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ACCUMULATION OF HEAVY METALS IN THE ROMAN SNAIL (*HELIX POMATIA*) AT SITES WITH DIFFERENT ANTHROPOGENIC IMPACTS IN KOSOVO AND METOHIJA (SERBIA)

Željko P. Bulatović[1]✉, Tatjana R. Jakšić[1], Nikola N. Đukić[1], Nikola R. Bačević[2]

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Abstract

Heavy metal concentrations in the environment have increased in recent decades, mainly due to anthropogenic activities. This study assessed the concentrations of As, Cu, Fe, Cd, Mn, Pb, and Zn in tissues of the Roman snail *Helix pomatia* (Linnaeus, 1758) and in soils from Kosovo and Metohija using AAS. Hepatopancreas, kidney, hemolymph and shell were analyzed together with soil from the same sites (Crkvena Vodica–Obilić, Vrbeštica–Štrpce, and Zubin Potok as a control site). Seventy-five individuals were collected and three composite samples per tissue were prepared (25 individuals per composite; $n = 3$) at each site. Data are presented as mean ± SD; differences among sites were tested by ANOVA and Tukey's test ($p < 0.05$). The strongest site-related differences occurred in the hepatopancreas, indicating that *H. pomatia* is a suitable bioindicator of local heavy-metal pressure.

Key words: *H. pomatia*, soil, accumulation, mine, thermal power plant

Introduction. Heavy metals occur naturally in soils through pedogenetic processes and are largely bound to parent material with limited bioavailability; however, anthropogenic inputs can increase their concentrations and bioavailability facilitating accumulation in biota [1]. Coal-based energy production and mining are major sources of environmental pressure and long-term soil degradation [2,

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3]. Kosovo and Metohija province faces legacy contamination from intensive industrial activities, including persistent sources such as industrial waste dumps and storage/storage-disposal sites [4]. Terrestrial molluscs are useful bioindicators because land snails can accumulate heavy metals due to limited excretion, with the hepatopancreas typically exhibiting the highest accumulation [5, 6]. This study determined concentrations of As, Cu, Fe, Cd, Mn, Pb, and Zn at three sites within the Kosovo and Metohija study area and assessed their accumulation in *H. pomatia* tissues (hepatopancreas, kidney, hemolymph, shell) and soils: a central site between two thermal power plants, a southern site near former mines and dumps, and a northern control site with lower expected anthropogenic influence.

Materials and methods. ***Study area.*** Sampling of snails and soils was conducted at three sites differing in anthropogenic impact: Crkvena Vodica (42°40.505′N, 021°03.396′E–42°40.396′N, 021°03.333′E), near the Obilić thermal power plants (Kosovo A and B), where ash disposal and plant operation may contaminate surrounding soils and waters [7]; Vrbeštica (42°14.467′N, 020°58.290′E–42°14.470′N, 020°58.392′E), with historically documented mining (1894–1960s), including mines in nearby Jažince and chromite tailings deposits ($\sim$ 1.5 ha) [8, 9]; and Zubin Potok (42°55.764′N, 020°40.599′E–42°55.788′N, 020°40.644′E), selected as a control site distant from major anthropogenic sources (Fig. 1). At each site, samples were collected along transects, and the biological and soil samples were transported to the laboratory for analysis.

Collection of samples and analysis. *H. pomatia* and soil sampling were conducted from late September to mid-October 2025 at three sites. At each site, 75 snails were collected; three soil subsamples were taken and pooled into one composite sample per site ($n = 1$). Snails were collected with glove-protected hands, transported in plastic boxes, measured for body mass, shell height and shell diameter, and dissected to isolate hemolymph, shell, hepatopancreas and kidney.

Snails ($n = 75$ per site) were divided into three groups of 25 ($n = 3$); for each group, four 10 g composite samples (one per matrix) were prepared, yielding 12 composites per site (36 in total), which were stored at $-80\,^{\circ}$C until analysis. Heavy metals were analyzed at the Institute of Public Health (Kosovska Mitrovica). Snail homogenates (10 g) were dried (105 °C), ashed (450 °C), digested with concentrated HNO_3 (0.5 mL; repeated to dryness), treated with HCl (1 mL), filtered and diluted to volume. Soils (5–10 g) were digested with aqua regia (HCl:HNO_3=3:1; 20 mL), heated at 95 °C for 2 h, filtered and diluted to 100 mL. Metals were determined by AAS (PinAAcle 900T) using flame and/or graphite furnace modes, following Atomic Absorption Data Book (Philips Analytical) [10].

