structure and epicuticular wax on the leaf surface, lime-trees have been used as suitable biomonitors of aerial trace elements in leaves in urban areas (*T. tomentosa* - Kovics and Podani, 1986; *T. cordata* - Bargagli, 1998; *Tilia tomentosa*, *Tilia cordata*, *Tilia platyphyllos* - Braun et al., 2007). According to Juranić et al., 1993 (from Šomšak et al., 2007), *T. cordata* seems to be a better bioaccumulator of heavy metals than *Acer platanoides*. Different authors (Bayuc et al., 2006; Popović et al., 2010; Oroian et al., 2012) reported that of the analyzed urban trees, *A. hippocastanum* accumulated the highest Pb concentrations in leaves and could be used as a monitor of concentrations and seasonal variations of heavy metals in air in urban areas. Recently, the use of plants as bioaccumulators or bioindicators has increased because it makes it possible to forecast air pollution for monitoring purposes.

A low-cost, practical method to assess the quality of the urban habitat consisted of determining the injured leaf area reflectance of *Tilia* spp. out of the leaf surface reflectance (Khavaninzadeh, 2014). The findings of many studies showed that metal accumulation in plants varied, depending on species, tissues and metals but it is obvious that *Tilia* spp. meet many of the requirements of a good bioindicator for heavy metals accumulation.

**Heavy metals accumulation in *Tilia* spp.**

There were significant differences (*p*<0.05) between lime-tree species in Cr, Cu, Fe, Ni content and non-significant differences in Pb and Mn content for leaves sampled in May and June 2014. When analyzing leaves sampled in August, the variance analysis showed significant differences between *Tilia* spp. in heavy metal accumulation except for Fe (*p*=0.10) and Ni (*p*=0.07). Our study indicated that only Pb was accumulated by all individuals irrespective of species and locations. The other heavy metals did not show a regular pattern during the growing season (Table 1).

Some metals could be used as micronutrients (Fe) or macronutrients (Cu, Zn) but generally, the interpretation of data related to accumulation of heavy metals from the air is difficult (Iordache, 2009). A continuous reduction of annual emissions of Pb has been recorded starting with 2009 in Timisoara. The highest heavy metal emissions were recorded for Pb, Zn and Ni, as coming mainly from fuel combustion (Romania, Timisoara Environment Protection Agency, 2014). The local soil was alkaline, pH > 8.0 (Tenceh-Constantinescu, 2011) and this causes lower element solubility and lower availability for plants (Tomašević et al., 2014; Serbula et al., 2013). Pb solubility in soils with various conditions was very low (Adriano, 1986; Alloway, 1995) and terrestrial plants accumulated less Pb from soil than from the atmosphere through stomata and the accumulated quantity depended on air pollution, particle size and climate. Under such environmental conditions, the atmospheric origin of the elements accumulated in leaves may be assumed: *Achillea millefolium* and *Hordeum vulgare* (Piegaard and Johnsen 1984), *Quercus ilex* (Allani et al., 1996), *Populus* spp. (Gregger, 1999), *Salix* spp. (Iordache, 2009), *Picea abies* (Hovmand et al., 2009) and *Tilia* spp. (Serbula et al., 2013).

There were highly significant differences in Pb accumulation (*p*<0.001) by comparing all species under similar traffic conditions at the end of the growing season (*C/AL/A, P/AL/A, T/AL/A*); different species or individuals of the same species, under different traffic conditions and in the same sampling period and different species or individuals of the same species, under the same traffic conditions and at different moments of sampling (seasonal accumulation) (Table 2). Moreover, we found significant differences in Pb accumulation (*p*<0.05) by comparing *T. platyphyllos* and *T. cordata* under different traffic conditions at the end of the growing season (*P/B/A, C/II/A*). However, no significant differences in Pb accumulation (*p*>0.05) were found when comparing species at the beginning of the growing season under different traffic conditions (*C/AL/M, C/I/M, P/AL/M, T/AL/M, T/AR/M*). The Pb accumulation in leaves at the beginning of the growing season was very low for all species and locations (minimum 0.01280 mg/kg Pb – T/AR/M and maximum 0.3631 mg/kg Pb – T/AL/M).

| Locations** | Heavy metals (mg/kg of dry material) |  
|-------------|------------------------------------|
| **Species*** | Cr | Cu | Fe | Mn | Ni |
| C/AL | -0.083 | -1.229 | 14.146 | -1.078 | -1.822 |
| C/II | 0.017 | 0.031 | 30.284 | 19.291 | -1.196 |
| T/AL | -0.302 | -2.372 | -36.484 | -13.667 | -2.917 |
| P/AL | -0.22 | -1.677 | -2.171 | -13.019 | -3.133 |
| T/AR | -0.01 | -1.824 | 62.075 | -22.947 | -0.248 |

*Species = C - T. cordata; P - T. platyphyllos; T - T. tomentosa.
**Urban locations in Timisoara = AL - Albinelor Street; II - Ion Ionescu de la Brad Street; perurban location = AR - Timisoara Airport.

