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Uptake and distribution of Cu, Pb, and Zn in *Tilia tomentosa* Moench: plant tissue and urban soil interactions

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Abstract: This study analyzed Cu, Pb, and Zn concentrations in roots and leaves of *Tilia tomentosa* Moench. and associated soil (at 0-10 cm and 10-30 cm) at Bulevar Nikola Tesla (BNT) and Park Ušće (PU) (Belgrade, Serbia), with Fruška Gora Mt. serving as the control. To evaluate its phytoremediation potential, the bioconcentration (BCF_{Root} and BCF_{Leaf}) and translocation factors (TF) were calculated. Site-dependent variations were observed for all analyzed parameters, especially at BNT at both depths, with Pb and Zn concentrations greater than 200 mg/kg. Leaf Pb concentrations indicated insignificant soil-leaf transfer. Photosynthetic efficiency measurements in *T. tomentosa* showed similar mean values within the optimum range for plants at all sites (Fv/Fm > 0.800). This indicates high overall vitality in urban habitats with elevated concentrations of potentially toxic elements, as shown by the absence of statistically significant differences in mean chlorophyll fluorescence values between sites. There was a positive correlation between Cu and Zn levels and Fv/Fm in leaves from all analyzed sites. Thus, the species appears well-adapted to the uptake and accumulation of elements that are vital for optimal photosynthesis and other physiological processes, while photosynthetic efficiency is not significantly impacted by their occasional deficiency.

Keywords: phytoremediation, *Tilia tomentosa* Moench., toxic element accumulation, bioconcentration and translocation factors, photosynthetic efficiency

INTRODUCTION

In an increasingly urbanized world, pollution mitigation is considered an important issue in urban planning [1]. As cities continue to expand, urban soils are becoming ever more modified by human activities, resulting in drastic changes to their composition and function, which includes both highly transformed and pseudo-natural soils [2]. Protecting urban soils from degradation and contamination, as a key measure for environmental preservation and pollutant reduction, is increasingly drawing the attention of the scientific community and institutions at national, regional, and global levels. The pollution of urban soils with potentially toxic elements, including heavy metals, has received great attention in the past decade with regard to numerous human health issues and challenges [3,4]. Heavy metals such as As, Cd, Hg, Ni, Pb, and Zn are the most frequent pollutants reported in urban soil samples around the world. Recent findings link metal contamination to one or more anthropogenic sources, such as industry, historic urbanization, domestic coal combustion, mining, and waste incineration. Traffic is the most common anthropogenic source of urban soil contamination, leading to elevated concentrations of Pb, Zn, and/or Cu [5,6]. Pb is linked to past urbanization, the historic use of leaded petrol, and the wear and tear of car parts [7]. Zn is commonly associated with brake linings, tire wear, corrosion, engine oil, and coolants,

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while Cu is linked to brake discs and engine wear. As metals tend to be relatively immobile once deposited on the soil's surface, accumulation occurs, and this can also be detected in plant tissues [8].

Many of the world's major cities have implemented tree planting programs based on the assumed environmental and social benefits of urban forests. Trees, as major components of the green infrastructure in urban environments, contribute significantly to a range of ecosystem services [9]. They help to improve air quality by facilitating the widespread deposition of airborne particles, and uptake of gases, both through the provision of large surface areas and through their influence on the microclimate and air turbulence 10]. Urban forests and trees support human health and well-being by providing multiple services, including the removal of air pollutants and particulate matter (PM) and the uptake of heavy metals from soil, thereby demonstrating local phytoremediation potential 3,10-13]. This benefit has encouraged urban planners to increase the areal extent of green space in cities, including the expansion of street tree populations.

Urban tree stocks are dominated by a limited number of species and genera, with recent surveys in European and North American cities confirming the continued prevalence of a few taxa. Several urban tree inventories from southern, northern, and central Europe have found that *Tilia* species are highly abundant in most of these regions, often as ornamental trees in streets and parks [14-16]. Silver lime (*Tilia tomentosa*), a flowering plant in the Malvaceae family, is native to southeastern Europe and southwestern Asia, ranging from Romania and the Balkans to western Turkey, and typically occurs at moderate altitudes. It is considered highly resistant to urban stresses, such as water scarcity, drought, pollution, and pruning [17]. As such, it has been successfully grown in urban areas where pollution, poor drainage, and compacted and polluted soil are common [18].

Unlike park soils, those along tree-lined avenues are often imported from various sources, including construction sites, and are additionally exposed to atmospheric deposition. Therefore, street trees and their soils in roadside pits can serve as indicators of the impacts of urban environments and vehicular traffic on both soils and trees. However, data on these soils remain scarce, as many studies have focused on park soils rather than street tree soils 19]. In addition, the selection of the most suitable tree species for a specific environment is a significant challenge 10,20]. This study evaluates concentrations of potentially toxic elements (PTEs) in *Tilia tomentosa* Moench. tissues and soils within planting pits located in a tree-lined avenue, a city park, and its natural habitat, the latter serving as the control site. We hypothesized that *T. tomentosa* along a tree-lined avenue in Belgrade would accumulate higher concentrations of PTEs in its tissues due to chronic exposure to traffic-derived pollutants, and that these elements would influence leaf photosynthetic efficiency. Specifically, concentrations of Cu, Pb and Zn were analyzed in roots and leaves and urban soils under each individual (at a depth of 0-10 cm and 10-30 cm) in the tree-lined Bulevar Nikola Tesla and the city park, Park Ušće, in Belgrade, Serbia, and in the species' natural habitat on Fruška Gora Mt. (control). Photosynthetic efficiency (Fv/Fm) and its relation to the PTE concentrations in leaves was also examined. To evaluate the potential of the selected species for the phytoremediation of PTEs, the bioaccumulation factors for roots and leaves (BCF_{Root} and BCF_{Leaf}) and translocation factors (TF) were calculated. Such ecophysiological research is suitable for assessing the effects of anthropogenic PTEs on tree functioning in urban habitats and evaluating the tolerance of urban trees to PTEs from vehicle emissions.