Data analysis. Statistical analyses were performed in the R software environment. Data on heavy-metal accumulation in *H. pomatia* and morphometric traits were presented as mean ± standard deviation (SD). Differences among sites were tested using one-way analysis of variance (one-way ANOVA) at a significance

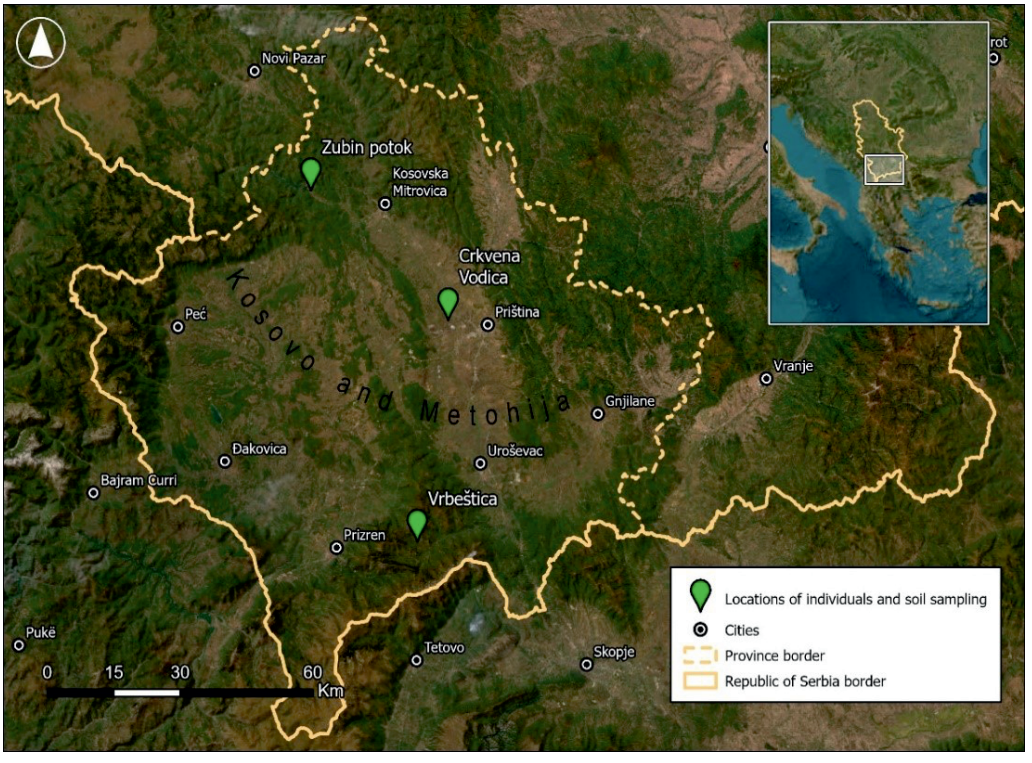


Fig. 1. Geographic location of the *H. pomatia* and soil sampling sites (Crkvena Vodica, Vrbeštica, and the control site), showing the study area within the region

level of $p < 0.05$, while Tukey's HSD post-hoc test was applied for subsequent pairwise comparisons. Data on heavy-metal concentrations in soil were presented descriptively, without applying statistical tests.

Results and discussion. Concentrations of Cu, Fe, Zn, Pb, Mn, As, and Cd in soils and tissues indicate anthropogenic influence across the studied sites, with the strongest contamination signal recorded at Crkvena Vodica and Vrbeštica compared with the control site (Zubin Potok). *H. pomatia* showed organ-specific metal accumulation, with the hepatopancreas exhibiting the highest concentrations among the analyzed tissues [11, 12]. The kidney also showed substantial accumulation, consistent with its role in excretion and detoxification [13], whereas lower levels were observed in hemolymph and shell [11].

