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## The potential of elm trees (*Ulmus glabra* Huds.) for the phytostabilisation of potentially toxic elements in the riparian zone of the Sava River

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### Abstract:

The use of trees to immobilise potentially toxic elements (PTEs) is a low-cost and effective method of soil remediation. Thus, the objective of this study was to assess the content of total and bioavailable As, Cd, Cr, Cu, Ni, Pb and Zn in soil samples, as well as their levels in the roots and leaves of elm (*Ulmus glabra* Huds.) in order to evaluate its potential for the phytostabilisation of PTEs in the riparian zone of the Sava River. Analysis of soils showed that the availability of PTEs ranged from low to medium, while the Pollution Load Index (PLI) and Potential Ecological Risk index (RI) showed that the examined soil fell into the category of uncontaminated to moderately contaminated, as well as into the category of low risk of PTEs contamination. However, the levels of Cr, Cu and Ni in soils were above the critical range for plants. The content of As and Cr measured in roots and leaves was in the toxic range for plants, while the content of Cd and Ni was elevated but not in the toxic range. Bioaccumulation (BCF) and translocation (TF) factors indicated that *U. glabra* is suitable for the phytostabilisation of As, Cu, Cr, Ni and Pb. Additionally, this species displayed the ability to transport most of the acquired Cu and Zn to the leaves. Correlation analysis showed that

PTE content in *U. glabra* roots was significantly positively correlated to their respective levels in soil (total and DTPA-extractable), except for Cu, indicating that PTE levels in soil strongly influence those in plants. This research into a successful phytoremediating species provides new possibilities when selecting PTE-tolerant native trees in riparian zones of large regional rivers such as the Sava.

**Keywords:** *Ulmus glabra*, potentially toxic elements, Sava River, bioavailability, phytostabilisation

## Introduction

Large river riparian zones are of immense importance because they provide the most diverse ecosystem services. At the same time, due to a high level of anthropogenic pressure and natural interaction between water and the mainland, these zones are very fragile and susceptible to negative changes in the environment (Coccosis 2004; Navarro-Ortega et al. 2015). Pollution of riparian zones by potentially toxic elements (PTEs) can, on the one hand, be the result of the occurrence of natural processes, like weathering of parent rocks, or, on the other, the result of human activity (Barceló and Sabater 2010; Kolditz et al. 2013; Xie et al. 2014; Navarro-Ortega et al. 2015; Čakmak et al. 2018; Pavlović et al. 2019). European legislation highlights eight PTEs (As, Cd, Cr, Cu, Hg, Ni, Pb, and Zn) as one of the main stressors affecting the quality of rivers (EC 2000). Essential micronutrients for living beings, like Cu and Zn, can have a toxic effect on plants and animals when they occur in high concentrations (Lu et al. 2015).

Plants are an important part of aquatic ecosystems because they readily take up chemical elements from soil solution (Zhang et al. 2011). Therefore, PTE content in plant parts can indicate the level of availability of these elements in the soil (Baker et al. 2000; Ma et al. 2001). A large number of plant species are used in environmental biomonitoring, in terms of pollution from PTEs (Piczak et al. 2003; Šerbula et al. 2013), while recent research has shown that trees can be used for the biomonitoring of large areas because they have wide areals and a

long lifespan (Sawidis et al. 2011). In addition, the latter can be used in environment management because, on the one hand, they can accumulate available elements in their aerial parts and foliage and then remove them by phytoextraction, and, on the other, with the aid of their large root mass they can further reduce the availability and toxicity of elements by accumulating them at root level (phytostabilisation) (Cheng 2003; Mahar et al. 2016; Oyuela Leguizamo et al. 2017). In the process of remediation, using native (autochthonous) species over introduced (allochthonous) ones has its advantages, which is reflected in the fact that native species are better adapted to environmental conditions in terms of survival, growth, and reproduction (Pavlović et al. 2004; Yoon et al. 2006; Mitrović et al. 2008; Gajić et al. 2016; Oyuela Leguizamo et al. 2017). Native species tolerant to high levels of PTEs attract the attention of researchers and present a continued challenge for research, although some authors have found positive effects when using non-native species (Madejón et al. 2017).

To the best of our knowledge, there has been no exhaustive assessment to date of the potential of *U. glabra* for the phytostabilisation of PTEs in riparian soil along the River Sava. In this regard, the objectives of this study were as follows: 1) to determine the content of total and bioavailable As, Cd, Cr, Cu, Ni, Pb and Zn in soil samples; (2) to determine levels of As, Cd, Cr, Cu, Ni, Pb and Zn in the roots and leaves of *Ulmus glabra* Huds.; and (3) to evaluate its potential for the phytostabilisation of PTEs in riparian soils affected by various sources of contamination in the riparian zone of the River Sava. The DTPA soil test, developed to assess pollution by trace metals in soils (Kabata-Pendias 2011), was used in this study. The starting hypotheses were that (i) there is a spatial distribution of total and bioavailable PTE content in soils in the riparian zone of the Sava, (ii) the examined elm species accumulates different levels of PTEs from soils at different habitats, and (iii) the content of PTEs differs in its roots and its leaves.

## **Materials and methods**

### *2.1 Study area*

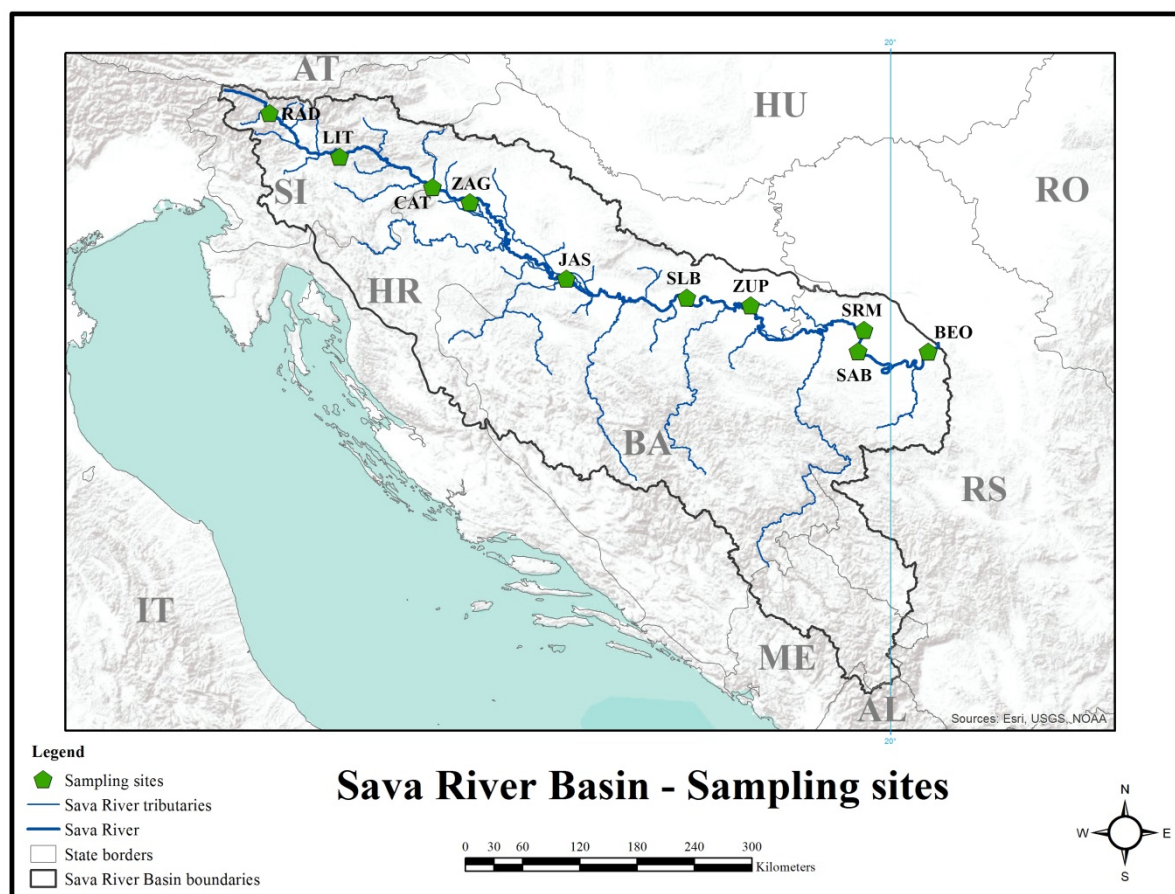
This study was conducted along the River Sava, at ten localities in Slovenia, Croatia, and Serbia, characterized by intense urbanisation and the development of economic and industrial

