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Differences in element accumulation in two groups of macrozoobenthos in the Javorinka River: Comparative analysis of two sites

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Abstract. This study focused on concentration of elements (P, S, Cl, K, Ca, Cr, Mn, Fe, Cu, Zn, Rb, Se, Sr, Mo, Ba, Pb) in the bodies of two species and functional feeding groups (Scrapes - Ephemeroptera and predators - Plecoptera) of water insects collected in the Javorinka stream (Slovakia). The primary goal was to determinate differences between the two sides of the stream downstream of Javorinka. L1 is situated near Tatranská Javorina and L2 at the Polish border in Podspády. We did not find significant differences between sites in the accumulation of most elements. Ephemeroptera accumulate significantly more Fe and Cu at L2, while Plecoptera accumulate significantly more Fe and Mn at L2 and more Pb on the L1 side. Synergy of some elements describe significant accumulation of Cl with Pb against Fe, Zn with Cr that show more accumulation of Cl and Pb on L2 side and Fe with Cr on L1 side. We can assume that lead, due to its weight, is more prevalent in L1 and does not washout downstream. Conversely, copper and iron, as lighter elements compared to lead, can be found downstream.

Key words: Plecoptera, Ephemeroptera, trace elements, mountain stream, site differences

Introduction

Biomonitoring is widely recognized as a crucial instrument for environmental scientists. It serves as an effective means to evaluate the condition of freshwater ecosystems, complementing other methods that assess the physical and chemical aspects of these habitats (Byl and Smith 1994). Additionally, it offers insights into the health of ecosystems by examining the species present in a water body or analyzing the pollutants within these organisms. To preserve and enhance water quality in bodies of water such as lakes, rivers, and streams, conservationists employ native species as markers of environmental shifts. The concept of using bioindicators for monitoring hinges on the fundamental idea that the presence and health of living organisms are key to gauging the state of the environment (Mandaville 2002).

Alterations in elemental concentrations within monitored organisms may serve as indicators of ecosystem contamination. In freshwater ecosystems, orders such as Ephemeroptera, Plecoptera, Trichoptera, and Diptera represent the most prolific aquatic invertebrates inhabiting riverbeds. These taxa are extremely sensitive to water quality fluctuations and thrive exclusively in minimally polluted, cool, and well-oxygenated streams (Chowdhury *et al.* 2023). Their presence signifies a high-quality aquatic resource, with substantial populations denoting a biologically robust community (USEPA 2005). Consequently, any variation in environmental element levels is mirrored by corresponding shifts in these insects' tissue concentrations. Prolonged exposure to elevated concentrations of certain pollutants can precipitate a decline in these species' populations or densities. Trace elements and prevalent contaminants in aquatic systems, when detected in organisms, provide insights into their environmental prevalence and serve as indirect markers of freshwater ecological health (Pastorino *et al.* 2019). These elements infiltrate aquatic organisms either directly from the water column or via dietary intake (Zhang and Wang 2006; Rainbow 2007). Water pollution remains a focal point of societal research and discourse, with climate change, industrialization, agriculture, and population growth contributing to the escalation of aquatic pollutants. Such contamination arises from both natural geogenic and anthropogenic sources (Giouri *et al.* 2010).

Differences in elemental concentrations in monitored species can vary between river reaches due to a variety of environmental factors such as water quality, sediment composition and human activities. Significant spatial differences in the distribution of heavy metals in river surface sediments also influence metal concentrations in macrozoobenthos (Wang *et al.* 2022). In pristine mountain rivers characterised by reference conditions, macrophytes have been observed to respond most strongly to factors such as altitude, pH, conductivity, alkalinity and total hardness, whereas macroinvertebrates are primarily dependent on dissolved oxygen, flow dynamics and channel material granulometry (Varadinova *et al.* 2023).

