Macedonian Journal of Ecology and Environment
Vol. 21, issue 1-2
pp. 65 - 71
Skopje (2019)
ISSN 1857 - 8330 (on-line)
Original scientific paper
Available online at www.mjee.org.mk

Heavy metals content (Zn, Cu, Mn and Cd) in edible Turkish snail (*Helix lucorum* L.) in Skopje, Republic of North Macedonia

Содржина на тешки метали (Zn, Cu, Mn and Cd) во јадливиот шумски полжав (*Helix lucorum* L.) во Скопје (Република Северна Македонија)

Trajče Mitev[1], Ivailo Dedov[2] & Slavčo Hristovski[1]

[1]*Institute of Biology, Faculty of Natural Sciences and Mathematics, Ss. Cyril and Methodius University, Skopje, Republic of Macedonia*

[2] *Bulgarian Academy of Sciences, Sofia, Bulgaria*

Abstract:

The results of heavy metals contents (Zn, Cu, Mn and Cd) in foot, shell and digestive gland (midgut gland or hepatopancreas) of Turkish snail (*Helix lucorum* L.) from 10 localities in Skopje region are presented in this paper and its value as food resource was assessed. Obtained results and their analysis shows that the distribution pattern of studied heavy metals in the organs of Turkish snail in different localities in Skopje follows basic physiological principles for this species. The highest content of Zn, Mn and Cu was detected in digestive gland as being the main storage organ. However, Cu content in digestive gland was comparable to the values in foot and shell pointing out that the digestive gland has lower potential to accumulate Cu. The values of Zn, Mn and Cu in our study are much lower than the ones recorded in other snail species in some polluted areas. Also, the concentration of Cd in foot was very low and thus it does not present any risk to human health in Skopje region when consummated.

Keywords: Turkish snail, *Helix lucorum*, heavy metals, Skopje

Во трудот се прикажани резултатите за содржината на неколку тешки метали (Zn, Cu, Mn и Cd) во стапало, черупка и хепатопанкреас на шумскиот (градинарски) полжав (*Helix lucorum* L.) собирани од 10 локалитети во Скопскиот регион. Врз база на резултатите е извршена проценка за квалитетот на полжавите како храна. Добиените резултати и нивната анализа покажуваат дека распоредот на тешките метали во органите на шумскиот полжав од Скопскиот регион е резултат на физиолошките механизми. Највисоки вредности за Zn, Mn и Cu беа констатирани во хепатопанкреасот кој служи како главен орган за депонирање на тешките метали. Специфичен е случајот со Cu каде беше утврдено дека хепатопанкреасот има помал потенцијал за негова акумулација што резултира со блиски вредности за содржината во

Submitted: 01.02.2019
Accepted: 08.04.2019

* Author for correspondence: trajcho.mitev@gmail.com

испитуваните органи. Вредностите за содржина на Zn, Mn и Cu се пониски отколку оние кои се објавени во литературата, а се однесуваат за некои загадени подрачја. Исто така, концентрацијата на Cd во стапалото беше многу ниска што наведува на заклучокот дека тој не претставува закана за човековото здравје при исхраната со полжави во скопскиот регион.

Клучни зборови: Шумски полжав, *Helix lucorum*, тешки метали, Скопје

Introduction

Turkish snail (*Helix lucorum* L.) is distributed from southern France, Italy, Balkan Peninsula Asia Minor to Iran (Neubert 2014). It is often used as food by local communities (Yildirim et al. 2004). It is believed that Turkish snail was introduced in France and Italy because of its edible features (Neubert 2014).

Five species of snails in Macedonia are used as food: Helix lucorum, H. pomatia, H. philibinensis, H. figulina and Cepea vindobonensis (Stojkoska and Jordanov 2004).

Turkish snail in Macedonia is also collected for food in restaurants and for export in significant quantities. Collected quantities reached over 1300 tons in 2001, valued at 3 million dollars when exported (MoEPP 2003) and 120 tons in 2002, 31 tons being collected from Skopje region (Stojkoska and Jordanov 2004). The Ministry of Environment and Physical Planning issued licenses for collection of 1760 tons of Turkish snails in the period 2011-2013, although exact collected quantities are not known (MoEPP 2014). According to some estimations its numbers have declined due to the uncontrolled collection (MoEPP 2003; Stojkoska and Jordanov 2004).