sectors, which are the major anthropogenic pressures on riparian habitats (Fig. 1). The River Sava is a moderately polluted European river in terms of pollution from PTEs in water and sediment (Milačić et al. 2010, 2017; Vidmar et al. 2017; Marković et al. 2018). In addition, the riparian ecosystems along the Sava's floodplains have been degraded, reduced, and significantly modified due to the expansion of agricultural areas and because of the development of a complex flood defence system that protects fertile agricultural land, settlements, and industrial facilities (ISRBC 2009). Hydrological conditions, topography, and the physical properties of soil are important factors for the differentiation of riparian communities, particularly willow (*Salix* sp.) and poplar (*Populus* sp.) forests in the riparian zone of the Sava (Jarić et al. 2011, 2015; Karadžić et al. 2015). According to Crnobrnja-Isailović et al. (2015), eighteen habitat types have been identified along the River Sava as being important for biodiversity conservation. Eight of these represent important riparian habitats. Riparian mixed forests of *Quercus robur*, *Ulmus laevis* and *U. minor*, *Fraxinus excelsior* or *F. angustifolia* are found in 40.8% of the representative localities (Crnobrnja-Isailović et al. 2015). Species of the *Ulmus* genus are present in forests of common alder with buckthorn (*Frangulo-Alnetum glutinosae* Rauš 1968) and oak and hornbeam forests (*Carpino betuli-Quercetum roboris* Rauš 1969) (Karadžić et al. 2015).

## 2.2 Plant and soil sampling

To identify the state of soil pollution in the riparian zone, field sampling was conducted in September 2015 during a sampling expedition as part of the GLOBAQUA project (Navarro-Ortega et al. 2015). In accordance with sampling protocols for the collection and preparation of samples for analyses, sampling was undertaken at ten sampling sites along the River Sava, having taken account of sample accessibility and representativeness in terms of different anthropogenic sources of pollution (e.g. traffic, and industrial, agricultural and urban activities), (Fig. 1). Soil and plant material samples were collected at the following sites: Radovljica (RAD), Litija (LIT) and Catež (CAT) in the upper stretch of the river, Zagreb (ZAG), Jasenovac (JAS) and Slavonski Brod (SLB) in the middle stretch, and at Zupanja (ZUP), Sremska Mitrovica (SRM), Šabac (SAB) and Belgrade (BEO) in the lower stretch. Each sampling site was exposed to different types and intensities of anthropogenic pollution, from

Radovljica (RAD) near the Sava's source right to its very mouth at the confluence with the Danube in Belgrade (BEO) (Table 1).



**Fig. 1** Sampling sites along the Sava River

**Table 1** Description of sampling sites

Abbr.	Full name	State	Latitude (°)	Longitude (°)	Distance from the mouth (rkm)	Contamination sources
<b>RAD</b>	Radovljica	Slovenia	46.339529	14.163860	908	Metal industry upstream of the sampling site
<b>LIT</b>	Litija	Slovenia	46.066067	14.850483	810	Agricultural activities, abandoned mining
<b>CAT</b>	Catez	Slovenia	45.890362	15.630107	736	Urban activities, wood processing

						industry, viticulture activities
<b>ZAG</b>	Zagreb	Croatia	45.785695	15.981591	664	Urban activities, industry
<b>JAS</b>	Jasenovac	Croatia	45.263670	16.894265	489	Agricultural activities, river traffic
<b>SLB</b>	Slavonski Brod	Croatia	45.144906	17.984106	360	Agricultural activities, river traffic, oil industry
<b>ZUP</b>	Zupanja	Croatia	45.075484	18.686883	262	Agricultural activities, river traffic, oil, metal and mining industry
<b>SRM</b>	Sremska Mitrovica	Serbia	44.913575	19.752491	118	Agricultural and urban activities, river traffic, heavy industry
<b>SAB</b>	Sabac	Serbia	44.769900	19.699400	106	Agricultural and urban activities, river traffic, chemical industry
<b>BEO</b>	Belgrade	Serbia	44.768511	20.355560	14	Urban activities, river traffic, thermoelectric plants, untreated municipal water, industry

Soil samples were taken at each sampling site at a distance of 10-15 m from the river bank because this area usually floods during high-water events. Composite samples for each site were obtained from 5 subsamples for each sampling point collected along the river bank, at depths of 0-30 cm, and transferred to PVC buckets and thoroughly homogenised before further treatment. Samples were kept in the dark at 4° before being analysed for trace element content. In the laboratory, all samples were air-dried, ground in a stainless steel mill, sieved through a 2 mm stainless steel sieve, and kept in clean polypropylene bags before analysis.

Plant samples were taken from elm trees (*Ulmus glabra* Huds.), which were selected as the target species since they are to be found along the entirety of the investigated stretch. Trees of approximately the same age were chosen. Thirty g of mature elm leaves were collected from three to five randomly chosen trees at each sampling site. Samples were taken from the trees at a height of 1.5-2 m above ground, from all sides of the tree. Root samples were taken from the very same tree individuals. Leaf and root samples were washed and dried to a constant weight.

### 2.3 Soil and plant analysis

Soil texture was determined according to the International Soil Texture Triangle (Soil Survey Staff 1951) and presented in terms of sand, silt and clay percentages (%). Soil pH was measured in H<sub>2</sub>O using a WTW (Germany) inoLab 7110 pH meter. Water soluble salts [EC(dSm<sup>-1</sup>)] were measured using a Knick (Germany) Portamess 911 Conductometer. Soil texture was determined using Atterberg's method of sedimentation with a combined pipette technique in 0.4 M tetrasodium diphosphate (Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub>) (Atterberg 1911). The soil organic matter content (%) was determined by means of a titration method, using (NH<sub>4</sub>)<sub>2</sub>Fe(SO<sub>4</sub>)<sub>2</sub> x 6H<sub>2</sub>O after digestion of samples with a dichromate-sulphuric acid solution, based on Simakov's modification of the Turin method (Simakov 1957).

To determine the total element content, 0.5 g of soil material was subjected to microwave assisted digestion (CEM Mars 6), using aqua regia ( 3ml HNO<sub>3</sub> and 9ml HCl), and concentrations of PTEs (As, Cd, Cr, Cu, Ni, Pb and Zn) were determined by ICP-OES (Spectro Genesis). The accuracy of the results was checked by analysing standard reference soil material (Loam soil - ERM-CC141, IRMM certified by EC-JRC). The recovery values found were within 95-110%, while the detection limits for the analysed elements in the soil samples were as follows (mg kg<sup>-1</sup>): As – 0.005, Cd – 0.0002, Cr – 0.001, Cu – 0.001, Ni – 0.0003, Pb – 0.004, and Zn – 0.006. Bioavailable content of PTEs was determined using diethylene-triamine-pentaacetic acid (DTPA) according to Lindsay and Norvell (1978). All measurements were conducted in 5 replicates. Element concentrations were expressed as mg per kilogram of dry leaf weight (mg kg<sup>-1</sup> d.w.).

