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Accumulation of metal trace elements in different body parts of terrestrial Roman snail *Helix pomatia* L., 1758 on three polluted sites in Serbia

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Abstract

Atmospheric pollution remains one of the growing concerns in the 21st century, with particular focus on metal trace elements (MTE) from anthropogenic sources, due to their adverse effects on biota. The concentration and type of MTE in the atmosphere and in the soil is diverse, depending on the origin of pollutants, which can cause diverse detrimental effects on organisms living in the nearby environment. Three sites in Central Serbia with different origins of MTE pollution (urban contamination, smelting and fly ash area) are assessed, using terrestrial Roman snails (*Helix pomatia*) as biomarker organisms. These snails are sentinel organisms and are known for their capacities for accumulation of MTE. Snails are sampled and their body is divided in three parts: viscera, foot and shell and concentrations of MTE are determined in each of these body parts using inductively coupled plasma optical spectrometry. Results showed contrasting MTE accumulation patterns in body parts of the snails. Of three studied sites, snails sampled in the vicinity of fly ash containment had lower concentrations of MTE comparing to other two polluted sites.

Keywords: terrestrial snails, *Helix pomatia*, biomarker, ICP-OES, bioaccumulation, smelting, fly ash containment, urban pollution

Introduction

Metal trace elements (MTE) are present in the environment and are essential for physiological processes in plants and animals. However, due to the anthropogenic activities, especially those related to traffic, agriculture, mining, smelting and coal burning, concentrations of MTE in the environment are elevated (Kapička et al. 1999; Wang et al. 2003; Yuan et al. 2013), which leads to adverse effects to various levels of organism organization (molecular, cellular, tissue or organ). Molluscs are known for accumulating high amount of metals from the environment (Berger and Dallinger 1993; Coughtrey and Martin 1977; Gimbert et al. 2008; Pihan and de Vaufleury 2000). Among them, terrestrial snails are used as sentinel organisms since the accumulation of MTE may indicate the bioavailability from the environment in their tissues (Berger and Dallinger 1993; Carbone and Faggio 2019; Dallinger and Wieser 1984; Madejón et al. 2013; Salih et al. 2021; Viard et al. 2004; Włostowski et al. 2016). Characteristics that define sentinel organisms are their ubiquitousness, abundance, relatively large body size, ease of sampling, and their role as primary consumers in the food web, which makes terrestrial snails perfect link for tracking the transfer of metals to higher trophic levels (Gomot de Vaufleury and Pihan 2000; Oehlmann and Schulte-Oehlmann 2003; Regoli et al. 2006). To be selected as a sentinel, species must be also capable of giving early indications to environmental hazards, but to show low level of toxic response and also to have a great capacity to quantify the magnitude of site differences (Beeby and Richmond 2002). According to Berger and Dallinger (1993), terrestrial snails meet the most important three prerequisites of a species to become an indicator: (1) practical prerequisites; (2) metal accumulation and influencing factors; (3) incorporation of the results in a reference system.

In Serbia, the emission of metals is monitored by the Serbian Hydrometeorological Institute (http://www.hidmet.gov.rs/) and the Agency for Environmental Protection (http://www.sepa.gov.rs). There are four national and international laws and number of regulations covering the emission of metals. However, there is no systematic monitoring of metal accumulation in living organisms, including terrestrial Roman snail, *Helix pomatia* L., 1758. This species is edible, with established farming technology and high potential for culture, which is developed in some European countries (Ligaszewski et al. 2007; Zucaro et al. 2016). It is known for its meat quality and appreciated by Western Europe cuisines, considered specialty in France, with consumation of *H. pomatia* peaking around Christmas period in many countries, including France, Italy, Spain, Greece, Germany and Portugal.

Since they are intended for consumption of humans and are readily eaten by animals, terrestrial snails can also be valuable tool in risk assessment (Madejón et al. 2013). In Serbia, at the beginning of the 21st century, attempts of establishing edible snail farming were not successful due to the uncontrolled and unsustainable exploitation of Roman snail (Poleksić et al 2004, 2005). Today, there is only a handful of operating snail farms in Serbia, while majority of exported snails are illegally collected from natural populations, which is also common in other Eastern European countries (Andreev 2006; Gheoca 2013). Although *H. pomatia* is not classified within IUCN Red List as an endangered species (Least Concern), it is a protected species in Serbia, with exploitation of its natural populations prohibited. Locations where snails were illegally collected remain unknown to consumers and may include habitats near traffic corridors, landfills, industrial areas or other contaminated sites. Therefore, there is reasonable concern that some of exported snails contain high concentrations of MTE in their bodies. For this reason we conducted field study at three sites contaminated with MTE, but with diverse sources of pollution: smelting, fly ash area and urban contamination.