Heavy metal content in other edible snail species was studied and it is generally recognized that higher levels were recorded in polluted areas and the value for biomonitoring has been shown (Martin and Coughtrey 1982; Pihan and de Vaufleury 2000; Roma et al. 2017; Mahmutovic et al. 2018).

Although it is important as food, the heavy metals' contents in Turkih snail have not been studied so far in Macedonia.

In this paper we aim to present the results of some heavy metals contents (Zn, Cu Mn and Cd) in foot, shell and digestive gland (midgut gland or hepatopancreas) of Turkish snail in Skopje region and assess its safety as food resource.

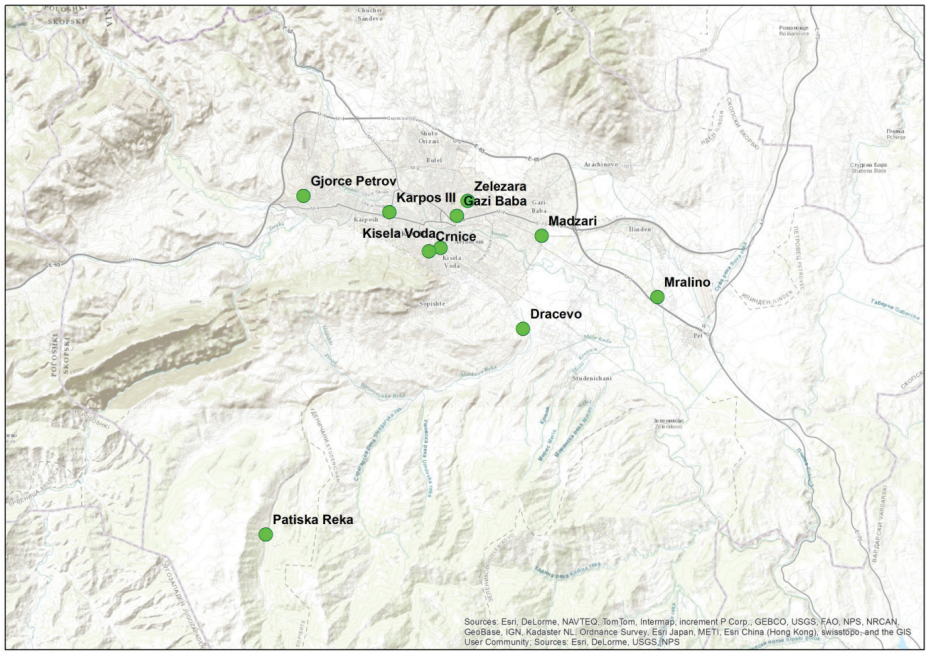

Fig. 1. Map of Skopje region with studied localities

Study area

Turkish snail specimens were collected from 10 localities in Skopje region (Fig. 1). Local population collects snail for consumption from all of these localities. 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. Dračevo (close to Pintija) and Mralino are considered as part of the rural area of Skopje region. Patiška Reka is a locality that is situated outside of the Skopje city area, in the foothills of Karadžica mountain.

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).

Materials and methods

Ten (10) 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 distilled water for 24h. Dead snails were dissected and three organs for analysis were extracted: digestive gland, foot and shell. The organs were immediately put in liquid nitrogen and stored in a refrigerator at -20 °C.

About 0.5 g of the freezed organs was measured on analytical scale with precision of 0.1 mg. Organs were transferred into Kjeldahl flasks and 8ml mixture of nitric, sulfuric and perchloric acid (10:2:1) were added and digested. Digested material was transferred into 25 ml flasks with hot distilled water. The content of heavy metals (Zn, Mn, Cu and Cd in digestive gland) was determined by flame atomic absorption spectrometry (Agilent 55A). Graphite furnace atomic absorption spectrometry (Agilent 240Z) was used for quantification of Cd content in foot and shell but the content of Cd was below the detection limit (3 μg·kg^{-1}). Same procedure was applied for blanks which were subtracted from the sample results.

