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MEĐUNARODNA KONFERENCIJA

MEETING POINT OF THE SCIENCE AND PRACTICE IN THE FIELDS OF CORROSION, MATERIALS AND ENVIRONMENTAL PROTECTION

STECIŠTE NAUKE I PRAKSE U OBLASTIMA KOROZIJE, ZAŠTITE MATERIJALA I ŽIVOTNE SREDINE

PROCEEDINGS

KNJIGA RADOVA

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Comparison of trace element accumulation and particulate matter deposition in leaves of *Aesculus hippocastanum* L. and *Platanus acerifolia* Willd. in three urban parks in Serbia

Marija Pavlović, Dragana Pavlović, Veljko Perović, Snežana Jarić, Milica Marković, Miroslava Mitrović, Pavle Pavlović

Department of Ecology, Institute for Biological Research "Siniša Stanković", University of Belgrade, Bulevar despota Stefana 142, 11000 Belgrade, Serbia

Abstract

Concentrations of four trace elements (Cu, Mn, Sr and Zn) were measured in leaf samples of Aesculus hippocastanum and Acer platanoides collected in urban parks in Pančevo and Belgrade, Serbia. The objectives were to assess the leaf tissue element content and to analyze the chemical composition of leaf surface particles. The results showed that the highest concentrations of trace elements for both plant species were measured at Pionirski Park sampling site which is exposed to severe traffic. Results for Cu and Zn in Narodna bašta for both species and for P. acerifolia from Topčider Park were below the normal range while Sr from all sampling sites was in toxic range for plants. The results of chemical composition of deposited particles (EDS analysis) showed that the most abundant elements found in particles deposited on the leaves of both species come from mineral dust or soil, or represent main constituents of plants. Deposition of particles was more efficient on A. hippocastanum in relation to P. acerifolia leaves, while sampling sites that stood out for the highest particle deposition were Narodna bašta for leaves of A. hippocastanum, and Pionirski Park for P. acerifolia.

Key words: *trace elements, urban parks, chemical characterization, particulate matter (PM), SEM-EDS analyses*

Introduction

Air pollution is a serious threat to the health and life quality of the urban population, especially the harmful particulate matter (PM) that comprises a mixture of heavy metals, black carbon, polycyclic aromatic hydrocarbons and other substances suspended in the atmosphere [1,2]. Urban pollution is mainly of anthropogenic origin [3,4]. That is why trees, particularly deciduous species with large, rough and complex leaves, have an important role in removing of PM and associated trace elements from the urban air [2,5,6,7]. Pollutants emitted into the atmosphere can be deposited on the leaves either by wet or dry deposition or accumulated in the leaf tissues [2,8]. The effectiveness of their retention is conditioned by specific anatomical and morphological features of the leaves (roughness, trichomes and epicuticular wax, etc.), particle size, chemical properties of the element, as well as climatic conditions such as temperature, humidity, wind and precipitation [7,9,10,11]. Deposition of atmospheric particles on the surface of leaves can inhibit physiological processes in plants, particularly transpiration and photosynthesis. Particles can also accumulate near stomata causing misbalance in gas flow and eventually in water balance. Furthermore accumulation of PM on leaf surface can raise the temperature of the leaf, especially during summer, which can lead to overheating [12]. Suspended particles are neither physically or chemically homogeneous, which is why it is important to have information on their physical characteristics and chemical composition [13], as well as possible harmful effects on people and environment [2,5,7,10].

The accumulation of elements by higher plants depends on the binding and solubility of particles deposited on leaf surfaces, as well as on concentrations and bioavailability of elements in the soil. Many plants accumulate trace elements in their above ground parts (leaves/barks) at quantities many times higher than contained in soil solution [10,14,15], however trees, as long-lived organisms reflect the cumulative effects of environmental pollution from both the soil and the atmosphere [10].

This study investigated two species *Aesculus hippocastanum* L. and *Platanus acerifolia* Willd., commonly planted in three urban parks in two cities in Serbia, in order to compare accumulation capacities for essential elements (Cu, Mn, Zn) and Sr in their leaves, and PM deposition on their leaf surfaces. It also examined the following: 1) How does the source of pollution affect the PM deposition on the leaves? 2) Which species is more efficient in accumulating essential elements? and 3) Which of two broad leaved species is more efficient in "capturing" PM particles and for what reason?

Materials and methods

Sampling sites

The research sites were urban parks in two cities in Serbia that are exposed to athmospheric element pollution from industrial activities and heavy traffic (Fig 1). In Pančevo, dominant pollution sources are the nitric fertilizer factory, the refinery and the petrochemical industry. Park Narodna bašta in Pančevo, where sampling was carried out is one of the most beautiful parks and urban green areas in Serbia that stretches over 14 ha.