The aim of the study was to determine MTE concentrations in different body parts (viscera, foot and shell) of the Roman snail collected at three sites in Serbia in order to assess: (i) potential differences among studied sites with certain level of MTE pollution and different pollution sources and (ii) MTE accumulation levels in different body parts.

Materials and methods

Study area

The Roman snail individuals were collected on 08 August 2011 from three sites in Central Serbia exposed to metal contamination (Fig. 1). Each of the sites was characterized by different sources of contamination, but with similar vegetation cover, in order to reduce variability of ecological factors, other than pollution.

Site 1 (Belgrade) is located in the centre of the Zemun administrative area, a part of the Belgrade urban area with estimated population of approximately 1.34 million people. Snails were collected from the park adjacent to the building of the Faculty of Agriculture, University of Belgrade (coordinates: 44°50'21.5"N 20°24'40.9"E) and the source of pollution was mainly traffic and urban pollution. At the time of sampling, leaded gasoline was still available at gasoline stations in Serbia. Program of monitoring of soil pollution in the area of Zemun in 2009 included analysis of As, Ni, Cr, Zn, Cu, Cd, Pb and Hg, as well as several other pollutants (Local Environmental Action Plan of the Municipality of Zemun 2011). Monitoring detected naturally increased Ni content in the proximity of the sampling site, caused by specific local geochemical composition of surface layers of soil, and not by anthropogenic contamination. On the other hand, in samples of soil taken in a wider area of busy roads in the center of Zemun Pb concentrations were greater than 124 μg g^{-1}. The content of Zn and Cu was not increased in samples from this area.

Site 2 (Smederevo) is located in the vicinity (7 km) of the city of Smederevo, in a small forest located within boundaries of a steel plant, the only such plant operating in Serbia. The site (coordinates: 44°36'09.6"N 20°57'47.6"E) is located approximately 200 m away from nearest plant facilities and approximately 400 m away from smoke stack of one of the two currently operating blast furnaces. Monitoring of soil pollution in the area during 2005-2009, in relation to prescribed maximum allowable concentrations (MAC) according to national legislation, detected exceeded concentrations of Ni in 100% of soil samples, moderately increased concentrations of Cu in 80% of samples, exceeded concentration of Pb in a single sample, while concentrations of Zn and Hg were close to MAC (Local Environmental Action Plan of the Municipality of Smederevo 2007). Measured concentrations of Cd, As, Cr, F and B in all tested samples were satisfactory in relation to domestic legislation that complies with international standards in this field. The contamination is estimated in line with the soil quality standards for Dutch target values (Ministry of Housing, Spatial Planning and Environment Directorate, Netherlands). Exceeding of remediation values was not registered in any sample. Measured concentrations of Ni (38.42 - 165.69 μg g^{-1}), Cu (19.5 to 40.8 μg g^{-1}) and Pb (33.23 - 120.04 μg g^{-1}) were significantly lower than the value that requires intervention measures according to standards (the environmental pollutant reference values of Dutch Standards are Ni 210 μg g^{-1}, Cu 190 μg g^{-1} and Pb 530 μg g^{-1}, respectively). The high frequency of the presence of Ni and Cu in soil is probably geologically conditioned. The estimated contamination is largely confirmed by subsequent monitoring of soil in the area (Dragović et al., 2014).

Site 3 (Kostolac) is located near (5 km) the city of Kostolac, where 1003 MW coal thermal power station is operating. Sampling site is situated 500 m away from “Ćirikovac” fly ash containment (44°40'53.4"N 21°10'58.4"E), with surface of approximately 0.76 km^2. According to the Annual Report on testing of soil pollution in the administrative district of Požarevac in 2009, in the municipality of Kostolac, in relation to prescribed MAC national legislation of Ni (50.0 $\mu g\ g^{-1}$), exceeded concentrations of Ni were found in 85% of soil samples. While high levels of Ni in soil are probably geologically conditioned, potential anthropogenic impact must be also taken into account. Measured concentrations of Ni (51.79 - 146.0 $\mu g\ g^{-1}$) were significantly lower than the value that requires intervention measures according to Dutch Standards. Small number of samples comprised increased concentrations of Cu, Zn and Pb, but very close to MAC.

Sampling and dissection

In order to avoid conditions that would represent acute or delayed reactions instead of indication of long-term MTE accumulation, sampling was performed during or near the end of the dormancy phase (Balzan et al., 2001; Beeby and Richmond 2002). In this case, sampling was carried out at the very end of aestivation, immediatelly following an extended period of summer droughts. Prior to collection, snails have been prevented to feed for a few weeks due to poor meteorological conditions (high temperatures and drought), and they resumed feeding couple of days before sampling. 30 active snails were collected, 10 specimens from each sampling site. Only adult (lipped) snails were collected, with shell diameter of 40.1 ± 2.8 mm while their fresh weight (with shell) was 24.5 ± 4.8 g. The three samples were statistically comparable, although individuals from the Site 1 sample were slightly smaller, which is typical for snail populations in urban areas (Dyduch-Falniowska et al. 2001).

