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TRACE ELEMENT CONTENT IN URBAN TREE LEAVES AND SEM-EDAX CHARACTERISATION OF DEPOSITED PARTICLES[†]

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Abstract. *Leaves of common deciduous trees: Aesculus hippocastanum and Tilia spp. from three parks within the urban area of Belgrade (Serbia) were studied as biomonitors of trace elements (V, Cr, Fe, Ni, Cu, Zn, As, Cd, and Pb) air pollution. Using a scanning SEM-EDAX, the size, size distribution, morphology and chemical composition of individual particles were examined on adaxial and abaxial surfaces of the leaves. Morphological and chemical composition indicated that the most abundant particles were soot and dust with minor constituents such as Pb, Zn, Ni, V, Cd, Ti, As, and Cu. Total element concentrations in the leaves were determined by ICP-OES and ICP-MS. This investigation included spatial, seasonal, and temporal variations in leaves of the selected species. The leaves of A. hippocastanum showed a significantly higher elements concentration and more consistency in trend of element accumulation during the vegetation season in the period 2002-2006 than Tilia spp., so it may be considered as a more suitable species for the assessment of trace element atmospheric pollution, especially Pb and Cu which correlated with the bulk deposition data.*

Key words: *deciduous tree leaves, particles deposition, SEM-EDAX, trace element, ICP-OES, ICP-MS*

1. INTRODUCTION

Direct effects of air pollutants on plants, animals and soil can influence the structure and function of ecosystems, including their self-regulation abilities, thus affecting the quality of life. Trace elements are released into the atmosphere by human activities, such as combustion of fossil fuels and wood, high temperature industrial activities and waste incineration. The combustion of fossil fuels constitutes the principal anthropogenic

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source of Ba, V, Co, Ni, Se, Mo, Sn, Sb, and Hg, and particularly of Cr, Mn, Cu, Zn, and As. High percentages of Ni, Cu, Zn, As, and Cd are emitted from industrial metallurgical processes. Exhaust emissions from gasoline may contain variable quantities of Ni, Cu, Zn, Cd, and Pb [1]. Several trace elements are emitted through the abrasion of tires (Cu, Zn, Cd) and brake pads (Sb, Cu), corrosion (V, Fe, Ni, Cu, Zn, Cd) lubricating oils (V, Cu, Zn, Mo, Cd) or fuel additives (V, Zn, Cd, Pb) [2-4]. The platinum group elements, Rh, Pd and Pt, represent a relatively new category of traffic related trace elements in the environment, specially urban one, due to their application in automobile catalytic converters since the beginning of the 1980s [5].

Studies of the transport and mobilization of trace elements up to now have attracted attention of many researchers [6, 7]. Trace elements are persistent and widely dispersed in the environment and interacting with different natural components result in toxic effects on the biosphere. The extensively employed direct collection of atmospheric deposition using bulk sampling devices offer a practical approach for monitoring of atmospheric trace elements deposition [8, 9]. However, studies on atmospheric contamination have frequently been limited by high cost of instrumental monitoring methods and difficulties in carrying out an extensive sampling in space and time. For these reasons, there is an increasing interest in using indirect monitoring methods such as the use of organisms that may act as bioaccumulators.

Biomonitoring of trace elements from atmospheric deposition can be currently evaluated by biomonitors such as mosses, lichens and higher plants [10, 11]. Although biomonitoring of air quality using plants has been practiced for many years, in many European countries, it has still not been applied at a satisfactory level due to different and even opposite results, depending, first of all, on plant species.

Trees are very efficient at trapping atmospheric particles, and they have a special role in reducing a level of fine, “high risk” particulate matter with < 2.5 μm in aerodynamic diameter ($PM_{2.5}$), with the potential to cause serious human health problems. Thus, the use of plant leaves, primarily, as accumulative biomonitors of trace metal pollution has attained great ecological importance [10-13]. Leaves of various tree species, both evergreen and deciduous, have been tested for this purpose in urban areas [14, 15] including a search for sensitive tree species and an approval of the validity of using such leaves as biomonitors. It is well known that trace elements air pollution leads to physiological disturbances in plants and affects the biogeochemical balance and stability of their habitats. An element uptake in higher vascular plants takes place through their root system, additionally through the leaves and, therefore, it is difficult to distinguish whether the accumulated elements originate from the soil or from the air [16, 17]. Research of trace elements contamination of vegetation requires the use of standardized methodological procedures [10, 11]. Representative sampling of plant material is one of the critical points within.

The aim of our research was to set up a reliable methodological approach for sampling and analytical procedures for further studies. This investigation also comprised physical and chemical characterization of particles deposited on leaf surfaces, and determination of element accumulation in leaf tissue. Investigations aimed at biomonitoring of trace element atmospheric contamination in Belgrade urban area using deciduous leaves of some common tree species and trace elements accumulation in leaves in relation to atmospheric bulk deposition measurements.