To determine the total element content, 0.3 g of plant material was subjected to microwave assisted digestion (CEM Mars 6) using a mixture of nitric (HNO<sub>3</sub> concentrated, 9 ml) and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>, 30%, 3 ml), and concentrations of PTEs were determined by ICP-OES (Spectro Genesis). The accuracy of the results was checked by analysing standard reference material (Beach leaves - BCR-100, IRMM certified by EC-JRC). Measurements were conducted in 5 replicates. The recovery values found were within 95-110%, while the detection limits for the analysed elements in the soil samples were as follows (mg kg<sup>-1</sup>): As – 0.005, Cd – 0.0002, Cr – 0.001, Cu – 0.001, Ni – 0.0003, Pb – 0.004, and Zn – 0.006.



### 2.3 Quantification of soil contamination, PTE availability in soil, and the transfer of PTEs from soil to plants

To assess the overall soil contamination level, the Pollution Load Index (PLI, Tomlinson et al. 1980) and Potential Ecological Risk index (RI, Hakanson 1980) were applied.

The value of the PLI was calculated by the n-root of the product of the concentration factor (CF) for n PTE:

$$PLI = \sqrt[n]{(CF1 \times CF2 \times CF3 \times \dots \times CFn)} \quad (1)$$

The CF was calculated as the ratio of the element content in soil ( $C_n$ ) and the average value of the local geochemical background of the element in soil ( $B_n$ ):

$$CF = \frac{C_n}{B_n} \quad (2)$$

PLI values close to 1 indicate heavy metal loads close to the background level, while values above 1 indicate soil contamination (Cabrera et al. 1999).

RI was calculated as the sum of the single risk factor  $E_i$  for PTE $_i$ :

$$RI = \sum E_i \quad (3)$$

$$E_i = T_i \times \frac{C_n}{B_n} \quad (4)$$

$T_i$  is the toxic response factor for the given PTE, which mainly reflects its toxicity level and the degree of environment sensitivity to PTE pollution.  $T_i$  values for As, Cd, Cr, Cu, Ni, Pb and Zn are 10, 30, 2, 5, 5, 5 and 1, respectively (Hakanson 1980). The potential ecological risk of each element is classified according to the following categories:  $E_i < 40$  indicates low potential ecological risk;  $40 \leq E_i < 80$ , moderate potential ecological risk;  $80 \leq E_i < 160$ , appreciable potential ecological risk;  $160 \leq E_i < 320$ , high potential ecological risk; and  $E_i \geq 320$ , serious potential ecological risk. On the basis of RI values, potential ecological risk is classified into four categories:  $RI < 150$  represents low risk;  $150 \leq RI < 300$ , moderate risk;  $300 \leq RI < 600$ , high risk; and  $RI \geq 600$ , significantly high risk.

The availability of PTEs in soil was assessed using the availability ratio index (AR; Massas et al. 2013), calculated as the ratio between the bioavailable DTPA content of the element and its total content in soil, expressed as a percentage.

$$AR = \frac{C_{DTPA}}{C_{tot}} \times 100 \quad (5)$$

The plant-to-soil bioconcentration ratio indices were used to evaluate the transfer of PTEs from soil to plant, comparing concentrations in roots and leaves to those in soil. In this study, the biological concentration factor (BCF; Yoon et al. 2006) was calculated as the ratio of the element concentration in elm roots to its total content (BCF) in soil:

$$BCF = \frac{C_{Root}}{C_{tot}} \quad (6)$$

where  $C_{Root}$  represents the content of the selected element in root samples and  $C_{tot}$  represents the total content of the same element in soil.

The translocation factor (TF) as the ratio of the element concentration in plant shoots to that in roots was used to evaluate the effectiveness of elm trees in translocating As, Cd, Cr, Cu, Ni, Pb and Zn from roots to leaves. TF was calculated as:

$$TF = \frac{C_{Leaf}}{C_{Root}} \quad (7)$$

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.

## 2.4 Data analysis

Correlations between the concentrations obtained for the analysed elements in soil, roots and leaves were performed using non-parametric Spearman's rank-order correlation. Correlation was assumed to be statistically significant at  $p < 0.05$  and is denoted by: \* for 0.05, \*\* for 0.01 and \*\*\* for 0.001. Statistical analysis was performed using Statistica 7.0.

## 3. Results and discussion

### 3.1 Physical and chemical soil properties

Some physical and chemical properties of the soils within the River Sava's riparian zone are presented in Table 2. Soil alkalinity ranged from pH 7.82 in the lower stretch to pH 8.26 in the upper stretch of the river. Texture was also variable, between loamy sand (sandy loam) in

the upper stretch and silty loam and silty clay loam in the lower stretch; the fact soils in the upper reaches of the river contained a greater proportion of sand is a common pattern in riparian soils (Jerolmack and Brzinski 2010). In the middle stretch of the river, the sand fraction in the soil texture decreased, while in the lower stretch, clay and silt prevailed (the stretch from SLB to BEO). Electrical conductivity exhibited an increasing trend from the source to the mouth of the river in relation to changing soil texture. Namely, in sandy soils in the upper and middle stretches, rainfall and flooding easily remove soil particles and soluble salts (Dvořák and Novák 1994). The content of overall soluble salts in the riparian soil was very low ( $EC=0.09-0.33 \text{ dS m}^{-1}$ ), far below the values which can affect growth and metabolic activity and enzyme and membrane activity in most plants ( $EC$  values of  $\geq 4 \text{ dS m}^{-1}$ , Maas and Grattan 1999). Therefore, we estimate that salts have no negative effects on riparian vegetation.

**Table 2** Physical and chemical characteristics of soil samples: electrical conductivity ( $\text{dSm}^{-1}$ ), pH, soil organic matter (%), sand, silt and clay content (%), and texture

	EC	pH	Soil organic matter	Sand	Silt	Clay	Texture
<b>RAD</b>	0.122	8.06	1.75	79.24	16.75	4.01	loamy sand
<b>LIT</b>	0.091	8.17	1.10	90.09	9.29	0.62	sand
<b>CAT</b>	0.123	7.98	1.77	55.34	37.94	6.72	sandy loam
<b>ZAG</b>	0.146	8.26	0.36	75.85	17.64	6.51	loamy sand
<b>JAS</b>	0.151	8.03	1.02	80.76	14.99	4.25	loamy sand
<b>SLB</b>	0.172	7.92	1.38	42.70	42.10	15.20	loam
<b>ZUP</b>	0.184	7.88	1.92	26.08	56.68	17.24	silt loam
<b>SRM</b>	0.333	7.88	1.71	22.26	55.57	22.17	silt loam
<b>SAB</b>	0.249	7.86	1.74	20.49	63.15	16.36	silt loam
<b>BEO</b>	0.203	7.82	2.90	7.91	64.75	27.34	silty clay loam

### 3.2. Spatial distribution of PTE total and bioavailable content in soils

Data on total trace element content in the riparian soil is shown in Table 3. In order to estimate the environmental status of riparian soils, several guidelines – the mean values of the

background levels of trace metals in two common soil types worldwide by Kabata-Pendias (2011), background values in European soils as proposed by Gawlik and Bidoglio (2006), and the critical range for plants by Alloway (2013) – were used. Concentrations above the critical range for plants are denoted in bold.

In the lower stretch of the river, from ZUP to BEO, total As content was higher than mean concentrations in global soils, which are generally below  $10 \text{ mg kg}^{-1}$  (Adriano 2001; Mench et al. 2009; Kabata-Pendias 2011); however, all the measured As levels in soils were below the proposed critical range for plants ( $20\text{-}50 \text{ mg kg}^{-1}$ , Alloway 2013). The availability ratio (AR) showed bioavailable DTPA fractions for As below 1%, indicating its low availability in the soils (Karak et al. 2011). Specifically, with the weak alkaline to alkaline soil conditions ( $\text{pH} > 7$ ) and the low clay and organic matter content, soluble As can leach into the deeper soil layers (Kabata-Pendias 2011; Alloway 2013).

Total Cd content in soil samples was  $< \text{DL}$  at all the sampling sites except BEO, where the level was  $0.98 \text{ mg kg}^{-1}$ , which is double the mean values for global soils ( $0.37\text{-}0.45 \text{ mg kg}^{-1}$ , Kabata-Pendias 2011). DTPA bioavailable content of Cd was only found at BEO, with this bioavailable fraction amounting to 43.82 % of total Cd. Because the total Cd content and the proportion of the available Cd fraction at this site is high, Cd could potentially represent an environmental burden.

