BIOACCUMULATION OF TRACE ELEMENTS IN THE MAROS AND SZAMOS RIVERS: FISH AS BIOINDICATORS OF AQUATIC POLLUTION

Thesis for the Degree of Doctor of Philosophy (PhD)

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Hereby, I declare that I prepared this thesis within the Doctoral Council of Natural Sciences and Engineering, Juhász-Nagy Pál Doctoral School, University of Debrecen in order to obtain a PhD Degree in Natural Sciences at Debrecen University.

The results published in the thesis are not reported in any other PhD theses.

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Hereby, I confirm that Nurfatin Zulkipli conducted his studies with my supervision within the Hydrobiology Doctoral Program of the Juhász-Nagy Pál Doctoral School between 2019 and 2025. The independent studies and research work of the candidate significantly contributed to the results published in the thesis.

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BIOACCUMULATION OF TRACE ELEMENTS IN THE MAROS AND SZAMOS RIVERS: FISH AS BIOINDICATORS OF AQUATIC POLLUTION

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1. INTRODUCTION AND OBJECTIVES

1.1. Introduction

"In wildness is the preservation of the world," famously observed by the naturalist and philosopher Henry David Thoreau (1862), highlighting the timeless truth that healthy ecosystems are the foundation of all life. This deep understanding highlights the critical importance of safeguarding our natural ecosystems, which sustain numerous species, including humanity. In the modern era, these ecosystems are increasingly threatened by three interconnected pressures—biodiversity loss, climate change and pollution—shaping the global crisis we face today. Over the past two decades, deaths attributable to modern forms of pollution—particularly ambient air pollution, lead exposure, and toxic chemical contamination—have increased by 66%, driven by industrialisation, rapid urbanisation, population growth, fossil fuel combustion, and weak chemical governance, with pollution now causing over 9 million deaths annually worldwide (Fuller et al., 2022).

We are currently living through the unprecedented 6th mass extinction, where there is an ongoing rapid loss of more than 75% of species, the most rapid and pervasive extinction since 66 million years ago (Barnosky et al., 2011). Studies show that water pollution and water extraction with the introduction of invasive species have endangered the freshwater fish species in Europe (Kottelat & Freyhof, 2007; Vié et al., 2009). Mankind has been depending on fish for food directly or indirectly, which makes fisheries one of the major contributors to the national economies (Rabo et al., 2014; Shamsuzzaman et al., 2020). The decline of the fish population not only results in the loss of one of the biggest food sources for humans but also affects the entire biological community. According to Gozlan et al. (2019), due to the lack of reliable data, the decrease in freshwater fish species in

Europe is probably underestimated, as population trends remain unknown for 76% of species throughout Europe; thus, immediate monitoring is essential to determine precise trends and improve the Red List evaluations. In 2008, a review of the IUCN Red List of Endangered Species highlighted man-induced activities as the main causes, including overexploitation, habitat loss and degradation, pollution, accidental by-catch, and human interference (Vié et al., 2009). In 2015, only around 53% of the rivers and lakes in Europe met good ecological standards according to the Water Framework Directive (WFD), as numerous lakes, ponds, and streams are vanishing due to agricultural intensification, ineffective irrigation, urbanisation, and the impacts of climate change (Gozlan et al., 2019). Globally, urbanisation has changed the surface features of the river channel and sediment mobility, where in Hungarian streams specifically, the diversity and abundance of living organisms are severely impacted (Szita et al., 2019).

Trace element pollution is the most overlooked latent hazard (C. Li et al., 2022). It has devastating environmental impacts due to its nature, which can occur from negligence or isolated incidents. Heavy metals in aquatic ecosystems originate from two main sources: natural geophysical processes of the Earth and human-induced actions, such as industrial and agricultural discharges, as well as air deposition (MacDonald et al., 2000). Due to their toxicity, long-term persistence, ability to bioaccumulate, and tendency to biomagnify within the food chain, heavy metals pose severe dangers to rivers as the metal toxicants are constantly introduced into the waterbody (Nyeste et al., 2019b; Sandu & Bloesch, 2008). Trace element pollution in rivers is concerning as it reduces the availability of world water supplies and hazard to human utilisation of the fishery products (Subotić, Spasić, et al., 2013a; Terra & Araújo, 2008).