2. MATERIALS AND METHODS

2.1. Study area

The study was conducted in Belgrade (φ = 44º 49’ N, λ = 20º 27` E, H_s = 117 m), the capital of Serbia, situated at the confluence of the rivers Sava and Danube with about 2 million inhabitants. There are many old buses and trucks on the streets, and leaded gasoline is still widely used. The total number of the registered vehicles increased from 2002 to 2006 (394 540 to 470 396, respectively). Of the total vehicles, the largest was the number of the passenger cars (83%, in 2006); most of them were either from 0-10 years old (41%), or 16-25 (40%). However, the percentage of new cars (0-10 years old) increased from 2002-2006, and so was the production of the unleaded gasoline (NIS oil refinery report, 2006). The survey was carried out at three representative locations, the city parks in heavy traffic areas: Students Park (SP); Botanic Garden (BG); Karađorđev Park (KP).

2.2. Sampling, sample preparation and analysis

Leaves were sampled from deciduous trees: *Aesculus hippocastanum* L. (horse chestnut), and *Tilia* spp. (linden: *Tilia tomentosa* L. being more frequently present, than *Tilia cordata* Mill.), at the beginning (May) and the end (September) of the seasonal vegetation cycles from 2002 to 2006. Wearing polyethylene gloves, leaves were cut off with stainless steel scissors from about 2 m height. Five subsamples (10 to 15 fully developed leaves) were taken randomly from all sides of a crown. The subsamples were packed in polyethylene bags.

In the laboratory, the leaves were washed with bi-distilled deionized water, dried in the oven at 40 ºC, pulverized using agate mortars, packed in polyethylene bags and kept in stable laboratory conditions till chemical analysis. Portions of approximately 0.4 g of leaves (dry weight) were digested for 2 h in a microwave digester (speed*wave*™ MWS-3$^+$, BERGHOF) with 3 mL of 65% HNO_3 (Suprapure, Merck) and 2 mL of 30% H_2O_2 using a temperature program for food (100-170 °C). The digested samples were diluted with distilled water to a total volume of 25 mL.

Analysis was performed by inductively-coupled plasma optical spectrometry, ICP-OES (SpectroGenesis EOP II, Spectroanalytical Instruments GmbH, Kleve, Germany), and concentrations of Cr, Fe, Ni, Cu, Zn, and Pb were determined. The following wavelength lines of the ICP-OES analysis were used: Cu 324.754 nm, Fe 259.941 nm, Zn 206.191 nm, Cr 205.552 nm, Ni 231.604 nm, and Pb 220.353 nm. The instrumental detection limits were: Cr 0.0001 mg L^{-1}, Fe 0.035 mg L^{-1}, Ni 0.001 mg L^{-1}, Cu 0.001 mg L^{-1}, Zn 0.0001 mg L^{-1}, and Pb 0.003 mg L^{-1}. The calibration standards were prepared for each analyzed element using ultra pure reference chemicals certified for ICP analyses (CertiPUR®, Merck KgaA, Darmstadt, Germany). Concentration of some trace elements (V, As, and Cd) in the extracts were analyzed by inductively coupled plasma-mass spectrometry (ICP-MS) using an Agilent 7500ce spectrometer equipped with Octopole Reaction System (ORS). With this method, low detection limits can be obtained for elements present in low concentration and for metalloid elements such as As with high ionization energies. Prior the ICP-MS analysis all the samples were filtered through a 0.45 μm pore diameter membrane filter. Calibration was performed with external standards obtained by appropriate dilution of Fluka Multielement Standard Solution IV. Blank and calibration standards were prepared in 2% nitric acid for all the measurements except for those used to determine the detection limits (As 0.2 μg L^{-1}, Cd 0.01 μg L^{-1}, and V 0.03 μg L^{-1}). Tuning solution containing 1 μg L^{-1} Li, Mg, Co, Y, Ce, and Tl (Agilent) was used for all instrument optimizations.

Quality control of both used methods (ICP-OES and ICP-MS) was performed using the standard reference material lichen-336 (IAEA). The concentrations found were within 90-115% of the certified values for all measured elements.

For Scanning Electron Microscopy and Energy Dispersive Spectroscopy (SEM-EDAX), samples were prepared for both adaxial and abaxial leaf surfaces at least ten leaves of several trees (3-5) of both tree species. Discs of 10 mm diameter were cut from unwashed leaves with a sharp device, wearing polyethylene gloves. Each leaf disc was mounted on an aluminum stub, over double-sided stick tape. The stubs were placed on a perforated round Teflon plate, cut to fit in a polycarbonate Petri dish. Leaf samples were dried in air in the clean room. To minimize charge build-up on the samples from exposure to the SEM electron beam the samples were coated with 100-150 Å layer of high purity carbon using vacuum evaporator (Balzers/Union FL-9496) prior to analysis. SEM Philips XL30 apparatus equipped with a thin-window EDAX DX4 system for energy dispersive X-ray microanalysis was used for physico-chemical characterization of particles deposited on the leaf samples. Three different leaf discs of the adaxial and abaxial surfaces for both tree species were examined in the same way. Ten photomicrographs were randomly taken of each 0.03 mm^2 area at 624·magnification and about 1800 particles per species were assessed to their morphology and about 900 for X-ray spectra analysis. For each tree species about 0.025% of the original leaf surface was examined. The elements observed were: Al, Si, C, S, N, Cl, P, K, Ca, Na, Mg, Cr, Fe, Cu, Zn, Ni, Cd, As, Ti and V with detection limit > 1 wt %. The relative elemental composition of the particles, were computed directly with EDAX software, using the atomic number, absorption and fluorescence (ZAF) correction.

