Arch Biol Sci. 2017;69(2):201-214 https://doi.org/10.2298/ABS161202005P

Seasonal variations of trace element contents in leaves and bark of horse chestnut (*Aesculus hippocastanum* L.) in urban and industrial regions in Serbia

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Received: December 2, 2016; **Revised**: January 12, 2017; **Accepted**: January 31, 2017; **Published online**: February 2, 2017

Abstract: In this study, we examined the ability of horse chestnut (*Aesculus hippocastanum* L.) to capture heavy metals, and whether its capacity to absorb metals from soil is associated with surrounding ecological characteristics and sources of pollution. We studied the seasonal accumulation of B, Cu, Sr and Zn in leaves and bark, and the chlorophyll content in the common deciduous tree *Aesculus hippocastanum* L. in four urban parks in Pančevo, Smederevo, Obrenovac and Belgrade (Serbia) affected by different anthropogenic activities. The research included plants from a control site located within the zone of a former oak forest. Our findings suggest that there are potential ecological risks around Smederevo, Belgrade and Obrenovac due to elevated concentrations of B and Zn relative to the average concentrations described for worldwide soils, as well as national regulations. Substantial and toxic foliar accumulation of B was observed in Smederevo and Belgrade, and of Sr in both plant tissues at all sites. However, the Cu and Zn contents in leaves were not enough to meet the physiological needs of plants. Chlorophyll a and the total carotenoid content peaked in August under the most unfavorable conditions of the year, which may be considered as an adaptive mechanism. The obtained results showed the remarkable complexity of environmental conditions and the difficulties *A. hippocastanum*, as a species, has to overcome. Under conditions of different types of urban and industrial pollution, *A. hippocastanum* showed great element accumulation potential and could be regarded as both an accumulator and a response indicator, since its leaves are quite susceptible to damage.

Key words: trace elements; urban parks; *Aesculus hippocastanum*; biomonitoring; soil pollution

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INTRODUCTION

The environment of large cities and industrial complexes is characterized by stressful factors including air and soil pollution, 'heat islands', disturbance of the soil/water balance and nutrient cycling [1,2]. Air and soil pollution with trace metals is of great interest because most of the trace metals and metalloids in urban areas are emitted from heavy traffic, industrial activities, wastewater effluents and waste disposal. Furthermore, urban pollution is apparent in pollution point sources such as mines, foundries and smelters, and other metal-based industrial operations [3,4].

Parks are a typical urban land use that involve mutual interactions among human activities, soil, air and plants [5]. The soil and vegetation in parks are strongly affected by anthropogenic factors. In the last decades, special attention has been paid to linear soil contamination by metals, petroleum hydrocarbons and de-icing salts along roadsides. Beside pollution, the pressure imposed by trampling causes soil compaction that reduces soil porosity, moisture and organic matter contents [6], as well as vegetation cover, height and species diversity [7].

The anthropogenic pollution of soils by trace elements represents a serious environmental problem, especially in zones near industrial areas as their emissions reach them by different routes, making urban soils a major sink for heavy metals, since they mostly do not undergo chemical or microbial degradation [8,9]. Unlike metals that are stably bound to silicate minerals and represent the background metal concentration that is

How to cite this article: Pavlović M, Rakić T, Pavlović D, Kostić O, Jarić S, Mataruga Z, Pavlović P, Mitrović M. Seasonal variations of trace element contents in leaves and bark of horse chestnut (*Aesculus hippocastanum* L.) in urban and industrial regions in Serbia. Arch Biol Sci. 2017;69(2):201-14.

not a environmental threat, metals that originate from human activities and are present in high concentrations can modify soil properties [10]. In polluted areas, the transfer of toxic elements from soils to plants is of great concern since excessive concentrations of heavy metals in soil may render it nonproductive because of phytotoxicity, and may cause the bioaccumulation of heavy metals in plants. The bioavailability of elements to plants is controlled by many factors associated with soil and climatic conditions, plant genotype and management, and include: active/passive transfer processes, sequestration and speciation, redox states, the type of plant root system and the response of plants to elements in relation to seasonal cycles [10-12].

Long-term soil contamination may be assessed by using plants as biomonitors, especially in areas where the sampling and chemical analysis of soil samples may be challenging and expensive [13]. The main advantages of using plants are the greater availability of biological material, simplicity of species identification, sampling and treatment, and ubiquity of some genera, which make it possible to cover large areas [14]. Trees could be a valuable tool in monitoring air pollution in cities and chemical foliar analysis is a widely-used tool to monitor the impact and extent of air and soil pollution [15-17]. Trees are also very efficient in trapping atmospheric particles, especially in urban areas, which is why the use of leaves as biomonitors that accumulate trace metals has acquired great ecological importance [18]. Various physiological and biochemical processes in trees are affected by metals: metal-polluted sites may cause altered metabolism, growth reduction, lower biomass production and toxic metals accumulation [19]. Furthermore, tree bark, due to its structural porosity and potential for efficient accumulation and retention of aerosol particles, is also considered to be a promising indicator in air pollution monitoring. The elemental composition of tree bark is influenced by different factors, including dry and wet deposition of aerosol particles from the atmosphere, transport of crown intercepted pollutants by stem flows and uptake of minor and trace elements via root nutrition, which is why a knowledge of these concentrations, as well as their variations, represents a basic prerequisite of using tree bark as a biomonitor [14,20].

For any single target element, the ratios of concentrations between plant parts provide useful information about the different accumulation in each part, which can suggest if there is a free movement of the elements between the parts [21]. In addition, bioaccumulation assessment is important in the scientific evaluation of risks chemicals may pose to humans and the environment. Plant-to-soil and plant-to-air bioconcentration ratios (BCRs) are used to relate the chemical concentrations measured in different plant tissues to concentrations in the soil supporting that vegetation. However, the distribution of elements in plant parts can vary because the total element contents in aboveground parts depend on root uptake and on element retranslocation from assimilation organs. Apart from the disadvantage that it is sometimes difficult to distinguish between the amounts of an element taken up from the soil and that are deposited from air in leaves, trees, as long-lived organisms, reflect the cumulative effects of environmental pollution from the soil and the atmosphere [22].

