Title: Assessment of heavy metal pollution of topsoils and plants in the city of Belgrade

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

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Извод: 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 током све четири сезоне са 15 локалитета у ужој градској зони Београда (у булеварима и градским парковима) и са три природна станишта ових врста. 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.

Keywords: soil, plants, *Buxus sempervirens*, *Mahonia aquifolium*, *Prunus laurocerasus*
Running title: HEAVY METAL POLLUTION IN BELGRADE

INTRODUCTION

The topsoil in urban and industrial areas is particularly exposed to heavy metal contamination. Determination of its heavy metal content is important for assessment of environmental pollution and the risk on plant, 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, 4, 5, 6, 7, 8, 9]. Therefore, the urban soils are in terms of their physical, chemical and biological properties, very different from the primary types of soil [10, 11].

The city of Belgrade, with about 2 million residents, is characterized by high urbanization dynamic, intense traffic volume and industrial zone that are located on the peripheral parts of the city within which many factories until recently operated with old technologies. The main part of the Belgrade is positioned on hilly landscape, whereas other parts, such as New Belgrade, lies on drained soil that was covered with sand more that 50 years ago. The main geological substrate in Belgrade consists of Neogen sediments that cover pre-Neogene rocks, such as clays, sands and carbonate rocks [12]. There is a large variety of natural soils; in the vicinity of the big rivers Sava and Danube and in the lower altitudes there are gley, semiglay and alluvial soils, whereas at higher altitudes there are different clay and skeletal soils. These soils in Belgrade were modified for many decades by different anthropogenic activities such as wetlands draining, covering with sand or introducing new materials during infrastructure building. The comprehensive and more recent surveys on the quality of urban soils in Belgrade were done by Vratuša [(13)] and Vratuša and Anastasijević [14].

Plants that are integral constituents of every urban ecosystem are exposed to complex adverse environmental conditions such as polluted air and soil, inadequate essential nutrient supply and most often unfavorable water regime. In the last decade the premature leaf fallout and high trees and shrubs mortality in urban area are the consequence of hydraulic failure, chronic exposure to polluted air, and finally pathogen attack [15]. Some plants that were used in urban landscaping are more sensitive to heavy metal pollution, such as conifers, whereas others are more tolerant. 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 therefore are under long-term negative impact of polluted air and soil all three species very successfully survive in numerous localities within Belgrade urban ecosystem.

Taking into account successful growth of above mentioned evergreen species the objectives of this work were twofold: 1) to assess heavy metal pollution in Belgrade urban area by investigation of heavy metal contamination in soil, 2) to determine concentrations of heavy metals in 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. Plants samples and their belonging topsoils were collected in the proximity to central urban boulevards (JNA Boulevard, Mihailo Pupin Boulevard and Ustanička Boulevard) and from urban parks (Botanical Garden, Kalemegdan and Sava Quay). In total, there are eighteen different sampling sites, positioned in six different quarts in central Belgrade urban zone. Their acronyms and positions are shown in Table I and Figure 1.

Table I Abbreviations of the sampling sites within the urban centre of the city of Belgrade

Sampling site	Botanical Garden	Kale-megdan	Sava Quay	JNA Boul.	Mihajlo Pupin Boul.	Ustanička Boul.
Species						
B. sempervirens	B1	B2	B3	B4	B5	B6
M. aquifolium	M1	M2	M3	M4	M5	M6
P. laurocerasus	P1	P2	P3	P4	P5	P6