In Belgrade, two urban parks were chosen for sampling - Pionirski Park which is within 500 m from several major traffic roads and about 1 km from the former industrial zone. Green areas in the park are significantly fragmented by large concrete surfaces. Heavy traffic exhaust is the main source of pollution. Second park is Topčider Park, located in the zone of mixed oak forest (*Quercus frainetto* and *Quercus cerris* Rud.) in an area without the direct source of pollution, 10km away from the city centre. This area has been proposed for the category of significant natural value with the establishment of a third-degree protection regime.

The research was carried out in October 2012, just before shedding in order to assure maximum element accumulation and deposition.

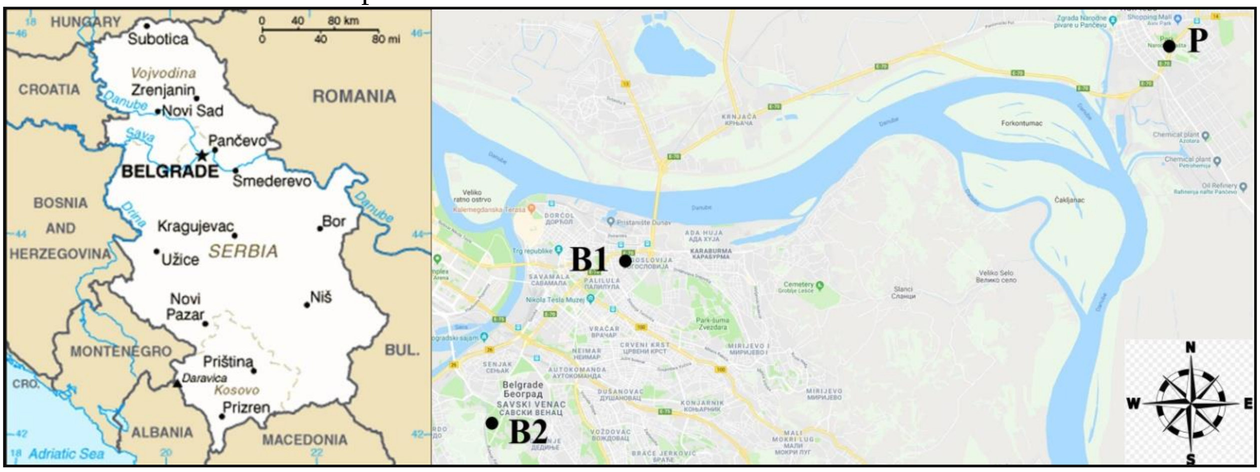


Figure 1. Selected urban sampling sites: P- Pančevo (Narodna bašta), B1- Belgrade 1 (Pionirski Park), B2- Belgrade 2 (Topčider Park)

Sample collection

At each urban park three sampling points were selected where leaves of three randomly chosen trees of A. hippocastanum (Fig 2a) and P. acerifolia (Fig 2b) were collected. Threes were of similar age (20-30 years old). Leaves samples were taken uniformly from the lower foliage (up to 2 m above the soil level) and from different quarters of the tree crown. Leaves samples from each tree and each sampling point were then mixed into a composite sample [16,17,18,19], which resulted in one composite sample of A. hippocastanum and P. acerifolia per sampling site (urban park).

For SEM analysis, leaves were collected and packed in plain white paper envelopes using polyethylene gloves and clean stainless steel scissors. In the laboratory, leaves were air dried on the white filter paper in order to avoid contamination. Leaf discs of 5x5 mm in size were cut from dry leaves, aside from the main veins and attached to the microscope stub with double-sided adhesive tape. Representative samples of both adaxial and abaxial leaf surfaces of the studied species were prepared.

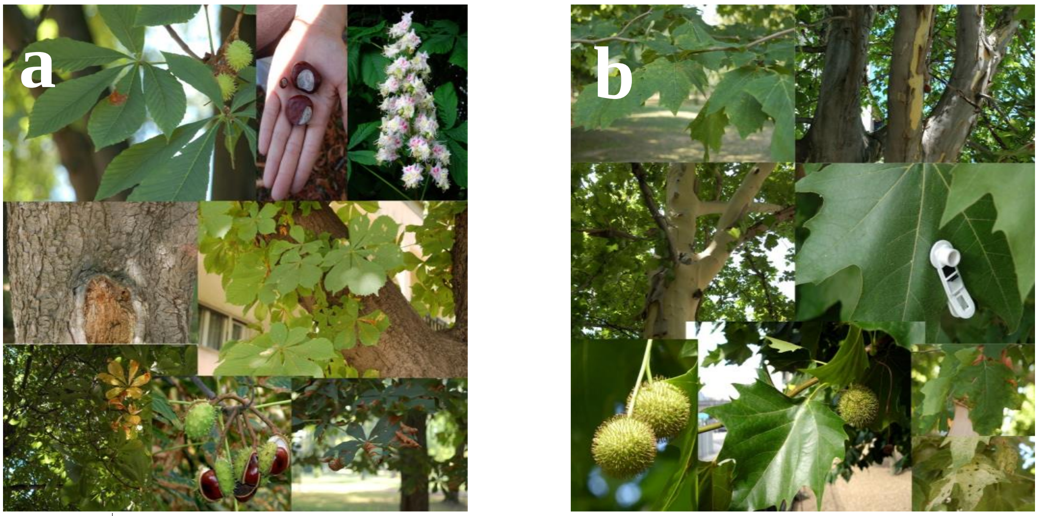


Figure 2. a) Aesculus hippocastanum L. b) Platanus acerifolia Willd.

