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Original Research

Determining As, Cd, Cu, Pb, Sb, and Zn in Leaves of Trees Collected near Mining Locations of Malé Karpaty Mts. in the Slovak Republic

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Abstract

The aim of the study was to compare accumulation capacity of selected biogenic and toxic metals (As, Cd, Cu, Pb, Sb, and Zn) in the leaves of 3 tree species (*Alnus glutinosa*, *Salix* sp., and *Sambucus nigra*) from 5 locations in the Malé Karpaty Mountains in the Slovak Republic near Trniansky, Stoličný, and Gidra streams. These observed locations (with accentuation to upper flows of mentioned streams) are characterized by mainly high occurrences of arsenic and antimony as a result of geological bedding and abandoned Sb mining deposits in the Pezinok area. Obtained results confirmed that each tree species in view varied in accumulation capacity for individual metals. While *Salix* sp. indicated in the leaves high levels of As, Cd, and Zn, *Sambucus nigra* accumulated from all spotted trees in the highest amounts mainly lead and antimony. The lowest cadmium accumulation was confirmed for *Alnus glutinosa*. Regression analysis confirmed significantly positive correlations between Pb–Cu ($p<0.001$), Cd–Cu ($p<0.01$), and Pb−Cd ($p<0.01$) concentrations in *Alnus glutinosa* leaves. However, for *Sambucus nigra* there was significantly positive correlation confirmed between As–Sb ($p < 0.001$).

Keywords: *Alnus glutinosa, Salix* sp., *Sambucus nigra*, heavy metal accumulation, bioconcentration factor (BCF)

Introduction

The Malé Karpaty Mountains (Mts.) in the Slovak Republic are characterized by the occurrence of arsenopyrite (FeAsS) and stibnite (also called antimonite, Sb_2S_3) in sites near towns Pezinok and Pernek (Fig. 1), as well as gudmundite (FeSbS) and galenite (PbS) [1-2]. Pezinok, Pernek, and Trnava areas are noted for their significant levels of As, Cd, Pb, Sb, and Zn [3-6]. Two prevailing ore types in the Malé Karpaty Mountains result in mineralization processes. The first type is pyrite pyrrhotite (FeS_2;

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Table 1. List of published data of selected adits in the studied area of Malé Karpaty Mts., mainly with As, Sb, Pb, and Zn occurrence [24].

Name of adit	Creek	(Semi)metal, minerals	Concentration ($\mu g.l^{-1}$ of water, $mg.kg^{-1}$ of soil)	Reference
Sirková	Pezinok	Sb-As minerals	15-33 μg $As.l^{-1}$, 1446 μg $Sb.l^{-1}$, <2 μg $Cu.l^{-1}$, 257 μg $Zn.l^{-1}$ of mine water with pH 6.87	[2, 5, 6]
Pyritová	Pezinok	Sb and As minerals (?)	17-104 μg $As.l^{-1}$, 330654 μg $Sb.l^{-1}$, <2 μg $Cu.l^{-1}$, 24 μg $Zn.l^{-1}$ of mine water with pH 7.99	[2, 5]
Budúcnosť	Pezinok	Sb and As minerals (?)	13-29 μg $As.l^{-1}$, 87150 μg $Sb.l^{-1}$, <2 μg $Cu.l^{-1}$, 81 μg $Zn.l^{-1}$ of mine water with pH 7.48	[2, 5]
	Pezinok		0.36 mg $Cd.kg^{-1}$, 55.2 mg $Pb.kg^{-1}$, 91.8 mg $Cu.kg^{-1}$, 55.7 mg $Zn.kg^{-1}$, 10.7 mg $As.kg^{-1}$ (all higher than limits for agricultural soils)	[4]
	Pezinok		<0.5 mg $Cd.kg^{-1}$, 63.1 mg $Cu.kg^{-1}$, 79.9 mg $Pb.kg^{-1}$, 52.7 mg $Zn.kg^{-1}$, 9760 mg $As.kg^{-1}$, 5900 mg $Sb.kg^{-1}$ of agricultural soil (A horizon); 0.862 mg $Cd.kg^{-1}$, 70.4 mg $Cu.kg^{-1}$, 1733.1 mg $Pb.kg^{-1}$, 181.4 mg $Zn.kg^{-1}$, 17.2 mg $As.kg^{-1}$, 870.2 mg $Sb.kg^{-1}$ of stream sediments	[3]
	Trnava		>0.3 mg $Cd.kg^{-1}$, <60 mg $Pb.kg^{-1}$, <40 mg $Zn.kg^{-1}$, <5 mg $As.kg^{-1}$ of agricultural soil	[4]
	Pernek		100-514 mg $As.kg^{-1}$, 121-894 mg $Sb.kg^{-1}$ of soil; 45-390 mg $As.kg^{-1}$, 48703 mg $Sb.kg^{-1}$ of stream sediment	[6]
	Pernek		0.224 mg $Cd.kg^{-1}$, 38.4 mg $Cu.kg^{-1}$, 21.7 mg $Pb.kg^{-1}$, 177.4 mg $Zn.kg^{-1}$, 12.2 mg $As.kg^{-1}$, 5.1 mg $Sb.kg^{-1}$ of agricultural soil (A horizon); 0.242 mg $Cd.kg^{-1}$, 29.1 mg $Cu.kg^{-1}$, 38.8 mg $Pb.kg^{-1}$, 157.5 mg $Zn.kg^{-1}$, 26.5 mg $As.kg^{-1}$, 20.3 mg $Sb.kg^{-1}$ of stream sediments	[3]

$Fe_{1-x}S$) mineralization, whose exploitation dates back to the late 18th century, and the second is hydrothermal Sb-As-Au mineralization [2]. Two Sb deposits bound to this mineralization were exploited: Pernek (1790-1922) and Kolársky Vrch (1790-1992) near Sirková adit [2]. The most intensive mining period was until 1940, and 15,000 tons of Sb was recovered [7].

Hydrothermal activity in the productive zones of the Malé Karpaty Mts. produced ores with gold-bearing pyrite and arsenopyrite and subsequently also antimony (Sb) ores [8]. Arsenic (As) is a fundamental constituent not only for sulfide mineral arsenopyrite, but also for other minerals such are löllingite (FeAs), realgar (AsS), and orpiment (As_2S_3). The predominant forms of inorganic As in terrestrial and aquatic plant tissues are arsenite (As^{3+}) and arsenate (As^{5+}), but their relative proportions vary among plant species [9-10]. The pH, redox conditions, and Fe-oxide contents in a soil are the most important features controlling As^{3+} adsorption [11]. Although ferrihydrite ($(Fe^{3+})_2O_3x0.5\ H_2O$) is an excellent material for capturing arsenic, its use as a medium for long-term storage of As could be dangerous for its possibility to release arsenic as it ages [8]. Arsenic has a rather long residence time in soils (from 1,000 to 3,000 years) and enriches topsoil horizons by cycling through vegetation, atmospheric deposition, and sorption by soil organic matter [12]. Its availability for uptake by plants is affected by several factors, such as the source, chemical speciation, and soil parameters. It is known that dangerous and toxic nonessential arsenate is capable of replacing biogenic and essential phosphate ions in biological materials. Similarly, arsenite has a high affinity to amino acids such as cysteine, and binds to sulfhydryl groups of enzymatic and structural proteins that play key roles in arsenic toxicity [13].

