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Contents of Zn, Cu, Mn and Cd in soil, plants and Turkish snails *Helix lucorum* Linnaeus, 1758 in Skopje, Republic of North Macedonia

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Abstract: Heavy metal pollution of the environment is especially apparent within or around urban areas and around industrial facilities. This paper deals with the concentration of zinc, copper, manganese and cadmium in the soil, leaves of fodder plants and in the body parts of the Turkish snail (*H. lucorum*). The study included ten localities, arranged in four groups, situated in and around the city of Skopje. The first group consisted of five urban localities that were in the central city area. The second group included two suburban localities. The third group consisted of two rural sites. The fourth group represented one site that was located away from the metropolitan area (control site). Soils, leaves of four plant species (*Cardaria draba, Lamium purpureum, Rumex crispus* and *Taraxacum officinale*) and a sample of 10 adult snails were collected from each locality. Heavy metals content was determined by wet combustion and atomic absorption spectroscopy. Digestive gland was an organ with the highest amount of all heavy metals and with proven bioaccumulation for Zn and Cd; it showed a prominent correlation with soil concentrations for the four heavy metals but not with the examined plant species. The foot and shell of the snails had limited indicator values since they exhibited weak correlation with soil concentrations of Cu and Mn.

Key words: Digestive gland, foot, shell, heavy metals

Introduction

Snails can be used as effective indicators of metal pollution (Meincke & Schaller 1974, Berger & Dallinger 1993, Van Gestel & Van Straalen 1994, Gomot & Pihan 1997, Roma et al. 2017). Digestive gland (hepatopancreas, mid gut gland) is of special indicator value while other organs (foot, kidney) have limited indicator values (Rabitsch 1996). There are obvious differences among species (Rabitsch 1996) and even subspecies (Gomot & Pihan 1997) in terms of contents of heavy metals in different body parts. Thus, species of *Helix* were proposed as a sentinel in temperate and sub-tropical habitats around the world quantifying metal exposure for other generalist herbivores (Beeby & Richmond 2002). Because of the great potential to accumulate heavy metals, snails were also used as biomonitors in phytoremediation experiments (Pihan & de Vaufleury 2000).

Caged snails positioned at different sites were successfully used to assess air pollution (Regoli et al. 2006, Sturba et al. 2020). Local populations (similar to those used in this study) are informative for the pollution from a longer period but they are known to adapt to environmental conditions, which may lead to underestimation of the contamination levels (Notten et al. 2006, Sturba et al. 2020). Heavy metals and micronutrients (Zn, Mn, Cu) can enter the snail organism through food ingestion, contact with soil and inhalation (Viard et al. 2004, Sturba et al. 2020). Laboratory experiments have provided valuable information on the percentages of assimilated metals from food (Laskowski & Hopkin 1996a, Scheifler 2002). Many terrestrial snails are known to be tolerant to high doses of heavy metals due to compartmentalisation and accumulation in the digestive gland, which makes them an important link in the food chain with an important role in biomagnification (Coughtrey & Martin 1976, Russell et al. 1981, Marigomez et al. 1986). Ingested pollutants are transported by blood and then detoxified in the digestive gland (Beeby & Richmond 2002, Regoli et al. 2006).

The food with excessive amounts of Zn (over 500 mg·kg^{-1}) was rejected by individuals of *Cornu aspersum* (O.F. Müller, 1774) (=*Helix aspersa*) (Laskowski & Hopkin 1996a). If such cases occur in nature, they may lead to extinction of snails due to starvation in combination with other factors such as delayed reproduction, decreased fecundity, prolongation of the juvenile phase, etc. (Laskowski & Hopkin 1996b, Notten et al. 2006). Nevertheless, the levels of experimental contamination can surpass the levels found under natural conditions, even at very contaminated sites (Gomot & Pihan 1997). It is interesting to note that snails feed on plants regardless of the Zn content in leaves and the hypothesis that high internal Zn concentration protects seedlings from predation or herbivory by snails was not confirmed (Huitson & Macnair 2003, Noret et al. 2004, 2007).