Urban and industrial areas in central Serbia are contaminated by several heavy metals and metalloids from local industrial emissions and heavy traffic. In the city of Belgrade, industrial facilities (chemical, pharmaceutical, metallic industry) are distributed at random in the central parts of the city and its surroundings, where there are large industrial complexes that represent, together with heavy city traffic, the sources of various types of pollutants. In the surroundings, within 50 km to the west, two thermoelectric power plants are located in Obrenovac; to the east there is a factory of nitric fertilizers and a refinery (Pančevo), and to the south there is an iron smelter (Smederevo). In all the cities, there are urban parks dominated by high trees, mainly deciduous species such as maple and chestnut, linden, sycamore, acacia, poplar, ash, birch, etc. The main conifer trees are pine, fir, larch, cypress, etc. From among these species, horse chestnut (*Aesculus hippocastanum* L., which belongs to the Sapindaceae family and the Hippocastanoideae subfamily, and originates from the southern Balkan Peninsula, northern Greece and southern Bulgaria) was chosen for this study. This is a rapidly growing tree with sessile leaflets that can reach a height of 36 m. In Serbia, it is commonly planted for ornamental purposes in urban parks and along roadsides. Likewise, it is extensively naturalized around Europe and in North America, even where there is a high level of urban pollution [23]. Previous studies have showed that *A. hippocastanum* has high capacity for accumulating trace elements in leaves [18,23-25].

The aim of this study was to analyze the composition and trace element concentrations in the topsoil of urban parks in four cities (Pančevo, Smederevo, Obrenovac and Belgrade) affected by different sources of pollution, and to determine the phytotoxic influence on horse chestnut. The second aim was to assess the seasonal gradient of heavy metal and metalloid content in *Aesculus hippocastanum* L. leaf and bark. The third aim was to evaluate the use of *Aesculus hippocastanum* L. as a biomonitor and to determine which plant parts are suitable as pollution biomonitors. Furthermore, the content and functionality of photosynthetic pigments were measured to assess the plant's response to pollution conditions. The results on elemental content in urban soils as well as in plants could be of interest to industrial and environmental authorities, since biomonitoring provides information on environmental quality and could serve as a baseline for future environmental impact assessment as well as urban park management.

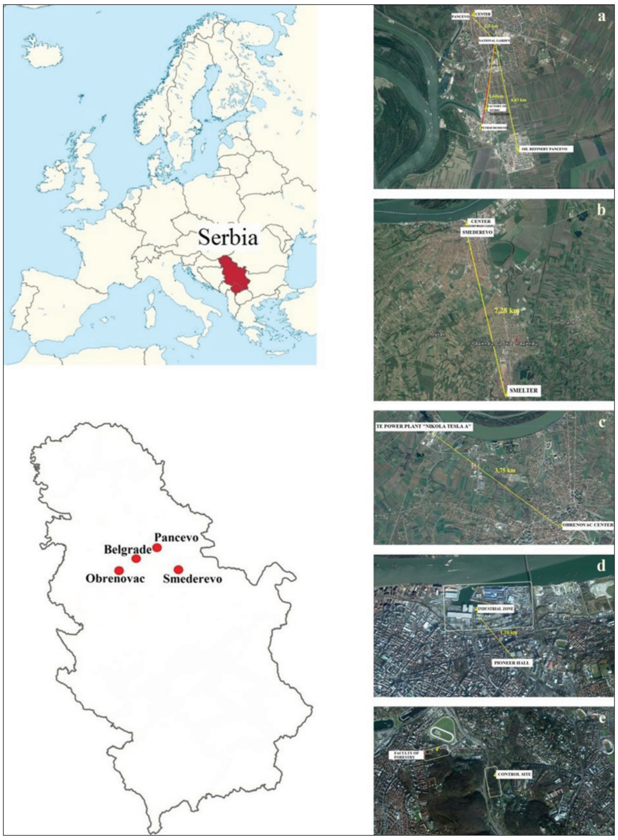

Fig 1. Selected urban sampling sites in Serbia: **a** – Pančevo; **b** – Smederevo; **c** – Obrenovac; **d** – Belgrade; **e** – Belgrade-control site.

MATERIALS AND METHODS

Study area

The sampling sites were urban parks in cities exposed to airborne trace element pollution from industrial activities, industrial waste disposal and traffic: Pančevo, Smederevo, Obrenovac, Belgrade. Topčiderski park in Belgrade, as part of a natural oak forest, served as a control site (Fig. 1).

Pančevo (φ 44°54'N, 20°40'E, H_s 77 m) is situated on the banks of the rivers Danube and Tamiš, in the autonomous province of Vojvodina with over 76000 inhabitants. It is an industrial center of Serbia with three large sources of pollution positioned in the northwest (a nitric fertilizer factory, a refinery and petrochemical industry). The National Garden, where sampling was carried out, is the largest and the oldest city park in Pančevo, founded in 1823. It is one of the most beautiful parks and urban green areas in Serbia that stretches over 14 ha. It is located at 44°51'54" north latitude and 20°39'23" east longitude, at an altitude of 77 m. The city center is only 1.3 km away. Its distance from the nitric fertilizer factory is 2.69 km, from the petrochemical plant 3.50 km and from the oil refinery 4.43 km. The nearest pollution monitoring station is "Vatrogasni dom", where SO_2, H_2S, B, T, Me-Me (benzene, toluene, methyl mercaptan), TNMHC (total non-methane hydrocarbons type), nitrogen oxides ($NO/NO_2/NO_x$), NH_3 and suspended particles smaller than 10, 2.5 and 1 μ, are measured.

Smederevo (φ 44°40'N, 20°56'E, H_s 73 m) is situated on the right bank of the Danube river with over 64000 inhabitants. Downtown Smederevo, where the sampling was performed, is located at 44°39'54" north latitude and 20°55'37" east longitude, at an altitude of 73 m. The central zone of the city has numerous parks and urban green areas that stretch over 9.25 ha. Samples were collected from the central zone of the "Tri heroja" Park. The industrial zone is located 7 km southeast of the city with a large smelter, "Železara Smederevo". The nearest monitoring station is in the city center, which measures the concentration of suspended particles <10 and 2.5 μm, NO_2 and SO_2.