Table 1. Differences in heavy metals content after the seasonal accumulation (May-August, 2014) in *Tilia* spp.
There were highly significant differences in Pb accumulation among all species of lime-tree studied under similar traffic conditions (Albinelor Street, Timisoara) at the end of the growing season. The differences between T. cordata and T. tomentosa, between T. platypyllus and T. tomentosa and also among individuals of the same species in different traffic conditions in the same sampling period, were also highly significant. The lowest average accumulation during the growing season was recorded in the area with low traffic for T. tomentosa (7.03 mg/kg Pb - T/AL) and the highest average accumulation was recorded in the area with the heaviest traffic for T. tomentosa (17.83 mg/kg Pb - T/AR). Similar results were reported in various deciduous broadleaves: Quercus ilex (Allafi et al., 1996), Tilia spp. and Aesculus hippocastanum (Sucur et al., 2010), Acer platanoides, Aesculus hippocastanum and Betula pendula (Petrova et al., 2014), in which Pb was mainly accumulated due to the traffic intensity in the sampling location. Recent studies indicated that Pb has exceeded the maximum admitted level in Cluj-Napoca, Romania, under different traffic conditions, in the leaves of Acer platanoides, A. pseudoplatanus, Aesculus hippocastanum, Betula pendula, Juglans regia, Picea pungens var. glauca, Picea abies, Pinus nigra, Tilia cordata, Robinia pseudoacacia, Thuja occidentalis (Vimran et al., 2011).

There were highly significant differences in Pb seasonal accumulation not only among all species of lime-tree studied but also among individuals within species, in the same traffic conditions and different sampling periods (seasonal accumulation). The Pb accumulation at the end of the growing season is high for all species and locations (minimum 7.4 mg/kg Pb - T/AL/A and maximum 17.84 mg/kg Pb - T/AR/A). The highly significant differences in Pb accumulation among the lime-tree species studied at the end of the growing season may be in relation with leaf shape and size (Hovmand et al., 2000; Alfini et al., 1996). Seasonal accumulation of other potential toxic metal, arsenic (As), in the leaves of Tilia spp. and Aesculus hippocastanum was reported in the Belgrade urban area (Sucur et al., 2010). However, As and Pb do not have any physiological function in plants and could be toxic (Adriano, 1986; Madejón et al., 2004). The seasonal accumulation of Pb may be explained as a detoxification process by passive sequestration of toxic metals in senescing foliage (Aznar et al., 2009 from Popovic et al., 2010). Significant seasonal accumulations of heavy metals in the leaves of Tilia spp. were reported (Bangghi, 1998; Piczak et al., 2003; Tomašević et al., 2004b; Anicic et al., 2011).

Table 2. Multiple comparisons of the average Pb content for Tilia spp. leaves at the beginning and at the end of growing season, in areas with different pollution level from Western Romania

| Dataset/Pb average content | C/AL/M | C/AL/A | C/IL/M | C/IL/A | C/BA | P/AL/M | P/AL/A | P/B/A | T/AL/M | T/AL/A | T/AR/M | T/AR/A |
|---------------------------|--------|--------|--------|--------|-------|--------|--------|-------|--------|--------|--------|--------|
| Pb (mg/kg dry mater)      | 0.0850 | 15.3640 | 0.2760 | 15.9580 | 0.0170 | 10.4540 | 15.0800 | 14.6860 | 0.3630 | 7.4000 | 0.01280 | 17.8400 |

(Duncan test, bold marked effects are highly significant at p < 0.0001; italic marked effects are significant at p < 0.05)
Species (C - T. cordata; P - T. platypyllus; T. tomentosa); locations (AL - Albinelor Street, Timisoara; II - I. I. de la Brad Street, Timisoara; AR - Airport, Timisoara; M - Borlova Forest; B - Borlova village; C - Căvărăș Forest); sampling data (M - May; A - August)

Conclusion

Lime tree species appeared to be highly resistant to abiotic and biotic stress. No major pest or disease of Tilia spp. was reported in the recent period in Western Romania. Our results indicated that the Pb content significantly increased during the growing season from very low values in May to much higher values in August in Tilia spp. leaves for all individuals studied and locations. The highest Pb content values were recorded for T. tomentosa in the area with the heaviest traffic and towards the end of the growing season. This supports the hypothesis of a self-detoxification mechanism of Tilia spp. by leaves fall. Other heavy metals analyzed (Cr, Cu, Fe, Mn, Ni) did not have regular accumulation in leaves during the growing season, probably due to different metabolic aspects. In spite of the increasing tendency to introduce exotic tree species in green areas, in the context of climate change, Tilia spp. are highly recommended to be used in urban landscapes. These tree species showed a high potential to improve the urban environment and also have an important ornamental, ecological and socio-economic value.

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