Macroinvertebrates are categorized into distinct Functional Feeding Groups (FFGs) based on their dietary preferences (Cummins and Klug 1979;

Merritt *et al.* 2008). This classification system is predicated on the behavioral strategies employed by these organisms to procure food, as described by Ramirez and Gutiérrez-Fonseca (2014). The FFGs paradigm serves to assign macroinvertebrates to roles pertinent to the decomposition and cycling of organic matter within aquatic ecosystems. It has been observed that certain macroinvertebrates, specifically shredders and scrapers, exhibit heightened sensitivity to environmental perturbations. Conversely, gatherers and filterers demonstrate a greater resilience to pollution, which may impact the availability of specific food resources (Barbour *et al.* 1996). Clements *et al.* (2000) noted a shift in the composition of FFGs among benthic macroinvertebrates, with a marked decrease in the populations of scrapers, shredders, collectors, and predators in locales with moderate to elevated levels of heavy metal contamination, in contrast to reference sites. The spatial distribution of FFGs within lotic systems is posited to mirror the functional attributes of aquatic ecosystems at the process level. Specialized feeders, such as shredders and scrapers, are hypothesized to be more susceptible to disturbances, whereas generalist feeders, like gatherers and filterers, are presumed to possess a higher tolerance to pollutants that may modify the accessibility of certain types of food (Barbour *et al.* 1996).

The aim of this study is to assess the spatio-temporal accumulation of elements in the bodies of aquatic insect larvae from FFG scrapers (order: Ephemeroptera, genus: *Ecdyonurus*, with *Ecdyonurus venosus* as the dominant species) and predators (order: Plecoptera, species: *Perla grandis*). Based on our knowledge to date, we expect that accumulation levels will vary between groups. We assume that element concentrations will vary from year to year, which may be related to changes in the environment and changes in the water regime.

Material and Methods

Study area

The Javorinka mountain stream (High Tatras, Slovakia) was selected for the study of element accumulation in the macrozoobetos of the river ecosystem. The Javorina valley, in which the stream originates and flows, is in the northern part of the Eastern Tatras. Javorinka has its source in the Žabie Javorové pleso, and it is a right-side tributary of the Białka.

It is a typical mountain stream, characterised by fast-flowing water and a narrow channel in the upper reaches at higher altitudes. The stream bed is made up of large boulders and sediment is deposited along the stream margins. The lower reaches, which flow through the mouth of the valley, passing through the village of Javorina, have a wider channel and the water flow slows down here. With decreasing altitude, a wide shallow channel is formed, dominated by smaller rocks and stones. More sediment is also deposited here. The rocks found in these parts of the stream are granite, limestone and shale as this stream flows between shale, flysch and granite belts.

Sampling

To Insect larvae were collected periodically at the end of the month from 2019 to 2023. Invertebrates, with a focus on Ephemeroptera (food guild: scrapers) and Plecoptera (food guild: raptors) species, were collected at two sites (Fig. 1) in the lower course of the river behind the village of Tatranská Javorina. The first site (L1) was located close to the village (N 49° 16’15.9; E 020° 09’11.4; 962 m a.s.l.) and the second site (L2) is located behind the village of Podspády (N 49° 17’52.4; E 020° 09’40.9; 855 m a.s.l.) close to the state border with Poland. The sites have different rheological characteristics and are about 4 km apart.

All samples were hand-picked with tweezers or collected by hand from the water, under rocks or from the aquatic substrate into plastic bottles filled with fresh water from the stream. 15-20 Ephemeroptera and 3-6 Plecoptera specimens were collected for each site, representing approximately 0.5 g dry weight.

Laboratory analysis

In the laboratory, the specimens were divided into two groups according to genera (Ephemeroptera, Plecoptera) and left to dry in petri dishes. Air drying was conducted over several days at room temperature.

The dried samples were ground in a ceramic mortar and analysed by X-ray fluorescence using a DELTA portable XRF spectrometer (Innov-X Technologies, USA). Analysis by XRF spectrometer is based on the principle of element identification by the characteristic wavelength of its radiation. The concentration of the measured elements in the sample is determined by measuring the intensity of their characteristic wavelength energy. The method is widely used for the quantitative identification of the composition of a wide range of materials in medicine, industry and science and allows the concentration of the following elements to be identi-

