REPORT

Plant parts of the apple tree (*Malus* spp.) as possible indicators of heavy metal pollution

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Abstract The content of Cu, Zn, Pb, As, Cd, and Ni was determined by ICP-OES in spatial soil and parts (root, branches, leaves, and fruit) of the apple tree (*Malus* spp.) from polluted sites near The Mining and Smelting Complex Bor (Serbia). The aim of this study was to examine if the obtained results can be used for biomonitoring purposes. Data recorded in plant parts, especially leaves, gave very useful information about the environmental state of the Bor region. Conveniently, these data described well the capability of investigated plant species to assimilate and tolerate severely high concentrations of heavy metals in its tissues, which may further allow the possibility for utilization of the apple tree for phytostabilization.

Keywords Apple tree · Heavy metals · Biomonitoring

INTRODUCTION

Heavy metals, which are often of anthropogenic origin, are very harmful substances. They are considered to be disturbing elements of ecosystems because of being non-biodegradable and due to their physiological effects on living organisms, including plants. Among these are Cd, Cr, Hg, Pb, as well as metalloid As, which are known to be toxic at even very low concentrations. Some of metals such as Fe, Mn, Cu, Zn, Ni, and Co are essential for plant metabolism in trace amounts but, when they are present at excessive levels, then they become toxic for plants (Lin and Aarts 2012). Many factors affect the bioavailability of

metals in the soil: the total metal concentration, pH, the organic matter content, redox conditions, and the presence of clays and hydrous oxides. However, some studies have found that different plant species or varieties grown on the same soil have different metal uptakes, confirming in that way that the plant genotype is the most important factor affecting the metals' adoption (Kabata-Pendias and Pendias 2001; Bhargava et al. 2012). Gallego et al. (2012) reported that the cell wall of the root was identified as one of the crucial places of metals assimilation and accumulation as well. The retention of metals in the root tissue represents one of the key mechanisms of heavy metal tolerance in plants that can survive and prosper in hard-contaminated fields. On the other hand, some plants have not only a good extraction but also a good root-to-shoot translocation, which make them able to accumulate extreme concentrations of metals into their above ground parts (100–1000 times higher than the concentrations normally present in plants). These plants are known as hyperaccumulators (Lin and Aarts 2012; Alagić et al. 2013; Maric et al. 2013). Both described tactics which were developed by different plants in their attempt to tolerate metal excess are often utilized for phytoremediation purposes.

At the same time, many plant species which had been permanently exposed to massive heavy metal pollution were successfully utilized for detection of accumulation, deposition, or distribution of metals in contaminated areas. Nowadays, higher plants are the most commonly used bioindicators in air quality biomonitoring studies. The main advantages of the use of higher plants for monitoring purposes are great availability of the biological material, simplicity of species identification, sampling, and treatment (Berlizov et al. 2007; Akosy 2008; Balasooriya et al. 2009).

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Unterbrunner et al. (2007) investigated Zn and Cd accumulation in tissues of different adult trees and associated herbaceous species collected from contaminated areas in Central Europe and found considerable amounts of Cd and Zn in leaves: 116 and 4680 mg kg⁻¹, respectively. Research on birch and lime from the contaminated area of the Bor region (East Serbia) showed that these plants interact with their local environment actively and reflect the level of local pollution (Alagić et al. 2013). Contents of heavy metals in birch leaves around a Ni/Cu smelter at Northwestern Russia showed that the birch resistance to pollution may favor it as an excellent indicator in environmental studies (Kozlov et al. 1995). To our knowledge, there are no literature data on the responses of the apple tree which had been exposed to massive industrial contamination by heavy metals, except the several studies about the content of heavy metals in the apple fruits (Bednarek et al. 2007; Park and Cho 2011; Aoyama and Tanaka 2013). However, regarding the fact that this tree species can be found at many locations in the Bor region, which is known as one of the most polluted areas in Serbia, we wanted to use this extraordinary possibility to explore whether the data on the content of some heavy metals in different parts of the apple trees can help in the assessment of the environmental quality of polluted areas. Additionally, the obtained results will give important information on the potential of the apple tree to accumulate and tolerate investigated heavy metals in its tissues (especially the root tissue) in the circumstances of a severe pollution.

MATERIALS AND METHODS

Description of the sampling area

Bor is a municipality in eastern Serbia and one of the largest copper mines in Europe. Two copper mines with surface mining (deposits “Veliki Krivelj” and “Cerovo”), an underground mining (mine “Jama”), and plants for mineral processing operate within The Mining and Smelting Complex Bor (RTB in Serbian). Wind direction influences the distribution of pollutants from the industrial facilities to the town of Bor and its surrounding areas.

The main factors which influenced the selection of the representing sites were the presence of the apple trees, the position of the industrial plants, the volume and the character of the emission, the type of settlement, and the meteorological and topographic parameters. The four sampling sites are an abandoned flotation tailings pond—Jalovište (FJ-wind direction W); a part of the town near the city hospital—Bolničko naselje (BN-wind direction E-ESE); and the two suburbs Slatinsko naselje (SN-wind direction WNW-NW) and Naselje Sunce (NS-wind

direction ENE-E), belong to the urban-industrial zone (UI) and are situated 0.7, 2.2, 2.3, and 2.5 km away from the copper smelter, respectively. The rural zone (R) includes three rural settlements Oštrej (O-wind direction W-WNW), Slatina (S-wind direction WNW-NW), and Dubašnica (D-wind direction E-ENE) which are located 4, 7, and 17 km away from the copper smelter as a main source of pollution, respectively. The control zone (C) is in an unpolluted area of rural settlement Gornjane (G-wind direction S) 19 km away from the copper smelter. This area is naturally protected from any pollution by mountain Veliki Krš (Fig. 1).