Ores with Sb include stibnite (also called antimonite, Sb_2S_3) and gudmundite (FeSbS), as well as berthierite ($FeSb_2S_4$), kermesite (Sb_2S_2O), and valentinite (Sb_2O_3). The abandoned Sb mining deposit near Pezinok, Slovakia is a significant source of As and Sb pollution that can be traced in the upper soil horizons several kilometres down the Blatina flow [8]. The pore solutions in the impoundment body above Pezinok contain up to 81 mg $As.l^{-1}$ and 2.5 mg $Sb.l^{-1}$. As and Sb concentrations in this area vary from 1.77 up to 9,760 and 0.03 up to 5,900 $mg.kg^{-1}$ soil, respectively. Both elements tend to concentrate in the upper soil horizons A0 and A. As described by Majzlan et al. [8], the contamination is probably also spread by mobilization of the fine-grained Fe-, Sb-, and As-rich oxides during periods of rain or snowmelt.

The results from studies of agricultural soils, stream sediments, or mine waters in Pezinok, Pernek, and Trnava are introduced in Table 1. However, results dealing with the accumulation of these metals in tree leaves from these locations were mentioned only in several conference materials [14-15]. The trees and shrubs growing on studied locations belong to perennial vegetation and can still absorb metals from the soil and

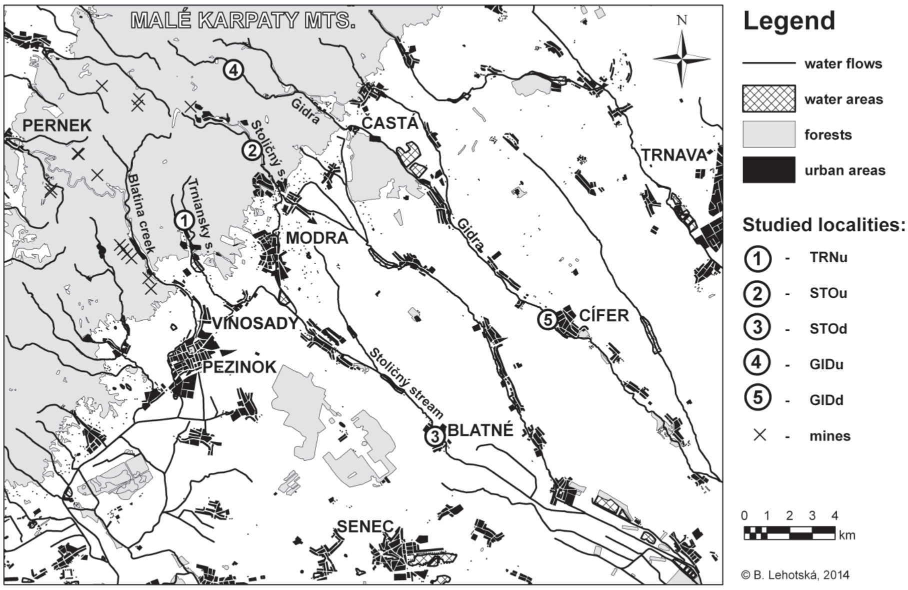


Fig. 1. Map of studied locations: ① TRNu – upper flow of Trniansky stream in Kučišdorf valley near Pezinok Village, ② STOu – upper flow of Stoličný stream above Modra-Harmónia, ③ STOd – downflow of Stoličný Stream in Blatné village, ④ GIDu – upper flow of Gidra near Píla village, and ⑤ GIDd – downflow of Gidra near Cífer village.

accumulate them in their tissues. During autumn, leaves falling from trees decomposing and absorbed metals could be returned from leaves to the upper layer of soil. Leaf litter with direct soil contact can act as a temporary pool for soil metals [16]. Earthworm bioturbation influences the mobility and phytoavailability of metals and could affect the proximity of pollutants to the roots and soil organic matter, too [17-18]. The metals content in the surrounding area strongly depends on geological bedding and anthropogenic activities in the location and markedly reflects in biota. The aim of this study was to compare the levels of selected (semi)metals – both toxic (As, Cd, Pb, Sb) and biogenic (Cu, Zn) in the leaves of the following plant species: European

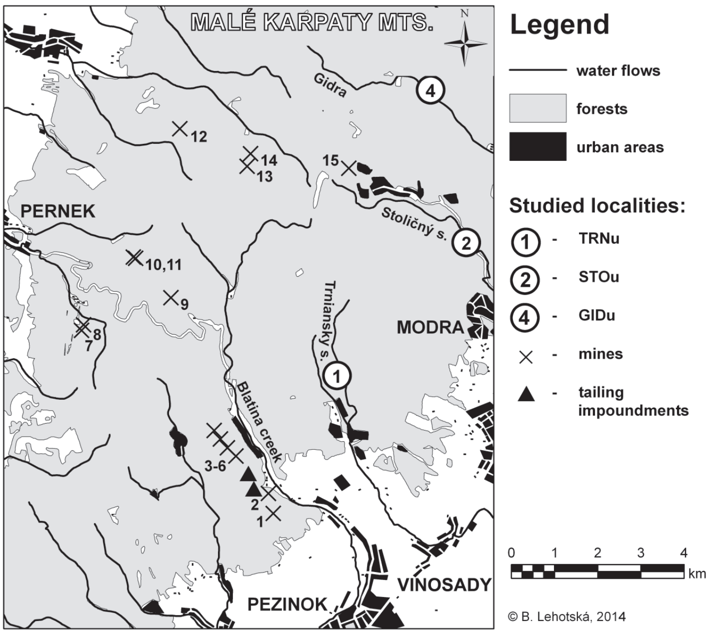


Fig. 2. The map of selected mines in the studied area of Malé Karpaty Mts., mainly with As, Sb, Pb, and Zn occurrence [2, 5, 7, 22]; the names of mines and occurrence of minerals: 1 – Sirková (Sb-As minerals); 2 – Budúcnosť (Sb-As minerals); 3 – Pyritová (Sb-As minerals); 4 – Nová Alexander (SbAs minerals); 5 – Antimónová (Sb-As minerals); 6 – Medzipatrová (Sb-As minerals); 7 – unnamed adit No. 6 (Pb and Zn minerals); 8 – Mikuláš (Pb and Zn minerals); 9 – Čmele II (pyrite – FeS_2, Sb minerals); 10 – Florián (pyrite, Sb minerals); 11 – Valentín (pyrite, Sb minerals); 12 – unnamed adit No. 3 (Pb minerals, galenite); 13 – unnamed adit No. 7 (Sb minerals); 14 – Trojičná (Sb, FeS minerals); and 15 – unnamed adit No. 8 (Sb minerals).

Table 2. The description of the studied locations with their GPS coordinates (N – north geographic latitude, E – east geographic longitude).