MATERIALS AND METHODS

Sampling sites and sampling procedure

For this study, field sampling was conducted in July 2022 at two urban sites located in central Belgrade, Serbia, Bulevar Nikola Tesla (BNT) and Park Ušće (PU), while the natural habitat of this species on Fruška Gora Mt. (FG) served as the control. The sampling locations are detailed in Supplementary Fig. S1. Bulevar Nikola Tesla is a 3,200-m-long avenue dominated by lime trees and located in the municipality of Novi Beograd. Park Ušće is situated at the confluence of the Sava and Danube rivers and extends over an area of approximately 80 ha. It is covered with deciduous and evergreen trees. Sampling was conducted in the section of the park that stretches from Park Prijateljstva to Brankov Most.

Fruška Gora Mt. is an isolated mountain in Serbia, covering an area of 255 km², and includes a national park. The highest peak is Crveni Čot (539 m), and elevations above 300 m are covered in dense deciduous forest. Fruška Gora Mt. has the largest concentration of lime forests in Europe.

At each of the three sampling sites, five *T. tomentosa* Moench. trees of similar age were randomly selected for the collection of roots, leaves, and associated soils. Root and leaf samples were collected from each tree (50 g per sample). Aerial samples were collected at the same height from all four cardinal directions. In the laboratory, they were washed with tap and distilled water, dried to constant weight, and stored in plastic bags until analysis. Before analysis, samples were crushed and passed through a 2-mm stainless steel sieve. Soil sampling was carried out in the root zone of each tree (250 g per tree) from a depth of 0-10 cm and 10-30 cm using stainless steel tools. In the laboratory, each soil sample was air-dried at room temperature to a constant mass and passed through a 0.02-mm stainless steel sieve.

Soil and plant analysis

The prepared samples were analyzed for PTE content (Cu, Pb, and Zn). Soil (0.5 g) and plant samples (0.3 g) were transferred to Teflon (iPrep) vessels. Soil mineralization was conducted using an aqua regia mixture (3 mL of 65% HNO_3 and 9 mL of 37% HCl), while plant samples were digested in a mixture of 9 mL of 65% HNO_3 and 3 mL of H_2O_2 (USEPA 3052 method, [21]) in a CEM MARS 6 Microwave Acceleration Reaction System microwave oven (Matthews, NC, USA). The final extracts were filtered and transferred into 50-ml flasks and diluted to the mark with deionized water. Concentrations of PTEs (mg/kg) in the root (C_{Root}), leaf (C_{Leaf}), and soil (C_{Soil}) were determined by inductively coupled plasma optic emission spectroscopy (ICP-OES, Spectro Genesis, Spectro-Analytical Instruments GmbH, Kleve, Germany). Each sample was analyzed in 3 replicates per tree (n=15).

All reagents used for plant digestion were of analytical grade (Merck, Darmstadt, Germany). The ICP-multi-element standard stock solutions (concentration of elements: 1000 mg L^{-1} in diluted nitric acid) used to prepare standard solutions for ICP-OES analysis were also obtained from Merck. Quality control and quality assurance of the analytical data were performed using standard reference material for leaves (beech leaves - BCR-100) and certified reference material for soil (clay ERM - CC141) (Institute for Reference Materials and Measurements (IRMM), Geel, Belgium), as well as by analyzing both reagent blanks and replicates. Recovery values ranged from 95-110% for plant material and 85.3-106% for soil, indicating close agreement between the measured and certified values. The detection limits for the analyzed elements in the soil samples were as follows (mg kg^{-1}): Cu - 0.0014, Pb - 0.005, and Zn - 0.0023.

Photosynthetic efficiency measurements

Photosynthetic efficiency was measured in July 2022, *in situ* on intact leaves still attached to plants, using a portable photosynthesis system (LI-6800, LI-COR, Lincoln, NE, USA) equipped with a fluorescent leaf chamber. Before measurements were taken, the leaves were fully dark-acclimated for approximately 20 min and then exposed to weak actinic light (0.05 μmol $m^{-2} s^{-1}$) to detect initial minimal fluorescence (Fo). A saturating light pulse (6,000 μmol $m^{-2} s^{-1}$) was applied for 2 s to detect maximum fluorescence (Fm). Variable fluorescence (Fv) was calculated as Fv=Fm-Fo, enabling the calculation of the Fv/Fm ratio (maximum quantum yield of PSII in dark-adapted leaves). For each of the five plants, at least three measurements were recorded between 9 and 11 AM at each site.