Obrenovac (φ 44°39'N, 20°12'E, H_s 76 m) is one of the 17 municipalities of Belgrade. It is suited at the confluence of the rivers Kolubara and Sava and has a little over 25000 inhabitants. Obrenovac City Square, "Trg Dr Zoran Djindjić", is one of the most important green areas in Obrenovac, built in 2005, and has over 100 trees and 50 shrubs, with a total of 22 different tree species. The City Square is about 4 km southeast from largest thermoelectric power plants in Serbia, "Nikola Tesla A", which burn about 12 Mt of low-caloric lignite coal and dispose approximately 2.4 Mt of ash per year, covering over 400 ha of the ash disposal site established on fertile agriculture soil [26]. The nearest measuring station is in the city center, where CO, NO_2, SO_2 and the concentrations of suspended particles <10 μm are measured.

Belgrade (φ 44°48'N, 20°27'E, H_s 117m) is the capital of Serbia, situated on the confluence of the rivers Sava and Danube, with about 1.7 million inhabitants. It covers 3.6% of Serbia's territory, and 22.5% of the country's population lives in the city. Traffic is one of the main sources of pollution because of large number of cars, trucks and buses. Of the total vehicles, the highest portion in 2012 (87.64%) was the number of the passenger cars, with 27273 vehicles passing by the Pioneer Hall park every day [27]. In addition, the vast majority of vehicles on the streets are older than 10 years. Sampling was conducted at two sites: Pioneer Hall park is located at coordinates 44°48'53" north and 20°29'70" east, at an altitude of 104.44 m. It is situated near several major traffic roads and about 1 km from the former industrial zone in the municipality of Palilula. Considering that industry has mostly ceased to operate, heavy traffic exhaust is the main source of pollution. Green areas in the park are significantly fragmented by large concrete surfaces. The nearest monitoring station is a Public Health Department unit, which measures NO_2, CO, as well as suspended particles <10 μm.

Topčiderski Park in Belgrade (44°46'59" north and 20°26'19" east), at an altitude of 80 m, 10 km from the city center, without any direct source of pollution, and located in a zone of mixed oak forest (*Quercus frainetto* and *Quercus cerris* Rud.) was chosen as the control site. This park, considered one of cultural and historical importance, is the first of its kind designed according to European standards on the territory of Serbia. The area has been proposed for the category of significant natural value with the establishment of a third-degree protection regime. The size of the area sampled is about 2.0 ha.

The research was carried out at three monthly intervals (June, August and October) during 2012. The studied year was the warmest and driest year compared to measurement from the period 1961-1990, with an extremely high number of hot days and nights. The mean summer air temperature was categorized as extremely warm, with 62 days of maximum daily temperatures higher than 30°C, and 52 nights with minimum temperatures higher than 20°C [28].

Sampling

Composite topsoil samples at 0-10 cm depth were obtained by mixing subsamples with a stainless-steel shovel at five random points within about 1 m^2 in each sampling site. Stones and foreign objects were removed by hand, and samples were packed in clean plastic bags. They were dried at 105°C, gently crushed and sieved to 2 mm.

Plant samples from representative *A. hippocastanum* trees of the same age were also collected from urban parks and compared with samples of the same species from a background site. Leaves were taken uniformly from the lower foliage and from different quarters of the tree crowns with stainless-steel scissors using polyethylene gloves, with an initial sample quantity of about 30 g. Flakes of the bark layer about 4-5 mm thick with maximum dimensions of about 1×3 cm^2, were carefully cut with a stainless-steel knife 1.2-1.5 m above ground level (in all directions around a tree). Sampling was performed in the central parts of the parks, avoiding proximity to the streets, in order to get a maximum dispersion. In the laboratory, unwashed leaves and bark were dried to a constant weight at 105°C (Binder, Tuttlingen, Germany). Dried samples were ground with a stainless-steel mill and sieved through 1.5-mm stainless-steel sieve.

Soil and plant sample analysis

The soil pH in aqueous solution was measured using a glass electrode (1:2.5 soil-water ratio) after shaking the samples to equilibrium for approximately 30 min

[29]. Organic matter was determined with a CNS analyzer, Vario model EL III (Elemental Analysis systems GmbH, Hanau, Germany). The physical properties of the soils entailed the determination of particle size distribution by combined pipette and sieve technique with a 0.4-N solution of sodium pyrophosphate. Fractionation was carried out according to Atterberg [30]. The texture classes were determined using a soil texture triangle [31].

For trace element analysis, leaves, bark and soil samples (0.3 g) were digested in a microwave (CEM, 39 MDS-2000) in advanced composite vessels using 12 ml of 65 % HNO_3 (Carlo Erba) and 4 ml 30% H_2O_2 (Lachner). The final extracts were filtered into 50-ml polyethylene volumetric flasks, and then diluted to the mark with deionized water. The concentrations of four elements (B, Cu, Sr and Zn) in the samples were measured by inductively coupled plasma optical emission spectrometry (ICP-OES) (SpectroGenesis Genesis Fee, Spectro-Analytical Instruments GmbH, Kleve, Germany). Quality control was performed using the standard reference material for leaves (Beech leaves – BCR-100; soil – Loam soil-ERM-CC141) obtained by the Institute for Reference Materials and Measurements (IRMM, Geel, Belgium) and certified by the European Commission Joint Research Centre (EC-JRC). The concentrations found were within 95-105% of the certified values for all measured elements. Detection limits were 0.000634 mg/kg for B, 0.00974 mg/kg for Cu, 0.00069 mg/kg for Sr and 0.00348 mg/kg for Zn, respectively. Measurements were done in 5 replicates. Element concentrations were expressed in milligram per kilogram of the dry leaf weight (mg/kg d.w.).

Plant-to-soil bioconcentration factor (BCF) estimates for the element accumulation ability of the plants from the substrate were also provided [32]. The BCF was calculated for leaves using the equation: BCF=metal in plant part/metal in substrate and expressed on a dry-weight basis.

Chlorophyll concentration in the leaf tissues was determined spectrophotometrically based on the light absorption of a solution obtained after extraction with dimethyl sulfoxide (DMSO) [33]. One disk (1 cm diameter) per leaf was harvested from 5 leaves. Chlorophylls and total carotenoids were extracted with 1 ml of DMSO. After incubation at 65°C until full extraction of chlorophyll was reached, the absorbance of extracts was measured at 663, 645 and 480 nm with UV-visible spectrophotometry (Shimdazy UV-160). Arnon's [34] equations were used to calculate chlorophyll a (Chl a) and chlorophyll b (Chl b). Total carotenoids (Tot carot) were calculated according to Wellburn [35]. Chlorophyll and carotenoid concentrations in leaves were expressed as mg per gram of the dry leaf weight (mg/g d.w.).

