

# BIOACCUMULATION OF HEAVY METALS IN THE ROMAN SNAIL (*HELIX POMATIA*) IN THE AREA OF KOSOVSKA MITROVICA

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## ABSTRACT

The bioaccumulation of heavy metals in the Roman snail (*Helix pomatia* Linnaeus, 1758) was investigated in October 2024. Animals were sampled at the location "Gornje Polje," which is situated near the former "Trepča" plant. The snails were collected by hand and then transported to the laboratory. A total of 34 medium-aged snails and 36 older snails were collected. The snails were dissected, and the content of heavy metals determined in the hemolymph, hepatopancreas, kidney and shell. In addition to analyzing the heavy metal content in the snails, an analysis of the heavy metals content in the soil was performed. The soil was collected in a quantity of 1 dm<sup>3</sup> using a shovel and transported to the laboratory. The content of seven elements was examined: Arsenic (As), Copper (Cu), Iron (Fe), Cadmium (Cd), Manganese (Mn), Lead (Pb), and Zinc (Zn). The highest level of accumulation is heavy metal observed in the hepatopancreas. Furthermore, other structures exhibit a significantly high level of accumulation. All of this suggests that snails are a highly useful tool for diagnostic purposes in environmental of heavy metal accumulation. The analysis of heavy metal content was performed at the Public Health Institute of Kosovska Mitrovica.

**Keywords:** Heavy metal, Hemolymph, Hepatopancreas, Shell, Kidney, Soil.

## INTRODUCTION

Bioaccumulation refers to the progressive accumulation of metals in biological organisms over time relative to ambient concentrations. This process occurs when the rate of metal uptake and storage exceeds its metabolism and excretion (Markich et al., 2001).

Early studies on metal accumulation in terrestrial invertebrates highlighted their significance in ecotoxicological research. Hopkin (Hopkin, 1989) proposed that "terrestrial invertebrates are key organisms in studying the accumulation of heavy metals due to their close association with the substrate and relative immobility."

Snails are particularly suitable for ecotoxicological research as they effectively reflect changes in metal bioavailability in the environment (Dallinger, 1994).

Subsequent studies have confirmed the ability of snails to accumulate heavy metals such as copper (Cu), lead (Pb), zinc (Zn), and cadmium (Cd). Beeby and Richmond (Beeby & Richmond, 2003) demonstrated that terrestrial snails could serve as reliable bioindicators of metal pollution, findings that were further supported by (Notten et al., 2005; Jordaens et al., 2006). Snails can accumulate heavy metals in their tissues and shells. Their ability for bioaccumulation makes them highly reliable for assessing environmental conditions. (Zalewski et al., 2010; Nica et al., 2012).

Various studies, including those conducted by different authors, have significantly expanded knowledge, emphasizing

atmospheric deposition as a key source of metal contamination in soils. These studies indicate that metals, which accumulate in soils through atmospheric deposition, can cause long-term negative effects in ecosystems. The process of atmospheric deposition contributes to the accumulation of these metals in the soil, thereby increasing their bioaccumulation in organisms at higher trophic levels, such as snails and other terrestrial invertebrates. Consequently, this contamination can impact soil quality, reducing its fertility and disrupting ecosystem balance. These findings underscore the importance of continuous monitoring of atmospheric metal deposition and research into its effects on biodiversity and ecological processes. (Gomot-deVaufleury, 2000; Azimi et al., 2004; Regoli & Giuliani, 2014).

The growing ecological implications of industrial metal pollution highlight serious long-term consequences for ecosystems, biodiversity, and human health. Continuous monitoring of metal concentrations is crucial for timely identification of distribution and accumulation changes. This approach aids in assessing and mitigating negative ecological impacts, while improving ecosystem adaptation. Monitoring is essential for environmental protection strategies, tracking trends, and identifying contamination sources, contributing to sustainable resource management and reduced pollution impacts. (Dixit et al., 2015).

The bioaccumulation process depends on both physicochemical and biological factors, with metal bioavailability in soil playing a critical role in their uptake and transfer through the food chain (Nica, 2012).