Location	GPS coordinates	Description
TRNu	48°20´1.42 N, 17°15´29.27 E	Upper flow of Trniansky stream in Kučišdorf valley near Pezinok village
STOu	48°22´22.02 N, 17°17´46.36 E	Upper flow of Stoličný stream upper of Modra-Harmónia village
STOd	48°15´54.75 N, 17°25´25.57 E	Downflow of Stoličný stream in Blatné village
GIDu	48°23´49.27 N, 17°19´0.89 E	Upper flow of Gidra near Píla village
GIDd	48°19´1.20 N, 17°28´44.70 E	Downflow of Gidra near Cífer village

black alder (*Alnus glutinosa*), willow (*Salix* sp.), and common elder (*Sambucus nigra*). These plants are quick-growing trees and shrubs generally present in all the selected locations. These trees could concentrate the studied (semi)metals from soil in higher amounts in the leaves. This increased concentration can be determined through bioconcentration factor (BCF). Our study was filled with copper (Cu) and zinc (Zn) determination as elements that are in nature mainly as bivalent forms and could enter into reciprocal interactions with other mostly bivalent toxic metals (i.e., cadmium (Cd) or lead (Pb)) during their uptake by trees. Elderberry (*Sambucus nigra* L.) is a widespread species that grows on sunlight-exposed locations in Europe, Asia, North Africa, and the USA [19]. Black alder is a prevalent tree in Europe, too. Post-agricultural black alder (*Alnus glutinosa*) woods represent the most fertile and the wettest forest habitats among the European temperate forest types [20]. For example, in Poland this tree is often planted on former meadows where agricultural practices have been abandoned because of the permanently wet soils [21].

Arsenic is a priority substance under the European Union's Water Framework Directive (WFD) 2000/60/EC throughout the European Union as well as cadmium (Cd) and lead (Pb). Copper (Cu) is relevant for Slovakia substances based on requirements of the same Water Framework Directive 2000/60/EC [22].

Five locations were selected for determination, and among them 3 are situated directly in the Malé Karpaty Mts. (upper flow of Trniansky, Stoličný, and Gidra streams). The next two locations were at downflows of Stoličný and Gidra streams near Blatné and Cífer villages. Near the source of Stoličný is situated unnamed adit No. 8 with Sb minerals (15 at Fig. 2), and we expected that in the soil near this stream and in this flow arsenic and antimony would be higher than in Gidra.

Material and Methods

Description of the Studied Locations

Five studied locations representing riparian vegetation were chosen on 3 streams, originating in the SW part of Malé Karpaty Mts. (Small Carpathian Mts.) and flowing to the adjacent Danube lowland (Fig. 1, Table 2). Three locations on the upper flows of Trniansky (TRNu), Stoličný (STOu), and Gidra (GIDu) streams are situated in natural, mainly wooded areas of the Malé Karpaty Mts. above the town of Pezinok and the village of Vinosady, the town of Modra, and the village of Častá, respectively. Trniansky stream later connects to Stoličný stream. Two locations located on the agricultural landscape of Danube lowland represent downflows of riparian vegetation of the mentioned streams. The downflow of Stoličný (STOd) is located near Blatné village and the downflow of Gidra (GIDd) near Cífer village (Fig. 1, Table 2).

The Malé Karpaty Mts. are known as an old mining site where several sources of arsenic and antimony contamination might be found [2, 7, 23-24]. These mining sites are shown in Fig. 2. The studied locations were not situated directly in mining sites, but in catchment areas of the introduced streams.

The species composition of riparian tree vegetation is dominated by European black alder (*Alnus glutinosa*) and common ash (*Fraxinus excelsior*) in a tree layer. Presented are also white willow (*Salix alba*), crack willow (*Salix fragilis*), European white elm (*Ulmus laevis*), small-leaved elm (*Ulmus minor*), and field maple (*Acer campestre*). The shrub layer is composed of blood twig dogwood (*Swida sanguinea*), European spindle tree (*Euonymus europaeus*), black elder (*Sambucus nigra*), and others.

Plant Materials and Chemicals

The following woody species were analyzed: European black alder (*Alnus glutinosa* L.) Gaertn., "AlnGlu"), willows (*Salix* sp., "Sal"), and common elder (*Sambucus nigra* L., "SamNig"). Whereas *Salix alba* L. did not grow on all the studied locations, *Salix caprea* L. was collected from the upper flow locations (TRNu, STOu and GIDu) and *Salix fragilis* L. from the downflow locations (STOd, GIDd). The tree and shrub leaves were acquired in 1-3 m height from different parts during October 2011. At each location we collected leaves of 4 individuals for every studied woody species (5-7 leaves per tree, depending on leaf size). All leaves were washed with redistilled water before drying at 60°C and then mechanically homogenized and electrochemically analyzed.

All chemicals were analytical grade. The electrolytes and standards were obtained from Istran, Bratislava,

Table 3. The parameters of sensitivity for electrochemical determination on EcaFlow 150 GLP.

(Semi)metal	Concentration range ($\mu g.dm^{-3}$)	Reproducibility
As	0.5 – 500	4.2% at 50 μg $As.dm^{-3}$
Sb	0.5 – 500	3.2% at 50 μg $Sb.dm^{-3}$
Cd, Cu, Pb, Zn	0.5 – 1000	1.5% at 50 μg $Pb.dm^{-3}$

Slovak Republic, and the Slovak Republic. Nitric acid was purchased from CentralChem, Bratislava, Slovak Republic, and hydrogen peroxide from CONLAC peroxides, Praha, Czech Republic.

Determining Water-Dissolved Soil Parameters

The samples were collected from 20 cm depth of soil followed by air drying at room temperature to constant weight, sieved through a 0.2 cm stainless sieve and well homogenized. The samples were dried 2 hours at 105ºC. Water-dissolved soil parameters (TS, VS, pH, and conductivity) in the studied soils were determined using extraction of 10 g of soil in 100 ml of deionised water with 0.5 ml of HNO_3. Extraction lasted 24 hours at room temperature and subsequently the samples were filtered through a membrane filter with a pore size 0.45 µm (millipore) and total solids (TS), volatile solids (VS), pH, and conductivity were measured. For determining dry matter the next 2 g of soil were used.

Accumulating Heavy (Semi) Metals in the Soil and Leaves

The accumulation of As, Cd, Cu, Pb, Sb, and Zn was determined in tree leaves after their drying at 60ºC. The minimum of 11 mg of leaf dry weight (DW) was mineralized in 5 ml of HNO_3:H_2O_2 mixture (4:1) for 60 minutes at 180ºC in a ZA-1 autoclave (Czech Republic). The minimum of 2 g of soil dried until constant weight were used for mineralization following the same procedure as the dry weight of leaves. After cooling, mineralized samples were diluted up to 25 ml with redistilled water and metals determination by galvanostatic-dissolved chronopotenciometry on an EcaFlow 150 GLP (Istran, Slovak Republic) [25]. This electrochemical method is comparable to the atomic absorption spectrophotometric (AAS) method in precision, accuracy, and sensitivity of the measured results (concentration range and reproducibility of selected (semi)metals in Table 3 originate from application lists of the Istran Company). Each sample was analyzed two times. Bioconcentration factor (BCF) was calculated as follows:

BCF = (M^+ concentration in the leaves) / (M^+ concentration in the soil), where M^+ is studied (semi) metal ion. Both parameters in the BCF ratio are in the same units (mg (M^+).kg^{-1} of dry weight).

Statistical Analyses

Average values with their standard deviations (SD) were plotted with Microsoft Excel 2010 software. The data were subjected to regression analyses that were calculated with formulas inserted in Microsoft Excel. Figures were finalized in Adobe Photoshop Elements 10, and maps using ArcMap, version 9.3.1.

