



J. Serb. Chem. Soc. 89 (10) S1–S10 (2024)

SUPPLEMENTARY MATERIAL TO
**Potentially toxic elements from different environmental
compartments of the River Watershed in Eastern Serbia –
Assessment of the human health risk**

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J. Serb. Chem. Soc. 89 (10) (2024) 1299–1309

STUDY AREA

Vlasina River is located in southeastern Serbia, flowing around 70 km from Vlasina Lake to South Morava River, belonging to the Dunav River watershed (Fig. S-I). Vlasina River basin covers a surface of around 1,000 km². The area is mostly hilly-mountainous (up to 1700 m a.s.l.) covered by forests, pastures, and agricultural crops. One of the main activities of the inhabitants of Vlasina is mountain cattle breeding (sheep, cattle and horses), mountain agriculture (rye, barley, oats, potatoes),¹ and also Vlasotince is the centre of the wine-growing region.² Lower quality arable land occupies a significant area in the Vlasina River basin, while around Vlasotince alluvial sediments in the Vlasina River valley are rich in humus. The majority of the area of the Vlasina River basin belongs to the Vlasina unit, which is a part of the vast Serbo-Macedonian Unit, stretching from the Panonian Basin in the north to the Aegean Sea in the south, in Serbia encompassing its central and southeastern parts, originating from the Carbon – Permian period (Paleozoic). Metamorphic rocks dominate the southern and western parts of the research area, while tertiary clastic sediments, Mesozoic carbonate rocks and flysch make the most of the eastern and northern parts.³

COLLECTION OF RIVER WATER AND SOIL SAMPLES

River water samples were taken at the following sampling sites (Fig. S-I): (1) Vlasina (upstream of the confluence Gradska river); (2) Gradska River (before its

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confluence with Vlasina); (3) Vlasina (upstream of the confluence with Tegošnička River); (4) Tegošnička River (stone pit); (5) Tegošnička River (near village Dobroviš); (6) Vlasina (downstream of the confluence with Tegošnička River, near village Gornji Orah); (7) Ljuberađa (middle course); (8) Ljuberađa (measuring profile); (9) Ljuberađa (confluence with Vlasina); (10) Vlasina (after receiving Ljuberađa); (11) Pusta River; (12) Vlasina (downstream of the confluence with Pusta River); (13) Bistrička River; (14) Rastavnica River; (15) Vlasina (upstream of the intake for water supply); (16) Vlasina (downriver from Vlasotince); (17) Zelenička River. Soil samples were taken at 15 sampling locations, close to the river water sampling locations. The soil was not sampled on locations (10) Vlasina after receiving Ljuberađa and (13) Bistrička River.

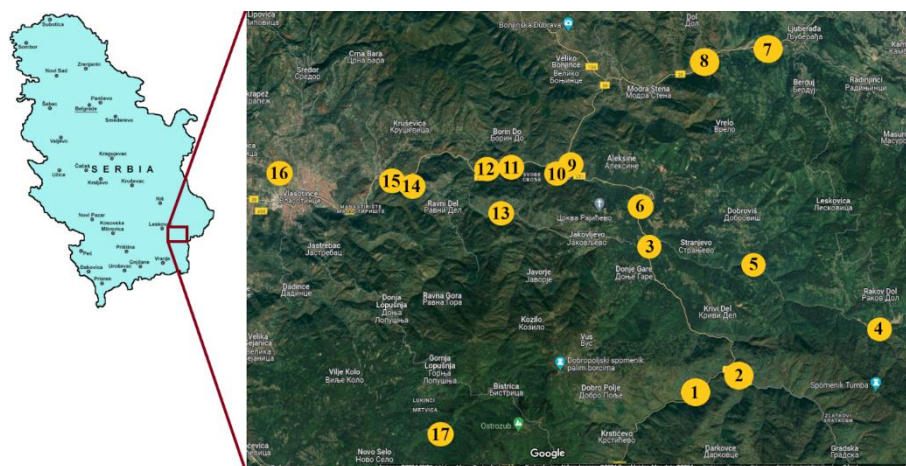


Fig. S-1. Location of the research area in Serbia and sampling sites in the Vlasina region.