Results

The majority of heavy metals is accumulated in the digestive glands (Fig. 2). The contents of Zn and Mn in digestive gland were more than 90 mg·kg^{-1} in average. The content of Zn in foot and shell was significantly lower, 7.9 and 4.5 mg·kg^{-1}, respectively. The content of Mn also showed much lower values in foot and shell (1.0 and 2.7 mg·kg^{-1}). The content of Cu was the highest in the digestive gland (8.0 mg·kg^{-1}), but the value in foot was comparable (4.7 mg·kg^{-1}) and the lowest values was recorded in the shell (1.1 mg·kg^{-1}).

Differences in the content of Zn, Mn and Cu in the investigated localities are presented

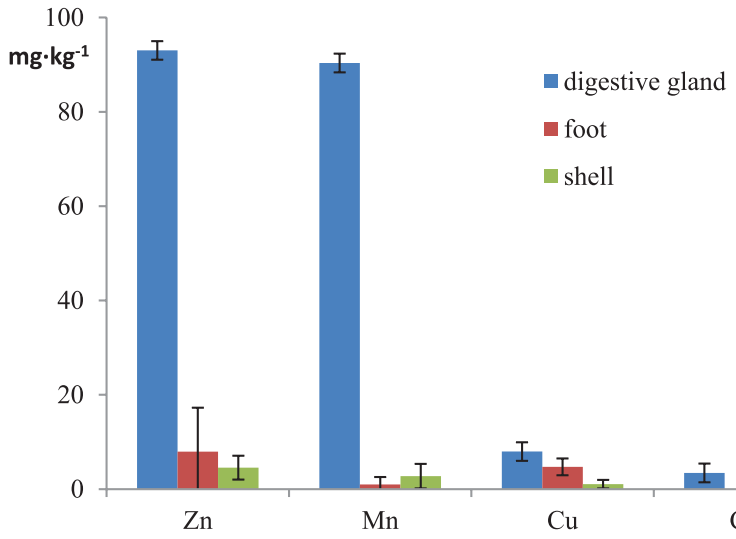

Fig. 2 Average contents of Zn, Mn, Cu and Cd in organs Turkish snail (*Helix lucorum* L.) in Skopje.

Tab. 1. Content of Zn in organs of Turkish snail (*Helix lucorum* L.) in different localities in Skopje (mg·kg^{-1}).

Locality	Digestive gland	Foot	Shell
Crniče	52.6 ± 5.5	5.1 ± 0.5	2.0 ± 0.4
Dračevo	56.2 ± 5.2	6.2 ± 0.3	1.5 ± 0.2
Gazi Baba	81.6 ± 8.6	6.2 ± 0.7	1.7 ± 0.4
Gjorče Petrov	74.7 ± 4.4	6.2 ± 0.4	5.3 ± 3.8
Karpoš III	127.8 ± 20.3	12.6 ± 1.3	14.3 ± 3.4
Kisela Voda	127.4 ± 18.7	5.9 ± 0.9	3.8 ± 1.1
Madžari	139.3 ± 11.2	11.0 ± 1.5	6.1 ± 0.8
Mralino	92.4 ± 17.9	11.3 ± 1.2	6.5 ± 1.1
Patiška Reka	42.5 ± 5.6	7.5 ± 0.4	1.7 ± 0.3
Železara	115.7 ± 4.3	8.1 ± 0.5	2.6 ± 0.4

Tab. 2 Content of Mn in organs of Turkish snail (*Helix lucorum* L.) in different localities in Skopje (mg·kg^{-1}).

