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RESEARCH PAPER

Heavy Metal Concentrations in Different Tissues of the Snail *Viviparus mamillatus* (Küster, 1852) from Lacustrine and Riverine Environments in Montenegro

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Abstract

The objective of this study was to analyze distribution of heavy metals (Cd, Cu, Pb, Fe and Zn) in the various tissues of a freshwater snail *V. mamillatus* from riverine and lacustrine environments in Montenegro. Significant differences between the different tissue of *V. mamillatus* were observed in concentrations of all studied metals (with exception of Pb). The results of correlation analysis revealed that head with tenatacles can be used as biomonitoring agents for Cd, Zn and Fe, mantle for Cd and Zn, while foot could reflect environmental Fe. Further, it was indicated that metal accumulation by *V. mamillatus* might affect its growth rate as we found revealed significant negative correlations between Pb, Cd, Cu and Zn in different tissues and allometric parameters. The described results showed suitability of *V. mamillatus* as bioindicator in regard to monitor environmental compartment.

Keywords: Freshwater snail; bioindicator; environmental monitoring; allometric parameter.

Introduction

Freshwater snails have been often used as bioindicators of contamination by heavy metals (Elder & Collins, 1991; Gundacker, 2000; Yap *et al*., 2009; Blagojević, Vukašinović-Pešić, Grudić, & Pešić, 2014). Important advantages of snails for biomonitoring research are their limited mobility and large size in comparison with other freshwater organisms (Elder & Collins, 1991). It is worth noting that bioaccumulation of metals in aquatic gastropods varied strongly according to sampling site, metal and the specific species (Yap & Cheng, 2013). Even within the same species, individual characteristics such as age, growth rate (Lau, Mohamed, Tan Chi Yen, & Su'Ut, 1998) and feeding (Mance, 1990) can have significant effects on responses to heavy metal contamination.

However, in most studies (Enzemonye, Enobakhare, & Ilechie, 2006; Kim & Kim, 2007; Zverkova, 2009; Astani, Vosoughi, Salimi, & Ebrahimi, 2012; Bhalchandra & Ram, 2013) on the distribution of heavy metals in freshwater gastropods, snails were dissected into soft body and shell, so their bioaccumulative potential should be interpreted with caution. As already stated by Elder and Collins (1991) metals in freshwater molluscs tend to be nonuniformly distributed among different organs. Only in a few studies (Pena & Poscidio, 2008; Yap *et al*., 2009) different soft tissue of freshwater snails were tested as bioindicators of the heavy metal contamination.

V. mamillatus (Küster, 1852) is an viviparous, endemic species known from Albania, Montenegro, Croatia and mainland Greece (Pešić & Glöer, 2013). This is a relatively common species that lives in both, lacustrine and river environments, and the largest (shell up to 60 mm in height) species of freshwater gastropods in Montenegro, fulfilling requirements for a possible good bioindicator species. Recently, Blagojević *et al*. (2014) used soft body and shell of *V. mamillatus* to monitor heavy metal pollution of Zeta river. They provide comparisons among freshwater snails of different distribution indicating that species with a restricted distribution (endemics) can be used as more suitable bioindicator for assessing heavy metal pollution.

Having all this in mind, the aims of this study were (1) to examines distribution of heavy metals (Pb, Cd, Zn, Cu and Fe) in different parts of *V. mamillatus* from lacustrine and riverine environments, where populations of this snail live, 2) to determine relationship between heavy metals in the different parts of *V. mamillatus* and its allometric parameters, and 3) test correlation between heavy metal concentration in different tissues and sediments

collected from riverine and lacustrine habitats in order to test the suitability of *V. mamillatus* as bioindicator in regard to its environmental compartment.

Materials and Methods

Sampling of *V. mamillatus* and surface sediment was conducted at the three sampling sites (Figure 1): 1) Zeta River (ZR) (42°28'7.46"N, 19°15'27.88"E); 2) Skadar Lake (SL) (42°18'50.10"N, 19°21'11.94"E) and 3) outlet stretches of the Matica River (MR) (42°27'38.40"N, 19°10'37.72"E).

The samples were collected in August 2015, at the time when water level were the lowest and abundance of sampled species high. The snails were collected from the shore by hand, washed with distilled water and transferred into clean plastic bags. Upon arrival at the laboratory, the samples were stored at –10°C until metal analysis.

