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Roman Snail (*Helix pomatia* L.) as Bioindicator of Heavy Metals Pollution in Mitrovica Town, Kosovo

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ABSTRACT

Our purpose was to monitor the environmental pollution with heavy metals, such as Pb, Zn, Cu, and Cd, and their accumulation in Roman snails (*Helix pomatia L.*) and the possibility of Roman using snail as a bioindicator of heavy metal pollution. For analyses, we were taken 22 specimens of roman snails in the Mitrovica town (area with the heavy metal pollution derived from the "Trepça" smelter) and 22 specimens in the Vernica village (control area), in which case is analyzed the concentration of heavy metals: Lead (Pb), cadmium (Cd), Zinc (Zn) and Copper (Cu) in the hepatopancreas, foot, and shell of snails. Our results show the high concentration of Pb, Zn, and Cd in the hepatopancreas compared to the other tissues (foot and shell), whereas Cu concentration was higher in the foot of snails from Mitrovica compared to the control group in a significant degree ($P < 0.001$). We have found a significant ($P<0.001$) positive correlation between all heavy metals (Pb/Cd; Pb/Cu; Pb/Zn; Cd/Cu; Cd/Zn and Cu/Zn) in the hepatopancreas tissue. In the foot, we have found the negative correlation for Pb/Cd and Pb/Cu and positive for the Pb/Zn; Cd/Cu; Cd/Zn, and Cu/Zn, whereas in the shell the correlation was positive for the Pb/Cd; Pb/Cu and Cd/Cu, and negative for the Pb/Zn; Cd/Zn and Cu/Zn. In the hepatopancreas, the concentration of metals ranged from higher to the lower concentration was: Zn>Cd>Pb>Cu; in the foot: Cu>Zn>Cd>Pb, whereas in the shell: Zn>Cu>Pb>Cd. The Mitrovica town is the area with heavy metals pollution, whereas the Roman snail accumulates relatively high values of these heavy metals and can serve as an indicator organism for pollution with the metals such as Pb, Cd, Zn, and Cu.

Keywords: Roman snail, Hepatopancreas, foot, shell, heavy metals.

INTRODUCTION

The pollution of the environment with heavy metals and their negative impact on the health of the living being is a problem that is treated seriously by the scientific community. As circumstances of this are very important to use the living organisms as a bioindicator of pollution, which provide us with data to identify (recognize) the effect of this pollution over a while, as well as to know much more precisely the bioavailability and mobility of pollutants. Human activities, very quick tecnic-technological, agricultural development, the transformation of energy, and the chemical industry have brought to the release of organic and inorganic wastes with the different chemical nature in the surrounding environment which consequently has caused extremely large environmental disturbances on our planet. In particular, heavy metal pollution is evident in many industrial areas, near the mine around the world, including our country. Heavy metals such as Mn, Co, Cu, Mo, V, Zn, Pb, Cd, Hg, As, Cr, etc., are concentrated in the environment as a result of anthropogenic activities, whose exploitation began thousands of years ago by man and continues until nowadays. In addition to this, for instance, calcium (Ca) and magnesium (Mg) are chemical essential elements, whereas other metals have negative effects on the health of living beings, some of these even in low doses. It has been proven that Pb, Cd, Hg, Cr, Ni, and As are powerful and poisonous pollutants even in low concentrations (Bolan et al., 2003), while Al, Zn, and Cu are considered not harmful to the health of living beings because they play an important role in metabolic processes, they have the effect in the activity of a large number of enzymes and also haemoglobin synthesis, etc. (Cachada et al., 2012). All of the heavy metals (in our case: Pb, Cd, Cu, and Zn), depending on the kind of compound are presented in the environment as a result of anthropogenic activities, such as mines, heavy industry, pesticides application, savages, etc. They enter into the organisms through the skin, respirative, or digestive tract and tend to accumulate in the so-colled target organs. In the high concentration, Pb atack many of stinks of organ systems, such as central nervous system, kidneys, immune system and reproductive system (WHO, 2019) and blood where Pb couse the anaemia and a stinks of the other disorders even in the very low doses in the humans and different animals (Elezaj et al. 2020, Trampel 2003). It was found out that the highest concentration of the Pb and Zn in the animals was accumulated in the bones (about 90 %, in which case Pb replaces the Ca) (Elezaj et al. 2011), Cd in the kidneys, etc. Also, along with the Pb as a co-contaminant is often found the Cd, which in the form of various compounds is increasingly present in the environment. Pb and Cd can be absorbed by living organisms through the water, various foods of the plant (through rice), and animals origine, and through the contaminated air (Genchi et al. 2020). Zn and Cu on the other hand have a function as a cofactor or as a structural component of many enzymes, nonenzymatic proteins, therefore playing a role in a wide range of biological processes (Gibson et al., 1995). Furthermore, the replacement of Zn with the Pb in Zn-dependent enzymes, or other proteins, may inhibit their function. For example, δ-aminolevulinic acid dehydratase is a Zn-dependent enzyme that was inhibited by the presence of Pb as a result of the replacement of Zn with Pb (Cachada et al., 2012, Çarkaj et al., 2021). However, organisms' toxicity with Zn is very rare. This toxicity can be expressed when the organism will be exposed to high doses of Pb and Cd, wherein this case as a protective effect against toxicity with Pb the organism tends to accumulate more Zn (Milaimi et al., 2016a, Cai & Calisi, 2016. Plum et al., 2010). Although they are essential metals for organisms, high concentrations of Zn and Cu can also lead to problems in the organs in which they are accumulated. Chronic poisoning in Zn and Cu also causes its deposition in various organs (Anant et al., 2018) thus leading to various histological, biochemical, and structural defects of organs (Milaimi et al., 2016b).

