Oecologia Montana 2024, **33,** 35-44

Heavy metals in droppings of Eurasian otters (*Lutra lutra***) from mountain stream Javorinka, the Tatra Mountains**

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Abstract. The research is focuses on the analysis of the content of heavy metals and other elements in the feces of river otter in the Javorinka mountain stream in the High Tatras in Slovakia. The aim of the study is to follow the cycles of concentrations of measured elements such as Hg, P, S, Cl, K, Ca, Ti, Cr, Mn, Fe, Cu, Zn, Se, Rb, Sr, Zr, Mo, Sb, Ba and Pb in otter feces, with a special focus on heavy metals, which were analysed at two sites, during four seasons and individually in each year. Comparison of the results from the two sites showed an almost twofold increase in the concentrations of the elements Ti, Mn, Fe and Sr at the site with the lower gradient and slower water flow. At the same time, seasonal variations are observed, with some elements such as P, S, Cl and K reaching their highest concentrations in the autumn months, while other elements such as Ti, Mn, Fe and Rb reach their peaks in the summer and autumn months. The concentration of the element Hg has also been found to vary with food availability, especially in the spring season. The average Hg concentrations reached 0.1 mg/kg in otter feces. Inter-annual variations in concentrations of heavy metals such as Hg, Pb, Ba and Cr may be related to a variety of factors including changes in otter diet, fish and otter breeding cycles and geological processes affecting water and sediment in the river. The results of this study contribute to a better understanding of the ecological stability of upland aquatic ecosystems and to the conservation of endangered species in these environments.

Key words: Lutra lutra, mountains stream, Javorinka, droppings, heavy metals

Introduction

The Eurasian otters (*Lutra lutra*) is an autochthonous species of the Slovak fauna, which ranks among the largest European species of the weasel family (Mustelidae) and carnivorous carnivores (Carnivora) (Urban and Kadlečík 2001). This mammal species is one of the apex predators of aquatic ecosystems, is semiaquatic and is an endangered species in Slovakia. Its presence serves as an indicator of the cleanliness and health of aquatic ecosystems.

This research follows and monitors the occurrence of the river otter in the Javorinka mountain stream in the Belianske Tatras. The river otter inhabits diverse habitats of standing and flowing waters and wetlands (mainly mountain, foothill and lowland streams, rivers, lakes, swamps and reservoirs) and adjacent bank structures. Throughout much of the range its occurrence is correlated with riparian vegetation, highlighting the importance of vegetation to otters (Mason and Macdonald 1987). In the past, it occurred throughout most of its range, with the exception of the upper parts of the high mountains (Urban *et al*. 2011). They also forage for food in a variety of habitats. They are able to make overland journeys often far from watercourses and sometimes move between water bodies (Roos *et al.* 2015). The presence of river otters is detected based on their resident signs. These include scats, tracks or hiding places (terrestrial or subterranean). However, for breeding purposes they need access to holes in river banks, cavities between tree roots, accumulated rocks, wood or debris. River otters show a strong attachment to linear habitat structure. Most of their activities are concentrated in a narrow band along the water-land interface (Kruuk 1995).

Mountain streams inhabited by otters are extremely sensitive to disturbances of the ecological balance caused by human activities as well as natural factors due to their specific characteristics. Consequently, they serve as sensitive indicators of climate change (Stone 1992; McGregor *et al.* 1995). To investigate the cycling of elements in otter droppings, the impact of pollution in high mountain areas and the effect of pollution on the fauna of the river ecosystem, the Javorinka stream was selected as a model stream that is little affected by humans.

Elemental elements, called "heavy metals", were named because of their metallic character and high density (more than 5g/cm³) (Pourret and Bollinger 2017). Heavy metals are considered important environmental toxicants due to their high toxicity, persistence, accumulation and non-biodegradability (Pujari and Kapoor 2021). They can alter enzyme and genetic systems and can also damage the nervous system (Kumar *et al.* 2020). Pollution comes from natural sources such as weathering of rocks containing metal ores, volcanic eruptions, as well as human activities including urbanization, industrialization and metal mining processes (Kar *et al.* 2008). While some metals such

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as copper (Cu), iron (Fe), manganese (Mn), nickel (Ni) and zinc (Zn) are vital micronutrients essential for the life processes of plants and microorganisms, others such as cadmium (Cd), chromium (Cr) and lead (Pb) have no known physiological functions and have been shown to be harmful above certain threshold levels (Marschner 1995; Bruins *et al.* 2000). These thresholds are particularly narrow for elements such as Cd (0.01 mg/L), Pb (0.10 mg/L) and Cu (0.050 mg/L) (ISI 1982). Exposure to heavy metals has been attributed to diseases with serious consequences, such as eyelid edema, tumors, nasal and pharyngeal mucosal blockages, headaches, gastrointestinal problems, muscular disorders, reproductive abnormalities, neurological disorders and genetic disorders (Abbasi *et al.* 1998; Johnson 1998). Therefore, monitoring for the presence of these metals is essential for the assessment of environmental safety and protection of human health (Kar *et al.* 2008).