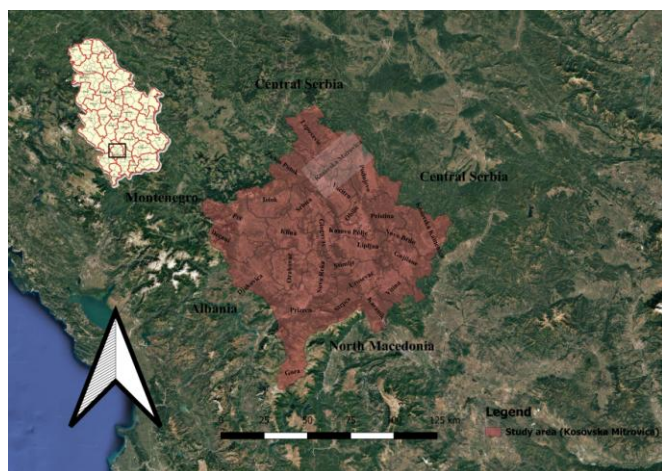
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In this study, soil and snail tissue samples were analyzed for the presence of as, Fe, Mn, Cd, Cu, Ni, Pb, and Zn to assess the level of accumulation under urban conditions. The results revealed significant differences in metal content across various anatomical and morphological structures, providing insight into the bioaccumulation process. Additionally, an analysis of heavy metal concentrations in the surrounding soil was conducted to better understand their environmental distribution.

The sampling site, "Gornje Polje," in Kosovska Mitrovica, was selected due to its proximity to potential pollution sources. This study aims to determine heavy metal concentrations in both snails and soil and, based on bioaccumulation patterns, to assess the ecological status of this region.

## THEORETICAL PART

The city of Kosovska Mitrovica is located in Kosovo and Metohija, in the southern part of Serbia (Figure 1). Its geographical coordinates are 42° 53' N latitude and 20° 52' E longitude. More precisely, the city is situated in the northern part of the Kosovo Basin, at the foot of the paleo-volcanic cone of Zvečan, at the confluence of the Sitnica River into the Ibar River, where the Ibar meanders before forming the second section of its gorge (Batočanin Srećković et al., 2020). The urban core of the city and its wider surroundings cover an area of 316 km<sup>2</sup>. Kosovska Mitrovica is surrounded by the Rogozna, Kopaonik, and Mokra Gora mountains to the north, east, and west, while the southern part of the area is bordered by the Čičavica mountain range. Two-thirds of the territory is characterized by mountainous relief, that is morphologically dominant. The average altitude of the city is 510 meters above sea level (Šukrija et al., 1979).



**Figure 1.** Physical-geographical map of Serbia and Kosovo and Metohija in Europe, with the geographical location of Kosovska Mitrovica specifically marked.

The entire territory of Kosovska Mitrovica holds a strategically significant position, particularly in terms of transit traffic, as it lies at the crossroads of routes connecting Belgrade in the north with Skopje and Thessaloniki in the south, Niš and Sofia in the east, and Podgorica and Bar in the west (Šukrija et al., 1979). The favorable geographical position and physical-geographical characteristics have facilitated the industrial development of Kosovska Mitrovica through various historical periods, from pre-Christian times to the modern era. This development has been primarily based on a highly developed mining industry, especially the extraction of silver-bearing, gold-bearing, lead, zinc, and copper ores. The "Trepča" mine in Stari Trg, along with surrounding mines, played a crucial role in the industrial development of this region (Jovanović, 2007). Following detailed research conducted in 1925, it was established that "Trepča" represents a grand-scale deposit and that its exploitation is highly profitable (Jovanović, 2008).

Sampling of the necessary individuals of *H.pomatia* for analysis was conducted at the Gornje Polje site (Figure 2), located within the territory of Kosovska Mitrovica, in close proximity to the Ibar River (Figure 3).



**Figure 2.** Locality Gornje Polje.



**Figure 3.** Material sampling site.

The samples were collected within the following geographical coordinates: from 42° 54' 07.6" N and 20° 51'

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53.8'' E to 42° 54' 08.1'' N and 20° 51' 57.2'' E. Additionally, soil sampling was conducted at the same site, with geographical coordinates of 42° 54' 10.8'' N and 20° 51' 38.8'' E.

This site represents an industrial waste deposit that was active from 1930 to 1965. The total amount of deposited waste, expressed in tons, is 26,344,212, while the total volume is 8,498,133 m<sup>3</sup> (Milentijević et al., 2014).

## EXPERIMENTAL

### *Materials and methods*

At the given site, few plant species were identified, including weeds, grasses, shrubs, and small-headed plants. Additionally, human generated waste was observed in the Ibar River, along with large amounts of waste in the vicinity of the river, including remnants of wood (branches and small trees). In the immediate vicinity of the Ibar River, two major chimneys were situated, whose previous activity contributed to the contamination of the Kosovska Mitrovica area and surrounding settlements.

