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Assessment of heavy metal pollution of topsoils and plants in the City of Belgrade

GORDANA ANDREJIĆ[1,2], TAMARA RAKIĆ[1], JASMINA ŠINŽAR-SEKULIĆ[1], NEVENA MIHAILOVIĆ[2], JASMINA GRUBIN[3]*, BRANKA STEVANOVIĆ[1] and GORDANA TOMOVIĆ[1]

[1]*Institute of Botany and Botanical Garden, Faculty of Biology, University of Belgrade, Takovska 43, 11000 Belgrade, Serbia,* [2]*Institute for the Application of Nuclear Energy – INEP, University of Belgrade, Banatska 31b, 11080 Belgrade, Serbia and* [3]*Ministry of Education, Science and Technological Development of the Republic of Serbia, Njegoševa 12, 11000 Belgrade, Serbia*

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Abstract: In order to assess heavy metal pollution in the City of Belgrade (Serbia), the concentrations of V, Cr, Mn, Co, Ni, Cu, Zn, Cd and Pb in 18 topsoil samples collected in the proximity of central urban boulevards and in urban parks were measured. In addition, the concentrations of the specified elements were determined in the leaves of three evergreen plant species *Buxus sempervirens* L., *Mahonia aquifolium* (Pursh) Nutt. and *Prunus laurocerasus* L. in order to estimate their sensitivity to heavy metal pollution. Even though various types of soils from different parts of Belgrade were sampled, their heavy metal contents were very similar, with somewhat higher concentrations of almost all elements detected in the proximity of heavy traffic roads. Generally, the concentrations of heavy metals in the leaves of the investigated plant species mirrored the heavy metal concentrations found in their respective soils and were higher in the plants sampled from the boulevards than from the urban parks. Since the investigated plants show no visible injuries induced by the detected heavy metal pollution, these species are suitable for successful urban landscaping.

Keywords: soil; plants; *Buxus sempervirens*; *Mahonia aquifolium*; *Prunus laurocerasus*.

INTRODUCTION

The topsoil in urban and industrial areas is particularly exposed to heavy metal contamination. Determination of the heavy metal content of topsoil is of importance for the assessment of environmental pollution and its risk on plant,

* Corresponding author. E-mail: jasmina.grubin@mpn.gov.rs
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animal and human health.[1,2] Heavy metal contamination in urban area mostly derives from vehicle exhaust gases, industrial gasses and coal combustion, as well as from new materials introduced during infrastructure and road engineering.[3–9] Therefore, urban soils are in terms of their physical, chemical and biological properties very different from the primary types of soil.[10,11]

Plants that are integral constituents of each urban ecosystem are exposed to complex adverse environmental conditions, such as polluted air and soil, inadequate essential nutrient supply and very often an unfavourable water regime. In the last decade, premature leaf fallout and high tree and shrub mortality in the urban area were found to be the consequence of hydraulic failure, chronic exposure to polluted air, and finally pathogen attack.[12] Some plants that were used in urban landscaping are more sensitive to heavy metal pollution, such as conifers, whereas others are more tolerant. The evergreen shrubs *Buxus sempervirens* L., *Mahonia aquifolium* (Pursh) Nutt. and *Prunus laurocerasus* L. are used very often in urban landscaping in Belgrade. Although their leaves last for several years and are, therefore, under the long-term negative impact of polluted air and soil, all three species very successfully survive in numerous localities within the Belgrade urban ecosystem.

Taking into account the successful growth of the above-mentioned evergreen species, the objectives of this work were threefold: 1) to assess the heavy metal pollution in the urban area of Belgrade by investigation of the heavy metal contamination in soil, 2) to determine the concentrations of heavy metals in the leaves of three evergreen species *B. sempervirens*, *M. aquifolium* and *P. laurocerasus* plants and 3) to assess the capacity of these plant species for tolerance and/or bioaccumulation of certain trace elements.

EXPERIMENTAL

Study area and sampling

The soil and plant samples were collected from the urban centre of Belgrade. In total, there were eighteen different sampling sites, positioned in six different quarts in central Belgrade urban zone. Details of the sampling sites, their acronyms and positions are given in Table S-I and shown in Fig. S-1 of the Supplementary material to this paper.