Instrumental monitoring was performed using the bulk atmospheric deposition (BD) collectors, i.e. the total, dry and wet deposits were collected monthly, at the same studied sites where the leaves were sampled, using open polyethylene cylinders fixed in baskets. The elemental composition in the atmospheric bulk deposits was determined by flame (FAAS; Perkin Elmer AA 200) and graphite furnace atomic absorption spectrometry (GFAAS). The details of sampling and analysis of bulk deposits are presented in our previous study [18].

2.3. Data analysis

Data handling included the basic statistics for As, V, Cd, Cr, Fe, Ni, Cu, Zn, and Pb concentrations in leaves within May and September samples of the years 2002-2006, and in monthly bulk depositions for the same period. Seasonal elements accumulation, spatial distribution and temporal trend during the investigated period for both plant species *A. hippocastanum* and *Tilia* spp., were studied.

Enrichment factor (EF) analysis was used to differentiate the elements mainly originating from human activities and those of natural origin and to assess the degree of anthropogenic influence [19]. EF represents the ratio of the element E and reference element R in the sample $(E/R)_{sample}$, and in the crust $(E/R)_{crust}$:

$$EF = \frac{\left(\frac{E}{R}\right)sample}{\left(\frac{E}{R}\right)crust} \tag{1}$$

3. Results and Discussion

3.1. Physical and chemical characterization of particles deposited on leaves

Scanning electron photomicrographs presented in Fig. 1 (e-g) show deposited particles/elements observed/detected on the surface of *A. hippocastanum* leaves, sampled in the Belgrade urban area: adaxial surface, general appearance (Fig. 1a); particles of different size and shape (Fig. 1 b-d); and chemical composition (Fig. 1 e-g). Particles were present at a higher density on the adaxial leaf surfaces. Thus, heavy loads were observed in some areas, mostly around the main veins. Particles were present in a wide range of diameters up to 50 μm, but the analysis of the particle size distribution for both species showed that 50-60% of the analyzed particles were of a diameter less than 2 μm (fine particles) mainly originating from anthropogenic activities. Two main particle categories were observed: particles of natural sources include materials of organic origin (pollen, bacteria, fungal spores etc.). This category also includes suspended soil dust (primarily soil mineral) such as the angular-shaped material. Particles from anthropogenic sources mostly emitted from high temperature combustion processes are characterized by their spherical shapes and smooth surfaces. This type of particles occurs as individual particles but also in aggregate form as agglomerates of similar-sized particles and individual large particles caring several smaller attached particles. Fine particles, were often observed around and over the 10 μm stomatal openings on abaxial leaf surfaces (Fig. 1c). Even an entrance of fine particles into leaves through the stomata is physically possible, it is not yet clear whether and to what extent this may occur. Both, fine and coarse particles (> 2 μm), were reported to be responsible for increased leaf temperature and decreased light absorption, thus also affecting photosynthesis. Similar observations of physico-chemical composition were also obtained for *Tilia* spp. (not shown).

The chemical composition of the particles deposited on leaves (Fig. 1e-g) plant species suggested that the most abundant particles were: soot (C) and soil dust with characteristic matrix elements (Si, Al, Fe, Mg, N, S, Ca, K, Cl); fuel oil particles rich in Al, Si, Ca, Ni, Fe, V and Pb; coal ash particles containing C, Al, Si, K, Ca; and particles liberated by the local industrial processes (Fe, Zn, Ni, Cu or Pb-rich). Among the particles containing trace metals, the most abundant were particles in aggregates form, where Pb is the major element associated with lower concentrations of S, Fe, Cd, Cu, As and Zn (Fig. 1g).

According to the particle morphology and chemical composition indicated by the SEM-EDAX procedure, it may be suggested that particles deposited on the leaves mostly originated from the traffic or from the resuspended particles and possibly local sources.

3.2. Total element content in leaves and comparison with bulk deposition measurement

The seasonal element accumulation, i.e. an increase of the element concentrations during the vegetation period for V, Cr, Fe, Ni, Cu, Zn, As, Cd, and Pb was more evident in leaves of *A. hippocastanum*, as presented in the Table 1. The Zn seasonal accumulation was distinguished in *A. hipposactanum*, but not in *Tilia* spp. leaves.