A soil sample measuring 10x10x10 cm (1 dm<sup>3</sup>) was collected along with vegetation. The soil was transported in a clean cardboard box to the laboratory. Geographical data of the site from which the soil was collected were labeled on the box.

The samples were collected in October 2024.

Snails of the species *H. pomatia* were manually collected using medical gloves. During collection, a high presence of snails was noticed on the surface of a shoot from the plant genus *Dipsacus* Linnaeus, 1753. Most of the snails were in a state of hibernation. A smaller number of individuals were found in the vicinity of plants, in the grass, and under rocks. Empty snail shells were also observed (Figure 4).

The shell of the Roman snail is firm, spherical in shape, with a diameter ranging from 38 to 43 mm.



**Figure 4.** *H. pomatia* collected in the study area.

The color of the shell is yellow-brown, with a tendency to transition into lighter shades under the influence of sunlight. The surface of the shell is characterized by spiral lines, which are unevenly distributed and faintly visible. The shell usually contains 4.5 to 5 turns, and the opening at its apex is round. For younger individuals, the number of turns is lower.

The surface of the head and foot of this species is granular and uneven, which represents a key morphological characteristic for its identification. During hibernation, snails secrete a calcareous operculum, a firm cover that seals the shell opening, protecting them from unfavorable external factors. (Monaco Nature Encyclopedia, no date).

During collection, the air temperature ranged from 18 °C to 22 °C, and the relative humidity was on average 72%. A total of 70 snails were collected, which were then placed in plastic containers and transferred to the laboratory for further processing.

After collection, the snails were divided into two groups: medium-aged snails and older snails. A total of 34 medium-aged snails and 36 older snails were dissected. The age of the snails was determined based on the number of whorls on the shell and body mass. Snails weighing over 20 g were classified as older, with a greater number of whorls on the shell, while snails weighing less than 20 g were classified as medium-aged, with fewer whorls on the shell. The procedure was carried out in the first half of November 2024.

The procedure for isolating specific anatomical structures was performed using laboratory equipment and utensils. In separate vials, the following were isolated: 10 ml of hemolymph, 10 g of hepatopancreas, 10 g of kidneys, and 10 g of macerated shell. The material was preserved in vials. Initially, the hemolymph was isolated, followed by the shell, hepatopancreas, and finally, the kidney.

The isolation procedure: Initially, HCl was used. The shell was treated in the region of the second whorl (decalcification of the shell near the heart) using a cotton swab. Afterward, the hemolymph was sampled with a medical syringe and transferred to a vial. Immediately after this procedure, the shell was removed from the body regions with tweezers and macerated in a mortar and pestle. It was transferred to a vial and preserved for further analysis. The next procedure involved the excision of the hepatopancreas and kidneys, which were then transferred to laboratory vials using surgical scissors for precise organ dissection. (Roljic & Nikolic, 2022).

Material was distributed into eight vials and stored in the refrigerator for two days.

The analysis of heavy metal content was performed at the Public Health Institute of Kosovska Mitrovica. The material of the snails were prepared using the following method: 10 g of homogenized sample was weighed on an analytical balance, after which the sample was dried at 105°C until completely

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dry. Following drying, the sample was placed in a muffle furnace. The furnace temperature was gradually increased by 50°C until reaching 450°C, and the sample was left to ash overnight. The sample was then removed from the furnace and treated with concentrated 0.5 ml of HNO<sub>3</sub>. It was evaporated to dryness on a hot plate and returned to the furnace, repeating this process until complete mineralization. Once cooled, 1 ml of concentrated HCl was added along the walls of the beaker, and the sample was transferred to a standard container via filter paper, then diluted with redistilled water to the mark.

The soil sample for analysis was prepared using aqua regia. Aqua regia was prepared the day before, consisting of 3 parts HCl and 1 part HNO<sub>3</sub>. A 5-10 g sample was weighed, and 20 ml of aqua regia was added, leaving the sample at room temperature for 24 hours. Afterward, the sample was evaporated in a water bath at 95°C for two hours. After cooling, the sample was filtered into a standard 100 ml container and diluted with demineralized water to the mark.

The concentration of heavy metals was determined using atomic absorption spectrophotometry (AAS), with the application of flame atomization and graphite furnace techniques, depending on the specific elements being analyzed. The procedure was carried out in accordance with the guidelines provided in the Atomic Absorption Data Book (Philips Analytical).