Statistical analysis

The results of the present study were analyzed using statistical analysis (ANOVA) by computing the statistical significance of the mean differences of photosynthetic pigments (Chl a, Chl b, Tot carot) and trace element concentrations with respect to their locations. Canonical discriminant analysis (CDA) was performed to detect differences between the analyzed sites based on the variations in Chl a, Chl b, Total carot and elements.

RESULTS AND DISCUSSION

Physicochemical properties of soil

The mobility and availability of heavy metals and metalloids are controlled by many chemical and biochemical processes such as precipitation-dissolution, sorption-desorption, complexation-dissociation and oxidation-reduction. The processes are not equally important for each element, but all of them are strongly affected by soil pH, and biological processes [36]. Furthermore, it should be emphasized that most of them are closely related, i.e. soil organic matter is determined by texture, pH, temperature, moisture, aeration, clay mineralogy and soil biological activities. On the other side, soil organic matter in turn influences or modifies many of these soil properties [37]. Soil texture influences a whole range of physical (structure, porosity, water-air, heat) and chemical (adsorption and buffering capacity) properties. The previous practice of forming urban parks in Serbia involved planting vegetation on surfaces that were filled with soils of various origin, including waste from construction works. This has led to a drastic deterioration in soil quality. The insufficient thickness of the soil horizon

Table 1. Selected properties of soil sampled from examined urban sites

Site	Depth	pH (H_2O)	Organic matter %	Clay (<0.002)	Silt (0.02-0.002)	Total sand (2.0–0.02)	Soil texture
Pančevo	0-10 cm	8.44	3.00	26.21	27.96	45.83	sandy-clay loam
Smederevo	0-10 cm	8.51	2.68	20.92	25.95	53.13	sandy-clay loam
Obrenovac	0-10 cm	8.44	2.10	29.02	34.71	36.27	clay loam
Belgrade	0-10 cm	8.47	5.56	20.34	27.43	52.23	sandy-clay loam
Control	0-10 cm	8.61	7.60	27.14	37.29	35.57	clay loam

The given values are the means, n=5

and small surface for plant nutrition when planting along asphalt walkways prevents the proper development of the root system.

In this study, soil pH in an aqueous solution at the examined park sites ranged from 8.44 to 8.61 (Table 1), designating them as alkaline [31]. High alkalinity represents a very negative property of substrate, since it results in deficits in some essential nutrients and trace elements, causing elements to form insoluble compounds [38].

The organic matter content in the tested soil was low and, depending on the site, ranged from 2.10 to 7.60% (Table 1). Given the importance of organic matter in soil as a "revolving nutrient fund" and an agent that improves soil structure and minimizes erosion [37], a low concentration of organic matter further exacerbates the already negative characteristics of anthropogenic soils, depriving soil organisms and plants of their primary energy source. The clay content (particles size <0.002 mm) was relatively uniformly distributed and ranged from 20.34% in Belgrade to 27.14% at the control site. The lowest total sand (fine and coarse fraction) content was measured at the control site (35.57%), while the highest were recorded in Belgrade (52.23%) and Smederevo (53.13%), respectively. Silt fraction varied from 25.95% in Smederevo to 37.29% at the control site (Table 1). This percentage share of clay, silt and total sand classifies urban soils in Pančevo, Smederevo and Belgrade as sandy-clay loam, while in city parks in Obrenovac and the control site, they are clay loam. These results confirm earlier reports of the existence of different soil classes: sand, sandy loam, clay, clay loam soils in Belgrade parks. Materials of anthropogenic origin: clay, gravel, bits of brick, concrete, plaster and metal, fragments of glass and ceramic, often dominate in soil texture, while the organic component is mainly present in the form of plant debris, coal, discarded paper, textile and other organic waste, which directly affect the physical or chemical properties of soil [39]. Similarly, Puskás and Farsang [40] also found in top soils of Szeged in Hungary a large number of artefacts, low and fluctuating humus and nitrogen levels, poor quality of humic material, high and fluctuating carbonate content, a concomitant variance in the pH and modified mechanical properties. All these characteristics referred to soils affected and transformed by human activities. In addition, some the urban soils studied in Szeged were regarded as Technosols.

Seasonal variations of trace element content in soil and plant material

In this study, the content of B in soil at all the examined sites was considerably higher than the average content described for worldwide soils (podzol, sandy soils, cambisols and loamy soils, 22-40 mg/kg) [41], with the highest level measured in Obrenovac (maximum value of 214.74 mg/kg), which was to be expected since the sampling site is in the vicinity (4 km southeast) of the Nikola Tesla A thermal power plant and its fly ash disposal site (Table 2). The area is exposed to emissions of different types of pollutants from coal combustion processes as well as fly ash disposal. Toxic elements from the fly ash deposit are leaching into the surrounding environment and can cause environmental risks for soil, vegetation and water [42]. Fly ash contains many elements in toxic concentrations, such as As, Cd, Cr and Pb, along with essential elements, such as B, Cu, Mn, Mo and Ni [43]. Boron originating from fly ash is highly mobile. James et al. [44] established that approximately 17-64% of B is immediately soluble in water, but a further 2 to 3 years is required for the amount of B to decrease to a concentration plants can tolerate. However, due to the low rainfall throughout the studied year, it could not be sufficiently leached and could therefore accumulate

Table 2. Average trace elements concentration (mg/kg d.w.) in soil samples

Site	B	Cu	Sr	Zn
Pančevo	155.01 (0.570)	15.882 (0.200)	30.172 (1.234)	61.937 (2.896)
Smederevo	129.73 (4.589)	35.840 (2.875)	40.247 (3.138)	152.07 (3.461)
Obrenovac	214.74 (2.598)	20.142 (0.826)	38.805 (1.792)	60.310 (0.448)
Belgrade	105.09 (4.955)	40.950 (0.254)	81.262 (3.664)	188.43 (6.775)
Control	139.74 (1.126)	20.547 (0.664)	30.405 (0.787)	68.707 (2.479)
Normal range[a]	1-134	1-100	5-1000	3.5-362
Average values in sandy and loamy soils[a]	22-40	13-23	87-210	45-60
MAC RS[b]		100		300

The given values are mean, with standard deviation in parentheses, n=5.aKabata-Pendias and Pendias, 2001; bOff. Gazette of the RS, No. 23/94

in plants in levels that could become toxic [45]. Soil pH is also an important factor that affects the availability of B to plants. For instance, a pH above 6.5 can cause interactions between soil pH and B uptake [46]. In alkaline soils, the availability of B increases with soil pH>7 [26,41], which was confirmed in this study.