Before industrialisation, Vadas and Ferenczi (2023) described that urban streams in Hungary played crucial roles in local economies with diverse water utilisation along the lower reaches of the streams in activities such as slaughterhouses, tanneries and industrial workshops, which already gave rise to several pollution concerns. As industrialisation took place in Europe, Hungary faced a massive geographical disadvantage regarding trace element pollution, as it is landlocked in the lowest part of the Carpathian basin. Rivers flowing from the west, north, and east carry contaminants from neighbouring countries, making this country more susceptible to anthropogenic pollution. According to Zhou et al. (2020), based on the collected studies in five decades across five continents, the leading causes of trace element pollution in Europe are mining and manufacturing, followed by rock weathering. Toxic metals are introduced into coastal environments through rivers and small streams, stemming from untreated wastewater, mining activities, and industrial, agricultural, municipal, household, and residential sources (Anandkumar et al., 2018).

In 2000, the cyanide-spilling disaster in northwestern Romania served as a wake-up call in Eastern Europe, portrayed as the worst disaster after the Chernobyl nuclear power plant incident. According to Koenig (2000), the plume that flowed into the Szamos River in Hungary also carried heavy metals (copper and zinc), where the concentration of the copper temporarily plummeted to 36 times higher than the safe level, with the zinc and lead also surging dramatically. In the aftermath, 200 tons of dead fish surfaced or washed ashore along the Tisza River, while a report by the World Wildlife Fund (WWF) has accounted for 1,240 tonnes of fish, with the toxic spill devastating fish populations, killing essential microbes and endangering predators (e.g. otters and eagles) reliant on contaminated prey (Koenig, 2000; WWF, 2002). The fish species in the affected river displayed varied

levels of vulnerability: "*Of the fish collected, 44.6% were herbivorous, with Silver carp (Hypophthalmichthys molitrix) particularly impacted, constituting a significant portion of the deaths. Other affected groups included 33.8% predatory fish, 13.5% carp, and 8.1% sturgeon, suggesting that cyanide toxicity disproportionately affected herbivorous fish in particular*" (WWF, 2002). Following severe cyanide-spill incidents, the Szamos River became the focus of long-term monitoring and extensive research, with subsequent studies showing that the fish community has largely recovered from pollution-related impacts in parallel with significant improvements in water quality (Antal et al., 2013).

As a result of the resilient properties of heavy metals and their ability to accumulate in living tissue, continuous and extensive monitoring is crucial to ensure the safety of fish consumption for humans and other living things. Following this, an international approach has been taken for ecosystem restoration, environmental protection and regular monitoring between neighbouring countries to prevent similar incidents from happening again in the future. The organisation was established in 2000, the International Commission for the Protection of the Danube River (ICPDR) was responsible for implementing the transboundary cooperation of the EU Water Framework Directive (WFD). "*ICPDR is the largest coalition of river basin management specialists in Europe, working to foster sustainable water management across the Danube Basin for the benefit of all its residents. The ICPDR unites both EU and non-EU Danube countries to collaborate on critical water management issues. All member countries have committed to complying with the EU WFD, a commitment further strengthened by the inclusion of the EU Floods Directive in 2007*", where ICPDR's goals are to have a cleaner, healthier and safer Danube (ICPDR, 2019).

In conjunction with the ICPDR's vision, it has driven our research on river monitoring, safeguarding water quality and promoting safe fish consumption for local communities. Besides that, our study area is strategically important to serve as a precursor for pollution from other countries and the local environment. Currently, it is evident that there are ongoing anthropogenic threats to the aquatic ecosystem in Hungary from heavy metals, mainly in the Maros River and Szamos River (Nyeste et al., 2019; Sandu & Bloesch, 2006, 2008; Szabó et al., 2008; Fleit & Lakatos, 2003). The current status of the Maros River is alarming; it carries substantial quantities of heavy metals, both dissolved and suspended, driven by its geological background as well as mining activities in the water catchment (György & Tímea, 2001; Kovács & Kiss, 2010; Sandu & Bloesch, 2008). Based on the social survey done with the locals by Sipos et al. (2022), the Maros River was identified as the second most significant natural asset, where the most commonly reported activities included walking along the riverside, fishing, and swimming in the river.(Sipos et al., 2022) Given this close human-river interaction, it may pose an indirect environmental risk to the local population through the food chain (Kovács & Kiss, 2010).