Most of the studies in Europe used two Helicidae species, *Helix pomatia* Linnaeus, 1758 and *C. aspersum* to asses or monitor the pollution with heavy metals. *Helix pomatia* in North Macedonia is distributed in the mountainous areas (Stankovic et al. 2006) and its usage as indicator species for pollution is limited. *Cornu aspersum* is present only as an alien species in North Macedonia (Cvetkovska-Gjorgievska et al. 2019). The native range of *H. lucorum* covers Balkan Peninsula, Asia Minor to Iran (Neubert 2014). However, its values as a bioindicator has not been assessed so far. This species can store high concentrations of heavy metals (with exception of Cu) in the digestive gland while foot (edible part) and shell have low concentrations (Mitev et al. 2019). Recently, the indicator values of *Helix vladica* (Kobelt, 1898) and *Helix secernenda* Rossmässler, 1837 were promoted (Vukašinović-Pešić et al. 2020).

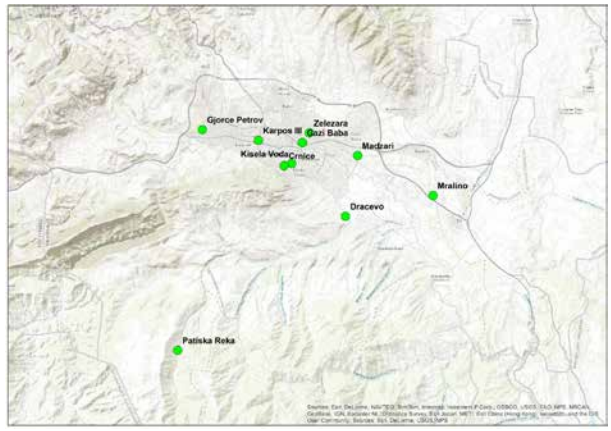

Fig. 1. Map of Skopje region with studied localities.

The pollution of the soils in Skopje with heavy metals has been recently studied (Stafilov et al. 2019). It was found that the distribution of the most of the studied elements (K, Na, Ba, Al, Cr, Ni, Mg, Fe, Sr and Ca) is a result of the complex geology and lithology of the region. The concentration of Zn, Cd and Cu is a result of anthropogenic activities while the concentration of Ag, As, Li, Mn and Pb could not be associated to any of the natural or anthropogenic factors. The study found that the average content of Zn was 230 mg·kg^{-1} (ranging 23–18000 mg·kg^{-1}), Cu was 40 mg·kg^{-1} (7.3–590 mg·kg^{-1}), Cd was 1.3 mg·kg^{-1} (0.05–77 mg·kg^{-1}) and Mn was 770 mg·kg^{-1} (340–5600 mg·kg^{-1}).

Air pollution, especially the pollution with particulate matters (PM), is one of the major environmental problems of Skopje city (Anttila et al. 2016, Martinez et al. 2018). Unfortunately, there are no reports on the concentrations of heavy metals in wild plants in Skopje area.The main aim of the study is to assess the potential of Turkish snail *H. lucorum* as a bioindicator of heavy metal pollution. We also assessed the relationships between soil, four species of fodder plants (*Cardaria draba, Lamium purpureum, Rumex crispus* and *Taraxacum officinale*) and snail parts such as digestive gland, foot and shell.

Materials and Methods

The study area is the city of Skopje and its vicinity.

This is the largest city in North Macedonia. It is situated in the Skopje Valley surrounded by the mountains of Vodno, Skopska Crna Gora and Zheden. The main river is Vardar, which flows through the city of Skopje. The climate in Skopje region is continental with strong Mediterranean influence that spreads through Vardar River valley. The average annual temperature is 11.5°C while annual precipitation varies between 500 and 600 mm (Lazarevski 1993). Skopje has 526,500 citizens and 194,000 transportation vehicles (SSO 2022). The major industrial capacities are iron smeltery Zhelezara, organic chemical industry OHIS near Pintija and the cement factory Usje in Kisela Voda.

Turkish snail specimens were collected from 10 localities in Skopje region (Fig. 1). Five localities (Crniče, Železara, Kisela Voda, Gazi Baba and Karpoš III) are part of the central (urban) area of Skopje City. Localities Gjorče Petrov and Madžari are part of the suburban city area. Pintija (Dracevo) and Mralino are considered as parts of the rural area of Skopje region. Patiška Reka is rural locality situated outside of the city area, in the foothills of Karadžica Mountain, and serves as a control site (no adjacent sources of pollution). Samples of soils, snails and four plant species were collected from each of the sites. The collection of samples was carried out during April and May 2012.