Soil analyses

Topsoil samples were collected beneath the ground projection of each of the species analyzed. Each soil was sampled at a depth from surface of 20 cm. From each sampling site, three different soil samples were taken for the analyses. The soil samples were dried at room temperature, sieved (pore size <200 μm), ground using a ceramic pestle and mortar and then dried at 105 °C to constant weight.

For the pH determination, 25 mL of double distilled water or 25 mL of 1 M KCl were added to 10 g of soil and stirred for 30 min. The pH was measured directly in the suspension.

The amount of organic matter in soil was determined after soil digestion in $K_2Cr_2O_7$ and H_2SO_4.[13]

The mineralization of soil samples for the determination of the total content of V, Cr, Mn, Co, Ni, Cu, Zn, Cd and Pb was performed by a slightly modified wet procedure described by ISO 11466, 1995 and ISO 11047 1998. Approximately 3 g of air-dried, ground and sieved (<0.2 mm) soil was weighed and transferred to a reaction vessel. Each sample was moistened with 0.5 to 1.0 mL of deionised water. First, hydrochloric acid was added (21 mL) and then 7 mL of nitric acid dropwise, and the contents were left overnight for slow oxidation. The next day, the reaction mixture was heated until the samples reached the boiling temperature. The samples were boiled for 2 h, ensuring that the steams did not pass 2/3 of the height of the reaction vessel. The mixture was allowed to cool. An additional 25 mL of nitric acid was added. The content was filtered through filter paper into a volumetric flask and the volume was adjusted to 100 mL.

Absorbances were determined by atomic absorption spectrophotometry (Shimadzu AA-7000). The series of standard solutions for investigated elements were made from 1 g L^{-1} solutions purchased from Carlo Erba, Italy. The concentrations were calculated using the Origin 7.0 program.

Plant material analyses

Two years old leaves were collected from the evergreen species *B. sempervirens*, *M. aquifolium* and *P. laurocerasus*. Plant voucher specimens (*B. sempervirens*, No. 26797 BEOU; *M. aquifolium*, No. 34944 BEOU; *P. laurocerasus*, No. 34942 BEOU) were deposited in the Herbarium of the Institute of Botany and Botanical Garden, Faculty of Biology, University of Belgrade (BEOU). All samples were collected in August 2012.

For the determination of the heavy metals and trace elements in the plants, the samples were thoroughly washed with tap water and than with deionised water. Air-dried plant material was ground with a ceramic pestle and mortar and then dried at 105 °C to constant weight. Dried and ground plant material was weighed (1 g) and transferred to reaction vessels for mineralization. The mineralization was performed by a slightly modified wet procedure described by the ISO standard 6636/2 1981. Absorbances were determined in filtered samples adjusted to 50 mL volume by the AAS method, using the same standards as those used for the determination of the metals in the soil samples. The concentrations were calculated using the Origin 7.0 program.

Statistical analyses

All data are expressed by the mean ± standard deviation (*SD*) of three replicates. Correlations between elements were evaluated using the bi-variation method with two-tailed significance and the Spearman rank correlation coefficient. All statistical analyses were performed in Statistica 5.1 (StatSoft 1996).

RESULTS AND DISCUSSION

General characteristics of analyzed soils and their heavy metal contents

The pH of all analyzed soils samples varied over a relatively narrow range from 7.0 to 7.7, measured in H_2O, or from 6.4 to 7.1 when measured in 1 M KCl (Table I). A pH around neutral does not indicate significant bioavailability of the investigated metals.[1,14] Despite this, it is expected that the plant root exudates could significantly change, increase or limit, heavy metal availability for root uptake and thus influence the metal content in the plant organs. The amount of soil organic matter (3.9–8.5 %) indicated that the investigated soils were well supplied with organic substances that considerably affect metal availability and the soil water regime. Namely, organic compounds decrease the availability of heavy metals for plant absorption due to their immobilization on the surface of the organic matter and assure the soil has a good water holding capacity, mainly due to the strong hydrophilic character of organic substances.[15]