In the upper stretch of the river, total Cr content fell within the range of mean values, while further downstream at ZUP, SRM, SAB and BEO, it was significantly higher than the average for global soils ( $47\text{-}51 \text{ mg kg}^{-1}$ , Kabata-Pendias 2011). Likewise, levels above the background values for European soils were measured at ZUP, SRM, SAB and BEO, probably due to a higher share of fine granulometric fractions in the soils from these sampling sites on the one hand and the impact of the geological substrate in the lower stretch of the Sava (Marković et al. 2018; Pavlović et al. 2019) on the other. Although the content of Cr at these sampling sites was above the critical range for plants ( $75\text{-}100 \text{ mg kg}^{-1}$ , Alloway 2013), the level of available Cr in DTPA solutions was  $< \text{DL}$  due to its low solubility at  $\text{pH} > 4$ , with complete precipitation occurring at  $\text{pH} > 5.5$  (Fuentes et al. 2006; Walter et al. 2006; Alloway 2013).

At CAT, as well as in the lower stretch of the river from ZUP to BEO, Cu content was higher than the mean values for global soils ( $13\text{-}23 \text{ mg kg}^{-1}$ , Kabata-Pendias 2011), but lower

than the background values for European soils (Gawlik and Bidoglio 2006). Levels above the copper threshold of  $100 \text{ mg kg}^{-1}$ , proposed by numerous scientific studies to denote polluted soils (Baize 1997; Adriano 2001), were measured at CAT. Moreover, copper at CAT ( $134.56 \text{ mg kg}^{-1}$ ) was above the critical range for plants ( $>125 \text{ mg kg}^{-1}$ , Alloway 2013). Elevated copper in this area is anthropogenically induced. Namely, CAT is located in the Posavska wine region, where fungicides are often used in grapevine cultivation, and this usage can cause the accumulation of Cu in soils, which leads to contamination of the environment and potential toxicity to plants (Fan et al. 2011; Miotto et al. 2017). Our previous research established that copper in the upper stretch of the river is of anthropogenic origin (Marković et al. 2018; Pavlović et al. 2019). Bearing in mind the fact that Cu is barely mobile in different types of soil conditions, elevated Cu levels may persist for a long time (Hutchinson 1979). The availability ratio for Cu varied from 14.07 % to 31.23 %, being highest in the upper stretch of the river. Copper availability in soil is affected significantly by the pH of the soil; specifically, it decreases as pH values increase and is lowest in alkaline conditions when it binds to organic complexes. Moreover, the presence of clay minerals can also lead to Cu binding (Kabata-Pendias 2011; Alloway 2013). However, although the investigated soils were alkaline, the organic matter and clay content in the upper and middle reaches of the river was low, meaning Cu binding is not possible (Adriano 2001; Alloway 2013; Kabata-Pendias 2011). Therefore, conditions at these sites result in the moderate availability of Cu.

Downstream from SLB, total Ni content was higher than the mean values for global soils ( $13\text{-}26 \text{ mg kg}^{-1}$  Kabata-Pendias, 2011). At ZUP and BEO, it was also above the critical range for plants ( $> 100 \text{ mg kg}^{-1}$ , Alloway, 2013), while at ZUP, SRM, SAB and BEO, it was higher than the background values for European soils ( $30\text{-}75 \text{ mg kg}^{-1}$ , Gawlik and Bidoglio 2006). Our earlier studies on the Sava revealed elevated Ni levels in riparian soils (Marković et al. 2018), which, similar to Cr, can be the result of the effects of the geological substrate in the lower reaches of the river, i.e. the Central Dinaric Ophiolite Belt, alluvial flooding by the River Bosna (Grba et al. 2015) at ZUP, and serpentine alluvial flooding by the River Kolubara from Mts. Maljen and Rudnik (Mrvić et al. 2009; Čakmak et al. 2018) at BEO. In general, low Ni availability is the result of alkaline conditions and the low content of organic matter (Bloomfield 1981; Alloway 2013). In terms of ZUP and BEO, while total Ni content in soils

was found to be above the critical range for plants, its availability is the lowest at these sites due to its binding to organic components and clay minerals.

At CAT, SRM, SAB and BEO, Pb levels were higher than the mean values for global soils (22-28 mg kg<sup>-1</sup> Kabata-Pendias, 2011; Alloway 2013), but far lower than the range proposed for European soils (Gawlik and Bidoglio 2006) and the critical range for plants proposed by Alloway (2013). The availability ratio in the soil in the upper stretch of the river was higher than in the middle and lower reaches, but in general it was low. Although the fine granulometric fraction may be the cause of the elevated Pb levels in soils at sites downstream from SRM, the alkaline substrate leads to its low availability (Adriano et al. 2004).

At the sites in the lower stretch of the river, as well as at CAT, total Zn content was higher than the mean values for global soils (45-60 mg kg<sup>-1</sup>, Kabata-Pendias, 2011) and within the critical range for plants (70-400 mg kg<sup>-1</sup>, Alloway, 2013). According to the calculated AR, Zn availability in soil is low (2.95 % - 7.10 %) (Table 3), which is mostly a result of the alkaline soil, the low organic matter content and the soil's predominantly sandy structure (Hafeez et al. 2013).

### *3.3 Comparison of PTE levels in riparian soils with reported studies*

The environmental status of the River Sava's riparian soils is comparable to other moderately polluted rivers in Serbia and in Europe. For instance, similar As and Pb levels were reported in earlier studies on the riparian soils of the Sava (Halamić et al. 2003; Marković et al. 2018), its tributary – the Kolubara (Čakmak et al. 2018), the Danube (Pavlović et al. 2016), and the Velika Morava (Jakovljević et al. 1997). Levels of arsenic were found to be lower than those measured in the riparian soils of the Elbe (Schulz-Zunkel et al. 2013) and the Guadiamar (Domínguez et al. 2016), but higher in comparison to earlier findings on the Sava (Pavlović et al. 2019). Cadmium and chromium levels in this study were lower than those reported in one of our previous studies (Marković et al. 2018) and in a study by Vertačnik et al. (1995) and Pavlović et al. (2019). Copper content was also found to be lower in comparison to earlier findings for the Danube (Pavlović et al. 2016), the Elbe (Schulz-Zunkel et al. 2013) and the Guadiamar (Dominguez et al. 2016). Likewise, lead content was lower than in the Elbe (Schulz-Zunkel et al. 2013) and the Guadiamar (Domínguez et al. 2016), while zinc levels

were lower in comparison to earlier findings on the Sava (Vertačnik et al. 1995; Marković et al. 2018). However, Cd and Cu levels were found to be higher than those in the riparian soils of the Velika Morava (Jakovljević et al. 1997) and the Kolubara (Čakmak et al. 2018). The copper range was also found to be higher in comparison to earlier findings for the River Sava (Halamić et al. 2003), while the nickel range was higher in comparison to results obtained for the Danube (Pavlović et al. 2016), the Kolubara (Čakmak et al. 2018), and the Elbe (Schulz-Zunkel et al. 2013).