Plant analysis

Element concentration

In the laboratory leaves were first air dried at room temperature, then dried to a constant weight at 65 C ° (Binder, Tuttlingen, Germany), grounded in a laboratory mill and sieved through 2 mm sieve. For the element concentration analysis, plant samples (0.3 g) were digested in a microwave (CEM, 39 MDS- 2000), using the USEPA 3052 method (USEPA 1996). Five replicates were performed for each sample. After cooling, the final extracts were filtered and transferred into 50 ml volumetric flasks, and then diluted with deionized water. Quality control was performed using standard leaf reference material—BCR-100 beech leaves obtained from the IRMM (Institute for Reference Materials and Measurements, Geel, Belgium) and certified by the ECJRC (European Commission-Joint Research Centre). Accuracy was in the range 90–110% of the certified reference material. The detection limits for the analyzed elements in the soil samples were as follows (mg kg^{-1}): Cu-0.00974, Mn-0.000157, Sr-0.00069 and Zn-0.00348.

SEM–EDS analyses

The physical and chemical characteristics of particles deposited on the leaves were analyzed by the electronic method microscopy (SEM) using JEOL, JSM-6460LV instrument an energy dispersive spectroscopy (EDS) detector for chemical composition analysis of PM found on the surface of the leaves and tungsten cathode. Instrument operated in conditions of high vacuum, at an acceleration voltage of 20 kV. In order to cool down EDS detector, liquid nitrogen was used. Leaf discs of 5x5 mm in size were gold coated prior to further characterization to ensure the conductivity of the samples and enable their morphology and characterization analysis. The sputtering was performed using the BALTEC, SCD 005 sputter coater. Approximately 15 randomly chosen fields were investigated per section with the different magnification. Adaxial leaf surface was observed at magnification of x 500 and x 1500, while abaxial leaf surface at magnification x 500. Displayed micrographs represent signal/snapshot of secondary signals (SE).

Results and discussion

Element concentration in leaves

Concentrations of Cu, Mn, Sr and Zn in leaves of *A. hippocastanum* and *P. acerifolia* from each sampling site are given in Table 1. Trace element accumulation in leaves varied depending on the element, sampling site, as well as on the species. For the majority of plant samples, the highest element accumulation was measured at Pionirski Park site.

Copper concentrations in leaves ranged from 4.39 mg kg^{-1} in Narodna bašta to 15.33 mg kg^{-1} in Pionirski Park. Results obtained in leaves for both species from Narodna bašta and in leaves of *P. acerifolia* from Topčider Park were below the optimum values for normal plant development (<5 mg kg^{-1} [20]), while Cu content in leaves from other sampling sites was around average values for plant tissues (10 mg kg^{-1} [21]). Copper belongs to a group of essential micro-nutrients. As a structural component of the regulatory protein, Cu is included in a series of processes such as photosynthesis, respiration, reduction and fixation N, carbohydrate metabolism, response on oxidative stress, etc [20,22,23]. That is why Cu content can vary significantly depending on the plant part and its' development stage [24]. However Cu has low mobility in relation to other elements in plants, which is why the majority of it remains in roots incorporated within the cell walls [20,22] and only small part translocates to the above-ground plant parts, which could cause its insufficient amount in leaves. In this study, leaves of *A. hippocastanum* better accumulated Cu in relation to leaves of *P. acerifolia.*

Optimum Mn concentration required for normal physiological functioning of the plants ranges between 30 and 300 mg kg^{-1} [20]. The results of this research showed that plants from all sampling sites, except for tress of *A. hippocastanum* from Topčider Park (22.92 mg kg^{-1}) contain optimum amount of Mn (38.74-98.93 mg kg^{-1}). Manganese has an irreplaceable function in plants given the fact that this essential micronutrient is necessary in photosynthesis, respiration, synthesis of chlorophyll and ATP etc. Like Cu, the efficiency of its accumulation, besides physico-chemical characteristics of the soil, depends on the plant species and development stage [20,25,26]. The deficit of Mn usually doesn't manifest in form of visible symptoms of leaves damage, which is why it cannot be clearly distinguished from other symptoms of deficiency or toxicity of trace elements. From the results obtained from this research leaves of *P. acerifolia* were more efficient in Mn accumulation in relation to *A. hippocastanum*, except in Pionirski Park.