For the analysis 25-30 specimens of snails with almost a similar size were randomly taken from the main sample and thawed at room temperature (about 30°C) on a clean tissue paper. The soft tissues were then separated from the shell and operculum, and dissected and pooled into four different parts, namely head with tentacles, mantle, visceral mass and foot. For the allometric studies, five specimens were selected and measured for the following parameters: shell height, shell weight, tissue wet weight, tissue dry weight, and water content. The samples were dried for 72 h at 60°C in an oven to constant dry weights (Yap, Ismail, Tan, & Abdul Rahim, 2003). The sediment samples were dried at 60°C for at least 72 h to constant dry weights.

A dry ashing method was used for destruction of organic matter to determine heavy metals from soft tissue (head with tentacles, mantle, visceral mass and foot) of snails. The crucibles with samples were partially covered and carefully dried on a burner. Than the samples were charred on a hot plate and ashed in a muffle furnace at 450°C for 12 h. After ashing the ash was dissolved with 1 M HCl and than completed to the volume of 25 mL with 1 M HCl for analysis (AOAC, 1997).

Preparation of shell and operculum for heavy metals analysis was performed as follows: the shell (and operculum) was digested with a mixture of HNO_3 and HCl with the addition of H_2O_2, so that approximately 4 g of sample is poured with 10 mL HNO_3 (1:1) in a glass partially covered and heated at 95-100 °C for 10-15 minutes. To the cooled sample 5 mL of concentrated HNO_3 is added, cover and heat for 30 min at the same temperature. After cooling, 2 mL of deionized water and 3 mL of 30 % H_2O_2 is added and slight heated. After the sample is cooled 7 mL of 30 % H_2O_2 is added. Then add 5 mL of concentrated HCl and 10 mL of deionized water, covered and heated 15 min. After cooling, the sample is transferred to a flask of 50 mL and completed to the volume with deionized water for analysis (AOAC, 1997).

Analytical methodologies used in this study were confirmed for accuracy using SRM 2976 mussel tissue, a biological standard reference materials (SRM) purchased from the National Institute of Standards and Technology (NIST). The obtained

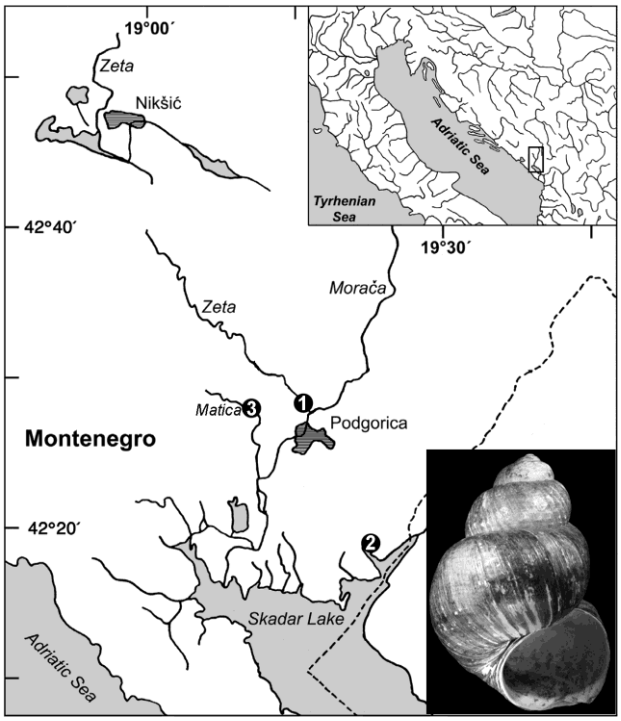

Figure 1. Map of the study area showing the sampling localities: 1 – Zeta River; 2 – Skadar Lake; 2 – Matica River. Inset top right: regional view; inset bottom right: photograph of *Viviparus mamillatus* Küster, 1852.

recovery data for all the metals analysed in the SRM were 95.0% for Pb, 102.5% for Cd, 98.5% for Zn, 105% for Cu and 95.7% for Fe.