**Table 3** Total ('aqua regia') concentrations, DTPA-extractable concentrations (mg kg<sup>-1</sup>), and the availability ratio (%) of PTEs in soils where *U. glabra* trees were sampled in the River Sava's riparian zone (mean ± standard deviation, range from minimum to maximum measured values). The availability of PTEs in soil was assessed using the Availability ratio index (the ratio between the bioavailable DTPA-extractable content of the element and its total content in soil; Massas et al. 2013)

Site/Element	Content		As	Cd	Cr	Cu	Ni	Pb	Zn
RAD	Total	Mean	3.13±0.85	<LoQ	21.87±0.7	16.27±0.49	15.45±0.44	24.72±1.31	59.89±0.97
		Range	2.30-4.17	<LoQ	20.90-22-36	15.95-16.91	14.88-15.79	23.13-26.01	58.96-61.08
	DTPA-extractable	Mean	<LoQ	<LoQ	<LoQ	5.08±0.21	1.03±0.05	4.66±0.11	4.25±0.11
		Range	<LoQ	<LoQ	<LoQ	4.88-5.33	0.98-1.09	4.54-4.81	4.15-4.38
	Availability ratio		/	/	/	31.23	6.67	18.85	7.10
LIT	Total	Mean	5.39±0.26	<LoQ	21.09±0.06	16.46±0.15	12.16±0.00	11.26±0.74	56.87±0.29
		Range	5.06-5.56	<LoQ	21.01-21.13	16.27-16.55	12.16-12.16	10.57-12.18	56.52-57.15
	DTPA-extractable	Mean	<LoQ	<LoQ	<LoQ	2.35±0.09	0.26±0.02	2.93±0.33	1.68±0.04
		Range	<LoQ	<LoQ	<LoQ	2.27-2.45	0.24-0.28	2.71-3.51	1.64-1.72
	Availability ratio		/	/	/	14.28	2.14	26.03	2.95
CAT	Total	Mean	8.24±0.24	<LoQ	35.69±0.21	<b>134.56±0.84</b>	20.19±0.06	29.19±2.30	99.12±0.47
		Range	8.04-8.53	<LoQ	35.45-35.93	133.92-135.64	20.15-20.26	27.27-32.11	98.59-99.64
	DTPA-extractable	Mean	0.01±0.01	<LoQ	<LoQ	30.29±1.67	0.51±0.01	4.02±1.80	5.74±0.13
		Range	0.00-0.03	<LoQ	<LoQ	28.31-30.89	0.49-0.53	2.79-7.20	5.54-5.88
	Availability ratio		0.12	/	/	22.51	2.35	13.77	5.79
ZAG	Total	Mean	5.02±0.63	<LoQ	22.82±0.12	16.12±0.05	12.22±0.06	16.00±0.52	51.41±0.00
		Range	4.26-5.65	<LoQ	22.66-22.90	16.06-16.53	12.15-12.25	15.39-16.54	51.41-51.41
	DTPA-extractable	Mean	0.01±0.01	<LoQ	<LoQ	2.32±0.16	0.32±0.01	1.77±0.14	3.25±0.10
		Range	0.00-0.03	<LoQ	<LoQ	2.10-2.48	0.31-0.33	1.62-1.94	3.15-3.39
	Availability ratio		0.20	/	/	14.39	2.62	11.06	6.32
JAS	Total	Mean	4.73±0.05	<LoQ	23.45±0.06	11.96±0.09	13.23±0.00	19.26±0.26	52.47±0.14
		Range	4.67-4.77	<LoQ	23.41-23.53	11.87-12.06	13.23-13.23	18.99-19.56	52.33-52.64
	DTPA-extractable	Mean	0.02±0.01	<LoQ	<LoQ	1.99±0.04	0.25±0.01	1.24±0.03	3.35±0.11
		Range	0.01-0.03	<LoQ	<LoQ	1.95-2.05	0.24-0.27	1.22-1.28	3.25-3.53

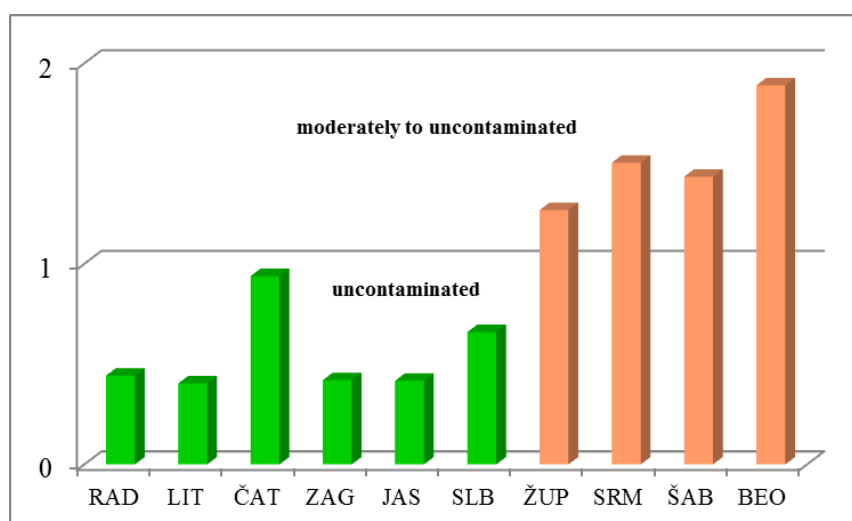


	Availability ratio		0.42	/	/	16.63	1.89	6.44	6.39
SLB	Total	Mean	6.88±0.21	<LoQ	51.43±0.06	20.87±0.09	32.97±0.10	20.06±1.22	57.20±0.29
		Range	6.75-7.15	<LoQ	51.35-51.47	20.77-20.96	32.86-33.07	19.22-21.64	56.85-57.49
	DTPA-extractable	Mean	0.01±0.01	<LoQ	<LoQ	3.15±0.09	0.47±0.02	1.27±0.03	2.14±0.04
		Range	0.00-0.03	<LoQ	<LoQ	3.08-3.27	0.46-0.49	1.25-1.30	2.07-2.19
	Availability ratio		0.15	/	/	15.10	1.43	6.33	3.74
ZUP	Total	Mean	11.58±0.27	<LoQ	<b>123.01±0.70</b>	36.18±0.00	<b>109.75±0.15</b>	25.52±0.87	96.93±0.33
		Range	11.32-11.91	<LoQ	122.17-123.73	36.18-36.18	109.57-109.89	24.40-26.13	96.55-97.28
	DTPA-extractable	Mean	0.01±0.01	<LoQ	<LoQ	5.34±0.09	1.39±0.03	2.66±0.04	3.98±0.06
		Range	0.00-0.03	<LoQ	<LoQ	5.20-5.43	1.34-1.42	2.59-2.70	3.89-4.04
	Availability ratio		0.26	/	/	14.76	1.27	10.42	4.11
SRM	Total	Mean	15.47±3.15	<LoQ	<b>114.63±0.49</b>	37.95±0.23	95.34±1.87	41.96±0.67	144.94±0.42
		Range	13.29-19.53	<LoQ	114.15-115.23	37.76-38.24	92.92-96.65	41.30-42.80	144.49-145.43
	DTPA-extractable	Mean	0.01±0.01	<LoQ	<LoQ	5.34±0.12	1.22±0.02	5.59±0.09	6.17±0.08
		Range	0.00-0.02	<LoQ	<LoQ	5.24-5.51	1.20-1.25	5.51-5.72	6.10-6.26
	Availability ratio		0.07	/	/	14.07	1.28	13.32	4.26
SAB	Total	Mean	14.99±0.27	<LoQ	<b>105.26±0.33</b>	37.68±0.13	94.00±2.17	37.21±1.70	141.05±0.38
		Range	14.66-15.25	<LoQ	104.94-105.66	37.55-37.84	92.15-96.74	35.01-38.36	140.56-141.30
	DTPA-extractable	Mean	0.01±0.01	<LoQ	<LoQ	6.77±0.07	1.22±0.01	4.85±0.10	7.90±0.09
		Range	0.00-0.02	<LoQ	<LoQ	6.67-6.86	1.21-1.24	4.74-4.98	7.82-8.02
	Availability ratio		0.07	/	/	17.97	1.30	13.04	5.60
BEO	Total	Mean	18.82±0.79	0.98±0.07	<b>116.74±2.44</b>	73.62±0.69	<b>103.15±1.69</b>	56.75±1.36	214.26±1.93
		Range	17.83-19.51	0.90-1.02	113.60-118.50	72.85-74.38	101.91-105.32	55.40-58.40	211.87-216.09
	DTPA-extractable	Mean	0.01±0.01	0.43±0.01	<LoQ	12.39±0.46	1.39±0.04	6.77±0.12	11.95±0.22
		Range	0.01-0.03	0.43-0.44	<LoQ	11.73-12.89	1.34-1.44	6.61-6.87	11.71-12.18
	Availability ratio		0.05	43.82	0.00	16.83	1.35	11.93	5.58
Average range for global soils <sup>a</sup>			4.4-8.4	0.37-0.45	47-51	13-23	13-26	22-28	45-60
Background for European soils <sup>b</sup>			-	1-3	50-100	50-140	30-75	50-300	150-300
Critical range for plants <sup>c</sup>			20-50	>2.5	75-100	60-125	>100	>100	70-400