Soil was collected from depth of 5–10 cm after removal of the top soil. Soil samples were air dried for two weeks and sieved through a mesh of 2 mm. After drying at 105°C to a constant weight, a 0.25 g of soil was measured on analytical scale with precision of 0.1 mg. For analyses of total heavy metal content process of hot digestion of 0.25 g soil was carried out with nitric (HNO_3), hydrofluoric (HF) and perchloric ($HClO_4$) acids (ISO, 2001). Digested material was transferred into 25 ml volumetric flask and filled with distilled water.

Leaves of four plant species were collected during the fieldwork: *Cardaria draba, Lamium purpureum, Rumex crispus* and *Taraxacum officinale*. Plant material was air dried and ground to a fine powder. After drying at 105°C to a constant weight, a 0.5 g of soil or leaves was measured on analytical scale with precision of 0.1 mg.

Ten adult specimens of Turkish snails were collected from each of the 10 studied localities. Snails were starved for three days in laboratory in order to empty their digestive tracts. Specimens were then put in a distilled water for 24 h. Dead snails were dissected and three body parts for analysis were extracted: digestive gland, foot and shell. The body parts were immediately put in liquid nitrogen and stored in a refrigerator at -20°C. About 0.5 g of the frozen body parts was measured on analytical scale with precision of 0.1 mg.

The samples of plants and snail body parts were wet digested in Kjeldahl flasks with 8 ml mixture of nitric, sulphuric and perchloric acid (10:2:1) at temperature of 200°C. Digested material was transferred into 25 ml volumetric flasks and filled with distilled water. The content of Zn, Cu, Mn and Cd in the digested material of soils, plants and snails was determined by Atomic Absorption Spectrometry (Agilent 55A). If necessary, diluted samples were prepared (1:10). Same procedure was applied for blanks, which were subtracted from the sample results.

The correlations between the obtained results for heavy metals concentration in the snails' body parts, plant leaves and soils were tested with simple regression analysis tool (linear and logarithmic models) in Statgraphic Plus for Windows 2.1.

Results

Zn

Among soils, the highest Zn content was recorded in the sites Kisela Voda and Zhelezara. The lowest values were recorded in Patishka Reka, Pintija, Gjorce Petrov and Crniche (Table 1). The area of Zhelezara shows high values of Zn due to the contamination, mainly by dust from the iron and steel works (Stafilov et al. 2019). Among plants, the lowest Zn content was measured in *Rumex crispus* and *Taraxacum officinale*, while the highest was measured in *Cardaria draba* (Table 1). Among sites, the highest values were recorded in Zhelezara, especially in *Cardaria draba* and *Lamium purpureum*. High values were also recorded in Kisela Voda, Crniche and Gjorce Petrov. The lowest values were recorded in Patishka Reka (Table 1). Among snail body parts, the highest values were recorded in the digestive gland and the lowest in the foot. The highest values of Zn in digestive gland were measured in Madzari, Karpos III, Kisela Voda and Zhelezara. The lowest value was recorded in Patishka Reka (Table 1). The values of Zn in the digestive gland were higher than the ones in soil (av. 1.22 times higher) pointing out to the bioaccumulation potential of the digestive gland.

The correlation between Zn content in soil and digestive gland is statistically significant (Table 2, Fig. 2). This correlation was stronger if individual data for Zn content in snails were plotted against the Zn soil content (same approach was used by Beeby & Richmond 2002). We found no correlation between

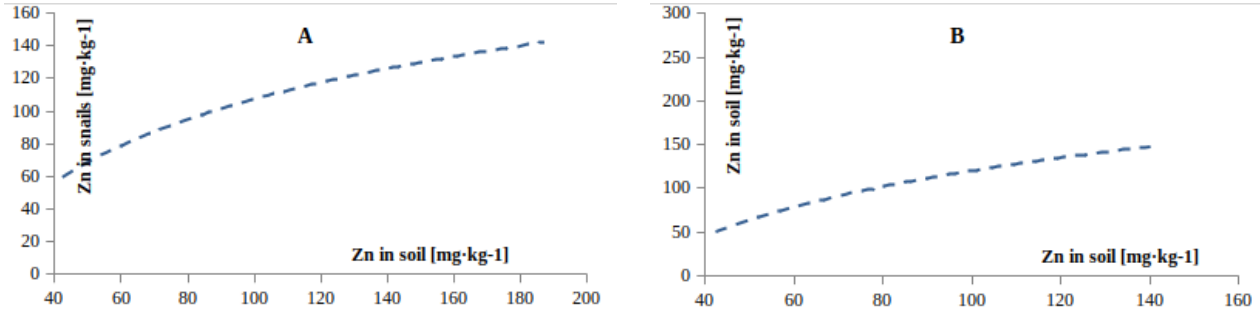

Fig. 2. Correlation between Zn content in digestive gland and soil. A. Correlation between the average values. B. Correlation between soil average values and all replicates for snails.