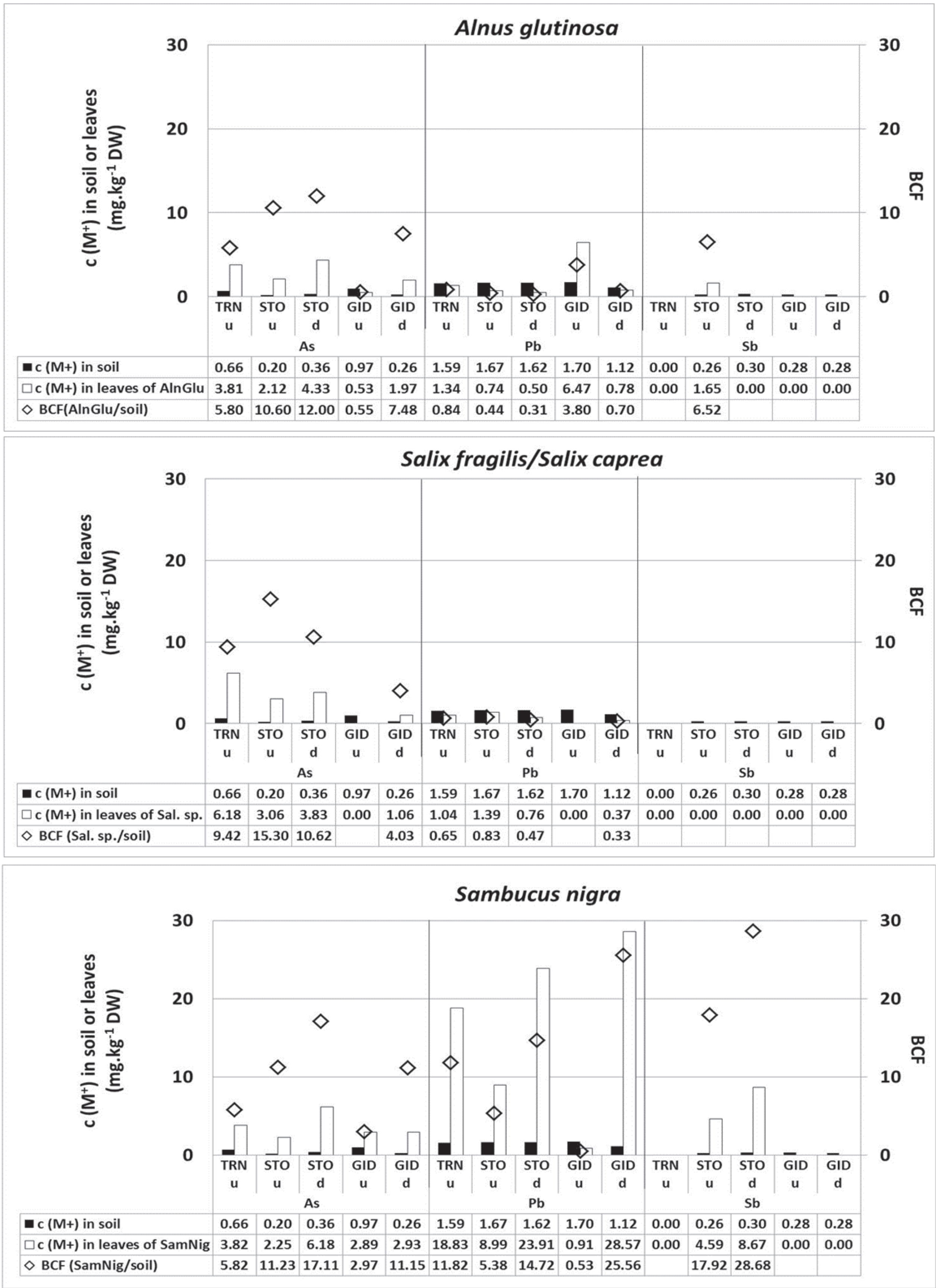
Results and Discussion

The concentrations of As, Cd, Cu, Pb, Sb, and Zn in the tree leaves dry weight are represented on Figs 3(a-b). As and Sb often occur in ensemble in the geological environment. Cd, Cu, Pb, and Zn occur in nature mainly as divalent forms. While Cu and Zn are essential for plants, Cd and Pb are toxic. Toxic nonessential metals in divalent oxidation forms can interact and compete with other essential divalent ions to entry into plants. It is known that different plant species do not transport the same comparable amounts of these metals to leaves.

The whole studied area is known through arsenic- and antimony-elevated occurrence from old mines (Fig. 2). Their concentrations in the water, sediments, or soils are shown in Table 1 and Table 4. Water soil extract from Stoličný upper stream (STOu) location was characterized by very acidic pH value (pH = 5.36)

Table 4. Heavy metals concentrations and water-dissolved parameters in the studied soils from 20 cm depth (nd – not dectected, TS – total solids, VS – volatile solids).

Studied site	TS (%)	VS (%)	pH (H_2O)	Conductivity (H_2O) ($mS.m^{-1}$)	As ($mg.kg^{-1}$ dry soil)	Cd ($mg.kg^{-1}$ dry soil)	Cu ($mg.kg^{-1}$ dry soil)	Pb ($mg.kg^{-1}$ dry soil)	Sb ($mg.kg^{-1}$ dry soil)	Zn ($mg.kg^{-1}$ dry soil)
TRNu	**98.2**	6.2	7.73	**62.6**	0.656	0.007	0.506	1.593	nd	**1.674**
STOu	94.5	4.9	5.36	22.2	0.200	0.004	0.241	1.669	0.256	0.184
STOd	**98.1**	6.1	**7.90**	50.2	0.361	**0.008**	**1.420**	1.624	**0.302**	1.261
GIDu	82.5	**8.2**	7.75	41.4	**0.972**	0.007	0.209	**1.704**	0.285	1.236
GIDd	89.4	4.3	7.48	37.2	0.263	0.007	0.855	1.118	0.280	1.313