The aim of the research was to highlight the importance of heavy metal accumulation relationships between predator and prey in general, but also to maintain environmental quality. The information provided can serve as a basis for conservation and nature management.

Material and Methods

Research area and samplings

Javorinka is a Tatra stream with a characteristic steep gradient in a spruce forest, a gently flowing stream with side channels and bays in the lower part of the stream. The study area lies in the Eastern Tatras, which include the High Tatras and the Belianske Tatras. The Eastern Tatras are part of the entire Tatra Mountains, which belong to the Western Carpathians. The Belianske Tatry form the peripheral part on the eastern side and are composed of limestones and dolomites with a distinctive karst topography, which contrasts with the granite and schist relief of the central High Tatras (Adamec and Roubal 1972). The study focuses on two sections of the river: the upper reaches and the lower reaches. The upper stream (Fig. 1.) approximately 3 km long, flows between the villages of Tatranská Javorina and Podspády. It is characterised by fast-flowing water and diverse habitats and serves as a spring area. The lower stream (Fig. 2.), approximately 1 km long, lies downstream of Podspády, with varied terrain including gravel banks and boulder-pebble-clay sediments. Monthly fecal sampling of river otter droppings was carried out upstream and downstream with regard to potential pollution from the Tatranská Javorina municipality. Sampling sites were selected based on regular monitoring of water, invertebrates and algae in the Javorinka stream.

Laboratory analysis

Air-dried samples were homogenized in the laboratory using a CryoMill for grinding and X-ray fluorescence spectrometry (spectrometer (Innov-X Delta XRF; Innov-X Systems, USA) for elemental detection. Mercury content was analyzed using a direct mecury analyzer DMA-80 (Milestone,Italy). NCS ZC

Fig. 1. View of the upper stream (Photo: A. Furendová, 2023).

Fig. 2. View of the lower stream (Photo:A. Furendová, 2023).

71001 Beef Liver certified reference material was used to check the accuracy of the measurements.

Statistical analysis

Statistica 12.0 (StatSoft, Prague, Czech Republic) was used for statistical analysis. Non-normal distribution of data was detected using Shapiro-Wilk test. The Mann-Whitney U test used when comparing two variables was used to compare the distribution of sites (upper and lower) and seasons within years (9/21 - 8/22 and 9/22 - 9/23). And for seasons (spring, summer, autumn, winter) the Kruskal-Wallis H test was used for more than two variables.

Results

The XRF spectrometer analyzed elements including Hg, P, S, Cl, K, Ca, Ti, Cr, Mn, Fe, Cu, Zn, Se, Rb, Sr, Zr, Mo, Sb, Ba and Pb. Other elements like Co, Ni, As and Ag were excluded due to concentrations below the detection limit (<LOD).

Different concentrations of heavy metals at the two sites

Differences in heavy metal concentrations were found between the upper and lower Javorinka site. In general, the lower site had higher concentrations of elements including P, S, Cl, K, Ca, Ti, Cr, Mn, Fe, Cu, Zn, Se, Rb, Sr, Mo, Sb, Ba and Pb, while Zr concentrations were higher at the upper site. Significant differences were confirmed in Ti (Fig. 3a), Mn (Fig. 3b), Fe (Fig. 3c) and Sr (Fig. 3d).

Different concentrations of heavy metals within seasons

Seasonal differences in heavy metal concentrations were monitored cumulatively for both sites. Significant differences were found for 10 elements. These differences were particularly significant for P, S, Cl, K, Ca, Ti, Cr, Mn, Fe and Rb (Table 1).

Seasonal trends observed in individual years

Within the seasonal changes, conditions vary from year to year. Significant differences in the heavy metal group were confirmed for Hg, P, S, Cl, K, Ca, Cr, Mn, Fe, Cu, Zn, Rb, Mo, Sb, Ba and Pb (Table 2).

Given the focus of this study, the elements Hg (Fig. 4a) and Pb (Fig. 4b) are most relevant for showing differences in accumulation.

In the case of year-to-year changes in the element Hg (Fig. 4a), the values, in the first period (9/21-8/22) compared to the values in the second period (9/22-9/23) are too different, even opposite.

Fig. 3. a) Titanium (Ti), b) manganese (Mn), c) iron (Fe) and d) strontium (Sr) accumulation depending on the point of collection (upper and lower). The square shows the mean value, the box shows the \pm SE and the span of the line shows the \pm SD.

Fig. 4. a) The accumulation of mercury (Hg) and b) lead (Pb) over one year during four seasons.