For the analysis of total metal concentrations in the sediment samples, the direct aqua-regia method was used. About 1 g of each dried sample was weighed and digested in a combination of concentrated nitric acid and perhloric acid in the ratio of 4:1. They were placed in a hot blok digester first at low temperature (40°C) for 1 h and were then fully digested at high temperature (140°C) for at least 3 h. The digested samples were then diluted to a volume of 50 mL with double distilled water and the sample was then filtered for analysis. The analytical accuracy was determined using a sertified standard reference material of the National Institute of Standards and Technology for trace elements in lake sediment (SRM 2709). The recoveries were within 89.7-110.3% of the certified values.

All parts of the snails samples (except operculum and head with tentacles) and sediments were prepared in triplicate and their average value was assessed. Blank solutions were added to the series of samples measured after every fifth sample determination. The concentrations of Pb, Cd, Cu, Zn and Fe were determined using inductively coupled plasma-optical emission spectroscopy (ICP-OES) according to the iCAP 6000 spectrometer method. The limits of detection and quantification of the analyzed heavy metals were, respectively, as follows: Pb (2.348 and 7.826 µg/l), Cd (0.100 and 0.333 µg/l), Cu (1.356 and 4.521 µg/l), Zn (0.119 and 0.396 µg/l) and Fe (0.590 and 1.967 µg/l).

All statistical analyses were performed using SPSS 17.0 (SPSS Statistics for Windows, Version 17.0. Chicago: SPSS Inc.). Data were expressed as mean ±standard deviation. They were analyzed by 2-way ANOVA with *tissue* and *site* as independent factors. 2-way ANOVA was also applied to test the effects of site and *tissue* on tissue metal concentration in snails. Bonferroni test, as posthoc test, was performed on the mean concentrations of the metals in order to determine if there any significant differences between pairs of tissues at the confidence level 0.05. The relationship of heavy metals between the tissues of snails and sediments were analyzed using the Spearman's correlation analysis.

Results and Discussion

Heavy metal concentrations in the different parts of *V. mamillatus* collected from the three sampling sites are given in Table 1.

In general, elevated concentrations of Pb were found in all parts of soft tissue (except foot) of the snails from all sites, wheares the highest concentration was noted in the operculum of snails from Zeta river. For the Cd, elevated concentrations were found in mantle and visceral mass from the snails of all sites, whilst the highest concentrations were observed in visceral mass of snails from Matica river. The highest Cu and Zn concentrations were found in visceral mass of the snails from all sites and for Fe, in operculum of snails from Skadar lake. Table 2 showing results of 2-way ANOVA testing the effects of sampling sites and snail tissues on metal concentrations. The results showed lack of a significant effect of *site* on tissue metal concentrations. On other hand, *tissue* had significant effects for all metals studied, with exception of Pb.

The mean concentrations of the five analyzed heavy metals in the different tissues (including shell

Table 1. Heavy metal concentrations in different parts of *V. mammilatus* collected on the three studied sites: Zeta river, Skadar lake and Matica river; mean values ± standard deviation

Site		Pb (mg/kg)	Cd (mg/kg)	Cu (mg/kg)	Zn (mg/kg)	Fe (mg/kg)
Zeta river	Head with tentacles	1.67±0.00	0.063±0.00	30±0.00	125±0.00	283±0.00
	Mantle	1.90±0.02	0.28±0.00	82±0.15	94±0.20	1500±1.56
	Visceral mass	1.71±0.07	1.07±0.01	152±5.57	819±2.50	914±0.64
	Foot	0.40±0.02	0.045±0.00	72±6.35	108±0.18	202±3.45
	Operculum	4.8±0.00	0.05±0.00	30±0.00	58±0.00	960±0.00
	Shell	0.15±0.01	0.006±0.00	5.4±0.55	1.8±0.00	52±0.23
Skadar lake	Head with tentacles	1.84±0.00	0.072±0.00	25±0.00	150±0.00	391±0.00
	Mantle	0.80±0.00	0.15±0.00	85±0.23	99±0.35	747±0.85
	Visceral mass	0.92±0.05	0.45±0.00	108±3.50	618±1.25	722±0.86
	Foot	0.44±0.08	0.026±0.00	39±1.65	87±0.24	232±4.35
	Operculum	1.20±0.00	0.03±0.00	11±0.00	121±0.00	4605±0.00
	Shell	0.19±0.00	0.005±0.00	4.6±0.28	1.5±0.00	192±2.45
Matica river	Head with tentacles	2.26±0.00	0.14±0.00	53±0.00	182±0.00	455±0.00
	Mantle	1.98±0.03	0.89±0.05	217±1.20	196±2.45	3130±5.67
	Visceral mass	1.97±0.04	1.90±0.03	309±1.20	806±0.95	1935±6.85
	Foot	0.71±0.12	0.16±0.04	102±5.35	138±0.12	462±5.87
	Operculum	1.40±0.00	0.10±0.00	15±0.00	178±0.00	3481±0.00
	Shell	0.14±0.02	0.005±0.00	2±0.08	2,8±0.03	66±1.37
Sediment	Zeta river	5.20±0.43	0.14±0.02	11.30±0.85	14±0.20	8300±105
	Skadar lake	14.10±0.190	0.28±0.05	16.30±0.75	36±0.35	11500±130
	Matica river	10.80±0.35	0.86±0.06	17±1.05	45±0.86	21500±245

