Evaluation of *Salix alba, Juglans regia* and *Populus nigra* as biomonitors of PTEs in the riparian soils of the Sava River

Zorana Mataruga[1], Snežana Jarić[1], Milica Marković[1], Marija Pavlović[1], Dragana Pavlović[1], Ksenija Jakovljević[2], Miroslava Mitrović[1], Pavle Pavlović[1]

[1] Department of Ecology, Institute for Biological Research 'Siniša Stanković', University of Belgrade, Bulevar despota Stefana 142, Belgrade, Serbia

[2] Faculty of Biology, Institute of Botany and Botanical Garden 'Jevremovac', University of Belgrade, Takovska 43, 11000 Belgrade, Serbia

Corresponding author:

Zorana Mataruga,

E-mail: zorana.mataruga@ibiss.bg.ac.rs

ORCID: https://orcid.org/0000-0003-3353-4810

Abstract: A large number of human activities result in the release of potentially toxic elements (PTEs) into the environment, which could lead to the degradation of riparian areas. This study aimed to evaluate the potential of *Salix alba*, *Juglans regia* and *Populus nigra* for the biomonitoring of PTEs in the riparian soils of the River Sava. Levels of seven PTEs (As, Cd, Cr, Cu, Ni, Pb and Zn) were measured in the soils, roots and leaves of plants at selected sampling sites and evaluated according to bioaccumulation and translocation factors. The obtained results showed that in riparian soils As, Cr, Cu, Ni and Zn were at levels considered to be critical for plants. The levels of As, Cd, Cr, Ni and Zn measured in roots of *Salix alba* and As, Cr, Ni and Zn in its leaves were toxic for plant tissue. Toxic levels of Cr were also measured in the roots of *Juglans regia* and As in its leaves, as well as As and Cr in the roots of *Populus nigra*, and Zn in its leaves. Bioconcentration and translocation factors showed that *S. alba* and *P. nigra* have potential for the phytoextraction of Zn and Cd, while *J. regia* has potential for the phytoextraction of As. In terms of phytostabilisation potential, *S. alba* proved to be good for the phytostabilisation of Cd and Cu, and *J. regia* for the phytostabilisation of Cr, As, Ni and Pb, while *P. nigra* showed potential for the phytostabilisation of Cr, Ni, Pb and Cu.

Keywords: *Salix alba*, *Juglans regia*, *Populus nigra*, riparian zone, potentially toxic elements, spatial variations

Introduction

Riparian areas are transitional zones between terrestrial and aquatic ecosystems which provide specific conditions for diverse life forms (Oelbermann and Raimbault 2015). They are habitats for a large number of aquatic and terrestrial species and can affect how they disperse, meaning they are important for biodiversity conservation (Pennington et al. 2010; Sabater and Elosegi 2013). Besides the benefits that riparian areas offer for wildlife, they also provide a large number of ecosystem services for humans, such as food, transportation, recreation and tourism, as well as power production and waste disposal (Naiman and Decamps 1997; Pennington et al. 2010). However, the rapid development of industrialisation, urbanisation, and agricultural practices has led to the degradation of riparian areas and a loss of riparian vegetation, which in turn has caused a deterioration in freshwater ecosystems (Sunil et al. 2010).

The ecological importance of the River Sava and its riparian zone can be seen in the significant number of protected areas. The River Sava is the third longest tributary of the River Danube and it extends across four countries (Slovenia, Croatia, Bosnia and Herzegovina, and Serbia) with a catchment area of 97,713 km^2. Around 36% of the floodplain and 64% of the River Sava's water body are protected by law (Schwarz 2016). There are 167 protected areas in total, including 6 Ramsar Sites and 8 national parks, as well as numerous important bird and plant areas, areas protected at the national level and Natura 2000 sites (ISRBC, 2009). Many human activities result in the release of various pollutants in the riparian zone of large rivers; of these, particular attention is paid to potentially toxic elements (PTEs) and the impact they have on the environment. Numerous studies have reported that riparian habitats have a large capacity to accumulate PTEs (Punshon et al. 2003; Du Laing et al. 2009; Milačič et al. 2017; Pavlović et al. 2019), with As, Cd, Cr, Cu, Ni, Pb and Zn having a damaging effect on living organisms, including plants (Gjorgieva et al. 2011; Zhang et al. 2011; Pavlović et al. 2016). Mainly originating from industrial and municipal waste waters and pollutants from agricultural activities, pollution of the Sava River with PTEs exhibits a spatial trend: in the upper reaches mineral weathering predominates, in the middle reaches there is pollution arising from agricultural activities, while high levels of pollutants originate from industrial processing, including untreated municipal wastewater discharge, in the lower reaches (Ščančar et al. 2015).

In order to quantify the quality of the environment, in terms of pollution from PTEs, a large number of higher plants are used as biomonitors (Piczak et al. 2003; Madejon et al. 2013). Trees are not the best indicators of environmental quality, but they have a wide distribution and are long living, so they can be used in the biomonitoring of large areas (Sawidis et al. 2011). The other advantages of using trees as biomonitors are the simplicity of sampling and identification, the ample supply of biological material for analyses, and the high biomass for PTE accumulation (Berlizov et al. 2007; Sawidis et al. 2011). Native species are more resistant than those introduced from other regions, bearing in mind their higher levels of survival, growth and reproduction success (Shu et al. 2002; Yoon et al. 2006). Therefore, there is continual interest in finding native species that are tolerant to increased PTE levels.

Previous research has shown that *Salix* and *Populus* species generally possess a high tolerance to PTE pollution, which is why they are proposed for the biomonitoring, stabilisation and removal of PTEs from contaminated soils (Mertens et al. 2004; Laureysens et al. 2005; Wu et al. 2010; Gaudet et al. 2011; Bhargava et al. 2012; Chen et al. 2014; Pavlović et al. 2016). Up to now, *Salix alba* L. and *Populus nigra* L. have not been evaluated as biomonitors of PTEs in the Sava River Basin. Both species are fast growing, easily propagated and tolerant to diverse types of soils (Kuzovkina et al. 2004). In addition to the dominant species of the *Salix* and *Populus* genera, another abundant species on the alluvial, well-drained soils of the River Sava is *Juglans regia* L. (Jovanović 1970). In addition to growing spontaneously in nature, this species is often cultivated, too. However, a large number of studies focus on PTE levels in the shells or kernels of *J. regia* (Arpadjan et al. 2013; Tošić et al. 2014; Feizi and Jalali 2015), while there is lack of data to confirm whether this species is suitable as a biomonitor of PTEs.

In this respect, the main objectives of this research were to evaluate the potential of native, naturally growing species in the River Sava riparian zone - *Salix alba*, *Juglans regia* and *Populus nigra* - for the uptake, accumulation and translocation of PTEs. These included: 1) determining PTE content in riparian soils; 2) determining soil enrichment with PTEs; 3) determining PTE content in the roots and leaves of *S. alba*, *J. regia* and *P. nigra*; 4) evaluating PTE uptake, accumulation and translocation through bioaccumulation and translocation factors; 5) highlighting variations between and discovering patterns of PTE accumulation in the analysed species; and 6) investigating the potential use of species as biomonitors of PTEs.

Materials and methods

Study area

This study was conducted along the River Sava, at 12 selected localities in Slovenia (Mojstrana (MOJ), Radovljica (RAD), Litija (LIT), Vrhovo (VRH), Catez (CAT)), Croatia (Zagreb (ZAG), Jasenovac (JAS), Slavonski Brod (SLB), Zupanja (ZUP)), and Serbia (Sremska Mitrovica (SRM), Sabac (SAB), Belgrade (BEO)) (Table 1; Fig. 1). The main characteristics of the Sava River Basin (SRB) have already been discussed extensively (Dragun et al. 2015; Ogrinc et al. 2015; Ščančar et al. 2015).

The SRB has a diverse geological composition, with magmatic (granite, diabase, dacite, andesite and peridotites), metamorphic (schist, gneiss, marble and quartzite) and sedimentary rocks (limestone, dolomites and conglomerates), and limestone as the dominant geological substrate (Simić et al. 2015; ISRBC 2016; Schwarz 2016). Soils in the upper section of the river are undeveloped skeleton soils, accompanied by poorly developed brown floodplain soils. In lowland parts, gley and semi-gley fluvisols are most dominant (Schwarz 2016). In terms of land use, forests and semi-natural areas cover 54.7% of the land, 42.4% is used for agricultural activities, while artificial surfaces (roads, buildings, etc.) cover 2.2% (ISRBC 2009).

The climate of the SRB is moderate continental, with the exception of the higher altitudes, where an alpine (mountainous) climate prevails. Average annual precipitation and air temperature differ along the course of the river. The annual mean air temperature in the upper stretches of the river is 6 °C, while in the lower stretches (near the confluence with the Danube) it reaches 13 °C. Annual precipitation varies from 660 mm $year^{-1}$ at the mouth of the River Sava up to 2000 to 3000 mm $year^{-1}$ in the alpine region. Maximum flows are usually recorded in spring and low ones in autumn, which is in line with precipitation and snow melting patterns (Ogrinc et al. 2015).

The floristic differentiation of riparian vegetation within the SRB is mainly affected by different hydrological conditions, such as intensity and duration of flooding and groundwater level, as well as topography and soil properties (maximum water capacity, aeration and water permeability). The woody vegetation consists of riparian forests of different willow species and white and black poplars (*Populus alba* and *P. nigra*) (Schwarz 2016). Besides the dominant species of the genera *Salix* and *Populus*, *Alnus glutinosa* (L.) Gaertn., *Fraxinus angustifolia* Vahl, *Ulmus laevis* Pall., *Ulmus glabra* Huds., *Quercus robur* L. and *Juglans regia* L. are frequent species in these forests (Karadžić et al. 2015). Willow communities along the River Sava belong to the alliances *Salicion eleagno-daphnoidis* (Moor 1958) Grass 1993 and *Salicion albae* Soó 1930 of the class *Salicetea purpureae* Moor 1958. The alliance *Salicion albae* grows on soils with a greater water capacity, mainly gravel and sandy littoral; it is directly impacted by streams and experiences frequent flooding, being just above the mean water level. White willow communities are dominated by *S. alba*, which is sometimes accompanied by *S. purpurea* and *S. triandra* (Karadžić et al. 2015). Forests of *P. nigra* are rare, but in mixed communities with willow (*Salici albae-Populetum nigrae* (R. Tx. 1931) Meyer Drees 1936) and white poplar (*Populetum nigrae-albae* Slavnić 1952), *P. nigra* is the dominant species of riparian forests. *Juglans regia* is found in a large number of localities in the study area as an accompanying species in willow and poplar forests.

