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Concentration of arsenic and heavy metals in snail tissues

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A b s t r a c t: The aim of this study was to determine arsenic and heavy metal concentrations (lead, mercury, copper, cadmium, zinc, iron and manganese) in snail tissues (foot and digestive gland) obtained from snail farms in Serbia (near urban areas). Snail samples were analysed using atomic absorption spectrophotometry. A total of 730 individual snail samples were included in this study. Snails were packed into plastic bags and transported to the laboratory at the Faculty of Veterinary Medicine, University of Belgrade. The levels of arsenic and mercury in the examined snail tissues were below the detection limit of the analytical method. Concentrations of heavy metals were higher in digestive gland tissues than in foot tissues.

Keywords: *elements, foot, digestive gland, monitoring, environmental pollution.*

Introduction

The city of Belgrade and the nearby region situated on two rivers (Sava and Danube) is one of the most economically developed regions in Serbia. Rivers play an important role in the economic and social development in this region. Rapid development of industrialisation and urbanisation in recent decades has resulted in significant negative impacts on ecosystems. Heavy metal concentrations have rapidly grown in the urban environments from these anthropogenic sources (*Milanov et al*., 2016; *Janjic et al*., 2015; *Ivanovic et al*., 2016; *Jovanovic et al*., 2017; *Ciric et al*., 2018). In spite of that, heavy metals are natural substances, so are also considered as environmental contaminants (*Gawad*, 2018). In general, they not degrade but accumulate throughout the trophic chain (*Gupta and Singh*, 2011). When the body accumulates heavy metals and does not metabolise them, they can be toxic (*Gawad*, 2018). Copper, manganese, zinc and iron are essential metals for many organisms. Cadmium and lead are non-essential metals, and their toxic effect can be relatively high in comparison to other metals (*Zhiyou et al*., 2016).

Land snails (*Helix pomatia*) accumulate heavy metals, and this property can be utilised for heavy metal monitoring in urban environments. Snails are a very suitable tool for diagnostics of regions contaminated with different heavy metals. Many studies (*Beeby and Richmond*, 2003; *Notten et al*., 2005; *Dallinger et al*., 2005; *Nica et al*., 2012) showed that land snails can accumulate high levels of copper, zinc, cadmium and lead in their foot and digestive gland tissues. The land snails have the potential to be used in environmental monitoring as model invertebrates for Cu, Zn, Cd and Pb accumulation in terrestrial ecosystems (*Gomot-De Vaufleury*, 2000).

Previous studies in Serbia showed accumulation of heavy metals in fish tissues from different locations (*Jaric et al*., 2011; *Subotic et al*., 2013; *Janjic et al*., 2015; *Milanov et al*., 2016; *Ivanovic et al*., 2016; *Jovanovic et al*., 2017). However, data on concentrations of different heavy metals in snail tissues are very limited in Serbia. This study investigated the concentration of arsenic and heavy metals (lead, mercury, copper, cadmium, zinc, iron and manganese) in snail tissues (foot and digestive gland) taken from snail farms in Serbia.

Materials and methods

Individuals of the adult land snail, *Helix pomatia* (35.1±1.1 mm in shell diameter and 19.6±1.00 g in body weight) were collected during 2017 from five different farms (F1, F2, F3, F4, F5) located near

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Belgrade city, Serbia. F1, F2, F3 and F4 were located near traffic roads, and F5 was near the Danube River. Altogether, 730 individual snails were randomly collected (146 from each farm). Animals were packed into plastic bags and transported to the laboratory for heavy metal determinations, performed at the Faculty of Veterinary Medicine, University of Belgrade. The animals were washed with deionised water, steam treated (at 95–100°C for 5 min), and the digestive glands and foot were rapidly dissected out. Half of the sample size of these tissues was used for heavy metal determination.

The concentrations of heavy metals were measured in the snail tissues. The digestive gland and foot samples were dried at 60°C for at least 48 h in Petri dishes. Half a gram of each dried sample was transferred into a 50 mL Erlenmeyer flask and 10 mL of concentrated nitric acid (65 % HNO_3, Merck, Germany) was added. The flask was heated on an electric plate at low temperature until digestion was completed. When the digested solution became clear, the solution was evaporated to dryness. The sample was diluted with 10 mL of deionised water (18.2 MΩ cm^{-1}) and filtered through a Whatman filter (Sigma-Aldrich, USA). Heavy metals were measured in triplicate by atomic absorption spectrophotometry (GBC 932 plus atomic absorption spectrometer, GBC Scientific Equipment, USA). All reagents and chemicals were of the highest purity grade available from Merck or Sigma-Aldrich, Germany.