Strontium accumulation by plants depends on the physico-chemical characteristics of soil such as organic matter, pH, ionic composition etc [27]. Despite the fact that it is not an essential element, it shares similar chemical and physical characteristics with essential Ca, and therefore it can be absorbed following the plants' metabolic requirements for Ca [28]. The content of Sr in the plants is species-specific and very variable and there are contradictory data about the tolerance of plants to this element. For instance, Shacklette et al. [29], considers Sr concentration of 30 mg kg^{-1} in plant tissue as toxic. From the results obtained it is evident that all plants accumulated Sr in quantities that could be regarded as toxic (36.67-118.55 mg kg^{-1}) and that leaves of *P. acerifolia* are better in Sr accumulation in relation to leaves of *A. hippocastanum*. Despite the fact that Sr is not readily transported from roots to shoots, most often leaves contain relatively high concentrations of this element [20]. Earlier studies showed that most of this accumulated Sr in urban areas is airborne [18,19], while Tsukada et al. [30] proved that prolonging exposure periods led to Sr accumulation in the aboveground plant parts.

Zinc is one of the most important essential elements that acts as an active component of enzymes, and has a key role in the metabolism of carbohydrates, proteins etc. It also influences the permeability of membranes and stabilizes cellular components [31]. Optimal Zn levels in plant tissues according to Kabata-Pendias and Pendias [20] are considered to be from 27-150 mg kg^{-1}, while the critical deficiency concentrations are below 15-20 mg kg^{-1} [32]. Despite the ability of plants to accumulate Zn in different forms, it seems that alkaline soil conditions [18,19,26], various site conditions and different individual traits of examined tree species conditioned that *A. hippocastanum* and *P. acerifolia* from Narodna bašta (10.52-22.53 mg kg^{-1}) and Topčider Park (16.55-21.82 mg kg^{-1}) sampling sites accumulated Zn in quantities that were in deficit range, or below the range that is considered to be sufficient for the normal functioning of plants. Such conditions did not present limiting factor for the trees from Pionirski Park whose Zn content in leaves was in normal range (30.60-56.42 mg kg^{-1}). The Zn content in plant samples from Pionirski Park indicate that they are most likely the results of traffic pollution rather than uptake from soil [19,33]. Furthermore, leaves of *A. hippocastanum* proved to be better in Zn accumulation compared to leaves of *P. acerifolia*.

Table 1. Content of Cu, Mn, Sr and Zn in leaves of *A. hippocastanum* and *P. acerifolia* (mg kg^{-1} d.w.)

Sampling site	**Cu**		**Mn**		**Sr**		**Zn**	
	A. hipp	*P. acer*	*A. hipp*	*P. acer*	*A. hipp*	*P. acer*	*A. hipp*	*P. acer*
Narodna bašta Pančevo	5.28± 0.29	4.39± 0.37	66.35± 0.84	76.91± 3.17	36.67± 1.53	58.68± 2.17	22.53± 0.96	10.52± 1.24
Pionirski Park Belgrade 1	15.33± 0.32	13.49± 0.35	98.93± 2.72	38.74± 1.14	107.56± 2.27	118.55± 3.47	56.42± 1.78	30.60± 3.52
Topčider Park Belgrade 2	10.58± 0.49	5.59± 0.26	22.92± 0.28	64.31± 2.29	54.46± 1.76	53.07± 3.21	21.82± 0.84	16.55± 3.06

Morphological and chemical characterization of tree leaf surfaces and deposited particles

In order to assess potential of *A. hippocastanum* and *P. acerifolia* leaves for trapping airborne pollutants, as well as chemical composition of deposited particles, SEM-EDS analysis was performed. Peripheral structures of the leaves were analyzed in October, to assure maximum deposition of pollutants and particles at the end of vegetation season. Analysis included SEM micrographs of adaxial and abaxial leaf surfaces, primarily the changes on the epidermis and the cuticle, ie cuticle waxes, but also changes of stomata and extent of atmospheric deposition on leaf surfaces (Figures 3 and 4). Furthermore, analysis included measuring the shape, the size and to some extent the chemical composition of the atmospheric particles deposited on adaxial and abaxial leaf surfaces.

Both examined plant species have large epidermal cells, dense trichomes and wavy wrinkled structure of the cuticle waxes on adaxial and abaxial leaf surface which enables large deposition of atmospheric particles, pollen grains, spores, hyphae of different fungi, but also pathogenic species and other biogenic material. However, *A. hippocastanum* leaves are characterized by the presence of glandular trichomes along the central nerve, while leaves of *P. acerifolia* have candelabriformes trichomes with whorls of arms across the whole leaf surface area and major veins [10]. Both species have thin cuticule with stomata distributed in areoles and raised ledges around stomata [34].