Prior to dissection, snails were washed under tap water, starved for 2 days and sacrificed by anesthetization with ethyl acetate in a sealed jar during 90 minutes. During anesthetization, 9% of weight (body fluids) is lost, compared to the total weight of the snail with shell. Snails were dissected and split into three sections: viscera, foot and shell. The shell was removed and crushed for shell content analysis. Soft tissues of each snail were dissected into two components - the foot and the viscera. The foot contained head and muscular sole, mantle edge, esophagus, salivary glands and anterior parts of genital system and ovispermiduct. The viscera comprised hepatopancreas, kidney, heart, intestines and the rest of the genitals. During dissection another 10-15% body fluids is commonly lost, compared to the total weight of the snail with shell.

Elemental accumulation analysis

Prior to elemental accumulation analysis, samples were lyophilized using Gamma 1–16 LSC freeze dryer rotational-vacuum concentrator (Christ, Germany) and afterwards digested in a Speedwave MWS-31microwave digester (Berghof Products Instruments, Germany), at a food temperature program (100–170 °C). Samples were prepared by mixing 0.2–0.5 g dry weight sample portions with 6 mL of 65% HNO_3 (Suprapur; Merck, Germany) and with 4 mL of 30% H_2O_2 (Suprapur; Merck, Germany). After digestion, samples were left at a room temperature for an hour and distilled water was added up to a volume of 25 mL. In addition, blank samples were also made in order to resolve presence of trace elements in utilized chemicals. Samples were analyzed by inductively coupled plasma optical spectrometry (ICP-OES), with assessment of concentrations of 19 elements, presented in Table 1. As the reference materials, BCR-185R of bovine liver and IAEA-336 Lichen were used, showing that the obtained concentrations were within 90–115% of the certified values for studied elements. No certified values were provided for B. Metal and other elements concentrations were expressed as mg g^{-1} dry weight (dw).

Statistical analysis

Metal concentrations were compared among sampling localities. Variable distributions were assessed for normality of distribution using the Kolmogorov–Smirnov test. Since datasets lacked normality of distribution, nonparametric tests were applied. Assessment of the differences among groups was performed with Kruskal–Wallis H test, and followed by comparisons of particular pairs of samples by Mann–Whitney U test. In order to assess the differentiation of the three analyzed tissues based on metal accumulation, tissues were also compared by means of the Canonical Discriminant Analysis. Untreated data for metal concentrations in each tissue were used as input variables.

Results

No significant differences among samples were observed for shell diameter and body mass of snails (Table 2). Mean MTE concentrations in each of the three body parts are presented in Table 2. Some concentrations were below detection thresholds and less frequently detected in shells than in the two body parts (As, Cd, Co, Ni). On the other hand, Li was detected only in the shell, while Pb was detected only in the viscera of sampled snails. Large number of metals showed significant differences among sampling sites. The majority of metals had concentrations significantly higher at Belgrade and Smederevo sites (Al, Cr, Cu, Fe, Mg, Mn, Mo, Ni, Zn), while only Sr and Li showed significantly higher concentrations in Kostolac site. B showed varied results in shell, viscera and foot. The greatest differences among sampling sites were found in MTE concentrations in shells. Large number of MTE showed distinct concentrations in foot, viscera and shell, and in some cases, these differences were up to 15-fold.

Concerning results obtained using multivariate statistics, Figure 2 shows differentiation among metal concentrations in shell, foot and viscera. All three tissues had fair separation along canonical functions (CV), decreasing in the following order: shell > foot > viscera. When shells were compared, first two CV accounted for 100% of the total heterogeneity (CV1 - 84.0% and CV2 - 16.0%), with the largest differentiation among samples by Mg, Mo and Mn concentrations. In, foot, first two CV explained 100% of the total heterogeneity (CV1 - 80.3% and CV2 - 19.7%), with the largest differentiation among samples by Sr, Cu and Mg concentrations. In viscera, first two CV also explained 100% of the total heterogeneity (CV1 - 63.3% and CV2 - 36.7%), with samples mostly differentiated by concentrations of Fe, Zn and B.