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Table 1. Seasonal difference in elemental concentrations (in mg/kg) by season. Significant differences are marked in bold. **38**

While Hg was higher in the fall and winter in period one, it was lower in the spring and summer. So in the second period it was the opposite. The interaction is significant. Variability of environmental conditions is important especially in winter. As far as the uptake of biogenic elements is concerned, the variability of environmental conditions is important in spring and summer. It varies mainly according to food.

In the case of interannual changes in Pb (Fig. 4b), concentrations varied at the end of the autumn period when values increased in the first period and reached a maximum in winter, with a gradual decrease in spring and summer. In the second period, the results were reversed, with values multiplying in summer. The interaction between years is significant.

Discussion

The main objective of the study was to investigate the process of element accumulation in the final link of the food chain - the river otter - using fecal samples. Metals include a wide range of elements that can have different effects on mammals. These elements include Hg, Cd, Pb and Cr, which are considered toxic, but there are also essential metals such as Zn, Fe and Cu. The concentrations of these metals and their behaviour can vary considerably in different environments. Intake of heavy metals even at very low concentrations can be harmful to living organisms (Egila and Daniel 2011).

Different concentrations of heavy metals at the two sites

Significant differences in metal concentrations were found between two sites, with the lower site showing slightly higher pollution, possibly due to contamination near Podspády and the influence of slope gradients on particle transport (Young *et al*. 1978; Brussock *et al.* 1985; Ward and Aumen 1986). Ti and Fe, likely from soil contamination rather than diet, were notable, aligning with geological data of

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Table 2. Elemental concentrations (in mg/kg) and their differences within seasons over separate years. Significant differences are marked in bold.

the Javorinka stream basin (Zacher 2022; Turekian and Wedepohl 1961). Ti concentrations ranged from 277.3 to 293.2 mg/kg and Fe from 2243.9 to 2643.0 mg/kg, with transport linked to Mg, Mn and Ti (Reimann and De Caritat 2012). Terrestrial habitat changes can affect metal leaching into rivers, impacting river otter populations (Calmano *et al.* 1993; Černohous 2020). Mn concentrations averaged 254.9 mg/kg in lower reaches and 166.8 mg/kg in upper reaches, likely reflecting otters' diet and environment. Sr was deficient in summer, with fish being a potential source (Schroeder *et al.*

1972; Jackman *et al.* 1984; Perinajová 2019). Sr values were 183.3 mg/kg at the lower site and 152.2 mg/kg at the upper site, related to diet and surrounding waters (Bodiš *et al*. 2011).

Different concentrations of heavy metals within seasons

We have classified the individual months into seasons of autumn, winter, spring and summer. Seasonal differences were reflected on 10 significant elements; P, S, Cl, K, Ca, Ti, Cr, Mn, Fe, Rb. The other elements Hg, Cu, Zn, Se, Sr, Zr, Mo, Sb, Ba, Pb did not show as significant.

The element P showed significant differences between seasons ($p < 0.05$), with the highest concentration in spring. Similarly, for elements S, Cl and K also show significant differences between seasons, with the highest concentrations observed in autumn months. Remarkable results were also found in our Javorinka stream, where Perinajová (2019) observed the same increase in elements S, Cl and K in the heads of Alpine bullhead (*Cottus poecilopus*). Consistency with our research is also confirmed by Palková (2018), who reported that the amount of K in the Javorinka stream was lower during winter and higher during warmer months. Potassium occurs naturally in compounds with chlorine and chloride is the major anion of gastric juice and blood (Lall 2022).

For the elements Ti, Mn, Fe and Rb we see a significant difference between seasons and the highest concentrations are observed in summer and autumn months. Rb is an important element that occurs especially in young individuals in higher abundance (Kapitola and Vilimovská 1984). Alpine bullhead fish are born from April to June, which corresponds to the breeding season (Kotusz *et al.* 2011). It can be argued that the intake of juvenile fish by otters causes an increased accumulation of the element Rb, especially in the summer and autumn months. However, this claim is refuted by the fact that in the case of Ca, calcium uptake decreases with ageing (Aloia *et al.* 2010), as the lowest measured values were just in the summer season. These claims may be influenced by various factors, such as increasing body size, which is accompanied by a decrease in calcium, rubidium, molybdenum and vice versa (Downhower 1990; Perinajová 2019). In adult fish, we encounter less chromium due to their ability to excrete it from the body (Dara 1995), independent of the size of the fish, which is also argued by Perinajová (2019).

Measured Cr levels were higher in the first year at 2336.0 mg/kg with the highest measured values in summer and autumn. This could again confirm the consumption of older fish in the winter months and reduced levels of the heavy metal Cr, possibly replacing the diet with amphipods. The accumulation of this metal in the droppings of river otters depends on the fish species consumed and their lifestyle. Chromium is required for normal carbohydrate and lipid metabolism (Lall 2022). The benthic lifestyle of fish can increase the concentration of elements such as Cr (Kahl *et al.* 2001) and it mainly affects the gills, which it can damage (Mattia *et al.* 2004).