Sampling

Sample collection was conducted in September 2015 during the GLOBAQUA expedition. Sampling sites were selected based on sample accessibility, as well as site representativeness in terms of sources of pollution (e.g. agricultural and urban activities, industry, traffic, etc.) (Fig 1; Table 1). Soil samples were collected along the river bank, with five subsamples taken at each sampling site, from a depth of 0 - 10 cm. At each sampling site, these five subsamples were mixed in order to form a representative composite sample. The composite samples were collected in PVC buckets and stored at 4 °C, in the dark. Soil samples were air-dried and sieved through a 0.2 mm stainless sieve for chemical analyses.

Plant samples were collected from three to five individuals of *S. alba*, *J. regia* and *P. nigra* trees of approximately the same age by random selection. Approximately 30 g of mature leaves were collected from each tree at a height of 1.5 - 2 m above the ground, from all sides of the tree. A composite plant (leaves and roots) sample for each examined species was formed by mixing (three to five) subsamples at each sampling site. *Salix alba* was found at all 12 sampling sites, forming a total of 12 composite samples. *Populus nigra* was found at the following localities: MOJ, VRH, CAT, ZAG, JAS, ZUP, SRM, SAB and BEO, forming a total of 9 composite samples, while *J. regia* was found at MOJ, RAD, LIT, VRH, CAT, JAS and SRM, forming a total of 7 composite samples (Fig. 1). Root samples were taken from the same tree individuals in the zone of the rhizosphere. Leaf and root samples were washed with tap and deionised water in order to remove all soil residues from their surfaces and then dried to a constant weight at 75 °C (Binder, Tuttlingen, Germany). Samples were sieved with a laboratory mill (Polymix, Kinematica AG) through a 1.5 mm stainless steel sieve before being analyzed for PTE content.

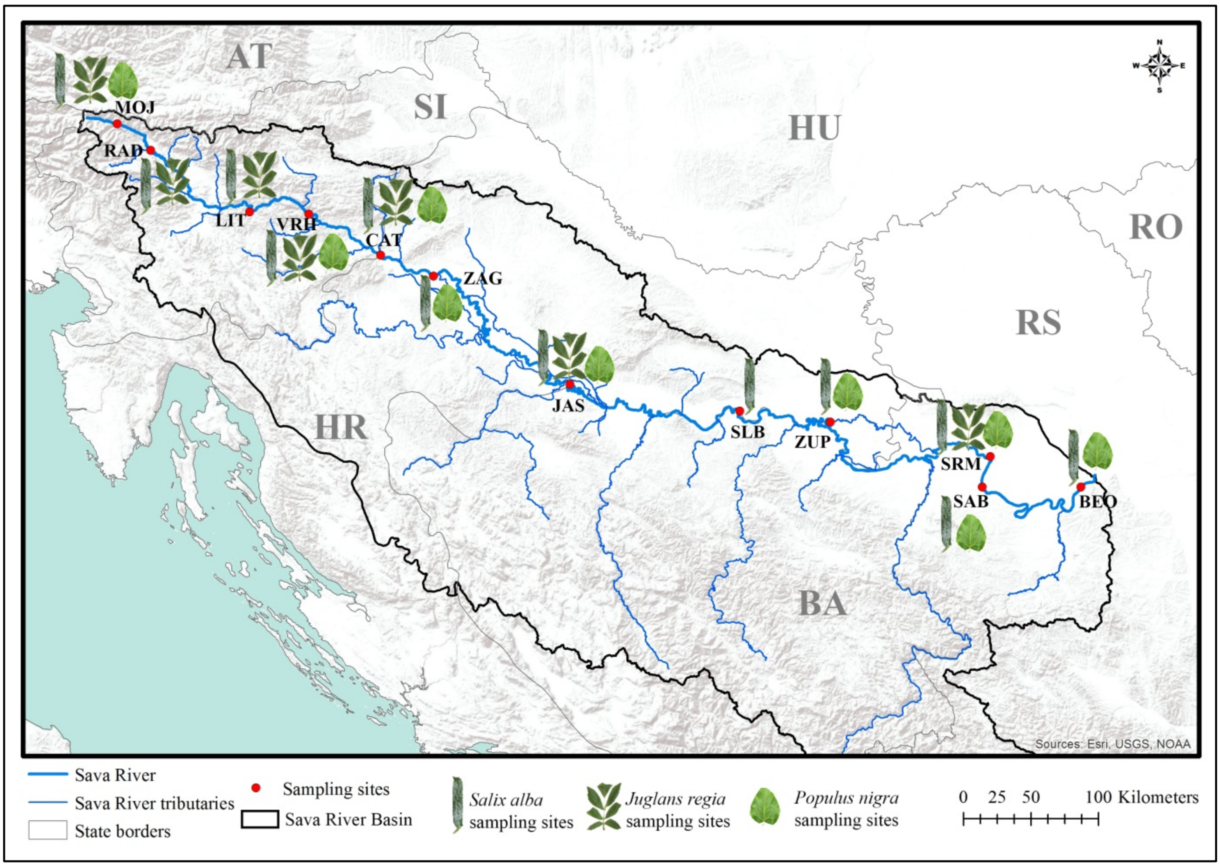

Fig. 1 Sampling sites along the Sava River

Table 1 Description of sampling sites

Abbreviation	**Full name**	**State**	**Latitude (°)**	**Longitude (°)**	**Elevation (m asl)**	**Distance from the mouth (rkm)**	**Pollution sources**
MOJ	Mojstrana	Slovenia	46.459967	13.940096	661	930	No direct sources of pollution; Elevated content of PTEs is from parent rock weathering
RAD	Radovljica	Slovenia	46.339529	14.163860	409	908	Metal industry is located upstream of the sampling site
LIT	Litija	Slovenia	46.066067	14.850483	225	810	Abandoned mining and agricultural activities
VRH	Vrhovo	Slovenia	46.042900	15.226300	194	776	Dam, hydromorphological change to river flow
CAT	Catez	Slovenia	45.890362	15.630107	137	736	Viticulture and urban activities, wood processing industry
ZAG	Zagreb	Croatia	45.785695	15.981591	112	664	Industrial and urban activities
JAS	Jasenovac	Croatia	45.263670	16.894265	90	489	River traffic and agricultural activities
SLB	Slavonski Brod	Croatia	45.144906	17.984106	82	360	Oil industry, river traffic and agricultural activities

ZUP	Zupanja	Croatia	45.075484	18.686883	77	262	Oil, metal and mining industry, river traffic and agricultural activities
SRM	Sremska Mitrovica	Serbia	44.913575	19.752491	72	118	Heavy industry, urban and agricultural activities, river traffic
SAB	Sabac	Serbia	44.769900	19.699400	71	106	Chemical industry, urban and agricultural activities, river traffic
BEO	Belgrade	Serbia	44.768511	20.355560	69	14	Untreated municipal water, thermal power plant, industrial and urban activities, river traffic

Soil and plant analysis

Soil sample mineralisation was carried out by wet digestion in a microwave (CEM Mars 6) using an aqua regia mixture (3 ml of 65% HNO_3 and 9 ml of 37% HCl), while the content of PTEs (As, Cd, Cr, Cu, Ni, Pb and Zn) was determined by inductively coupled plasma optic spectrometry (ICP-OES, Spectro Genesis). The accuracy of the results dwas confirmed by analysing standard reference soil material (Loam soil - ERM-CC141, IRMM certified by EC-JRC). The recovery values found were within 90 - 110%.

Plant sample mineralisation was also carried out by wet digestion in a microwave (CEM Mars 6) using a mixture of 9 ml of 65% HNO3 and 3 ml of 30% H2O2. The accuracy of the measured results was confirmed by analysing the standard reference material (Beach leaves - BCR-100, IRMM certified by EC-JRC). The recovery values found were within 91.7 - 108.9%

All samples were analyzed in 6 replicates and results were presented as mean values, with standard deviation (SD). The detection limits for the analysed elements in soil and plant samples were as follows (mg kg^{-1}): As – 0.005, Cd – 0.0002, Cr – 0.001, Cu – 0.001, Ni – 0.0003, Pb – 0.004, and Zn – 0.006.

Data analysis

The enrichment factor (EF) has been widely used to assess the environmental burden of elements accumulated in soils (Hu et al. 2013; Čakmak et al. 2018), as well as to evaluate possible anthropogenic inputs of PTEs in soil (Swarzenski et al. 2006; Bai et al. 2015). The EF was calculated as:

$$EF = \frac{\left(\frac{C_x}{C_{Mn}}\right) soil}{\left(\frac{C_x}{C_{Mn}}\right) background}$$

where (C_x/C_{Mn}) soil is the ratio between concentrations of the potentially enriched element C_x and Mn concentrations (C_{Mn}) in the same soil sample, and (C_x/C_{Mn}) background is the ratio of the referenced background values. The use of Mn as the reference background element has been referred to in studies by Barbieri (2016), Kowalska et al. (2018), and Pavlović et al. (2019). To calculate the EF, the background values published in Marković et al. (2018) were used. According to Sutherland (2000), five contamination categories are recognised based on the enrichment factor: EF < 2 represents deficiency to minimal enrichment; EF 2 – 5, moderate enrichment; EF 5 – 20, significant enrichment; EF 20 – 40, very high enrichment; and EF > 40, extremely high enrichment. In addition, an EF value of around 1 could indicate the predominant natural origin of the element in soil (Mil-Homens et al. 2006).

In order to evaluate (potential) toxic element tolerance in selected plant species, the bioaccumulation (BCF) and translocation (TF) factors were calculated as follows:

$$BCF = \frac{C_{root}}{C_{soil}}$$

where C_{root} represents the content of the selected element in roots and C_{soil} represents the content of the same element in soil (Zayed et al. 1998), and

$$TF = \frac{C_{leaf}}{C_{root}}$$

where C_{leaf} represents the content of the selected element in leaves and C_{root} represents the content of the same element in root samples (Li et al. 2007; Malik et al. 2010).