Locality	Digestive gland	Foot	Shell
Crniče	46.6 ± 8.0	0.9 ± 0.1	2.5 ± 0.5
Dračevo	45.3 ± 12.0	0.8 ± 0.1	1.4 ± 0.2
Gazi Baba	113.3 ± 28.0	1.1 ± 0.1	3.5 ± 0.6
Gjorče Petrov	59.2 ± 7.7	1.1 ± 0.2	1.0 ± 0.2
Karpoš III	111.5 ± 18.1	1.2 ± 0.2	2.2 ± 0.5
Kisela Voda	39.1 ± 4.7	0.9 ± 0.0	1.9 ± 0.3
Madžari	164.7 ± 26.0	1.3 ± 0.1	7.2 ± 0.8
Mralino	154.4 ± 75.0	0.8 ± 0.1	4.6 ± 0.8
Patiška Reka	34.8 ± 8.8	0.7 ± 0.1	0.6 ± 0.1
Železara	118.9 ± 18.3	0.9 ± 0.1	1.8 ± 0.4

in Table 1-3. It can be noticed that there is certain variation in the values across localities, especially in the case of Zn and Mn. Cu content seems to be more homogenous in all studied organs in the 10 studied localities.

Cd content is only presented in digestive glands (Fig. 3) since the Cd content in foot and shell was below the limit of detection of 3 μg kg^{-1}. The highest Cd content was recorded in Železara and the lowest in Karpoš III.

Discussion

In general, the content of Zn and Cu decreased in the following order: digestive gland > shell > foot. I Mn content was the lowest in foot and higher in the shell.

The highest content of Zn, Mn and Cu was detected in digestive gland as being the main storage organ (Dallinger and Wieser 1984). However, the value of Cu content in digestive gland was comparable to the values in foot and shell (Fig. 2) pointing out that the digestive gland has lower potential to accumulate Cu (Rabitsch 1996; Menta and Parisi 2001; Beeby and Richmond 2002). Dallinger and Wieser (1984) presented similar results in the case of *Helix pomatia* and explained that Cu is easily lost from all organs and more evenly distributed in the body of the snail. The values for Cd content in foot and shell were very low (below the detection limit) as was the case in the other studies concerning *Helix pomatia* (Ćirić et al. 2018; Mahmutovic et al. 2018). Obviously, digestive gland performed as an effective storage organ for Cd, as well (Fig. 3).

The values of Zn, Mn and Cu in our study (especially in foot) are generally lower than the ones recorded in other snail species in some polluted areas (Gomot and Pihan 1997; Rabitsch 1996; Viard et al. 2004; Ćirić et al. 2018). The variation of the studied heavy

Tab. 3 Content of Cu in organs of Turkish snail (*Helix lucorum* L.) in different localities in Skopje ($mg \cdot kg^{-1}$).

Locality	Digestive gland	Foot	Shell
Crniče	5.4 ± 2.9	3.7 ± 1.0	1.2 ± 0.5
Dračevo	2.3 ± 0.4	2.6 ± 0.3	0.5 ± 0.0
Gazi Baba	7.1 ± 1.6	5.1 ± 0.9	0.8 ± 0.1
Gjorče Petrov	2.1 ± 0.4	2.3 ± 0.4	0.8 ± 0.1
Karpoš III	4.7 ± 0.6	4.0 ± 0.5	0.7 ± 0.1
Kisela Voda	12.3 ± 2.5	6.0 ± 0.7	1.3 ± 0.1
Madžari	8.9 ± 1.3	4.5 ± 0.8	1.3 ± 0.3
Mralino	24.9 ± 7.3	9.2 ± 2.4	2.9 ± 1.4
Patiška Reka	4.0 ± 0.6	4.0 ± 0.6	1.0 ± 0.2
Železara	8.1 ± 1.1	6.6 ± 0.8	0.6 ± 0.0