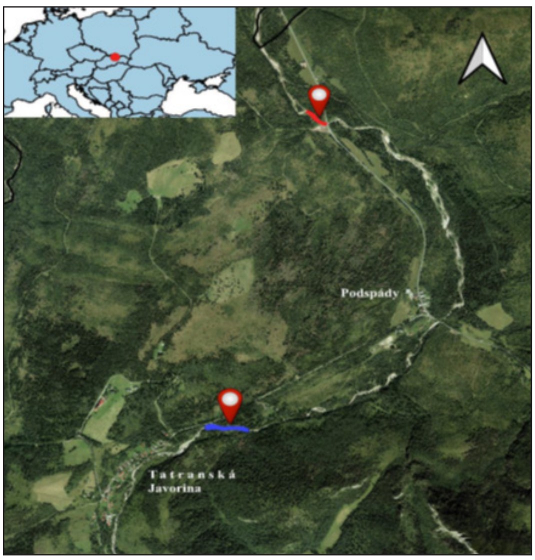

Fig. 1. Research sides on Javorinka stream. L1 (blue color) and L2 (red color), (Author: M. Hudák, ArcMac 10.4 version).

fied: Cl, S, K, Ca, P, Rb, Zn, Mn, Mo, Fe, Ti, Sn, Co, Ni, Cu, As, Se, Pb, Sb, Ba, Hg, Cr, Ag, and Cd.

The default mode was selected for the analysis. Each sample was measured for 120 seconds in three repetitions and the arithmetic mean was calculated for the measured values of the element levels.

Statistical analysis

Only elements that were regularly measured above the detection limit of the instrument were included in the analyses. Statistical analyzes were performed using the Statistica Ver. 12. (TIBCO Software Inc.) software. One-way ANOVA at the 95% confidence level ($p < 0.05$) was used for statistical comparison in species between localities. Principal component analysis (PCA) was used to determine the relationships between the accumulated elements. The variables (elements) that contribute the most to the significance of the factor were arranged according to the percentage contribution to the total variation of the components.

Results

Significant differences in the accumulation between localities

To assess differences in element accumulation in the two groups of functional feeding guilds (scrapers and predators) of aquatic insects, we analyzed the element (P, S, Cl, K, Ca, Cr, Mn, Fe, Cu, Zn, Rb, Se, Sr, Mo, Ba, Pb) content in whole bodies of the larvae. We compared the elemental abundances between sites L1 and L2. In the FFG scrapers, only the elements Fe and Cu differed significantly between sites. Element accumulation between sites differed significantly for the elements Fe, Cu, which were measured in higher concentrations at L2 (Fig. 2). In the FFG of predators, Fe, Mn, Pb concentrations differed significantly between sites. While Fe and Mn were higher in L2, Pb reached higher concentrations in L1 (Fig. 3). Levels of the other analyzed FFGs did not differ significantly between localities.

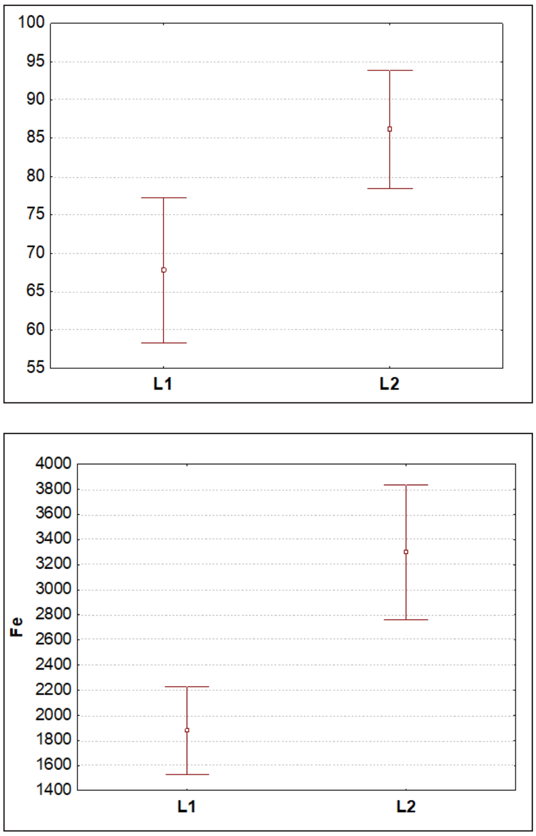

Fig. 2. Comparison of element concentrations of scrapers (Ephemeroptera) in two sites. a) Difference in Fe: K-W H (1; 114) = 17.521; p = 0.00003. b) Difference in Cu: K-W H (1; 114) = 11.3469; p = 0.0008. Middle points: means, Whiskers: +/- standard derivation of means.