The Cu content in Smederevo and Belgrade was higher than the mean values of the background concentrations described for worldwide soils (13-23 mg/kg) [41] (Table 2). However, the levels did not exceed maximum allowable concentrations (MAC) of heavy metals in soils that has been enacted by Serbian regulations, amounting to 100 mg/kg [47,48]. Elements such as Cu, Co, Cr, and Pb have low transfer coefficients and are stably bound to the soil structure, which is in accordance with obtained results for Cu in this study, given the fact that the content in leaves was close to, or in the deficit range for optimal plant development [12] (Table 3). Although Cu is one of the least mobile heavy metals in soil, this metal is abundant as free and complexed ions in soil solutions of all soil types. However, the binding of Cu by soils is related to the formation of organic complexes and is highly dependent on soil pH, and the overall solubility of both cationic and anionic forms decreases at about pH 7-8 [41].

Strontium is a metal found in the environment after both degradation of minerals naturally containing Sr^{2+}, such as celestite and strontianite [49], and anthropic activities such as nuclear weapon tests or nuclear accidents [50]. In this study, the Sr content in soil was within the normal values for worldwide soils (87-210 mg/kg) proposed by Kabata-Pendias and Pendias [41]. It varied in a wide range, from 30.17 mg/kg in Pančevo to 81.26 mg/kg in Belgrade (Table 2). The uptake of Sr by plant roots is species-specific and is also strongly affected by soil properties such as organic matter content, pH and ionic composition [51].

Zn concentration in the soils was above average values for soils worldwide (45-60 mg/kg) [41] and within the maximum allowed contents proposed by the Official Gazette of the RS, no 23/94 [47]. Significant deviations were recorded in Smederevo (152.07 mg/kg) and Belgrade (188.43 mg/kg), respectively (Table 2). Although Kloke et al. [12] stated that Zn, together with Cd and Tl, have the highest soil-to-plant transfer coefficients, in part because of their relatively poor sorption in the soil, our study showed that Zn most probably is not present in an available form for plants because of soil alkalinity. Namely, alkaline soils with a low organic matter content, sandy-clay texture and high content of phosphate those examined in this study, contain low levels of bioavailable Zn [52].

Boron is unique among the essential plant nutrients in terms of its restricted mobility in many plant species and free mobility in others. No other element is known to vary so greatly in mobility [53]. In our study, B showed increasing accumulation tendency. Namely, throughout the vegetation season levels continuously increased and reached maximum levels that are considered toxic (>100 mg/kg) [40] in October (Table 3). At the beginning of the season, boron levels ranged from 16.83 mg/kg in Obrenovac to 104.48 mg/kg in Smederevo, whereas in August, B ranged from 31.71 mg/kg in Pančevo to 162.67 mg/kg in Smederevo and 157.27 mg/kg in Belgrade. By the end of vegetation season, the range was found to be from 43.30 mg/kg in Pančevo to 264.05 mg/kg in Smederevo. The poor transfer of B from soil to leaves in Obrenovac is likely a consequence of the clay loam

Table 3. Average trace elements concentration (mg/kg d.w.) in leaves and bark of *A. hippocastanum*

LEAF												
Site	**June**				**August**				**October**			
	B	**Cu**	**Sr**	**Zn**	**B**	**Cu**	**Sr**	**Zn**	**B**	**Cu**	**Sr**	**Zn**
Pančevo	21.558 (0.867)	5.588 (0.180)	31.130 (0.566)	10.846 (0.692)	31.710 (0.885)	5.161 (0.576)	42.059 (0.935)	14.309 (1.150)	43.304 (0.181)	5.284 (0.290)	36.679 (1.530)	22.537 (0.965)
Smederevo	104.48 (1.183)	3.101 (0.277)	41.627 (0.693)	10.007 (0.634)	162.67 (0.579)	2.490 (0.257)	53.405 (1.004)	18.355 (1.166)	264.05 (1.328)	2.927 (0.275)	50.270 (1.184)	17.282 (1.010)
Obrenovac	16.836 (0.679)	6.982 (0.590)	30.689 (0.187)	10.230 (0.741)	59.269 (0.844)	3.100 (0.516)	35.123 (1.067)	15.554 (0.170)	64.890 (0.455)	4.809 (0.524)	27.821 (0.492)	16.627 (1.121)
Belgrade	51.700 (0.920)	23.512 (0.898)	86.275 (0.517)	30.987 (1.479)	157.27 (0.297)	9.271 (0.155)	79.471 (1.990)	34.942 (2.866)	171.50 (0.855)	15.338 (0.324)	107.56 (2.274)	56.429 (1.789)
Control	18.667 (0.724)	10.788 (0.438)	39.353 (0.722)	15.525 (0.367)	50.370 (0.296)	9.823 (0.354)	56.675 (2.683)	17.709 (0.452)	89.185 (0.394)	10.583 (0.496)	54.468 (1.765)	21.828 (0.840)

BARK												
Site	**June**				**August**				**October**			
	B	**Cu**	**Sr**	**Zn**	**B**	**Cu**	**Sr**	**Zn**	**B**	**Cu**	**Sr**	**Zn**
Pančevo	7.057 (0.151)	7.371 (0.217)	58.537 (1.463)	12.145 (0.645)	10.560 (0.144)	8.744 (0.416)	58.451 (1.387)	24.610 (1.431)	11.517 (0.482)	9.585 (0.517)	48.528 (1.082)	20.531 (1.418)
Smederevo	21.310 (0.277)	9.290 (0.274)	67.620 (0.905)	49.504 (1.689)	25.922 (0.944)	8.751 (0.484)	66.071 (2.260)	44.504 (2.686)	34.899 (0.957)	9.366 (0.398)	41.537 (0.843)	55.189 (2.147)
Obrenovac	10.609 (0.285)	5.600 (0.427)	70.027 (1.308)	16.417 (0.395)	14.192 (0.494)	7.481 (0.507)	65.758 (1.821)	24.747 (2.746)	15.928 (0.612)	7.034 (0.435)	41.823 (0.717)	23.745 (1.714)
Belgrade	18.458 (0.397)	40.118 (0.695)	245.41 (3.603)	104.607 (2.231)	15.536 (0.593)	28.892 (1.205)	229.62 (2.158)	58.872 (1.808)	28.063 (0.824)	63.977 (0.951)	129.00 (2.390)	145.12 (1.708)
Control	8.717 (0.048)	16.521 (0.231)	69.918 (0.943)	12.785 (0.799)	10.407 (0.299)	22.890 (0.603)	74.473 (2.402)	13.534 (0.952)	19.715 (0.985)	18.609 (0.593)	62.028 (1.748)	35.960 (2.710)