Discussion

Distinct accumulation patterns observed in the three studied sampling sites were likely due to site-specific pollution characteristics. A number of studies showed low to moderate positive correlations between MTE accumulation in terrestrial snails and concentrations in soil and/or vegetation (Beeby and Richmond 2002; Boshoff et al. 2015; Nica et al. 2012; Notten et al. 2005). On the other hand, correlation patterns depended on investigated elements, snail species and tissues. Terrestrial snail species and subspecies tend to differ in metal bioaccumulation rates (Fritsch et al. 2011; Gomot and Pihan 1997). Seasonal effects can also be significant, since higher activity in summer commonly leads to lower accumulation levels, probably due to faster turnover and excretion rates (Ćirić et al. 2018). Tissues that are commonly studied include whole-body snail samples or hepatopancreas (synonyms: digestive gland, midgut gland), and sometimes also kidney. We opted for viscera, shell and foot, in order to be able to assess the health risks for consumers, who are usually consuming only the foot of the terrestrial snails.

High MTE accumulation levels were reported for tissues of terrestrial snails sampled, caged or reared in a vicinity of smelters (Fritsch et al. 2011; Rabitsch 1996; Włostowski et al. 2016), which are known to produce significant negative impacts on terrestrial mollusk populations (Nesterkov 2013). Elevated concentrations of Cu, Zn, Pb and Cd were detected in snails from smelting-impacted areas, at levels able to produce toxic effects. Accumulation gradients are commonly observed in such areas, with accumulation levels dependent on a type of smelter, usually dominated by Zn and Pb concentrations. One study, conducted by using whole-body samples of Roman snail as a sentinel species, detected substantially higher concentrations of Zn, Pb and Cd compared to those observed in the present study (Włostowski et al. 2016). Since Smederevo sampling site was located in the vicinity of a steel plant, elevated concentrations of Fe in shells were expected, but such pattern was not reflected in other tissues. There were no differences in foot concentrations among sites, with concentrations in snails from the urban area in Belgrade similar to those in snails living nearby the steel plant.

Urban pollution and proximity to areas with high traffic levels, such as highways, are important factors in MTE bioaccumulation in snails (Massadeh et al. 2016). Concentrations of Zn and Pb in viscera decreased as *H. aspersa* were caged and placed in increasing distance from a highway in France (Viard et al. 2004). Streets with higher traffic induced higher pollution and accumulation of Cr, Cu, Fe, Pb, Mn, Ni and Zn in digestive gland of *H. aspersa* in Ancona, Italy (Regoli et al. 2006) and Cu, Cd, Pb, Zn and Mn in shells of Roman snail in Krakow, Poland (Aleksander-Kwaterczak and Gołas-Siarzewska 2015). In the present study, snails from urban site (Belgrade) overall had significantly higher MTE concentrations taking into consideration all three locations, but the majority of elevated concentrations were observed in shells. Study of MTE bioaccumulation in Roman snail sampled during June in the city of Pančevo, about 25 km northeast from Belgrade, showed that concentrations of Cd, Cu, Zn, Fe and Mn in foot and hepatopancreas were all below concentrations from Belgrade, found in the present study (Ćirić et al. 2018). While Pančevo is a considerably smaller city compared to Belgrade (approx. 76.000 residents), it is recognized as one of black ecological spots in Serbia due to a heavy industry complex located in the area (including oil refinery, petrochemical and artificial fertilizer factories). Pančevo city area is known to have elevated MTE concentrations in soil and river sediments (Mitrović et al. 2019; Sakan et al. 2015). Of importance is higher concentration of Ni in snail viscera determined in Belgrade, compared to other sites. Ni is moderately toxic to snails (Boyd et al. 2002), and is naturally present in higher concentrations in soils from all sites, but is also known as hazard in traffic pollution (Johansson et al. 2009), which probably caused higher accumulation in snails from the urban area.

In contrast to a number of studies focused on MTE bioaccumulation in snails from urban settlements and in vicinity of smelters, studies of snail populations near functional fly ash landfills are quite rare, even though living conditions in those areas is considered to be unfavourable for molluscs (Pech and Fric 2013). Fly ash is a by-product of coal burning in thermal power stations and heating plants. In Kostolac site, fly ash is mixed with water to prevent spreading by wind and is disposed in the landfill, near the sampling site. Typically, fly ash is characterized by high levels of pH and soluble salts, and is rich in Ca, Ba, Pb, Mo, Se, S, B and Sr (Haynes 2009). High levels of MTE in fly ash could be toxic to plants, and B toxicity is usually highlighted as the main threat (Hammermeister et al. 1998), although toxicity of B is considerably lower for animals. Main MTE ingestion route in terrestrial animals is via food (Berger et al. 1993), which is the most likely reason for highest concentrations of B in viscera (compared to Belgrade) and foot (compared to Smederevo) of snails sampled at Kostolac site. Apart from elevated concentrations of B, as well as of Li in shell and Sr in foot and shell, no other MTE was peaking at Kostolac site, which indicates that Roman snail populations from Kostolac were comparatively less impacted by environmental pollution.