Device model: Atomic Absorption Spectrophotometer PinAAcle 900T..

## NUMERICAL RESULTS

The results of the heavy metal content analysis in the area of Kosovska Mitrovica show a high level of heavy metals both in the soil (Figure 5) and in terrestrial snail.

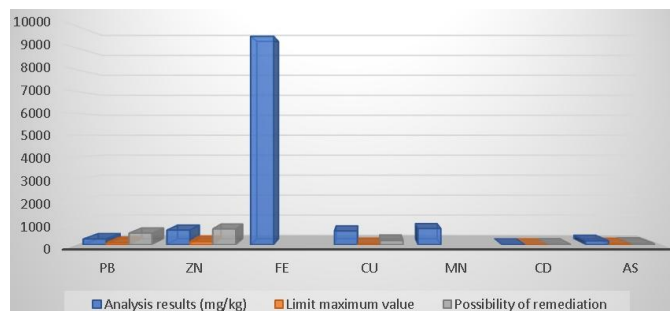
Heavy metals are considered one of the major soil pollutants and contributors to soil contamination. These metals have toxic effects on soil microorganisms by altering population size, biodiversity, and the various activities of microbial communities in the soil (Ashraf and Ali, 2007). The results of the analysis show the following quantities of heavy metals: Lead (Pb) is present at a concentration of 260 mg/kg, significantly exceeding the threshold value of 85 mg/kg. The remediation capacity for Pb is 530 mg/kg of absolute dry matter.

Zinc (Zn) is present at a concentration of 686.5 mg/kg, which is far above the threshold value of 140 mg/kg. The remediation capacity for Zn is 720 mg/kg.

Iron (Fe) reaches the highest concentration at 9520 mg/kg.

Copper (Cu) is present at a concentration of 628 mg/kg, with a threshold value of 36 mg/kg, while the remediation value is 190 mg/kg.

Manganese (Mn) is present at a concentration of 737 mg/kg.



**Figure 5.** Heavy metal in the soil.

Cadmium (Cd) is present in the soil at a concentration of 1.59 mg/kg. The threshold value for Cd is 0.8 mg/kg, while the remediation capacity is 12 mg/kg.

Arsenic (As) is present at a concentration of 177 mg/kg, with a threshold value of 29 mg/kg, and the remediation capacity is 55 mg/kg (Figure 5).

Based on the conducted analyses, the results show that the concentrations of several heavy metals in the investigated soil significantly exceed the threshold values. The highest concentrations were recorded for iron (Fe), zinc (Zn), copper (Cu), and lead (Pb), while the concentrations of cadmium (Cd) and arsenic (As) were also above the threshold values. The concentration of lead (Pb) was 260 mg/kg, more than three times the permissible threshold value of 85 mg/kg. Zinc (Zn) was present at a concentration of 686.5 mg/kg, also high above the permissible limit of 140 mg/kg. Copper (Cu), at a concentration of 628 mg/kg, exceeded the threshold value of 36 mg/kg, and manganese (Mn), with 737 mg/kg, indicates high levels of contamination. Although the concentrations of cadmium (Cd) and arsenic (As) were above the threshold values, they were not as pronounced as the other metals.

The remediation capacities indicate that reduction of metal concentrations is possible in all cases, but values are still higher than the maximum allowable concentrations. Remediation appears particularly necessary for elements such as lead, zinc, and copper, where concentrations are several times higher than the threshold values, while cadmium and arsenic require less intensive measures.

The maximum threshold values are defined by the Regulation on Threshold Values of Pollutants, Harmful, and Hazardous Substances in Soil (Official Gazette of RS, No. 30/2018 and 64/2019).

### *Analysis of heavy metal content in middle-aged snails*

Hemolymph is the primary circulatory fluid in mollusks, similar to blood in vertebrates, and plays a crucial role in the transport of nutrients, gases, and metals throughout the organism. Hemolymph can reflect the current exposure of snails to environmental contaminants, as "metals ingested into the organism rapidly reach the hemolymph before being distributed to target organs or excreted" (Vlahogianni et al., 2007).

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The results of heavy metal content in medium-aged snails are presented in Tables 1-4, which detail the distribution of various metals in the snail's organism.

These tables contain data on the concentration of heavy metals in four different examined parameters: hemolymph, shell, kidneys, and hepatopancreas.