The amount of deposited particles on leaves of both species was not necessarily related to the occurrence of leaf damage, giving the fact that no damages of epidermis were recorded. However particle deposition varied with leaf surface, particles characteristics, as well as weather conditions [2,7,35]. Some previous researches showed that ultrafine or fine particles can penetrate to leaf tissues trough stomata and affect the physiology of the plants [7,36,37].

The particles deposited on leaves of *A. hippocastanum* had different size and shape (spherical and non-spherical) and were unevenly distributed across the whole leaf surface, and at Narodna bašta and Pionirski Park sampling sites they formed aggregates that were larger than 30 µm (Fig 3B; Fig 3E).

On adaxial surface of leaves *P. acerifolia*, fewer particles and their aggregates were deposited in relation to leaves of *A. hippocastanum*, possibly because of the loss of trichomes during the course of the season. Epidermal cells are finer and the cuticle is less wrinkled, however prominent veins and stomata on the lower epidermis enables the particle trapping as well [10,34].

The results of chemical composition of deposited particles (EDS analysis) showed that the most abundant elements found in particles deposited on the leaves of both species at all sampling sites were Al, C, Ca, Fe, K, O and Si (Fig 5, 6; Table 2). These elements are thought to come from mineral dust or soil [7, 38] since they are incorporated in aluminosilicate particles or form oxides. However C, Ca, Fe, K, Mg and O are also main constituents of plants, and they represent essential micro and macro nutrients that are necessary for normal plant functioning.

Chlorine was present in two samples from Belgrade and probably originates from trichome vacuoles [7].

In general, particle deposition was unequal, which was also observed in other studies [7,37,39,40], particles were present individually or formed aggregates which depended on the type of pollution, deposition was higher on adaxial surface and near rougher surfaces or close to main veins. Furthermore deposition of particles was more efficient on *A. hippocastanum* in relation to *P. acerifolia* leaves, and sampling sites that stood out for the highest deposition were Narodna bašta for leaves of *A. hippocastanum*, and Pionirski Park for *P. acerifolia*, respectively.