The Roman snail (*Helix pomatia*) is a good animal model to research and assess an area contaminated with heavy metals. Previous studies (Nica et al., 2012, Vukašinovic-Pešic et al., 2020, Çarkaj et al., 2021) show that the Roman snails, which were lived in areas contaminated with heavy metals can accumulate high concentrations of Cu, Zn, Cd, and Pb in the leg muscle and glands of the digestive tract, such as hepatopancreas. According to various authors (Gomot-De & Pihan 2000), the analysis of the aforementioned metals (Cd, Pb, Cu, and Zn) in the tissues of snails provide abundant information on the bio-availability of heavy metals in the environment. Animal model – *Helix pomatia* can be useful for monitoring heavy metal environmental pollution, as being a first-class consumer, it comes in contact with higher concentrations of these metals and is capable of surviving and growing in such an environment.

STUDY AREA

Mitrovica is already known as an area polluted with heavy metals that were derived from the lead mine. Various authors (Elezaj et al., 2013) in the wild populations of urban pigeon (*Columba livia* – forma Urbana) in Mitrovica found out that the concentration of lead in the blood was 27.7 times higher (149.6; 50.5 μg/g DW) compared to the concentration of Pb in the blood of pigeons of the control area (5.4 (μg/g DW). A very high presence of heavy metals in Mitrovica has been found in the plants (Imeri et al. 2019, Cakaj Krasniqi et al., 2020) and animals (Elezaj et al. 2013; Elezaj et al. 2011). The area even nowadays (Trepça smelter is not active nowadays, since 1999) are polluted, and the level of Pb in the environment is 2–3 times higher than the limit permission from EU for the heavy metals (Dehari-Zeka et al. 2021, Memishi et al., 2021). However, data on the concentration of these metals in various snail tissues are very scarce (Çarkaj et al., 2021). In our studies, we wanted to investigate the concentration of Pb, Cd, Zn, and Cu in the hepatopancreas, leg muscle, and shells of the Roman snails in Mitrovica, very close to the "Trepça" smelter. This is done to ascertain the level of the metals (Pb, Cd, Cu, and Zn) and which of the organs has the highest ability to accumulate these metals.