In order to determine the differences in the content of the chemical elements obtained in soil and plant material, a one-way ANOVA was carried out, with Tukey's honest significant difference (HSD) post-hoc test. ANOVA was conducted for each examined species separately. Correlations between the levels of the examined elements in soil, root and leave samples were obtained using non-parametric Spearman rank-order correlation. Canonical discriminant analysis (CDA) was performed for element contents in roots and leaves separately in order to classify species according to different accumulation abilities. Descriptive and multivariate statistical analyses were performed using Statistica 10.0 software.

Results and discussion

PTEs in soil

The element levels established in soil are presented in Table 2. The trend for the average element content for the entire study area was as follows: Zn > Cr > Cu > Ni > Pb > As > Cd. Compared to the average element content in world soils (Kabata-Pendias 2011), a higher element content was measured for: As (> 8.4 mg kg^{-1}) at the ZUP, SRM, SAB and BEO sampling sites, Cd (> 0.45 mg kg^{-1}) at BEO, Cr (> 51 mg kg^{-1}) at ZUP, SRM, SAB and BEO, Cu (> 23 mg kg^{-1}) at CAT, ZUP, SRM, SAB and BEO, Ni (> 26 mg kg^{-1}) at SLB, ZUP, SRM, SAB and BEO, Pb (> 28 mg kg^{-1}) at MOJ, SRM, SAB and BEO, and Zn (> 60 mg kg^{-1}) at MOJ, VRH, CAT, ZUP, SRM, SAB and BEO. The background values for European soils (Gawlik and Bidoglio 2006) were exceeded for: Cr (> 100 mg kg^{-1}) at ZUP, SAB and BEO, Cu (> 140 mg kg^{-1}) at CAT, and Ni (> 75 mg kg^{-1}) at ZUP, SRM, SAB and BEO. Compared to the background values for this area recorded in the study by Marković et al. (2018), a higher content was measured for: As (> 11.53 mg kg^{-1}) at SRM, SAB and BEO, Cd (> 0.68 mg kg^{-1}) at BEO, Cr (> 72.52 mg kg^{-1}) at ZUP, SRM, SAB and BEO, Cu (> 24.12 mg kg^{-1}) at CAT, ZUP, SRM, SAB and BEO, Ni (> 41.33 mg kg^{-1}) at ZUP, SRM, SAB and BEO, Pb (> 44.03 mg kg^{-1}) at MOJ, SAB and BEO, and Zn (> 91.64 mg kg^{-1}) at MOJ, CAT, SRM, SAB and BEO. Levels in the critical range for plants (Alloway 2013) were measured for: As (20 - 50 mg kg^{-1}) and Cr (75 - 100 mg kg^{-1}) at SRM, and for Zn (70 - 400 mg kg^{-1}) at MOJ, CAT, ZUP, SRM, SAB and BEO, while levels above the suggested critical range for plants were measured for: Cr (> 100 mg kg^{-1}) at ZUP, SAB and BEO, Cu (> 125 mg kg^{-1}) at CAT, and Ni (> 100 mg kg^{-1}) at ZUP, SAB and BEO. In addition, a statistically significant difference was found between these sites in terms of the obtained levels of As, Cd, Cr, Cu, Ni, Pb and Zn (Table 2).

PTE levels in the analysed soil samples were lower than those from our earlier survey on the riparian soil of the River Sava, except for Cu (Marković et al. 2018). The reason for this is probably due to differences in the analytical methods used for determining element content, as well as the different depths of the riparian soil samples. However, in comparison to the study by Pavlović et al. (2019), the results for As, Cd and Pb were lower or similar, while the measured content of Cr, Cu, Ni and Zn was higher than previously reported. The differences in the measured element content could be a consequence of water level. Specifically, soil samples in the study conducted by Pavlović et al. (2019) were taken during a high water event, while samples in the present study were taken during a low water event.

When compared to some European rivers with similar anthropogenic pressures (urbanisation, industry, agriculture, etc.), the As, Pb and Zn levels recorded in the riparian soils of the Sava were lower than those in the riparian soils of the Elbe in Germany (Schulz-Zunkel et al. 2013), but higher than in the riparian soils of the Don in Russia (Minkina et al. 2017) and the Kolubara in Serbia (Čakmak et al. 2018). Moreover, the content of Cu and Ni measured in this study was similar to that in the riparian soils of the Don (Minkina et al. 2017), the Elbe (Schulz-Zunkel et al. 2013) and the Kolubara (Čakmak et al. 2018) rivers. On the other hand, levels of Cd and Cr in the riparian soils at the examined sampling sites were lower than those reported in the riparian soils at all the rivers mentioned above.

Table 2 PTE levels in riparian soils at the examined sampling sites (in mg kg^{-1}, with SD in brackets); values in and above the critical range for plants are denoted in bold

Site	As	Cd	Cr	Cu	Ni	Pb	Zn
MOJ	3.67 (0.46) [f]	< LoQ [d]	15.91 (1.07) [i]	14.65 (0.43) [g,h]	7.20 (0.42) [l]	60.12 (4.23) [b]	**105.67 (1.61) [d]**
RAD	4.60 (0.35) [f]	< LoQ [d]	29.66 (0.58) [e]	21.10 (0.08) [f]	20.71 (0.10) [f]	25.18 (0.62) [f]	58.32 (0.54) [h]
LIT	4.84 (0.25) [f]	< LoQ [d]	19.52 (0.17) [h]	12.67 (0.07) [h,i]	11.86 (0.11) [j]	24.19 (0.49) [f]	47.55 (1.72) [j]
VRH	4.45 (0.30) [f]	< LoQ [d]	26.76 (0.19) [f]	20.14 (0.34) [f]	16.03 (0.05) [h]	20.07 (1.08) [g]	67.77 (0.80) [g]
CAT	7.30 (0.42) [e]	< LoQ [d]	31.63 (0.19) [e]	**382.26 (3.90) [a]**	18.20 (0.12) [g]	27.34 (0.78) [e]	**97.30 (0.45) [e]**
ZAG	4.96 (0.32) [f]	< LoQ [d]	16.08 (0.12) [i]	11.43 (0.09) [i]	10.04 (0.08) [k]	10.74 (0.23) [i]	41.48 (0.33) [k]
JAS	4.37 (0.30) [f]	< LoQ [d]	23.72 (0.26) [g]	12.19 (0.16) [i]	13.70 (0.26) [i]	12.53 (1.38) [h,i]	50.92 (0.42) [j]
SLB	7.21 (0.21) [e]	< LoQ [d]	43.67 (0.40) [d]	15.62 (0.11) [g]	29.86 (0.54) [e]	13.06 (1.15) [h]	46.27 (0.31) [i]
ZUP	8.89 (0.26) [d]	< LoQ [d]	**123.88 (0.69) [a]**	30.28 (0.14) [e]	**101.07 (1.28) [c]**	25.60 (0.89) [e,f]	**87.70 (0.30) [f]**
SRM	13.93 (0.21) [c]	0.12 (0.00) [c]	**99.46 (1.54) [c]**	32.77 (0.27) [d]	81.17 (0.21) [d]	30.46 (0.71) [d]	**114.47 (0.73) [c]**
SAB	16.54 (0.29) [b]	0.14 (0.00) [b]	**123.38 (2.25) [a]**	42.55 (0.14) [c]	**113.37 (1.25) [a]**	44.07 (0.58) [c]	**159.03 (2.00) [b]**
BEO	**21.86 (0.30) [a]**	0.72 (0.00) [a]	**119.22 (2.04) [b]**	58.52 (0.18) [b]	**106.16 (0.40) [b]**	74.40 (1.11) [a]	**223.38 (1.09) [a]**
Average	8.55	0.33	56.07	54.52	44.11	30.65	87.19
Average range in world soils[a]	4.4-8.4	0.37-0.45	47-51	13-23	13-26	22-28	45-60
Background for European soils[b]	-	1-3	50-100	50-140	30-75	50-300	150-300
Background values for the study area[c]	11.53	0.68	72.52	24.12	41.33	44.03	91.64
Critical range for plants[d]	20-50	> 2.5	75-100	60-125	> 100	> 100	70-400

Reference values for element content in soil: [a] Kabata-Pendias, 2011; [b] Gawlik and Bidoglio 2006; [c] Marković et al. 2018; [d] Alloway 2013

< LoQ – below the Limit of Quantification

Values with the same letter were not significantly different.

Enrichment factor

In order to assess pollution in soils in terms of the sources of PTEs, the enrichment factor (EF) was calculated and the results are presented in Table 3. Calculating the soil enrichment factor is important given the fact that PTEs can present a risk to vegetation and, consequently, the living world through food chains (Chojnacka et al. 2005). The results revealed deficiency to minimal enrichment (EF < 2) for most elements at most of the sampling sites. Deficiency to minimal enrichment in soil could indicate that the origin of PTEs is predominantly natural (Mil-Homens et al. 2006). Moderate enrichment (EF 2 - 5) was found for Cu at MOJ and RAD, for Ni at ZUP, SRM, SAB and BEO, and for Zn at MOJ and CAT (Table 3). Significant enrichment was calculated for Pb at MOJ (EF = 5.60), while Cu enrichment at CAT was very high (EF = 33.41). Soil enrichment with Pb and Zn at MOJ is most likely due to the chemical weathering of parent rock and ore deposits (Marković et al. 2018; Pavlović et al. 2019). Furthermore, enrichment with Pb, Zn and Cu in the upper stretch of the river could be related to anthropogenic sources, such as mining or the application of different types of fertilizers (Adriano 2001; Alloway 2013). Very high enrichment with Cu in the riparian soils at CAT probably occurs due to agricultural (the application of grapevine fungicides that contain Cu; Fan et al. 2011) and industrial activities (the paper and wood processing industry; ISRBC 2016, Marković et al. 2018). In the lower stretch of the river, soils are moderately enriched with Ni, but this elevated EF is predominantly a result of the geological substrate (Zovko and Romić 2011; Pavlović et al. 2019) and, to some extent, different anthropogenic activities (Milačič et al. 2017; Marković et al. 2018).