Bioconcentration and translocation factors

Bioconcentration patterns were calculated as the ratios between soil and plant parts (roots and leaves), while translocation patterns were determined as the ratios between aboveground (leaves) and underground (roots) tissues [22. The bioconcentration factor (BCF) was calculated using Equations (1) and (2), and the translocation factor (TF) was calculated using Equation (3):

$$BCFRoot = \frac{CRoot}{CSoil} \quad (1)$$

$$BCFLeaf = \frac{CLeaf}{CSoil} \quad (2)$$

$$TF = \frac{CLeaf}{CRoot} \quad (3)$$

Data analysis

One-way analysis of variance (ANOVA) was performed to test for differences in PTE accumulation in soil samples and *T. tomentosa* roots and leaves. Means were separated using the Bonferroni test at the following levels of significance: *P<0.05; **P<0.01; and ***P<0.001 (ns - not significant). Spearman's rank order correlation coefficients were calculated using Statistica 8.0 software. The correlations were calculated for three data sets (soil and plant material), as well as for each analyzed element in the different media. Correlation was assumed to be statistically significant at P<0.05. Descriptive and multivariate statistical analyses were performed using Statistica 12.0 and OriginPro 2023b software. The map of the sampling sites was created using Google Earth Pro.

RESULTS

Copper, lead, and zinc concentrations in soils

In this study, Cu, Pb, and Zn concentrations in the topsoil (0-10 cm) and subsoil layers (10-30 cm) at the Belgrade sites were higher than at the control site. In topsoil, Cu concentrations ranged from 15-78 mg/kg, with the highest level measured in samples from BNT. In the deeper soil layer, Cu concentrations showed a similar range (13-84 mg/kg). Compared to Cu, Pb levels in soil samples were higher, ranging from 32-233 mg/kg in topsoil and 31-283 mg/kg in the deeper layer. Zn concentrations in topsoil varied from 65-197 mg/kg and from 52-213 mg/kg in the subsoil. The highest levels of all the examined elements were measured in samples from Bulevar Nikola Tesla (P>0.001).

Significant differences in Cu concentrations were found in both soil layers across all samples and sites (P<0.001). In contrast, significant differences in Pb concentrations in both soil layers were found between the urban sites (P<0.001), while there were no differences between samples from the urban park (PU) and the control site (FG; ns). Similarly, Zn concentrations differed significantly between samples from the urban sites (P<0.001), while topsoil concentrations showed no significant difference (ns) between PU and FG, unlike the deeper layer (P<0.01). For all elements, higher concentrations were generally found in the subsoil (10-30 cm) at most sites, especially Pb at the urban locations (Table 1).

Copper, lead, and zinc concentrations in lime roots and leaves

Cu, Pb, and Zn concentrations in plant samples varied depending on location. In the analyzed lime root samples, Cu concentrations ranged from 5-9 mg/kg, with the highest level measured in samples collected from Bulevar Nikola Tesla. However, no significant differences in Cu between root samples from Park Ušće and the control site at Fruška Gora Mt. were detected. Cu concentrations measured in leaves were around 7 mg/kg and were similar at all the sites. Pb concentrations in roots ranged from 1 to 6 mg/kg, with the highest levels at BNT, while Pb in PU roots and in leaves from all sites was below the detection limit. Concentrations of Zn in root samples ranged between 10 and 16 mg/kg, with

Table 1. Mean values and standard deviation of total Cu, Pb, and Zn concentrations (mg/kg dry weight) in topsoil (0-10 cm) and subsoil (10-30 cm) at Bulevar Nikola Tesla (BNT), Park Ušće (PU), and Fruška Gora Mt. (FG).

	Site	topsoil 0-10 cm				subsoil 10-30 cm				topsoil/subsoil
		M (SD) (mg/kg)	**BNT**	**PU**	**FG**	**M** (SD) (mg/kg)	**BNT**	**PU**	**FG**	
Cu	**BNT**	**78.58** (4.41)	/	***	***	**84.93** (13.37)	/	***	***	ns
	PU	**31.53** (3.99)	***	/	***	**34.30** (0.90)	***	/	***	*
	FG	**15.61** (2.40)	***	***	/	**13.91** (2.29)	***	***	/	ns
Pb	**BNT**	**233.68** (43.37)	/	***	***	**283.16** (36.02)	/	***	***	**
	PU	**39.65** (6.97)	***	/	ns	**50.44** (6.51)	***	/	ns	***
	FG	**32.58** (3.30)	***	ns	/	**31.58** (1.56)	***	ns	/	ns
Zn	**BNT**	**197.15** (28.93)	/	***	***	**213.58** (43.68)	/	***	***	ns
	PU	**72.99** (14.24)	***	/	ns	**83.17** (3.88)	***	/	**	*
	FG	**65.05** (8.64)	***	ns	/	**52.12** (2.72)	***	**	/	***

ANOVA, mean (SD), n=15, levels of significance: ***P<0.001, **P<0.01, *P<0.05, ns - not significant

Table 2. Mean values and standard deviation in parenthesis of total Cu, Pb, and Zn concentrations (mg/kg d.w.) in *T. tomentosa* roots and leaves at Bulevar Nikola Tesla (BNT), Park Ušće (PU), and Fruška Gora Mt. (FG).