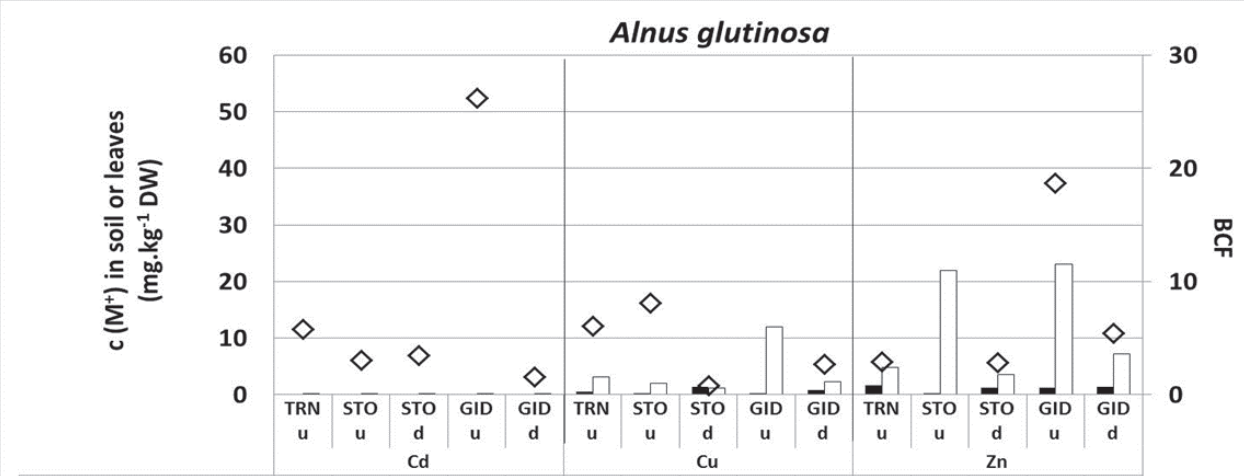


	TRN u	STO u	STO d	GID u	GID d	TRN u	STO u	STO d	GID u	GID d	TRN u	STO u	STO d	GID u	GID d
	As					Pb					Sb				
■ c (M+) in soil	0.66	0.20	0.36	0.97	0.26	1.59	1.67	1.62	1.70	1.12	0.00	0.26	0.30	0.28	0.28
□ c (M+) in leaves of AlnGlu	3.81	2.12	4.33	0.53	1.97	1.34	0.74	0.50	6.47	0.78	0.00	1.65	0.00	0.00	0.00
◇ BCF(AlnGlu/soil)	5.80	10.60	12.00	0.55	7.48	0.84	0.44	0.31	3.80	0.70		6.52			

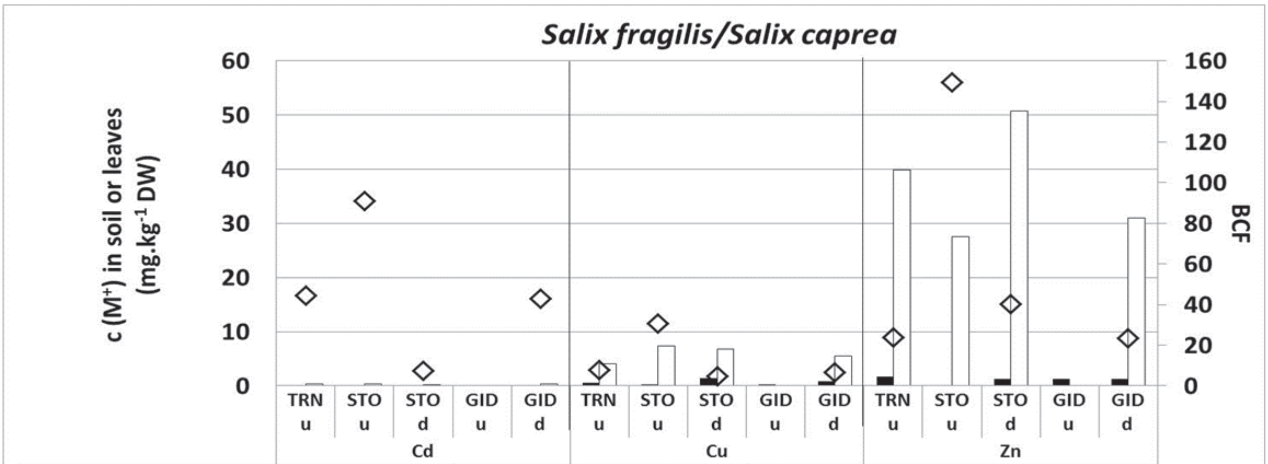
	TRN u	STO u	STO d	GID u	GID d	TRN u	STO u	STO d	GID u	GID d	TRN u	STO u	STO d	GID u	GID d
	As					Pb					Sb				
■ c (M+) in soil	0.66	0.20	0.36	0.97	0.26	1.59	1.67	1.62	1.70	1.12	0.00	0.26	0.30	0.28	0.28
□ c (M+) in leaves of Sal. sp.	6.18	3.06	3.83	0.00	1.06	1.04	1.39	0.76	0.00	0.37	0.00	0.00	0.00	0.00	0.00
◇ BCF (Sal. sp./soil)	9.42	15.30	10.62		4.03	0.65	0.83	0.47		0.33					

	TRN u	STO u	STO d	GID u	GID d	TRN u	STO u	STO d	GID u	GID d	TRN u	STO u	STO d	GID u	GID d
	As					Pb					Sb				
■ c (M+) in soil	0.66	0.20	0.36	0.97	0.26	1.59	1.67	1.62	1.70	1.12	0.00	0.26	0.30	0.28	0.28
□ c (M+) in leaves of SamNig	3.82	2.25	6.18	2.89	2.93	18.83	8.99	23.91	0.91	28.57	0.00	4.59	8.67	0.00	0.00
◇ BCF (SamNig/soil)	5.82	11.23	17.11	2.97	11.15	11.82	5.38	14.72	0.53	25.56		17.92	28.68		

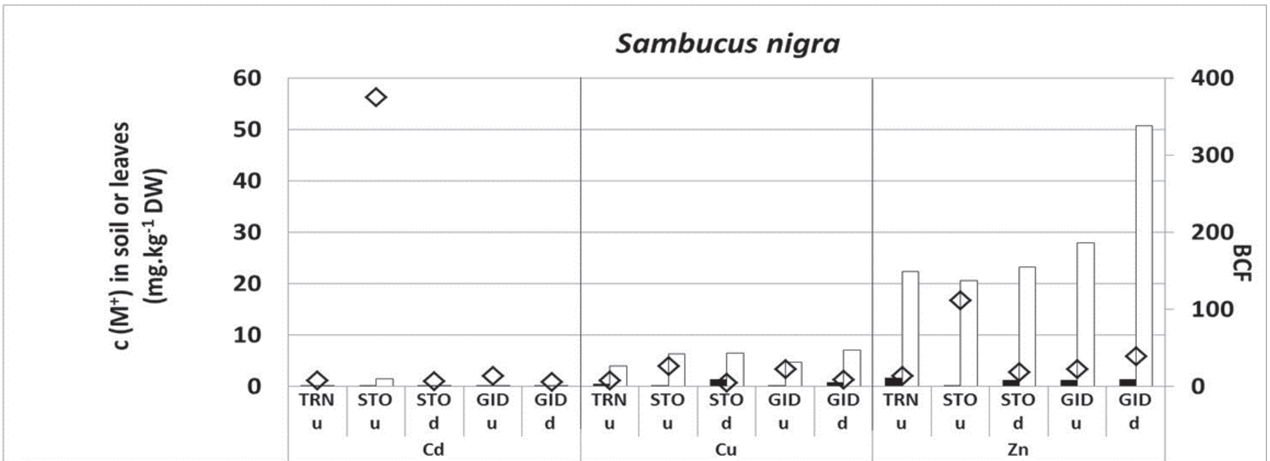
Fig. 3a). Concentrations of As, Pb, and Sb in $mg.kg^{-1}$ of the dry soil or $mg.kg^{-1}$ of dry weight (DW) of leaves in *Alnus glutinosa* (AlnGlu), *Salix* sp. (SalFra/SalCap) and *Sambucus nigra* (SamNig) of the following places: TRNu – the upper flow of Trniansky stream in Kučišdorf valley near Pezinok village, STOu – upper flow of Stoličný stream above Modra-Harmónia village, STOd – downflow of Stoličný stream in Blatné village, GIDu – upper flow of Gidra near Píla village, and GIDd – the downflow of Gidra near Cífer village; BCF – bioconcentration factor, M^+ – (semi)metal ion.