The rationale of the study is to secure the safety of fish intake in the Szamos River and Maros River for humans and living things in the surrounding area. There are research gaps in the existing literature on trace element bioaccumulation in fish. As far as we are aware, no studies have been conducted that compare the accumulation of trace elements between young and adult fish in a river system, especially in Hungary (Szamos River and Maros River) in recent years. It is important to understand these differences when we think about how age influences bioaccumulation. Moreover, even though many studies have documented the occurrence of trace metals in fish tissues, only a few exist on information-based health

hazards related to the intake of specific species from these rivers being inhabited, considering contamination to varied tissues like muscle, gills and liver. In addition, research often overlooks whether and how different environmental conditions (water chemistry, sediment type, and feeding behaviour) jointly function to impact the bioavailability of trace elements in fish. The present study is focused on the common fish species that are usually caught by anglers and are also commercially important, since there is a lack of published information on the species' bioaccumulation studies in both Szamos River and Maros River.

The results of this study also aimed for a deeper insight into the connection between trace element bioaccumulation and the trophic level of each species. This study emphasises fish as the main subject in obtaining information about the safety of human consumption and the adaptability of fish exposed to organic pollutants, which will be useful in solving ecological, social, and economic problems. It will provide baseline data for the current status of the safety of human consumption of the common fish species in the Szamos River and Maros River, which are popular spots for recreational fishing. Lastly, the data on the toxic metal accumulation in fish tissues can become a good precursor of water quality, as fish can serve as bioindicators, which will be useful for the development of protection and conservation efforts.

1.2. Objectives

This study aims to achieve the following primary objectives, designed to address specific research questions:

(i) *To analyse the concentrations of macroelements (calcium, potassium, magnesium, and sodium) and microelements (cadmium, chromium, copper, iron, manganese, lead, strontium, and zinc) in different fish tissues (gills, liver, and muscle) to assess tissue-specific bioaccumulation patterns:* (a) Which macroelements and microelements exhibit the highest accumulation in gill, liver, and muscle tissues? (b) How do tissue-specific bioaccumulation patterns vary among fish species within each river system?

(ii) *To evaluate the influence of environmental conditions and species-specific traits on trace element bioaccumulation in juvenile fish from the Szamos River and adult fish from the Maros River*: (a) How do trace element bioaccumulation patterns vary within juvenile fish populations of the Szamos River and within adult fish populations of the Maros River? (b) How do water and sediment quality parameters influence trace element accumulation within each river system? (c) How do species-specific traits, including habitat preference, feeding habits, and species-level trophic position, contribute to observed bioaccumulation patterns? (d) Which fish species serve as the most reliable bioindicators of trace element pollution? (e) Do trace element concentrations in fish muscle exceed food safety thresholds and pose potential health risks to consumers?

2. LITERATURE REVIEW

2.1. Overview of Fish Biodiversity in the Tisza River Basin

The Tisza River is among the most important affluent rivers of the Danube River Basin, with the longest and the largest catchment of 965km and 156 087 km^2, respectively (ICDPR, 2008). The primary activities of the Tisza River watershed are agriculture and grazing, followed by forestry, which is shared between five countries (Hungary, Romania, Slovakia, Serbia, and Ukraine) and is populated by 14 million people. The Tisza River loses 1600 meters in elevation over the first 260 kilometres from its source until it meets the Szamos/Szumeș River. According to Sandu & Bloesch (2008), the middle Tisza continues until it converges with the Maros/Mureș River, which has exceptional natural hydromorphology. The Körös/Crișul and Maros/Mureș rivers, which flow through regions of Transylvania in Romania, are the Tisza's major tributaries, while the lower Tisza is the downstream zone of the river from the confluence of the Maros/Mureș River (Sommerwerk et al., 2009).