	Site	Root				Leaf			
		M (SD) (mg/kg)	**BNT**	**PU**	**FG**	**M** (SD) (mg/kg)	**BNT**	**PU**	**FG**
Cu	**BNT**	**9.59** (2.22)	/	***	***	**7.87** (1.46)	/	ns	ns
	PU	**5.90** (1.14)	***	/	ns	**7.32** (0.99)	ns	/	ns
	FG	**5.55** (0.98)	***	ns	/	**7.54** (0.46)	ns	ns	/
Pb	**BNT**	**6.18** (1.23)	/	/	***	/	/	/	/
	PU	/	/	/	/	/	/	/	/
	FG	**1.62** (0.23)	***	/	/	/	/	/	/
Zn	**BNT**	**16.92** (3.36)	/	***	***	**12.81** (2.03)	/	**	***
	PU	**10.24** (2.01)	***	/	ns	**11.09** (1.20)	**	/	*
	FG	**10.46** (2.50)	***	ns	/	**9.57** (0.49)	***	*	/

ANOVA, mean (SD), n=15, levels of significance: ***P<0.001, **P<0.01, *P<0.05, ns - not significant

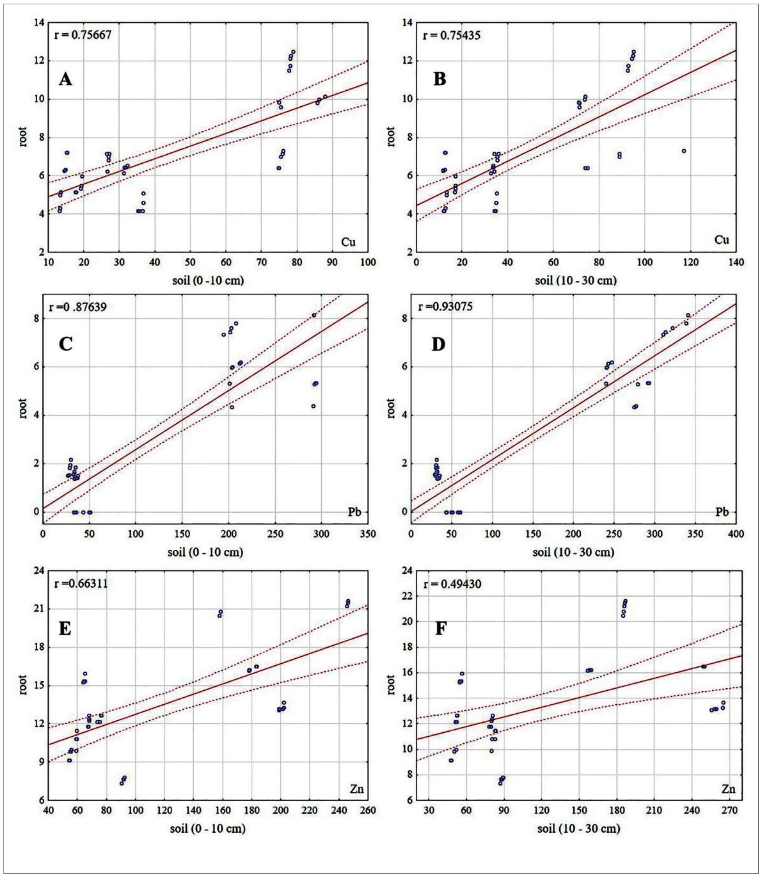

Fig. 1. The relationship of the total content of trace metals Cu, Pb, and Zn in soil-root samples: **A** - Cu in soil-root samples (0-10 cm); **B** - Cu in soil-root samples (10-30 cm); **C** - Pb in soil-root samples (0-10 cm); **D** - Pb in soil-root samples (10-30 cm); **E** - Zn in soil-root samples (0-10 cm); **F** - Zn in soil-root samples (10-30 cm); (r - correlation coefficients; confidence level of 95%; significance level *P<0.05; n=15).

the highest levels once more measured in samples from Bulevar Nikola Tesla, and similar concentrations at PU and FG. Zn concentrations in leaves ranged from 9 to 12 mg/kg, with the highest at BNT and the lowest at FG (Table 2).

Relationship of copper, lead, and zinc concentrations in soils and plants

In this study, Spearman's rank correlation coefficients were calculated for PTE contents across all datasets, including total element concentrations in soil-root and root-leaf samples from all locations.

Significant positive correlations were found between soil (at both depths) and roots for all the analyzed elements. Similar positive correlations in Cu concentrations were observed between topsoil and plant roots (r=0.757, P<0.05), as well as between subsoil and roots (r=0.754, P<0.05). Strong positive correlations were observed between Pb in topsoil and roots (r = 0.876, P < 0.05) and between Pb in the deeper soil layer (10-30 cm) and roots (r = 0.931, P < 0.05), indicating a substantial influence of subsoil on Pb uptake by roots. A significant positive correlation was also observed between Zn content in soil and plant roots (Fig. 1). In addition, positive correlations were found between root and leaf samples, particularly for Cu (r=0.639, P<0.05) (Fig. 2).

Copper, lead, and zinc transfer in the soil-root-plant system: Bioconcentration and translocation factors

Results showed BCF <1 for Cu, Pb, and Zn in lime roots and leaves at both soil depths across all sites, indicating phytostabilization potential, while TF >1 was observed for Cu (Park Ušće and Fruška Gora Mt.) and Zn (Park Ušće). In contrast, the translocation factor for Pb was

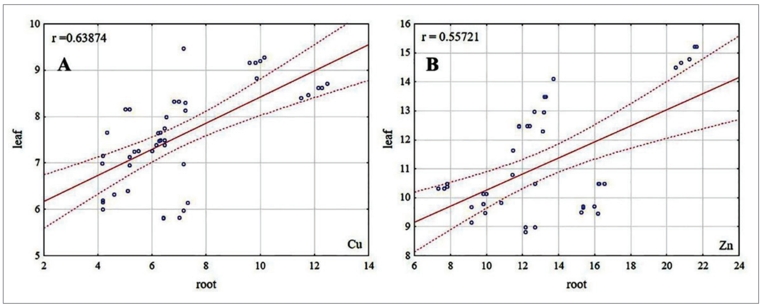

Fig. 2. The relationship of the total content of trace metals Cu and Zn in root-leaf samples: **A** - Cu in root-leaf samples; **B** - Zn in root-leaf samples; (r - correlation coefficients; confidence level of 95%; significance level *P<0.05; n=15).