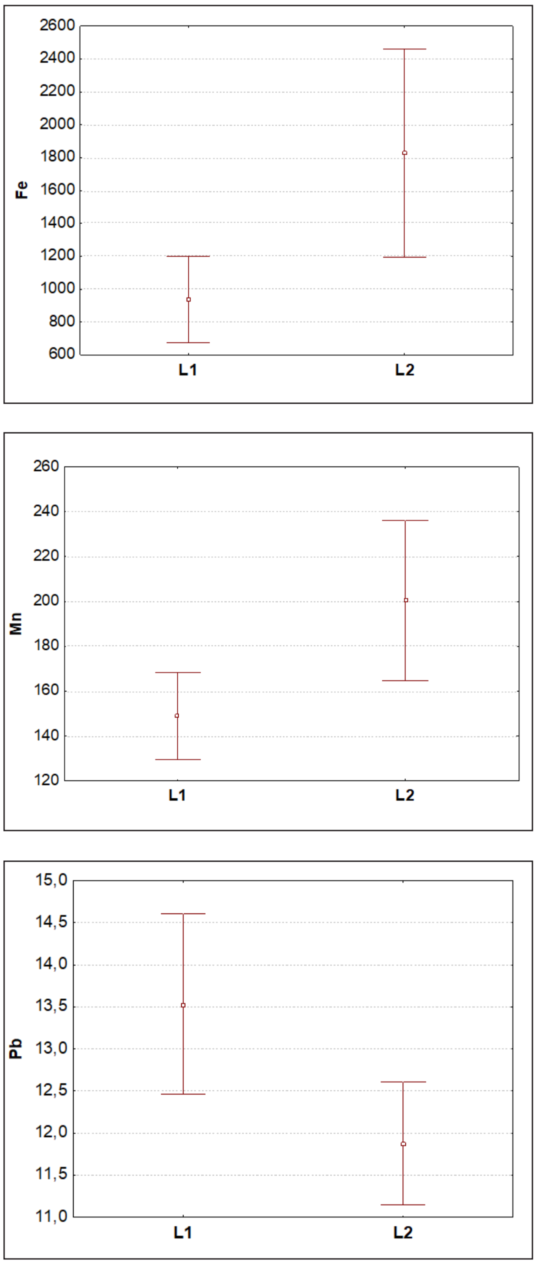

Fig. 3. Comparison of element concentrations of predators (Plecoptera) in two sites. a) Difference in Fe: K-W H (1; 78) = 5.2971; p = 0.0214. b) Difference in Mn: K-W H (1; 78) = 4.6724; p = 0.0307. c) Difference in Pb: K-W H (1; 78) = 4.2344; p = 0.0396. Middle points: means, Whiskers: +/- standard derivation of means.

Multivariate analysis of element accumulation

We used principal component analysis (PCA) to better understand the relationship between the accumulation of individual elements in the bodies of selected FFGs in between sites. This analysis helps us to better understand the relationships between accumulated elements, allowing us to find new common factors that influence the accumulation pattern. Correlation analysis is also more sensitive to outliers in the data.

PCA in FFG scrapers

For accumulated elements in the FFG of scrapers analyzed collectively from both sampling locations (L1, L2), the first 3 factors are significant (total data variation above 70%) (Table 1). The first factor represents the common accumulation of elements. Thus, the level of all elements in the bodies of scrapers either increases or decreases. Factor 2 represents the common accumulation of the elements Zn, Rb and Pb, whose concentration decreases compared to the increasing concentration of P and S. Factor 3 represents the joint accumulation of Ca, Fe and Sr.

Although there were significant differences in Fe and Cu accumulation in FFG scrapers between sites when comparing means, no significant differences between sites were found for either factor when testing for factors characterising the common relationship between accumulated elements (F1 $p = 0.65$, F2 $p = 0.738$, F3 $p = 0.9$). Thus, element accumulation as defined by each factor is not related to site L1 and L2.