Starting from 2002 to 2006, the Pb concentrations in leaves of *A. hippocastanum* at the beginning and the end of vegetation seasons showed a decreasing trend at the studied sites (Table 1). The concentrations of Cu in the leaves were significantly higher at the SP site as compared to other studied sites, and with a decreasing trend through the investigated years, but more regular for *A. hippocastanum* (Fig. 2) than for *Tilia* spp. (not shown). At the KP and BG sites, the Cu concentrations in leaves of both, *A. hippocastanum* and *Tilia* spp., remained at about the same concentration level throughout the years, and mostly higher in May than in September (Table 1). The Cr, Fe, Ni, and Zn concentrations remained at about the same level in the studied species throughout the investigated years. The observed temporal decrease of Cu and Pb concentrations in *A. hippocastanum* leaves was in agreement with trends in the atmospheric bulk deposition (BD) data [18]. The enrichment factors (EFs) were calculated for the trace element concentrations in the leaves of *A. hippocastanum*, *Tilia* spp., and in the bulk atmospheric deposition (Fig. 3). The results are based on the median concentrations for all sites and years. In this study, Fe was used as the reference element and the upper continental average crustal composition given by Mason [19]. The EFs for leaves, as well as for BD, were the highest for Pb, than followed by Zn and Cu, and according to the EF scale, belong to the class of intermediately enriched ($10 < EF < 100$) elements, while Ni and Cr were among the least enriched ($EF < 10$) [20].

Seasonal accumulation trends of elements concentration in leaves, often referred to as accumulative biomonitors, have been well known from the literature, and reported for many plant species [11, 21, 22]. An increase of the element concentrations ($p < 0.001$) from May to September was evident in all samples of *A. hippocastanum* troughout the investigated years for: Cr, Fe, Ni, Zn, and Pb, as well as in *Tilia* spp. leaves for: Cr, Fe, Ni, and Pb, but was not so regular, i.e. absent at some sites and in some years of the investigation. On the other hand, in *A. hippocastanum* leaves there was no regularity in the seasonal accumulation of Cu ($p < 0.15$) and in *Tilia* spp. leaves for Cu ($p < 0.2$) and Zn ($p < 0.09$). For *A. hippocastanum*, such seasonal discrepancy for the Cu and Zn concentrations was also noted previously by Kim and Fergusson [21], pointing that these elements were the highest in new leaves, and decreased along vegetation season. Thus, a variation in seasonal accumulation of Cu and Zn in some leaf samples of *A. hippocastanum* and *Tillia* spp. obtained in this study, may be due to a physiological role of these elements as essential constituents of plant tissue. It is considered that the Cu remobilization to non-senescent parts occurs before the senescence, and leaf fall takes place.