The presented values are means with standard deviation in parentheses, n=5. Kabata-Pendias and Pendias, 2001: deficit for plant tissues: B 3-30 (mg/kg); Cu 2-5 (mg/kg); Zn 10-20 (mg/kg); normal range for plant tissues: B 10-100 (mg/kg); Cu 5-30 (mg/kg); Sr 1-10 (mg/kg); Zn 27-150 (mg/kg); toxic range for plant tissues: B 50-200 (mg/kg); Cu 20-100 (mg/kg); Sr>30 (mg/kg); Zn 100-400 (mg/kg)

texture, as well as the low level of Zn in the soil. In general, boron uptake to toxic levels in plants could be expected in soils with a low level of available Zn and high level of B in the rooting zone [54]. The highest concentration of B in leaves was measured at the sites where the total contents of both B and Zn were high in the rooting zone (Smederevo and Belgrade), which can be linked to the effects of the pH reaction of the examined soils (pH>8). There is a narrow range between deficit and toxic B levels in plants; however, most toxicity symptoms occur above 100 mg/kg. They include changes in metabolism, reduction of chlorophyll, photosynthetic intensity and the appearance of chlorotic and/or necrotic areas on the leaf surface [55]. A similar increasing seasonal trend was observed with the tree bark, with the lowest concentrations measured in June at all sites and the highest in October (Table 3). Nonetheless, bark also showed a spatial pattern for B, in a sense that throughout the season the lowest concentrations were recorded in Pančevo (7.05 mg/kg in June, 10.56 mg/kg in August and 11.517 mg/kg in October), and the highest in Smederevo (21.31 mg/kg in June, 25.92 mg/kg in August and 34.89 in October). The B content in bark was several times lower compared to that in leaves.

Unlike B, the Cu content in chestnut leaves showed a decreasing temporal trend from June to August, with the lowest concentrations measured in Smederevo 3.10 mg/kg and the highest in Belgrade 23.51 mg/kg in June (Table 3). It remained relatively uniform through the rest of the season at all the examined sites, except Belgrade. The lowest Cu content in leaves was measured during August at all sites and varied from only 2.49 mg/kg in Smederevo to 9.82 mg/kg at the control site. These results are consistent with the findings of Kim and Fergusson [56] and Aničić et al. [25], who stated that copper concentrations decrease through the vegetation season, with the highest in new leaves, suggesting that the main source of elements is uptake from soil. Unlike leaves, the dynamics of Cu in bark did not show a seasonal pattern

(Table 3). The obtained measurements for Cu in bark varied between 5.60 mg/kg in Obrenovac in June to 63.97 mg/kg in Belgrade in October. In Pančevo, Smederevo and Obrenovac the concentration of Cu was relatively constant throughout the growing season, except for Belgrade, where toxic (>30 mg/kg) concentrations for plant tissues were measured in June (40.11 mg/kg) and October (63.97 mg/kg) [41]. In Belgrade, the concentrations of Cu in both leaves and bark are most likely the result of traffic pollution rather than uptake from soil [57]. It should be noted that most of the obtained results for Cu in leaves were well below the optimal values for plant development suggested by Kabata-Pendias and Pendias [41] (5-30 mg/kg). Baker and Seneft [58] showed that the average Cu content in plant tissues is around 10 mg/kg, while toxicity occurs at levels over 30 mg/kg. In our study, a deficit in Cu (values below 5 mg/kg) was measured in Smederevo throughout the season and in Obrenovac during August and October. Copper deficiency affects physiological processes and therefore plant production, affecting mostly young leaves and reproductive organs.

The content of Sr in plants is highly variable and there are different opinions about the potential tolerance of plants of this element. In this study, there was an obvious spatial trend for leaves, where the lowest Sr content was always measured in Obrenovac (in October, 27.82 mg/kg) and the highest in Belgrade (also October, 107.56 mg/kg). Furthermore, maximum concentrations were reached in the peak of the growing season at all the sites, except for Belgrade (Table 3). It is important to mention that Shacklette et al. [59] set the toxic Sr level for plants at 30 mg/kg. Although Sr is not readily transported from roots to shoots, most often the content in plant leaf tops is relatively high [41]. Given the fact that the soil concentration of Sr was mostly lower compared to leaves, it is reasonable to assume that most of the Sr content in *A. hippocastanum* leaves is airborne and the result of aerial deposition. It was previously established that prolonging exposure periods with high levels of Sr led to accumulation in the aboveground parts of plants [60]. Toxic concentrations of Sr were also measured in bark at all the examined sites and throughout the season. Unlike leaves, maximum concentrations were observed in June and ranged from 58.53 mg/kg in Pančevo, up to 245.41 mg/kg in Belgrade (Table 3), decreasing over the season. The highest decline in Sr content was observed in the October samples from Belgrade, which were almost two times lower compared to those at the beginning of the season. Samples from Smederevo (41.53 mg/kg) and Obrenovac (41.82 mg/kg) in October contained the lowest amount of strontium. Given the fact that bark contained significantly higher concentrations of Sr in relation to soil and leaves, it could be concluded that it originated mainly from the atmosphere. This decrease could be attributed to changes in atmospheric conditions.