PCA in FFG predators

Even in the FFG of predators, the first 3 factors appear significant in the PCA, which explain more than 62% of the data variation (Table 2). Accumulated elements were evaluated collectively from both sampling locations (US, DS). These factors differ from those of Ephemroptera. Factor 1 represents the joint accumulation of elements P, S, Cl, Cr, Mn, Fe and Ba. Factor 2 represents the joint accumulation of Ca, Zn, Rb and Sr. Factor 3 is a bipolar factor and represents an increasing accumulation of Cr and Fe and a concomitant decreased accumulation of Cl, Zn and Pb.

Factors 1 and 2 did not differ significantly between locations (F1: $p = 0.946$, F2: $p = 0.678$). Only Factor 3 differed significantly between locations ($p = 0.016$), with the accumulation of Cr and Fe elements increasing on the L1, and the accumulation of Cl and Pb elements simultaneously decreasing. On the contrary, accumulation of Cl and Pb elements increased at L2 and accumulation of Cr and Fe elements simultaneously decreased (Fig. 4).

Discussion

The accumulation of elements in aquatic invertebrates along watercourses can vary due to several factors, including the stream's position from source to mouth, the type of invertebrates, and environmental conditions. The River Continuum Concept suggests that biological communities, including invertebrates, respond to physical changes along the lengths of rivers (Cummins 2019). For instance, headwater streams often have a different inverte-

	Factor 1	Factor 2	Factor 3
P	**-0.666020**	**0.562185**	-0.069111
S	**-0.604657**	**0.605688**	0.034763
Cl	**-0.566025**	0.474130	0.455729
K	**-0.705165**	0.445847	0.320502
Ca	**-0.480966**	0.173477	**-0.690121**
Cr	**-0.609974**	0.422220	-0.033460
Mn	**-0.860121**	-0.092746	-0.018926
Fe	**-0.512127**	-0.333808	**-0.547339**
Cu	**-0.635509**	-0.437348	0.357219
Zn	**-0.563538**	**-0.587314**	0.215710
Rb	**-0.510454**	**-0.721640**	0.186033
Sr	**-0.371957**	-0.343121	**-0.669318**
Mo	**-0.624365**	-0.178500	0.307947
Ba	**-0.707002**	0.137214	-0.411856
Pb	**-0.546918**	**-0.538179**	0.172423
Total variance	**36.94%**	**19.67%**	**13.63%**

Table 1. Factor coordinates of variables with percentage of variation in PCA of the most and regularly detected elements in the invertebrate body from FFG scrapers (Ephemeroptera). Variables that most contributed to value of factor are in bold.

	Factor 1	Factor 2	Factor 3
P	**0.860785**	0.024049	-0.170575
S	**0.661267**	-0.379056	-0.045234
Cl	**0.704945**	-0.263953	**-0.448770**
K	**0.763609**	-0.285765	-0.367573
Ca	0.581518	**0.539675**	0.126653
Cr	**0.770748**	0.085573	**0.422307**
Mn	**0.878998**	0.050356	0.112580
Fe	**0.741324**	0.231129	**0.477999**
Cu	0.174048	0.361768	0.313855
Zn	0.191690	**0.617536**	**-0.420162**
Rb	0.073448	**0.601910**	-0.148501
Sr	-0.086809	**0.713252**	-0.146507
Mo	0.576694	-0.193951	-0.307827
Ba	**0.899862**	-0.041091	0.173776
Pb	0.068768	0.349664	**-0.485994**
Total variance	**38.26%**	**14.63%**	**9.89%**

Table 2. Factor coordinates of variables with percentage of variation in PCA of the most and regularly detected elements in the invertebrate body from FFG predators (Plecoptera). Variables that most contributed to value of factor are in bold.