In a study by Nowakowska et al. (2012), conducted on Roman snail populations from non-poluted sites, distinct Zn, Cd and Mg bioaccumulation pattern in hepatopancreas, foot, shell and kidney was observed: hepatopancreas > kidney > foot > shell, which corresponds to results in the present study. Such accumulation pattern for Zn and Cd was also confirmed in laboratory and semi-field assays (Berger et al. 1993; Nica et al. 2015). In the present study, majority of elements in soft tissues were higher in viscera compared to foot, except for Ba and Cu. Dallinger and Wieser (1984) described specific pattern of accumulation of some elements in Roman snail, and showed that >70% of Pb, Zn and Cd accumulation occurs in hepatopancreas, while Cu tends to be evenly distributed among organs, which is in line with the present study. Nutritional assay with Roman snail showed that 97% of ingested metals remain in the snail, mostly in the foot (Moser and Wieser 1979). According to Beeby and Richmond (2002), Cu concentrations in soft tissues of *H. aspersa* are not correlated with concentrations in plants or soil.

Use of shells of terrestrial snails in MTE accumulation monitoring is not common, in contrast to the use of soft tissues. Snails are incorporating MTE in shells during growth, which makes them more suitable for monitoring chronic exposure and historical information than for the current status of population and acute pollution (Laskowski and Hopkin 1996). Moreover, concentrations of Zn, Cu, Pb and Cd in shells rarely exceed 5% of concentrations found in soft tissues (Laskowski and Hopkin 1996), which is in line with the data from the present study. This could be a probable reason for significant differentiation of samples indicated, using the canonical discriminant analysis. However, while shells should be able to provide most reliable information on chronic MTE exposure that was present in all three investigated sites, they are characterized by specific MTE accumulation patterns that are substantially different from those in soft tissues. For example, Sr concentrations in shells were several times higher than in viscera or foot of Roman snail, which is common for calcified structures since Sr has similar chemical properties to Ca and therefore gets easily incorporated in the shell (Maurer et al. 2012). This element is rarely reported in studies of MTE accumulation in snail shells. Lower Sr concentrations observed at Smederevo site, were either due to unbalanced Ca/Sr ratios or lower precipitation and water content in the soil (Brand et al. 1986). Mg has similar accumulation properties to Sr and Ca and should follow similar accumulation patterns (Brand et al. 1986), which is in contrast to results of the present study. If compared with other studies where Roman snail was used as a sentinel species, mean concentrations of Cu, Mn and Fe in shells from Smederevo were considerably higher. For example, their concentrations were two to three times higher compared to those in snail shells from the town of Krakow, likely due to the presence of steel factory (Aleksander-Kwaterczak and Gołas-Siarzewska 2015).

To conclude, different MTE accumulation patterns were observed in snails in this study. Of the three investigated localities, Roman snail from Belgrade and Smederevo had higher overall accumulation levels compared to Kostolac site. Thus, results indicate that MTE accumulation in Roman snail is strongly driven by the presence of heavy industry and urban areas, while fly ash landfills seem to have lower pollution potential, and can be considered as moderate source of pollution. Concentrations of MTE were in line with other studies of polluted localities in Europe that were based on monitoring with terrestrial snails, and confirm good potential of Roman snail as a sentinel species of terrestrial MTE pollution.

Ethical Approval

Not applicable, due to the fact that there is no suitable ethical guidelines for mollusks. However, all animals were anesthetized prior to sacrifice, using standardized protocol, as reported in the Material and methods chapter.

Consent to Participate

All authors have given consent to their contribution.

Consent to Publish

All authors have agreed with the content and all have given explicit consent to publish.

Authors Contributions

Bojan Stojnić, Božidar Rašković and Vesna Poleksić contributed to the study conception and design. Material preparation, data collection and analysis were performed by Bojan Stojnić, Božidar Rašković, Ivan Jarić, Stefan Skorić and Goran Topisirović. The first draft of the manuscript was written by Božidar Rašković and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Availability of data and materials

MTE concentrations raw data are available from Supplementary Table 1. All other data that support the findings of this study are available from the corresponding author, [B.R.], upon reasonable request.