<sup>a</sup>Kabata-Pendias 2011; <sup>b</sup>Gawlik and Bidoglio 2006; <sup>c</sup>Alloway 2013

### 3.4 Contamination indices

Contamination within the study area was also varied, with uncontaminated soils ( $PLI \leq 1$ ) in the upper stretch from RAD to SLB and contaminated soils ( $PLI > 1$ ) in the lower stretch from ZUP to BEO, while RI values placed all sites in the category of low risk from PTE contamination (Fig. 2, Table 4). Our findings confirm those from previous studies on the River Sava which place it in the category of moderately polluted rivers (Milačić et al. 2010; Vidmar et al. 2017; Marković et al. 2018).



**Fig. 2** Assessment of overall soil contamination levels at the investigated sites on the basis of the pollution load index (PLI)

**Table 4** Individual ( $E_i$ ) and total potential ecological risk ( $R_i$ ) from PTE contamination in riparian soils in the study area

Site/Element	As	Cd	Cr	Cu	Ni	Pb	Zn	$R_i$	
	$E_i$								
<b>RAD</b>	2.72	0.00	0.60	3.37	1.87	2.81	0.65	12.02	Low
<b>LIT</b>	4.68	0.00	0.58	3.41	1.47	1.28	0.62	12.04	Low
<b>CAT</b>	7.14	0.00	0.98	27.89	2.44	3.31	1.08	42.86	Low
<b>ZAG</b>	4.36	0.00	0.63	3.34	1.48	1.82	0.56	12.18	Low
<b>JAS</b>	4.10	0.00	0.65	2.48	1.60	2.19	0.57	11.59	Low
<b>SLB</b>	5.97	0.00	1.42	4.33	3.99	2.28	0.62	18.60	Low
<b>ZUP</b>	10.05	0.00	3.39	7.50	13.28	2.90	1.06	38.17	Low
<b>SRM</b>	13.42	0.00	3.16	7.87	11.53	4.76	1.58	42.32	Low

<b>SAB</b>	13.00	0.00	2.90	7.81	11.37	4.23	1.54	40.85	Low
<b>BEO</b>	16.32	43.24	3.22	15.26	12.48	6.44	2.34	99.31	Low

### 3.5 PTE levels in plant

The contents of PTEs measured in plant material are presented in Table 5 with those that are considered to be toxic for plants denoted in bold.

Toxic levels of As ( $>5 \text{ mg kg}^{-1}$ , McBride 1994; Kabata-Pendias 2011) were measured in leaves from ZAG and in root samples from ZAG, JAS and SAB, despite the low content of DTPA-extracted fraction. Namely, enhanced plant uptake is species-specific, but can also be a result of dynamic soil reactions affecting As biogeochemical behaviour, such as oxidation-reduction, sorption-desorption, precipitation/dissolution, and volatilization, which can contribute to As transformation and speciation in the rhizosphere soil in the riparian zone (Fitz and Wenzel 2002; Alloway 2013). This is confirmed by a comparison with other species growing in riparian habitats, such as *Salix* species (Zimmer et al. 2012; Delplanque et al. 2013; Pavlović et al. 2016) and *Populus alba* L. (Madejón et al. 2004), which accumulated lower amounts of As. At the same time, the levels of As measured in roots and leaves were similar to those for *U. laevis* from uncontaminated sites, obtained by Budzyńska et al. (2017) and Mleczek et al. (2017), also with a higher content in roots than in leaves.

Normal Cd levels in plants are considered to be in the range of  $0.05\text{--}0.2 \text{ mg kg}^{-1}$  (Kabata-Pendias 2011; van der Ent et al. 2013), while toxic levels are  $>5 \text{ mg kg}^{-1}$  (McBride 1994; Mrvić et al. 2009; Kabata-Pendias 2011; Alloway 2013; van der Ent et al. 2013). All the Cd levels measured in root and leaf samples were elevated compared to normal levels in plants, but lower in comparison to different *Salix* and *Populus* species, which can accumulate considerable amounts of Cd and Zn in their aboveground organs (Vervaeke et al. 2003; Meers et al. 2003, 2005; Madejón et al. 2004; Bedell et al. 2009; Zimmer et al. 2012; Delplanque et al. 2013; Pavlović et al. 2016). Likewise, they were lower than in *Ulmus carpinifolia* Gled. leaves (Miri et al. 2016) from urban habitats and the leaves and roots of *Ulmus pumila* L. (Saba et al. 2015; Yakun et al. 2016) growing in soils contaminated by industrial activities.

Chromium content  $>5 \text{ mg kg}^{-1}$  is considered to be toxic for plants (McBride 1994; Cicek and Koparal 2004; Kabata-Pendias 2011), while some authors consider concentrations  $>1 \text{ mg}$

kg<sup>-1</sup> as toxic (Mrvić et al. 2009; Vamerali et al. 2010). Following these recommendations, it can be concluded that *U. glabra* accumulated toxic levels of Cr in leaves at CAT, JAS and SLB and in roots at all sampling sites except LIT. In general, chromium is slightly available to plants and not easily translocated within plants; thus, it is mainly concentrated in roots (Kabata-Pendias 2011), as confirmed by our results. The chromium levels measured in the leaves and roots of *U. glabra* in the present study were higher than those previously reported for *U. pumila* (Saba et al. 2015; Yakun et al. 2016) growing in polluted soils, as well as for different *Salix* species growing in riparian areas of the River Danube (Pavlović et al. 2016).

Copper is one of the seven micronutrients indispensable for plant growth and usually 5–30 mg kg<sup>-1</sup> of Cu in plant tissues is regarded as adequate (Adriano 2001; Pugh et al. 2002; Kabata-Pendias 2011). As far as Cu accumulation in elm vegetative organs is concerned, it is clear that the content of this element fell within a range considered normal in plants; however, in a large number of samples, its levels were close to the range of deficiency because the binding of Cu by soils is related to the formation of organic complexes and is highly dependent on soil pH, with its solubility decreasing at about pH >7 (Kabata-Pendias 2011). Copper content in the analysed plant samples was higher than earlier findings for *U. carpinifolia* (Miri et al. 2016) and *U. pumila* (Yakun et al. 2016) growing at uncontaminated sites. Similar Cu levels were measured in *P. alba* (Madejón et al. 2004) and *Salix viminalis* L. (Vervaeke et al. 2003), but were lower in comparison to other species of the genus *Salix* from riparian forests (Delplanque et al. 2013; Pavlović et al. 2016).

According to McBride (1994), Kabata-Pendias (2011) and Alloway (2013), normal Ni levels in plants are in the range of 0.1-5 mg kg<sup>-1</sup>, while toxic levels are >10 mg kg<sup>-1</sup>. The obtained results showed that Ni content in leaves was in a range considered normal in plants. Elevated Ni levels in root samples were found at JAS, SLB, ZUP, SRM and BEO. Compared to similar research, the Ni levels measured in *U. glabra* leaves were higher than those previously reported for *U. glabra* (Baslar et al. 2009) and *U. pumila* (Saba et al. 2015; Yakun et al. 2016) growing in urban and industrial zones. The obtained levels are also higher than those reported for *P. alba* (Madejón et al. 2004) and different species of *Salix* (Pavlović et al. 2016) growing in riparian habitats.