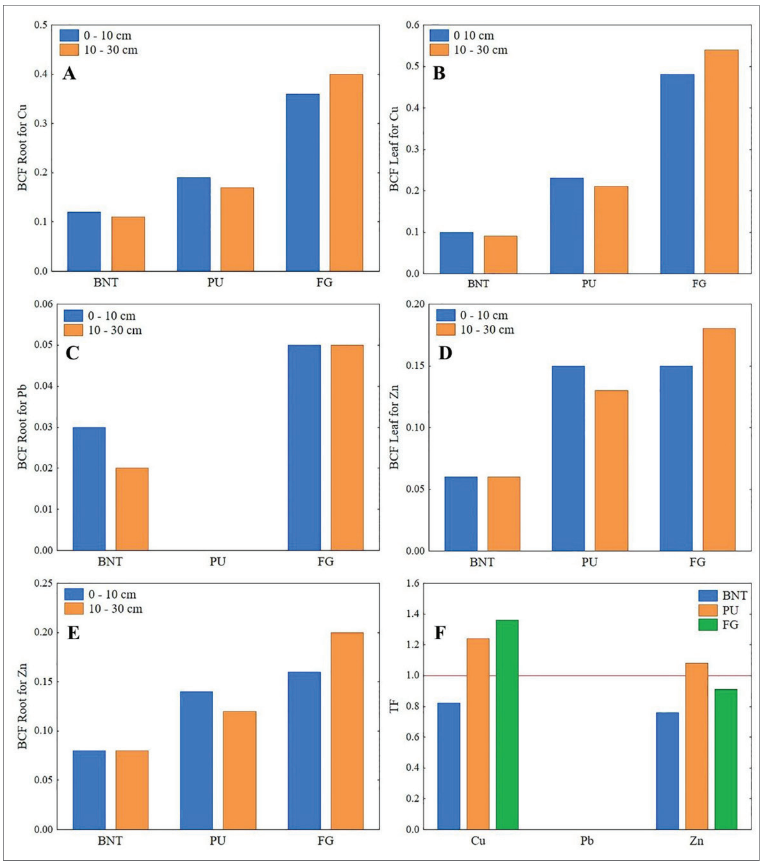

Fig. 3. Bioconcentration factors (BCF) and translocation factors (TF) for Cu, Pb and Zn on all locations, Bulevar Nikola Tesla (BNT); Park Ušće (PU), and Fruška Gora Mt. (FG): **A** - BCF root for Cu in both soil depths on all locations; **B** - BCF leaf for Cu in both soil depths on all locations; **C** - BCF root for Pb in both soil depths on BNT and FG locations; **D** - BCF leaf for Zn in both soil depths on all locations; **E** - BCF root for Zn in both soil depths on all locations; **F** - TF for Cu and Zn on all locations.

very low at all sites because leaf concentrations were below the detection limit, indicating that the species retains soil-absorbed Pb in roots (BCF <1) without transporting it to leaves. This species exhibited the greatest ability to absorb Cu and Zn in its natural habitat (FG) and in the urban park (PU). It also transported absorbed Cu and Zn from its roots to its leaves (TF>1 at PU and FG). This is to be expected given that Cu and Zn are essential elements required for the key physiological processes in plants, such as photosynthesis and pigment synthesis (Fig. 3).

Table 3. Photosynthetic efficiency (Fv/Fm) of *T. tomentosa* at different sites at Bulevar Nikola Tesla (BNT), Park Ušće (PU), and Fruška Gora Mt. (FG).

Locality	Fv/Fm	Min-Max
BNT	0.801 (0.025)^{ns}	0.747 - 0.825
PU	0.802 (0.016)^{ns}	0.766 - 0.814
FG	0.812 (0.011)^{ns}	0.790 - 0.826

ANOVA, mean (SD), n=15, levels of significance: ns - not significant

Photosynthetic efficiency (Fv/Fm) of lime trees

There were no statistically significant differences in the mean values of the chlorophyll fluorescence parameter (Fv/Fm ratio) among the sites, although lower values were measured in leaves from the urban sites (Table 3). Greater variability in photosynthetic efficiency values was also determined in samples from the urban sites, with the greatest variation measured in the tree-lined street (BNT).

Low positive correlations were observed between total Cu and Zn contents and Fv/Fm in leaves across all sites (Fig. 4). Correlation analysis indicated that photosynthetic efficiency was not significantly affected by the uptake and transfer of these essential elements, particularly Zn.