**Table 1.** Heavy metal content in the hemolymph of medium-aged snails.

Testing Parameter	Value
Arsenic (As)	0.00482 mg/kg
Copper (Cu)	10.578 mg/kg
Iron (Fe)	0.7153 mg/kg
Cadmium (Cd)	0.0012 mg/kg
Manganese (Mn)	0.1242 mg/kg
Lead (Pb)	0.0005 mg/kg
Zinc (Zn)	0.5121 mg/kg

**Table 2.** The content of heavy metals in the shells of medium-aged snails.

Testing Parameter	Value
Arsenic (As)	1.5641 mg/kg
Copper (Cu)	0.1695 mg/kg
Iron (Fe)	1.1248 mg/kg
Cadmium (Cd)	0.00079 mg/kg
Manganese (Mn)	0.0916 mg/kg
Lead (Pb)	0.0751 mg/kg
Zinc (Zn)	0.09184 mg/kg

**Table 3.** Heavy metal content in the kidney of medium-aged Snails.

Testing Parameter	Value
Arsenic (As)	0.0005 (mg/kg)
Copper (Cu)	0.3481 (mg/kg)
Iron (Fe)	0.9151 (mg/kg)
Cadmium (Cd)	0.0124 (mg/kg)
Manganese (Mn)	0.2764 (mg/kg)
Lead (Pb)	0.141 (mg/kg)
Zinc (Zn)	0.3878 (mg/kg)

**Table 4.** Heavy metal content in the hepatopancreas of medium-aged snail.

Testing Parameter	Value
Arsenic (As)	0.0594 (mg/kg)
Copper (Cu)	7.5241 (mg/kg)
Iron (Fe)	8.9057 (mg/kg)
Cadmium (Cd)	0.02695 (mg/kg)
Manganese (Mn)	0.8768 (mg/kg)
Lead (Pb)	1.284 (mg/kg)
Zinc (Zn)	0.79289 (mg/kg)

The snail shell primarily consists of calcium carbonate and can act as a reservoir for certain metals, particularly those that have an affinity for carbonate structures. According to studies, "snail shells can accumulate metals from the environment, serving as a long-term indicator of pollution" (Rainbow, 2002).

The kidneys play a major role in detoxifying the organism and excreting metabolic waste, including heavy metals. Research has shown that "mollusk kidneys play a key role in metal homeostasis, given their ability to accumulate and excrete toxic elements" (Dallinger et al., 1997).

The hepatopancreas is the most metabolically active organ in snails, responsible for digestion, nutrient storage, and metal detoxification. It is considered that the "hepatopancreas is the main organ for metal accumulation in snails, often showing high concentrations of heavy metals due to active removal and storage" (Regoli & Principato, 1995).

Copper (Cu) is present at the highest concentration in the hemolymph (10.578 mg/kg), reflecting its function as a respiratory pigment in the snail's circulatory system. Elevated copper levels were also detected in the hepatopancreas (7.5241 mg/kg), indicating significant accumulation in this metabolically important organ. In contrast, copper concentrations in the shell (0.1695 mg/kg) and kidneys (0.3481 mg/kg) were much lower, suggesting these tissues are not major copper reservoirs.

Iron (Fe) reaches an exceptionally high concentration in the hepatopancreas (8.9057 mg/kg), underscoring its key role in enzymatic reactions and metabolism. High iron levels were also found in the shell (1.1248 mg/kg), while the hemolymph (0.7153 mg/kg) and kidneys (0.9151 mg/kg) contained moderate amounts.

Arsenic (As) occurs at very low concentrations in the hemolymph (0.00482 mg/kg) and kidneys (0.0005 mg/kg), with a marked increase observed in the shell (1.5641 mg/kg). The hepatopancreas contained measurable arsenic (0.0594 mg/kg), indicating possible detoxification or partial storage of this metal.

Cadmium (Cd) is generally low across all tissues, with the highest levels recorded in the hepatopancreas (0.02695 mg/kg) and kidneys (0.0124 mg/kg). These data suggest some cadmium accumulation capacity in these organs, while the shell showed extremely low values (0.00079 mg/kg).

Manganese (Mn) is most abundant in the hepatopancreas (0.8768 mg/kg), consistent with its metabolic function. Lower concentrations were recorded in the hemolymph (0.1242 mg/kg) and shell (0.0916 mg/kg), whereas the kidneys (0.2764 mg/kg) contained moderate levels.