Zinc showed an increasing tendency in leaves during the vegetation season, with the lowest concentrations measured in June at all the examined sites (minimum 10.00 mg/kg in Smederevo), and the highest in October (maximum 56.42 mg/kg in Belgrade) (Table 3). Optimal Zn levels in plant tissues, according to Kabata-Pendias and Pendias [41], are considered to be from 27-150 mg/kg, deficit occurs in the range of 10-20 mg/kg, while toxicity develops from 100-400 mg/kg. Consequently, a deficit in Zn was observed at all the sites throughout the vegetation period, except for Belgrade. Since Zn is not readily translocated in plants, deficiency occurs primarily in younger leaves, and therefore our results are consistent with the findings of Tomašević et al. [23] and Aničić et al. [25]. Nonetheless, the existence of seasonal discrepancies in Zn levels for horse chestnut in other literature sources should be emphasized. For instance, Scheffer et al. [61] reported the highest Zn content in barley leaves, sheaths and internodes is always during the phase of intense growth. Kim and Ferguson [56] noted that the highest concentration of Zn in horse chestnut leaves was measured in new leaves and decreased along the vegetation season. Similarly, Baycu et al. [24] showed that the highest Zn levels for chestnut were mostly recorded at the beginning of the season. This disagreement in the literature clearly reflects Zn fluctuations within the plant during the vegetative period, which is probably the result of various site conditions and different genotypes [41]. The seasonal dynamics of Zn in bark did not show any temporal or spatial regularity (Table 3). For instance, its content in bark ranged from 12.45 mg/kg in Pančevo during June to 145.12 mg/kg in Belgrade in October. A deficit in Zn in plant tissues was observed in Pančevo and Obrenovac throughout the season and at the control site during June and August. In Belgrade, however, the Zn content was notably higher compared to all the other sites, and reached a maximum in October (145.12 mg/kg). The results from Belgrade are probably due to motor vehicle emissions and abrasions of car tires, because zinc is used as a catalyst in the manufacture of tires and its additives are often used in lubricating motor oil [62].

The analysis of trace elements in leaves and bark showed toxic concentrations of the following elements: boron in leaf tissue in Smederevo throughout the season, as well as in Belgrade during August and October; strontium both in leaves and bark at all sampling locations throughout the season. An interesting pattern was observed for Cu and Zn. Namely, the Cu contained in the leaves was deficient throughout the season in Smederevo, as well as in Obrenovac during August and October; however toxic concentrations (>30 mg/kg) were observed in bark during June and October in Belgrade. A similar situation applies to Zn, where foliar concentration remained in a deficit range during the whole season, except in samples from Belgrade. Also, bark samples from Belgrade contained a toxic amount of Zn during June and October.

Bioconcentration factor (BCF)

BCF values were used to evaluate the potential of plant species for phytoextraction and phytostabilization of metals in soil whereby a plant's leaves/soil ratio (BCF) was used for the qualification of plant species for phytostabilization of contaminated soils. BCF>1 indicated the potential of plants for phytostabilization [63]. In our study, the BCF for B and Sr in leaves was greater than 1 in all sites except for Obrenovac. For Cu and Zn, the BCF was lower than 1, indicating that they are not easily accumulated in leaves. The low accumulation of the examined elements in leaves in Obrenovac could be attributed to soil texture (clay loam), but also other soil parameters, such as pH or organic content.

Photosynthetic pigments

Photosynthetic pigments are usually determined in plants to evaluate the effects of environmental stresses because changes in pigment concentrations are often associated with photosynthetic activity and visual symptoms of plant damage [64]. Measurements of chlorophyll a and b are important indices of the effects of pollution and heavy metal stress on plants [65]. Whereas certain pollutants induce an increase in total chlorophyll content in higher plants, others cause their decline [66]. Numerous studies indicate a reduction in chlorophyll content under pollution stress: Tripathi and Gautam [67] recorded a decline in the leaves of *Cassia fistula* and *Eucalyptus hybrid* exposed to intensive air pollution; Joshi and Swami [65,68] found a decline in the total chlorophyll content that ranged from 3.44% up to 48.73% in a large number of economically important plants; Giri et al [69] who also noted a severe decline in total chlorophyll, Chl a and Chl b contents in the leaves of *Azadirachta indica*, *Nerium oleander*, *Mangifera indica* and *Dalbergia sissoo* affected by air pollution. There are also frequent reports on the increase in chlorophyll contents in plants exposed to various types of pollutants, such as that of Tripathi and Gautam [67], who reported an increase of 12.8% in *Mangifera indica* leaves subjected to air pollution; Keser [70] in leaves of *Lepidium sativum* treated with wastewater; Dezhban et al. [71] in *Robinia pseudoacacia* leaves treated with Cd and Pb; Nabeela et al. [72] in *Brassica napus* seedlings; Sai Kachout et al. [73] in *Atriplex hortensis* and *Atriplex rosea* growing in soils treated with different concentrations of metals.

In the present study, *A. hippocastanum* responded to pollution stress by increasing the content of Chl a during August (Fig. 2a). This could be attributed to the high content of B and Zn in soil [72,74], as well as the deficiency of Zn in leaves, which coincided with unfavorable climatic conditions (extremely high temperatures and lack of precipitation). On the other hand, Chl b content was highest in June and apparently less sensitive to changes than Chl a, since its content remained relatively uniform during the whole season (Fig. 2b).

Carotenoids act as alternative antennas to capture light, absorbing the blue region of the spectrum (400-600 nm) and transferring the energy to the chlorophylls. Nevertheless, the main function of carotenoids is to protect the photosynthetic apparatus, dissipating energy to avoid photooxidation [75]. These pigments have been involved in plant tolerance against different types of stress, such as drought, UV-B light excess or heavy metal toxicity [76]. In our research, carotenoids showed slight variations over the season, but it is important to note that the maximum content

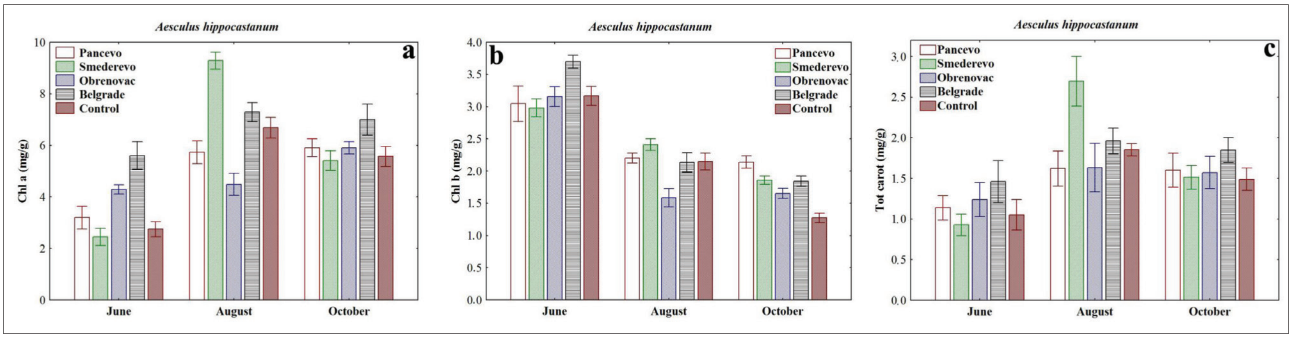

Fig 2. Average Chl a (**a**), Chl b (**b**) and Tot carotenoids (**c**) (mg/g d.w.) in *A. hippocastanum* sampled from examined urban sites (Pančevo, Smederevo, Obrenovac, Belgrade and control site) in June, August and October 2012.

corresponds with Chl a in August (Fig. 2c). Given their protective nature and the previously mentioned conditions, this outcome was expected, and is in accordance with earlier studies [77].