The statistical analysis was performed using the GraphPad Prisma version 7.00 software. Means were compared for significance of differences by the Student's t-test at the probability of 0.05. The samples were analysed in triplicate for each farm.

Results and discussion

Heavy metal concentrations in the different snail tissues (foot and digestive gland) of *H. pomatia* collected at the five snail farms are given in Table 1. These elements were chosen due to their frequency in environmental pollution (due to urban area, industrial area, traffic road etc.). Also, previous studies have shown that land snails are capable

Table 1. Heavy metal concentrations (μg g^{-1}) from snail (*Helix pomatia*) tissues from five different farms, F1-F5 (N=30; mean±standard deviation)

Heavy metal	F1	F2	F3	F4	F5
	Foot				
Cd	$0.19{\pm}0.01^{x}$	$0.17{\pm}0.03^{x}$	$0.22{\pm}0.02^{x}$	$0.13{\pm}0.01^{x}$	$0.11{\pm}0.02^{x}$
Hg			BLD		
Pb			BLD		
As			BLD		
Cu	$12.46{\pm}1.86^{a}$	$26.58{\pm}3.67^{b}$	$21.60{\pm}3.52^{b}$	$9.74{\pm}1.44^{ax}$	$21.01{\pm}3.91^{bx}$
Zn	$11.49{\pm}1.64^{x}$	$12.83{\pm}1.58^{x}$	$12.51{\pm}1.26^{x}$	$12.11{\pm}1.12^{x}$	$13.09{\pm}1.65^{x}$
Fe	$10.25{\pm}1.33^{ax}$	$12.30{\pm}2.50^{bx}$	$10.66{\pm}2.69^{ax}$	$9.77{\pm}0.62^{ax}$	$10.34{\pm}1.78^{ax}$
Mn	$2.42{\pm}0.22^{ax}$	$1.69{\pm}0.11^{bx}$	$1.65{\pm}0.17^{bx}$	$2.05{\pm}0.19^{ax}$	$1.80{\pm}0.11^{bx}$
	Digestive gland				
Cd	$0.66{\pm}0.03^{ay}$	$1.14{\pm}0.10^{by}$	$1.25{\pm}0.08^{by}$	$0.90{\pm}0.02^{ay}$	$0.92{\pm}0.08^{ay}$
Hg			BLD		
Pb			BLD		
As			BLD		
Cu	$11.68{\pm}0.65^{a}$	$26.62{\pm}1.97^{b}$	$23.33{\pm}1.64$	$19.00{\pm}1.98^{y}$	$11.41{\pm}1.01^{ay}$
Zn	$35.42{\pm}4.53^{y}$	$67.23{\pm}5.90^{ay}$	$46.06{\pm}3.15^{y}$	$29.78{\pm}1.28^{by}$	$30.49{\pm}2.92^{by}$
Fe	$77.66{\pm}8.57^{y}$	$130.67{\pm}9.43^{ay}$	$86.76{\pm}6.54^{y}$	$49.18{\pm}3.84^{by}$	$49.64{\pm}5.57^{by}$
Mn	$104.43{\pm}10.61^{y}$	$296.08{\pm}39.68^{ay}$	$172.99{\pm}10.75^{y}$	$79.48{\pm}4.49^{by}$	$76.65{\pm}7.92^{by}$

Legend: BLD - below the limit of detection; Within a row, means with different lower-case superscript letters (^{a,b}) differ significantly ($p<0.05$); Within a column, means with different lower-case superscript letters (^{x,y}) per heavy metal ($p<0.05$) differ significantly.

of accumulating high levels of different heavy metals (*Beeby and Richmond*, 2003; *Notten et al*., 2005; *Dallinger et al*., 2005; *Nica et al*., 2012). Cadmium concentrations in foot tissues ranged between 0.11±0.02 μg g^{-1} (F5) and 0.22±0.02 μg g^{-1} (F3). In digestive gland tissues, Cd concentrations ranged between 0.66±0.03 μg g^{-1} (F1) and 1.25±0.08 μg g^{-1} (F3). The farm location had a significant influence on heavy metal concentrations in the digestive gland tissues ($p<0.05$). Similar, highly significant differences were found when comparing the higher Cd concentrations in snail digestive glands with the lower Cd concentrations in snail foot tissues (Table 1). *Massadeh et al*. (2016) showed that snail tissues are very good indicators for Cd accumulation. Similar results were presented in a study by *Ciric et al*. (2018). Our study suggests that variances in Cd level are indicative of environmental exposure differences between the farms (data not shown). The concentrations of Mg, Pb and As were below the limit of detection in the examined snail tissues.