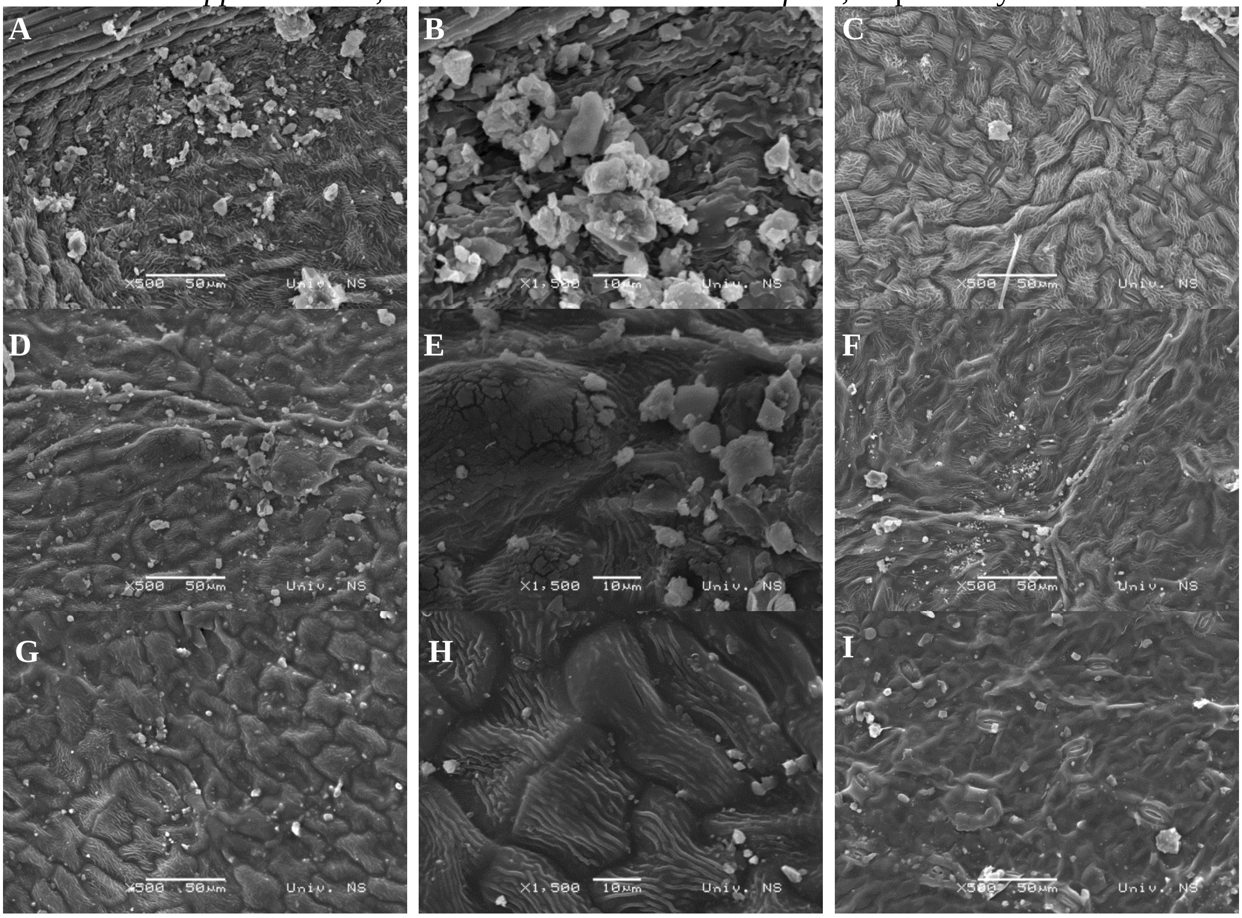


Figure 3. SEM micrographs of adaxial (D) and abaxial (B) leaf surface of A. hippocastanum in October: Pančevo A (Dx500), B (Dx1500), C (Bx500); Belgrade 1 D (Dx500), E (Dx1500), F (Bx500), Belgrade 2 G (Dx500), H (Dx1500), I (Bx500)

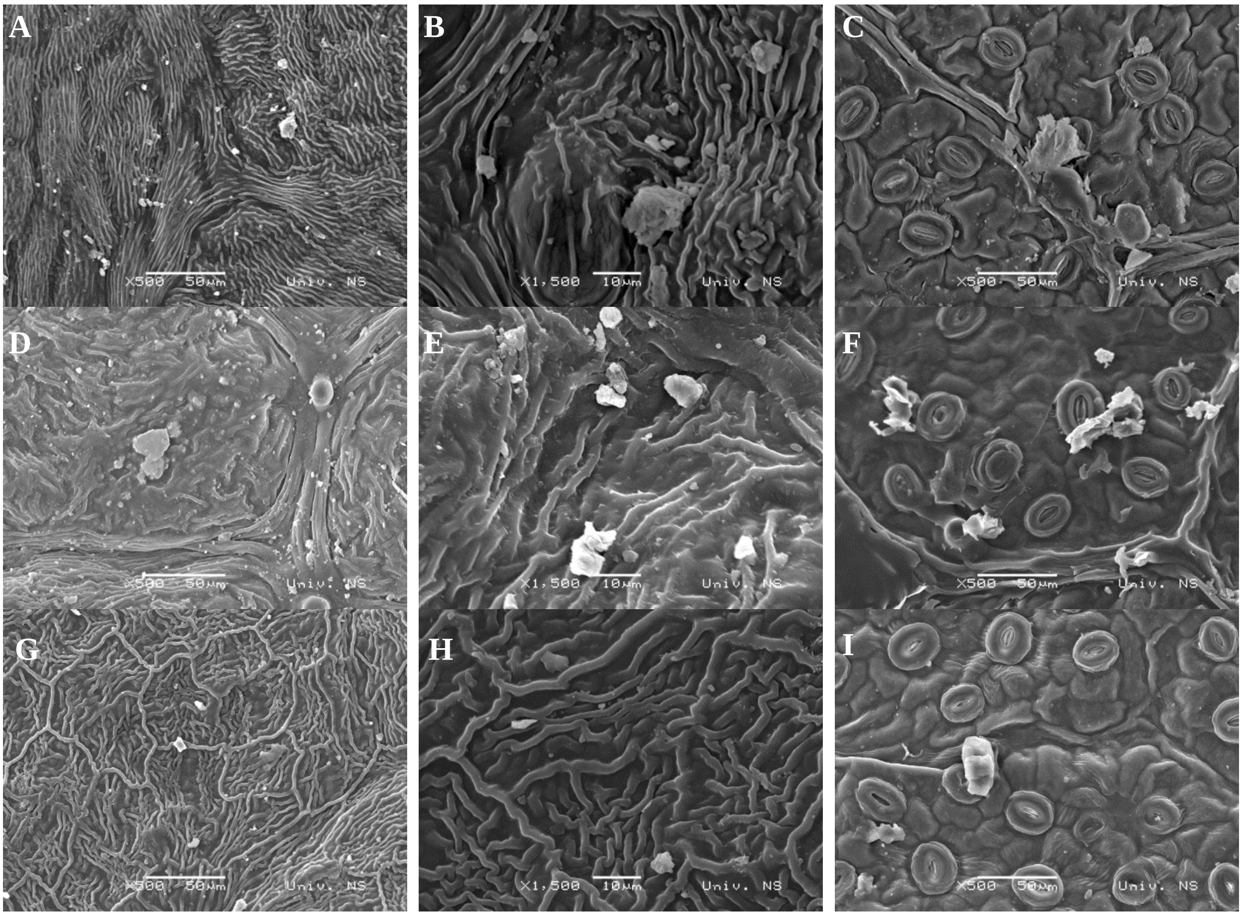
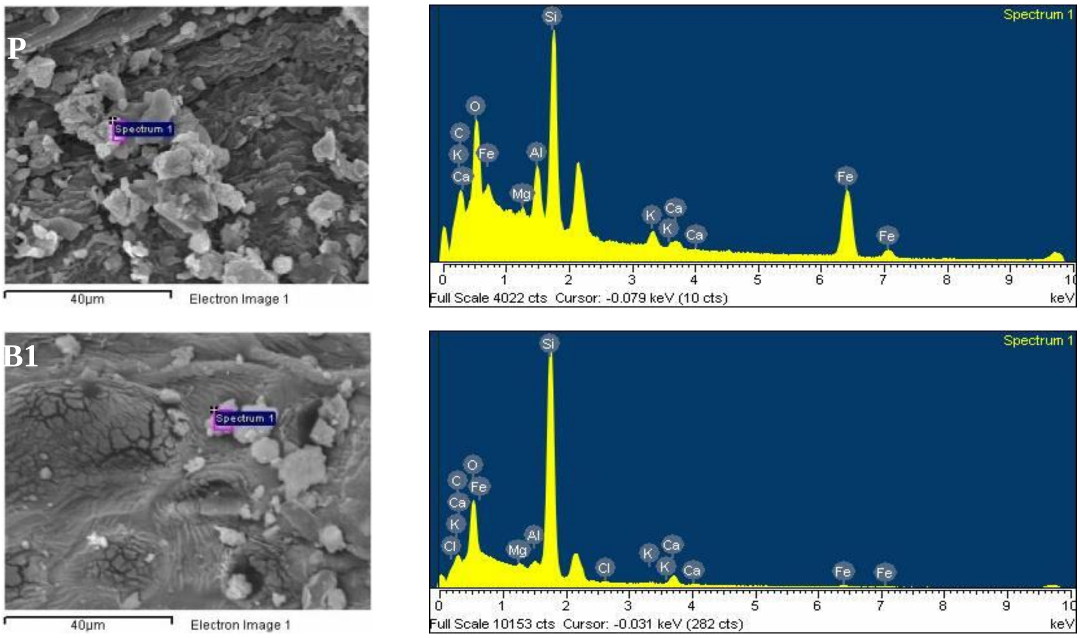