As stated by Kottelat and Freyhof (2007), the Danube is the most biodiverse river in the European river basin, containing approximately 20% of the freshwater fish fauna of Europe, with 129 native species found in the Danube River Basin (DRB). The Tisza River boasts a biodiversity that surpasses that of most other European rivers. For fish such as nase [*Chondrostoma nasus* (Linnaeus, 1758)], barbel [*Barbus barbus* (Linnaeus, 1758)] and sterlet [*Acipenser ruthenus* (Linnaeus, 1758)], the upper Tisza basin is a crucial migration route for survival. At the same time, the critically endangered species that shows sporadic appearance, the Russian sturgeon (*Acipenser gueldenstaedtii*), also seeks refuge in the Tisza River. The Baia Mare cyanide spill in 2000, which lasted for 2 months, caused massive destruction in the ecosystem and left tons of fish dead. Possible long-lasting consequences on living things are one of the

main concerns of the research and monitoring work for years due to trace element accumulation in the sediments.

2.2. Ecological and Physiological Characteristics of Fish

Fish serve as an important food source for humans. To combat the global surge in trace element contamination, it is vital to comprehend the interplay between the biological and ecological traits of fish species and their trace element concentration (Luzardo et al., 2022; Morales, 2022; Zaghloul et al., 2024; Zaynab et al., 2022). Heavy metals can enter the body of a fish mainly through its gills, skin and digestive tracts (Zaynab et al., 2022). Deposition of heavy metals in the liver and gills may result in cytotoxicity, immunomodulation and oxidative damage (Luzardo et al., 2022; Zaghloul et al., 2024). Understanding the consequences of heavy metals will aid in assessing the environmental condition of an aquatic ecosystem as well as identifying potential human health risks from eating products contaminated with these substances, hence, protecting public and environmental health (Luzardo et al., 2022; Morales, 2022; Zaghloul et al., 2024).

The physiological state of a fish species plays a big role in trace element bioaccumulation levels. An early study by Hodson (1988) shows that metal-binding proteins aid in their resistance to heavy metals. Heavy metals impact the blood chemistry, immunological system, and tissue health (Shahjahan et al., 2022a). A study by Wood (2011) described the mechanism of the uptake, process and modifications of discarding heavy metals. Golovanova (2008) added that heavy metals affect fish and aquatic organisms' physiology and biochemistry, specifically through their absorption and detoxification. These studies highlight the complex physiological processes during the uptake, dispersion and excretion of trace elements in fish.

Moreover, ecological factors such as habitat type, trophic level, and feeding behaviours can affect the bioaccumulation of heavy metals. For habitat type, a study by Li et al. (2015) found that cadmium (Cd), zinc (Zn) and lead (Pb) in water accumulate more in benthic species due to the direct interaction with polluted sediments. Regarding trophic level and feeding behaviours, predator species positioned at the top of the food chain usually obtain more heavy metals through biomagnification as harmful substances accumulate along the chain (Liang et al., 2016). Other than that, temperature and salinity ultimately caused variability in the metabolic rate for fish, which affected the metal uptake rate and rate of elimination. Fish living in polluted habitats may struggle to detoxify and excrete metals effectively, leading to a higher accumulation of trace elements in their bodies (El-Moselhy et al., 2014).

2.3. Overview of the Ecological Diversity of Fish Species

To explore this further, ten common species were chosen to be studied. Each species has unique physiological characteristics that can influence the bioaccumulation of heavy metals in its tissue. Firstly, the *Leuciscus aspius* (Linnaeus, 1758), often referred to as asp, is a potamodromous cyprinid fish (Abbasi et al., 2021). It inhabits the European rivers and the Caspian Sea basin (Gordon & Michael, 2022). It can be distinguished by its upper caudal-fin length and maximum body depth from the diverse morphological traits across its populations (Abbasi et al., 2021). It is a piscivorous fish; juveniles and adults usually feed on smaller fish such as bleak or roach (Kottelat & Freyhof, 2007; Krpo-Cetkovic et al., 2010). Asp is prone to bioaccumulation due to its predatory behaviour. It is considered valuable in aquatic ecosystems and a popular recreational fish. According to Fredrich (2003), there are individual variations in the migratory behaviour of asp, whereby some occupy home ranges of 1-100 stream km, and others migrate up to 166 km away. They typically

inhabit main river channels but may use harbours or oxbows as winter habitats (Fredrich, 2003).