	TRN u	STO u	STO d	GID u	GID d	TRN u	STO u	STO d	GID u	GID d	TRN u	STO u	STO d	GID u	GID d
	Cd					Cu					Zn				
■ c (M+) in soil	0.01	0.00	0.01	0.01	0.01	0.51	0.24	1.42	0.21	0.86	1.67	0.18	1.26	1.24	1.31
□ c (M+) in leaves of AlnGlu	0.04	0.01	0.03	0.18	0.01	3.08	1.94	1.16	11.90	2.32	4.86	21.97	3.56	23.09	7.16
◇ BCF(AlnGlu/soil)	5.74	3.07	3.45	26.23	1.56	6.09	8.07	0.82	56.96	2.71	2.90	119.3	2.82	18.68	5.45



	TRN u	STO u	STO d	GID u	GID d	TRN u	STO u	STO d	GID u	GID d	TRN u	STO u	STO d	GID u	GID d
	Cd					Cu					Zn				
■ c (M+) in soil	0.01	0.00	0.01	0.01	0.01	0.51	0.24	1.42	0.21	0.86	1.67	0.18	1.26	1.24	1.31
□ c (M+) in leaves of Sal. sp.	0.31	0.36	0.06	0.00	0.30	4.03	7.41	6.86	0.00	5.55	39.82	27.51	50.65	0.00	30.97
◇ BCF (Sal. sp./soil)	44.43	91.00	7.44		42.81	7.96	30.73	4.83		6.49	23.79	149.5	40.17		23.58



	TRN u	STO u	STO d	GID u	GID d	TRN u	STO u	STO d	GID u	GID d	TRN u	STO u	STO d	GID u	GID d
	Cd					Cu					Zn				
■ c (M+) in soil	0.01	0.00	0.01	0.01	0.01	0.51	0.24	1.42	0.21	0.86	1.67	0.18	1.26	1.24	1.31
□ c (M+) in leaves of SamNig	0.05	1.50	0.05	0.09	0.04	4.02	6.28	6.41	4.69	7.06	22.28	20.53	23.16	27.89	50.68
◇ BCF (SamNig/soil)	7.43	375.5	6.38	13.14	5.43	7.94	26.06	4.51	22.44	8.25	13.31	111.5	18.36	22.56	38.60

Fig. 3b). Concentrations of Cd, Cu, and Zn in mg.kg^{-1} of the dry soil or mg.kg^{-1} of dry weight (DW) of leaves in *Alnus glutinosa* (AlnGlu), *Salix* sp. (SalFra/SalCap), and *Sambucus nigra* (SamNig) in the following places: TRNu – upper flow of Trniansky stream in Kučišdorf valley near Pezinok village, STOu – upper flow of Stoličný stream above Modra-Harmónia village, STOd – downflow of Stoličný stream in Blatné village, GIDu – upper flow of Gidra near Píla village, and GIDd – downflow of Gidra near Cífer village; BCF – bioconcentration factor, M^+ – (semi)metal ion.

commonly connected with the lowest conductivity, and the lowest As, Cd, Sb, and Zn concentrations in this soil extract (Table 4). No Sb was detected at Trniansky upper stream (TRNu). Soils from the other locations had slightly alkaline pH with higher content of volatile solids (VS). The lower total solids (TS) were observed in the soils from Gidra upper stream (GIDu) but contained the highest amount of VS (Table 4).

Arsenic and antimony were considered the most toxic (semi)metals in the evaluated area. Arsenic together with a few other elements has been classified as one of the priority pollutants by the U.S. Environmental Protection Agency. Since As accumulates during weathering and translocates in colloid fractions, its concentration is usually higher in soil than in parent rocks [26]. Although in observed locations As concentration in the soil was low, it accumulates in the leaves of all selected tree species (Fig. 3a). Only in the leaves of *S. caprea* on Gidra upper stream (GIDu) was it not detected. The highest concentration reached As in the leaves of *A. glutinosa* and *S. nigra* from the STOd location, and for *Salix* sp. from TRNu place (Fig. 3a). While the highest bioconcentration factor BCF was at the same location as the highest As concentration in the leaves *A. glutinosa* and *S. nigra* (STOd), the highest BCF for *Salix* sp. were obtained from STOu location with the lowest As in the soil (Fig. 3a). Majzlan et al. [8] observed higher As content in Blatina Creek above the impoundments near Pezinok, which contains 2-12 μg $As.l^{-1}$ and 2-5 μg $Sb.l^{-1}$. Mentioned impoundments are close to the TRNu location (Figs 1-2), and can also contribute to higher As levels in tree samples from the STOd area where (semi)metals could be washed down from the mountains to the valley. Arsenic accumulation in the leaves of trees could present a closed cycle by which this (semi)metal persists in the assessed locations and creates potential danger for biota. Plants accumulate As primarily in inorganic forms and account for its toxicity by damage of their tissues [27].

Arsenic so as antimony are chalcophilic metalloids that share numerous similarities in biogeochemical properties [28]. Both can be volatilised (Sb as SbH_3), or methylated in the environment [29], and plants utilize these mechanisms for restriction of the possibilities of their cell damage. Arsenic and antimony content in mine waters are mentioned in Table 1. Mines with these elements are located mainly near Pezinok or Modra (Fig. 2). As introduced by Bergqvist et al. [30], arsenic concentrations in plant shoots grown on mine sites increased up to 34.3 mg $As.kg^{-1}$ DW of herbs. Although our research was farther from adit No. 8 (adit signed as 15 on Fig. 2), we observed up to 6.18 mg $As.kg^{-1}$ DW in the leaves of *Salix* sp. and *S. nigra* (Fig. 3a), which also confirmed the accumulation and translocation of this (semi)metal by trees. These results could be compared with those published by Vaculík et al. [14], who also determined arsenic content in the leaves of trees growing in Pernek. These authors introduced this in *Acer pseudoplatanus*, *Salix caprea*, and *Sambucus nigra* were As concentrations in the leaves up to 3, 2, and 1 $mg.kg^{-1}$ DW, respectively. However, our results introduced for upper flows of studied locations were for As higher than those introduced by mentioned authors. As shown in Fig. 3a), As concentrations in *Salix caprea*, occurring at upper flows of studied locations, reached values up to 6.18 mg $As.kg^{-1}$ DW, and locations could be arranged in the order: TRNu > STOu > GIDu (with no As in the leaves). The lowest value of arsenic in the leaves of *S. nigra* was observed on the upper flow of Stoličný stream (2.25 mg $As.kg^{-1}$ DW at STOu) and at the downflow of the same stream this value increased 2.75-times (STOd, Fig. 3a). The location around Pernek (Fig. 2) is characterized by increased arsenic levels (average value 523 mg $As.kg^{-1}$ soil), and its content in plant shoots varied 0.69-7.83 $mg.kg^{-1}$ DW [15]. This is close to our results introduced for leaves in Fig. 3a (0.53-6.18 mg $As.kg^{-1}$ DW). As shown on the map on Fig. 2, around Pernek are mostly the adits with Pb and Zn minerals, while the adit with Sb minerals is near the upper flow of Stoličný stream (15 at Fig. 2), where our samples were collected. Location characterization can explain higher As levels determined in our samples. Although As occurs in this area together with Sb, any antimony was observed in the leaves of all tree species from Gidra stream (GIDu, GIDd), and the downflow of Stoličný stream merged with Trniansky stream (STOd) (Figs 1, 3a), except of *S. nigra* leaves from STOd location. As and Sb accumulated very well (mainly in *S. nigra*), while in *A. glutinosa* it prevailed in the leaves As (Fig. 3a). While As was presented in *Salix* sp. leaves in higher concentrations, Sb was completely absent in the leaves of this tree species from studied locations. The highest concentrations of As were observed in *Salix* sp. from the upper flow of Trniansky stream (TRNu), and *S. nigra* from the downflow of Stoličný stream (STOd). At the same area (STOd) we also determined the highest concentration of Sb in the leaves of *S. nigra*. Although Sb level was 1.15-times lower on the upper flow of Stoličný (STOu), any presence of this element was confirmed in the leaves of *S. nigra* grown on the location of Trniansky upper stream (TRNu) (Fig. 3a). While any presence of Sb was acquired from the leaves of *Salix* sp., *A. glutinosa* leaves contained 1.65 mg $Sb.kg^{-1}$ DW only on the upper flow of Stoličný stream (STOu). Because no Sb was observed in the leaves of *Salix* sp. and for *A. glutinosa* leaves only on one studied location (STOu), interdependence between As and Sb was confirmed only for *S. nigra* in regression analysis as significant positive correlation ($p<0.001$, $r = 1.000$ in Table 5). While for *A. glutinosa* it was on STOd location determined in the leaves of the highest concentration of As, no Sb content was determined in this case. Concentration of arsenic increased with downflow of Gidra stream for all studied tree species, and downflow of Stoličný stream, except of *Salix* sp. (Fig. 3a).