Sample collection and preparation

The analyzed samples were taken from selected sites during September/October 2012, when the maximum of enrichment was expected. Plant material was taken from three to five adult apple trees which were of about the same age. These sub-samples were bulked to obtain a representative sample for each investigated site. Leaves and fruits were taken from different quarters of canopy. Root samples were washed with tap water followed by deionized water whereas the above ground plant parts remained unwashed (for the sake of evaluating the real shape of atmospheric pollution). The samples of soil were taken from the topsoil, from which root samples were taken, too. All the samples were air-dried to a constant weight in a well-ventilated room protected from any additional contamination. Plant samples were homogenized in a laboratory mill. Soil samples were griddled through 2 mm stainless steel sieve. For complete mineralization of the samples, a representative 1 g of each sample is treated with repeated additions of the nitric acid (20 mL 65 % HNO₃, Merck, Darmstadt) and hydrogen peroxide (4 mL 10 % H₂O₂, Merck, Darmstadt), according to a microwave-assisted strong acid digestion method recommended by US Environmental Protection Agency (known as USEPA method 3052) (Toselli et al. 2009). The digestion was performed in a microwave digestion system ETHOS 1 (Milestone, Bergamo, Italy) with the temperature program: ramping time 10 min up to 180 °C, and holding time 15 min at 180 °C (constant). The obtained solutions were filtered, diluted to a volume of 50 mL with deionized water, and stored in polyethylene bottles at 4 °C.

Instrumentation

The iCAP 6000 inductively coupled plasma optical emission spectrometer (Thermo Scientific, Cambridge, UK) was used for analysing the metals content. The multi-element standard solution of about 20.00 ± 0.10 mg L⁻¹ (Ultra scientific, USA) was used as a stock solution for

Fig. 1 Map of the study area showing measuring sites in Bor and its surroundings with wind rose diagram (%) for 2012

calibration. All metals contents were calculated on a dry weight basis (mg kg^{-1} DW).

The pH and EC (electrical conductivity) values of the soil samples (solid:distilled water = 1:2.5) were measured using a pH meter (3510 Jenway, UK) and an EC meter (4510 Jenway, UK), respectively. The organic matter (OM) was determined by LOI (loss-on-ignition) method at 550°C (Jolivet et al. 1998).

Statistical analysis

ICP-OES measurements were carried out in triplicate and presented as mean $\pm$ standard deviation (SD). In order to investigate the relations between investigated parameters, the two-way ANOVA with post hoc Tukey's multiple comparison test, Pearson's correlation study, and hierarchical cluster analysis were used (Al-Khashman and Shawabkeh 2006; Simon et al. 2011, 2014; Alagic et al.

2015). All of statistical analyses were performed using a statistical package IBM SPSS 20, USA (Miller and Miller 2005).

RESULTS

The results of soil ICP-OES analysis are presented in Table 1 together with maximum allowed concentrations (MACs) for heavy metals determined by Serbian Regulation about allowable quantities of hazardous and harmful substances in soil (The Off. Gazette of RS No. 23/94). The common abundance of investigated heavy metals as well as their phytotoxic levels in various topsoils, given by different authors are also included in Table 1. The results obtained in the present work were marked on the basis of Tukey's post hoc test. Additionally, Table 1 also contains the results of measurements of soil parameters that may

Table 1 The contents of heavy metals (mg kg⁻¹ DW) in soil samples, pH, EC, and OM

Sampling site	Cu	Zn	Pb	As	Cd	Ni	pH	EC ($\mu\text{S cm}^{-1}$)	OM (%)
FJ	1929 $\pm$ 9 ^g	234.1 $\pm$ 0.7 ^e	125.6 $\pm$ 0.2 ^e	45.42 $\pm$ 0.09 ^f	5.22 $\pm$ 0.01 ^d	13.08 $\pm$ 0.03 ^a	7.37	321.0	7.6
BN	2162 $\pm$ 16 ^h	285 $\pm$ 2 ^g	159 $\pm$ 1 ^f	67.8 $\pm$ 0.5 ^h	7.46 $\pm$ 0.04 ^f	31.7 $\pm$ 0.1 ^f	7.00	139.2	8.6
SN	1046 $\pm$ 22 ^e	240 $\pm$ 2 ^f	85 $\pm$ 1 ^d	29.86 $\pm$ 0.04 ^e	3.74 $\pm$ 0.02 ^c	23.8 $\pm$ 0.2 ^d	7.13	199.5	10.4
NS	1604 $\pm$ 10 ^f	235.30 $\pm$ 0.05 ^e	162.6 $\pm$ 0.5 ^g	48.4 $\pm$ 0.7 ^g	6.925 $\pm$ 0.005 ^e	45.8 $\pm$ 0.2 ^g	5.22	78.3	18.0
O	814 $\pm$ 5 ^d	132 $\pm$ 2 ^c	67.9 $\pm$ 0.4 ^c	23.5 $\pm$ 0.3 ^d	3.11 $\pm$ 0.02 ^b	22.6 $\pm$ 0.2 ^c	6.81	148.9	11.8
S	401 $\pm$ 2 ^c	98 $\pm$ 2 ^b	49.5 $\pm$ 0.9 ^b	19.5 $\pm$ 0.2 ^c	3.16 $\pm$ 0.04 ^b	31.8 $\pm$ 0.4 ^f	6.69	200.0	13.8
D	267 $\pm$ 2 ^b	140.2 $\pm$ 0.3 ^d	49.3 $\pm$ 0.2 ^b	11.3 $\pm$ 0.2 ^b	3.11 $\pm$ 0.01 ^b	27.71 $\pm$ 0.08 ^e	6.99	153.6	11.6
G	21.7 $\pm$ 0.3 ^a	52.2 $\pm$ 0.8 ^a	21.5 $\pm$ 0.4 ^a	2.88 $\pm$ 0.05 ^a	1.16 $\pm$ 0.02 ^a	15.66 $\pm$ 0.06 ^b	6.76	83.2	10.0
MAC ^D	100	300	100	25	3	50			
Common abundance in topsoils	2–50 ^E 13–24 ^F	10–100 ^E 45–100 ^F	22–44 ^F	0.1–55 ^E 0–9.3 ^F	0.1–1 ^E 0.37–0.78 ^F	20–50 ^E 12.0–34 ^F			
Phytotoxic levels of metals in soils ^E	36–698 ^A	100–1000		40 ^B 200 ^C		100			

Data are presented as the mean $\pm$ standard deviation (SD) for triplicate determinations; mean values in the same column with different letters indicate significant differences ($p < 0.05$)—*a* is always lower than *b* and *b* is always lower than *c*, etc.