TABLE I. Properties of the sampled topsoils

Parameter	Plant					
	B. sempervirens					
Sampling site	B1	B2	B3	B4	B5	B6
pH (H_2O)	7.5	7.5	7.5	7.5	7.0	7.5
pH (1 M KCl)	6.9	7.0	6.9	6.8	6.6	6.8
Org. comp. content, %	5.5	4.8	5.8	4.8	8.5	3.9
	M. aquifolium					
Sampling site	M1	M2	M3	M4	M5	M6
pH (H_2O)	7.7	7.5	7.5	7.2	7.7	7.6
pH (1 M KCl)	7.1	7.0	6.8	7.0	7.0	6.8
Org. comp. content, %	4.5	4.8	5.4	4.8	5.5	3.9
	P. laurocerasus					
Sampling site	P1	P2	P3	P4	P5	P6
pH (H_2O)	7.3	7.3	7.5	7.2	7.6	7.1
pH (1 M KCl)	6.7	6.8	6.9	7.0	6.9	6.4
Org. comp. content, %	7.0	4.0	5.4	3.7	5.3	3.9

The analyzed soil samples showed greatest variations in the concentrations of Pb (33.50 mg kg^{-1} – B4 to 510.0 mg kg^{-1} – M5), Cu (34.55 mg kg^{-1} – P3 to 102 mg kg^{-1} – P2) and Zn (101 mg kg^{-1} – B4 to 342 mg kg^{-1} – M5), Table II. Moderate variations were found for the concentrations of Ni (45.15 mg kg^{-1} – M1 to 83.14 mg kg^{-1} – B3), Cr (38.72 mg kg^{-1} – M1 to 64.8 mg kg^{-1} – B3) and Cd (1.34 mg kg^{-1} – B4 to 2.41 mg kg^{-1} – P5). The concentrations of Fe, Mn, Co and V varied within the narrowest range. The concentrations of Mn ranged from 810 mg kg^{-1} – B5 to 1222 mg kg^{-1} – P6, whereas the concentrations of Co varied between 17.39 mg kg^{-1} – M5 and 22.21 mg kg^{-1} – B3. The amount of V reached up to 43.72 mg kg^{-1} in Ustanička Boulevard – B6. All the obtained results indicated heavy metal contamination of the investigated surface soils from the central urban area of Belgrade.

When compared with data previously reported for urban soils originating from different parts of central zone in Belgrade,[16–21] the results found in the present study were mostly within similar ranges of values. Comparing the results of the heavy metal concentrations in Belgrade urban area soils with data reported for other cities (Table S-II of the Supplementary material), the amounts of V, Cr and Cu were similar to those found in urban soils of other industrial cities and were within the world range.[14,15] On the other hand, the concentrations of Co, Ni and Pb significantly exceeded the world average concentrations and values detected

TABLE II. Heavy metal concentrations in the topsoils (mg kg^{-1}). The concentrations are expressed as mean ± standard deviation (n = 3; <UDL – under the detection limit