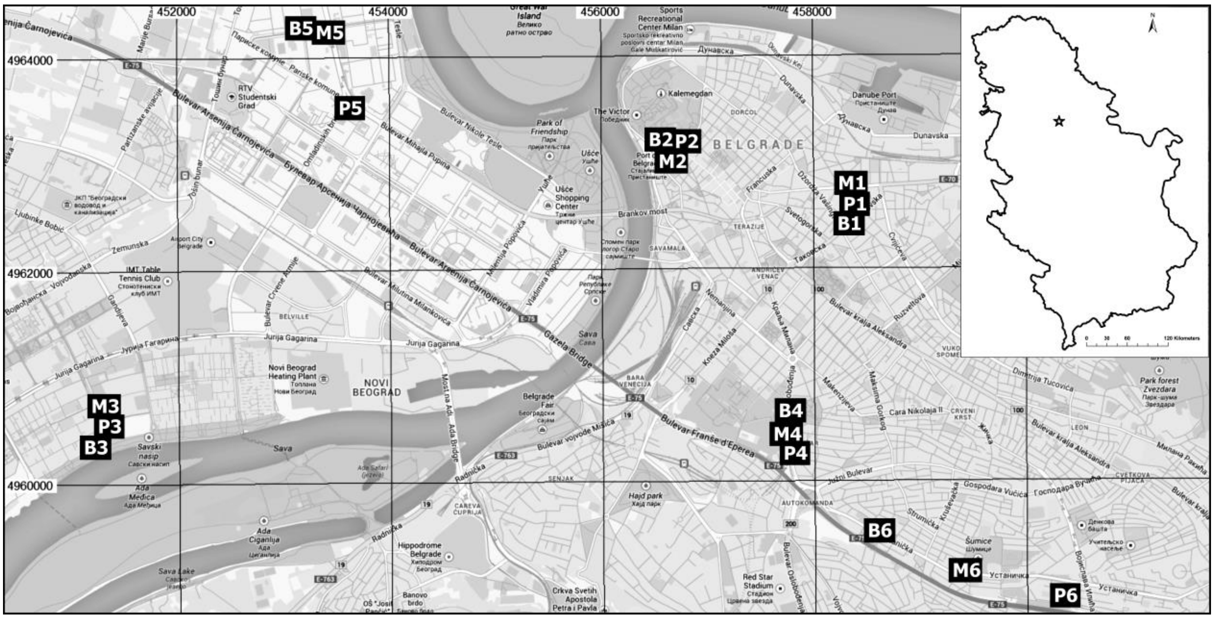

Figure 1. Map of the studied area within the urban centre of the city of Belgrade and sampling sites (for the abbreviations of the sampling sites see Table I)

Soil analyses

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

For the pH determination 25 ml of double distilled water or 25 ml 1M KCl were added to 10 g of soil and stirred for 30 minutes. 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 [16].

The extraction of elements for determination of the total mineral elements in soil (V, Cr, Mn, Co, Ni, Cu, Zn, Cd, Pb) was performed in aqua regia, according to ISO 11466 (Soil quality – extraction of trace elements soluble in aqua regia), and determined by ISO 11047 (1998) (Soil quality – determination of cadmium, chromium, cobalt, copper, lead, manganese, nickel and zinc in aqua regia extracts in soil). The mineral elements concentrations in the sample solutions were determined using the atomic absorption spectrophotometer (Shimadzu AA-7000) and standards of known concentrations (Carlo Erba, Italy).

Plant material analyses

Two years old leaves were collected from 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) are 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.

Plant samples were thoroughly washed in tap water and than in deionized water. Air dried plant material was ground with ceramic mortar and pestle and then dried at 105°C until the constant weight. Plant material was digested in HNO_3:H_2SO_4 (2:1, v/v) and the total mineral elements concentrations (V, Cr, Mn, Co, Ni, Cu, Zn, Cd, Pb) in solutions were determined by AAS Shimadzu AA-7000 and standards of known concentrations.

Statistical analyses

All data are expressed by the mean+standard deviation (SD) of three replicates. Correlations between elements were evaluated using bi-variation method with two-tailed significance and Spearman's 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 content

The pH of all analyzed soils samples varies in relatively narrow range from 7.0 to 7.7, measured in H_2O, or from 6.4 to 7.1 when measured in 1M KCl (Table II). The pH around neutral does not indicate significant bioavailability of investigated metals [17-18]. Despite this, it is expected that the plant root exudates could significantly change, increase or limit, heavy metal availability for the root uptake and thus influence the metal content in plant organs. The amount of soil organic matter (3.9% to 8.5%) indicates that investigated soils are well supplied with organic substances that considerably affect metal availability and soil water regime. Namely, organic compounds decrease availability of heavy metals for plants absorption due to their immobilization on the organic matter surface and assure the soil well water holding capacity mainly due to the strong hydrophilic character of organic substances [18].

Table II Properties of the sampled topsoils

	B. sempervirens					
Sampling site	B1	B2	B3	B4	B5	B6
pH (H2O)	7.5	7.5	7.5	7.5	7.0	7.5
pH (1M KCl)	6.9	7.0	6.9	6.8	6.6	6.8
% org.comp.	5.5	4.8	5.8	4.8	8.5	3.9
	M. aquifolium					
Sampling site	M1	M2	M3	M4	M5	M6
pH (H2O)	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.	4.5	4.8	5.4	4.8	5.5	3.9
	P. laurocerasus					
Sampling site	P1	P2	P3	P4	P5	P6
pH (H2O)	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.	7.0	4.0	5.4	3.7	5.3	3.9

The analyzed soil samples show greatest variations in the concentrations of Pb (33.50 mg kg^{-1}– B4 to 511.0 mg kg^{-1}– B5), Cu (34.55 mg kg^{-1} – B3 to 102 mg kg^{-1} – P2) and Zn (101 mg kg^{-1}– B4 to 342 mg kg^{-1} – M5) (Table III). Moderate variations are 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). Concentrations of Fe, Mn, Co and V vary within the narrowest range. Concentrations of Mn range from 810 mg kg^{-1}– B5 to 1222 mg kg^{-1} – P6, whereas concentrations of Co vary between 17.39 mg kg^{-1} – M5 and 22.21 mg kg^{-1} – B3. The amount of V reaches up to 43.72 mg kg^{-1} in Ustanička Boulevard – B6. All the obtained results indicate heavy metal contamination of investigated surface soils from the central urban area of Belgrade.