Water grab samples were taken at around 10 cm beneath the water surface and filtered through 0.2 μM nylon syringe filters into the high-density polyethylene bottles. Aliquot of each sample for the analysis of elements was subsequently acidified with HNO_3 to a pH below 2 and all samples were stored at 4°C until.³⁻⁵

Soil samples were collected into polyethylene bottles with a plastic spatula/shovel⁷⁻⁹ and transported to the laboratory. Stones and plant debris were removed from the samples in the laboratory, the samples were subsequently homogenized and kept in the refrigerator at 4° C. Samples were air dried for 8 days before analysis.^{10, 11}

DETERMINATION OF ELEMENT CONCENTRATIONS IN RIVER WATER SAMPLES AND SOIL EXTRACTS BY ICP-OES AND ICP-MS

Element concentrations in river water and soil extracts obtained at each of BCR extraction steps were determined using techniques of Inductively Coupled Plasma-Optical Emission spectrometry (Thermo Scientific ICP-OES iCap 6500

Duo) and Inductively Coupled Plasma-Mass spectrometry (Thermo Scientific ICP-MS iCap Q). The analytical data quality was controlled by using laboratory quality assurance and quality control methods, including the use of standard operating procedures, calibration with standards, and analysis of both reagent blanks and replicates.¹² The blank solutions were prepared in the same way as the samples during the extraction procedure. The quality of data was assessed by estimations of accuracy and precision. The accuracy and precision of the obtained results were checked by analyzing sediment reference material (BCR 701) for three-step sequential extraction. Acceptable accuracy (80–120%) and precision ($\leq 20\%$) of metals was achieved for all steps of sequential extraction. Values of detection limits (μgL^{-1}) for elements in river water were: 0.09 for Zn, 0.025 for Ni, 0.055 for Cu, 0.006 for Cr, 0.021 for As, 0.056 for Pb, and 0.029 for Cd.

DETERMINATION OF ANION CONCENTRATION IN RIVER WATER BY ION CHROMATOGRAPHY

The concentrations of anions (Cl^- , NO_3^- , SO_4^{2-} and PO_4^{3-}) were measured by ion chromatograph Metrohm 761 Compact IC, with a conductometric detector, Metrosep A Supp 1-250 column (particle size $7\ \mu\text{m}$, column dimensions $4.6 \times 250\ \text{mm}$) and guard precolumn Metrosep A Supp 1 Guard. The used eluent was $3\ \text{mM}\ \text{Na}_2\text{CO}_3$ (Fluka, Switzerland). Every analytical run started with calibration standards in order of increasing concentration, followed by water blank and samples. Reagent blank, which followed all procedure steps as samples, was also analyzed. The check calibration standard was analyzed after every ten analyses. Detection limit values (mgL^{-1}) for anions in river water were: 0.02 for Cl, NO_3^- , SO_4^{2-} and PO_4^{3-} .

HEALTH RISK ASSESSMENT

Human health risks of PTEs via ingestion and dermal absorption of river water, which represent the main routes of exposure, were assessed for residential receptors (direct ingestion of water, dermal absorption during showering) and recreational receptors (incidental water ingestion and dermal contact with water during swimming), both adults and children. Although direct ingestion of river water is the less probable route of exposure, assessing health risks by direct ingestion in this study is justified because the river Vlasina is the source of raw water for the water supply of Vlasotince town and this assessment can offer information on requirements for drinking water treatment. To estimate human exposure to PTE through ingestion and dermal contact with river water average daily doses (ADDs) were calculated. Non-cancer health risks of individual PTE in river water for every considered exposure pathway and receptor were assessed through the calculation of hazard quotients (HQs). Non-cancer risks due to exposure to all PTE in river water for each exposure scenario were assessed by calculating the sum of HQs of individual PTE. Non-cancer risks for different

receptors caused by all routes of exposure were assessed using hazard index (HI), which is calculated as a sum of HQs. Cancer health risks (CR) for each exposure pathway and receptor were calculated for elements having cancer slope factors, which in the case of this study are Cr and As. Summation of CR of all exposure routes for each receptor yielded the total cancer risks (TCR).

To assess the human health risk of PTE in soil, ingestion, dermal contact and inhalation exposure pathways were considered. Following the health risk assessment methodology applied for river water, hazard quotients (HQs), hazard indices (HI), cancer risks (CR) and total cancer risks (TCR) were calculated.