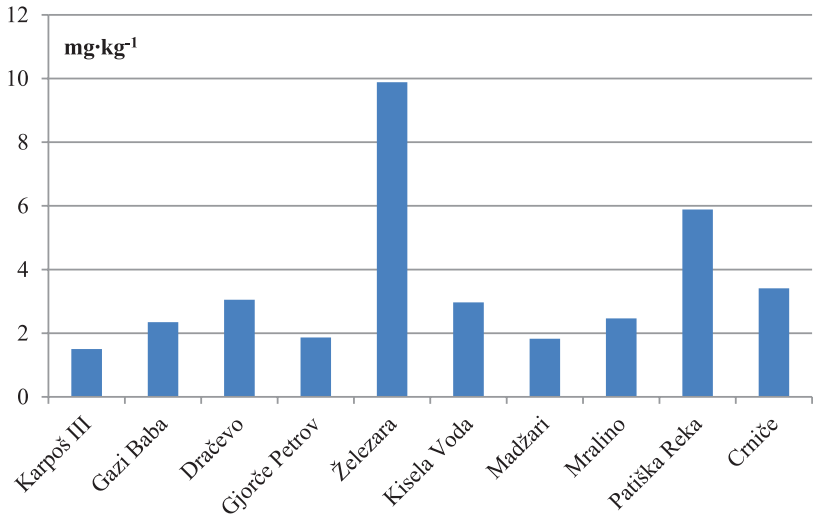

Fig. 3. Content of Cd in digestive gland of Turkish snail (*Helix lucorum* L.) in different localities in Skopje

metals' content is probably due to different environmental factors (pollution, natural soil chemistry) and other factors such as species specifics, age, diet, seasonal changes etc. (Ireland 1984; Laskowski and Hopkin 1996a; b; Gomot and Pihan 1997; Ćirić et al. 2018) have to be taken into account.

It is well known the Ca makes most of the shell. In *Cepaea vindobonensis* Ca was represented by 98% (Mierzwa 2008). Zn, Mn and Cu were present in low contents in the shell in studied Turkish snail populations with exception of Zn in Karpoš III and Cu in Mralino.

The concentration of Cd in foot was very low and thus it does not present any risk to human health when consummated. The value is below the limits prescribed for meat products (FAO 2012; EC Commission 2006).

National legislation for food safety sets limit values for Cd in livestock meat, fish and shell meat of 0.05, 0.05 and 1 $mg \cdot kg^{-1}$, respectively. Limit values for Pb in livestock meat, fish and shell meat are 0.1, 0.2 and 1.0 $mg \cdot kg^{-1}$, respectively (Ministry of Health 2005). All of the recorded values for Cd and Pb Turkish snail foot are well below the limit values.

However, thermal meat processing during food production can significantly increase the concentration of Cd in the snail meet as a result of water loss and contamination from digestive gland (Ziomek et al. 2018).

Obtained results and their analysis shows that the distribution pattern of studied heavy metals in the organs of Turkish snail (*Helix lucorum* L.) in different localities in Skopje

follows basic physiological principles for this species. The concentration of heavy metals in the foot which is used as a food are below the limit values and can be used as a safe food source.

References

Beeby, A., Richmond, L. (2002). Evaluating *Helix aspersa* as a sentinel for mapping metal pollution. *Ecological Indicators* **1**(4): 261–270.

Ćirić, J., Cerić, O., Marković, R., Janjić, J., Spirić, D., Popović, M., Pećanac, B., Baltić, B., Baltić, M. Ž. (2018). Seasonal distributions of heavy metal concentrations in different snail (Helix pomatia) tissues from an urban environment in Serbia. *Environmental Science and Pollution Research* **25**(33): 33415–33422.

Dallinger, R., Wieser, W. (1984). Patterns of accumulation, distribution and liberation of Zn, Cu, Cd and Pb in different organs of the land snail *Helix pomatia* L. *Comparative Biochemistry and Physiology Part C: Comparative Pharmacology* **79**(1): 117–124.

EC Commission (2006). *Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuff*. 2006R1881-EN-01.09. 2014-014.001-1.

FAO (2012). *Codex Alimentarius. Joint FAO/ WHO food standards programme Codex Committee on contaminants in foods. Working document for information and use in discussions related to contaminants and toxins in the GSCTFF (Prepared by Japan and the Netherlands). Sixth Session Maastricht, The Netherlands, 26-30 March 2012*. Food and Agriculture Organization of the United Nations.

Gomot, A., Pihan, F. (1997). Comparison of the Bioaccumulation Capacities of Copper and Zinc in Two Snail Subspecies (*Helix*). *Ecotoxicology and environmental safety* **38**(2): 85–94.

Ireland, M. P. (1984). Seasonal changes in zinc, manganese, magnesium, copper and calcium content in the digestive gland of the slug *Arion ater* L. *Comparative Biochemistry and Physiology Part A: Physiology* **78**(4): 855–858.