Table 3 Enrichment factors (EF) for PTEs in soils at selected sampling sites

	EF_{As}	EF_{Cd}	EF_{Cr}	EF_{Cu}	EF_{Ni}	EF_{Pb}	EF_{Zn}
MOJ	1.31	0.00	0.90	**2.49**	0.71	**5.60**	**4.73**
RAD	1.04	0.00	1.06	**2.27**	1.30	1.49	1.65
LIT	1.40	0.00	0.90	1.75	0.95	1.83	1.73
VRH	0.85	0.00	0.81	1.84	0.85	1.00	1.63
CAT	1.33	0.00	0.92	**33.41**	0.93	1.31	**2.24**
ZAG	1.22	0.00	0.63	1.34	0.69	0.69	1.28
JAS	0.90	0.00	0.77	1.20	0.78	0.67	1.31
SLB	0.78	0.00	0.75	0.81	0.90	0.37	0.63
ZUP	0.81	0.00	1.80	1.32	**2.58**	0.61	1.01
SRM	1.31	0.19	1.49	1.47	**2.13**	0.75	1.35
SAB	1.14	0.16	1.36	1.41	**2.19**	0.80	1.38
BEO	1.48	0.82	1.28	1.89	**2.00**	1.32	1.44

EFs > 2 are denoted in bold

PTEs in plants

PTE levels in the roots and leaves of the examined plants are presented in Tables 4 and 5. Those that are considered to be toxic for plants (Marschner 1995; Pugh et al. 2002; Kabata-Pendias 2011; Alloway 2013) are denoted in bold. The trend for the average element content in roots was as follows: Zn > Cu > Cd > Ni > Pb > Cr > As in *S. alba*; Zn > Cr > Cu > Pb > As > Ni > Cd in *J. regia*; and Zn > Cu > Cr > Ni > Pb > As > Cd in *P. nigra*. In leaves, the trend was as follows: Zn > Cu > Ni > Cr > Cd > Pb > As in *S. alba*; Zn > Cu > As > Cr > Pb > Ni > Cd in *J. regia*; and Zn > Cu > As > Cd > Pb > Ni > Cr in *P. nigra*.

Copper and Zn are essential elements in plants, which is why they are the most abundant elements in samples. However, in some samples their concentrations were very low, especially in the roots and leaves of *J. regia* and *P. nigra*. In terms of Cu, the roots of some species have the ability to retain it and to prevent its transport to shoots. The levels of other elements in plants, combined with the low mobility of Cu, could lead to Cu deficiency in plant leaves (Kabata-Pendias 2011). Additionally, the binding of Cu by soils is highly dependent on soil pH, which is why Cu availability decreases at about pH 7–8 (Kabata-Pendias 2011). Since the study by Pavlović et al. (2019) reported alkaline pH for this research area, this could be the reason for Cu deficiency in the analysed plants. As for Zn, its availability is highly dependent on clay content in the soil; thus the low Zn accumulation in the leaves of *J. regia* may be a result of this as soils in our research area contain up to 35% clay (Pavlović et al. 2019).

Table 4 Potentially toxic elements in roots, presented as mean values with SD in brackets, in mg kg^{-1} on a dry mass basis

Species	Site	As	Cd	Cr	Cu	Ni	Pb	Zn
Salix alba	**MOJ**	2.05 (0.51) [f,g]	0.22 (0.00) [e]	< LoQ [e]	2.28 (0.00) [g]	< LoQ [f]	2.19 (0.12) [e,f,g]	36.99 (0.48) [e]
	RAD	3.19 (0.57) [d,e]	0.22 (0.00) [e]	< LoQ [e]	2.66 (0.00) [g]	< LoQ [f]	2.33 (0.26) [d,e,f]	18.35 (0.25) [g]
	LIT	2.42 (0.43) [e,f]	0.22 (0.00) [e]	< LoQ [e]	5.19 (0.07) [f]	1.83 (0.26) [d,e]	3.14 (0.13) [d]	20.29 (1.15) [g]
	VRH	1.33 (0.52) [g]	0.25 (0.00) [e]	0.64 (0.07) [e]	7.41 (2.59) [e]	0.27 (0.08) [f]	2.19 (0.07) [e,f,g]	33.17 (1.75) [e]
	CAT	2.78 (0.33) [e,f]	0.44 (0.00) [d,e]	1.00 (0.00) [d,e]	6.50 (0.00) [e,f]	0.55 (0.14) [e,f]	1.83 (0.15) [f,g]	53.07 (0.33) [c,d]
	ZAG	< LoQ [h]	0.25 (0.00) [e]	1.33 (0.00) [d,e]	7.33 (1.28) [e]	0.61 (0.14) [e,f]	2.22 (0.09) [d,e,f,g]	48.89 (2.16) [d]
	JAS	2.58 (0.31) [e,f]	0.44 (0.00) [d,e]	0.58 (0.09) [e]	5.28 (0.25) [f]	0.39 (0.09) [e,f]	1.33 (0.21) [g]	26.59 (0.11) [f]
	SLB	3.86 (0.81) [c,d]	0.67 (0.00) [d]	2.61 (0.31) [d]	7.75 (0.09) [e]	2.70 (0.34) [d]	2.95 (0.52) [d,e]	35.82 (2.97) [e]
	ZUP	4.72 (0.45) [b,c]	1.22 (0.12) [c]	**14.57 (2.96) [a]**	14.58 (1.09) [d]	**19.08 (2.40) [a]**	7.36 (1.29) [b]	48.37 (1.81) [d]
	SRM	**5.53 (0.13) [b]**	1.33 (0.00) [c]	**5.55 (0.61) [c]**	17.44 (0.62) [c]	**10.47 (1.13) [b]**	5.50 (0.30) [c]	56.72 (1.29) [c]
	SAB	**6.42 (0.25) [a]**	4.48 (0.09) [b]	**7.25 (0.82) [b,c]**	22.14 (0.58) [b]	**10.17 (0.49) [b]**	9.89 (0.49) [a]	**125.55 (6.55) [b]**
	BEO	**5.50 (0.23) [b]**	**86.96 (0.63) [a]**	**7.65 (0.57) [b]**	26.46 (0.41) [a]	8.16 (0.14) [c]	10.48 (0.46) [a]	**278.18 (1.71) [a]**
	Average	3.37	8.06	3.43	10.42	4.52	4.28	65.17
Juglans regia	**MOJ**	2.99 (0.43) [b,c]	< LoQ [c]	**6.17 (0.24) [c,d]**	4.75 (1.55) [c]	0.24 (0.08) [e]	6.07 (0.27) [a]	26.21 (1.72) [c]
	RAD	2.12 (0.66) [c,d]	< LoQ [c]	**6.80 (0.29) [c]**	8.27 (0.15) [b]	1.14 (0.10) [d]	1.62 (0.23) [e]	25.19 (0.67) [c]
	LIT	2.48 (0.09) [b,c,d]	< LoQ [c]	**6.00 (0.06) [c,d]**	7.96 (0.04) [b]	0.77 (0.01) [d]	1.26 (0.03) [e]	27.70 (0.23) [b]
	VRH	1.95 (0.65) [d]	< LoQ [c]	4.96 (0.53) [d]	1.92 (0.46) [e]	< LoQ [e]	1.41 (0.12) [e]	1.60 (0.00) [f]
	CAT	3.31 (0.55) [b]	0.25 (0.00) [b]	**24.37 (1.67) [a]**	7.20 (0.17) [b]	3.53 (0.55) [b]	4.91 (0.47) [b]	18.78 (0.47) [d]
	JAS	4.91 (0.80) [a]	0.25 (0.00) [b]	**12.24 (1.67) [b]**	3.53 (0.54) [d]	2.22 (0.18) [c]	2.89 (0.18) [d]	8.12 (0.62) [e]
	SRM	1.62 (0.37) [d]	0.45 (0.24) [a]	**11.50 (0.55) [b]**	10.05 (0.35) [a]	7.66 (0.22) [a]	3.57 (0.51) [c]	45.19 (0.31) [a]
	Average	2.77	0.14	10.29	6.24	2.22	3.10	21.83
Populus nigra	**MOJ**	2.12 (0.44) [b]	0.26 (0.00) [e]	**5.34 (1.11) [b]**	10.33 (0.86) [a]	2.46 (0.47) [e]	4.04 (0.26) [a]	19.67 (2.17) [e]
	VRH	**5.38 (0.17) [a]**	0.26 (0.00) [e]	4.22 (1.30) [b,c]	5.84 (0.55) [e]	0.75 (0.38) [g]	1.56 (0.69) [d,e]	11.05 (0.86) [f]
	CAT	**5.19 (0.24) [a]**	0.30 (0.11) [d,e]	3.90 (0.00) [c]	4.03 (0.07) [f]	0.75 (0.11) [g]	3.04 (0.61) [b]	25.54 (0.26) [d]
	ZAG	< LoQ [c]	0.69 (0.13) [b]	2.27 (0.12) [e]	7.08 (0.09) [d]	0.96 (0.11) [g]	1.23 (0.23) [e]	49.68 (0.46) [c]
	JAS	< LoQ [c]	0.69 (0.13) [b]	3.93 (0.21) [c]	5.25 (0.09) [e]	1.50 (0.11) [f]	1.41 (0.18) [d,e]	61.16 (4.26) [b]
	ZUP	< LoQ [c]	0.43 (0.13) [c,d]	2.60 (0.00) [d,e]	8.50 (0.15) [c]	4.71 (0.00) [d]	1.26 (0.23) [e]	27.19 (0.43) [d]
	SRM	< LoQ [c]	0.52 (0.00) [c]	4.75 (0.00) [b,c]	7.40 (0.08) [d]	6.34 (0.22) [b]	2.59 (0.28) [b,c]	25.81 (0.52) [d]
	SAB	< LoQ [c]	1.03 (0.00) [a]	**7.35 (0.79) [a]**	9.79 (0.00) [b]	7.94 (0.08) [a]	4.33 (0.39) [a]	53.11 (3.99) [c]
	BEO	< LoQ [c]	0.52 (0.00) [c]	3.61 (0.56) [c,d]	8.45 (0.13) [c]	5.36 (0.08) [c]	2.11 (0.23) [c,d]	85.93 (1.73) [a]
	Average	1.41	0.52	4.22	7.41	3.42	2.40	39.90
Deficit		-	-	-	2-5[a]	-	-	10-20[a]
Normal range		1-1.7[a]	0.002-1[a]	0.1-0.5[a]	5-30[a,c]	0.1-5[a,b]	0.2-10[a,c]	27-150[a,d]
Toxic range		5-20[a,b]	5-30[a,b]	5-30[a,b]	20-100[a,c]	10-100[a,b]	30-300[a,b]	100-400[a,b,d]

Values for deficit, normal and toxic range: [a] Kabata-Pendias 2011; [b] Alloway 2013; [c] Pugh et al. 2002; [d] Marschner 1995; < LoQ – below the Limit of Quantification

Values with the same letter were not significantly different.