^A Dicotyledon plants

^B Sand and loam soils

^C Clay soils

^D The Off. Gazette of RS No. 23/94

^E Alloway (2013)

^F Kabata-Pendias and Pendias (2001)

affect the metal solubility and availability to plant roots such as soil pH, OM, and EC.

The content of investigated metals in plant parts (root, branches, leaves, and fruit) is represented in Table 2, simultaneously with their normal and phytotoxic concentrations in different plant tissues given by different authors. However, due to the fact that the above ground parts were processed as unwashed, some considerations in this work related to these parts should be taken with caution, because the contents in them do not represent a true bioaccumulation. Namely, the contribution of a simple atmospheric deposition at the aerial surface to the detected metals concentrations may be significant in polluted areas. The results of Tukey's post hoc test that had been performed after the two-way ANOVA analysis are also displayed in Table 2.

Previously, the two-way ANOVA applied to the apple upper organs, showed that the two factors: "Location" (FJ, BN, SN, NS, S, O, D, and G) and "Part of the plant" (Branches, Leaves, and Fruit) have different impacts on elements' concentrations. In the case of Cu and Pb concentrations, "Location" has the highest impact, whereas in the case of Zn, As, and Ni, "Part of the plant" has the highest impact. In the case of Cd, none of the variables has the dominant impact. It is noticeable that very often leaves were the "Part of the plant" which had positive impact on concentrations of all elements, except in the case of Pb.

In order to establish the possible correlations, the Pearson's correlation study was applied between the content of each metal in all analyzed samples and distance from the copper smelter as the main source of pollution; between the content of each metal in plant parts and related contents in soil samples; between metal contents in individual organs and between metal contents in roots and soil parameters (Table 3). The Pearson's correlations between different metals in the soil and, again, between different metals in the same plant part are given in the Supplementary Material (Table S1).

Several biological factors were used for the estimation of plant/risk metal interactions: Bioconcentration factor (BCF) expressed as the ratio of metal concentration in washed plant root to that in soil (the values of BCF > 1 point to a good accumulation of a particular metal in roots); Mobility ratio (MR) as the ratio of heavy metal in above ground plant part to that in soil; and Translocation factor (TF) was calculated as a ratio of heavy metal in the above ground plant part to that in plant root (Mingorance et al. 2007; Alagić et al. 2013). BCF is calculated for all locations (Table 4), whereas MR and TF factors were calculated only for uncontaminated samples from the control site (G) (Supplementary Material Table S2).

Enrichment factor (EF) expressed as the ratio of metal concentration in plant parts from the polluted and the