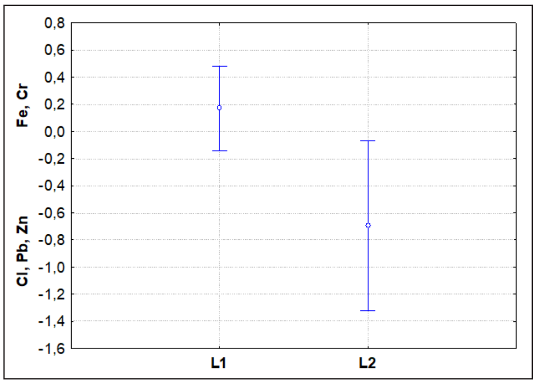

Fig. 4. While L1 is dominated by Fe and Cr accumulation, L2 is dominated by Cl and Pb accumulation p= 0.016. Values are in ppm. Middle pionts: means, Whiskers: +/- standard derivation of means.

brate community composition compared to midstream and downstream sections due to variations in food availability, water temperature, and oxygen levels (McCabe 2011). The accumulation of elements in invertebrate groups also differs based on their feeding habits and habitat preferences (Cummins 2019). Environmental conditions such as water temperature, flow rate, and substrate type can influence the abundance and distribution of invertebrates, which in turn affects element accumulation. Seasonal changes can also play a role, as they can alter the thermal metrics and thus impact the invertebrate communities (Arai *et al.* 2015).

Our research took place in the lower reaches of the river and we hypothesized that element accumulation would differ between FFGs. The results show that even at a short flow distance there are differences in the accumulation of elements within the same food group.

Scrapers showed higher average values of Fe and Cu accumulation at L2, which differs from L1 in terms of rheological properties and a more stable height of the water level throughout the width of the stream. Predators in L2 also accumulated more Fe and Mn. Pb accumulation was higher in L1 in this group. However, significant differences in the concentrations of FFGs were not confirmed in most elements. Thus, the spatial distance of L1 and L2 locations does not play a significant role in the accumulation of elements from the environment.

Iron

In aquatic ecosystems, iron (Fe) is a key element with a decisive role in the biogeochemical cycle of the main elements - carbon, nitrogen, phosphorus and various trace elements. Increases in Fe concentrations in aquatic environments reduce species diversity (Rasmussen and Lindegaard 1988), and most often this decrease is attributed to inhibition of periphyton growth and a consequent decrease in the number of grazers (McKnight and Feder 1984; Wellnitz *et al.* 1994), respiratory stress, and reductions in oxygen uptake, with decreases in food intake and amobility of mayflies (Sykora *et al.* 1972). We hypothesize that increased iron concentrations in the bodies of aquatic insect larvae in L2 may be caused by several environmental factors. The physical and chemical parameters of the water, as well as the heavy metal content in the sediment, can impact the bioavailability of iron to macroinvertebrates (Taborda *et al.* 2022).

Sources of ferrous iron are mostly iron oxides attached to bedrock such as granodiorites (Romanescu *et al.* 2016). Iron dissolves and leaches out of soil and rocks during precipitation and snowmelt. Fe comes primarily from watershed sources (Durand *et al.* 1994), including soils with a high organic content that contribute more Fe than mineral soils, as evidenced by a strong correlation between Fe and dissolved organic matter in surface waters (Ekström *et al.* 2016). Ekström *et al.* (2016) reported that seasonal changes in river water chemistry, flow, and air temperature indicated that increases in iron (Fe) concentrations were primarily influenced by the prevalence of reducing conditions in the catchment. In general, elevated levels of Fe were associated with elevated levels of methylmercury and low levels of sulfate, indicating a reducing environment. Increasing temperature and flow rates in the basin can also lead to the occurrence of anaerobic conditions and thus to increased transport of Fe into aquatic ecosystems. Therefore, a greater number of permanent and temporary inflows from forest ecosystems to Javorinka at L2 explains the increased concentration of Fe in the water environment.

Copper

Copper is naturally present and bioavailable in soils and surface waters from processes such as rock and soil weathering or atmospheric deposition (Sullivan *et al.* 2022; Leybourne and Cameron 2008). A comprehensive understanding of how copper is distributed in surface waters requires considering numerous contributing factors and biogeochemical processes (Fekiacova *et al.* 2015). The presence and behaviour of copper in surface waters, especially at base levels, are intricately linked to low-temperature inorganic reactions (such as physico-chemical reactions between minerals and fluids) and biological uptake. Consequently, these processes are influenced by existing redox conditions as well as the presence of essential nutrients and the rate of organic matter production (Thomas *et al.* 2017; Schoenfuss *et al.* 2020).