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FIGURES

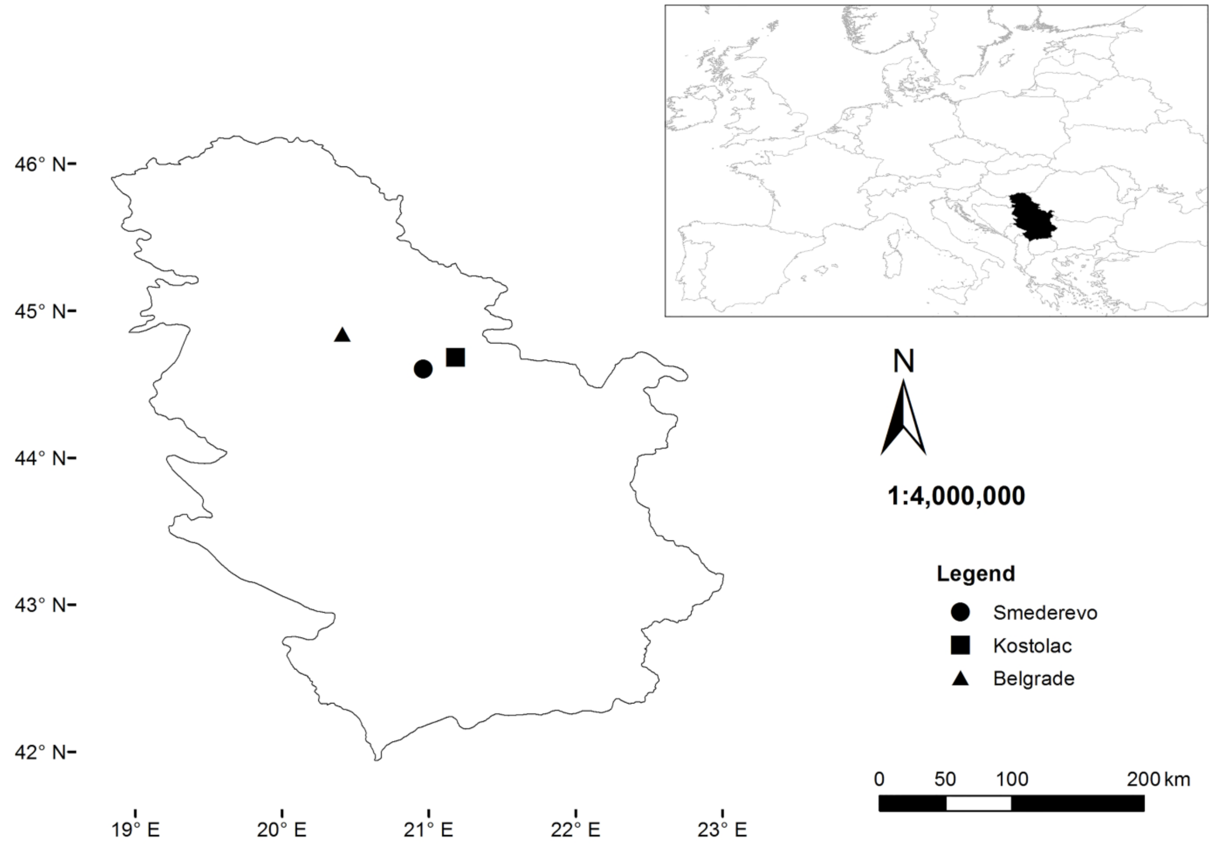

Fig. 1 The location of sampling sites on the territory of Central Serbia. Three locations were chosen in the vicinity of following cities and their exact location is given in the brackets: Belgrade (44°50'21.5"N 20°24'40.9"E), Smederevo (44°36'09.6"N 20°57'47.6"E) and Kostolac (44°40'53.4"N 21°10'58.4"E); inserted image represents location of Serbia within Europe