Table 2 The contents of heavy metals (mg kg⁻¹ DW) in plant parts

Sampling site	Plant part	Cu	Zn	Pb	As	Cd	Ni
FJ	Root	459 ± 7 ^f	65.4 ± 0.6 ^e	25.5 ± 0.4 ^g	8.48 ± 0.05 ^e	1.400 ± 0.005 ^g	7.2 ± 0.7 ^d
	Branches	291 ± 2 ^g	71 ± 2 ^e	53.9 ± 0.4 ^g	5.90 ± 0.06 ^f	1.06 ± 0.01 ^g	1.58 ± 0.07 ^b
	Leaves	419 ± 3 ^h	125.3 ± 0.4 ^h	47.1 ± 0.3 ^h	18.7 ± 0.2 ^h	1.91 ± 0.01 ^g	9.38 ± 0.06 ^f
	Fruit	19.0 ± 0.2 ^e	12.00 ± 0.02 ^f	1.5 ± 0.2 ^d	2.40 ± 0.07 ^h	0.2250 ± 0.0000 ^e	2.84 ± 0.02 ^e
BN	Root	380 ± 3 ^e	52.6 ± 0.5 ^d	33.3 ± 0.6 ^h	11.70 ± 0.05 ^g	1.44 ± 0.01 ^h	5.2 ± 0.2 ^b
	Branches	226 ± 4 ^f	70.8 ± 0.3 ^e	68.6 ± 0.6 ^h	6.48 ± 0.06 ^g	1.120 ± 0.005 ^h	2.35 ± 0.06 ^d
	Leaves	267.2 ± 0.4 ^g	96.4 ± 0.4 ^g	38.6 ± 0.4 ^g	17.8 ± 0.2 ^g	1.990 ± 0.005 ^h	4.22 ± 0.09 ^c
	Fruit	34.0 ± 0.2 ^g	9.68 ± 0.04 ^e	2.44 ± 0.06 ^e	1.90 ± 0.04 ^{fg}	0.195 ± 0.005 ^d	3.69 ± 0.06 ^f
SN	Root	374 ± 3 ^e	101.2 ± 0.3 ^f	22.3 ± 0.2 ^f	9.80 ± 0.09 ^f	0.965 ± 0.005 ^e	6.6 ± 0.2 ^{cd}
	Branches	118.2 ± 0.5 ^e	49.6 ± 0.1 ^d	9.7 ± 0.2 ^d	3.13 ± 0.05 ^d	0.450 ± 0.005 ^d	1.88 ± 0.04 ^c
	Leaves	203 ± 2 ^f	52.4 ± 0.2 ^e	15.5 ± 0.2 ^d	8.62 ± 0.03 ^e	0.825 ± 0.005 ^d	4.13 ± 0.05 ^c
	Fruit	24.1 ± 0.1 ^f	6.230 ± 0.005 ^d	1.38 ± 0.05 ^{cd}	1.64 ± 0.03 ^{bc}	0.160 ± 0.005 ^b	1.55 ± 0.01 ^d
NS	Root	47.1 ± 0.2 ^b	20.24 ± 0.02 ^a	4.80 ± 0.02 ^a	2.06 ± 0.04 ^a	0.333 ± 0.005 ^a	2.02 ± 0.07 ^a
	Branches	65.7 ± 0.5 ^c	29.8 ± 0.2 ^b	7.0 ± 0.2 ^c	2.04 ± 0.08 ^b	0.3700 ± 0.0000 ^c	1.36 ± 0.07 ^a
	Leaves	66.2 ± 0.8 ^c	38.6 ± 0.2 ^c	12.3 ± 0.2 ^c	4.86 ± 0.08 ^c	0.66 ± 0.01 ^c	2.98 ± 0.03 ^a
	Fruit	7.02 ± 0.04 ^b	3.050 ± 0.005 ^b	0.955 ± 0.002 ^{ab}	1.46 ± 0.09 ^a	0.135 ± 0.005 ^a	0.76 ± 0.02 ^b
O	Root	117.7 ± 0.8 ^d	52 ± 2 ^d	11.27 ± 0.05 ^b	2.90 ± 0.04 ^c	0.710 ± 0.005 ^b	5.29 ± 0.02 ^b
	Branches	69.2 ± 0.6 ^c	30.28 ± 0.08 ^b	4.8 ± 0.2 ^b	2.18 ± 0.03 ^b	0.340 ± 0.005 ^b	1.18 ± 0.03 ^a
	Leaves	62.40 ± 0.05 ^b	25.6 ± 0.2 ^b	9.4 ± 0.1 ^b	4.28 ± 0.04 ^b	0.52 ± 0.02 ^b	3.54 ± 0.09 ^b
	Fruit	9.06 ± 0.06 ^c	4.62 ± 0.04 ^c	1.11 ± 0.08 ^{bc}	1.73 ± 0.04 ^{bd}	0.170 ± 0.005 ^{bc}	0.67 ± 0.05 ^{ab}
S	Root	96.1 ± 0.1 ^c	36.50 ± 0.06 ^c	13.7 ± 0.2 ^d	4.77 ± 0.07 ^d	0.8250 ± 0.0000 ^d	8.30 ± 0.08 ^e
	Branches	86.7 ± 0.7 ^d	31.9 ± 0.3 ^c	12.3 ± 0.2 ^e	3.74 ± 0.09 ^e	0.70 ± 0.01 ^e	2.0 ± 0.2 ^c
	Leaves	192.4 ± 0.4 ^e	78.2 ± 0.6 ^f	32.8 ± 0.3 ^f	14.9 ± 0.2 ^f	1.66 ± 0.01 ^f	4.54 ± 0.05 ^d
	Fruit	6.7 ± 0.2 ^b	3.36 ± 0.01 ^b	0.84 ± 0.07 ^a	1.56 ± 0.06 ^{ac}	0.165 ± 0.005 ^{bc}	0.64 ± 0.03 ^a
D	Root	90.8 ± 0.6 ^c	146.4 ± 0.3 ^g	12.1 ± 0.1 ^c	8.48 ± 0.04 ^e	1.34 ± 0.01 ^f	14.60 ± 0.02 ^f
	Branches	47.8 ± 0.3 ^b	120.3 ± 0.4 ^f	17.30 ± 0.02 ^f	2.81 ± 0.08 ^c	0.725 ± 0.005 ^f	1.96 ± 0.04 ^c
	Leaves	119.7 ± 0.6 ^d	55.2 ± 0.4 ^d	23.5 ± 0.5 ^e	8.0 ± 0.2 ^d	1.200 ± 0.000 ^e	5.07 ± 0.02 ^e
	Fruit	11.86 ± 0.05 ^d	4.7 ± 0.2 ^c	1.22 ± 0.06 ^c	1.76 ± 0.02 ^{beg}	0.165 ± 0.005 ^{bc}	1.02 ± 0.04 ^c
G	Root	18.5 ± 0.4 ^a	33.3 ± 0.4 ^b	15.20 ± 0.07 ^e	2.62 ± 0.06 ^b	0.7400 ± 0.0000 ^c	5.96 ± 0.04 ^{bc}
	Branches	11.2 ± 0.4 ^a	16.00 ± 0.01 ^a	2.4 ± 0.2 ^a	1.42 ± 0.05 ^a	0.225 ± 0.005 ^a	1.92 ± 0.07 ^c
	Leaves	14.31 ± 0.07 ^a	13.2 ± 0.3 ^a	1.9 ± 0.2 ^a	2.06 ± 0.03 ^a	0.230 ± 0.005 ^a	3.56 ± 0.02 ^b
	Fruit	5.12 ± 0.01 ^a	2.0 ± 0.4 ^a	0.89 ± 0.05 ^a	1.89 ± 0.07 ^{def}	0.1750 ± 0.000 ^c	0.75 ± 0.05 ^b
Normal range in plant tissues		4–15 ^{B,C}	60 ^C	0.1–10 ^D	0.009–1.5 ^{C,D}	0.1–2.4 ^E	0.05–10 ^C
		1–X0 ^D	8–100 ^E	1–13 ^E			1 ^E
Phytotoxic concentration in plant tissues		15–20 ^{A,B,D}	100–500 ^C	10–20 ^B	>~20 ^B	5–10 ^{A,B,D}	20–30 ^B
		5–40 for leaves and 100–400 for root ^C	150–200 ^{A,B,D}		1–20 ^D		>10 ^{A,C}
							10–100 ^D

Data are presented as the mean ± standard deviation (SD) for triplicate determinations; mean values for the same plant part with different letters indicate significant differences ($p < 0.05$)— a is always lower than b and b is always lower than c , etc.