Heavy metals in soil. Site-specific differences were observed in the soil, most pronounced for Cu and Cd, with instances of limit values being exceeded recorded at sites under anthropogenic influence (Fig. 2). Cu was 38.81 mg/kg at Crkvena Vodica and 55.35 mg/kg at Vrbeštica, compared with 33.05 mg/kg at the control site (Zubin Potok); the limit value for Cu is 36 mg/kg and the remediation value is 190 mg/kg, according to the Rulebook on limit values of polluting, harm-

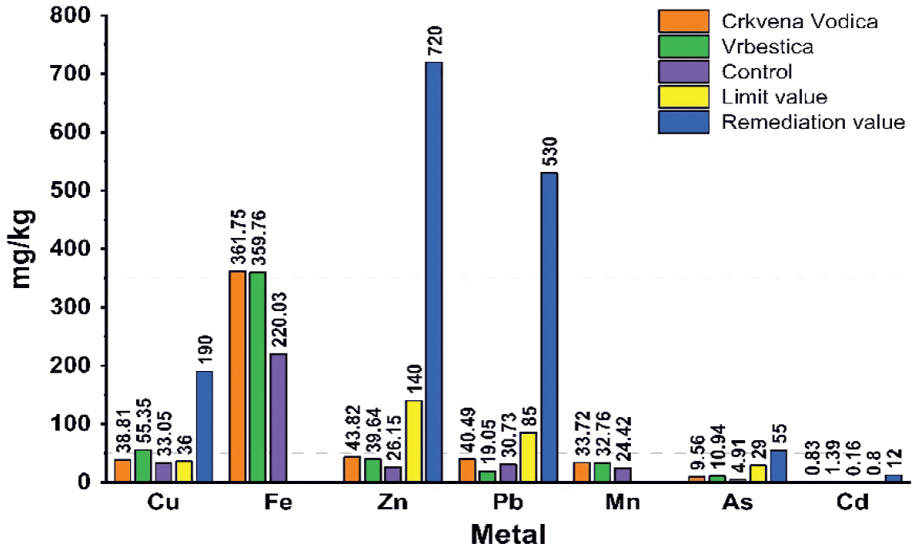


Fig. 2. Soil heavy-metal concentrations (mg/kg) at Crkvena Vodica, Vrbeštica, and the control site, with indicated limit and remediation values

ful and hazardous substances in soil ("Official Gazette of RS", Nos. 30/2018 and 64/2019) [14]. Cd was 0.83 mg/kg (Crkvena Vodica) and 1.39 mg/kg (Vrbeštica), versus 0.16 mg/kg at the control site; relative to the limit value of 0.8 mg/kg, Cd exceeded the limit at Vrbeštica and was slightly above the limit at Crkvena Vodica, while both remained far below the remediation value (12 mg/kg). In contrast, Zn (43.82/39.64/26.15 mg/kg), Pb (40.49/19.05/30.73 mg/kg) and As (9.56/10.94/4.91 mg/kg) (Crkvena Vodica/Vrbeštica/Zubin Potok) were below their respective limit values (Zn 140 mg/kg; Pb 85 mg/kg; As 29 mg/kg) in all samples. For Fe (361.75/359.76/220.03 mg/kg) and Mn (33.72/32.76/24.42 mg/kg), no reference regulatory values are defined in the cited Rulebook [14].

Metal accumulation in* H. pomatia *tissues. The strongest and statistically supported site differences occurred in the hepatopancreas, consistent with its role in accumulation and primary detoxification in terrestrial gastropods [15] (Table 1). Cu differed significantly in the hepatopancreas (ANOVA $p = 0.00246$), with higher values at Crkvena Vodica (25.19 ± 2.97^{b}) and Vrbeštica (25.93 ± 6.33^{b}) than in the control (7.85 ± 0.40^{a}), while differences were not significant in the kidney ($p = 0.0973$), shell ($p = 0.208$) or hemolymph ($p = 0.446$). The largest overall effect was recorded for Pb in the hepatopancreas ($p = 0.000052$): Crkvena Vodica 6.44 ± 1.18^{b}, Vrbeštica 0.05 ± 0.04^{a}, and control 0.73 ± 0.20^{a}, consistent with emissions and deposition from thermal power plants and ash disposal in the Crkvena Vodica area [4]. In contrast, Pb in the kidney showed a reversed pattern ($p = 0.0024$), with the highest value in the control (0.74 ± 0.22^{b}) and lower values at Crkvena Vodica (0.15 ± 0.14^{a}) and Vrbeštica (0.02 ± 0.03^{a}), as important exception. Fe differed significantly only in the hepatopancreas ($p = 0.0168$), with the

Table 1

Heavy metal (mg/kg) in the hepatopancreas, kidney, shell, and hemolymph of *H. pomatia* from Crkvena Vodica, Vrbeštica and the control site

Note: mean ± SD ($n = 3$); one-way ANOVA p-values; different superscript letters (a–c) within a row indicate significant site differences (Tukey HSD, $p < 0.05$)