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Table 5 Potentially toxic elements in leaves, presented as mean values with SD in brackets, in mg kg^{-1} on a dry mass basis

Species	Site	As	Cd	Cr	Cu	Ni	Pb	Zn
Salix alba	**MOJ**	3.06 (0.46) ^{b}	0.44 (0.00) ^{e}	< LoQ ^{d}	4.92 (0.09) ^{f}	0.44 (0.09) ^{e,f}	< LoQ ^{c}	**149.61 (0.59) ^{f}**
	RAD	< LoQ ^{d}	0.22 (0.00) ^{f}	< LoQ ^{d}	5.11 (0.09) ^{f}	< LoQ ^{f}	< LoQ ^{c}	**157.07 (0.28) ^{e}**
	LIT	< LoQ ^{d}	0.22 (0.00) ^{f}	0.44 (0.09) ^{c}	6.31 (0.22) ^{e}	0.42 (0.09) ^{e,f}	0.71 (0.06) ^{b}	44.41 (1.00) ^{l}
	VRH	< LoQ ^{d}	1.55 (0.00) ^{b}	< LoQ ^{d}	5.05 (0.14) ^{f}	< LoQ ^{f}	< LoQ ^{c}	**227.91 (2.24) ^{b}**
	CAT	< LoQ ^{d}	**5.13 (0.09) ^{a}**	< LoQ ^{d}	6.28 (0.31) ^{e}	< LoQ ^{f}	< LoQ ^{c}	**308.03 (2.67) ^{a}**
	ZAG	< LoQ ^{d}	0.63 (0.09) ^{d}	< LoQ ^{d}	5.33 (0.18) ^{f}	< LoQ ^{f}	< LoQ ^{c}	83.48 (0.48) ^{k}
	JAS	< LoQ ^{d}	0.22 (0.00) ^{f}	< LoQ ^{d}	7.96 (0.07) ^{c}	0.50 (0.00) ^{e}	< LoQ ^{c}	**102.08 (1.94) ^{j}**
	SLB	< LoQ ^{d}	0.66 (0.00) ^{d}	2.39 (0.31) ^{b}	6.83 (0.37) ^{d}	1.42 (0.09) ^{d}	1.03 (0.19) ^{b}	**133.30 (2.50) ^{g}**
	ZUP	1.07 (0.23) ^{c}	0.66 (0.00) ^{d}	2.58 (0.09) ^{b}	8.00 (0.18) ^{c}	4.14 (0.07) ^{b}	1.00 (0.24) ^{b}	**105.34 (0.69) ^{i}**
	SRM	<LoQ ^{d}	0.66 (0.00) ^{d}	< LoQ ^{d}	6.17 (0.61) ^{d,e}	< LoQ ^{f}	< LoQ ^{c}	**175.41 (0.44) ^{d}**
	SAB	**6.31 (0.59) ^{a}**	1.33 (0.00) ^{c}	**11.48 (0.60) ^{a}**	11.34 (0.21) ^{a}	**13.26 (0.84) ^{a}**	8.34 (0.67) ^{a}	**204.55 (2.17) ^{c}**
	BEO	< LoQ ^{d}	0.44 (0.00) ^{e}	< LoQ ^{d}	10.39 (0.25) ^{b}	2.33 (0.00) ^{c}	< LoQ ^{c}	**127.52 (0.39) ^{h}**
	Average	0.87	1.01	1.41	6.97	1.88	0.92	151.56
Juglans regia	**MOJ**	1.39 (0.12) ^{b}	< LoQ	1.43 (0.84) ^{c}	6.53 (0.52) ^{b}	< LoQ ^{d}	1.34 (0.32) ^{a,b}	19.66 (0.23) ^{c}
	RAD	1.71 (0.14) ^{b}	< LoQ	2.20 (0.24) ^{b}	9.64 (0.20) ^{a}	0.44 (0.08) ^{c}	1.11 (0.18) ^{a,b}	22.74 (0.54) ^{b}
	LIT	**5.26 (0.63) ^{a}**	< LoQ	1.18 (0.18) ^{c,d}	5.19 (0.07) ^{c}	0.44 (0.08) ^{c}	< LoQ ^{c}	25.55 (0.11) ^{a}
	VRH	**5.69 (0.22) ^{a}**	< LoQ	1.10 (0.20) ^{c,d}	4.33 (0.18) ^{d}	< LoQ ^{d}	< LoQ ^{c}	1.60 (0.00) ^{f}
	CAT	**5.59 (0.85) ^{a}**	< LoQ	0.62 (0.09) ^{d}	3.44 (0.14) ^{e}	< LoQ ^{d}	< LoQ ^{c}	8.32 (0.12) ^{e}
	JAS	1.54 (0.55) ^{b}	< LoQ	4.63 (0.00) ^{a}	4.75 (0.09) ^{d}	0.94 (0.10) ^{b}	1.44 (0.33) ^{a}	8.24 (0.00) ^{e}
	SRM	2.10 (1.13) ^{b}	< LoQ	1.62 (0.18) ^{b,c}	4.33 (0.18) ^{d}	1.55 (0.10) ^{a}	1.09 (0.14) ^{b}	12.74 (0.12) ^{d}
	Average	3.33	/	1.83	5.46	0.48	0.71	14.12
Populus nigra	**MOJ**	< LoQ ^{c}	< LoQ ^{g}	< LoQ ^{c}	5.62 (0.38) ^{c}	< LoQ ^{d}	0.73 (0.29) ^{b}	42.88 (4.74) ^{g}
	VRH	< LoQ ^{c}	< LoQ ^{g}	0.22 (0.00) ^{b}	6.79 (0.17) ^{a}	< LoQ ^{d}	0.28 (0.09) ^{b}	24.08 (1.88) ^{h}
	CAT	< LoQ ^{c}	2.06 (0.00) ^{a}	0.22 (0.00) ^{b}	4.78 (0.08) ^{d}	0.20 (0.00) ^{c}	3.15 (1.12) ^{a}	**135.23 (0.68) ^{b}**
	ZAG	< LoQ ^{c}	1.29 (0.00) ^{c}	< LoQ ^{c}	4.36 (0.16) ^{e}	< LoQ ^{d}	0.30 (0.08) ^{b}	**122.00 (1.05) ^{c}**
	JAS	2.56 (0.23) ^{a,b}	1.03 (0.00) ^{d}	0.32 (0.12) ^{a}	7.06 (0.14) ^{a}	0.27 (0.11) ^{c}	0.30 (0.08) ^{b}	74.59 (0.34) ^{e}
	ZUP	2.40 (0.89) ^{a,b}	1.29 (0.00) ^{c}	< LoQ ^{c}	6.27 (0.13) ^{b}	2.04 (0.13) ^{a}	< LoQ ^{b}	94.73 (0.97) ^{d}
	SRM	2.82 (0.94) ^{a}	0.52 (0.00) ^{f}	< LoQ ^{c}	4.17 (0.00) ^{e}	0.20 (0.00) ^{c}	< LoQ ^{b}	10.43 (0.89) ^{i}
	SAB	1.76 (0.28) ^{b}	1.55 (0.00) ^{b}	< LoQ ^{c}	6.03 (0.16) ^{b}	0.51 (0.00) ^{b}	< LoQ ^{b}	**207.77 (3.54) ^{a}**
	BEO	3.17 (0.49) ^{a}	0.77 (0.00) ^{e}	< LoQ ^{c}	4.06 (0.08) ^{e}	< LoQ ^{d}	< LoQ ^{b}	50.93 (1.21) ^{f}
	Average	1.41	0.95	0.08	5.46	0.36	0.53	84.74
Deficit		-	-	-	2-5[a]	-	-	10-20[a]
Normal range		1-1.7[a]	0.002-1[a]	0.1-0.5[a]	5-30[a,c]	0.1-5[a,b]	0.2-10[a,c]	27-150[a,d]
Toxic range		5-20[a,b]	5-30[a,b]	5-30[a,b]	20-100[a,c]	10-100[a,b]	30-300[a,b]	100-400[a,b,d]

Values for deficit, normal and toxic range: [a] Kabata-Pendias 2011; [b] Alloway 2013; [c] Pugh et al. 2002; [d] Marschner 1995; < LoQ – below the Limit of Quantification

Values with the same letter were not significantly different.

PTEs in Salix alba

Salix alba accumulated various PTEs in its roots at levels considered toxic for plants: As (5 - 20 mg kg^{-1}) at SRM, SAB and BEO, Cd (5 - 30 mg kg^{-1}) at BEO, Cr (5 - 30 mg kg^{-1}) at ZUP, SRM, SAB and BEO, Ni (10 - 100 mg kg^{-1}) at ZUP, SRM and SAB, and Zn (100 - 400 mg kg^{-1}) at SAB and BEO (Marschner 1995; Pugh et al. 2002; Kabata-Pendias 2011; Alloway 2013). Statistically significant differences between the obtained values of Cd, Cr, Ni and Zn in roots were found between the investigated stands (Table 4). On the other hand, there was a deficit of Cu (2 - 5 mg kg^{-1}) measured at MOJ and RAD and of Zn (10 - 20 mg kg^{-1}) at RAD (Kabata-Pendias 2011). In *S. alba* leaves, toxic levels were measured for As (6.31 mg kg^{-1}), Cr (11.48 mg kg^{-1}) and Ni (13.26 mg kg^{-1}) at SAB, Cd (5.13 mg kg^{-1}) at CAT, and Zn at all sampling sites except LIT and ZAG (Table 5). All the sites mentioned above differ from one another in terms of the obtained content of As, Cd, Cr, Ni and Zn in *S. alba* leaves (Table 5).

The results from this study show that *S. alba* is a good accumulator of Cd and Zn, a fact supported by numerous previous studies (Vandecasteele et al. 2002; Vysloužilová et al. 2003; Dos Santos Utmazian et al. 2007; Bedell et al. 2009; Han et al. 2013). In *S. alba* roots at BEO, besides toxic levels of Cd and Zn, the content of other elements was elevated, too, or even at toxic levels for plants, which might be connected to specific point sources of pollution at this sampling site, leading to the release of PTEs in a form that is more available for plant uptake (Pavlović et al. 2019).