Copper concentrations in snail foot tissues ranged between 9.74±1.44 μg g^{-1} (F4) and 26.58±3.67 μg g^{-1} (F2) (Table 1). The highest Cu levels were measured in digestive gland tissues from F2, and foot tissue levels from this farm were similarly high. In fact, the Cu concentrations from F2 were significantly higher compared to F1 and F4 ($p<0.05$) (Table 1). Also, significant differences in Cu levels were found between foot and digestive gland tissues from both F4 and F5. Copper is an essential element for snails (*Yap and Noorhaidah*, 2012). Similar results were presented in studies by *Coeurdassier et al*. (2007), *Massadeh et al*. (2016) and *Ciric et al*. (2018).

Zinc concentrations in snail foot tissues ranged between 11.49±1.64 μg g^{-1} (F1) and 13.09±1.65 μg g^{-1} (F5). The concentration of Zn in foot tissues was significantly lower compared to digestive gland tissues, from all examined farms. *Nica et al*. (2012) and *Ciric et al*. (2018) found the heavy metal concentration in different snail tissues depends on the farm and investigated snail tissue. These authors also showed that digestive gland tissues accumulated higher amounts of heavy metals compared to other snail tissues. Similar results were determined in our study.

Iron concentrations in snail foot tissues ranged between 9.77±0.62 μg g^{-1} (F4) and 12.30±2.50 μg g^{-1} (F2), and in snail digestive gland tissues were between 49.18±3.84 μg g^{-1} (F4) and 130.67±9.43 μg g^{-1} (F2). The results obtained show that Fe concentrations were significantly ($p<0.05$) higher in digestive gland tissues compared to foot tissues. Higher concentrations of Fe are probably due to an environmental pollution or other anthropogenic sources. According to *Gomot and Pihan* (1997), *Gomot de Vaufleury and Pihan* (2000) and *Ciric et al*. (2018), snails are target organisms that could be utilised for measuring environmental pollution with industrial waste.

Manganese concentrations in snail foot tissues ranged from 1.65±0.17 μg g^{-1} (F3) to 2.42±0.22 μg g^{-1} (F1). In snail digestive gland tissues, Mn concentrations ranged from 76.65±7.92 μg g^{-1} (F5) to 296.08±39.68 μg g^{-1} (F2). Significant differences were observed between tissues (foot and digestive gland tissues) and between the farms (Table 1). The levels of Mn reported in this study are similar to results of *Sivaperumal et al*. (2007) and *Iwegbue et al*. (2008).

Conclusions

Based on the present study, snails are a very good species for use in biomonitoring of various environmental pollutants. In particular, *Helix pomatia* accumulated high amounts of Fe and Mn (digestive gland tissues).

Koncentracija arsena i teških metala u tkivu puževa

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A p s t r a k t: Cilj ovog ispitivanja bio je utvrđivanje koncentracije arsena i teških metala (olova, žive, bakra, kadmijuma, cinka, gvožđa i mangana) u tkivima puževa (stopalo i digestivni trakt) poreklom sa odabranih farmi u Srbiji (u blizini urbanih područja). Uzorci puževa su analizirani pomoću atomskog apsorpcionog spektrofotometra. U ovu studiju uključeno je ukupno 730 pojedinačnih uzoraka puževa. Uzorci su upakovani u plastične kese, dopremljeni u laboratoriju Veterinarskog fakulteta, Univerziteta u Beogradu. Nivo arsena i žive u ispitivanim uzorcima puževa bio su ispod granice detekcije analitičke metode. Koncentracija teških metala bila je veća u digestivnom tkivu u poređenju sa koncentracijom teških metala u stopalu puževa.

Ključne reči: *elementi, stopala, digestivni trakt, monitoring, zagađenje životne sredine.*

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