Sampling site	Element								
	Mn	Ni	Zn	Cr	Co	Cu	Cd	Pb	V
B. sempervirens									
B1	932±30	59.60±1.50	227±4	46.61±1.37	18.07±0.56	75.35±1.72	1.95±0.09	149±2	23.09±2.22
B2	988±29	61.60±0.83	134±4	47.92±0.76	19.51±0.67	85.61±1.62	2.05±0.09	118±1	16.74±2.18
B3	1117±29	83.14±3.16	295±4	64.80±1.14	22.21±0.52	48.66±1.68	2.15±0.07	73.47±0	22.3±2.02
B4	976±30	55.09±1.50	101±4	47.70±0.66	19.87±0.67	39.31±1.72	1.34±0.06	33.50±0.64	38.17±2.22
B5	810±27	56.24±0.88	270±4	49.24±1.37	19.56±0.46	61.66±1.68	2.05±0.07	104±1	23.88±1.59
B6	870±28	52.28±1.50	138±4	47.7±1.31	20.09±0.44	36.21±1.72	1.44±0.05	49.27±1.10	43.72±1.87
M. aquifolium									
M1	908±30	45.15±1.50	134±4	38.72±0.76	17.55±0.67	89.22±1.72	1.80±0.07	54.03±0.64	25.07±2.26
M2	988±29	61.60±0.83	134±4	47.92±0.76	19.51±0.67	85.61±1.62	2.05±0.09	118±1	16.74±2.18
M3	1012±29	63.19±1.34	190±4	55.59±0.66	20.39±0.74	37.33±0.69	1.54±0.06	41.07±0.63	24.28±1.98
M4	955±29	60.96±2.33	132±4	44.85±1.00	19.86±0.67	90.98±1.68	1.70±0.08	47.80±0.64	37.77±2.38
M5	985±29	62.3±1.16	342±4	61.51±1.14	17.39±0.63	96.23±1.63	2.21±0.09	510±5	16.34±2.22
M6	904±29	49.11±0.88	111±4	45.07±1.14	19.43±0.67	34.67±1.64	1.59±0.07	53.30±0.64	33.41±2.06
P. laurocerasus									
P1	988±29	54.66±2.49	159±4	45.07±0.66	18.39±0.6	56.86±1.73	1.70±0.07	66.65±0	19.12±2.02
P2	1031±30	64.75±1.70	198±4	46.83±1.00	20.23±0.74	102±2	2.31±0.11	138±1	<UDL
P3	1012±29	63.19±1.34	190±4	55.59±0.66	20.39±0.74	34.55±1.62	1.54±0.06	41.07±0.63	24.28±1.98
P4	1028±30	54.17±2.03	137±4	45.07±0.66	18.12±0.69	54.76±1.69	1.65±0.08	59.34±0.63	14.36±2.38
P5	1130±30	68.98±2.35	322±4	57.13±0.38	19.07±0.69	70.48±1.63	2.41±0.10	124±7	4.04±2.26
P6	1222±29	55.30±0.83	162±4	46.61±0.76	20.35±0.52	45.12±1.65	1.54±0.08	53.13±0.63	27.85±2.22
Allowable limit[a]	<2000	<50	<300	<100	<50	<100	<3	<100	–

[a]The maximum allowable limits of heavy metals in soils according to the National legislation

in topsoils of various cities.[14] Moreover, concentrations of Mn and Zn in investigated topsoils were two-fold higher and that of Cd was even more than three-fold higher than world average values and the amounts detected in topsoils from other urban centres. The heavy metal pollution of soil in Belgrade derives from multiple sources, as is the case with other large industrial cities. According to previous reports, the main portion of soil heavy metal contamination in the Belgrade urban areas derives from atmospheric deposition.[17,18]

Despite various soil types that were sampled from different parts of Belgrade for these analyses, their heavy metal contents were very similar, with the highest concentrations of all elements, with exception of Cu, detected in the proximity of busy traffic roads. This indicates that in all sampling sites that were located either along main traffic routes with heavy daily traffic or in parks that were close to busy roads, air pollution represents the main source of their heavy metal contamination. This pollution can be mostly attributed to the burning of fossil fuels that are sources of different heavy metals, such as Pb, Cd, Co or Zn.[22] For many decades, Pb was gradually accumulated in urban soils mostly due to the extensive car fuel combustion, as well as due to its low mobility and low leaching through the soil profile.[1,14,15,22,23] Despite the fact that Pb additives in fuel have not been in use for many years, there are still some other existing sources of Pb pollution. Numerous heating plants and individual house heating systems in Belgrade frequently use oil fuel or charcoal, which are known to be significant sources of Pb, Ni and V.[24–26]

Heavy metal concentration in plants

As a consequence of growing in polluted environment, all the investigated plants showed elevated concentrations of several heavy metals in their leaves. The average heavy metal concentrations in leaves and their standard deviations are summarized in Table III. Heavy metals that occur within the widest range of concentrations were Cr (from 1.47 mg kg^{-1} in *P. laurocerasus* to 5.51 mg kg^{-1} in *M. aquifolium*), Zn (from 3.20 to 26.84 mg kg^{-1} in *M. aquifolium*) and Mn (from 23.43 mg kg^{-1} in *P. laurocerasus* to 559 mg kg^{-1} in *M. aquifolium*). Moderate variations in amounts were found for Cu (from 2.77 to 7.93 mg kg^{-1} in *P. laurocerasus*) and Ni (from 5.77 mg kg^{-1} in *P. laurocerasus* to 20.68 mg kg^{-1} in *M. aquifolium*), whereas Cd and Pb showed variations within a narrow range. The concentrations of Co were lower than the detection limit in most samples, whereas the contents of V were under the detection limit in all plant samples (Table III).