The results obtained in our research in terms of the content of the analysed PTEs in the leaves of *S. alba* are similar to those from earlier research undertaken along the River Danube (Pavlović et al. 2016). On the other hand, in a study conducted by Zimmer et al. (2012) on the floodplains of the River Elbe, while As, Cu, Ni and Pb levels in *Salix spp*. leaves were similar to those in our present study, Cd and Zn content was much higher. The lower uptake of Cd and Zn in our study is probably due to the lower PTE content in soils compared to the riparian soils of the Elbe, as well as due to the characteristics of the *Salix* hybrid clone that Zimmer et al. (2012) used for analysis. Vandecasteele et al. (2002) also reported higher levels of Zn in *S. alba* leaves and roots at polluted dredged sediment disposal sites along the Scheldt and Leie rivers in Belgium, while the content of Cd in leaves was similar to that recorded in the present study. Meers et al. (2003) also conducted research on different *Salix* sp. clones growing at dredged sediment disposal sites on the River Leie, where higher levels of Cd and Zn were recorded, while Cu, Ni and Pb content in leaves was similar to the present study. As in the case of the Elbe, a large area along the River Leie is also affected by PTE pollution, so a higher uptake of elements in *Salix* sp. leaves is possible, but it is also dependent on the characteristics of the *Salix* clones used in the research. In soils experimentally enriched with high loads of As, Cd, Pb and Zn, *S. alba* accumulated higher amounts of Cd and Zn in comparison to the results obtained in our study, while leaf uptake of As and Pb was similar (Vysloužilová et al. 2003).

PTEs in Juglans regia

Juglans regia accumulated Cr in its roots at levels considered toxic for plants at all sampling sites except VRH (5 - 30 mg kg^{-1}; Kabata-Pendias 2011; Alloway 2013) (Table 4). In addition, there was found to be a statistically significant difference between VRH and the other sites in terms of the obtained content of Cr in *J. regia* roots (Table 4). Copper deficiency (2-5 mg kg^{-1}) was observed in its roots at MOJ, VRH and JAS and Zn deficiency (10-20 mg kg^{-1}) at VRH, CAT and JAS (Kabata-Pendias 2011) (Table 4).

In *J. regia* leaves, toxic levels of As were measured at LIT, VRH and CAT (5 - 20 mg kg^{-1}; Kabata-Pendias 2011; Alloway 2013), while a deficit of Cu in leaves (2 - 5 mg kg^{-1}) was found at VRH, CAT, JAS and SRM, and of Zn (10 - 20 mg kg^{-1}) at all sampling sites except LIT and RAD (Kabata-Pendias 2011) (Table 5). The LIT, VRH and CAT sampling sites stand out from the others in terms of the obtained content of As in the leaves of this species (Table 5). The deficiency of Cu and Zn observed in the roots and leaves of *J. regia* is probably due to their antagonism with As and Cr (Kabata-Pendias 2011), which were accumulated in a toxic range.

Compared to the results of the present study, Antonijević et al. (2012) measured similar As levels in roots near the flotation tailings pond of the copper mining and smelting complex in Bor, Serbia, but a slightly higher content in leaves (up to 8 mg kg^{-1}). The latter is to be expected, bearing in mind that flotation tailings generally contain higher amounts of PTEs potentially available to plants (Antonijević et al. 2012). The available literature on Cr accumulation by *J. regia* mostly relates to its accumulation in branches, shells and kernels. In a study conducted by Arik and Yaldiz (2010) in an area with different types of ore deposits in western Turkey, Cr levels in *J. regia* branches were lower than those measured in the roots in our study. The same authors measured a lower As content in branches compared to the level in leaves in our research. Furthermore, numerous studies have shown that Cr levels in shells and kernels were lower than those obtained for *J. regia* leaves and roots in our study (Özcan 2008; Arpadjan et al. 2013; Tošić et al. 2014). From the current results, it is impossible to say with certainty whether the high Cr content measured in *J. regia* roots is species specific, demonstrating the need for further research. In terms of the accumulation of the other examined PTEs, similar levels of Cu and Zn were reported in a study by Sawidis et al. (2002) in *J. regia* leaves growing on deposits of lignite in Ptolemais, Greece. Moreover, Ghaderian and Ghotbi Ravandi (2012) reported similar Zn levels in *J. regia* leaves from the Sarcheshmeh copper mining area in Iran. In research conducted by Nečemer at al. (2008), Cd and Pb levels in the leaves of this species from a non-polluted site in Slovenia (Zaplana near Vrhnika) were determined using different methods, but the results revealed similar levels as in the present study.

PTEs in Populus nigra

Populus nigra accumulated various PTEs in its roots at levels considered toxic for plants: As (5 - 20 mg kg^{-1}) at VRH and CAT and Cr (5 - 30 mg kg^{-1}) at MOJ and SAB (Kabata-Pendias 2011; Alloway 2013). The ANOVA test revealed a significant difference between VRH and CAT and the other sites in terms of As content, as well as between SAB and the other sites when it came to Cr content in *P. nigra* leaves (Table 4). A deficit of Cu (2 - 5 mg kg^{-1}) was measured at CAT and of Zn (10 - 20 mg kg^{-1}) at MOJ and VRH (Kabata-Pendias 2011) (Table 4).

In *P. nigra* leaves, toxic levels were measured for Zn at CAT, ZAG and SAB (Table 5), while it was in the deficit range for plant tissues at SRM (10.43 mg kg^{-1}). In addition, a copper deficit was measured at CAT (4.78 mg kg^{-1}), ZAG (4.36 mg kg^{-1}), SRM (4.17 mg kg^{-1}) and BEO (4.06 mg kg^{-1}) (Table 5). There is a statistically significant difference between the CAT, ZAG and SAB sites and the other locations in terms of Zn content in *P. nigra* leaves (Table 5).

At CAT, the deficit of Cu in roots is probably the result of the toxic levels of As due to the possible antagonistic relationship between these elements (Tang and Miller 1991). However, it could also be a result of the elevated levels of Cr in *P. nigra* roots (> 0.5 mg kg^{-1}; Kabata-Pendias 2011), bearing in mind the antagonistic relationship between Cu and Cr in plants. The Cu deficit in leaves at CAT and ZAG is most probably related to the toxic content of Zn because of the antagonistic relationship between these elements (Kabata-Pendias 2011). Plants most likely uptake these elements using the same mechanism and therefore one may inhibit the uptake of the other (Kabata-Pendias 2011). Copper levels are closely associated with soil texture and are usually the lowest in sandy soils (Kabata-Pendias and Mukhjeree 2007). Since the study by Pavlović et al. (2019) reported the texture of soils at CAT as being sandy loam, soil texture could also contribute to the lower uptake of Cu in the analysed plants.

At those sites where As content in roots was below the level of detection, Zn uptake was found to be at optimum levels for plant growth and development. Even so, at VRH and CAT, where As is in the toxic range, *P. nigra* accumulated lower amounts of Zn, which may be a consequence of the antagonistic relationship between them (Kabata-Pendias 2011).

As with the genus *Salix*, species of the genus *Populus* have been shown to be good accumulators of Cd and Zn (Djingova et al. 1999; Sawidis et al. 2002; Baslar et al. 2005; Stobrawa and Lorenc-Plucińska 2007; Domínguez et al. 2008; Zacchini et al. 2011), which is in accordance with the results from this study. Namely, our results for Zn content in the leaves of *P. nigra* are similar to those obtained in research by Sawidis et al. (2002) on *P. nigra* leaves in an area with large coal-fired plants in Greece, as well as research conducted by Djingova et al. (1999) on the leaves of *P. nigra 'Italica'* growing near Cu smelter, metallurgical, cement and chemical plants. Meanwhile, Baslar et al. (2005) measured similar or lower Zn levels in *P. nigra* leaves in industrial (metallurgical, electronic, textile, and cement plants and a petroleum refinery), urban and suburban areas in Turkey.

On the other hand, our results point to elevated or toxic Cr content, as well as toxic As content in *P. nigra* roots at VRH and CAT. Similar Cr levels were reported in the leaves of this species growing in a copper mine area along the Nure river valley (Dinelli and Lombini 1996), as well as in the roots of this species, very often near the Cu smelter in Glogow, Poland (Stobrawa and Lorenc-Plucińska 2007). Moreover, similar As levels were reported in the leaves of *P. nigra 'Italica'*, near metallurgical, chemical and cement plants in Bulgaria (Djingova et al. 1999), while Cr content was lower compared to our results.

When comparing the accumulation capacity of these three species, it was established that *S. alba* accumulated the highest content of PTEs in roots, except for Cr in the roots of *J. regia*. A similar accumulation pattern was observed for PTE levels in the leaves of the analysed plants. *Salix alba* accumulated the highest levels of Cd, Cu, Ni, Pb and Zn, while the leaves of *J. regia* accumulated the highest amounts of As and Cr. *Populus nigra* has been shown to be the least effective for As accumulation in roots and Cr, Ni and Pb accumulation in leaves.

Bioconcentration factor (BCF) and translocation factor (TF)

The phytostabilisation process reduces the mobility of PTEs, their leaching into ground water, and their bioavailability for entry into the food chain (Chojnacka et al. 2005), while through the phytoextraction process, plants transport and concentrate PTEs from the soil into their aerial parts (Yoon et al. 2006; Cui et al. 2007). The use of tolerant plant species to stabilise PTEs in soils could improve conditions for the natural attenuation or stabilisation of these pollutants. In order to evaluate the potential of the selected plants for the phytoextraction and phytostabilisation of PTEs, the bioaccumulation (BCF) and translocation (TF) factors were calculated (Table 6). If plants are able to retain PTEs in their roots, the TF has to be lower than 1, while the BCF can be either lower or higher than 1 indicating their potential for phytostabilisation. Plants can be regarded as phytoextractors if the TF is higher than 1, which indicates the transport of PTEs from roots to leaves (Fitz and Wenzel 2002). Leaves are considered to be the main sink for pollutants and therefore they are usually more sensitive to their effects than other plant parts (Bargagli 1998), which is important from the environmental standpoint due to leaf-fall which could further enrich soils with PTEs, especially in the case of TF > 1, and the existence of significant correlations of element levels in soils and leaves (Robinson et al. 2003).