Table 1. Content of Zn (mg·kg^{-1}) in soils, plants and snails in the 10 investigated sites in Skopje (n.a. = not assessed).

Locality	**Gazi Baba**	**Zhe-lezara**	**Kar-posh III**	**Kisela Voda**	**Crniche**	**Gjorce Petrov**	**Pin-tija**	**Madzari**	**Mrali-no**	**Patish-ka Reka**	**Aver-age**
Soil	62.94	107.57	76.77	138.18	57.33	55.24	53.28	76.57	63.68	52.27	**74.38**
Plants											
Cardaria draba	43.97	113.48	37.98	96.90	100.18	70.96	57.26	41.13	42.44	23.02	**62.73**
Lamium purpureum	38.20	128.95	n.a.	59.11	40.44	54.65	25.28	17.60	41.75	-	**45.11**
Rumex crispus	20.92	48.49	n.a.	64.98	37.76	48.36	24.73	44.37	18.23	24.90	**36.97**
Taraxacum officinale	39.23	65.58	n.a.	39.21	38.57	37.99	48.57	10.15	28.11	27.04	**37.16**
Average in plants	35.58	89.12	n.a.	65.05	54.24	52.99	38.96	28.31	32.63	18.74	**45.36**
Snails											
Digestive gland	81.56	115.67	127.77	127.44	52.57	74.66	56.23	139.27	92.36	42.49	**91.00**
Foot	6.16	8.06	12.58	5.87	5.06	6.22	6.17	11.02	11.29	7.48	**7.99**
Shell	1.74	2.63	14.34	3.85	2.03	5.32	1.52	6.08	6.49	1.65	**4.56**

Table 2. Correlation coefficients between Zn contents in soils, plants and snails ($p<0.05$). Legend: * – significant correlation obtained only when all values for individual replicates were analysed. n. s. – not significant correlation.

	Soil	***Cardaria draba***	***Lamium purpureum***	***Rumex crispus***	***Taraxacum officinale***
Soil	X				
Cardaria draba	0.674	X			
Lamium purpureum	0.855	0.755	X		
Rumex crispus	0.673	0.688	n.s.	X	
Taraxacum officinale	n.s.	0.685	0.767	n.s.	X
Digestive gland	0.779	n.s.	n.s.	0.417*	n.s.
Foot	n.s.	n.s.	n.s.	n.s.	n.s.
Shell	n.s.	n.s.	n.s.	n.s.	n.s.

the Zn soil content and contents in foot or shell.

Cu

The concentration of Cu in soils ranged between 11.86 mg·kg^{-1} (Patishka Reka) and 27.86 mg·kg^{-1} (Kisela Voda). High values were also recorded in Madzari and Mralino (Table 3). The variation of Cu in plants was even smaller compared to soils. Among species, the lowest values were recorded in *Cardaria draba* and the highest in *Taraxacum officinale*. Among sites, the highest values were recorded in Zhelezara. The values of Cu content in digestive gland varied between 2.09 and 24.86 mg·kg^{-1} (Mralino). The most striking feature of Cu in snails is the small difference between the content in digestive gland and foot or shell. The lowest values were recorded in shells of the snails. Among sites, the highest values for all the three body parts were recorded in Mralino. The Cu contents in digestive gland and shell was positively correlated with the Cu content in the soil. Similar pattern is observed for foot although the correlation was not statistically significant (Fig. 3). However, by analysing the values for each specimen separately the statistical parameters for correlation soil-foot improved (Table 4).

There was positive and significant correlation between soil Cu content and content of Cu in *Cardaria draba*, *Rumex crispus* and *Taraxacum officinale* (Fig. 4). No correlation was observed in the case of *Taraxacum officinale* (Table 4). There was also a statistically significant correlation between the Cu content in *Cardaria draba* and Cu content in the digestive gland and foot. It seems that the Cu content in shell is also positively correlated, although not statistically significant (Table 4, Fig. 5). There were no significant correlations between Cu content in *Lamium purpureum, Rumex crispus* or *Taraxacum officinale* with any of the snail body parts' Cu content (Table 4).