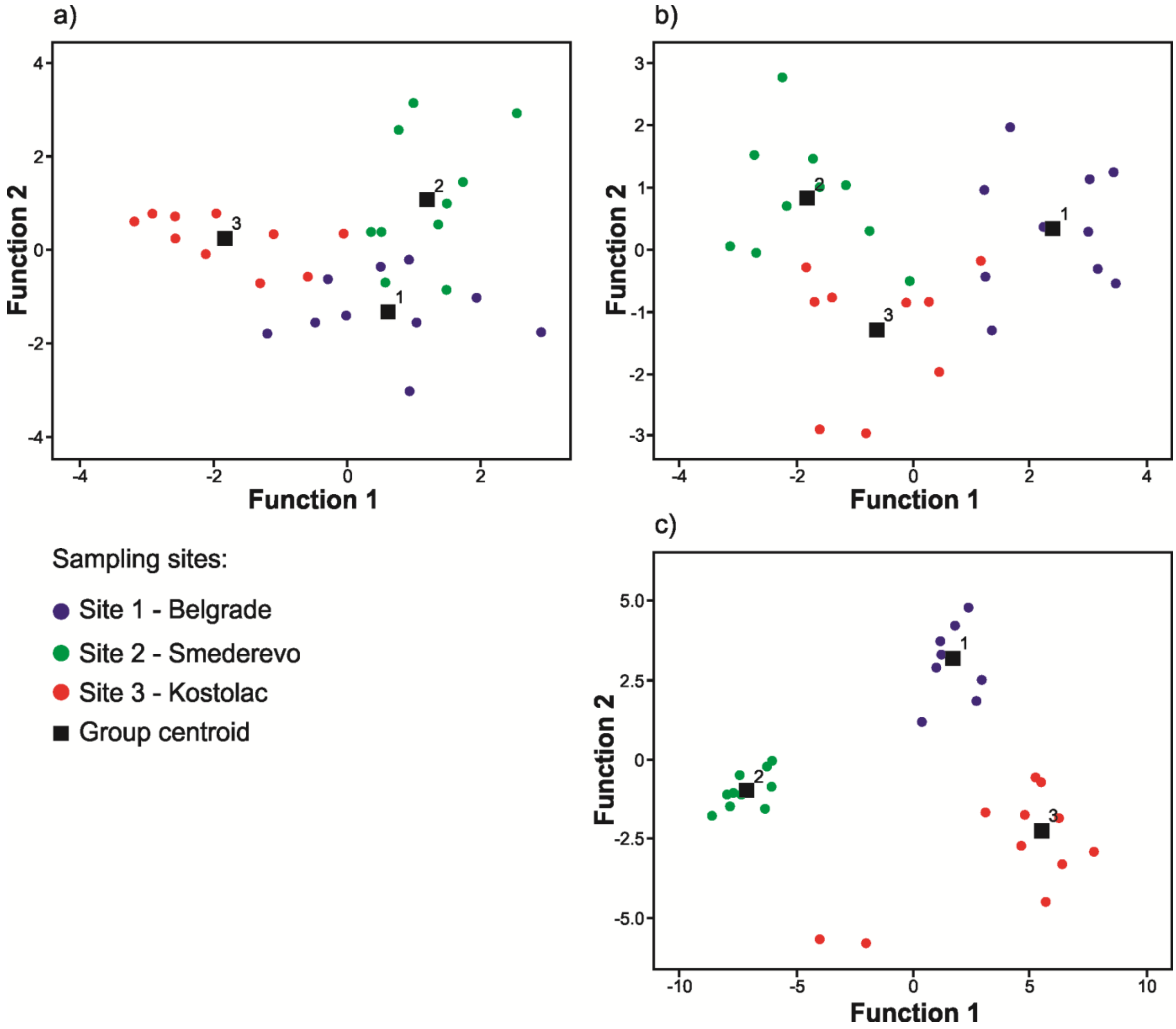

Fig. 2 Plots of canonical discriminant analysis for different body parts of Roman snail (*Helix pomatia*): a) viscera; b) foot; c) shell

Table 1. Analyzed elements, wavelength lines and detection threshold of ICP-OES

Element	Wavelength line (nm)	Detection threshold (mg g^{-l})
Al	394.401	0.125
As	189.042	0.223
B	249.773	0.074
Ba	233.527	0.042
Cd	228.802	0.010
Co	228.616	0.019
Cr	205.552	0.029
Cu	324.754	0.046
Fe	259.941	0.044
Hg	184.950	0.437
Li	460.289	3.320
Mg	279.079	0.048
Mn	259.373	0.032
Mo	202.095	0.062
Ni	231.604	0.090
Pb	220.353	0.271
Se	196.090	0.156
Sr	460.733	1.265
Zn	206.191	0.031

Table 2. Shell diameter, body mass (including shell) and metal trace element (MTE) concentrations in different body parts of Roman snail, *Helix pomatia* (mean value ± standard deviation) from the three studied localities. MTE concentrations are expressed as μg g^{-1} dry weight, while ND indicates values below the detection threshold. Hg and Se were below detection threshold in all samples and are not presented in table.