DISCUSSION

Anthropogenic pollution in urban and industrial areas is a major environmental concern at global, regional, and local levels. Urban pollution varies between cities, reflecting their specific historical urban and industrial activities. Urban soils have

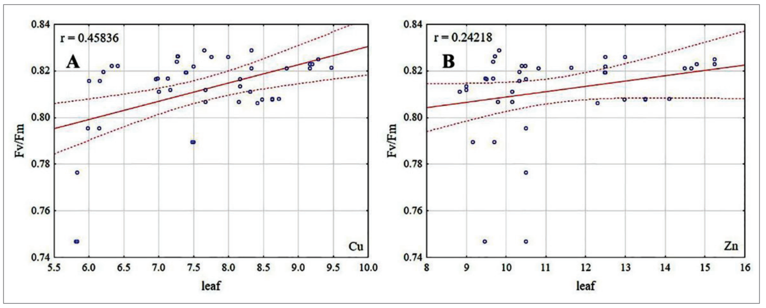

Fig. 4. Spearman correlations between leaf Cu content (mg/kg d.w.), and Fv/Fm(**A**), and leaf Zn content (mg/kg d.w.) and Fv/Fm (**B**); r - correlation coefficients; confidence level of 95%; significance level *P<0.05; n=15).

been extensively altered by human activities through mixing, import, export, and contamination in urban and suburban areas [2]. Urban surface soils act as the primary sink for potentially toxic metals and other pollutants, while deeper layers can reflect historical contamination [23,24]. These subsoils are typically affected by a long history of industrial activities, atmospheric deposition, reconstruction, and backfilling with materials of often unknown composition [7]. Generally, topsoils and subsoils contain natural levels of potentially toxic metals, with concentrations determined by the composition of the parent rock material [3,25,26], while the anthropogenic origins of potentially toxic metals, including Cu, Pb, and Zn, are mainly attributed to traffic, vehicle emissions, brake and tire wear, and street-level industrial activities [3,27,28].

This study assessed concentrations of three potentially toxic elements (Cu, Pb, and Zn) in urban soils at 0-10 cm and 10-30 cm depths, their accumulation in roots and leaves, and their effects on photosynthetic efficiency. Soil measurements showed a wide range of values depending on both location and element. For all elements, higher concentrations were generally observed in the 10-30 cm soil layer at most sites, especially Pb at urban locations, indicating possible historical pollution. Spatially, the highest concentrations of all analyzed elements were found at Bulevar Nikola Tesla in Belgrade, while the lowest occurred at the control site, Fruška Gora Mt.

It was established that Zn and Pb are the most abundant elements in urban soils with concentrations higher than reference concentrations for world soils (90 mg/kg for Zn and 35 mg/kg for Pb) [29]. At the same time, these concentrations fell within the upper range of values proposed for European soils (150-300 mg/kg for Zn and 50-140 mg/kg for Pb) [30]. Cu levels at the urban sites exceeded the global average soil concentration of 30 mg/kg [29]. Considering that previous studies indicate urban soils in Serbia and elsewhere are generally alkaline [12,26], and in accordance with Directive 86/278/EEC on soil pH, Gawlik and Bidoglio [30] proposed threshold values for Cu, Pb, and Zn in such soils: 100 mg/kg for Cu and Pb, and 200 mg/kg for Zn. The results of this study show that there are higher concentrations of Pb and Zn at urban sites at both soil depths, implying potential toxic effects on plants.

Significant differences in Cu concentrations were observed in both soil layers across all samples and sites. In contrast, Pb concentrations in both soil layers differed only between urban sites, with no differences between the urban park and control site. Similarly, Zn concentrations in topsoil differed between urban sites, while topsoil levels at the urban park and natural site were similar; differences were observed in subsoil. Such heterogeneity in concentrations reflects the local influence of road traffic on soil along the tree-lined avenue. These findings align with previous studies of Belgrade's urban parks, which reported slightly elevated Cu, Pb, and Zn levels above European and national regulatory limits, attributed to intensive traffic and nearby industrial activity [26]. The same study reported that heavy metal concentrations in Belgrade's urban soils were comparable to those in other European cities, although EU regulations currently do not control urban soil metal contamination, and data on its extent remain limited [6,31]. In the current study, average concentrations for the three elements were far lower than for urban soils in European countries (Italy, Spain, the UK, Poland, Norway, Portugal, Greece, etc.), where data indicate that Cu, Pb, and Zn exceed national safety thresholds. Specifically, Cu, Pb, and Zn concentrations in Belgrade's urban soils were generally lower than in other European cities: Cu was lower than in Spain (mean 386.6 mg/kg), the UK (mean 140 mg/kg), and Italy (57-163 mg/kg, mean 138.3 mg/kg); Pb was lower than in the UK (195-971 mg/kg, mean 488.7 mg/kg) and Poland (148-782 mg/kg, mean 331 mg/kg) but higher than in Spain (100-364 mg/kg, mean 209 mg/kg); Zn was lower than in Poland (364-844 mg/kg, mean 603 mg/kg), Italy (140-406 mg/kg, mean 226.8 mg/kg), and the UK (178-364 mg/kg, mean 268.3 mg/kg) [6]. Furthermore, Cu concentrations in both the tree-lined avenue and park were much lower than in Paris urban forest soils, whereas Pb and Zn levels were higher in the avenue soil [32]. Nonetheless, the concentrations measured in Belgrade soils fell within the upper range of mean values reported for other European urban soils (46 mg/kg for Cu, 102 mg/kg for Pb, and 130 mg/kg for Zn), as summarized in [33] for 34 urban soils across more than ten European countries. The elevated concentrations of Pb and Zn measured in urban soils (BNT) imply that, while industrial facilities and city heating plants contribute to metal pollution in urban areas, traffic emissions are still a significant source, and in the case of Pb, this is despite petrol now being lead-free [4]. Nevertheless, legacy Pb from leaded petrol can be re-released from urban soils, dust, and sediments into the atmosphere and other environmental compartments, serving as a secondary pollution source. Additionally, unleaded petrol and diesel may still contain a certain amount of Pb and other metal pollutants [24]. Therefore, Pb contamination from old (leaded petrol) and new (unleaded petrol) vehicle exhaust emissions cannot be ignored. Moreover, human activities have increased Zn levels in topsoil through atmospheric deposition, fertilization, and sewage sludge application. While Zn-toxic soils are less common than Zn-deficient soils, excess Zn can affect soil organisms, including plants, invertebrates, and microorganisms [34].