Figure 4. SEM micrographs of adaxial (D) and abaxial (B) leaf surface of P. acerifolia in October: Pančevo A (Dx500), B (Dx1500), C (Bx500); Belgrade 1 D (Dx500), E (Dx1500), F (Bx500), Belgrade 2 G (Dx500), H (Dx1500), I (Bx500)



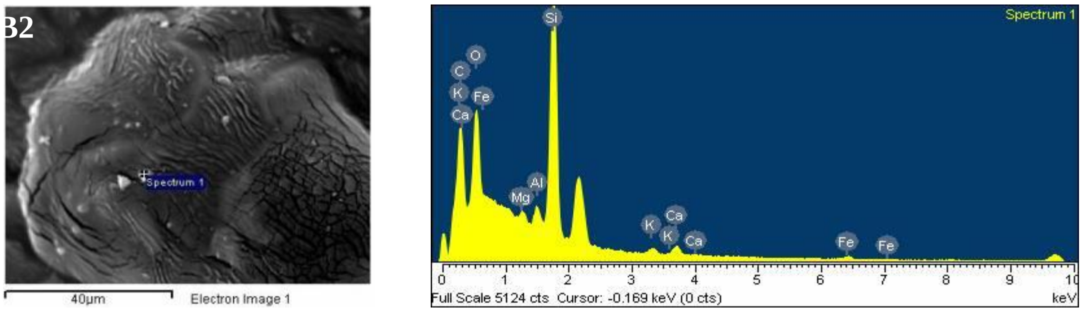


Figure 5. SEM-EDS analysis of PM chemical composition deposited on A. hippocastanum leaves in October: Narodna bašta (P), Pionirski Park (B1) and Topčider Park (B2)