Higher Pb accumulation in roots is common in most plants, with as much as 90 % of total Pb being accumulated in the roots of some species, where it is localised mainly in cell walls (Kumar et al. 1995; Piechalak et al. 2002). All the lead levels measured in the leaves and roots of *U. glabra* were in a range that is considered normal in plants ( $< 10 \text{ mg kg}^{-1}$ , Pugh et al. 2002; Kabata-Pendias 2011), except for root samples from JAS, where Pb content was elevated, but did not fall within the toxic range. The lead levels measured in *U. glabra* leaves are higher than those previously reported for *U. glabra* (Baslar et al. 2009), but similar to those for *U. carpinifolia* (Miri et al. 2016). Compared to different species of *Salix*, Pb content in *U. glabra* roots and leaves was higher (Zimmer et al. 2012; Delplanque et al. 2013) or similar (Vervaeke et al. 2003; Pavlović et al. 2016). Similar results were also reported for the leaves of *P. alba* in riparian habitats (Madejón et al. 2004).

According to some authors (Marschner 1995; Karolewski et al. 2005; Mrvić et al. 2009), normal Zn levels in plants are  $>15 \text{ mg kg}^{-1}$ , while others consider a content of  $>20 \text{ mg kg}^{-1}$  as normal (Taiz and Zeiger 2002), or even  $>27 \text{ mg kg}^{-1}$  (Kabata-Pendias 2011), while concentrations  $<20 \text{ mg kg}^{-1}$  are considered as deficient (Kabata-Pendias 2011). Deficient levels were found in both *U. glabra* roots and leaves at most of the examined sites. Zinc deficiency in plants occurs in soils with a pH  $>7$ ; however, a higher proportion of sand in the soil structure can also contribute (Alloway, 2013). Even so, the content of Zn in the leaves of *U. glabra* was higher than that previously reported by Baslar et al. (2009). Similar results for Zn content in roots and leaves were reported by Yakun et al. (2016) for *U. pumila*. On the other hand, higher Zn levels have been reported by Miri et al. (2016) for *U. carpinifolia* leaves, as well as for various *Salix* and *Populus* species, well-known accumulators of Cd and Zn (Vervaeke et al. 2003; Madejón et al. 2004; Bedell et al. 2009; Zimmer et al. 2012; Delplanque et al. 2013; Pavlović et al. 2016).

**Table 5** Concentrations of PTEs (mg kg<sup>-1</sup> dry matter) in the roots and leaves of *U. glabra* trees from the riparian forests of the River Sava

Site	As		Cd		Cr		Cu		Ni		Pb		Zn	
	root	leaves	root	leaves	Root	leaves	root	leaves	Root	leaves	root	leaves	Root	leaves
<b>RAD</b>	1.62 ±0.51	2.35 ±1.81	<DL	<DL	<b>5.20</b> <b>±0.20</b>	<DL	5.27 ±0.78	6.37 ±0.14	2.74 ±0.44	0.12 ±0.11	4.64 ±0.33	<DL	2.65 ±0.13	3.89 ±0.32
<b>LIT</b>	<DL	4.31 ±1.26	<DL	<DL	0.97 ±0.30	<DL	6.57 ±0.67	5.86 ±0.14	0.32 ±0.11	0.20 ±0.00	1.82 ±0.54	<DL	5.86 ±0.12	11.08 ±0.13
<b>CAT</b>	1.99 ±0.61	1.79 ±0.32	<DL	<DL	2.08 ±0.49	1.10 ±0.00	6.90 ±0.98	6.13 ±0.27	<DL	0.69 ±0.11	1.46 ±0.42	1.78 ±0.18	13.83 ±0.37	15.48 ±0.50
<b>ZAG</b>	<b>5.25</b> <b>±0.47</b>	<b>5.55</b> <b>±0.78</b>	<DL	<DL	1.41 ±0.12	<DL	6.13 ±0.73	6.36 ±0.07	<DL	<DL	<DL	<DL	14.04 ±1.38	13.00 ±0.19
<b>JAS</b>	<b>5.64</b> <b>±1.83</b>	2.64 ±0.54	0.98 ±0.00	0.24 ±0.00	<b>14.69</b> <b>±0.33</b>	4.62 ±0.21	11.78 ±0.60	8.09 ±0.24	8.85 ±0.22	3.79 ±0.17	12.19 ±0.60	4.07 ±0.36	14.61 ±0.29	30.45 ±0.22
<b>SLB</b>	2.18 ±0.28	3.97 ±0.76	0.49 ±0.00	0.25 ±0.00	<b>11.50</b> <b>±1.55</b>	<b>5.78</b> <b>±0.82</b>	8.52 ±0.17	8.50 ±0.00	5.64 ±0.54	5.61 ±0.22	5.20 ±1.38	3.94 ±0.28	10.33 ±0.10	25.09 ±0.31
<b>ZUP</b>	2.30 ±0.28	1.88 ±0.18	<DL	<DL	<b>7.85</b> <b>±0.33</b>	0.53 ±0.11	6.06 ±0.42	5.97 ±0.14	6.61 ±0.17	4.96 ±0.22	3.12 ±0.12	<DL	16.75 ±0.30	23.97 ±0.38
<b>SRM</b>	4.18 ±0.96	2.26 ±0.39	0.74 ±0.35	<DL	<b>6.04</b> <b>±1.63</b>	<LoQ	10.03 ±0.28	6.90 ±0.09	5.73 ±0.76	2.91 ±0.11	3.86 ±0.94	1.00 ±0.12	25.32 ±0.34	16.03 ±0.42
<b>SAB</b>	<b>6.57</b> <b>±0.18</b>	1.58 ±0.12	0.49 ±0.00	<DL	4.90 ±0.36	0.75 ±0.12	6.24 ±0.09	7.13 ±0.22	3.63 ±0.00	3.95 ±0.11	5.72 ±0.12	1.26 ±0.24	26.34 ±0.85	25.35 ±0.87
<b>BEO</b>	3.84 ±0.61	1.58 ±0.24	0.83 ±0.13	<DL	<b>10.40</b> <b>±0.67</b>	0.44 ±0.00	9.93 ±0.15	5.47 ±0.18	8.59 ±0.37	0.81 ±0.00	7.85 ±0.18	1.17 ±0.12	21.43 ±1.19	26.96 ±0.11
<b>Deficit<sup>a</sup></b>	-	-	-	-	-	-	-	2-5	-	-	-	-	-	10-20
<b>Normal range<sup>a</sup></b>	1-1.7		0.05-0.2		0.1-0.5		5-30		0.1-5		0.2-10		27-150	

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<b>Toxic range<sup>a</sup></b>	5-20	5-30	5-30	20-100	10-100	30-300	100-400
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<sup>a</sup>Kabata-Pendias 2011

### 3.6 The potential of elm for the phytostabilisation of PTEs

In polluted areas, the transfer of potentially toxic elements from soils to plants is of great concern. In our study, regression analysis showed that PTE contents in *U. glabra* roots were significantly positively correlated to their respective levels in soil (total and DTPA-extractable), except for Cu and Pb (Table 6). This demonstrates that *U. glabra* plants acquire more PTEs with increasing soil contamination, indicating that PTE levels in soil strongly influence those in plants. The absence of a positive correlation for leaves (except for Cr and Ni) indicates that the highest content of the absorbed PTEs was in the roots. With the proportion of the total PTE content extracted with aqua regia (Table 6) being a good indicator of PTE availability to plants (He and Singh 1993), the BCF was calculated in comparison to their total content in soil.