Many municipalities are increasing urban tree planting to harness ecosystem services, including air pollution reduction, mitigation of heat islands, and retention of metals and nutrients in soils [8,35]. These services contribute to mitigating global urban environmental changes, including through biomonitoring and phytoremediation. However, urban trees are exposed to severe abiotic stresses, such as contamination by PTEs. This can have a negative effect on the ecological services they provide, particularly in tree-lined streets. Degradation of the physical and chemical properties of soil due to management practices or pollution is just one of the constraints that can affect plant growth, development, and functioning, including plant-soil relations [36,37]. Trees are at particular risk from climatic and environmental threats due to their size and the length of time their growth and reproductive phases last. A key challenge for urban green-space managers is to have accessible, cost-effective, and reliable methods to monitor tree health and identify tolerant species [38]. This study was conducted in Belgrade to expand knowledge and evaluate the potential of silver lime (*T. tomentosa* Moench.) as a suitable tree for urban sites, particularly along heavily trafficked streets. Site-dependent variations in Cu, Pb, and Zn concentrations were found in plant samples. In roots, the highest Cu, Pb, and Zn levels were found at Bulevar Nikola Tesla, while Cu and Zn concentrations did not differ between Park Ušće and the control site at Fruška Gora Mt. In leaf samples, there was no difference in Cu content between the sites, in contrast to Zn, which exhibited significant differences between BNT and the other two sites. Correlation analysis in this study showed that soil chemical properties, specifically PTE concentrations, strongly influence elemental levels in plant tissues through absorption of water and minerals, including essential elements (Cu and Zn) for optimal plant function and non-essential, potentially toxic elements (Pb). The highest concentrations of all three elements at both investigated soil depths were recorded along the tree-lined Bulevar Nikola Tesla.

Cu concentrations in all leaf samples across sites were approximately 7 mg/kg, within the normal range for plants. Cu levels in plant shoots typically range from 4 to 15 mg/kg dry weight and are well regulated across a wide soil Cu range [34], while the sufficient-to-normal range is 5-30 mg/kg. [39]. In contrast, Zn concentrations in leaves differed significantly between sites, ranging from 9 to 12 mg/kg, within the deficiency range of 10-20 mg/kg [39]. All examined elements in Belgrade were lower compared to a heavily trafficked street in Budapest, Hungary, where leaf Cu ranged from 15.40 to 26.10 mg/kg, Pb from 0.88 to 2.11 mg/kg, and Zn from 19.30 to 20.85 mg/kg [40]. However, previous studies reported similar leaf Cu and Zn accumulation, and to a lesser extent Pb, throughout most of the growing season at various Belgrade sites with differing pollution levels (e.g., Aničić et al. [41]), as well as for Cu in other European cities (e.g., Piczak et al. [42]; Quénéa et al. [19]), suggesting a species-specific pattern. Earlier findings established that concentrations of elements such as Pb, Mn, Fe, and Zn in leaves are primarily influenced by genotype, supporting this assertion [43].

Spearman's rank correlations for soil-plant transfer of Cu, Pb, and Zn were significant, with strong positive correlations observed between soil and root metal contents for all elements. Cu concentrations showed positive correlations between both soil depths and roots. High positive correlations were also observed between Pb in topsoil and roots, and between Pb in subsoil and roots, highlighting the strong influence of the root zone on Pb uptake. The lowest correlation between soil and root concentrations was observed for Zn. Correlation analysis indicated that the 0-30 cm soil layer strongly influences the chemical properties of plant tissues, with positive correlations observed between roots and leaves, especially for Cu.

Plant species vary in their capacity to accumulate or tolerate metals in their aerial parts and roots. It is something that is determined by soil metal concentrations, the physiological characteristics of the species, and their selectivity for specific elements [44,45]. Phytostabilization is a form of phytoremediation aimed at immobilizing pollutants in a contaminated substrate by establishing vegetation, while phytoextraction involves hyperaccumulator plants absorbing metals from the soil through their root systems and translocating them to the harvestable shoot, making it possible to recover metals from the harvestable parts of plants [46,47]. A key aspect of phytoremediation is selecting suitable species, which requires evaluating their phytoextraction or phytostabilization capacities. The results obtained in this study revealed BCFRoot<1 for both soil depths and BCFLeaf<1 for Cu, Pb, and Zn at all the study sites, while TF>1 was calculated for Cu (Park Ušće, Fruška Gora Mt.) and for Zn (Park Ušće). It was also determined that there was no transfer of absorbed Pb from roots to leaves at any of the locations. This is consistent with the known behavior of Pb, which is less soluble, less mobile, and has low phytoaccumulation potential, with soil concentrations of 10-30 mg/kg typically not affecting plant growth [34,39]. The absorption of studied elements in *T. tomentosa* roots (BCF<1) indicates its phytostabilization potential, while Cu and Zn translocation (TF>1) at natural sites, where soil properties are unaltered (FG) or minimally altered (PU), is expected, as these elements are essential for physiological processes like photosynthesis and pigment synthesis. This is in line with previous research recommending *T. tomentosa* as a bioindicator of anthropogenic pollution and for phytoremediation of metal-contaminated soils [48]. However, our results suggest the species may be more effective for biomonitoring, specifically in quantifying urban soil quality and pollution.