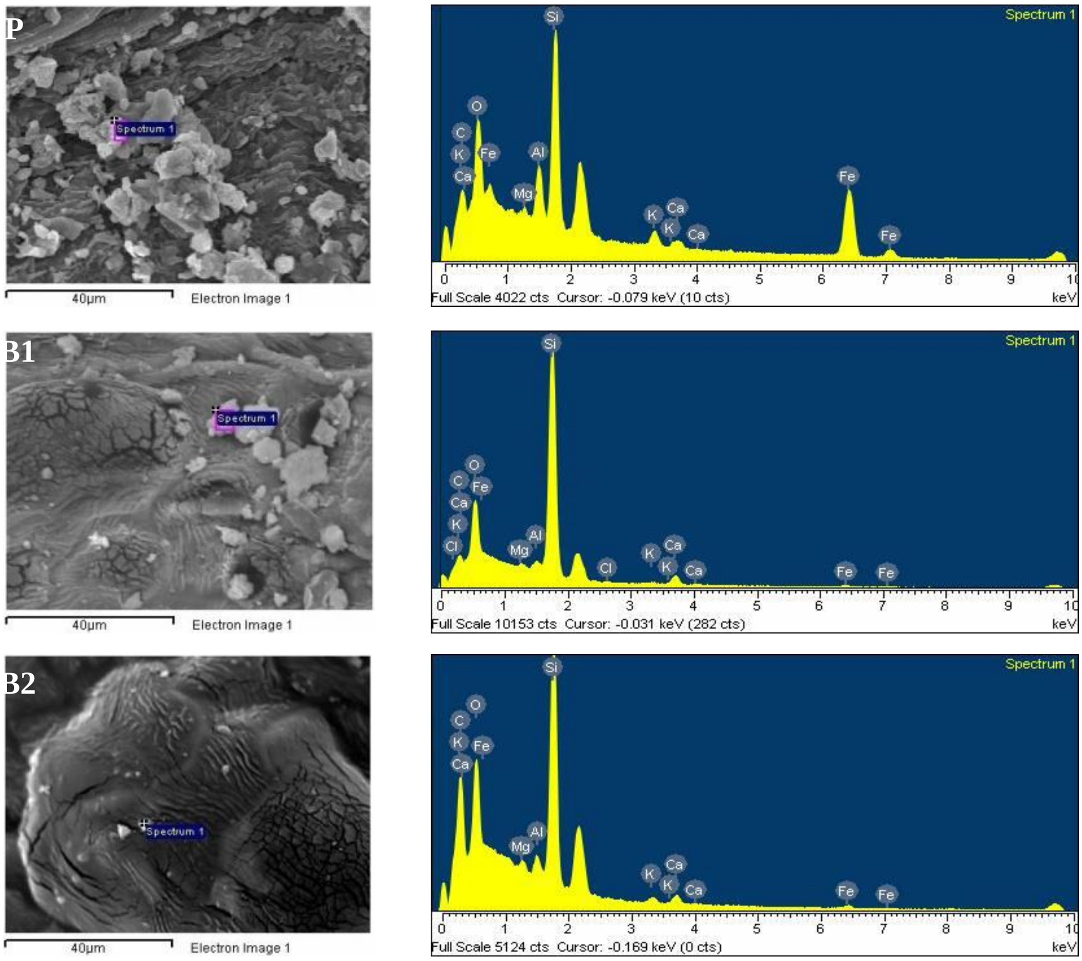


Figure 6. SEM-EDS analysis of PM chemical composition deposited on P. acerifolia leaves in October: Narodna bašta (P), Pionirski Park (B1) and Topčider Park (B2)

Table 2. Elemental composition of PM deposited on the leaf surface of the investigated species in Narodna bašta (P), Pionirski Park (B1) and Topčider Park (B2) in October

Trace elements %										
Sp	Site	Al	C	Ca	Cl	Fe	K	Mg	O	Si
A. hipp	P	3.45	26.24	0.73		15.71	1.29	0.62	39.60	12.35
	B1	0.59	26.78	1.53	0.21	0.71	0.23	0.55	46.95	22.44
	B2	0.71	47.24	0.59		0.41	0.31	0.58	40.56	9.60
P. acer	P	3.23	42.99	1.40		1.66	0.96	0.70	42.46	6.60
	B1	1.26	42.52	15.38		0.59	0.57		36.77	2.92
	B2	1.73	61.71	1.83	0.24	1.53	0.51	1.38	27.00	4.06

Conclusions

Trace element accumulation in leaves varied depending on the element, sampling site, as well as on the species. For the majority of plant samples, the highest element accumulation was measured at Pionirski Park sampling site which is exposed to severe traffic.

Results for the trace element showed that neither of the species was more efficient in accumulation which is probably the result of ecophysiological characteristics of the species, as well as element availability in soil and air. Results for Cu and Zn in Narodna bašta for both species and for *P. acerifolia* from Topčider Park were below the optimum range for plants. On the opposite side, results for Sr from all sampling sites were in toxic range, which indicates potential risk for the examined species and environment.

The results of chemical composition of deposited particles (EDS analysis) showed that the most abundant elements found in particles deposited on the leaves of both species mostly originate from mineral dust or soil, or represent the main constituents of plant tissues.

Particle deposition on leaf surfaces was unequal, in form of single particles or in form of aggregates which was mainly related to the source of pollution. PM deposition was higher on adaxial surface and near rougher surfaces, or close to main veins. Results of this study showed more efficient deposition of particles on leaf surfaces of *A. hippocastanum* in relation to *P. acerifolia* because leaves of *P. acerifolia* have finer epidermal cells, less wrinkled cuticle and lose trichomes during the course of the season. In regard to sampling sites, it was found that the highest deposition of PM particles was at Narodna bašta for leaves of *A. hippocastanum*, and Pionirski Park for leaves of *P. acerifolia*, respectively.

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