Table III. Heavy metal concentration in topsoils (mg kg-1). Concentrations are expressed as mean ± standard deviation (n=3) (<UDL – Under Detection Limit)

Element	Mn	Ni	Zn	Cr	Co	Cu	Cd	Pb	V
Sampling site					*B. sempervirens* – soils				
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
Sampling site					*M. aquifolium* – soils				
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	34.55±1.62	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
Sampling site					*P. laurocerasus* – soils				
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

When compaired with data reported by Vratuša and Anastasijević [(13)] for several other urban park soils from Belgrade, we found that in almost all investigated soil samples concentrations of Pb were much lower, whereas concentrations of Zn and Cd much higher than those previously reported. Contents of Cu in our soil samples were similar to those reported by the same authors for other Belgrade urban parks. Comparing our results of heavy metal concentrations in Belgrade urban area soils with data reported for other cities (Table IV) we found that amounts of V, Cr and Cu are similar to those found in urban soils of other industrial cities and are within the world range [18-19]. On the other hand, concentrations of Co, Ni and Pb significantly exceed the world average concentrations and values detected in topsoils of various cities [18]. Moreover, concentrations of Mn and Zn in investigated topsoils are two-fold higher, whereas that of Cd is even more than three-fold higher than world average values and amounts detected in topsoils from other urban centers. The soil heavy metal pollution in Belgrade derives from multiple sources, as is the case with other big industrial cities. According to previous reports, the main portion of soil heavy metal contamination in Belgrade urban areas derives from atmospheric deposition [14].

Table IV Minimum, maximum and average concentrations of heavy metals (mg kg-1) ± standard deviation in urban soils of several cities in the world.

City	Mn	Ni	Zn	Cr	Co	Cu	Cd	Pb	V
Belgrade	810−1223	45−83	101−343	39−65	17.4−22.2	35−103	1.3−2.4	34−511	74−127
London[1]			424				1-17	654	
Hamburg[2]		62.5	516	95.4		146.6		168	
Bangkok[3]	340+210	24.8±13.1	118±185	26.4±13.6	-	41.7±54.2	0.29±0.49	47.8±52.7	-
Mexico city[4]	-	39.8	306.7	117	-	100.8	-	140.5	97
Palermo[5]	519±250	17.8±7.7	138±71	34±19	5.2±3.5	63±54	0.68±0.35	202±111	54±24
Hong Kong[6]			168+74.8			24.8+12	2.18+1.02	93.4+37.3	
Zagreb[7]	597±266	35.2±23.8	77.9±33.6	54.6±21.1	10.9±3.6	56.1±117	0.40±0.34	23.2±14.4	-
Damask[8]	-	39±9	103±54	57±24	13±5	34±16	-	17±18	-
Naples[9]	-	-	251±253	11±9	-	74±56	-	262±337	-
World average*	488	13-37	7-89	60	6-14	14-109	0.4	27	150

Sources: 1 garden topsoils and in brackets public garden topsoils (0– 5 cm)30, 2 surface soils (0– 5 cm)31, 3 topsoil9, 4 topsoil32, 5 urban topsoil33, 6 urban parks topsoil34, 7 urban topsoil35, 8 urban topsoil36, 9 urban topsoil36, * world average19.

Despite various soil types that were sampled for these analyses from different parts of Belgrade their heavy metal contents are very similar, with the highest concentrations of all elements, with exception of Cu, detected in the proximity to high traffic roads. This indicates that in all sampling sites that are located either along main traffic routes with heavy daily traffic or in parks that are close to busy roads, the air pollution represents the main source of their heavy metal contamination. This pollution can be mostly attributed to burning of fossil fuels that are source of different heavy metals such as Pb, Cd, Co or Zn [20]. 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 [16-20]. Despite the fact that Pb additives in fuel are not 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 and charcoal, which are known to be the significant sources of Pb, Ni and V [22, 23, 24]. The common positive correlations between Ni-Cr, Mn-Cr, Zn-Cd and Cu-Pb detected in all soils indicate that 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.