Values of HQ and HI > 1 indicate that detrimental non-carcinogenic effects on human health could be expected.¹³ If the values of CR and TCR are < 10⁻⁶, there is generally no concern for increased cancer risk, values in the range 10⁻⁶ - 10⁻⁴ indicate potential risk, while values > 10⁻⁴ are considered unacceptably high risk.¹⁴

The following equations were used for the assessment of the health risk of PTEs in river water for residential (res) or recreational (rec) receptors:

$$ADDw_{ingestion\ res/rec} = \frac{C_w \times IRw_{res/rec} \times EF_{res/rec} \times ED}{BW \times AT} \quad (1)$$

$$ADDw_{dermal\ res/rec} = \frac{C_w \times SA \times K_p \times ET_{res/rec} \times EF_{res/rec} \times ED}{BW \times AT_{res/rec} \times 10^3} \quad (2)$$

$$HQw_{ingestion\ res/rec} = \frac{ADDw_{ingestion\ res/rec}}{RfD_o \times 10^3} \quad (3)$$

$$HQw_{dermal\ res/rec} = \frac{ADDw_{dermal\ res/rec}}{RfD_o \times GIABS \times 10^3} \quad (4)$$

$$HIw = HQw_{ingestion} + HQw_{dermal} \quad (5)$$

$$CRw_{ingestion\ res/rec} = \frac{ADDw_{ingestion\ res/rec} \times CSF_o}{10^3} \quad (6)$$

$$CRw_{dermal\ res/rec} = \frac{ADDw_{dermal\ res/rec} \times CSF_o}{GIABS \times 10^3} \quad (7)$$

$$TCRw = CRw_{ingestion} + CRw_{dermal} \quad (8)$$

The calculation of the human health risk of TE in soil, caused by ingestion, dermal contact and inhalation of soil, was as follows:

$$ADDs_{ing} = C \times \frac{I_{ingR} \times EF \times ED}{BW \times AT} \times CF \quad (9)$$

$$ADDs_{dermal} = C \times \frac{AF \times SA \times ABS \times EF \times ED}{BW \times AT} \times CF \quad (10)$$

$$ADDs_{inhalation} = C \times \frac{I_{inhR} \times EF \times ED}{PEF \times BW \times AT} \quad (11)$$

$$HQ = \frac{ADD}{RfD} \quad (12)$$

$$HI = NCR = \sum HQ = HQ_{ing} + HQ_{der} + HQ_{inh} \quad (13)$$

$$CR = ADD \times CSF \quad (14)$$

$$TCR = CR_{total} = CR_{ing} + CR_{der} + CR_{inh} \quad (15)$$

The values of exposure factors and chemical specific and toxicity associated parameters related to assessment of human exposure to PTE in river water and soil are given in TABLES S-I and S-II, and TABLES S-III and S-IV, respectively.

TABLE S-I. Exposure factors and their values used in the assessment of exposure to PTE through water ingestion and dermal contact with water for residential (res) and recreational (rec) receptors¹⁵⁻²⁰

Parameters	Unit	Adult	Children
C_{water} : Element concentration in water	μgL^{-1}	/	/
IR_{res} : Resident water ingestion rate	Lday^{-1}	2	0.64
IR_{rec} : Recreator water ingestion rate	Lday^{-1}	0.11	0.12
EF_{res} : Exposure frequency	day year^{-1}	350	350
EF_{rec} : Exposure frequency	day year^{-1}	45	45
ED: Resident/recreator exposure duration	years	24	6
BW: Body weight	kg	70	15
AT: Averaging time	days	8760	2190
SA: Resident/recreator skin surface area	cm^2	18000	6600
ET_{res} : Resident exposure time	h day^{-1}	0.58	1
ET_{rec} : Recreator exposure time	h day^{-1}	1	1

TABLE S-II. Chemical specific and toxicity-associated parameter values for trace elements used for health risk assessment (exposure pathway - ingestion and dermal absorption of water): dermal permeability coefficient (K_p), reference dose oral (RfD_o), cancer slope factor oral (CSF_o) and gastrointestinal absorption coefficient (GIABS)^{20, 21}

Element	K_p (cm h^{-1})	RfD_o ($\text{mg kg}^{-1}\text{day}^{-1}$)	CSF_o ($\text{mg kg}^{-1}\text{day}^{-1}$) ⁻¹	GIABS (unitless)
Cr	0.002	0.003	0.5	0.025
Ni	0.0002	0.02		0.04
Cu	0.001	0.04		1
Zn	0.0006	0.3		1
As	0.001	0.0003	1.5	1