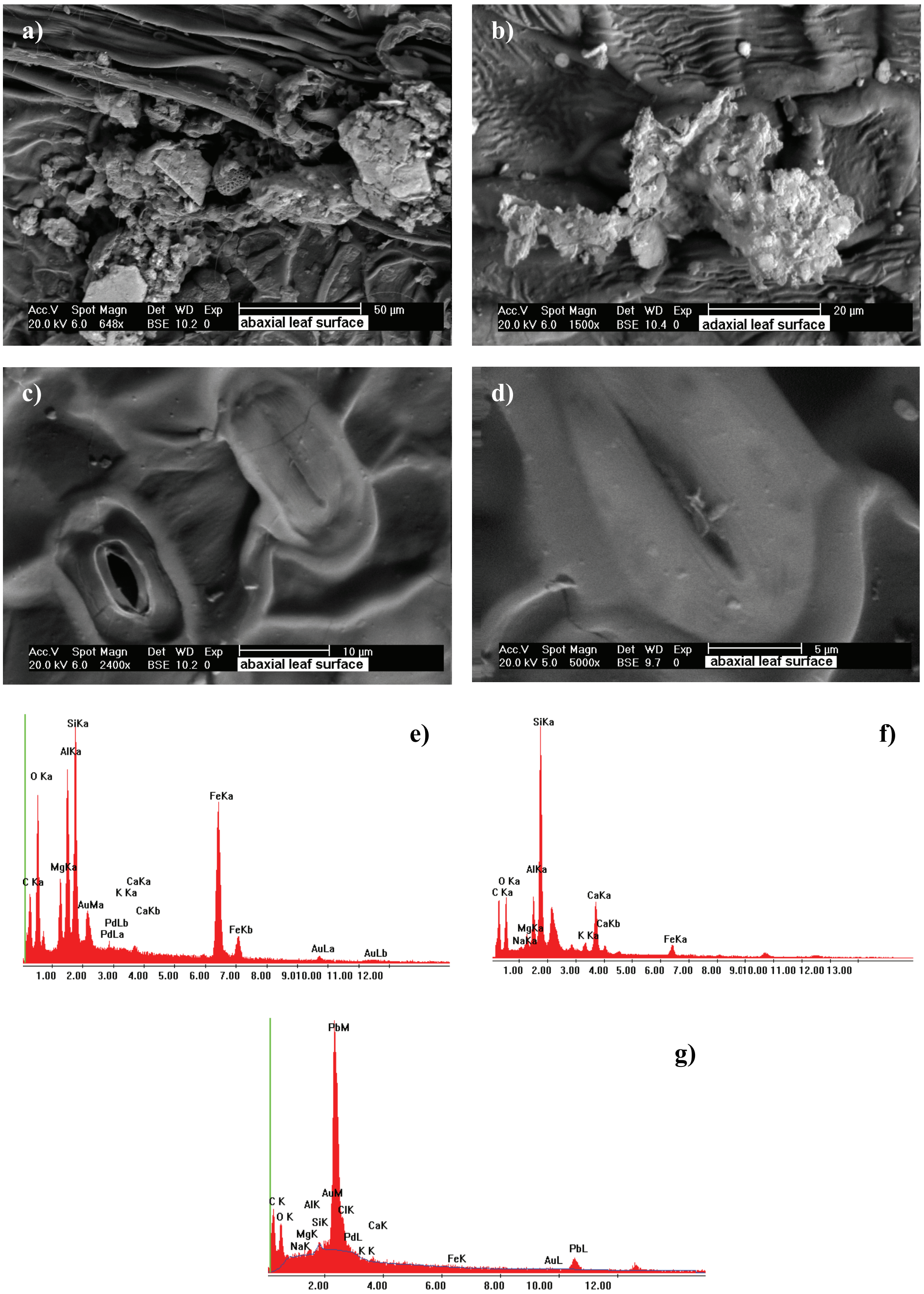

Fig. 1. SEM-EDAX photomicrographs of *A. hippocastanum* leaf surfaces (a-d) and spectra presenting the chemical composition of most frequently observed particles (e-g)

Table 1. Median concentration [μg g^{-1}] of V, Cr, Fe, Ni, Cu, Zn, As, Cd, and Pb in deciduous tree leaves, sampled from the urban area of Belgrade in May and September from 2002 to 2006