Secondly, it is a European cyprinid fish called barbel, scientifically known as *Barbus barbus* (Linnaeus, 1758), which can live over 20 years and grow to 8 kg (Bašić & Britton, 2016). Barbel river zones are the swift, deep upper reaches with rocky or gravel substrates. The bottom shallow coastal settings are the refuge of the larvae and juveniles, and migration will take place where they grow to fast-flowing waters (Kottelat & Freyhof, 2007). The diet of barbel usually consists of benthic invertebrates, including crustacean zooplankton, insect larvae, bivalves, mayfly and midge hatchlings, juvenile fish, and sometimes algal species (Froese & D. Pauly, 2022). The species were observed to have trophic niche partitioning with other cyprinids, such as *Squalius cephalus*, across various habitats and life stages (Bašić & Britton, 2016). Studies have shown that barbel can accumulate hazardous levels of heavy metals and mercury, posing risks to human consumption (Mensoor & said, 2018). Accumulation patterns are influenced by age and feeding habits, with younger and piscivorous individuals being particularly sensitive to genotoxic effects (Onita et al., 2021; Sunjog et al., 2012).

Thirdly, it is one of the largest freshwater fish in the world, originating from the east of Europe, known as European catfish or wels [*Silurus glanis* (Linnaeus, 1758)], it can reach a body length of roughly 2.7 meters with a mass exceeding 130 kg (Cucherousset et al., 2017). Known for its boneless textured body, catfish is considered to be one of the most popular foods in Hungary. Its diet range varies with habitats (littoral and pelagic) and is highly adaptable where it feeds from fish to crayfishes (Santis & Volta, 2021). It exhibits multiple feeding strategies, alternating between generalism and specialization, and—compared to native predators such as the Northern pike—plays a distinct

role in ecosystem coupling due to its broader niche and individual dietary specialisation (Vejřík et al., 2017).

Another notable species is *Squalius cephalus* (Linnaeus, 1758), known as chub is a freshwater cyprinid that inhabits slow and moderate water flows and is widely spread in European waters. Chub is native to Hungary, but its high fecundity, rapid growth, and tolerance to anthropogenic stressors have enabled it to become invasive in other regions, often facilitated by recreational fishing (Mònica & Alberto, 2022). The juveniles are gregarious and inhabit shallow shorelines, feeding on various animal and plant materials, but as they grow larger, they become solitary, often occupying the pelagic zones and primarily piscivorous (Kottelat & Freyhof, 2007). It is regarded as an important species for environmental monitoring and has been the subject of numerous ecotoxicological studies due to its wide distribution (Mònica & Alberto, 2022; Nyeste et al., 2019, 2024; Pekol & Arslan, 2015)

Among other species is the common carp [*Cyprinus carpio* (Linnaeus, 1758)], a widely distributed riverine fish belonging to the Cyprinidae family. It has a relatively high resistance to pollution and is one of the key aquaculture species in Eastern Europe (Harangi et al., 2017a; Váradi, 2022). Carp farming has ancient roots and has become a major global industry, with production reaching 2.5 million metric tons in 1998, estimated to be worth US$2.8 billion, with China as the leading producer (Takeuchi et al., 2002). It thrives in slow-moving rivers, marshes, and weedy areas with muddy bottoms (Takeuchi et al., 2002). While young carp feed on zooplankton, adults are omnivorous benthic feeders and also act as bioturbators in the aquatic ecosystem (Harangi et al., 2017a; Takeuchi et al., 2002). Common carp serves as an excellent indicator species due to its susceptibility to waterborne pollutants and suspended surface sediments, containing both organic and inorganic contaminants that are likely to accumulate at elevated levels (Harangi et al., 2017a).