^A In sensitive plant species

^B Vamerali et al. (2010)

^C Alloway (2013)

^D Kabata-Pendias and Pendias (2001)

^E Nagajyoti et al. (2010)

control site, respectively, was used to assess the degree of anthropogenic influence (Table 4). As the EF values increase, the contribution of the anthropogenic influence

also increases. EFs have been evaluated usually by using the local background values. Values of $EF > 2$, point to the enriched samples (Mingorance et al. 2007).

Table 3 The results of Pearson's correlations studies

Metal	Sample	pH	OM	EC	Distance	Soil	Root	Branches	Leaves
Cu	Soil				−0.822**	1			
	Root	0.499*	−0.608**	0.688**	−0.643**	0.715**	1		
	Branches				−0.673**	0.822**	0.907**	1	
	Leaves				−0.571**	0.649**	0.886**	0.947**	1
	Fruit				−0.499*	0.691**	0.854**	0.726**	0.646
Zn	Soil				−0.768**	1			
	Root	−0.651**	0.523**	−0.138	0.268	0.089	1		
	Branches				0.128	0.318	0.836**	1	
	Leaves				−0.511*	0.558**	0.117	0.467*	1
	Fruit				−0.571**	0.689**	0.203	0.465*	0.858**
Pb	Soil				−0.759**	1			
	Root	0.376	0.601**	0.358	−0.338	0.321	1		
	Branches				−0.423*	0.605**	0.837**	1	
	Leaves				−0.447*	0.432*	0.629**	0.839**	1
	Fruit				−0.410*	0.588**	0.859**	0.876**	0.568**
As	Soil				−0.792**	1			
	Root	−0.028	−0.156	0.240	−0.264	0.459*	1		
	Branches				−0.535**	0.721**	0.767**	1	
	Leaves				−0.501*	0.596**	0.691**	0.956**	1
	Fruit				−0.078	0.188	0.388	0.581**	0.498*
Cd	Soil				−0.713**	1			
	Root	−0.191	−0.085	0.224	−0.016	0.158	1		
	Branches				−0.364	0.555**	0.836**	1	
	Leaves				−0.412*	0.523**	0.729**	0.965**	1
	Fruit				−0.168	0.072	0.739**	0.673**	0.569**
Ni	Soil				−0.234	1			
	Root	−0.814**	0.697**	−0.195	0.552**	−0.276	1		
	Branches				0.232	0.084	0.301	1	
	Leaves				−0.231	−0.553**	0.321	0.136	1
	Fruit				−0.470**	−0.173	−0.085	0.400	0.505*

* Correlation is significant at the 0.05 level (2-tailed)

** Correlation is significant at the 0.01 level (2-tailed)

Finally, for a full emphasizing of possibility for using apple parts for biomonitoring purposes, a hierarchical cluster analysis was performed. Three different hierarchical dendrograms were obtained for identification the existing similarities among contaminated locations (UI and R zones) using Ward linkage method and squared Euclidean distance as measure interval (Figs. 2, 3, 4).

DISCUSSION

Soil material

The soil samples (Table 1) from all investigated locations from urban-industrial zones had high amounts of determined heavy metals. These amounts were above related

MAC (The Off. Gazette of RS No. 23/94) except in the case of Zn and Ni whose concentrations were under the MAC at all investigated sites. Only the control location G had the amount of all elements under the MAC and these amounts are at the level of common abundance in topsoils. The concentration of Cu reached to 2162 mg kg^{-1} and the greatest variation in the content of this element was present in the investigated areas. The concentrations of Cu in soils from the urban areas are several times greater than the upper limit of the phytotoxicity in soil. In the case of Zn, all detected concentrations were under the MAC and only G and S locations have a level of this metal below the phytotoxic level. Of course, the fact that the Zn content in the areas which are positioned close to the main sources of pollution is slightly below the MAC should not be ignored. Although elevated (above the MAC), the contents of As in

Table 4 Bioconcentration factor (BCF) and element enrichment factor (EF)

Sampling site	Plant part	Cu		Zn		Pb		As		Cd		Ni	
		BCF	EF	BCF	EF	BCF	EF	BCF	EF	BCF	EF	BCF	EF
FJ	Root	0.24	24.81	0.28	1.96	0.20	1.68	0.19	3.24	0.27	1.89	0.55	1.21
	Branches		25.98		4.30		22.46		4.15		4.71		0.82
	Leaves		29.28		9.49		24.79		9.08		8.30		2.63
	Fruit		3.77		6.00		1.67		1.27		1.29		3.79
BN	Root	0.18	20.54	0.18	1.58	0.21	2.19	0.17	4.47	0.19	1.95	0.16	0.87
	Branches		20.18		4.29		28.58		4.56		4.98		1.22
	Leaves		18.67		7.30		20.32		8.64		8.65		1.19
	Fruit		6.64		4.84		2.71		1.01		1.11		4.92
SN	Root	0.36	20.22	0.42	3.04	0.26	1.47	0.33	3.74	0.26	1.30	0.28	1.11
	Branches		10.55		3.01		4.04		2.20		2.00		0.98
	Leaves		14.19		3.97		8.16		4.18		3.59		1.16
	Fruit		4.71		3.12		1.53		0.87		0.91		2.07
NS	Root	0.03	2.54	0.09	0.61	0.03	0.32	0.04	0.79	0.05	0.45	0.04	0.34
	Branches		5.87		1.81		2.92		1.44		1.64		0.71
	Leaves		4.63		2.92		6.47		2.36		2.87		0.84
	Fruit		1.37		1.52		1.11		0.77		0.77		1.01
O	Root	0.14	6.36	0.39	1.56	0.17	0.74	0.12	1.11	0.23	0.96	0.23	0.89
	Branches		6.18		1.84		2.00		1.54		1.51		0.61
	Leaves		4.36		1.94		4.95		2.08		2.26		0.99
	Fruit		1.77		2.31		1.23		0.92		0.97		0.89
S	Root	0.24	5.19	0.37	1.10	0.28	0.90	0.24	1.82	0.26	1.11	0.26	1.39
	Branches		7.74		1.93		5.12		2.63		3.11		1.04
	Leaves		13.45		5.92		17.26		7.23		7.22		1.28
	Fruit		1.31		1.68		0.89		0.82		0.94		0.85
D	Root	0.34	4.91	1.04	4.4	0.25	0.80	0.75	3.24	0.43	1.81	0.53	2.45
	Branches		4.27		7.29		7.21		1.98		3.22		1.02
	Leaves		8.36		4.18		12.37		3.88		5.22		1.42
	Fruit		2.32		2.35		1.36		0.93		0.94		1.36
G		0.85		0.64		0.71		0.91		0.64		0.38	