A. hippocastanum											
			V	Cr	Fe	Ni	Cu	Zn	As	Cd	Pb
2002	May	Median	0.448	0.57	247.71	0.59	11.21	23.08	0.252	0.302	7.49
		SD	*0.118*	*0.13*	*71.38*	*0.29*	*17.05*	*3.07*	*0.074*	*0.250*	*2.96*
	September	Median	1.104	1.43	564.51	1.12	8.34	29.64	0.530	0.032	16.01
		SD	*0.459*	*0.26*	*137.73*	*0.79*	*45.81*	*4.06*	*0.304*	*0.037*	*4.97*
2003	May	Median	0.158	0.44	114.01	0.49	14.78	18.65	0.142	0.014	2.14
		SD	*0.131*	*0.19*	*32.71*	*0.07*	*3.67*	*2.67*	*0.063*	*0.005*	*0.27*
	September	Median	0.405	0.78	331.30	0.92	8.74	23.10	0.296	0.075	6.69
		SD	*0.285*	*0.24*	*72.43*	*0.12*	*38.34*	*6.21*	*0.277*	*0.023*	*6.80*
2004	May	Median	0.277	0.42	162.79	0.44	11.87	15.70	0.133	0.020	2.39
		SD	*0.064*	*0.16*	*32.60*	*0.14*	*4.84*	*2.94*	*0.112*	*0.006*	*0.52*
	September	Median	0.664	1.23	394.38	1.20	15.82	26.60	0.346	0.027	10.55
		SD	*0.335*	*0.36*	*123.13*	*0.25*	*28.88*	*7.42*	*0.528*	*0.003*	*2.86*
2005	May	Median	0.336	0.30	111.75	0.51	16.60	18.14	0.146	0.066	1.28
		SD	*0.303*	*0.03*	*16.07*	*0.15*	*6.73*	*1.57*	*0.043*	*0.140*	*0.83*
	September	Median	0.494	0.96	345.65	1.19	8.79	25.69	0.352	0.271	5.64
		SD	*0.449*	*0.26*	*110.46*	*0.55*	*6.78*	*7.61*	*0.251*	*0.791*	*0.52*
2006	May	Median	0.339	0.40	175.14	0.75	12.08	21.14	0.216	0.014	1.31
		SD	*0.280*	*0.18*	*60.49*	*0.20*	*2.86*	*1.67*	*0.227*	*0.007*	*0.85*
	September	Median	0.518	0.79	338.13	1.06	9.23	18.73	0.244	0.033	4.57
		SD	*0.075*	*0.29*	*93.01*	*0.44*	*3.42*	*8.13*	*0.293*	*0.015*	*1.07*
Tillia spp.											
			V	Cr	Fe	Ni	Cu	Zn	As	Cd	Pb
2002	May	Median	0.405	0.77	346.01	0.92	10.97	26.38	0.193	0.758	12.92
		SD	*0.236*	*0.40*	*153.88*	*0.55*	*1.73*	*14.76*	*0.080*	*0.957*	*7.90*
	September	Median	0.745	0.57	276.50	1.17	7.59	21.95	0.293	0.023	6.67
		SD	*0.587*	*0.27*	*103.01*	*0.52*	*11.03*	*5.94*	*0.272*	*0.019*	*2.74*
2003	May	Median	0.089	0.34	83.20	0.48	14.22	25.03	0.063	0.025	1.09
		SD	*0.080*	*0.13*	*24.12*	*0.13*	*2.75*	*3.03*	*0.064*	*0.014*	*0.38*
	September	Median	0.408	0.67	285.21	1.06	10.53	21.03	0.294	0.029	6.43
		SD	*0.277*	*0.01*	*23.60*	*0.19*	*21.33*	*2.16*	*0.093*	*0.019*	*1.84*
2004	May	Median	0.235	0.36	145.51	0.64	10.32	15.36	0.077	0.014	1.48
		SD	*0.102*	*0.25*	*58.03*	*0.26*	*2.85*	*4.29*	*0.018*	*0.013*	*0.80*
	September	Median	0.661	1.81	391.30	1.38	16.33	24.67	0.213	0.052	7.20
		SD	*0.180*	*0.54*	*164.63*	*0.41*	*22.22*	*7.04*	*0.111*	*0.049*	*3.22*
2005	May	Median	0.082	0.34	99.46	0.50	12.09	16.38	0.060	0.038	1.13
		SD	*0.017*	*0.09*	*29.74*	*0.02*	*4.84*	*2.42*	*0.030*	*0.024*	*0.11*
	September	Median	0.460	0.36	144.74	0.70	8.91	15.58	0.233	0.554	1.95
		SD	*0.052*	*0.05*	*27.46*	*0.16*	*1.98*	*1.52*	*0.534*	*1.464*	*0.46*
2006	May	Median	0.162	0.31	130.17	0.65	7.96	18.05	0.105	0.016	0.86
		SD	*0.235*	*0.18*	*60.38*	*0.19*	*1.69*	*4.89*	*0.030*	*0.004*	*0.54*
	September	Median	0.235	0.57	220.64	0.89	8.16	20.17	0.249	0.020	2.60
		SD	*0.443*	*0.10*	*13.63*	*0.08*	*1.37*	*1.78*	*0.156*	*0.005*	*1.23*

In case of the walnut trees, the concentration of Cu in old leaves was just 8% of the maximum Cu value measured in the younger mature leaves [23]. Also, some recent data for the black spruce needles, supporting a previous hypothesis, confirmed that an active translocation of essential metals, particularly Cu, takes place from senescent to nonsenescent parts of a plant [24]. The authors also pointed that, on the contrary, the results obtained for Pb, as nonessential metal, were in accordance with a hypothesis that the passive sequestration of toxic metals was attained in the senescing foliage as a detoxification process.

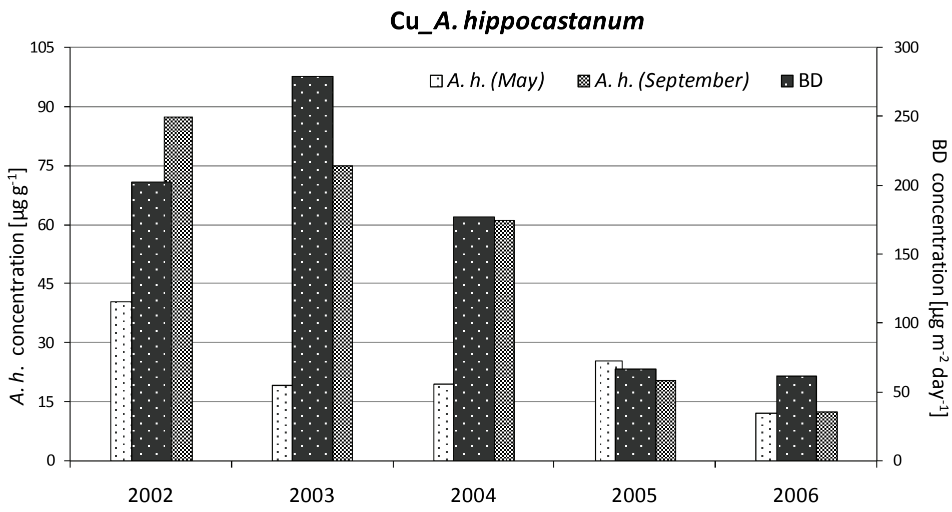

Fig. 2. The Cu concentrations in A. hipposactanum leaves in May and September (2002-2006), and bulk deposition (BD) at the SP site.