Another most abundant bottom-dwelling cyprinid species is the common nase [*Chondrostoma nasus* (Linnaeus, 1758)]. It thrives in moderate or fast-flowing, oxygen-rich rivers and streams with a life expectancy of up to 12 years (Froese & D. Pauly, 2022; Kottelat & Freyhof, 2007). The juvenile finds refuge in the shallow shoreline benthic region and leaves for fast-flowing water as it grows (Froese & D. Pauly, 2022). Nase is characterised by a pronounced slit, complete with a thick, horny, and pointed lower lip ideal for scraping the bottom substratum, which enabled a diet consisting of small invertebrates, benthic algae, detritus, insect larvae, snails, and fish eggs (Ovidio & Nzau Matondo, 2024; Subotić et al., 2019). Although nase is not widely consumed and holds minimal economic value, it plays an important role as a bioindicator for riverine ecosystem health, with its population declining due to various anthropogenic factors (Ovidio & Nzau Matondo, 2024).

Originally introduced in European and North American waters, this invasive species known as silver carp [*Hypophthalmichthys molitrix* (Valenciennes, 1844)], poses ecological risks due to its filter-feeding habits (Boros et al., 2015; Jawdhari et al., 2022a). The silver carp primarily feeds on phytoplankton and organic material, with the specialised gill structure that allows it to sieve particles smaller than 20 μm (Spataru, 1977). Tumolo & Flinn (2019) added that zooplankton and heterotrophic flagellates are also part of their diet. The species' sensitivity to environmental conditions underscores its suitability as a bioindicator of aquatic pollution (Jeffrey et al., 2019).

Common bream or *Abramis brama* (Linnaeus, 1758) is widely distributed in the lakes and medium and large-sized rivers where it mostly inhabits the backwaters (Froese & D. Pauly, 2022). It has a generalised foraging pattern, mainly ingesting chironomid larvae, detritus, and Caspian mud shrimp, scientifically known as *Corophium curvispinum* (Specziár et al., 1997). However, in gravel

pit lakes, bream demonstrates flexibility by feeding on chironomid larvae, bivalves, and zooplankton (Giles et al., 1990). Its ability to filter-feed on zooplankton is facilitated by its branchial sieve and mucus encapsulation of particles (Hoogenboezem, 2000). Bream is a bioindicator of metal pollution, accumulating higher concentrations of toxic metals in gills and liver than in muscle, with tissue-specific and age-related variations (Farkas et al., 2001).

The benthos-feeding white-eye bream [*Ballerus sapa* (Pallas, 1814)] is native to the Ponto-Caspian and Aral Sea regions but has spread to European rivers, including the Danube, making it relevant for monitoring metal pollution (Kakareko & Kobak, 2014; Skóra & Skóra, 2013). It is a rheophilic species associated with main lowland channels and estuaries (Kakareko & Kobak, 2014; Kottelat & Freyhof, 2007). The diet is comprised of benthic invertebrates and these species are thought to be nocturnal, where they hunt at night and spawn on the gravel bottom and submerged vegetation (Kakareko & Kobak, 2014; Kottelat & Freyhof, 2007). This semi-anadromous species is considered a rare species with low economic value and is currently threatened by water pollution (Kakareko & Kobak, 2014; Kottelat & Freyhof, 2007).

The notorious predatory species called zander or pikeperch [*Sander lucioperca* (Linnaeus, 1758)] can be found in large, murky rivers and nutrient-rich lakes. The larvae are attracted to light (positive phototactic) and feed on pelagic organisms, and adults feed mainly on gregarious pelagic fishes (Kottelat & Freyhof, 2007). The populations can thrive in regulated water bodies, where they play a crucial role in ecosystem balance and have significant commercial value (Marenkov, 2018). A study in French rivers by Kopp et al. (2009) has shown that zander occupies an elevated trophic level compared to other predatory fish species. It is also well known among anglers due to the challenging nature of catching it as it is more elusive than the other freshwater

species. The lean white flesh is favoured for its mild and firm texture and is often a popular choice amongst locals in Hungary.

2.4. Trace Element Contamination in Aquatic Ecosystems

Trace element contamination in aquatic ecosystems is a growing global concern, posing significant risks to both the environment and human health. These metals originate from industrial waste, mining, urban runoff, and natural sources (Saravanan et al., 2024). It can accumulate in aquatic animals, especially fish, leading to negative impacts on their physiology and immune systems (Authman et al., 2015; Vajargah, 2021). The contaminated fish will reach the final consumer, such as birds, cats or humans and cause severe complications, as we can see, in the case of Minamata disease (Howlett, 2019).