		Sampling site		
		Belgrade	Smederevo	Kostolac
Shell diameter (mm)		38.8 ± 2.4^{a}	40.6 ± 3.7^{a}	40.8 ± 1.6^{a}
Body mass (g)		22.3 ± 4.4^{a}	25.0 ± 5.8^{a}	26.1 ± 3.3^{a}
	Tissue			
Al	shell	32.78 ± 7.40^{a}	62.75 ± 38.89^{b}	35.21 ± 16.27^{a}
	foot	6.63 ± 4.89^{a}	7.44 ± 2.48^{a}	7.72 ± 4.59^{a}
	viscera	15.77 ± 11.17^{a}	33.71 ± 30.31^{a}	24.48 ± 19.56^{a}
As	shell	ND	ND	ND
	foot	0.23 ± 0.17^{a}	0.58 ± 0.25^{b}	0.48, 0.50**
	viscera	0.40 ± 0.26^{a}	0.38 ± 0.26^{a}	0.34*
B	shell	1.10 ± 0.20^{a}	2.14 ± 1.15^{b}	0.86 ± 0.14^{c}
	foot	4.44 ± 2.04^{a}	2.46 ± 0.71^{b}	4.18 ± 1.61^{a}
	viscera	8.17 ± 1.93^{a}	13.20 ± 4.70^{b}	11.38 ± 2.45^{b}
Ba	shell	17.26 ± 1.78^{a}	19.35 ± 3.74^{a}	15.57 ± 2.53^{a}
	foot	241.96 ± 241.38^{a}	165.44 ± 128.06^{a}	101.35 ± 101.16^{a}
	viscera	72.81 ± 78.79^{a}	176.94 ± 224.94^{a}	84.02 ± 106.70^{a}
Cd	shell	ND	ND	ND
	foot	0.46 ± 0.46^{a}	0.52 ± 0.15^{a}	0.59 ± 0.31^{a}
	viscera	5.88 ± 2.17^{a}	11.16 ± 5.95^{a}	6.83 ± 4.73^{a}
Co	shell	ND	ND	ND
	foot	0.21 ± 0.27^{a}	0.09 ± 0.11^{a}	0.07, 0.25**
	viscera	0.20 ± 0.16^{a}	0.29 ± 0.24^{a}	0.13 ± 0.10^{a}
Cr	shell	0.09 ± 0.07^{a}	0.09 ± 0.11^{a}	0.04, 0.07**
	foot	0.34 ± 0.12^{a}	0.19 ± 0.08^{b}	0.16 ± 0.10^{b}
	viscera	0.27 ± 0.13^{a}	0.35 ± 0.31^{a}	0.16 ± 0.10^{a}
Cu	shell	7.08 ± 1.48^{a}	8.54 ± 3.79^{a}	3.35 ± 1.24^{b}
	foot	116.06 ± 52.34^{a}	79.35 ± 33.67^{a}	62.03 ± 27.15^{a}
	viscera	78.52 ± 41.73^{a}	63.63 ± 27.95^{a}	47.49 ± 28.87^{a}
Fe	shell	51.62 ± 14.41^{a}	122.50 ± 48.27^{b}	44.53 ± 14.83^{a}
	foot	95.46 ± 44.92^{a}	73.64 ± 25.51^{a}	88.25 ± 48.68^{a}
	viscera	168.10 ± 64.89^{a}	181.37 ± 55.45^{a}	96.45 ± 30.53^{b}
Li	shell	9.83 ± 0.88^{a}	11.12 ± 1.36^{b}	12.11 ± 2.16^{b}
	foot	ND	ND	ND
	viscera	ND	ND	ND
Mg	shell	174.14 ± 28.07^{a}	133.02 ± 31.37^{b}	114.77 ± 23.30^{b}
	foot	1699.15 ± 612.00^{a}	974.33 ± 481.37^{a}	1272.62 ± 504.63^{a}
	viscera	3244.07 ± 2325.82^{a}	2354.48 ± 1176.88^{a}	2118.77 ± 958.17^{a}
Mn	shell	7.40 ± 0.67^{a}	11.55 ± 1.91^{b}	5.96 ± 0.81^{c}
	foot	7.07 ± 1.39^{a}	7.91 ± 4.68^{a}	7.30 ± 1.52^{a}
	viscera	90.96 ± 75.06^{a}	115.61 ± 159.73^{a}	130.22 ± 144.38^{a}
Mo	shell	2.89 ± 0.48^{a}	2.35 ± 0.17^{b}	2.31 ± 0.22^{b}

	foot	2.88 ± 0.21^{a}	3.68 ± 0.91^{a}	3.13 ± 0.43^{a}
	viscera	3.43 ± 0.49^{a}	3.35 ± 0.47^{a}	3.40 ± 0.40^{a}
Ni	shell	ND	ND	ND
	foot	0.16 ± 0.11^{a}	0.13 ± 0.10^{a}	0.12 ± 0.11^{a}
	viscera	1.00 ± 0.33^{a}	0.20 ± 0.18^{b}	0.32 ± 0.20^{b}
Pb	shell	ND	ND	ND
	foot	ND	ND	ND
	viscera	1.78 ± 1.32^{a}	2.43 ± 1.45^{a}	ND
Sr	shell	368.98 ± 26.99^{a}	289.69 ± 34.72^{b}	380.85 ± 28.67^{a}
	foot	62.38 ± 33.85^{a}	14.43 ± 7.49^{b}	24.26 ± 9.93^{c}
	viscera	29.36 ± 13.39^{a}	44.73 ± 23.62^{a}	55.16 ± 41.58^{a}
Zn	shell	2.15 ± 0.63^{a}	2.14 ± 0.95^{a}	1.06 ± 0.90^{b}
	foot	37.91 ± 5.08^{a}	32.02 ± 13.35^{a}	38.44 ± 6.50^{a}
	viscera	135.68 ± 64.81^{a}	213.58 ± 179.65^{a}	88.77 ± 39.89^{a}

^{a,b,c} The value with a different letter in the same row is different (Mann–Whitney U test, $p<0.05$)

*Concentrations above detection threshold only in one sample

**Concentrations above detection threshold only in two samples