The amount of Fe in water can also be influenced with water pH (Hem and Cropper 1959), but Fe can also enter river ecosystems through weathering processes (Vuori 1995). When rivers are deforested and rebuilt, there can be a significant increase in iron leaching into rivers, which can affect water quality (Calmano *et al.* 1993) and the abundance of Fe in otter droppings.

Seasonal trends observed in individual years

We divided the elements by year into the periods of 9/21 - 8/22 and 9/22 - 9/23, revealing significant differences in the heavy metal group, including Hg, P, S, Cl, K, Ca, Cr, Mn, Fe, Cu, Zn, Rb, Mo, Sb, Ba and Pb. Elements Ti, Se, Sr and Zr did not show significant seasonal changes, as indicated by p-values greater than 0.05 in the MW-U tests. The most relevant heavy metals were Hg, Pb and Cr.

In July 2018, a significant flood and bridge break affected the upper abstraction point of the Javorinka stream, impacting nutrient and sediment distribution and causing contamination (Bayley and Sparks 1989; Hrivnáková *et al.* 2020). Most elements, particularly mercury, showed higher concentrations between September 2021 and August 2022, with mercury bioaccumulation decreasing until spring 2023. This suggests that the 2018 flood had lasting effects on bioaccumulation, especially in the first year of the study (Janiga *et al*. 2024).

Phosphorus and potassium exhibited significant seasonal changes, with higher concentrations from September 2021 to August 2022 compared to the following year. Phosphorus is crucial for living organisms (Ji *et al.* 2011), while calcium levels, maintained by soil and rock weathering (Ohta *et al.* 2018), were higher in the first year, potentially due to flooding (Munn and Meyer 1990). Increased calcium levels in smaller fish correlate with higher molybdenum and rubidium (Perinajová 2019). Molybdenum and rubidium are found in higher amounts in juveniles (Ward 1973; Kapitola and Vilimovská 1984; Davies *et al.* 2005; Perinajová *et al.* 2020; Janiga and Janiga 2023). Rubidium, essential for many animals, can accumulate in fish due to its similarity to potassium (Patterson and Settle 1987; Campbell *et al.* 2005), with higher concentrations potentially toxic (Clayton and Clayton 1994; Espinoza-Quiñones *et al*. 2011; Plessl *et al.* 2017; Alti *et al.* 2022).

Sulfur levels were higher from September 2021 to August 2022, likely due to acid rain (Pond *et al.* 2008). Chloride's bioaccumulation in river otter food chains requires further study. Manganese, abundant after iron, varies seasonally due to factors like absorption from food and interactions with calcium and phosphorus (Remy and Pineau 1977; Salem *et al.* 2014; Lall 2022). Strontium can replace calcium in bones, explaining its presence in certain river parts (Crossgrove and Yokel 2005). Essential heavy metals like copper, zinc and iron play crucial roles in metabolism, while others like mercury and lead are toxic (Canli and Atli 2003). Zinc, vital for physiological functions, showed higher levels from September 2021 to August 2022 (Espinoza-Quiñones *et al.* 2011; Rosseland *et al*. 2017). Barium and iron levels were also higher in the first year, influenced by cyanobacteria and weathering of rocks like granite (Erel *et al.* 1991; Romanescu *et al.* 2016).

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Higher concentrations of lead and copper were noted from September 2021 to August 2022, with copper peaking in autumn. Lead variability may be influenced by reproduction and food availability, with higher birth rates in certain periods leading to increased lead transfer from mothers to young (Gorman *et al.* 1978; Scheuhammer 1991; Mead *et al*. 1989; Hauer *et al.* 2002; Basu *et al.* 2005; Croteau *et al.* 2005; Yates *et al.* 2005). Mercury poses significant threats, requiring close monitoring due to its harmful effects on river otters and entire ecosystems (Bodaly *et al.* 1993; Farris *et al.* 1993; Hyvarinen *et al.* 2003; Scheuhammer *et al.* 2007; Lemarchand *et al.* 2010; Peng *et al.* 2012; Janiga and Haas 2019; Pitoňáková 2022; Janiga *et al.* 2024). Comparatively, mercury levels in Javorinka's otters were similar to those found in other studies (Wren 1986; Josef *et al.* 2008; Klenavic *et al.* 2008; Sleeman *et al.* 2010).

Continued monitoring of toxic elements like mercury and lead is essential to assess their impact on river otter populations (Mason and O'Sullivan 1993; Gutleb *et al*. 1998; Ruiz-Olmo *et al.* 2001).

Acknowledgements

We thank all those who contributed in any way to this study.

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Received 23 April 2024; accepted 5 June 2024.