Majzlan et al. [8] observed very low amounts of PbO (<0.18% (w/w)) in weathering rims of the primary sulfide minerals near the Blatina Creek area. The low

Pb amount following with BCF values lower than one were determined in the leaves of *Salix* sp. and *A. glutinosa* collected from all observed locations except of *A. glutinosa* leaves from the GIDu location (Fig. 3a). However, its higher levels (>18.8 mg Pb.kg^{-1} DW) were observed in the leaves of *S. nigra*, mainly from the TRNu, STOd, and GIDd areas (Fig. 3a). It is known that *Alnus firma* is a metal hyperaccumulator plant that endophytic bacteria from their roots could potentially reduce heavy metal phytotoxicity and increase Pb accumulation in these plants [31]. While leaves of *A. glutinosa* contained the highest Pb level on the upper flow of Gidra stream (GIDu) in our experiments, no Pb content was detected in the leaves of *Salix* sp., and its very low content was observed in *S. nigra* leaves from this area (Fig. 3a). Although in some areas Pb accumulation in *S. nigra* was high enough, Yanqun et al. [32] introduced for leaves of *Salix cathayana* (95.92 mg Pb.kg^{-1} DW) and *Sambucus chinensis* (89.84 mg Pb.kg^{-1} DW) growing in Lanping lead-zinc mining area concentrations that many times overreached those determined during our experiments. These differences can be explained through variability of studied locations, because for our experiments plant material was not collected directly from mining sites, but near the streams of catchment areas. During our studies relatively low Pb concentrations were determined in *Salix caprea* (maximum 1.39 mg.kg^{-1} DW) from STOu and *Salix fragilis* (maximum 0.76 mg.kg^{-1} DW) from STOd location (Fig. 3a). The highest Pb concentrations were observed in the leaves of *S. nigra* from lower (GIDd 28.57 mg.kg^{-1} DW), and the lowest from the upper (GIDu 0.91 mg.kg^{-1} DW) flow of Gidra stream. Pb occurrence can originate from galenite (PbS). For *A. glutinosa* we also confirmed positive correlation between lead and copper ($p<0.001$) or cadmium ($p<0.01$), as is shown in Table 5.

Cadmium is present as greenockite (CdS), which commonly occurs with zinc minerals [32]. A solo mineral creates cadmium rarely, but due to very close chemical properties is observed mainly in polymetallic Pb-Zn-Cu ores in sfalerite (ZnS), where the supply of zinc is in a ratio between 1:1,000 to 1:100 [33]. Although cadmium is not essential for plants, it is effectively absorbed by roots and leaves. Berndes et al. [34] found that *Salix* sp. cultivation offers an effective option for addressing cadmium accumulation, especially when the topsoil has high cadmium content (about 0.025 mg Cd.kg^{-1} of soil) due to anthropogenic inflows, and the subsoil naturally contains little cadmium. In our study were increased levels of cadmium in the tree leaves, except for *Salix* sp. leaves from GIDu location, observed on the upper flow of Trniansky (TRNu), Stoličný (STOu), and Gidra (GIDu) streams (Fig. 3b). However, Yanqun et al. [32] observed 11.26 mg Cd.kg^{-1} DW for *Salix cathayana* and 1.69 mg Cd.kg^{-1} DW for *S. chinensis* in the leaves from Lanping lead-zinc mining area, cadmium levels in leaves of *Salix* sp. and *Sambucus nigra* during our experiment reached no more than 0.36 and 1.50 mg Cd.kg^{-1} DW, respectively (Fig. 3b). It was observed higher Cd content in elderberry flowers and fruits from road traffic [35]. In our experiments, *S. nigra* leaves from STOu location contained the highest measured Cd concentration (1.50 mg.kg^{1} DW) from all collected samples; however, here studied locations are not described in the literature as locations with high cadmium concentrations. It has been shown that *Salix* sp. has the ability to accumulate more cadmium than most agricultural crops [34]. Good Cd accumulation in *Salix* sp. was also confirmed in our studies, when accumulated Cd amounts in the leaves from most locations overreached those in leaves of two other studied species (Fig. 3b). While the STOu location was the lowest Cd concentration in the soil (0.004 mg.kg^{-1}), we acquired the highest accumulation in the leaves of *Salix* sp. (BCF = 91.00) and *S. nigra* (BCF = 375.5) (Fig. 3b). *Salix* sp. and *S. nigra* naturally invade dredged sediment landfills and are commonly encountered on substrates contaminated

Table 5. Correlation matrixes with Pearson's coefficient of the selected elements in the leaves from *Alnus glutinosa*, *Salix* sp., and *Sambucus nigra*; asterisks are statistical significance of Pearson's coefficient (*$p<0.05$; **$p<0.01$; ***$p<0.001$).

Heavy metal	Cd	Cu	Pb	Sb	Zn
Alnus glutinosa					
As	-0.633	-0.750	-0.726	-[a]	-0.816
Cd		**0.983****	**0.991****	-[a]	0.553
Cu			**0.999*****	-[a]	0.623
Pb				1.000	0.619
Sb					-[a]
Salix sp.					
As	0.062	-0 104	0.519	-	0.224
Cd		0.715	0.301	-	0.737
Cu			0.240	-	0.859
Pb				0.581	0.079
Sb					-
Sambucus nigra					
As	-0 504	0.060	0.474	1.000*** [b]	-0 244
Cd		0.241	-0.390	-1.000*** [b]	-0 388
Cu			0.506	1.000*** [b]	0.515
Pb				0.853	0.506
Sb					1.000*** [b]

[a]at only one location did woody species contain Sb in their leaves (STOu),

[b]at only two locations did woody species contain Sb in their leaves (STOu, STOd)

with heavy metals. Foliar concentrations of Cd as other bivalent cation Zn in four *Salix* species and elder were explored along the rivers Scheldt and Leie in Flanders, Belgium [36]. These authors described that willows grown on polluted dredged sediment landfills showed elevated foliar Cd and Zn concentrations (>6.6 mg $Cd.kg^{-1}$ DW and >700 mg $Zn.kg^{-1}$ DW). This was not the case for elder. For willow, a significant dependence was found between total soil and foliar Zn or Cd content, regardless of age, species, or clone. Willows proved to be useful bioindicators. However, this is not applicable for our data (Tables 4-5). We can make the following rank for Zn in the soil: TRNu > GIDd > STOd ~ GIDu > STOu, but this dependence is observed for any studied trees (Table 4, Fig. 3b). Among studied bivalent cations, the highest Cd concentration was observed for *S. nigra* from the STOu location, but only the second-lowest Pb concentration was confirmed for this location (STOu) and tree leaves of *S. nigra*. This negative correlation between Cd and Pb was observed also by correlation matrix (Table 5), where correlation was negative but not significant ($r = -0.390$).