In this study, highly significant correlations were found between As, Ni and Zn levels in soils and leaves (Table 7). Although the correlation between As content in soils and the leaves of *J. regia* was not statistically significant (ns), the high TF and toxic level of As in leaves could contribute to soil enrichment with this element in the future.

Table 6 Bioconcentration (BCFs) and translocation (TFs) factors for PTEs in plants at selected sampling sites

	Elements	**BCFs**							**TFs**						
	Sites	**As**	**Cd**	**Cr**	**Cu**	**Ni**	**Pb**	**Zn**	**As**	**Cd**	**Cr**	**Cu**	**Ni**	**Pb**	**Zn**
Salix alba	**MOJ**	0.56	/	/	0.16	/	0.04	0.35	**1.49**	**2.00**	/	**2.16**	/	/	**4.04**
	RAD	0.69	/	/	0.13	/	0.09	0.31	/	**1.00**	/	**1.92**	/	/	**8.56**
	LIT	0.50	/	/	0.41	0.15	0.13	0.43	/	**1.00**	/	**1.22**	0.23	0.23	**2.19**
	VRH	0.30	/	0.02	0.37	0.02	0.11	0.49	/	**6.20**	/	0.68	/	/	**6.87**
	CAT	0.38	/	0.03	0.02	0.03	0.07	0.55	/	**11.66**	/	0.97	/	/	**5.80**
	ZAG	/	/	0.08	0.64	0.06	0.21	**1.18**	/	**2.52**	/	0.73	/	/	**1.71**
	JAS	0.59	/	0.02	0.43	0.03	0.11	0.52	/	0.50	/	**1.51**	**1.29**	/	**3.84**
	SLB	0.54	/	0.06	0.50	0.09	0.23	0.77	/	0.99	0.92	0.88	0.53	0.35	**3.72**
	ZUP	0.53	/	0.12	0.48	0.19	0.29	0.55	0.23	0.54	0.18	0.55	0.22	0.14	**2.18**
	SRM	0.40	**11.08**	0.06	0.53	0.13	0.18	0.50	/	0.50	/	0.35	/	/	**3.09**
	SAB	0.39	**32.00**	0.06	0.52	0.09	0.22	0.79	0.98	0.30	**1.58**	0.51	**1.30**	0.84	**1.63**
	BEO	0.25	**120.78**	0.06	0.45	0.08	0.14	**1.25**	/	0.01	/	0.39	0.29	/	0.46
Juglans regia	**MOJ**	0.81	/	0.39	0.32	0.03	0.10	0.25	0.46	/	0.23	**1.37**	/	0.22	0.75
	RAD	0.46	/	0.23	0.39	0.06	0.06	0.43	0.81	/	0.32	**1.17**	0.39	0.69	0.90
	LIT	0.51	/	0.31	0.63	0.06	0.05	0.58	**2.12**	/	0.20	0.65	0.57	/	0.92
	VRH	0.44	/	0.19	0.10	/	0.07	0.02	**2.92**	/	0.22	**2.26**	/	/	**1.00**
	CAT	0.45	/	0.77	0.02	0.19	0.18	0.19	**1.69**	/	0.03	0.48	/	/	0.44
	JAS	**1.12**	/	0.52	0.29	0.16	0.23	0.16	0.31	/	0.38	**1.35**	0.42	0.50	**1.01**

	SRM	0.12	**3.75**	0.12	0.31	0.09	0.12	0.20	**1.30**	/	0.14	0.43	0.20	0.31	0.28
Populus nigra	**MOJ**	0.58	/	0.34	0.71	0.34	0.07	0.19	/	/	/	0.54	/	0.18	**2.18**
	VRH	**1.21**	/	0.16	0.29	0.05	0.08	0.16	/	/	0.05	**1.16**	/	0.18	**2.18**
	CAT	0.71	/	0.12	0.01	0.04	0.11	0.26	/	**6.87**	0.06	**1.19**	0.27	**1.04**	**5.29**
	ZAG	/	/	0.14	0.62	0.10	0.11	**1.20**	/	**1.87**	/	0.62	/	0.24	**2.46**
	JAS	/	/	0.17	0.43	0.11	0.11	**1.20**	/	**1.49**	0.08	**1.34**	0.18	0.21	**1.22**
	ZUP	/	/	0.02	0.28	0.05	0.05	0.31	/	**3.00**	/	0.74	0.43	/	**3.48**
	SRM	/	**4.33**	0.05	0.23	0.08	0.09	0.23	/	**1.00**	/	0.56	0.03	/	0.40
	SAB	/	**7.36**	0.06	0.23	0.07	0.10	0.33	/	**1.50**	/	0.62	0.06	/	**3.91**
	BEO	/	0.72	0.03	0.14	0.05	0.03	0.38	/	**1.48**	/	0.48	/	/	0.59

Table 7 Correlations of PTE levels in the roots and leaves of the examined species with the soils in which they grow

	Soil						
Plants	**As**	**Cd**	**Cr**	**Cu**	**Ni**	**Pb**	**Zn**
Roots							
Salix alba	**0.764*****	**0.752*****	**0.811*****	**0.570*****	**0.732*****	**0.493*****	**0.682*****
Juglans regia	**-0.348***	**0.615*****	**0.571*****	**0.434****	**0.627*****	**0.658*****	0.296
Populus nigra	**-0.519*****	**0.443*****	0.162	0.041	**0.654*****	**0.684*****	0.148
Leaves							
Salix alba	0.108	0.083	**0.498*****	**0.399*****	**0.517*****	-0.026	**0.532*****
Juglans regia	0.247	/	-0.135	**-0.451****	**0.407****	0.154	-0.240
Populus nigra	**0.590*****	0.018	**-0.276***	**-0.373****	**0.525*****	**-0.307***	-0.082

$^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$

The results showed that despite significant positive correlations between the levels of all the examined elements in soil and roots, the BCF for *S. alba* was less than 1 (BCF < 1) at all the sampling sites for all the elements except Cd at SRM (11.08), SAB (32.00) and BEO (120.78) and Zn at ZAG (1.18) and BEO (1.25). Similarly, significant positive correlations existed between PTE levels in the roots and leaves of *S. alba*, with a TF for Cd higher than 1 recorded at MOJ, RAD, LIT, VRH, CAT and ZAG, while the values for Zn were also higher than 1 at all sampling sites except BEO (Table 6). In addition to Cd and Zn, *S. alba* also transferred Cu to leaves at MOJ, RAD, LIT and JAS, Ni at JAS and SAB, Cr at SAB, and As at MOJ (Table 6).

The results indicate the potential of *S. alba* for the phytoextraction (in the upper stretch of the river) and phytostabilisation (in the middle and lower stretches) of Cd and Cu. Furthermore, its potential for the phytoextraction of Zn was shown at all sampling sites except BEO. Previous research on *Salix* sp., especially on *Salix viminalis* and *Salix dasyclados*, has shown that these species have good Cd and Zn accumulation capacity both in leaves (Punshon and Dickinson 1997; Meers et al. 2003, 2007) and roots (Stobrawa and Lorenc-Plucińska 2007; Zacchini et al. 2011). Additionally, the results of our research indicate that *S. alba* possesses the capacity for the phytostabilisation of Pb (at all sampling sites), and Cr and Ni (at all sampling sites except SAB). Vandecasteele et al. (2005) had similar results in their research on *Salix* sp. clones.

Significant correlations were established between the levels of all the examined elements in soil and the roots of *J. regia*, except As (-0.348*) and Zn (ns) at all the sampling sites. However, the BCF was less than 1 for all the examined elements except As at JAS (1.12) and Cd at SRM (3.75) (Table 6). Even though significant positive correlations were also established between roots and leaves for Zn (0.742), Ni (0.608) and Pb (0.403) (Table 8), only As at LIT, VRH, CAT and SRM, Cu at MOJ, RAD, VRH and JAS, and Zn at VRH and JAS was efficiently transported to leaves (TF > 1) (Table 6)

The results indicate the potential of this species for the phytoextraction of As, as well as for the phytostabilisation of Cr, Ni and Pb. Similarly, Saqib et al. (2013) and Ozen and Yaman (2016) in their research demonstrated the uptake capacity of *J. regia* for As and Pb in its leaves, while Marmiroli et al. (1999, 2005) showed that this species accumulates Cr and Pb in its roots. However, Antonijević et al. (2012) previously reported high BCFs and TFs for Zn, which does not coincide with the results from our study, given the fact that Zn content in roots and leaves was low, and even deficient at most of the sampling sites.

For *P. nigra* significant correlations were established between the levels of Cd (0.443), Ni (0.654) and Pb (0.684) in soil and roots, while for As, there was a significant negative correlation (-0.519**) (Table 7). However, as with *S. alba*, the BCF for *P. nigra* was higher than 1 for Cd at SRM (4.33) and SAB (7.36), for Zn at ZAG and JAS (1.25), and for As at VRH (1.21) (Table 6). In addition, significant positive correlations were established for Cd (0.348*), Ni (0.345) and Zn (0.413*) in its roots and leaves (Table 8), with *P. nigra* efficiently transporting Cd at all sampling sites, Zn at all sites except SRM and BEO, Cu at VRH (1.16), CAT (1.19) and JAS (1.34) and Pb at CAT (1.04) from its roots to its leaves (Table 6).

The results obtained during our research indicate the potential of *P. nigra* for the phytoextraction of Cd and Zn. This species was also proven to be good for the phytostabilisation of Cr, Ni and Pb, and to some extent Cu. Similarly, in their research Jakovljević et al. (2014) showed that *P. nigra* also possesses potential for Cd phytoextraction, while Baldantoni et al. (2014) found high TFs for Cd and Zn, and the potential for Pb phytostabilisation in *P. nigra* clones.

The *S. alba* and *P. nigra* species demonstrated that they possess the potential for the phytoextraction of Cd and Zn, while *J. regia* has the potential for the phytoextraction of As. On the other hand, *S. alba* shows potential for the phytostabilisation of Cd and Cu in the middle and lower reaches of the river. *J. regia* is a phytostabiliser for As, Cr, Ni and Pb. *P. nigra* also proved to be good for the phytostabilisation of Cr, Ni and Pb, and to some extent Cu. The results also showed similar variations in the BCFs and TFs for Cu in all the examined species, which can be linked to the physiological mechanisms of uptake and accumulation of Cu as an essential element (Domínguez et al. 2008).