Table 2. Results of a 2-way ANOVA for the effects of '*sites*', and '*tissues*' on metal concentrations in snail tissues. Significant effects were only considered if P<0.05

	Source of variation	MS	F-ratio	Sig.
Pb	Sites	1.16	1.52	0.265
	Tissues	2.,26	2.98	0.067
Cd	Sites	0.26	3.06	0.092
	Tissues	0.58	6.73	**0.005**
Cu	Sites	8260.95	4.06	0.051
	Tissues	15424.62	7.59	**0.003**
Zn	Sites	7962.75	3.05	0.093
	Tissues	216117.59	82.66	**0.000**
Fe	Sites	1316666.89	1.60	0.249
	Tissues	3785986.86	4.61	**0.019**

and operculum) of *V. mamillatus* from the three studied sites are summarized in Table 3. No significant differences were observed in accumulation of Pb by different tissues. On other hand we found significant differences in concentrations of Cd, Cu, Zn and Fe between different tissues of *V. mamillatus.* The level of Zn in visceral mass was significantly higher than the other parts of the snail.

Our study confirmed that the levels of heavy metal in the shell were significantly lower than in gastropod soft tissue. This is in agreement with many studies which showed that shells of mollusk accumulate lower concentrations of Cu, Zn and Fe than soft tissue (Szefer *et al*., 2002; Hoang, Rogevich, Rand, & Frakes, 2008; Yap *et al*., 2009). However, some studies showed that shells can be used as biomonitoring agents for Cd and Zn (Jordaens, De Wolf, Vandecasteele, Blust, & Backeljau, 2006). According to Lau *et al*. (1998) the shell of *Melanoides tuberculata* would be most suitable for assessing Cu in the aquatic environment, whilst Yap *et al*. (2009) found that the shell of freshwater snail *Pomacea insularum* can reflect environmental Zn. The results of the Spearman's correlation analysis used in our study showed that there was no correlation found between shell and sediment for any of the studied metals.

Heavy metal concentrations in sediments sampled on studied sites are given in Table 1. The highest concentrations of Cd, Zn, Cu and Fe were found in sediment of Matica River, and for the Pb in sediment from Skadar Lake. Concentrations of all heavy metals investigated in the sediment from all sites were below the maximum permissible levels allowed by the national regulations (Official Gazette of Montenegro, 1997).

Results of Spearman's correlation analysis for heavy metal concentrations in tissues and sediments are given in Table 4. The results revealed that the head with tentacles can be used as biomonitoring agent for assessing environmental Cd as the perfect positive correlation (Rs=1 p=0) was found for the pair of Cd_sediment-Cd_head with tentacles. In addition, head with tentacles can reflect environmental Zn as the perfect correlation (Rs=1 p=0) was found between Zn in sediment and head with tentacles. According to Yap and Edward (2010) tentacles are considered responsible for the metal transfer to the organism.

Moreover, for Zn, the perfect positive correlations (R=1 p=0) were found between the pairwises for Zn_sediment–Zn_mantle and Zn_sediment–Zn_operculum. The current data indicate that mantle and operculum can be used as biomonitoring agents for environmental Zn. The epidermis of the mantle secretes conchiolin and calcium carbonate, including heavy metals, and creates a shell what can be explanation for the high concentration of Zn and Cu in mantle (Yap & Cheng, 2013).