Silver lime is one of the most widely used ornamental trees in urban environments, with its broad foliage offering deep shade. In this study, photosynthetic efficiency (Fv/Fm) showed similar mean values across all sites (above 0.800), with the highest at the natural control site (0.790-0.826; mean 0.812) and the lowest, with the greatest variability, at the tree-lined BNT site in Belgrade (0.747-0.825; mean 0.801). All values were within the optimal plant range (0.750-0.850) and slightly below the empirically determined mean for deciduous trees (0.843 ± 0.012) [49], indicating high overall vitality of *T. tomentosa* in urban habitats despite exposure to abiotic stresses, including potentially toxic pollutants. No statistically significant differences in mean chlorophyll fluorescence values were established between the sites, although relatively lower values were measured in leaves from the urban sites. However, this analysis showed that photosynthetic efficiency is not significantly influenced by uptake and transfer, i.e., the content of essential elements in leaves, particularly zinc. In other words, this species is well-adapted to absorbing and accumulating elements that are essential for optimal photosynthesis and other physiological processes, but an occasional deficit of some of these elements does not significantly affect photosynthetic efficiency. This result supports earlier findings on the stress tolerance of *T. tomentosa*. Previous research suggested that this species is a better alternative to more moisture-demanding lime trees for urban habitats [50]. *T. tomentosa* has exhibited superior capacity to adapt to the climatic conditions of the urban environment, enabling its robust development in the face of potential environmental change due to its higher net photosynthesis, transpiration rate, and water use efficiency in comparison to other tree species, such as *Fraxinus excelsior* L. and *Acer platanoides* L. [51]. Its higher leaf area index indicates a denser, more efficient canopy, enhancing light interception, photosynthesis, and growth, while increasing interaction with environmental pollutants, thereby boosting physiological activity and ecosystem services, including urban thermal regulation [52]. Silver lime adapts to heat and drought by orienting its leaves with undersides outward, reflecting more solar radiation, lowering leaf temperature, and reducing transpiration and water los. [53].

Its high vitality and resistance to the stressful conditions in urban habitats are also closely related to its leaf surface morphology, including trichomes, and a granulated epicuticular wax layer. These features reduce transpiration and uncontrolled water loss during summer droughts, as occur in Belgrade, and protect the leaf surface, particularly the abaxial side, from air pollutants and particulate matter (PM). Simultaneously, these morphological traits help trap and retain significant amounts of fine particulate matter on the leaf surface. From a fine PM retention perspective, *T. tomentosa* is a favorable choice for urban greening due to its resilience and capacity to capture particulate matter from polluted air [51]. Combined with tolerance to low light (from building shade) and aphids, and its capacity to uptake and accumulate PTEs without visible damage, *T. tomentosa* is a preferred urban species, often planted in densely paved areas such as tree-lined avenues [12,53-56].

CONCLUSIONS

The study indicates both current and potential historical urban soil contamination, with road traffic as a major source of Cu, Zn, and Pb. *T. tomentosa* exhibited strong physiological stability and the ability to stabilize these elements in the soil. Its resilience and adaptability, evidenced by limited Pb soil-to-leaf transfer and tolerance of essential element deficiencies (e.g., Zn), make *T. tomentosa* suitable for phytoremediation and urban greening in long-term polluted areas. Additionally, *T. tomentosa* could serve as roadside vegetation, contributing to the mitigation of environmental pressures in street corridors and representing an ideal candidate for such purposes.

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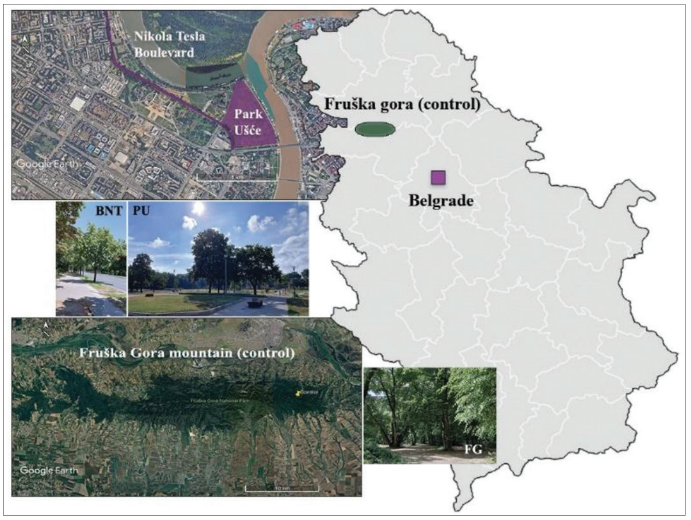
SUPPLEMENTARY MATERIAL



Supplementary Fig. S1. Location map showing the control Fruška Gora Mt., FG; Bulevar Nikola Tesla, BNT, and Park Ušće PU study sites.