Element mg/kg	Snail organs/tissues	Crkvena Vodica	Vrbeštica	Control	p (ANOVA)
Cu	Hepatopancreas	25.19 ± 2.97^{b}	25.93 ± 6.33^{b}	7.85 ± 0.40^{a}	0.00246
	Kidney	14.14 ± 4.10^{a}	7.71 ± 4.41^{a}	6.85 ± 2.09^{a}	0.0973
	Shell	0.17 ± 0.10^{a}	0.18 ± 0.10^{a}	0.06 ± 0.03^{a}	0.208
	Hemolymph	29.97 ± 1.57^{a}	24.86 ± 7.34^{a}	26.86 ± 2.82^{a}	0.446
Fe	Hepatopancreas	34.47 ± 1.58^{b}	19.39 ± 7.61^{a}	30.17 ± 1.41^{ab}	0.0168
	Kidney	32.25 ± 1.69^{a}	24.17 ± 9.53^{a}	22.67 ± 1.38^{a}	0.162
	Shell	0.16 ± 0.18^{a}	0.76 ± 1.17^{a}	0.01 ± 0.01^{a}	0.421
	Hemolymph	11.82 ± 3.07^{a}	10.59 ± 2.83^{a}	13.20 ± 1.42^{a}	0.496
Zn	Hepatopancreas	5.75 ± 0.20^{a}	5.49 ± 0.08^{a}	4.65 ± 1.07^{a}	0.165
	Kidney	5.39 ± 0.60^{a}	5.67 ± 0.47^{a}	5.15 ± 0.26^{a}	0.44
	Shell	0.60 ± 0.35^{b}	0.09 ± 0.07^{ab}	0.04 ± 0.02^{a}	0.03
	Hemolymph	3.04 ± 0.01^{c}	1.20 ± 0.30^{b}	0.63 ± 1.02^{a}	0.01
Pb	Hepatopancreas	6.44 ± 1.18^{b}	0.05 ± 0.04^{a}	0.73 ± 0.20^{a}	0.000052
	Kidney	0.15 ± 0.14^{a}	0.02 ± 0.03^{a}	0.74 ± 0.22^{b}	0.0024
	Shell	0.04 ± 0.02^{a}	0.04 ± 0.02^{a}	0.03 ± 0.02^{a}	0.367
	Hemolymph	$1.33\times10^{-3} \pm 1.55\times10^{-3\,a}$	$4.03\times10^{-3} \pm 4.04\times10^{-3\,a}$	$1.50\times10^{-4} \pm 2.09\times10^{-4\,a}$	0.23
Mn	Hepatopancreas	3.27 ± 0.35^{a}	3.35 ± 0.28^{a}	1.98 ± 0.61^{b}	0.0143
	Kidney	2.36 ± 0.88^{a}	2.85 ± 1.30^{a}	1.00 ± 0.40^{a}	0.115
	Shell	0.04 ± 0.06^{a}	0.20 ± 0.32^{a}	0.01 ± 0.00^{a}	0.44
	Hemolymph	0.74 ± 0.46^{a}	0.49 ± 0.67^{a}	0.34 ± 0.31^{a}	0.643
As	Hepatopancreas	$2.07\times10^{-3} \pm 1.76\times10^{-3\,a}$	$1.63\times10^{-3} \pm 1.36\times10^{-3\,a}$	$0.60\times10^{-3} \pm 0.26\times10^{-3\,a}$	0.42
	Kidney	$0.87\times10^{-3} \pm 0.15\times10^{-3\,a}$	$0.90\times10^{-3} \pm 0.17\times10^{-3\,a}$	$0.30\times10^{-3} \pm 0.30\times10^{-3\,a}$	0.0382
	Shell	0.33 ± 0.34^{a}	0.83 ± 1.07^{a}	0.08 ± 0.05^{a}	0.41
	Hemolymph	$5.03\times10^{-3} \pm 1.12\times10^{-3\,a}$	$2.88\times10^{-2} \pm 3.67\times10^{-2\,a}$	$1.63\times10^{-3} \pm 1.29\times10^{-3\,a}$	0.30
Cd	Hepatopancreas	0.22 ± 0.01^{a}	0.19 ± 0.03^{ab}	0.12 ± 0.05^{b}	0.03
	Kidney	0.18 ± 0.02^{a}	0.12 ± 0.03^{b}	0.11 ± 0.03^{b}	0.02
	Shell	$3.57\times10^{-3} \pm 5.48\times10^{-3\,a}$	$1.37\times10^{-3} \pm 1.67\times10^{-3\,a}$	$0.04\times10^{-3} \pm 0.06\times10^{-3\,a}$	0.466
	Hemolymph	$10.70\times10^{-3} \pm 8.06\times10^{-3\,a}$	$3.43\times10^{-3} \pm 5.69\times10^{-3\,a}$	$0.07\times10^{-3} \pm 0.11\times10^{-3\,a}$	0.14