Comparing the results obtained for the investigated plant species, it could be noticed that the leaves of *M. aquifolium* contained the highest concentrations of Mn (M6), Ni (M5), Zn (M5), Cr (M5) and Pb (M6), whereas the highest amount of Cu was detected in *P. laurocerasus* (M6).

TABLE III. Heavy metal concentration in plant leaves collected from sampling sites (mg kg^{-1}). The concentrations are expressed as means ± standard deviations (n = 3); <UDL – under the detection limit

Sampling site	Element								
	Mn	Ni	Zn	Cr	Co	Cu	Cd	Pb	V
					B. sempervirens				
B1	59.90±0.37	13.55±0.85	6.98±0.37	1.78±0.05	2.32±0.24	6.36±0.40	1.85±0.06	21.62±1.65	<UDL
B2	66.36±0.40	10.25±0.92	3.92±0.41	2.11±0.09	0.65±0.26	3.95±0.44	1.48±0.15	21.07±0.95	<UDL
B3	50.69±0.32	13.42±0.72	5.20±0.32	1.78±0.05	<UDL	4.08±0.34	1.55±0.21	19.97±1.65	<UDL
B4	57.53±0.40	16.45±0.9	6.63±0.4	2.57±0.09	0.70±0.25	5.64±0.43	1.58±0.13	19.97±1.65	<UDL
B5	47.56±0.40	16.78±0.90	7.23±0.40	3.92±0.05	<UDL	5.93±0.43	1.55±0.16	21.07±0.95	<UDL
B6	48.00±0.39	13.60±0.90	5.20±0.40	2.94±0.09	2.35±0.25	4.90±0.42	1.64±0.06	19.97±1.65	<UDL
					M. aquifolium				
M1	189±1	10.61±0.87	17.59±0.38	3.00±0.05	<UDL	3.79±0.41	1.78±0.07	22.72±0.95	<UDL
M2	128±1	11.49±0.85	3.20±0.37	2.11±0.09	1.22±0.24	3.27±0.40	1.57±0.10	21.62±1.65	<UDL
M3	119±1	15.94±0.95	12.94±0.42	3.83±0.05	<UDL	4.10±0.45	1.38±0.10	19.42±0.95	<UDL
M4	158±1	10.12±0.70	8.85±0.31	2.11±0.09	0.66±0.19	5.42±0.33	1.71±0.09	22.72±0.95	<UDL
M5	190±1	20.68±0.62	26.84±0.27	5.51±0.09	0.66±0.17	4.63±0.29	1.26±0.13	22.72±0.95	<UDL
M6	559±26	10.61±0.62	21.65±0.27	2.57±0.09	3.44±0.17	4.45±0.29	1.62±0.08	23.82±0.95	<UDL
					P. laurocerasus				
P1	32.68±0.38	5.77±0.87	6.98±0.38	1.47±0.09	<UDL	4.49±0.41	1.64±0.08	19.97±1.65	<UDL
P2	50.41±0.38	8.39±0.87	4.31±0.38	2.27±0.05	0.66±0.24	2.77±0.41	1.63±0.07	17.22±0.95	<UDL
P3	23.43±0.41	10.08±0.92	3.94±0.41	2.24±0.05	<UDL	3.19±0.44	1.66±0.09	20.52±0.95	<UDL
P4	92.46±0.32	8.31±0.72	8.70±0.32	1.56±0.09	<UDL	4.05±0.34	1.61±0.10	18.87±0.95	<UDL
P5	33.04±0.41	8.39±0.92	7.29±0.41	2.42±0.14	<UDL	3.92±0.44	1.35±0.09	16.67±0	<UDL
P6	42.51±0.36	10.35±0.83	5.01±0.37	1.81±0.05	<UDL	7.93±0.39	1.55±0.16	17.77±0.95	<UDL
Normal range[a]	30–300	0.1–5	27–150	0.1–0.5	0.02–1	5–30	0.05–0.2	5–10	0.2–1.5
Excessive level[a]	400–1000	10–100	100–400	5–30	15–50	20–100	5–30	30–300	5–10

[a]Data of trace elements in mature leaf tissue is generalized for various plant species and are not given for very sensitive or tolerant plants[19]