For Fe, the most significant positive correlations (R=1 p=0) were found between the pairs for Fe_sediment-Fe_head with tentacles and Fe_sediment-Fe_foot. Thus, it may be concluded that foot and head with tentacles can be used a biomonitoring agents for assessing Fe in the aquatic environment. However, the concentration of Fe as well the concentration of the apparently less bioavailable Pb in all tissues (including operculum and shells) of *V. mamillatus* is significantly lower than in sediment from all sites. On other hand, Cd, Cu and Zn loads of the mollusks exceeded environmental concentrations, which indicate the presence of bioaccumulation process (Gundacker, 2000).

For Pb there was no significant correlation found between any of pairs of tissues and sediments. The operculum in our study accumulated the highest concentration of Pb and Fe. Our results are not consistent with Yap *et al*. (2009) who reported low heavy metal concentrations in operculum and foot of *Pomacea insularum* from polluted and unpolluted freshwater ecosystems in Malaysia, suggesting that these two organs functionally are not major metal storage or metal detoxifying organ.

The correlations between the heavy metal concentrations in different tissue of *V. mamillatus* and its allometric parameters are shown by Spearman's correlation coefficient in Table 5. Our study confirmed that concentrations of heavy metals in

Table 3. Mean heavy metal concentrations in the different parts of *V. mamillatus*. Values shown as mean±standard deviation, followed by the same small letter(s) within the same column are not significantly different from one another (two-way ANOVA, Bonferroni test, α=0.05)

	Pb	Cd	Cu	Zn	Fe
Head with tentacles	1.92±0.26^{a}	0.09±0.04^{a}	36.00±12.62$^{a.b}$	152.33±24.25^{a}	376.33±73.47$^{a.b}$
Mantle	1.56±0.56^{a}	0.44±0.33$^{a.b}$	128.00±65.15$^{a.b}$	129.67±48.60^{a}	1792.33±1029.48$^{a.b}$
Visceral mass	1.53±0.46^{a}	1.14±0.61^{b}	189.67±89.30^{b}	747.67±95.07^{b}	1190.33±551.05$^{a.b}$
Foot	0.52±0.14^{a}	0.08±0.06^{a}	71.00±26.63$^{a.b}$	111.00±21.66^{a}	298.67±120.22^{a}
Operculum	2.47±1.71^{a}	0.06±0.03^{a}	18.67±8.47^{a}	119.00±50.73^{a}	3015.33±1557.55^{b}
Shell	0.16±0.02^{a}	0.00±0.00^{a}	4.00±1.50^{a}	2.03±0.58^{a}	103.33±65.17^{a}

Table 4. Spearman's correlation coefficient between heavy metal concetrations in the different parts of *V. mamillatus*, their allometric parameters (n = 10) and environmetal surface sediments