BCF C_{root}/C_{soil} , EF $C_{polluted}/C_{control}$

urban areas are below the phytotoxic levels. Ni is the only metal whose concentrations are at the level of common abundance in topsoils at all investigated sites and also below the phytotoxic level. All measured Cd concentrations were above the MAC (except at the control site G) and over the common abundance in topsoils. The content of Pb was present in concentrations above the MAC at the sites near the source of pollution. The location BN which is more distant from the source of pollution in comparison with location FJ was the place with the highest amount of Cu, Cd, As, Zn, while the location NS had the highest amount of Pb and Ni. This situation may be a consequence of influence of several factors such as direction of the winds, additional impact of traffic, geological origin of some elements (Alloway 2013).

The results of Tukey's post hoc test show statistically significant differences between the metal content at all investigated locations for Cu and As, and in most cases for Zn, Pb, and Ni. In the case of Cd, there is the lowest number of statistically significant differences between locations; even three rural locations O, S, and D do not significantly differ in its content. The results of the Pearson's correlation study for relations between the metal contents in topsoils and distance from the main source of pollution (Table 3) show very significant negative correlations for all metals except in the case of Ni. The decreasing of metal concentrations with distance increasing, points that the presence of metals in soils (except Ni) is most likely of the atmospheric i.e., anthropogenic origin (Kozlov et al. 1995; Alagic et al. 2013).

Fig. 2 Hierarchical dendrogram for polluted sites based on metals' contents in soils

Fig. 4 Hierarchical dendrogram for polluted sites based on metals' contents in apple upper organs

Fig. 3 Hierarchical dendrogram for polluted sites based on metals' contents in apple roots

Additionally, there is an extremely high positive correlation between the contents of the elements in the soils (Table S1) except in the case of Ni; its content is weakly correlated with other metals which also indicate a different (i.e., geogenic) origin of Ni in the soil (Al-Khashman and Shawabkeh 2006).

Plant material

In the case of plant parts, the most abundant metal, at most locations is Cu (Table 2). Its content increases in the following order: fruit < branches < leaves < root with some exceptions at locations such as NS, S, and D, where the

highest Cu content was found in unwashed leaves. The contents of Cu in roots from sites FJ, BN, SN, and O and in branches from sites FJ, BN, and SN are above phytotoxic concentrations as well as the contents in leaves from all investigated sites. Zn is the next abundant element in plant parts and its level was within normal range in plant tissues except some slightly increased contents in a few plants' parts from the sites FJ and D which may be considered as phytotoxic. The Pb content at urban locations nearest to the source of pollution is above the phytotoxic values for all plant parts except the fruit. In rural zone, these values were exceeded only in leaves from the sites S and D and in root from the control zone G. This may be due to the other sources of Pb pollution such as traffic (Duong and Lee 2011; Simon et al. 2014). In other words, it can be said that the mining and metallurgical processes are not the only source of Pb pollution in investigated areas. This is confirmed by the fact that there is no order of Pb content in plant parts at investigated locations. The Pb content in apple fruits is below the maximum allowable value in the dry fruit (3 mg kg^{-1}) provided by Serbian regulation (The Off. Gazette of RS No. 5/92, 11/92, 32/2002, 25/2010, and 28/2011). The contents of As at urban sites FJ, BN, and SN are up to ten times higher than normal values in plant tissues. All of the detected amounts may be considered as phytotoxic according to Kabata-Pendias and Pendias (2001). A worrying fact is that the contents of As in the unwashed dry fruits are above the MAC defined by national regulation ($1 \text{ mg kg}^{-1} \text{ DW}$) (The Off. Gazette of RS No. 5/92, 11/92, 32/2002, 25/2010, and 28/2011). At most locations, the order of the contents of this contaminant is as follows: fruit < branches < root < leaves. The greatest amounts of Cd were found for leaves at locations

FJ and BN while the lowest in fruits. Concentrations in fruits are under the MAC ($0.3 \text{ mg kg}^{-1} \text{ DW}$) (The Off. Gazette of RS No. 5/92, 11/92, 32/2002, 25/2010, and 28/2011). All measured Cd contents were within the normal range in plant tissues and below the phytotoxic concentrations. Some similar situation is observed in the case of Ni also. Only the maximal measured value of Ni content in the root from the rural site D is above the normal range and also phytotoxic for this plant tissue (Alloway 2013).