MATERIAL AND METHODS

The research was conducted in two different localities: in Mitrovica (locality with industrial-urban pollution) where 22 individuals of the natural population of the Roman snail (*Helix pomatia L.*) were taken, as well as in 22 other individuals in the village of Vernica – 25 km away from the polluted area of the city of Mitrovica (control area). The individuals have been of approximate size. After sampling, the snails were placed in plastic bags and transported to the laboratory which was kept at room temperature. They are then dissected by first releasing them from the shell and then isolating the hepatopancreas and snail foot. During the experiment, ethical guidelines were followed where we tried to get a few samples as possible in the study. The weight of the snails (live weight) and the weight of the tissues in question were then measured. The concentration of heavy metals (Pb, Cd, Zn, and Cu) was determined in the homogeneity of the isolated tissues. Initially, the tissues were dried at 75˚C for 48 hours. Each sample was weighed by 0.3 grams, then digested with royal water (7 ml HNO_3 and 3 ml H_2O_2) in the microwave. The total concentration of heavy metals was determined was analyzed with the ICP-OES device, Optima 2100 DV (Perkin Elmer Optima 2100 DV ICP-OES). The detection threshold for metals was: for Zn: 0.2 ppb, Pb: 1 ppb, Cd: 0.1 ppb, and Cu: 0.4 ppb. With the Sigma Stat, 3.5 software calculated the arithmetic mean, standard deviation, significance, and correlation (Spearman Correlation) between the concentration of metals in tissues and between tissues. The result is expressed in mg/kg of dry weight.

RESULTS AND DISCUSSION

Table 1 represents our results regarding to the concentration of heavy metals in the hepatopancreas, leg muscle and the shell of snails of the city of Mitrovica and in Vernica (control area). These results indicate a higher concentration of all metals analyzed in Mitrovica: in hepatopancreas (Pb = 39.5 ± 2.1, Zn = 598.03 ± 299, Cd = 53.4 ± 26.6, Cu = 9.3 ± 5.2), leg muscle (Pb = 0.44 ± 0.40, Zn = 11.45 ± 2.58, Cd = 2.95 ± 2.37, Cu = 44.41 ± 16.83) and shell (Pb = 2.159 ± 4.46, Zn = 9.68 ± 9.10, Cd = 0.075 ± 0.145, Cu = 3.524 ± 1.171) compared to control group: in hepatopancreas (Pb = 3.2 ± 3.8, Zn = 47.0 ± 25.7, Cd = 2.8 ± 2.1, Cu = 6.2 ± 5.7), leg muscle (Pb = 0.22 ± 0.18, Zn = 9.22 ± 1.47, Cd = 0.00, Cu = 4.98 ± 1.43) and shell (Pb = 0.00, Zn = 0.356 ± 0.276, Cd = 0.00, Cu = 1.081 ± 1.62) with the significant degree $P < 0.001$, with the exception of Pb in the leg muscle ($P = 0.164$) and Zn in shell ($P = 0.019$) which differences are non-significant.

Our results regarding the higher concentration of heavy metals in the samples analyzed in Mitrovica are in line with the results of the other authors who have researched the bioaccumulation of heavy metals in various animal and plant models, such as birds (Elezaj et al., 2013), mammals (Krasniqi-Cakaj et al., 2020) and plants (Imeri et al., 2019) in Mitrovica. The high levels of heavy metals in Mitrovica have also been reported on agricultural lands (Mensur et al. 2018) finding a high concentration of Pb in the food chain (soil, grass, blood, and milk of cows) (Krasniqi-Cakaj et al., 2020). The other authors (Çarkaj et al., 2021) have found out the higher concentration of metals (Pb, Zn, Cd, and Cu) in hepatopancreas of snails on a significant degree ($P < 0.001$) for all metals in Mitrovica (Pb = 37.6 ± 2.1; Zn = 605.2 ± 308; Cd = 51.7 ± 27.8; Cu = 8.5 ± 6.3) compared to the control group (Pb = 3.7 ± 3.8; Zn = 49.22 ± 25.7; Cd = 3.9 ± 2.8; Cu = 7.3. 6.8).