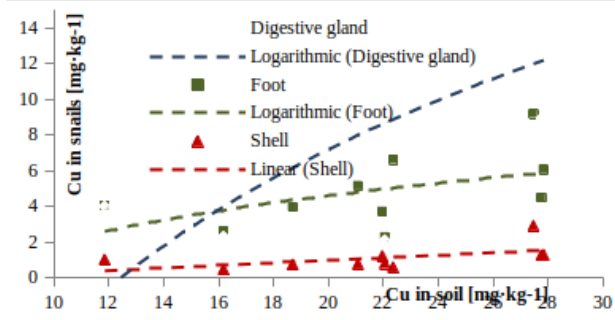

Fig. 3. Correlation between Cu content in the soil and Cu content in digestive gland, foot (not significant) and shell.

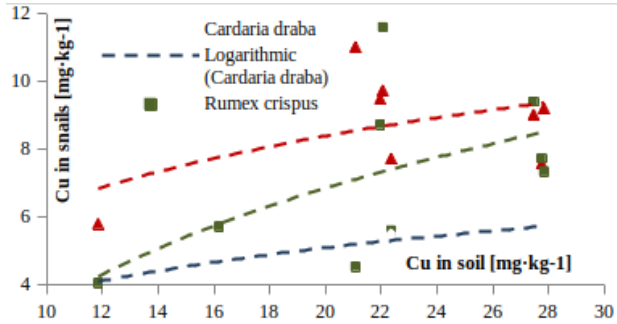

Fig. 4. Correlation between soil Cu content and Cu content in *Cardaria draba*, *Rumex crispus* and *Taraxacum officinale.*

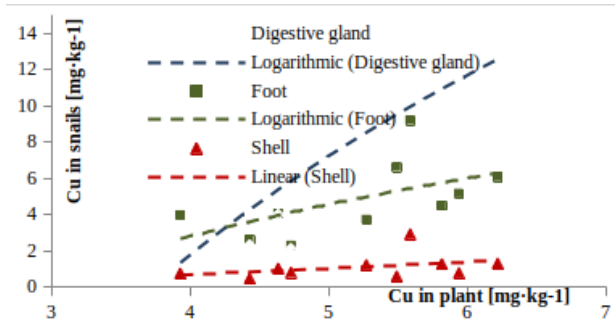

Fig. 5. Correlation between Cu content in *Cardaria draba* and Cu content in digestive gland, foot and shell (not significant).

Mn

The lowest Mn soil content was recorded in Zhelezara, while the highest in Madzari, Gazi Baba and Patishka Reka (Table 5). The soils of Patishka Reka (control site) are formed over Quaternary bedrock, which are known to have higher concentrations of Mn (Stafilov et al. 2019). The content in plant species was much lower than that in soil (except for Zhelezara). The differences between species were twofold: the lowest values were recorded in *Rumex crispus* and the highest in *Lamium purpureum*. Among sites, the highest values were found in Zhelezara, which is close to the large metal processing facility. The content of Mn in digestive gland varied between 39 and 165 mg·kg^{-1}. The highest values were recorded in Madzari and Mralino, while the lowest in Patishka Reka, Kisela Voda, Pintija and Crniche. However, the values of Mn content in foot varied considerably less (0.74–1.31 mg·kg^{-1}). Variation in shell was the highest with ratio of 11.4 between maximum and minimum value (0.63–7.19 mg·kg^{-1}). Among sites, the highest values were recorded in Zhelezara. The lowest were found in the control and, presumably, the least polluted site (Patishka Reka). There is no statistically significant relationship between Mn soil content and average values of Mn content in any of the body parts. How-

Table 3. Content of Cu ($mg \cdot kg^{-1}$) in soils, plants and snails in the 10 investigated sites in Skopje. Legend: n. a. – not assessed.