Lead (Pb) shows notably high concentrations in the hepatopancreas (1.284 mg/kg), suggesting significant toxicity potential and bioaccumulation. Elevated levels were also found in the shell (0.0751 mg/kg) and kidneys (0.141 mg/kg), while hemolymph levels were extremely low (0.0005 mg/kg).

Zinc (Zn) is most concentrated in the hepatopancreas (0.79289 mg/kg), reflecting its important role in metabolic processes. Moderate zinc levels were measured in the hemolymph (0.5121 mg/kg), whereas the shell (0.09184

mg/kg) and kidneys (0.3878 mg/kg) showed lower concentrations.

#### *Analysis of heavy metal content in older snails*

Observed variations in metal concentrations across different organs particularly the hemolymph, shell, kidneys, and hepatopancreas may reflect both systemic distribution and organ-specific mechanisms of metal storage and excretion. These findings emphasize aging as a key factor affecting the dynamics of heavy metal bioaccumulation. Detailed data on metal content in older snails are presented in Tables 5–8.

**Table 5.** Heavy metal content in hemolymph of older snails.

Testing Parameter	Value
Arsenic (As)	0.00094 mg/kg
Copper (Cu)	8.088 mg/kg
Iron (Fe)	1.0198 mg/kg
Cadmium (Cd)	0.00125 mg/kg
Manganese (Mn)	0.2348 mg/kg
Lead (Pb)	0.0005 mg/kg
Zinc (Zn)	0.3799 mg/kg

**Table 6.** Heavy metal content in shell of older snails.

Testing Parameter	Value
Arsenic (As)	1.8964 mg/kg
Copper (Cu)	0.276 mg/kg
Iron (Fe)	0.086 mg/kg
Cadmium (Cd)	0.00016 mg/kg
Manganese (Mn)	0.0005 mg/kg
Lead (Pb)	0.01772 mg/kg
Zinc (Zn)	0.02848 mg/kg

**Table 7.** Heavy metal content in kidney of older snails.

Testing Parameter	Value
Arsenic (As)	0.0005 mg/kg
Copper (Cu)	3.3581 mg/kg
Iron (Fe)	4.8477 mg/kg
Cadmium (Cd)	0.0231 mg/kg
Manganese (Mn)	0.4099 mg/kg
Lead (Pb)	0.0091 mg/kg
Zinc (Zn)	0.5706 mg/kg

**Table 8.** Heavy metal content in hepatopancreas of older snails.

Testing Parameter	Value
Arsenic (As)	0.005 mg/kg
Copper (Cu)	9.5475 mg/kg
Iron (Fe)	9.9886 mg/kg
Cadmium (Cd)	0.0385 mg/kg
Manganese (Mn)	1.0756 mg/kg
Lead (Pb)	1.4324 mg/kg
Zinc (Zn)	0.9199 mg/kg

Copper (Cu) reaches the highest concentration in the hepatopancreas of older snails (9.5475 mg/kg), highlighting its role in the accumulation of this metal during metabolism. A

moderate level of copper was recorded in the kidneys (3.3581 mg/kg), while the hemolymph contained 8.088 mg/kg and the shell showed a very low concentration (0.276 mg/kg), which contrasts with medium-aged snails, where copper levels in the shell were much lower than in other organs.

Iron (Fe) reaches an exceptionally high concentration in the hepatopancreas of older snails (9.9886 mg/kg), underscoring its key role in enzymatic reactions and metabolism. High iron levels were also found in the kidneys (4.8477 mg/kg), while the hemolymph (1.0198 mg/kg) and shell (0.086 mg/kg) contained significantly lower amounts.

Arsenic (As) occurs at very low concentrations in the hemolymph (0.00094 mg/kg) and kidneys (0.0005 mg/kg), with the highest accumulation observed in the shell (1.8964 mg/kg). The hepatopancreas contained a moderate level of arsenic (0.005 mg/kg), indicating possible detoxification or storage of this metal.

Cadmium (Cd) is present at relatively high concentrations in the hepatopancreas (0.0385 mg/kg), with lower concentrations in the kidneys (0.0231 mg/kg) and hemolymph (0.00125 mg/kg). The shell shows extremely low cadmium levels (0.00016 mg/kg), similar to the findings in medium-aged snails.

Manganese (Mn) reaches the highest concentration in the hepatopancreas (1.0756 mg/kg), reflecting its importance in enzymatic activities and metabolism. Manganese levels in the kidneys (0.4099 mg/kg) are higher than in the hemolymph (0.2348 mg/kg) and shell (0.0005 mg/kg), consistent with the metal's role in these organs.