TABLE S-III. Parameters of health risk assessment for exposure to PTE in soil^{17, 22-28}

Parameters	Unit	Adult	Children
I_{inh} : Inhalation Intake rate	$\text{m}^3 \cdot \text{day}^{-1}$	12.8	7.63
BW: Body weight	kg	70	15
AT: Averaging time	days	8760	2190
EF: Exposure frequency	$\text{day} \cdot \text{year}^{-1}$	350	350
ED: Exposure duration	years	24	6
PEF: particle Emission factor	$\text{m}^3 \cdot \text{kg}^{-1}$	1.36E+09	1.36E+09
SL(AF): Skin Adherence Factor	$\text{mg} \cdot \text{cm}^{-2} \cdot \text{day}^{-1}$	0.07	0.2
SA: Skin Area	$\text{cm}^2 \cdot \text{day}^{-1}$	5700	2800
ABS: Dermal absorption factor	Unitless	0.001	0.001
IngR	$\text{mg} \cdot \text{day}^{-1}$	100	200
InhR	$\text{m}^3 \cdot \text{day}^{-1}$	20	7.65
CF	Unitless	0.000001	0.000001

TABLE S-IV. Reference dose (RfD) and cancer slope factor (CSF) of potentially toxic metals via the three main pathways of human exposure to soil ^{24, 25 28, 29}

	Reference dose (RfD)		
	RfD _{ing}	RfD _{der}	RfD _{inh}
Cr	3.00E-03	6.00E-05	2.86E-05
Ni	2.00E-02	5.40E-03	2.06E-02
Cu	4.00E-02	1.20E-02	4.02E-02
Zn	3.00E-01	6.00E-02	3.00E-01
As	3.00E-04	1.23E-04	3.00E-04
Cd	1.00E-03	1.00E-05	1.00E-03
Pb	3.50E-03	5.25E-04	3.52E-03
	Cancer slope factor (CSF)		
	CSF _{ing}	CSF _{der}	CSF _{inh}
Cr	5.00E-01	2.00E+01	4.20E+01
Ni	1.70E+00	4.25E+01	8.40E-01
As	1.50E+00	3.66E+00	1.51E+01
Cd	6.10E+00	6.10E+00	6.30E+00
Pb	8.50E-03	8.50E-03	4.20E-02

TABLE S-V. Pollutant limit values for surface water quality classes³⁰

	Surface water quality classes according to the Regulation Off. Gaz. RS 50/2012				
	I	II	III	IV	V
	Limit values				
Fe / $\mu\text{g L}^{-1}$	200	500	1000	2000	>2000
Cr / $\mu\text{g L}^{-1}$	25 (or natur. level)	50	100	250	>250
Mn / $\mu\text{g L}^{-1}$	50	100	300	1000	>1000
Ni / $\mu\text{g L}^{-1}$	4			34	>34
Cu / $\mu\text{g L}^{-1}$	5 (H=10) ^a	5 (H=10) ^a			
	22 (H=50)	22 (H=50)			
	40 (H=100)	40 (H=100)	500	1000	>1000
	112 (H=300)	112 (H=300)			
Zn / $\mu\text{g L}^{-1}$	30 (H=10) ^a	300 (H=10) ^a			
	200 (H=50)	700 (H=50)			
	300 (H=100)	1000 (H=100)	2000	5000	>5000
	500 (H=500)	2000 (H=500)			
As / $\mu\text{g L}^{-1}$	<5 (or natur. level)	10	50	100	>100
		0.08(H<40) ^a	0.45(H<40) ^a	>0.45(H<40) ^a	
Cd / $\mu\text{g L}^{-1}$ *		0.08(H=40-50)	0.45(H=40-50)	> 0.45(H=40-50)	
		0.09(H=50-100)	0.6(H=50-100)	> 0.6(H=50-100)	
		0.15(H=100-200)	0.9(H=100-200)	> 0.9(H=100-200)	
		0.25 (H>200)	1.5(H>200)	> 1.5(H>200)	
Pb / $\mu\text{g L}^{-1}$ *	1.2		14		>14
Cl ⁻ / mg L^{-1}	50 (or natur. level)	100	150	250	>250
NO ₃ ⁻ / mgN L^{-1}	1.0-1.5	3.0	6	15	>15
PO ₄ ³⁻ / mgP L^{-1}	0.02	0.05-0.10	0.2	0.5	>0.5
SO ₄ ²⁻ / mg L^{-1}	50 (or natur. level)	100	200	300	>300

^aH – water hardness / mgL^{-1} CaCO₃.