Locality	Gazi Baba	Zhe-lezara	Kar-posh III	Kisela Voda	Cr-niche	Madzari	Gjorce Petrov	Pin-tija	Mrali-no	Patish-ka Reka	Aver-age
Soil	21.08	15.99	18.70	27.83	21.97	27.76	22.06	16.18	27.46	11.86	**21.09**
Plants											
Cardaria draba	5.94	4.72	5.49	3.92	6.22	5.81	5.58	4.63	4.43	5.27	**5.20**
Lamium purpureum	6.54	7.14	7.62	n.a.	7.77	3.94	8.76	7.61	6.30	10.88	**7.40**
Rumex crispus	4.53	11.60	5.61	n.a.	7.34	7.74	9.41	4.05	5.72	8.72	**7.19**
Taraxacum officinale	11.02	9.74	7.73	n.a.	9.21	7.58	9.02	5.80	10.85	9.48	**8.94**
Average in plants	7.01	8.30	6.61	n.a.	7.63	6.27	8.19	5.52	6.82	8.59	**7.18**
Snails											
Digestive gland	7.09	8.14	4.67	12.26	5.44	8.91	2.09	2.28	24.86	3.97	**7.97**
Foot	5.14	6.59	3.95	6.04	3.69	4.48	2.26	2.60	9.16	4.03	**4.79**
Shell	0.75	0.57	0.74	1.28	1.20	1.25	0.78	0.45	2.89	1.01	**1.09**

Table 4. Correlations coefficients between Cu contents in soils, plants and snails ($p<0.05$). Legend: * – significant correlation obtained only when all values for individual replicates were analysed; n.s. – not significant correlation.

	Soil	***Cardaria draba***	***Lamium purpureum***	***Rumex crispus***	***Taraxacum officinale***
Soil	X				
Cardaria draba	0.730	X			
Lamium purpureum	n.s.	n.s.	X		
Rumex crispus	0.693	n.s.	n.s.	X	
Taraxacum officinale	0.729	n.s.	n.s.	n.s.	X
Digestive gland	0.703	0.700	n.s.	n.s.	n.s.
Foot	0.314*	0.620	n.s.	n.s.	n.s.
Shell	0.333*	n.s.	n.s.	n.s.	n.s.

ever, when individual values for each of the specimens were analysed against the soil concentrations (see Beeby & Richmond 2002), we observed significant relationship in the case of digestive gland shell, but not for foot (Table 6). In all four plants, we found no significant positive correlation between Mn soil content and content of Mn in leaves. There were also no statistically significant relationships between Mn content in snail body parts (digestive gland, foot) and shell) and Mn content in four studied plants (*Cardaria draba*, *Lamium purpureumm, Rumex crispus* and *Taraxacum officinale*).

Cd

The content of Cd in soils varied between 0.14 and 1.08 $mg \cdot kg^{-1}$ (Table 7). Among plants, higher values were recorded in *Cardaria draba* and *Taraxacum officinale* compared to *Lamium purpureum* and *Rumex crispus*. Karposh III had notably higher Cd content in plants compared to other localities (Table 7). The content of Cd in the digestive gland varied between 1.50 and 9.88 $mg \cdot kg^{-1}$ (Table 7). These values are 2.7-16.8 times higher than the respective soil concentrations and show the potential of digestive gland for bioaccumulation of Cd. As in the case of plants, the highest value was recorded in Karposh III. Statistical analyses showed positive and significant correlation between Cd content in the digestive gland and Cd content in leaves of *Lamium purpureum* and *Taraxacum officinale*. However, when all measured replicates for Cd in digestive gland were analysed against the content of Cd in leaves we found sig-

Table 5. Content of Mn ($mg \cdot kg^{-1}$) in soils, plants and snails in the 10 investigated sites in Skopje. Legend: n. a. = not assessed.

Locality	Gazi Baba	Zhe-lezara	Kar-posh III	Kisela Voda	Cr-niche	Gjorce Petrov	Pintija	Madzari	Mrali-no	Patish-ka Reka	Aver-age
Soil	603.82	69.53	425.20	431.75	318.99	468.43	425.21	698.82	545.27	612.65	**459.97**
Plants											
Cardaria draba	72.67	158.82	44.46	52.22	84.44	93.62	58.33	52.54	63.32	60.51	**74.09**
Lamium purpureum	93.32	255.51	n.a.	55.10	90.33	60.70	72.93	64.36	84.06	42.51	**90.98**
Rumex crispus	32.43	95.65	n.a.	28.70	35.24	48.73	46.43	39.61	30.14	17.99	**41.66**
Taraxacum officinale	67.12	104.84	n.a.	51.75	52.14	59.43	61.12	39.30	38.72	41.58	**57.33**
Average in plants	66.39	153.71	n.a.	46.94	65.54	65.62	59.70	48.95	54.06	40.65	**66.02**
Snails											
Digestive gland	113.33	118.94	111.47	39.07	46.59	59.22	45.28	164.66	154.45	34.77	**88.78**
Foot	1.07	0.95	1.24	0.85	0.90	1.05	0.84	1.31	0.81	0.74	**0.98**
Shell	3.48	1.80	2.16	1.89	2.55	0.98	1.36	7.19	4.61	0.63	**2.67**

Table 6. Correlation coefficients between Mn contents in soils, plants and snails ($p<0.05$). Legend: * – significant correlation obtained only when all values for individual replicates were analysed. n. s. – not significant correlation.