The results of Tukey's post hoc test showed statistically significant differences between the contents of metals in leaves from all locations, except in the case of Ni. The contents of Pb and Cd in roots and branches are also statistically significantly different at all tested locations. It is evident that the fruit is the plant part which shows the least number of statistically significant differences between all metal contents. Also, Ni was the metal with the smallest differences between the contents in all analyzed plant parts. The results obtained from ICP-OES and the two-way ANOVA analysis followed by the Tukey's post hoc test pointed that the apple leaves have an excellent capability to display the status of metals in the environment as well as that they can be also very useful for identification of differences in metals' levels. Some studies confirm this standpoint (Simon et al. 2011, 2014). Obviously, the lowest capability in this sense has the fruit of the apple tree which makes it less useful for biomonitoring purposes. On the other hand, the obtained results also point that this tree has pretty successful mechanisms to protect its fruit from overdoses of toxic metals; only the contents of As were at the elevated levels.

The results of Pearson's correlation analysis between metals contents in plant parts and the distance from the copper smelter (Table 3) showed that there is a significant negative correlation for Cu in all the cases; for Zn in the cases of leaves and fruits; for As and Pb in the cases of leaves and branches, while Cd and Ni show generally weak correlations in all investigations. This points that the content of elements in plant parts is not always influenced by the pollution which comes from the atmosphere (Alagic et al. 2013). The correlations between the content of investigated elements in the soil and plant parts (Table 3) have positive values for Cu, Zn, Pb, As, and Cd, very often at the significant level, except for Pb in root, As in fruit, Zn in root and branches, and Cd in root and fruit (Alagic et al. 2013). Again, Ni shows irregular behavior. High positive correlation between the contents of Cu in soil and root is of particular importance, because it indicates that the uptake of this essential metal by roots of apple tree is dose dependent. This observation is valid because the detected root concentrations can be treated as a real bioaccumulation, due to the fact that root samples were analyzed as washed parts. On the other hand, the consideration of

correlations related to the above ground parts should not ignore the reality that the contents in these unwashed parts do not reflect a factual bioaccumulation. The relations between metals' contents in different plant parts (Table 3) show high positive correlation for Cu, Pb, As, and Cd in nearly all investigated parts; slightly lower values for Zn; and generally weak correlations in the case of Ni. The Pearson's correlation analysis for the relations between the metals' contents in root and soil parameters (Table 3) suggests that soil pH, OM, and EC affected the assimilation of metals by plant roots differently. For example, the uptake of Cu decreased significantly with increasing OM, and increased with pH and EC raise. With increasing of soil pH, the Zn content decreases in the root but with increasing of soil OM, the Zn content also increases. Due to high positive correlation between the OM and root Pb content it can be said that soil OM has dominant influence whereas the influence of the EC and pH values was not such significant. Root Ni content is in a very high negative correlation with soil pH but in a good positive correlation with the soil OM. Unlike the previously mentioned elements, the contents of As and Cd in roots show weak correlations with all measured soil parameters. The obtained correlations between the contents of elements in the same plant parts (Supplementary Material Table S1) show the existence of high positive correlations in all cases except for Ni in branches and sometimes in the case of root (for Zn and Cd). Also, the obtained results show the low correlations between pairs such as Cu–Zn and Zn–Pb in root and Cu–Zn in branches. This may indicate that there were some competition processes between the mentioned elements in the root of the apple tree (Kabata-Pendias and Pendias 2001; Bhargava et al. 2012).

Data analysis

The greatest MR and TF values (Table S2) are found for Cu in the case of branches and leaves (MR 0.52 and 0.66; TF 0.61 and 0.77, respectively) and for As in the case of branches, leaves, and fruits (MR 0.49, 0.72 and 0.66; TF 0.54, 0.79 and 0.72, respectively) but there was no any value close to 1, or higher which may suggest that the apple tree is not so successful in heavy metal transfer from soil or root to above ground parts.

The uptake of metals as well as their accumulation rates in plant roots varied among investigated locations (Table 4). BCF values for Cu and As at the unpolluted site G, suggest that the root of the apple tree has a pretty good potential to adopt these two elements from the soil. However, these values are much lower for highly contaminated sites which mean that this plant species may activate some of the defense strategies in the hostile environment, acting as a plant excluder (Bhargava et al. 2012; Lin and Aarts

2012). BCF values for all elements are the lowest in the urban location NS although high level of pollution at this site is present. Also, this site has the most acidic soil, the highest content of organic matter and the lowest value of conductivity, so that it was expected that some of these factors should help in the uptake of heavy metals by root (Bhargava et al. 2012). However, the extremely low BCF values for this site point to an extremely low rate of assimilation which further allows a possibility of speculation that some of specific soil microorganisms have helped the plants in restriction of metals uptake (Vamerali et al. 2010). The next clue that this speculation is reasonable can be found in calculated element EFs for this location (Table 4). Namely, it is obvious that the values of EFs for the NS site are much lower than EF values for the sites FJ, BN, and SN from the same, UI zone, characterized by enormous pollution.

The EF values for Cu are the greatest among all elements and much greater than 2 (except for some fruits), which points to a high level of pollution in investigated plant organs from different locations of the Bor region. The highest EF values for Zn were observed mostly in leaves. One of the farthest sites, site D, has unexpectedly large EF values for Zn which may be a consequence of the influence of winds, that spread waste gases from industrial facilities of RTB Bor. The highest EF values for Pb are calculated for branches and leaves from urban sites FJ and BN. Some great values for Pb are calculated for the leaves from the sampling sites S and D too, which can be ascribed to the additional traffic pollution. EF values for As were often high showing the order at all tested locations as follows: fruit < root < branches < leaves. EF for As in leaves is greater than 2 at all locations. Based on calculated EF values for Cd, it can be concluded that, the greatest deposition of Cd was on the leaves especially in urban areas FJ and BN. The fact should not be ignored that the considerable EF values for leaves are calculated in the case of rural sites. Ni shows a specific behavior and only in a few cases, the values of EF are greater than 2. Overall, the values of these coefficients indicate that no serious contamination of this metal is present. Some similar findings were documented also during the analysis of Ni content in roots and leaves of lime and birch trees from the same region of the Bor's municipality (Alagić et al. 2014).