The detected amounts of Cr, Cd, Co and Pb in the leaves of the investigated plants were above the normal range for plants, but were still below the concentrations that are considered to be toxic for most plants.[1] Similar values were detected in evergreen plants *Ilex aquifolium*, *Mahonia aquifolium* and *Rhododendron catawbiense* from Wroclaw Botanical Garden in Poland and in grass and clover, but they were far higher than those reported for some cereals and vegetables.[15,26] High concentrations of cadmium were found in all three plant species and are similar to those detected in Brussels sprouts (1.2–1.7 mg kg^{-1}) and cabbage outer leaves (1.1–3.8 mg kg^{-1}) from Great Britain and in lettuce (0.9––7.0 mg kg^{-1}) from the USA.[15] Despite its slight mobility in soil, Cd is known to be very readily absorbed by plants and translocated to their aboveground parts, even though it has no physiological significance. Thus, the elevated amounts of Cd found in the investigated topsoils from Belgrade were directly reflected in the elevated amounts of Cd in the plant leaves. Furthermore, high concentrations of lead in soils, which is very slightly soluble at neutral pH values and not easily absorbed by plants,[15] was reflected in elevated Pb concentrations in the leaves of all three investigated plant species. The high levels of Ni found in all three plant species were close to the threshold toxicity levels of Ni for sensitive plants (>10 mg kg^{-1} dw) but far lower than those for moderately sensitive plants (>50 mg kg^{-1} dw).[27] The amounts of Mn detected in the leaves of the investigated plant species were within the range that is normal for plants that are neither very sensitive nor highly tolerant to elevated levels of Mn, as reported by Kabata-Pendias.[15]

In general, the concentrations of heavy metals in the leaves of the investigated plant species corresponded to the heavy metal concentrations found in their respective soils and were higher in plants sampled from the boulevards than in those from urban parks (Table IV). However, in comparison to the other two

TABLE IV. Ranges of heavy metal concentrations in the surface soils and in plant material sampled from urban parks and boulevards

Site	Element							
	Mn	Ni	Zn	Cr	Co	Cu	Cd	Pb
				Soils				
Parks (*n* = 27)	908−1031	45−64	134−227	38.7−47.9	17.6−20.2	56−102	1.7−2.3	54−138
Blvds. (*n* = 27)	810−1222	42−83	101−342	45.1−64.8	17.4−22.2	34−96	1.3−2.4	33−510
				B. sempervirens				
Parks (n = 9)	59.9−66.4	10.3−13.6	3.9−7.0	1.8−2.1	0.7−2.3	4.0−2.7	1.5−1.8	21.1−21.6
Blvds. (*n* = 9)	47.5−57.5	13.4−16.8	5.2−7.2	1.8−3.9	0.7−2.4	4.1−6.0	1.6−1.6	20.0−21.1
				M. aquifolium				
Parks (*n* = 9)	128.8−189.5	10.6−11.5	3.2−17.6	2.1−3.0	0.1−1.2	3.3−3.8	1.6−1.8	21.6−22.7
Blvds. (*n* = 9)	119.6−559.4	10.1−20.7	8.9−26.8	2.1−5.5	0.7−3.4	4.1−5.4	1.3−1.7	19.4−23.8
				P. laurocerasus				
Parks (*n* = 9)	32.7−50.4	5.8−8.4	4.3−7.0	1.5−2.3	0.1−0.7	2.8−4.5	0.0−1.6	17.2−20.0
Blvds. (*n* = 9)	23.4−92.5	8.3−10.3	3.9−8.7	1.56−2.42	0.1−0.1	3.2−7.9	1.4−1.7	16.7−20.5

investigated plant species, *M. aquifolium* accumulated the highest concentrations of Cr, Mn, Co, Ni, Zn and Pb in their leaves.

The common positive correlations between Ni–Cr, Mn–Cr, Zn–Cd and Cu–Pb detected in all soils indicated that the specified metals could derive from the same pollution sources that are non-site related, such as emission along traffic routes (Table V).

Table V. Spearman's rank correlation coefficients (*rs*) for soil and leaves of *B. sempervirens*, *M. aquifolium*, *P. laurocerasus*. The upper right part is for leaves, the lower left part is for soils. Significant correlation coefficients are in bold (* p <0.05; ** p <0.01and *** p<0.001).