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***	-

Heavy metal concentration in plants

As a consequence of growing in polluted environment all investigated plants show elevated concentrations of several heavy metals in their leaves. The average heavy metal concentrations in leaves and their standard deviations are summarized in Table VI. Heavy metals that occur within the widest range of concentrations are Cr (from 1.47 mg kg^{-1} in P. laurocerasus to 5.51 mg kg^{-1} in *M. aquifolium*), Zn (from 3.20 mg kg^{-1} 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 are 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 show variations within the narrow range. Concentrations of

Co are under detection limit in most samples, whereas the contents of V are under detection limit in all plant samples (Table VI).

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

Table VI Heavy metal concentration in plant leaves collected from sampling sites (mg kg-1). Concentrations are expressed as means ± standard deviations (n=3) (<UDL – Under Detection Limit)

Element	Mn	Ni	Zn	Cr	Co	Cu	Cd	Pb	V
Sampling site	*B. sempervirens* – leaves								
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
Sampling site	*M. aquifolium* – leaves								
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
Sampling site	*P. laurocerasus* – leaves								
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*	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*	400-1000	10-100	100-400	5-30	15-50	20-100	5-30	30-300	5-10

Detected amounts of Cr, Cd, Co and Pb in leaves of investigated plants are above the normal range in plants, but are still below concentrations that are considered to be toxic for most plants [18]. 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 are far higher than those reported for some cereals and vegetables [19, 25]. High concentrations of cadmium are 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 U.S. [19]. 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 that are found in investigated topsoils from Belgrade directly reflect in elevated amounts of Cd in plant leaves. Also, high concentrations of lead in soils, that is very slightly soluble at neutral pH values and not easily absorbed by plants [19], reflect in elevated Pb concentrations in leaves of all three investigated plant species. High levels of Ni found in all three plant species are 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) [26]. Amounts of Mn detected in leaves of investigated plant species are within the range that is normal for those plants that are neither very sensitive nor highly tolerant to elevated Mn, as reported by Kabata-Pendias [19].

In general, concentrations of heavy metals in leaves of investigated plant species correspond to heavy metal concentrations found in their respective soils and were higher in plants sampled from boulevards then from urban parks (Table VII). However, in comparison to the other two investigated plant species, *M. aquifolium* accumulated highest concentrations of Cr, Mn, Co, Ni, Zn and Pb in leaves.

Table VII Ranges of heavy metal concentrations in surface soils and in plant material sampled from urban parks and boulevards

	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
Boulevards (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
Boulevards (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
Boulevards (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
Boulevards (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

The most significant positive correlations ($p<0.001$) are found for Ni-Cu in leaves of *B. sempervirens*, and for Cu-Pb in its corresponding soil (Table V). Regarding *M. aquifolium*, the most significant positive correlations are 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 are negative and are found for Cr-Pb, whereas in the corresponding soil the most significant correlations between elements are positive and are 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 are very different among investigated species, it indicates that they differ in their affinities for the absorption of the same element.

In order to obtain to which of four categories of plant-heavy metal relationships (excluders, indicators, accumulators and hyperaccumulators) investigated evergreen plants can be classified [27] we calculated their accumulation potential for Mn, Ni, Zn, Cr, Co, Cu, Cd, Pb and V. The accumulation factor (AF), calculated as the ratio between metal concentration in leaves and the total concentration in the corresponding soil, in most samples is much lower then 1 (data not shown), except for V, since the average AF values in *B. sempervirens* is 1.31, in *M. aquifolium* is 1.34 and in *P. laurocerasus* is 1.50. Also, average AF values slightly below 1 are found for Cd: in *B. sempervirens* – 0.91, in *M. aquifolium* – 0.87 and in *P. laurocerasus* – 0.88. Therefore, all three plant species studied here can be categorized as plants tolerant to almost all investigated heavy metals and can 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 such as V and Cd, these three evergreen plants could 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 [27-28].

Since all analyzed plants grow on the contaminated soils in the vicinity to the high traffic roads in Belgrade and are 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 or Co, might have entered leaf tissues through stomata, as already reported by Dalenberg and van Driel [29] for different plant species. Although concentrations of heavy metals are elevated in leaves of investigated plant species there is clear lack of any visible injury on leaves external appearance. It might be suggested that all three species posses mechanisms that provide them successful tolerance of higher concentrations of heavy metals in their tissues.

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

CONCLUSIONS

Detected concentrations of heavy metals in topsoils from urban parks and boulevards indicate their heavy metals contamination. Concentrations of Cd are even more than three-fold higher, those of Mn and Zn are two-fold higher, whereas concentrations of Co, Ni and Pb are significantly higer then the world average concentrations and values detected in topsoils of various cities. These heavy metals are accumulated in surface soil during long-term pollution from various sources such as air pollution that originates from fuel combustion, but also 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 contain elevated amounts of heavy metals such as Cd, Pb and Ni as the consequence of environmental heavy metal pollution. Yet, they show no visible injuries induced by heavy metal pollution and because of such features these species are suitable for the successful urban landscaping.

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