Lead (Pb) accumulates at very high concentrations in the hepatopancreas (1.4324 mg/kg), which is higher than in medium-aged snails (1.284 mg/kg). The kidneys show lower levels (0.0091 mg/kg), while the hemolymph (0.0005 mg/kg) and shell (0.01772 mg/kg) contain much lower concentrations.

Zinc (Zn) reaches the highest concentration in the hepatopancreas (0.9199 mg/kg), indicating its role in metabolic processes. Moderate zinc levels were recorded in the kidneys (0.5706 mg/kg), while the hemolymph (0.3799 mg/kg) and shell (0.02848 mg/kg) contained lower amounts.

Comparing the results for medium-aged and older snails, significant differences in metal concentrations in various organs are observed. The hepatopancreas of older snails shows considerably higher concentrations of copper, iron, manganese, lead, and zinc, which may indicate an increased accumulation of these metals in older snails, likely due to prolonged exposure to pollution or metabolic changes occurring with age. The shell of older snails accumulates high concentrations of arsenic, suggesting long-term exposure to pollutants. Additionally, the higher concentrations of lead in the hepatopancreas and kidneys indicate potential toxic effects of this metal, especially in older snails, which may be more susceptible to toxic influences.

## CONCLUSION

The study of heavy metal accumulation in terrestrial snails of the species *Helix pomatia*, as well as in soil samples from the Gornje Polje site, enabled the assessment of the ecological condition of the area, particularly in the context of industrial contamination.

Samples of medium-aged and older snails, along with soil samples from specific geographical coordinates near the Ibar River and former industrial plants, were analyzed.

The results indicate the presence of certain concentrations of heavy metals such as copper (Cu), zinc (Zn), cadmium (Cd), and lead (Pb) in the tissues of snails, particularly in: hemolymph, hepatopancreas, kidneys, and shell. These concentrations suggest significant bioaccumulation, confirming the use of terrestrial snails as indicators for ecological monitoring of heavy metal contamination. Considering the limited ability of this species to excrete metals, terrestrial snails prove to be an efficient model for assessing long-term exposure to toxic substances in urban and industrially contaminated areas.

Furthermore, the soil analysis revealed significant concentrations of the same metals, suggesting long-term contamination caused by industrial activities such as mining and metallurgy, particularly between 1930 and 1965, when the industrial landfill was active. The contamination may pose a serious risk to the ecosystem and human populations in the vicinity, as heavy metals can enter the food chain, increasing the risk of chronic poisoning.

Taking all these factors into account, the study highlights the need for continued monitoring and the implementation of strategies to reduce pollution in order to protect both ecosystems and human populations in this area. Given the high concentrations of heavy metals in both biological and abiotic samples, additional environmental protection measures are required, along with more detailed studies on the impact on the health of local communities.