During the 1950s and 1960s in Japan, the world was shocked by industrial pollution resulted in methylmercury poisoning, which exhibited severe neurological symptoms in both humans and animals (Howlett, 2019; Saito, 2020). Howlett (2019) added that the unfortunate incident underscores the significance of early identification and exploration of environmental dangers, as prompt detection of the "cat dancing disease" could have averted human deaths. The contamination affected not only adults but also caused congenital cases through prenatal exposure, with new research showing that even minimal methylmercury exposure during pregnancy can result in lasting neurological and cognitive issues later in life (Saito, 2020; Yorifuji et al., 2023). Consequently, there have been actions around the globe to protect public health and concern from subsequent effects of mercury including the Minamata Convention on Mercury (Sly & Kayama, 2020).

Even today, the Minamata disease serves as a benchmark in research relevant to the effects of environmental media on human health. Nevertheless, there are numerous heavy metals disasters reported throughout the world, including the Fuyang River Incident in China, Danube River in Eastern Europe,

and Kabwe Lead-Zinc Mine Legacy in Zambia (Mufalo et al., 2021; WWF, 2002; J. Zhao et al., 2021). Similarly, Malaysia faces its own challenges with trace element contamination in freshwater and marine ecosystems, arising from poor waste management, industrial discharge, agricultural run-off and mining operations, which threaten aquatic biodiversity and human health (Mohd Najib et al., 2025; Ruzi et al., 2024; Yap et al., 2019; Zanuri et al., 2020). This results in increased policies, laws and higher global collaboration to prevent it in the future. Many attempts have been made to solve the problem, for example, through physicochemical adsorption and advanced oxidation processes (Aziz et al., 2023). However, the practical use of some of the methods is restricted by the fact that they are quite expensive. Recommendations suggested for controlling heavy metals include proper treatment of the water before discharge, enforcing policies to protect the environment, and searching for cheap removal methods (Authman et al., 2015; Aziz et al., 2023).

2.4.1. Common Types of Heavy Metals in Aquatic Environments

What constitutes heavy metals remains controversial in scientific literature. Traditionally, heavy metals are characterised by high atomic mass and density, typically exceeding 5 g/cm³ (H. Ali & Khan, 2018; Koller & Saleh, 2018; Matthess, 1984). According to Koller and Saleh (2018), alternative definitions that rely on atomic mass or atomic number have been put forward, creating confusion and inconsistency. Some researchers propose discontinuing the term because of its lack of precision (Ali & Khan, 2018). Appenroth (2010) suggests defining heavy metals according to the periodic table, encompassing transition metals, rare earth elements, and a diverse group that includes elements such as bismuth (Bi), aluminium (Al), and arsenic (As). There is a lot of disagreement about using the term in plant science and environmental literature, but it still shows up frequently and is associated with pollution and toxicity (Ali & Khan,

2018; Appenroth, 2010). Heavy metals can be grouped into macroelements or microelements. Macronutrients like calcium and magnesium are usually required in significantly large quantities, whereas micronutrients such as copper, iron, and zinc are essential in very small quantities (Rotter et al., 2015). However, Kabata-Pendias (2000) pointed out that excessive intake or presence of these crucial components due to environmental pollution can lead to toxicity for both humans and ecosystems.

Calcium (Ca), being the most abundant mineral, is crucial for numerous physiological functions in the human body (Tandoğan & Ulusu, 2005). According to Pravina et al. (2013), some of the crucial roles of Ca in the framework of bodily operations are blood coagulation, sending nerve impulses, rhythm regulation of the heart, muscle contraction, and development of bones and teeth. Fish studies elicited that the concentration of Ca in the tissue depends on the species, size of the fish and the surrounding environment (Brucka-Jastrzębska et al., 2009; Davis & Boyd, 1978). According to Loewen et al. (2016), most of the Ca is associated with biomineralized structures, including otoliths, scales, fins, and bones. Besides, the intake of dietary components and the composition of water influence in absorption and retention of Ca and other minerals. Ca deficiency results in poor bone development and lower bone density, reflecting mild skeletal deformities (Lall & Kaushik, 2021). Ca and its substitutes, strontium and barium, integrate into the biomineral based on environmental factors, the rate of absorption, and physiological processes (Loewen et al., 2016).