Laskowski, R., Hopkin, S. P. (1996a). Accumulation of Zn, Cu, Pb and Cd in the garden snail (*Helix aspersa*): Implications for predators. *Environmental Pollution* **91**(3): 289–297.

Laskowski, R., Hopkin, S. P. (1996b). Effect of Zn, Cu, Pb, and Cd on fitness in snails (*Helix aspersa*). *Ecotoxicology and Environmental Safety* **34**(1): 59–69.

Lazarevski, A. (1993). *Climate in Macedonia*. Kultura, Skopje, 253 p.

Mahmutovic, H., Markovic, R., Janjic, J., Glamoclija, N., Baltic, B., Katanic, N., Ciric, J. (2018). Concentration of Arsenic and Heavy Metals in Snail Tissues. *Meat Technology* **59**(2): 75–79.

Martin, M. H., Coughtrey, P. J. (1982). *Biological Monitoring of Heavy Metal Pollution: Land and Air.* Applied Science Publishers, London, 485 p.

Menta, C., Parisi, V. (2001). Metal concentrations in *Helix pomatia*,*Helix aspersa* and *Arion rufus*: a comparative study. *Environmental Pollution* **115**(2): 205–208.

Mierzwa, D. (2008). Chemical composition and structure of the shell of *Cepaea vindobonensis* (Férussac, 1821) (Gastropoda: Pulmonata: Helicidae). *Folia Malacologica* **16**(1): 17–20.

MoEPP (2003). *Country Study for Biodiversity of the Republic of Macedonia (First National Report)*. Ministry of Environment and Physical Planning, Skopje, 217 p.

MoEPP (2014). *Fifth National Report to the Convention on Biological Diversity of the Republic of Macedonia*. Ministry of Environment and Physical Planning, Skopje, 118 p.

Ministry of Health (2005). Rulebook on general requirements for food safety. Official Gazette of the Republic of Macedonia, 118 (1639): 30-275.

Neubert, E. (2014). Revision of *Helix* Linnaeus, 1758 in its eastern Mediterranean distribution area and reassignment of *Helix godetiana* Kobelt, 1878 to *Maltzanella* Hesse, 1917 (Gastropoda, Pulmonata, Helicidae). *Contributions to Natural History* **26**: 1–100.

Pihan, F., de Vaufleury, A. (2000). The Snail as a Target Organism for the Evaluation of Industrial Waste Dump Contamination and the Efficiency of Its Remediation. *Ecotoxicology and Environmental Safety* **46**(2): 137–147.

Rabitsch, W. B. (1996). Metal accumulation in terrestrial pulmonates at a lead/ zinc smelter site in Arnoldstein, Austria. *Bulletin of environmental contamination and toxicology* **56**(5): 734–741.

Roma, A. D., Neola, B., Serpe, F. P., Sansone,

D., Picazio, G., Cerino, P., Esposito, M. (2017). Land Snails (Helix aspersa) as Bioindicators of Trace Element Contamination in Campania (Italy). *OALib* **4**(2): 1–12.

Stojkoska, E., Jordanov, S. (2004). The protection problems with the edible snails diversity and the survey on the situation of their collection, buying up in the Republic of Macedonia. *Ekol. Zašt. Život. Sred* **9**(1–2): 69–74.

Viard, B., Pihan, F., Promeyrat, S., Pihan, J.-C. (2004). Integrated assessment of heavy metal (Pb, Zn, Cd) highway pollution: bioaccumulation in soil, Graminaceae and land snails. *Chemosphere* **55**(10): 1349–1359.

Yildirim, M. Z., Kebapçi, Ü., Gümüş, B. A. (2004). Edible Snails (Terrestrial) of Turkey. *Turkish Journal of Zoology* **28**(4): 329–335.

Ziomek, M., Drozd, Ł., Chałabis-Mazurek, A., Szkucik, K., Paszkiewicz, W., Valverde Piedra, J. L., Bełkot, Z., Maćkowiak-Dryka, M., Gondek, M., Knysz, P. (2018). Concentration levels of cadmium and lead in the raw and processed meat of Helix pomatia snails. *Polish journal of veterinary sciences* **21**(3): 483–489.