B. sempervirens							
	Mn	Ni	Zn	Cr	Cu	Cd	Pb
Mn	–	**–0.59****	0.13	**–0.55***	**–0.44***	**0.48***	0.09
Ni	0.10	–	**0.49***	**0.47***	**0.74*****	–0.01	–0.05
Zn	0.01	0.42	–	0.12	0.32	**0.65****	0.10
Cr	–0.18	**0.63****	0.41	–	0.20	–0.27	–0.06
Cu	0.01	**0.66****	0.30	0.13	–	0.08	0.13
Cd	**0.61****	0.16	**0.57****	–0.02	0.38	–	–0.17
Pb	–0.12	**0.58****	**0.44***	0.10	**0.93*****	0.33	–
M. aquifolium							
	Mn	Ni	Zn	Cr	Cu	Cd	Pb
Mn	–	–0.01	**0.72*****	0.24	0.34	0.19	**0.66****
Ni	**0.66****	–	0.14	**0.81*****	–0.06	**–0.57****	–0.06
Zn	0.19	**0.64****	–	**0.45***	**0.54***	–0.19	0.24
Cr	**0.68*****	**0.69*****	**0.44***	–	–0.04	**–0.49***	0.06
Cu	–0.22	0.26	**0.65****	–0.06	–	0.07	0.25
Cd	0.04	0.25	**0.57****	0.07	**0.83*****	–	**0.46***
Pb	–0.30	0.05	**0.51***	0.03	**0.70*****	**0.84*****	–
P. laurocerasus							
	Mn	Ni	Zn	Cr	Cu	Cd	Pb
Mn	–	0.38	0.17	0.22	0.35	0.06	**–0.57****
Ni	**0.59****	–	–0.34	**0.54***	0.41	0.16	–0.36
Zn	**0.58****	**0.97*****	–	–0.38	0.24	–0.15	0.12
Cr	**0.63****	**0.89*****	**0.89*****	–	–0.12	–0.12	**–0.69*****
Cu	0.43	**0.66****	**0.68*****	0.41	–	–0.05	–0.22
Cd	**0.48***	**0.78*****	**0.76*****	**0.55***	**0.96*****	–	**0.49***
Pb	–0.03	0.32	0.38	0.05	**0.76*****	**0.69*****	–

The most significant positive correlations (p < 0.001) were found for Ni–Cu in the leaves of *B. sempervirens*, and for Cu–Pb in their corresponding soils (Table V). Regarding *M. aquifolium*, the most significant positive correlations were found for Zn–Mn and Ni–Cr in leaves and for Cr–Mn, Ni–Cr, Cu–Cd, Cu–Pb and Pb–Cd in the corresponding soil. In the leaves of *P. laurocerasus*, the most significant correlations were negative and were found for Cr–Pb, whereas in the corresponding soil, the most significant correlations between the elements were positive and detected for Ni–Zn, Ni–Cr, Ni–Cd, Zn–Cr, Zn–Cu, Zn–Cd, Cu–Pb, Cu–Cd, Cu–Pb and Pb–Cd. Since correlations between elements were very different among the investigated species, it indicates that they differ in their affinities for the absorption of the same element.

In order to determine to which of the four categories of plant–heavy metal relationships (excluders, indicators, accumulators and hyperaccumulators) the investigated evergreen plants could be classified,[1] their accumulation potential for Mn, Ni, Zn, Cr, Co, Cu, Cd, Pb and V were calculated. The accumulation factor (*AF*), calculated as the ratio between the metal concentration in the leaves and the total concentration in the corresponding soil was in most samples much lower than 1 (data not shown), except for V, for which the average *AF* value for *B. sempervirens* was 1.31, for *M. aquifolium* 1.34 and for *P. laurocerasus* 1.50. In addition, the average *AF* values for Cd were only slightly below 1, *i.e.*, for *B. sempervirens*, 0.91; for *M. aquifolium*, 0.87 and for *P. laurocerasus*, 0.88. Therefore, all three plant species studied herein could be categorized as plants tolerant to almost all the investigated heavy metals and be generally considered as excluders – plants that restrict transport of metals to the shoot and maintain relatively low metal concentrations in the leaves over a wide range of soil metal concentrations. For only two trace elements, *i.e.*, V and Cd, could these three evergreen plants be treated as indicators – plants that show an intermediate response to high soil trace elements concentrations with the element concentration in the plants reflecting the soil concentration.[28,29]