In natural waters, copper uptake by biota is more dependent on the activity of the free copper ion (Cu^2) than on the total Cu concentration (Brooks *et al.* 2007). Dissolved copper in rivers is very strongly bound to organic ligands (Vance *et al.* 2008). These molecules are often involved in various biological processes and play a significant role in the behavior and bioavailability of copper. The bioavailability of copper is modified by the source and environmental history of dissolved organic matter (DOM), and in wetlands, exposure to solar radiation increases the complexation of Cu with DOM. (Brooks *et al.* 2007). In other words, more available copper for the biota is in the aquatic environment, which is exposed to sunlight, has a slower flow and contains more DOM, which, compared to the sites of our research, is more characteristic of L2. In this location, copper significantly accumulated in the FFG of scrapers, likely related to their food requirements, as mayfly larvae accumulate more metals than caddisflies (Trichoptera). The importance of metal absorption by larvae through dissolved substances or food varies greatly depending on the specific rates of metal absorption characteristic of individual insect families and on the level of concentration of metals in their food and water environment (Balistrieri *et al.* 2020). In addition, essential trace metals (copper, zinc, iron) are not biomagnified through the food chain (Jeong *et al.* 2023). Therefore, their increased intake was not manifested even in the group of predators.

Manganese

Manganese is an essential trace element that acts as a metal co-factor in diverse biochemical and cellular functions (Ben-Shahar 2018). Like vertebrates, all insects require Mn as a metal cofactor for the catalytic action of various enzymes (Burnell 1988; Schramm 2012; Holley *et al.* 2012). Mn also plays a direct role in various molecular and physiological processes specifically associated with insect development and behavior (Ben-Shahar 2018). It occurs in watercourses from natural sources or because of human activities such as mining and industrial discharges. In river water, manganese can persist for longer distances downstream from the pollution source compared to iron from the same tributaries (Wong *et al.* 2022). Manganese plays a critical role in river water quality because Mn oxides serve as sorption sites for contaminating metals (Bryant *et al.* 2020). The transport of manganese (Mn) in the river flow is a complex process influenced by various environmental factors. Transport and cycling of Mn in rivers is affected by seasonal changes, such as snowmelt, which can affect river water and groundwater interactions and Mn transport (Bryant *et al.* 2020). In addition, seasonal components, such as a period with increased precipitation, can lead to Mn and Fe introduction into rivers and thus to higher concentrations of these elements in the river, as confirmed by Geochemical simulations of the dissolution of MnO_2 and FeOOH through the oxidation of organic matter (Gödeke *et al.* 2022). The oxidation state of manganese can also significantly affect its distribution, transport, and accumulation in water (Li *et al.* 2019). However, these factors were not monitored in our study, so the cause of increased Mn accumulation in the L2 location in the bodies of FFG predators cannot be clearly determined. Souto *et al.* (2018) noted that manganese was less efficiently transferred to upper trophic levels. We hypothesize that increased levels of manganese in the bodies of FFG predators (Plecoptera) are not related to intake from the environment or food. Besser *et al.* (2001) found wide variation in the bioaccumulation of different metals in aquatic invertebrates, suggesting that the routes of exposure for the biota differ among different metals.

In aquatic environments, Fe and Mn can compete for similar uptake pathways due to their chemical similarities. This competition can affect the bioavailability and uptake rates of both metals. On the other hand, their similarity can also be the cause of increased accumulation of both elements in bodies in the L2 location.

Lead

Lead tends to accumulate in the sediments of streams and rivers, which can be a concern for benthic organisms - those living on or in the bottom substrates. Pb has no confirmed biological role, and there is no confirmed safe level of lead exposure (World Health Organization 2018). The primary cause of its toxicity is its predilection for interfering with the proper function of enzymes. It does so by binding to the sulfhydryl groups found on many enzymes or mimicking and displacing other metals which function as cofactors in many enzymatic reactions (Dart *et al.* 2004). The essential metals that lead interacts with include calcium, iron, and zinc (Kosnett *et al.* 2007). Elevated levels of calcium and iron tend to provide some protection from lead poisoning, though low levels cause increased susceptibility (Venugopal 1977). This conclusion may explain the reversed levels of Fe and Pb in L1 and L2 in FFG predators in our research. Moreover, Pb toxicity can cause oxidative stress in organisms, which may increase the physiological demand for Mn due to its role in the antioxidant enzyme Mn-superoxide dismutase (MnSOD) (Poteat *et al.* 2012). In addition, Ephemeridae (FFG scrapers) have weak accumulation relationships with aqueous Pb and Cu and with Zn bound to the sediment (Goodyear and McNeill 1988), therefore, Pb accumulation was not manifested significantly in them.