	Soil	*Cardaria draba*	*Lamium purpureum*	*Rumex crispus*	*Taraxacum officinale*
Soil	X				
Cardaria draba	n.s.	X			
Lamium purpureum	n.s.	0.913	X		
Rumex crispus	n.s.	0.892	0.904	X	
Taraxacum officinale	n.s.	0.883	0.887	0.887	X
Digestive gland	0.422*	n.s.	n.s.	n.s.	n.s.
Foot	n.s.	n.s.	n.s.	n.s.	n.s.
Shell	0.509*	n.s.	n.s.	n.s.	n.s.

nificant relationships for all the four plant species (Table 8).

Correlations between elements in snails

Zn, Cu and Mn are well correlated in the digestive gland, foot and shell. Cd showed only weak correlation of Cu in the digestive gland (Table 9).

Discussion

The obtained results for content of Mn, Cd, Cu and Zn in body parts of *H. lucorum* are presented as $mg \cdot kg^{-1}$ fresh weight. These results are not easy to compare with the results in literature, which are usually presented per dry weight (after drying at 60 or 80°C). In addition, it is well known that the drying of biological materials at different temperatures leads to different results (Allen 1989). Unfortunately, the data on the water content in snails are scarce. It is known that the water content of the whole body of *C. aspersum* varies between 65 and 72 %, about 70 % during April and May (Klein-Rollais & Daguzan 1990).

Out of four metals examined (Pb, Cd, Cu and Zn) in *Arianta arbustorum* (Linnaeus, 1758), it was found that Pb showed the best distinction between the urban (polluted) sites and control (unpolluted) site. It was followed by Cd and Cu while the differences in Zn contents were the weakest (Berger & Dallinger 1993).

Table 7. Content of Cd in soils, plants and snails in the 10 investigated sites in Skopje. Legend: n. a. = not assessed.

Locality	Gazi Baba	Zhe-lezara	Kar-posh III	Kisela Voda	Cr-niche	Gjorce Petrov	Pintija	Madzari	Mrali-no	Patish-ka Reka	Aver-age
Soil ($mg \cdot kg^{-1}$)	0.140	0.482	0.893	0.553	0.459	0.372	0.476	1.083	0.342	0.747	**0.555**
Plants: ($\mu g \cdot kg^{-1}$)											
Cardaria draba	114.3	197.5	365.2	95.0	137.9	203.8	443.7	234.9	83.8	428.9	**230.5**
Lamium purpureum	38.2	23.0	401.6	n.a.	41.1	13.9	48.7	13.5	52.5	61.6	**77.1**
Rumex crispus	5.8	58.5	112.3	n.a.	93.2		27.4	14.1	11.9	15.0	**37.6**
Taraxacum officinale	127.7	101.2	509.8	n.a.	108.4	52.2	258.5	324.3	116.1	175.2	**197.0**
Average in plants	71.5	95.0	347.2	n.a.	95.1	67.5	194.6	146.7	66.1	170.2	**134.9**
Snails ($mg \cdot kg^{-1}$)											
Digestive gland	2.349	1.867	9.885	1.504	2.969	1.828	2.468	5.888	3.050	3.411	**3.522**

Table 8. Correlation coefficients between Cd contents in soils, plants and snails ($p<0.05$).

	Soil	*Cardaria draba*	*Lamium purpureum*	*Rumex crispus*	*Taraxacum officinale*
Soil	X				
Cardaria draba	n.s.	X			
Lamium purpureum	n.s.	n.s.	X		
Rumex crispus	n.s.	n.s.	n.s.	X	
Taraxacum officinale	0.740	n.s.	0.803	n.s.	X
Digestive gland	0.720	0.331*	0.867	0.452*	0.929

Table 9. Correlation coefficients between elements in body parts of *H. lucorum* ($p<0.05$).