Copper and zinc are present as biogenic (mainly bivalent) ions in the environment. Their total concentrations in all three plant species was measured to compare with other nonbiogenic mostly bivalent metals, such as cadmium and lead. Mentioned biogenic and nonbiogenic ions due to similar valence can mutually reciprocally interact during their uptake from soil to roots as well as during their translocation through the plant. The copper concentrations in the leaves varied from 1.16 to 11.90 mg $Cu.kg^{-1}$ DW (Fig. 3b) according to plant species and location. In our collected material any Cu level was determined only in the leaves of *Salix* sp. from the GIDu location. Yanqun et al. [32] observed in Lanping lead-zinc mine the Cu concentration in *Salix cathayana* and *S. chinensis* leaves 34.49 and 15.37 mg $Cu.kg^{-1}$ DW, while soils contained 16.63 and 18.59 mg $Cu.kg^{-1}$ DW, respectively. In our study, leaves from *Salix* sp. collected from evaluated locations, except for the GIDu location, contained Cu in the range 4.03-7.41 $mg.kg^{-1}$ DW and for *Alnus glutinosa* it was less. The highest concentration of Cu was observed in the soil from the STOd location, while for Zn from the TRNu location (Table 4). All other locations had lower content than 1.0 mg $Cu.kg^{-1}$. Analogous to Cd with the lowest soil content and the highest BCF value, we observed the highest bioconcentration in the leaves of trees from the STOu location (Fig. 3b). Obtained results confirmed, similarly to Yanqun et al. [32], that Zn accumulation depended on both plant species and location character (Table 1). Enough high accumulation of Cd, Cu, and Zn for *A. glutinosa* was observed from the GIDu location (Fig. 3b). Similarly, the highest concentrations of Cu, Pb, and Zn were observed for *S. nigra* from downflow of Gidra (GIDd), where the lowest Cd concentration was observed, too. Determining Cu, Cd, Pb, and Zn in *Alnus* sp. is not generally often described in scientific literature. Moreover, we confirmed highly positive correlation in regression analysis between accumulations of biogenic Cu and nonessential Pb ($r = 0.999$, $p<0.001$) or Cd ($r = 0.983$, $p < 0.01$) in the leaves of *A. glutinosa* (Table 5). The results of Butkus and Baltrėnaitė [37] showed that black alders grown on sludge accumulated about 2 times larger amounts of Zn in leaves than control alders. According to Lorenc-Plucińska et al. [38], *A. glutinosa* accumulated Cu, Pb, Cd, and Zn in roots and nodules, and transferred only small proportions of Cu, Pb, and Cd to aboveground parts, including leaves, which means that the species is suitable for phytostabilization of Cu, Pb, and Cd in soils heavily polluted by copper smelters.

When we concentrated on the leaves of *Salix* sp. from Trniansky and Stoličný streams (TRNu, STOu, STOd), the highest Cd, Cu, and Pb concentrations were confirmed for the upper flow of Stoličný stream (STOu), where concurrently the lowest Zn concentration was measured (Figs 3a-b). Moreover, the highest values of BCF for Cd and Zn were determined for leaves of *Salix* sp. collected from all studied locations.

Plant roots with symbiotic microbial populations may alter the physicochemical properties of the rhizosphere through their metabolite excretion [39]. Many metals in the soil are often in insoluble form, but plants might enhance the metal deportation from the soil matrix by the acidification of the rhizosphere resulting from the action of plasma membrane proton pumps and the secretion of ligands to chelate the metal out of the binding sites [40]. Dissolved metals enter into the roots and translocate to the leaf cells by the xylem through membrane pump or channel [39]. Only soil from the upper flow of Stoličný stream (STOu) had acidic value of pH (Table 4). Many metals (except arsenic) are more bioavailable under acidic conditions. Among TRNu, STOu, and STOd locations were the lowest values of arsenic observed in the leaves of all studied trees just in STOu location with acidic pH of soil, but the highest concentrations of Cd, Cu, and Pb were observed at this location in the leaves of *Salix* species (Figs 3a-b, Table 4). *Alnus* sp. is known as a species that cooperated with mycorrhizal fungi (ectomycorrhizal and arbuscular endomycorrhizal), which effect heavy metal bioavailability for *A. glutinosa* [41-42]. The highest pH value was observed in the downflow of Stoličný stream (STOd, Table 4) and truly we observed the highest As concentration in this location in *A. glutinosa* and *S. nigra* (Fig. 3a). As is known, in aerobic soils the predominant As species is arsenate (As^{5+}), while in anaeorobic soils (such as flooded paddy soils) the reduced form, arsenite (As^{3+}) [27]. The mobility of As in soil determines its bioavailability and it is influenced by many factors (redox potential, pH value, the presence of adsorbent-like oxides and hydroxides of Fe, Al, Mn, humic substances, and clay minerals). While As concentration in *A. glutinosa* leaves was on the downflow of Stoličný stream, no Sb content in these tree leaves was observed (Fig. 3a). In contrast, in *S. nigra*, Sb concentrations were the highest from all observed samples (Fig. 3a).

Obtained results confirmed high levels of accumulated heavy metals (As, Cd, Cu, and Zn) – mainly in *Salix* sp., which seems a good metal accumulator from contaminated areas. This is in agreement with data introduced by other authors [43-49]. As for Pb, its accumulation by this species was the lowest from all evaluated trees (Fig. 3a).

Conclusions

The Malé Karpaty Mts. in the Slovak Republic are known for the high occurrence mainly of arsenic and antimony due to their mineral composition and abandoned Sb deposits in Pezinok. During evaluation of 5 locations bounded with water streams in this area, metals accumulation in trees leaves depended on local contamination as much as on woody plant species. While the greatest content of As, Cd, and Zn was detected in *Salix* sp., in *S. nigra* it was lead and antimony. For *A. glutinosa* leaves the lowest cadmium accumulation was determined and positive correlations between Cu and Pb, Cd and Cu, and Cd and Pb were confirmed. These correlations refer to reciprocal interactions among bivalent cations that are essential (Cu, Zn) and those introduced as nonessential or toxic (Cd, Pb) for plants. *S. nigra* appears as the best antimony and lead accumulator in the leaves. A higher level of antimony was observed also in *A. glutinosa* from the upper stream location of Stoličný stream in the Malé Karpaty Mts. The results show that woody plant species play a substantial role in the cycle of heavy metals, and their function depends on the environment and plant species characteristics.

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