Rs (p)	Shell height	Shell width	Wet weight	Dry weight	Pb_sed	Cd_sed	Cu_sed	Zn_sed	Fe_sed
Pb_headwithtentacles	0.49 (0.062)	0.52 (0.049)	0.51 (0.052)	0.47 (0.075)	0.5 (0.058)	1.00 (0.000)	1.00 (0.000)	1.00 (0.000)	1.00 (0.000)
Pb_mantle	-0.41 (0.132)	-0.4 (0.138)	-0.34 (0.215)	-0.36 (0.188)	-0.5 (0.058)	0.5 (0.058)	0.5 (0.058)	0.5 (0.058)	0.5 (0.058)
Pb_visceralmass	-0.41 (0.132)	-0.4 (0.138)	-0.34 (0.215)	-0.36 (0.188)	-0.5 (0.058)	0.5 (0.058)	0.5 (0.058)	0.5 (0.058)	0.5 (0.058)
Pb_foot	0.49 (0.062)	0.52 (0.049)	0.51 (0.052)	0.47 (0.075)	0.5 (0.058)	1.00 (0.000)	1.00 (0.000)	1.00 (0.000)	1.00 (0.000)
Pb_operculum	-0.9 (0.000)	-0.92 (0.000)	-0.85 (0.000)	-0.83 (0.000)	- 1.00 (0.000)	-0.5 (0.058)	-0.5 (0.058)	-0.5 (0.058)	-0.5 (0.058)
Pb_shell	0.41 (0.132)	0.4 (0.138)	0.34 (0.215)	0.36 (0.188)	0.5 (0.058)	-0.5 (0.058)	-0.5 (0.058)	-0.5 (0.058)	-0.5 (0.058)
Cd_headwithtentacles	0.49 (0.062)	0.52 (0.049)	0.51 (0.052)	0.47 (0.075)	0.5 (0.058)	1.00 (0.000)	1.00 (0.000)	1.00 (0.000)	1.00 (0.000)
Cd_mantle	-0.41 (0.132)	-0.4 (0.138)	-0.34 (0.215)	-0.36 (0.188)	-0.5 (0.058)	0.5 (0.058)	0.5 (0.058)	0.5 (0.058)	0.5 (0.058)
Cd_visceralmass	-0.41 (0.132)	-0.4 (0.138)	-0.34 (0.215)	-0.36 (0.188)	-0.5 (0.058)	0.5 (0.058)	0.5 (0.058)	0.5 (0.058)	0.5 (0.058)
Cd_foot	-0.41 (0.132)	-0.4 (0.138)	-0.34 (0.215)	-0.36 (0.188)	-0.5 (0.058)	0.5 (0.058)	0.5 (0.058)	0.5 (0.058)	0.5 (0.058)
Cd_operculum	-0.41 (0.132)	-0.4 (0.138)	-0.34 (0.215)	-0.36 (0.188)	-0.5 (0.058)	0.5 (0.058)	0.5 (0.058)	0.5 (0.058)	0.5 (0.058)
Cd_shell	-0.8 (0.000)	-0.83 (0.000)	-0.79 (0.001)	-0.75 (0.001)	-0.87 (0.000)	-0.87 (0.000)	-0.87 (0.000)	-0.87 (0.000)	-0.87 (0.000)
Cu_headwithtentacles	-0.41 (0.132)	-0.4 (0.138)	-0.34 (0.215)	-0.36 (0.188)	-0.5 (0.058)	0.5 (0.058)	0.5 (0.058)	0.5 (0.058)	0.5 (0.058)
Cu_mantle	0.49 (0.062)	0.52 (0.049)	0.51 (0.052)	0.47 (0.075)	0.5 (0.058)	1.00 (0.000)	1.00 (0.000)	1.00 (0.000)	1.00 (0.000)
Cu_visceralmass	-0.41 (0.132)	-0.4 (0.138)	-0.34 (0.215)	-0.36 (0.188)	-0.5 (0.058)	0.5 (0.058)	0.5 (0.058)	0.5 (0.058)	0.5 (0.058)
Cu_foot	-0.41 (0.132)	-0.4 (0.138)	-0.34 (0.215)	-0.36 (0.188)	-0.5 (0.058)	0.5 (0.058)	0.5 (0.058)	0.5 (0.058)	0.5 (0.058)
Cu_operculum	-0.9 (0.000)	-0.92 (0.000)	-0.85 (0.000)	-0.83 (0.000)	- 1.00 (0.000)	-0.5 (0.058)	-0.5 (0.058)	-0.5 (0.058)	-0.5 (0.058)
Cu_shell	-0.49 (0.062)	-0.52 (0.049)	-0.51 (0.052)	-0.47 (0.075)	-0.5 (0.058)	- 1.00 (0.000)	- 1.00 (0.000)	- 1.00 (0.000)	- 1.00 (0.000)
Zn_headwithtentacles	0.49 (0.062)	0.52 (0.049)	0.51 (0.052)	0.47 (0.075)	0.5 (0.058)	1.00 (0.000)	1.00 (0.000)	1.00 (0.000)	1.00 (0.000)
Zn_mantle	0.49 (0.062)	0.52 (0.049)	0.51 (0.052)	0.47 (0.075)	0.5 (0.058)	1.00 (0.000)	1.00 (0.000)	1.00 (0.000)	1.00 (0.000)
Zn_visceralmass	-0.9 (0.000)	-0.92 (0.000)	-0.85 (0.000)	-0.83 (0.000)	- 1.00 (0.000)	-0.5 (0.058)	-0.5 (0.058)	-0.5 (0.058)	-0.5 (0.058)
Zn_foot	-0.41 (0.132)	-0.4 (0.138)	-0.34 (0.215)	-0.36 (0.188)	-0.5 (0.058)	0.5 (0.058)	0.5 (0.058)	0.5 (0.058)	0.5 (0.058)
Zn_operculum	0.49 (0.062)	0.52 (0.049)	0.51 (0.052)	0.47 (0.075)	0.5 (0.058)	1.00 (0.000)	1.00 (0.000)	1.00 (0.000)	1.00 (0.000)
Zn_shell	-0.41 (0.132)	-0.4 (0.138)	-0.34 (0.215)	-0.36 (0.188)	-0.5 (0.058)	0.5 (0.058)	0.5 (0.058)	0.5 (0.058)	0.5 (0.058)
Fe_headwithtentacles	0.49 (0.062)	0.52 (0.049)	0.51 (0.052)	0.47 (0.075)	0.5 (0.058)	1.00 (0.000)	1.00 (0.000)	1.00 (0.000)	1.00 (0.000)
Fe_mantle	-0.41 (0.132)	-0.4 (0.138)	-0.34 (0.215)	-0.36 (0.188)	-0.5 (0.058)	0.5 (0.058)	0.5 (0.058)	0.5 (0.058)	0.5 (0.058)
Fe_visceralmass	-0.41 (0.132)	-0.4 (0.138)	-0.34 (0.215)	-0.36 (0.188)	-0.5 (0.058)	0.5 (0.058)	0.5 (0.058)	0.5 (0.058)	0.5 (0.058)
Fe_foot	0.49 (0.062)	0.52 (0.049)	0.51 (0.052)	0.47 (0.075)	0.5 (0.058)	1.00 (0.000)	1.00 (0.000)	1.00 (0.000)	1.00 (0.000)
Fe_operculum	0.9 (0.000)	0.92 (0.000)	0.85 (0.000)	0.83 (0.000)	1.00 (0.000)	0.5 (0.058)	0.5 (0.058)	0.5 (0.058)	0.5 (0.058)
Fe_shell	0.9 (0.000)	0.92 (0.000)	0.85 (0.000)	0.83 (0.000)	1.00 (0.000)	0.5 (0.058)	0.5 (0.058)	0.5 (0.058)	0.5 (0.058)