The high concentration of metals, in this case, was followed by inhibition of enzymes and reduction of the amount of total proteins in the hepatopancreas and the hemolymph of snails of the Mitrovica group (ALAD: 3.25 ± 1.25; Prot. Hepat. = 65.4 ± 7.46 µg/ml, Prot. Hemol. = 78.2 ± 14.6 µg/g) compared to the control group. This data serves to justify that the high values of metals in our study (Pb, Zn, Cd, and Cu) in all studied tissues are at toxic levels for

Table 1. The concentration of heavy metals (mg/kg of dry weight) in Roman snail (Helix pomatia) in Mitrovica (study area) and Vernica (control area)

Location	Pb	Zn	Cd	Cu
The concentration of heavy metals in the Hepatopancreas				
Vernica	3.2 ± 3.8 (22)	47.0 ± 25.7 (22)	2.8 ± 2.1 (22)	6.2 ± 5.7 (22)
Mitrovica	39.5 ± 2.1(22)	598.03 ± 299(22)	53.4 ± 26.6 (22)	9.3 ± 5.2 (22)
Significant (P <)	P = < 0.001	P = < 0.001	P = < 0.001	P = < 0.001
The concentration of heavy metals in the leg muscle				
Vernica	0.22 ± 0.18 (22)	9.22 ± 1.47 (22)	0.00 – ND (22)	4.98 ± 1.43 (22)
Mitrovica	0.44 ± 0.40 (22)	11.45 ± 2.58 (22)	2.95 ± 2.37 (22)	44.41 ± 16.83 (22)
Significant (P <)	NS; (P = 0.164)	(P = 0.001)	(P = <0.001)	(P = <0.001)
The concentration of heavy metals in the shell				
Vernica	0.00; ND (22) <0,1ppb*	0.356 ± 0.276 (22)	0.00; ND (22) <1ppb*	1.081 ± 1.62 (22)
Mitrovica	2.159 ± 4.46 (22)	9.68 ± 9.10 (10)	0.075 ± 0.145 (8)	3.524 ± 1.171 (22)
Significant (P <)	(P = <0.001)	NS; (P = 0.019)	(P = <0.001)	(P = <0.001)

Note: Values are expressed as means, ± SD – standard deviation; NS – nonsignificant; ND – not detected; in parenthesis – number of animals; * level of detection with ICP-OES

Roman snails and undoubtedly these values will have a very negative impact on the physiology and biochemistry of such organisms, in this case, the Roman snail.

Pb metal accumulated significantly more in all snail tissues from the Mitrovica group compared to the control area is observed to be more concentrated in the hepatopancreas (Pb = 39.5 ± 2.1) followed by a lower concentration in the shell (Pb = 2.159 ± 4.46) and then on the leg muscle (Pb = 0.44. 0.40). The higher accumulation in the hepatopancreas compared to the other tissues speaks to a persistent poisoning of this species with the lead. Giving into account that Pb target organs are solid tissues (Milaimi et al., 2016, Commission Regulation (EC), 2006) in which tissues it accumulated more, as well as the fact that Pb is less mobile in the tissues in question, therefore it can be said that the Pb concentration tends to increase more over the time. As a result, snail specimens appear to have undergone chronic lead intoxication, as we have a higher concentration of Pb in the shell compared to snail leg muscle. In Mitrovica, although nowadays the Trepça smelter does not work, still the concentration of Pb and other heavy metals remains above the high limits allowed in the soil by EU directives and instructions of the Republic of Kosovo, which are as follows: for Zn = 150–300 mg/kg; Zn = 300 mg/kg; Cu = 50–140, Cu = 100; Pb = 50–300, Pb = 50; Cd = 1–3, Cd = 2. In Mitrovica, this concentration of metals in soil, grass, and cow's milk is at least twice as high as the permitted limits (Memishi et al. 2020). Also, the allowed metal limits in the hepatopancreas of snails in the city of Mitrovica are exceeded which poses a risk to human populations which consume snails from this area (Çarkaj et al., 2021).