Since all the analyzed plants were growing on contaminated soils in the vicinity of heavy traffic roads in Belgrade and were therefore exposed to intensive air pollution, it is expected that some of the heavy metals, primarily those whose main source is atmospheric pollution – Ni, Cr, Cd and Co, might have entered the leaf tissues through stomata, as already reported by Dalenberg and van Driel[30] for different plant species. Although the concentrations of heavy metals were elevated in the leaves of the investigated plant species, there was a clear lack of any visible injury to the leaves in term of their external appearance. It might be suggested that all three species possess mechanisms that provide them with successful tolerance to higher concentrations of heavy metals in their tissues.

Despite the absence of some structural leaf damage, more precise investigations, primarily those related to leaf anatomy, photosynthesis and chlorophyll *a* fluorescence and determination of activities of specific enzymes, are required for an accurate assessment of their sensibility or tolerance to heavy metal pollution.

CONCLUSIONS

The detected concentrations of heavy metals in topsoils from urban parks and boulevards indicated their heavy metals contamination. The concentrations of Cd were even more than three-fold higher, those of Mn and Zn were two-fold higher, whereas the concentrations of Co, Ni and Pb were significantly higher than the world average concentrations and the values detected in topsoils of various cities. These heavy metals were accumulated in the surface soil during long-term pollution from various sources, such as air pollution that originated from fuel combustion, and additionally from abrasion of tires and asphalt and from car lubricants. Leaves of evergreen species *Buxus sempervirens*, *Mahonia aquifolium* and *Prunus laurocerasus* from the central urban area contained elevated amounts of heavy metals, such as Cd, Pb and Ni, as the consequence of environmental heavy metal pollution. Yet, they showed no visible injuries induced by the heavy metal pollution and because of such features, these species are suitable for successful urban landscaping.

SUPPLEMENTARY MATERIAL

The details about study area and sampling procedure,as well as a comparison of the present with previous worldwide results are available electronically from http:// //www.shd.org.rs/JSCS/, or from the corresponding author on request.

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И З В О Д

ПРОЦЕНА ЗАГАЂЕНОСТИ ЗЕМЉИШТА И БИЉАКА ТЕШКИМ МЕТАЛИМА НА ТЕРИТОРИЈИ БЕОГРАДА

ГОРДАНА АНДРЕЈИЋ[1,2], ТАМАРА РАКИЋ[1], ЈАСМИНА ШИНЖАР-СЕКУЛИЋ[1], НЕВЕНА МИХАИЛОВИЋ[2], ЈАСМИНА ГРУБИН[3*], БРАНКА СТЕВАНОВИЋ[1] и ГОРДАНА ТОМОВИЋ[1]

[1]*Институт за ботанику и ботаничка башта, Биолошки факултет, Универзитет у Београду, Таковска 43, 11000 Београд,* [2]*Институт за примену нуклеарне енергије – ИНЕП, Универзитет у Београду, Банатска 31б, 11080 Београд и* [3]*Министарство образовања, науке и технолошког развоја Републике Србије, Његошева 12, 11000 Београд*

Концентрације V, Cr, Mn, Co, Ni, Cu, Zn, Cd и Pb су одређене у узорцима земљишта на којима расту биљне врсте *Buxus sempervirens* L., *Mahonia aquifolium* (Pursh) Nutt. и *Prunus laurocerasus* L. као и у њиховим листовима. Земљиште и биљни материјал су сакупљени са 18 локалитета у ужој градској зони Београда (у булеварима и градским парковима). Количине испитиваних елеманата у узорцима земљишта из уже градске зоне су биле међусобно веома сличне, при чему су детектоване више концентрације метала у земљиштима сакупљеним крај великих градских саобраћајница. Концентрације тешких метала у листовима биљака су показале зависност од количине датог метала у земљишту и биле су више у биљкама које су расле у великим градским булеварима у односу на градске паркове. С обзиром да истраживане биљке нити на једном од градских локација нису показивале видљиве знаке оштећења узрокованим вишом концентрацијом тешких метала може се констатовати да су ове врсте погодне за сађење у специфичним условима градског екосистема.

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