Differences in chemical elements between examined sites

Differences in the total content of chemical elements (B, Cu, Sr and Zn) and photosynthetic pigments between examined sites in leaves of *Aesculus hippocastanum* were determined by canonical discriminant analysis (CDA). The analysis showed that the variance was explained by two canonical axes (Fig. 3).

The DC1 axis, explaining 78.6% of the variance, clearly separated Smederevo and Belgrade from the other sites, showing that these two sites are more polluted due to the toxic level of B and Sr in chestnut leaves at both sites. The DC2 axis, explaining 21.4% of the variance, also separated Smederevo. The overlapping distribution of points representing samples in Pančevo and Obrenovac indicates a similar pollution level at these sites. The parameters that contribute most to this separation are B, Sr and Chl b.

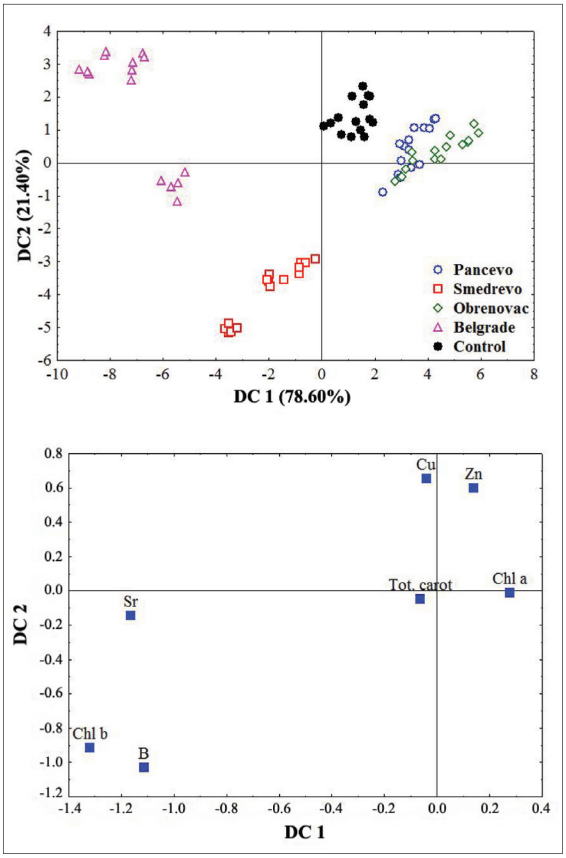

Fig 3. Canonical discriminant analysis (CDA) for the examined sampling sites based on element concentration and chlorophyll content in leaves of *Aesculus hippocastanum.*

CONCLUSION

Soils acts as a natural sink for pollutants that retain metals, but under anthropogenic influence these retained pollutants can lead to harmful effects. Although the elemental composition in the tested urban soils did not surpass maximum allowed concentrations for soils on a national scale set by Serbian regulations, our findings suggest that there are potential ecological risks in the areas of Smederevo, Belgrade and Obrenovac due to elevated concentrations of B and Zn compared to average values of the background concentrations described for worldwide soils.

Seasonal variations in the accumulation of chemical elements in plants were observed in all the examined elements. An increasing tendency was observed for B, Sr and Zn and decreasing temporal trend for Cu in leaf, while for bark a similar pattern was found for B, a decreasing tendency for Sr, with Cu and Zn showing no spatial or temporal pattern. Analysis of trace elements in leaves and bark showed toxic concentrations of B in leaf tissue in Smederevo during the whole season, and in Belgrade during August and October, and Sr in leaves and bark at all the sampling locations throughout the season. An interesting pattern was observed for Cu and Z. Namely, Cu in leaves was deficient throughout the season in Smederevo, as well as in Obrenovac during August and October; however, toxic concentrations were observed in bark during June and October in Belgrade. The situation for Zn was similar, where foliar concentration remained in a deficit range during the whole season, except in samples from Belgrade. Likewise, bark samples from Belgrade contained a toxic amount of Zn during June and October.

As regards photosynthetic pigments, chestnut responded to pollution by increasing the content of Chl a in August during the most unfavorable conditions of the year. Total carot content corresponded with Chl a and was also reached in August. On the other hand, Chl b content was the highest in June.

In recent years, the use of plants as biomonitors or bioindicators has increased since it enables the prediction of pollution for monitoring purposes in urban and environments where traffic and industry are major sources of heavy metal pollution. Our results suggest that under conditions of different sources of urban and industrial pollution with heavy metals, *A. hippocastanum* could be regarded as a potential biomonitor for B and Sr.

In testing plants growing in urban sites, this study has provided information of the potential risk of pollution in urban parks. This highlights the importance of using plants as biomonitors as tools for decision making, especially in the planning and assessment of planting actions, making this approach necessary in determining the success of urban parks management.

Acknowledgments: This work was supported by the Ministry of Science and Education of Serbia, Grant No 173018.

Author's contribution: Marija Pavlović is the principal author who, in cooperation with Dragana Pavlović, carried out all the analyses, measurements and calculations and wrote the manuscript. Miroslava Mitrović and Pavle Pavlović participated in the design of this study and helped in the critical revision of the manuscript. Tamara Rakić helped in work related to the research. Snežana Jarić participated in sample collection and plant identification. Olga Kostić participated in data interpretation and writing the manuscript. Zorana Mataruga contributed to the literature research. All authors have read and approved the final version of manuscript.

Conflict of interest disclosure: The authors declare that they have no competing interests.

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