Depending on selected material, the hierarchical dendrograms obtained from hierarchical cluster analysis showed a different grouping of locations (Figs. 2, 3, 4). Hierarchical dendrogram for polluted sites based on metals' contents in soil material (Fig. 2) shows the grouping of locations into two primary clusters. The first cluster is formed of sites (sub-clusters) which are situated in the UI zone (FJ, BN, and NS) while the second one is formed of sites (sub-clusters) from the R zone (S, O, and D) and the

site SN from the UI zone. The presence of high buildings, which surround and protect the sampling site SN, may be the reason of the reduced metals contents in comparison with other sites from the UI zone which consequently classifies this location in the same group with the three rural settlements. The difference between these two primary clusters, in terms of measure interval, is significant, which suggests that the level of soil pollution is significantly higher at the FJ, BN, and NS sites.

Dendrogram based on metals' contents in apple roots (Fig. 3) is an excellent illustration that the contents of metals in roots, although dependent on soil concentrations, cannot reflect an authentic profile of the present pollution in the soil, so cannot be used as a secure tool in monitoring soil pollution. Obviously, the assemblage of polluted locations in this dendrogram is different in comparison to the dendrogram based on metals' contents in soils: the first main cluster is constituted of the sites FJ, SN, and BN, while the second main cluster includes all rural sites and the site NS from the UI zone. This may be a consequence of numerous factors (soil pH, OM, EC) which have affected the uptake of metals by the apple root, as it can be seen from the results of Pearson's correlation study. The influence of some unknown soil microorganism(s) at the site NS cannot be excluded also (Alagić et al. 2015).

Some dissimilar cluster patterns are also obtained in the hierarchical dendrogram based on the study of upper parts (Fig. 4). Namely, branches, leaves, and fruits have separated the investigated locations into two main clusters: the first of them consists of the sites FJ and BN from the UI zone, whereas the sites NS and SN from this zone and all the sites from the R zone are joined together as the second main cluster with the following arrangement of sub-clusters S-SN and D/O-NS. The obtained arrangements may suggest a better suitability of upper parts to recording seasonal variations. Namely, it is well known that in highly polluted areas, a simple atmospheric deposition may represent a significant source of metals in upper plant organs, which is used very often and very conveniently in different biomonitoring studies dealing with both washed and unwashed parts. It is very likely that the dominant directions of winds during 2012 influenced significantly the obtained classification of polluted sites. (Unterbrunner et al. 2007; Alagić et al. 2013).

Obviously, the differences between obtained dendrograms are a consequence of influence of many factors which may affect the metals' contents in plant parts and soils as well. Data obtained from the soil analysis represent the time-integrated information, rather than the evidence of the current state of pollution, because metals can stay in soils for years. A current state of environmental pollution is better reflected in the upper parts of the apple tree, especially in leaves as it can be seen also from the calculated

EFs and from the results obtained during the Pearson's correlation study, the two-way ANOVA analysis, and Tukey's post hoc test. In general, it can be concluded that the combination of the results obtained from all methods that were applied in this work (ICP-OES, statistical analyses, and calculation of biological and EFs) illustrated soundly and very profoundly all the capabilities of the apple tree that could be useful for biomonitoring or phytoremediation procedures.

CONCLUSIONS

The results obtained from chemical and statistical analyses, as well as from calculated EFs confirm that all organs of the apple tree can be useful for a relevant biomonitoring. However, the safest data are kept in leaves which reflect the level as well as the character of atmospheric pollution in a great extent. The leaves of the apple tree successfully pointed that the content of metals such as Zn, Pb, As, Cd, and Cu in this apple organ is caused by massive airborne pollution which comes from the copper smelter, whereas geology contributes significantly to the Ni content. But it should be noticed that leaves have recorded even that slight Ni load which came from the atmosphere. Also, the results obtained from the Pearson's correlation analysis of aerial parts suggest that the enrichment by Pb, Cd, and As may come from some other sources than the copper smelter, such as traffic, combustion of fossil fuels, or some minor agricultural doings which are present, more or less, across the investigated area and which was not so obvious from the soil analysis. The data obtained from the root analysis were affected by many factors such as plant managing under concrete environmental circumstances, different influences of soil pH, OM and EC on metal uptake, as well as possible interaction with specific soil microbes at the site NS, so its interpretation is too complicated and it cannot be recommended as a simple and secure tool for monitoring purposes. Even so, the results obtained from the apple parts analysis confirm that the environmental quality of ecosystem of Bor and its surrounding area is at the very low level. The consummation of fruits produced in the circumstances of severe pollution can be considered risky only regarding As contents which were above the Serbian maximum allowed limit for dry fruits.

The most abundant element in all plant and soil samples was Cu, except some rare examples, where the content of Zn was the highest. The lowest metal contents were always found in the fruit sample, which means that the smooth fruit surface was not so susceptible for the retention of airborne particulate matter, but this also indicates that investigated plant species has some effective ways to protect its fruit from high concentrations of heavy metals which come also from the soil, via root. Simultaneously, it

is evident that this plant species can tolerate large concentrations of heavy metals (sometimes at the levels of phytotoxicity) in its other tissues, especially root tissue, despite the fact that plants had the low rates of uptake from polluted soils as it can be seen from the low BCF values for all polluted sites. This suggests that the investigated plant species has some very effective means for heavy metals detoxification and tolerance to the stress induced by heavy metals, including different tactics of limitation of metals' adoption from the soil, as well as the retention of some of assimilated metals in the root tissue. According to this, the apple tree can be recommended for phytostabilization purposes. Indeed, all the trees which grow at highly polluted soils (at the level of phytotoxicity for Cu) had only trivial visible symptoms of metals' toxicity: a couple of dead branches and a diminutive quantity of chlorotic leaves were found during sampling, which is a fact more that favors this plant species as a suitable candidate for both biomonitoring and bioremediation purposes.

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