PCA-based feature accumulation relationship

The concentrations of elements in the water and their subsequent transfer to the biota can vary even over smaller distances along the stream, which can be influenced by factors including stream rheology, sediments, flows, dissolved organic substances, etc.. These factors are primarily related to environmental conditions and differences in seasons. Evaluation of other factors, however, was not the aim of this study. Principal component analysis (PCA) found no significant differences in the accumulation of element groups between L1 and L2 sites in FFG scrapers. In this group of predators, only the divergent accumulation of elements Cl, Pb and Fe, Cr (factor 3) differed significantly between L1 and L2 sites. We attempted to explain the opposite accumulation of Fe and Pb in the compared localities based on the comparative tests above. Research on the role of Pb in aquatic ecosystems has focused on its bioaccumulation and biomagnification in aquatic food webs (Gerhardt 1994) and toxicity studies have mostly been done with fish, crustaceans, molluscs, or insects as test organisms (Wren and Stephenson 1991; Gerhardt 1993). Local variations in Pb concentration in mayfly larvae were found in a study by Fialkowski *et al.* (2003). Chlorine, an element with a key role in osmoregulation, appears to accumulate more in predator bodies than in scraper bodies. Our results support the research of Sarkka *et al.* (1978), who noted that predators of the zoobenthos contained significantly more organic chloride compounds than herbivores or detritus feeders. However, chlorine can enter freshwater systems during winter road de-icing (mixtures of salts such as NaCl, KCl). Salinity level (NaCl content) is often monitored in studies on the toxicological effects of chlorine on aquatic invertebrates (Wallace and Biastoch 2016). Principal component analysis (PCA) found no significant differences in the accumulation of element groups between sites L1 and L2 in FFG scrapers. In the group of predators, only the divergent accumulation of elements Cl, Pb and Fe, Cr (factor 3) differed significantly between L1 and L2 sites. We tried to explain the opposite accumulation of Fe and Pb in the compared localities based on the comparative tests above.

Factor 3 (Table 2) shows a higher concentration of Cr and Fe upstream and lower concentrations downstream. However, a larger accumulation of Cl and Pb (Fig. 4) was observed upstream in Plecoptera bodies. Lead interacts with chlorine to form lead chloride $PbCl_2$. In the aquatic environment, $PbCl_2$ can form when lead meets chloride ions, which are abundant in natural waters, especially seawater. L2 is situated close to a busy road, therefore it is possible to assume anthropogenic pollution in this location, such as increased levels of salts from road anti-icing. $PbCl_2$ is used in the production of asbestos clutch or brake linings, acrylonitrile, olefin polymers (Williams 2013), which is another potential source from transport. In the aquatic environment, both Fe and Cr are present in various forms and can be taken up by organisms including insects. Iron is an essential nutrient that plays a key role in oxygen transport and metabolic processes.

Chromium, especially in its trivalent form (Cr(III)), is also considered an essential trace element involved in the metabolism of sugars and lipids. However, the hexavalent form (Cr(VI)) is highly toxic and is a known carcinogen. The movement of elements along the flow can also be affected by their density. For comparison, $PbCl_2$ has a density of approximately 5.85 g/cm^3. In comparison, elemental iron (Fe) has a density of about 7.87 g/cm^3 and elemental chromium (Cr) has a density of about 7.19 g/cm^3. Therefore, both Fe and Cr are denser than $PbCl_2$. This means that on a volume basis, Fe and Cr would be heavier than the same volume of $PbCl_2$. Thus, heavier elements move more slowly along the flow, while lighter ones, (e.g. $PbCl_2$) can slide to the lower side of the stream. Bouchelouche and Arab (2020) also document higher Pb concentrations in the lower reaches of the stream.

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