The results in our study show for a very high concentration of Zn in Mitrovica (hepatopancreas: Zn = 598.03 ± 299; leg muscle: 11.45 ± 2.58 and shell: 9.68 ± 9.10) compared to the control group (hepatopancreas: Zn = 47.0 ± 25.7; leg muscle: 9.22 ± 1.47 and shell: 0.356 ± 0.276), where the hepatopancreas was the target organ of Zn accumulation. Also, Cu metal in snail samples from the city of Mitrovica is in higher concentration (hepatopancreas: Cu = 9.3 ± 5.2; leg muscle: 44.41 ± 16.83 and shell: 3.524 ± 1.171) compared to the control group (hepatopancreas: Cu = 6.2 ± 5.7; leg muscle: 4.98 ± 1.43 and shell: 1,081 ± 1.62). The metals Zn and Cu are known to be essential elements, without which the function of many enzymes and the construction of various protein structures in living organisms would be impossible.

It is already known that some of the enzymes, such as delta-aminolevulinic acid dehydratase as coenzyme have Zn and in cases of intoxication with Pb comes to the competitive role of Zn and Pb for the same binding site in the enzyme (Milaimi et al., 2016). In these cases, the organism tends to accumulate more Zn as a protective effect against Pb contamination (Çarkaj et al., 2021). Otherwise, Zn and Cu poisonings are very rare in the living world, except when the metals in question are found to be highly concentrated in the environment.

Cd metal in snail samples from the city of Mitrovica is in higher concentration (hepatopancreas: Cd = 53.4 ± 26.6; leg muscle: 2.95 ± 2.37 and shell: 0.075 ± 0.145) compared to the control group (hepatopancreas: Cd = 2.8 ± 2.1; leg muscle: 0.00 and shell: 0.00). Cd even at very low concentrations is extremely toxic to living organisms. It has been found that the target organ has soft tissues, with special emphasis on kidney tissue and testicles in higher animal organisms (Milaimi et al. 2016a). Cd was also found in the hepatopancreas of snails near the Pb mine, where the level of Pb and Cd was 2 times higher than the maximum concentration of metals allowed in the environment (Çarkaj et al., 2021, Dehari Zeka et al., 2021).

Tables 2 and 3 show the correlation between metals, where it was found that in Mitrovica there is a positive correlation to a significant degree between all metals compared in hepatopancreas; Pb/Zn, Cd/Cu, Cd/Zn, and Cu/Zn in the leg muscle and Pb/Cd, Pb/Cu, Cd/Cu in shell, comparison of the other metals resulted in negative correlation for Pb/Cd and Pb/Cu in hepatopancreas, and Pb/Zn, Cd/Zn, Cu/Zn in the shell. A significant positive correlation between Pb and the other metals in hepatopancreas (Table 3); of Pb with Cu and Zn in the leg muscle and of Pb with Cu in the shell refers to a chronic intoxication of snails with Pb. From the data of the other authors (Elezaj et al., 2011, Milaimi et al., 2016, Hutton & Godman, 1980) it is known that during Pb intoxication the body accumulates high concentrations of Zn as a protective effect against Pb poisoning. On the other hand, the very high concentration of Pb and Zn in the hepatopancreas has led to a low concentration of Cu which show a competitive role of these metals for binding sites in proteins and enzymes, which will have led to the inhibition of absorption of Cu by snail tissues and organs. This is complemented by the fact that in the leg muscle tissue, where a lower concentration of Pb was found, a lower concentration was also found for Zn, while the concentration of Cu was higher compared to Cu in the hepatopancreas. Positive correlations between Pb with Zn, Cd, and Cu in hepatopancreas and shell show a higher accumulation tendency of Zn, Cu, and Cd as a result of the accumulation of high Pb values.