**Table 6** Correlation coefficients between total and available (DTPA-extracted) PTEs in soil and their content in the roots and leaves of elm (significance levels are \*\*\*  $p < 0.001$ , \*\*  $p < 0.01$ , \*  $p < 0.05$ )

	DTPA available elements in soil		Total element contents in soil	
	Roots	Leaves	Roots	Leaves
As	<b>0.299*</b>	-0.187	<b>0.311*</b>	<b>-0.488***</b>
Cd	<b>0.397**</b>	-0.165	<b>0.402**</b>	-0.165
Cr	/	/	<b>0.452***</b>	<b>0.267*</b>
Cu	-0.112	<b>-0.341**</b>	0.103	<b>-0.330*</b>
Ni	<b>0.343**</b>	<b>0.285*</b>	<b>0.541***</b>	<b>0.616***</b>
Pb	0.086	-0.203	<b>0.371**</b>	0.206
Zn	<b>0.697***</b>	<b>0.277*</b>	<b>0.620***</b>	0.223

Significant values are marked in bold

In order to evaluate the potential of *U. glabra* for the phytoextraction and phytostabilisation of PTEs, the bioaccumulation (BCF) and translocation (TF) factors were applied in this study (Table 7). Excluder plants are of prime importance for the



phytostabilisation of PTEs; their adaptive strategy is based on excluding PTEs from aerial parts by keeping them in their roots, reducing their uptake, or immobilising them in the rhizosphere through the secretion of root exudates (Blaylock and Huang, 2000). For plants to be excluders, the BCF in roots can be higher or lower than 1, but the TF must always be below 1. On the other hand, plant species which have both a BCF and TF >1 are suitable for phytoextraction (Fitz and Wenzel 2002; Yoon et al., 2006).

At the study sites, the level of PTE uptake from the soil via the roots of *U. glabra* was low (BCF <1), except for As at ZAG (1.04) and JAS (1.19). The low BCF values indicate that *U. glabra* cannot be viewed as an accumulator of PTEs, but more an excluder of them, and may be a result of the alkaline soil (Table 2), which results in the immobilisation of PTEs in soil, with which their availability to plants decreases (Shahid et al., 2017). In addition, the mechanical composition of soil, the organic matter content in soil, and the content of Fe and Mn oxides in soil play an important role in the process of PTE uptake via roots. The BCF for Cd was not calculated due to its low levels in soil and roots (<DL at all sites except BEO).

The translocation factor for As, Cr, Ni and Pb was lower than 1 (TF <1) at all sites, except for As at RAD (1.45), ZAG (1.06) and SLB (1.82), Ni at SAB (1.09), and Pb at CAT (1.22). On the other hand, the TF for Cu and Zn at most of the sampling sites was higher than 1 (TF >1). These results indicate the ability of *U. glabra* to activate binding mechanisms in roots, thereby preventing the transport of PTEs from the roots to the aboveground parts of the plants on the one hand, while ensuring the efficient translocation of essential elements (Cu and Zn) on the other, despite their low availability in soil (Table 3).

On the basis of the BCF and TF values obtained, it can be concluded that *U. glabra* shows potential for the phytostabilisation of As, Cu, Ni, and Pb. Additionally, the studied species displays the ability to obtain the essential elements necessary for basic metabolic processes to take place even in unfavourable habitat conditions (high pH, high proportion of sand, and low content of available Cu and Zn). Such an ability indicates that *U. glabra*

might be considered an interesting alternative for the phytoremediation of potentially toxic elements. Up to now, *Ulmus* species have been studied for this purpose focusing on As (Budzyńska et al. 2017). Phytoremediation potential based on metal translocation patterns has revealed the considerable tolerance of riparian tree species, e.g. willows, to heavy metals (Evlard et al., 2014). Similar to *U. glabra*, Cloutier-Hurteau et al. (2013) found that *S. purpurea* accumulates Zn in its leaves and As, Cu, Ni and Pb in its roots. Likewise, Bart et al. (2016) also noted this species' ability to retain Pb in its roots. Meanwhile, *Salix miyabeana* (Desjardins et al. 2015), *Salix caprea* (Chen et al. 2013) and *S. alba* (Pavlović et al. 2016) have been shown to accumulate higher amounts of Zn in their aboveground organs.

**Table 7** Transfer coefficients (BCF and TF) of PTEs from soil to roots and leaves for *U. glabra*

Site		As	Cd	Cr	Cu	Ni	Pb	Zn
RAD	BCF	0.52	0.00	0.24	0.32	0.18	0.19	0.04
	TF	<b>1.45</b>	0.00	0.00	<b>1.21</b>	0.04	0.00	<b>1.47</b>
LIT	BCF	0.00	0.00	0.05	0.40	0.03	0.16	0.10
	TF	0.00	0.00	0.00	0.89	0.62	0.00	<b>1.89</b>
CAT	BCF	0.24	0.00	0.06	0.05	0.00	0.05	0.14
	TF	0.90	0.00	0.53	0.89	0.00	<b>1.22</b>	<b>1.12</b>
ZAG	BCF	<b>1.04</b>	0.00	0.06	0.38	0.00	0.00	0.27
	TF	<b>1.06</b>	0.00	0.00	<b>1.04</b>	0.00	0.00	0.93
JAS	BCF	<b>1.19</b>	0.00	0.63	0.98	0.67	0.63	0.28
	TF	0.47	0.25	0.31	0.69	0.43	0.33	<b>2.09</b>
SLB	BCF	0.32	0.00	0.22	0.41	0.17	0.26	0.18
	TF	<b>1.82</b>	0.50	0.50	<b>1.00</b>	0.99	0.76	<b>2.43</b>
ZUP	BCF	0.20	0.00	0.06	0.17	0.06	0.12	0.17
	TF	0.82	0.00	0.07	0.98	0.75	0.00	<b>1.43</b>
SRM	BCF	0.27	0.00	0.05	0.26	0.06	0.09	0.17
	TF	0.54	0.00	0.00	0.69	0.51	0.26	0.63
SAB	BCF	0.44	0.00	0.05	0.17	0.04	0.15	0.19
	TF	0.24	0.00	0.15	<b>1.14</b>	<b>1.09</b>	0.22	0.96

<b>BEO</b>	BCF	0.20	0.85	0.09	0.13	0.08	0.14	0.10
	TF	0.41	0.00	0.04	0.55	0.09	0.15	<b>1.26</b>

Values >1 are marked in bold

#### 4. Conclusion

In this study, the total contents of all the examined elements in riparian soil were higher than the mean values for global soils, while concentrations of Cr and Ni were higher than the background values for European soils at some sites (for Cr at ZUP, SRM, SAB and BEO and for Ni at ZUP and BEO), which can pose a potential environmental risk. However, the examined soils were characterised by low- to mid-availability of PTEs due to their low solubility, which is linked to the alkaline nature of the examined soils. The exception was Cd at BEO with a high total and available Cd fraction at this site, which could potentially represent an environmental risk.

This study also showed that element translocation from soils to roots and leaves varied greatly from element to element and from site to site. In the roots of *U. glabra*, levels of As at sites along the middle and lower stretches, as well as of Cr in the entire stretch, were in the toxic range for plants. In addition, Zn was in the deficient range in plants growing at sites in the upper and lower stretches of the river. With regard to the bioaccumulation and translocation factors, it can be concluded that *U. glabra* is suitable for the phytostabilisation of As, Cu, Ni, and Pb and could potentially reduce the ecological risks of these elements in the River Sava riparian zone.

Elms are the dominant species in the mixed broadleaf forests of many areas in Europe, mainly distributed near rivers or on floodplains. Accordingly, these species are good candidates for phytoremediation. Although other species of *Ulmus* have been studied for remediation purposes focusing on individual elements, this study is the first on *U. glabra*, providing an insight into the ability of this native tree species to interact with PTEs present in soil in the riparian zone of the large regional river, the Sava.

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## **Author's contributions**

Zorana Mataruga and Snežana Jarić participated in the design of this study, data collection, data interpretation and manuscript preparation. Olga Kostić and Ksenija Jakovljević participated in the design of this study and data collection. Milica Marković contributed to literature research. Miroslava Mitrović and Pavle Pavlović participated in data interpretation and helped in drafting and writing the manuscript, including a critical revision of the manuscript. All the authors have read and approved the final version of the manuscript.

## **Declaration of interest**

The authors declare that they have no competing interests.

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