In order to assess anthropogenic origin of elements in leaves, enrichment factors (EFs) were calculated on the basis of the average crustal values given by Mason [19]. The EFs calculated for the elements in leaves of *A. hippocastanum* and in *Tilia* spp., as well as for the bulk deposition [18], followed the same pattern. The highest enrichment was displayed for Pb, then for Zn and Cu, indicating their anthropogenic origin. It is in agreement with a recent report for the Belgrade urban area, stating that most of the trace element atmospheric pollution originated from traffic and road dust resuspension [25]. Lower EFs were obtained for Cr and Ni, and there were no significant variations throughout the investigated years, possibly due to a lack of the main emission sources of these elements - fossil fuel combustion - during heating season [26].

Temporal decrease of the Pb concentrations in leaf tissue of both species, observed in the Belgrade urban area might be a consequence of diminished usage of leaded gasoline, along the investigated years, in favor of the unleaded kind. This is in accordance to the concentration trends reported for other European countries. As suggested from a long-term study (1992-2004), the phasing-out of leaded gasoline had a major effect on reduced Pb contamination in Warsaw [27]. The authors reported decreasing trends of Pb concentrations for two types of biomonitors: moss bags (*Sphagnum fallax*), and tree leaves of

Crimean linden (*Tilia euchlora*). In Italy, from a long-term monitoring of metal pollution by urban trees, Gratani *et al.* [28] showed decreasing trends for Pb, among other elements (Al, Fe, Cu, Zn) in leaves of *Q. ilex.* Also, as shown from another recent long-term study, though atmospheric Pb declined by a factor of 7 from 1980 to 2007, airborne Pb was named as still a major pathway to vegetation and topsoils [29]. Uncertainty in element uptake pathways has generally been seen as a disadvantage for the use of the vascular plant leaves as biomonitors of trace element atmospheric pollution. However, studying the origin of Pb in leaves, Hovmand *et al.* [29] showed that less than 2% of the Pb content of needles and twigs of Norway spruce comes from root uptake, and approximately 98% is of the atmospheric origin. The previous study in the Belgrade urban area also pointed to a good correlation of the Pb leaf content of *A. hippocastanum* with significantly increased level of atmospheric Pb in suspended particles during the two successive years (1996-1997), as reported by the Health Institute in Belgrade. So, the Pb concentration in the leaves of *A. hippocastanum* reflected changes in atmospheric trace metal pollution [30].

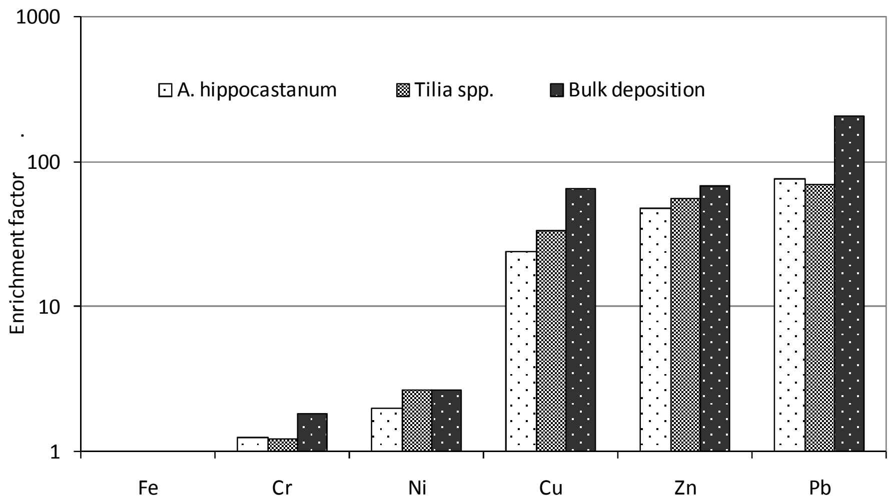

Fig. 3. Enrichment factor of the elements in leaves of *A. hippocastanum*, *Tilia* spp., and bulk deposits

Among the studied parks, the highest Cu concentrations were obtained at one of the studied sites (SP), as also indicated by some other monitoring techniques - BD, PMs [18] and moss [31] pointing to some additional local source. Presumably, a local Cu emitter (metal arts and crafts manufacturing) contributed to a much higher atmospheric Cu levels in 2002, 2003 and 2004 at this site, tending to decrease throughout the years, when it closed down. Namely, the Cu concentration was the highest in September of 2002, and this accumulation level was about nine times higher in *A. hippocastanum* leaves (87.5 μg g^{-1}) than the "reference plant" value (10 μg g^{-1}), given by Markert [32]. At the same time, the Cu concentrations in bulk deposits were 3-4 time higher at the beginning comparing to the final year of the study. Thus, the temporal trend for the Cu accumulation in leaves of *A. hippocastanum* is in accordance with the Cu contents in the BD for SP site. The Cu content in *Tilia* spp. leaves neither showed clear neither seasonal nor temporal dependence.