Table 8 Correlations of PTE levels in the roots and leaves of the examined species

Roots / Leaves	***Salix alba***						
	As	**Cd**	**Cr**	**Cu**	**Ni**	**Pb**	**Zn**
As	**0.299****	0.139	**0.248***	0.119	**0.234***	**0.325****	**0.269***
Cd	0.089	**0.339****	**0.429*****	**0.407*****	**0.276***	0.081	**0.508*****
Cr	**0.461*****	**0.348****	**0.420*****	**0.330****	**0.619*****	**0.535*****	0.087
Cu	**0.737*****	**0.815*****	**0.711*****	**0.721*****	**0.773*****	**0.569*****	**0.524*****
Ni	**0.571*****	**0.528*****	**0.515*****	**0.431*****	**0.570*****	**0.606*****	**0.299***

Pb	**0.452*****	**0.336****	**0.401*****	**0.317****	**0.606*****	**0.531*****	0.078
Zn	**0.234***	0.189	0.071	0.119	-0.091	-0.043	**0.288***
Juglans regia							
	As	**Cd**	**Cr**	**Cu**	**Ni**	**Pb**	**Zn**
As	-0.154	-0.183	-0.178	-0.183	-0.226	**-0.365***	-0.299
Cd	/	/	/	/	/	/	/
Cr	0.109	0.086	0.101	0.131	0.125	-0.120	0.103
Cu	-0.001	**-0.599*****	**-0.358***	0.198	**-0.355***	-0.179	**0.312***
Ni	-0.129	**0.520*****	**0.357***	**0.579*****	**0.608*****	-0.188	**0.450****
Pb	0.206	0.154	0.175	0.084	0.190	**0.403****	0.151
Zn	-0.136	**-0.366***	-0.104	**0.644*****	-0.010	-0.213	**0.742*****
Populus nigra							
	As	**Cd**	**Cr**	**Cu**	**Ni**	**Pb**	**Zn**
As	**-0.694*****	**0.324***	0.060	0.177	**0.652*****	-0.124	**0.606*****
Cd	-0.218	**0.348****	-0.229	-0.240	-0.030	0.038	**0.416****
Cr	**0.478*****	-0.239	-0.080	**-0.826*****	**-0.640*****	-0.225	-0.119
Cu	0.217	-0.080	0.075	-0.191	-0.248	-0.214	-0.218
Ni	**-0.310***	0.097	0.076	-0.001	**0.345***	0.034	0.173
Pb	**0.656*****	**-0.339***	-0.118	**-0.465*****	**-0.779*****	0.042	**-0.368****
Zn	-0.152	**0.424****	-0.162	-0.045	-0.042	0.100	**0.413****

Significance is presented as: * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$

Differences in PTE accumulation capacity between the examined species

In order to highlight the variations between PTE accumulation in *S. alba*, *J. regia* and *P. nigra* and discover patterns, canonical discriminant analysis (CDA) was performed separately for element content in the species' roots and leaves (Fig 2 and 3; Table 9).

Table 9 Standardised Coefficients for Canonical Variables

	CDA roots		**CDA leaves**	
	DC 1	**DC 2**	**DC 1**	**DC 2**
As	0.085	**-0.899**	-0.315	-0.126
Cd	0.153	-0.292	0.239	0.195
Cr	**-0.508**	-0.299	-0.042	-0.404
Cu	0.245	-0.291	0.191	-0.325
Ni	0.167	-0.077	0.127	-0.245
Pb	0.148	**-0.599**	0.029	-0.116
Zn	0.281	-0.216	**0.541**	-0.171
Eigenval	1.597	0.261	3.274	0.621
Cum.Prop	0.859	1.000	0.840	1.000

Values below -0.5 and above 0.5 are denoted in bold

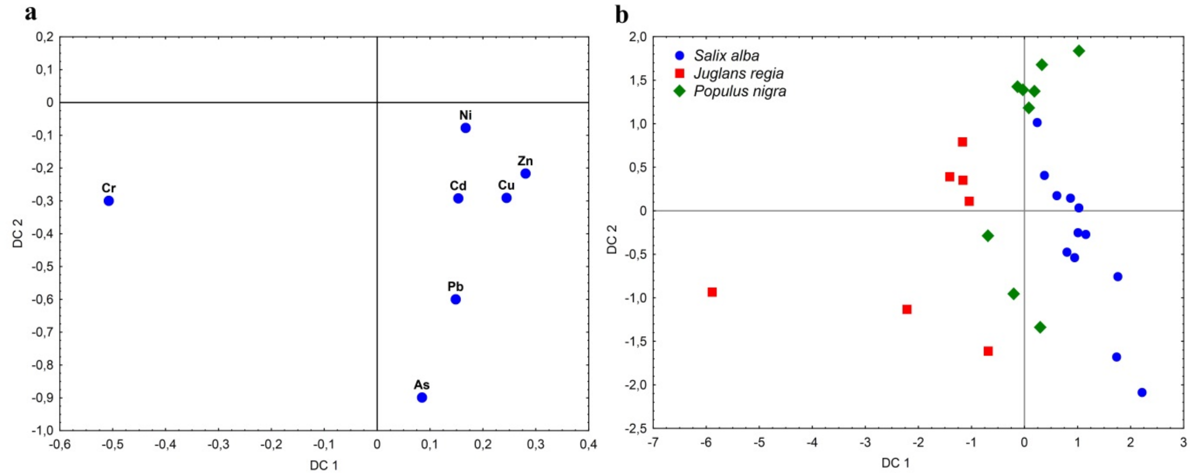

Fig. 2 Differences in the accumulation patterns of the examined species in roots a) elements b) species

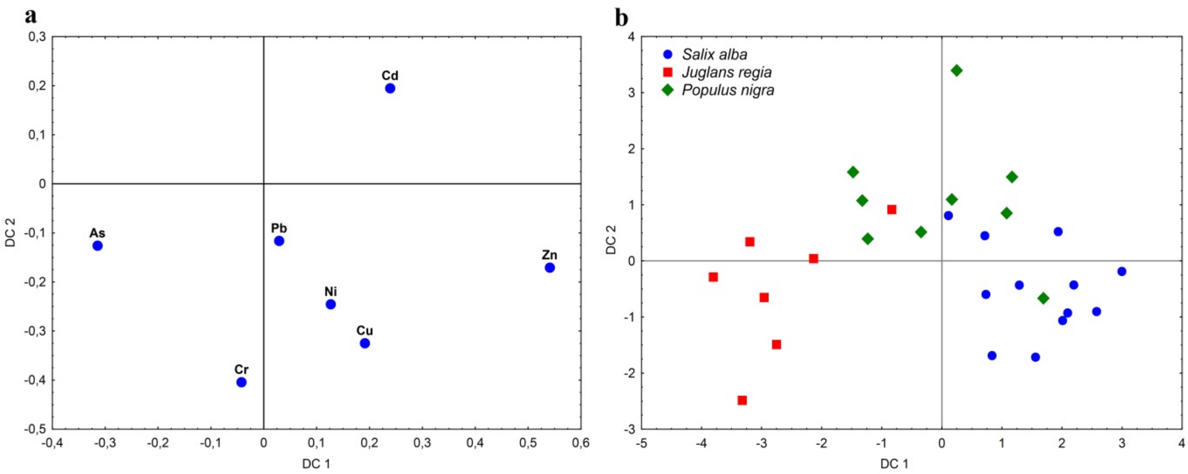

Fig. 3 Differences in the accumulation patterns of the examined species in leaves a) elements b) species

Based on the first component (DC 1), which explains 85.9% of variance, and the second component (DC 2), which explains 14.1 % of differences, *J. regia* roots are separated from the other two species, probably due to higher Cr accumulation. The separation between *S. alba* and *P. nigra* is mainly impacted by the higher levels of As, Pb, Cd, Cu, Ni and Zn in the roots of the former. In leaves, the first component (DC 1) explained 84% of variance and separates *S. alba* from the other two species due to a higher leaf uptake of Zn, Cu, Ni and Pb, as well as *J. regia* due to a higher uptake of As and Cr. The second component (DC 2), which explains 16% of variance, separates the leaves of *P. nigra* from the other examined species due to Cd accumulation (Fig. 3).

Conclusion

The present study focused on the potential of *Salix alba*, *Juglans regia* and *Populus nigra* to accumulate PTEs (As, Cd, Cr, Cu, Ni, Pb and Zn) in the riparian zone of the River Sava. Their levels were determined in soils at selected sampling sites with specific anthropogenic sources of pollution, as well as in the roots and leaves of the selected species.

Despite the fact that levels above the background values for European soils were measured for Cr, Cu and Ni in some soils of the River Sava's riparian zone, with moderate (Zn), significant (Pb) and even very high (Cu) enrichment, the uptake, accumulation and transfer of the elements in the aboveground parts of the examined species differed from species to species. The results obtained in the present study revealed close similarity in the accumulation of PTEs in the leaves of *S. alba* and *P. nigra*. Both species predominantly showed phytoextraction potential for Cd and Zn in the examined soils, with *S. alba* being slightly more efficient than *P. nigra*. However, these two species differ in their phytostabilisation potential, given the fact that *S. alba* demonstrated potential for Cd and Cu phytostabilisation, while P. *nigra* revealed potential for the phytostabilisation of Cr, Ni and Pb, and to some extent Cu. On the other hand, *J. regia* showed potential for the phytoextraction of As and the phytostabilisation of As, Cr, Ni and Pb. The phytostabilisation and phytoextraction potential of the species was additionally confirmed through the BCFs and TFs, correlations and CDA analyses. Through comparing the accumulation capacity of these three species, it was established that *S. alba* accumulated the highest levels of PTEs in roots, except for Cr in the roots of *J. regia*. A similar accumulation pattern was observed in terms of PTE levels in the leaves of the analysed plants. *Salix alba* accumulated the highest amounts of Cd, Cu, Ni, Pb and Zn, while the leaves of *J. regia* accumulated the highest levels of As and Cr. *Populus nigra* was shown to be the least effective in terms of the accumulation of As in roots, and the accumulation of Cr, Ni and Pb in leaves.

The positive correlations of element levels in soils and leaves indicated the possibility of the additional enrichment of soils with PTEs (especially As, Ni and Zn) due to leaf-fall, which can be a focus of future research.

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Conflict of interest

The authors declare that they have no conflict of interest.

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