Given the fact that snail meat is consumed because it has high concentrations of Selenium, Cu, and Zn (Nowakowska et al., 2012, Toader-Williams & Golubkina., 2009), as well as the fact that *Helix pomatia* is the species most commonly used for this purpose (Vukašinovic-Pešic et al., 2020) then very little, is known about the mineral composition and ability to accumulate metals from the environment polluted with metals that have an extremely negative impact on the environment and live beings. The data in our paper show for exceeding the concentration of heavy metals (especially Pb, Cd, and Cu in the hepatopancreas, Zn, Cd, and Cu in the leg muscle and Pb, Zn, and Cu in the shell) above the limits allowed by the European Commission 1.5 mg/kg of heavy metals is the maximum allowed in food (molluscs) (Ziomek et al., 2018), which shows that snails sampled in this area contain levels of heavy metals dangerous to the health of the people who consume them. According to research conducted in Kosovo (Mensur et al., 2018), it turned out that there are significant differences (P = 0.001) in the countries around the Industrial Zone in Mitrovica. The highest value of metals (Cd, Cu, Pb, and Zn) was found in the soil in North Mitrovica areas near industrial sites in Mitrovica. The authors emphasize that this high level of heavy metals poses an extraordinary risk for the pollution of the ecosystem, even of the agricultural land in the territory of Mitrovica. On the other hand, research shows that a high

Table 2. Correlation coefficient (r; Spearman correlation) between the concentration of heavy metals (Pb, Zn, Cd, Cu) in hepatopancreas, leg muscle, and snail shell in the city of Mitrovica

Metals	Hepatopancreas	Leg muscle	Shell
	r; (P<)	r; (P<)	r; (P<)
Pb/Cd (22)	0.568 (0.005)	-0.491 (P < 0.050)	0.891; (P < 0.050)
Pb/Cu (22)	0.745 (<0.001)	-0.345; NS (P > 0.050)	0.414; NS (P > 0.050)
Pb/Zn (22)	0.839 (<0.001)	0.152; NS (P > 0.050)	-0.489; NS (P > 0.050)
Cd/Cu (22)	0.684(<0.001)	0.503; (P < 0.050)	0.201; NS (P > 0.050)
Cd/Zn (22)	0.981 (<0.001)	0.273; NS (P > 0.050)	-0.579; (P < 0.050)
Cu/Zn (22)	0.677 (<0.001)	0.321; NS (P > 0.050)	-0.0821; NS (P > 0.050)

Note: The results are expressed as correlation values according to Spearman (r). In parentheses is the number of specimens.

Table 3. Correlation coefficient (r; Spearman correlation) between the concentration of heavy metals (Pb, Zn, Cd, Cu) in the tissues (hepatopancreas, leg muscle, and shell) of the snail in the city of Mitrovica

Pb	Hepatopancreas (r)	Leg muscle (r)	Shell (r)
Hepatopancreas (r)	1		
Leg muscle (r)	8.047; (P = <0.001)	1	
Shell (r)	-4.691; (P = <0.001)	-3.538; (P = <0.001)	1
Zn			
Hepatopancreas (r)	1		
Leg muscle (r)	-8.604; (P = <0.001)	1	
Shell (r)	9.058; (P = <0.001)	-0.840; NS ((P = 0.406)	1
Cd			
Hepatopancreas (r)	1		
Leg muscle (r)	-7.808; (P = <0.001)	1	
Shell (r)	7.808; (P = <0.001)	0.000; NS (P = 1.000)	1
Cu			
Hepatopancreas (r)	1		
Leg muscle (r)	-9.530; (P = <0.001)	1	
Shell (r)	3.584; (P = <0.001)	-11.648; (P = <0.001)	1

amount of lead of 151,000 mg/kg was found in the samples near the former Trepça smelter, where these figures exceed the standard of the US Environmental Protection Agency (Longtong et al., 2016). Given the fact that the Roman snail is an organism that comes in close contact with pollutants since it is also herbivorous, then the high concentrations of metals in the soil, through the food chain can be easily introduced by plants to the snail. It has been interesting for us to investigate the survival ability of this animal species at relatively high concentrations of heavy metals, previously reported (Çarkaj et al., 2021). In this context, the possibility of a broader assessment should be considered and it should be borne in mind that such a situation negatively affects human health.

CONCLUSION

In Mitrovica, despite the non-functioning of the Trepça smelter nowadays, the level of Pb, Cd, Zn, and Cu in the environment continues to be high, disturbing, and dangerous for the environment and living organisms. Since our results show that the Roman snail (*Helix pomatia*) can accumulate relatively high concentrations of heavy metals and survive despite these levels then it can be proposed as a good animal model for monitoring the environment contaminated with heavy metals.

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