Correlations	Digestive gland	Foot	Shell
Zn-Cu	0.519 (95)	0.278 (94)	0.209 (90)
Zn-Mn	0.640 (95)	0.278 (92)	0.261 (92)
Zn-Cd	n. s.	-	-
Cu-Mn	0.381 (95)	n. s.	0.278 (92)
Cu-Cd	0.228 (95)	-	-
Mn-Cd	n. s.	-	-

The highest content of all heavy metals was detected in the digestive gland as being the main storage organ (Dallinger & Wieser 1984). Juvenile snails can already accumulate heavy metals in their digestive gland but this potential increases towards the adult stage (Scheifler et al. 2002). *Helix pomatia* has 2.2 times more Zn in digestive gland compared to foot; this ratio in *C. aspersum* is 5 to 10 (Menta & Parisi 2001, Regoli et al. 2006). In *H. lucorum* we found that this ratio varied between 6 and 22 with an average of 11.7 (Table 1). This ratio for Mn was much higher and ranged from 46 to 190 with an average of 89.1 (Table 3). With great certainty, we can assume that ratio for Cd between digestive gland and foot is very high, too (Table 2). The only exception is Cu with a range between 0.9 and 2.7, 1.5 in average (Table 4). Obviously, digestive gland is not the main storage of Cu, which was evidenced in other *Helix* species (Laskowski & Hopkin 1996b, Beeby & Richmond 2002, Vukašinović-Pešić 2020).

Laboratory experiments undoubtedly show the relation between the Zn content in the plant diet with the Zn content in the reared snails (Dallinger & Wieser 1984, Laskowski & Hopkin 1996b, Gomot & Pihan 1997). Such experiments with *H. pomatia* proved that ingested Zn-enriched plant food resulted

in quick increase in anterior and posterior gut, salivary gland and kidneys followed by steady release through faeces as well as steady and longer-lasting increase in the mid-gut (=digestive) gland up to 1300 ppm (Dallinger & Wieser 1984). Another laboratory experiment confirmed that Zn content in food proportionally increases the Zn content in soft tissues with about 60% of the Zn in the food assimilated through digestion (Laskowski & Hopkin 1996a).

The changes in heavy metals contents in the digestive gland occur seasonally although these changes are species- and site-dependent. In *Arion rufus* (Linnaeus, 1758), it was found that Zn, Mn and Cu content was higher in October-November compared to May-June (Ireland 1984). In whole body of *A. arbustorum*, some changes in Zn content were observed with general decrease from spring towards autumn and no changes in Cu (Berger & Dallinger 1993). Significant increase (June vs October) for Cu and no changes for Zn and Cd were found in *H. pomatia* in Pancevo, Serbia (Ćirić et al. 2018).

Zn, Mn, Cd and Cu contents in digestive gland of *H. pomatia* are good predictors to pollution (Berger & Dallinger 1993, Ćirić et al., 2018). Pb, Cd, Cu and Zn contents were higher in more polluted site, which was closer to a Pb-Zn smelter in Austria (Rabitsch 1996). Furthermore, viscera of *C. aspersum* contained more Pb, Cd and Zn closer to a highway in France with Pb being the most sensitive to pollution (Viard et al. 2004). A correlation between Zn, Pb and Cd content in snail components (hepatopancreas and "rest") and in *Taraxacum* or soil was confirmed in a study of 23 populations of *C. aspersum*, while Cu was not significant (Beeby & Richmond 2002). Our results also support the validity of Zn, Cd and Cu content in digestive gland as an indicator of Zn load in soils and footer plants. As far as Mn is concerned, there are studies suggesting that digestive gland is sensitive to metal pollution (Ćirić et al. 2018) but we found only weak correlation between soil and shell contents.

From laboratory experiments, it was concluded that soil contributes by 30-40 % and food (lettuce) with 70-60 % of the Zn content in the snail viscera; the values for Cd are 40 % from soil and 60 % from food (Scheifler 2002). We also found that the Zn content in the digestive gland is dependent on both the Zn soil content and Zn plant content (Fig. 5). Heavy metals concentrations in soft tissues differ between slugs and snail as well as between tissues of the same species (Samiullah 1990, de Vaufleury & Pihan 2000). Nevertheless, soft tissues are preferred over shell in monitoring studies since high calcium concentrations in shells affects the results of heavy metals (Graham 1972, Stenner & Nickless 1974). Our results clearly show that the shell of *H. lucorum* does not accumulate significant quantities of heavy metals, which makes it unsuitable for monitoring.

It is obvious that the digestive gland of all gastropods is powerful accumulator of heavy metals. This tissue should be removed from snails collected as food in the wild, especially from more polluted environments (urban, suburban and agricultural areas).

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