highest concentration at Crkvena Vodica (34.47 ± 1.58^{b}) vs. Vrbeštica (19.39 ± 7.61^{a}), while control values were intermediate (30.17 ± 1.41^{ab}). Mn also differed in the hepatopancreas ($p = 0.0143$), showing higher values at Crkvena Vodica (3.27 ± 0.35^{a}) and Vrbeštica (3.35 ± 0.28^{a}) and the lowest level at the control site (1.98 ± 0.61^{b}). Zn showed a clear site gradient in hemolymph ($p = 0.01$): Crkvena Vodica (3.04 ± 0.01^{c}) > Vrbeštica (1.20 ± 0.30^{b}) > control (0.63 ± 1.02^{a}), with a significant difference in the shell ($p = 0.03$). Cd was highest at Crkvena Vodica in the hepatopancreas ($p = 0.03$; 0.22 ± 0.01^{a}) and kidney ($p = 0.02$; 0.18 ± 0.02^{a}), supporting its indicator value for anthropogenic pressure. For As, concentrations were generally low and differences among sites were modest; kidney levels showed a significant site effect ($p = 0.0382$) but without post-hoc separation.

Combining soil and tissue data. The data indicate that Crkvena Vodica shows the most pronounced contamination signal (soil Cu/Cd reach or exceed limit values; hepatopancreatic Cu, Pb, Fe and Mn are elevated), whereas Vrbeštica exhibits high soil Cu/Cd and increased hepatopancreatic Mn, consistent with legacy mining and tailings influence [9]. The control site generally showed lower levels, but Pb concentration in the kidney suggests baseline variability. Morphometrics follow the same gradient (Vrbeštica > control > Crkvena Vodica; Table 2), and larger size may reflect older individuals and longer exposure [16].

Table 2

Morphometric characteristics (g; mm) of *H. pomatia* from Crkvena Vodica, Vrbeštica and the control site

Note: mean ± SD ($n = 75$); one-way ANOVA p-values; different superscript letters (a–c) within a row indicate significant site differences (Tukey HSD, $p < 0.05$)

	Crkvena Vodica	Vrbeštica	Control	p (ANOVA)
Body mass (g)	16.55 ± 2.33^{a}	24.63 ± 5.04^{b}	18.37 ± 2.44^{c}	7.4e-34
Shell height (mm)	28.95 ± 2.64^{a}	36.84 ± 3.93^{b}	30.85 ± 2.49^{c}	8.7e-39
Shell diameter (mm)	35.11 ± 2.38^{a}	39.25 ± 3.01^{b}	38.85 ± 2.94^{b}	1.08e-18

Conclusions. The results show a clear site gradient in heavy-metal levels, with the strongest anthropogenic impact at Crkvena Vodica, followed by Vrbeštica, while the reference site had the lowest concentrations. With regard to the soils, differences were most pronounced for Cu and Cd concentrations, with slight exceedances of limit values at Crkvena Vodica and Vrbeštica, whereas Zn, Pb, and As concentrations remained below limits. *H. pomatia* showed organ-specific accumulation, most evident in the hepatopancreas (Cu, Pb, Fe, Mn, and Cd concentrations), while the kidney exhibited substantial accumulation, particularly for Cd content hemolymph differed significantly in Zn concentration, with the exception of kidney Pb concentration peaking at the control site. Overall, *H. pomatia* proved to be a reliable bioindicator; future studies should expand sampling across more sites and seasons and include distance gradients from potential pollution sources.

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Faculty of Sciences and Mathematics, University of Priština in Kosovska Mitrovica, Kosovska Mitrovica, Serbia
[1] *Biology;* [2] *Geography*
e-mails: zeljko.bulatovic@pr.ac.rs (https://orcid.org/0009-0009-8094-1030)
tatjana.jaksic@pr.ac.rs (https://orcid.org/0000-0001-7091-5343)
nikola.djukic@pr.ac.rs (https://orcid.org/0000-0003-1301-7066)
nikola.bacevic@pr.ac.rs (https://orcid.org/0000-0003-0369-3888)