## REFERENCES

- Ashraf, R. & Ali, T. A. 2007. Effect of heavy metals on soil microbial community and mung beans seed germination. *Pakistan Journal of Botany*, 39(2), pp. 629-636.
- Azimi, S., Cambier, P., Lecuyer, I. & Thevenot, D. 2004. Heavy metal determination in atmospheric deposition and other fluxes in northern France Agrosystems. *Water Air Soil Pollut.* 157, pp. 295-313. <https://doi.org/10.1023/B:WATE.0000038903.25700.5a>
- Beeby, A. & Richmond, L. 2003. Do the soft tissues of *Helix aspersa* serve as a quantitative sentinel of predicted free lead concentrations in soils? *Applied Soil Ecology*, 22(2), pp. 159-165.
- Dallinger, R., Prosi, F., Segner, H. & Back, H. 1997. Contaminated food and uptake of heavy metals by fish: A review and proposal for further research. *Oecologia*, 73(1), pp. 91-98.
- Dallinger, R., Berger, B., Hunzinger, P. & Kgi, J. H. 1997. Metallothionein in snail Cd and Cu metabolism. *Nature*, 388(6639), 237.
- Dallinger, R., Chabicozsky, M., Hodl, E., Prem, C., Hunziker, P. & Manzl, C. 2005. Copper in *Helix pomatia* (Gastropoda) is regulated by one single cell type: differently responsive metal pools in rhogocytes. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, 289(4), pp. 1185-1195.
- Dixit, R., Malaviya, D., Pandiyan, K., Singh, U. B., Sahu, A., Shukla, R., Singh, B. P., Rai, J. P., Sharma, P. K., Lade, H. et al. 2015. Bioremediation of Heavy Metals from Soil and Aquatic Environment: An Overview of Principles and Criteria of Fundamental Processes. *Sustainability*, 7, pp. 2189-2212.
- Gomot de Valfleury, A. & Pihan, F. 2000. Growing snails used as sentinels to evaluate terrestrial environment contamination by trace element. *Chemosphere*, 40, pp. 275-284.
- Gomot de Vaufleury, A. 2000. Standardized growth toxicity testing (Cu, Zn, Pb, and pentachlorophenol) with *Helix aspersa*. *Ecotoxicology and Environmental Safety*, 46(1), pp. 41-50.
- Hopkin, S. P. 1989. *Ecophysiology of Metals in Terrestrial Invertebrates*. Elsevier Applied Science.
- Jordaens, K., De Wolf, H., Vandecasteele, B., Blust, R. & Backeljau, T. 2006. Associations between shell strength, shell morphology and heavy metals in the land snail *Cepaea nemoralis* (Gastropoda, Helicidae). *Science of the Total Environment*, 363(1-3), pp. 285-293.
- Markich, S. J., Brown, P. L., Batley, G. E., Apte, S. C. & Stauber, J. L. 2001. Incorporating metal speciation and bioavailability into water quality guidelines for protecting aquatic ecosystems. *Australasian Journal of Ecotoxicology*, 7, pp. 109-122.
- Monaco Nature Encyclopedia. (n.d.) *Helix pomatia*. Available at: <https://www.monacatureencyclopedia.com/helix-pomatia/?lang=en>. Accessed March 15, 2025.
- Nica, D. V., Bura, M., Gergen, I., Harmanescu, M. & Bordean, D. 2012. Bioaccumulative and conchological assessment of heavy metal transfer in a soil-plant-snail food chain. *Chemistry Central Journal*, 15, pp. 1-15.
- Notten, M. J. et al., 2005. Accumulation of metals in the terrestrial snail *Cepaea nemoralis*. *Archives of Environmental Contamination and Toxicology*, 48(3), pp. 342-354.
- Official Gazette of the Republic of Serbia. 2018-2019. pp. Official Gazette of RS, Nos. 30/2018 and 64/2019. Available at: <https://www.pravno-informacioni-sistem.rs>. Accessed March 15, 2025.
- Rainbow, P. S. 2002. Trace metal concentrations in aquatic invertebrates: Why and so what? *Environmental Pollution*, 120(3), pp. 497-507.
- Regoli, F. & Principato, G. B. 1995. Glutathione, glutathione-dependent and antioxidant enzymes in mussel, *Mytilus galloprovincialis*, exposed to metals under field and laboratory conditions: Implications for the use of

- biochemical biomarkers. *Aquatic Toxicology*, 31(2), pp. 143-164.
- Regoli, F. & Giuliani, M. E. 2014. Oxidative pathways of chemical toxicity and oxidative stress biomarkers in marine organisms. *Marine Environmental Research*, 93(1), pp. 106-117.
- Roljić, R. & Nikolić, V. 2022. *Zoologija beskičmenjaka: praktikum sa radnom sveskom*. Banja Luka: Univerzitet u Banjoj Luci, Prirodno-matematički fakultet.
- Srećković-Batočanin, D., Bačević, N., Valjarević, A. & Memović, E. 2020. Paleovolcanic Cone Zvečan – Potential Monument of Nature or Geoheritage Site. “Smederevo Ecological City” – Fourth Ecological Conference with International Participation. Smederevo, October 2020. Proceedings, pp. 89-95. ISBN 978-86-919317-3-5. Local Ecological Movement.
- Šukrija, A. & Hadri, A. 1979. *Kosovska Mitrovica i okolina*. Priština: Institut za istoriju Kosova u Prištini.
- Vlahogianni, T., Dassenakis, M., Scoullos, M. J. & Valavanidis, A. 2007. Integrated use of biomarkers (superoxide dismutase, catalase, lipid peroxidation, and acetylcholinesterase) in *Mytilus galloprovincialis* for assessing heavy metal pollution in coastal areas from the Saronikos Gulf of Greece. *Marine Pollution Bulletin*, 54(9), pp. 1361-1371.
- Zalewski, M. et al., 2010. The impact of heavy metals on snail populations in polluted environments. *Ecotoxicology and Environmental Safety*, 73(4), pp. 287-294.