The soil Cu concentration at the SP site was also decreasing from 2002 (98 μg g^{-1}) [33] to 2008 (50 μg g^{-1}) [34]. The presence of the elements in soil does not imply that they are available to plants. Generally, plant-to-soil concentration ratio is far from a linear function [11, 35]. The results of a recent study showed that there was low correlation between the transfer factors of metals from polluted soils to plants [36]. Moreover, the measured pH of the soil samples at this site was 8.8, and it is not likely that the element availability for the root uptake would be considerable. Consequently, it may be supposed from the results of this study that the Cu content in leaves was substantially of atmospheric origin.

There were no substantial variations in accumulated content of Cr, Fe, Ni, and Zn thought the studied years, and no agreement in temporal trends with the bulk deposition measurements.

Evaluation of a validity of biomonitor appears as a complex task, and may require other aspects apart from elements accumulation level, such as temporal trend consistency in accumulation capability. Also, it would be affirmative for biomonitoring if a biomonitor shows a correspondence to some instrumental monitoring data.

4. CONCLUSIONS

The SEM-EDAX analysis of individual particles deposited on both side of the leaves showed that the 50-60% belong to a class of fine particles (D < 2 μm) singly or gathered in agglomerates of various shapes (spherical, flakes, irregular). The particles were distributed with higher density on the adaxial leaf surfaces. Fine particles, mainly of anthropogenic origin, were often observed around and over the stomata which may affect the physiological characteristics of leaves. According to their morphology and chemical composition investigated by the SEM-EDAX, the most abundant particles were soot (C) and dust (Si, Al, Fe, Mg, N, S, Ca, K, Cl) with minor constituents such as Pb, Zn, Ni, V, As, Ti, Cu, and Cd. The most abundant were particles in aggregates form, where Pb is the major element associated with lower concentrations of S, Fe, Cd, Cu, As, and Zn. It may be suggested that the particles deposited on leaves mostly originated from the traffic, or from the resuspended particulate matter. Chemical analyses of leaves total element content need a consideration the seasonal accumulation of Cr, Fe, Ni, Zn, and Pb in leaves of *A. hippocastanum* and *Tilia* spp. (except Zn). During the studied time span, Pb concentrations in the leaves showed a decreasing trend (more regular for *A. hippocastanum*), being in accordance with the bulk atmospheric deposition data. Also, the temporal concentration trend for the Cu in *A. hippocastanum* was decreasing and in accordance with the Cu trends in the bulk atmospheric deposition at the site with the highest atmospheric Cu loading. No agreement was observed between the accumulation trend of Cr, Fe, Ni, and Zn in leaves and the bulk deposition rates, i.e. the elements content in the leaves did not reflect atmospheric deposition directly. Accordingly, *A. hippocastanum* could be suggested as an appropriate biomonitor for Pb atmospheric pollution, and for Cu in highly polluted areas.

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SADRŽAJ ELEMENATA U TRAGOVIMA U LISTOVIMA DRVEĆA I SEM-EDAX ANALIZA FIZIČKO-HEMIJSKIH KARAKTERISTIKA DEPONOVANIH ČESTICA

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U ovom radu je ispitivana mogućnost primene listova listopadnog drveća divljeg kestena (Aesculus hippocastanum) i lipe (Tilia spp.), često zastupljenih u parkovima Beograda, u biomonitoringu zagađenosti vazduha elementima u tragovima (V, Cr, Fe, Ni, Cu, Zn, As, Cd i Pb). Ispitivanja oblika, veličine, kao i raspodele po veličinama čestica deponovanih na površinama listova rađena su korišćenjem metoda SEM-EDAX. Morfološka analiza, kao i analiza hemijskog sastava čestica, na licu i naličju listova, ukazala je na većinsko prisustvo čađi i prašine sa manjim doprinosom elemenata kao što su Pb, Zn, Ni, V, Cd, Ti, As i Cu. Ukupan sadržaj elemenata u listovima određivan je metodama ICP-OES i ICP-MS. Ovo istraživanje je obuhvatilo analizu sezonske, prostorne i vremenske promene sadržaja elemenata u listovima ispitivanih vrsta. Listovi A. hippocastanum su više akumulirali određivane elemente i pokazali veću pravilnost u akumulaciji tokom vegetacione sezone u višegodišnjem periodu (2002-2006) u odnosu na Tilia spp. Stoga se A. hippocastanum može smatrati pogodnijim biomonitorom za procenu zagađenosti vazduha elementima u tragovima, naročito sa Pb i Cu, što je u saglasnosti sa rezultatima merenja ukupne atmosferske depozicije.

Ključne reči: *listovi listopadnog drveća, atmosferska depozicija čestica, SEM-EDAX, elementi u tragovima, ICP-OES, ICP-MS*