TABLE S-VI. Average values of HQ and HI for PTE in the investigated rivers (residential receptors)

	Child			Adult		
	HQ _{ingestion}	HQ _{dermal}	HI	HQ _{ingestion}	HQ _{dermal}	HI
As	0.1319131	0.0013604	0.1332735	0.0883347	0.0004611	0.0887958
Cr	0.0012925	0.0010663	0.0023587	0.0008655	0.0003614	0.0012269
Zn	0.0001142	0.0000007	0.0001149	0.0000764	0.0000002	0.0000767
Ni	0.0006060	0.0000312	0.0006372	0.0004058	0.0000106	0.0004164
Cu	0.0004567	0.0000047	0.0004614	0.0003058	0.0000016	0.0003074
All elements	0.1343825	0.0024633	0.1368458	0.0899883	0.0008350	0.0908232

TABLE S-VII. Average values of HQ, and HI for PTE in the investigated rivers (recreational receptors)

	Child			Adult		
	HQ _{ingestion}	HQ _{dermal}	HI	HQ _{ingestion}	HQ _{dermal}	HI
As	0.0029150	0.0001749	0.0030899	0.0006814	0.0001022	0.0007837
Cr	0.0000286	0.0001371	0.0001657	0.0000067	0.0000801	0.0000868
Zn	0.0000025	0.0000001	0.0000026	0.0000006	0.0000001	0.0000006
Ni	0.0000134	0.0000040	0.0000174	0.0000031	0.0000023	0.0000055
Cu	0.0000101	0.0000006	0.0000107	0.0000024	0.0000004	0.0000027
All elements	0.0029696	0.0003167	0.0032863	0.0006942	0.0001851	0.0008793

TABLE S-VIII. Average hazard quotients (HQ) for non-carcinogenic risk in adults and children due to exposure (inhalation, dermal, and ingestion) of various PTEs in studied soils

	Adults			Children		
	HQ _{ing}	HQ _{der}	HQ _{inh}	HQ _{ing}	HQ _{der}	HQ _{inh}
As	0.046763	0.00045508	6.88E-06	0.4364558	0.011601	1.23E-05
Cd	0.000606	0.00024199	8.92E-08	0.0056606	0.006053	1.59E-07
Ni	0.001173	1.7333E-05	1.67E-07	0.0109470	0.000442	2.99E-07
Pb	0.006846	0.00018212	1.67E-07	0.0639005	0.004560	1.79E-06
Cr	0.008042	0.00160448	1.24E-04	0.0750633	0.041359	2.21E-04
Cu	0.008042	0.00160448	1.24 E-05	0.0065923	0.000209	1.84E-07
Zn	0.008042	0.00160448	1.24 E-05	0.0005378	0.000134	7.06E-07

TABLE S-IX. Average hazard index values (HI) and the total cancer risk (TCR) through PTE consumption in the studied soils.

PTE	HI		TCR	
	Adults	Children	Adults	Children
As	0.0472	0.4481	2.13E-05	2.02E-04
Cd	0.0008	0.0117	3.71E-06	3.49E-05
Ni	0.0012	0.0114	4.39E-05	4.74E-04
Pb	0.0070	0.0685	2.05E-07	1.92E-06
Cr	0.0098	0.1166	1.41E-05	1.62E-04
Cu	0.0007	0.0068	-	-
Zn	0.0003	0.0007	-	-

TABLE S-X. Mean values of carcinogenic human health risk (CR) for adults and children via inhalation, dermal, and ingestion

	Adults			Children		
	CR _{ing}	CR _{der}	CR _{inh}	CR _{ing}	CR _{der}	CR _{inh}
As	2.10E-05	2.05E-07	3.12E-08	1.96E-04	5.22E-06	5.56E-08
Cd	3.70E-06	1.48E-08	5.62E-10	3.45E-05	3.69E-07	1.00E-09
Ni	3.99E-05	3.98E-06	2.90E-09	3.72E-04	1.01E-04	5.17E-09
Pb	2.04E-07	8.13E-10	1.48E-10	1.90E-06	2.03E-08	2.64E-10
Cr	1.21E-05	1.93E-06	1.49E-07	1.13E-04	4.96E-05	2.66E-07

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