different tissues play an important role for the growth performance of the populations examined. The results of correlation analysis revealed very strong negative correlations between concentrations of Pb and Cu (in operculum), Cd (shell), Zn (visceral mass) and Fe (shall and operculum) with all allometric parameters examined, indicating that heavy metal accumulation of these metals by *V. mamillatus* might affect growth rate of its populations. Some studies (Leung, Grist, Morley, Morritt, & Crane, 2001; Yap & Edward, 2010) showed that gastropods which accumulated relatively higher concentrations of heavy metals have lower growth rates and consequently lower values of allometric parameters such as shell height and width. In this regard it is worth noting that the growth rates of freshwater gastropods depends on many environmental parameters such as temperature, oxygen availability and their interactions, but also on biotic interactions such as predation and competition (Jakubik, 2012; Jakubik, Koperski, & Lewandowski, 2014).

In general we found that population from lacustrine environment (Skadar lake) have larger dimensions of all allometric parameters (Table 5) than specimens from riverine environment; the specimens from outlet stretches (stream pool) of Matica river are larger than specimens from lotic habitat of Zeta river. The specimens from latter site were found to accumulate Cd and Zn to a higher extent than the populations from other two sites. Such results indicate moderate water pollution of Zeta River with Cd and Zn (Blagojević *et al*., 2013). However, we cannot exclude that this may be due to bioavailability of the metals in the studied habitat(s) which greatly depends on pH and the acid-volatile sulphide of the water (Baser, Ingersoll, & Giery, 1998) or because of the differences in the diet and/or trophic guild of the studied populations from lacustrine and riverine environments (Jakubik, 2009).

In conclusion, the results of this study showed

Table 5. Water contents and allometric parameters of *V. mammilatus* collected on studied sites; mean values ± standard deviation, n=10

No.	Site	Shell height (cm)	Shell width (cm)	Wet weight (g)	Dry weight (g)	Water content (%)
1.	Zeta river (ZR)	3.12±0.30	2.34±0.13	7.46±1.82	3.08±1.31	59.79±7.33
2.	Skadar lake (SL)	4.70±0.46	3.66±0.39	20.94±3.53	11.7±4.76	43.92±9.68
3.	Matica river (MR)	3.90±0.37	3.00±0.32	13.55±3.23	7.03±4.01	50.84±17.85

that *V. mamillatus* can be used for biomonitoring of Cd, Cu, Zn and Fe in the aquatic environment and should be included in the national monitoring program of freshwater ecosystems.

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