McLean & Wang (2021) highlighted that potassium (K) plays an important role in osmolality, fluid balance and cellular processes. Potassium is readily available in the earth's crust, however, with increased sales of processed foods, this nutrient is often lacking in one's diet (Bhaskarachary, 2011; He & MacGregor, 2008). For a long time, adequate potassium intake has been associated

with several health advantages such as reduced blood pressure, a lower risk of cardiovascular disease, and improved kidney function (He & MacGregor, 2008; J. A. McCormick et al., 2023). Potassium homeostasis is essential for appropriate cell function. According to Udensi and Tchounwou (2017), imbalances in potassium can result in several illnesses, such as hyperkalaemia, hypokalaemia, hypertension, and chronic renal disease. He and MacGregor (2008) stated that potassium also supports healthy bones, glucose tolerance, and it can help avoid fatal ventricular arrhythmias.

Magnesium (Mg) is an essential mineral crucial for numerous physiological processes in humans, animals, and plants. It is a crucial cofactor in more than 300 enzymatic reactions that is important for metabolic processes, bone strength, muscle performance, and heart health (Pinotti et al., 2021; Schwalfenberg & Genuis, 2017). In clinical medicine, magnesium levels are frequently disregarded despite their significance (Reddy et al., 2018). Even though magnesium deficiency is significant, it is common, as almost 66% of Western individuals do not consume the recommended daily amount (Schwalfenberg & Genuis, 2017). This lack of nutrients can result in a range of health problems, such as muscle weakness, exhaustion, and heart function impairment (Ismail & Ismail, 2016).

Sodium (Na), the second alkali metal on the periodic table, is extremely reactive and commonly utilised in multiple industries (Kale & Rajan, 2004). Sodium is used in various industries such as pharmaceuticals, herbicides, dyes, fragrances, and metal refining (Eggeman, 2007; Kale & Rajan, 2004). Sodium and potassium are crucial for the functioning of the nervous and muscular systems, and their levels are maintained by intricate physiological processes (Pohl et al., 2013). In the past, sodium in the form of salt was commonly used to preserve food and add flavour, but nowadays, many countries are working to decrease sodium levels in processed foods due to alarming health concerns

(Doyle & Glass, 2010). The high levels of salt consumption are linked to hypertension and other health problems, prompting suggestions for decreased intake (Doyle & Glass, 2010).

Microelements, or trace elements, are necessary in minute quantities but are just as important for metabolic purposes. Still, many microelements, particularly cadmium and lead, are harmful at high concentrations (Satarug et al., 2020). The tendency of these elements to bioaccumulate in living tissues leads to health problems that affect neurotoxicity, reproduction, and have negative effects on organs. Cadmium (Cd) is a silvery-blue, naturally occurring metal element that ranks 67th abundant in the lithosphere of the Earth and is broadly utilised in industry despite being toxic (Borsari, 2011, 2014). It is mainly present as a contaminant in copper, lead and zinc mineral deposits and is obtained as a secondary product while they are being processed (Morrow, 2010). Concerns regarding the environmental consequences and health dangers of Cd have resulted in attempts to limit its utilisation. According to Mainier et al. (2013), Cd is the 7th most harmful substance to human health, impacting various bodily systems. It builds up in the body over time, staying for a prolonged period and leading to the possibility of different types of cancer, kidney damage, weakened bone (osteoporosis), and various other health problems (Genchi et al., 2020; Jarup, 2003). Industrial use, improper disposal of Cd-containing products, and the utilization of phosphorus fertilisers and wastewater sludge in agriculture have led to a rise in environmental Cd contamination (Jarup, 2003). New research indicates that negative health impacts could happen at lower levels of exposure than previously believed, underscoring the importance of more stringent rules and awareness for the use of Cd (Jarup, 2003).

As stated by Aslam et al. (2022), chromium (Cr) does not exist purely in nature, is very stable, and is mainly present in the two oxidation states of chromium, Cr(III) and Cr(VI), in the environment. Even though Cr(III) is necessary

for regular metabolism, Cr(VI) is extremely harmful and has carcinogenic properties (Andleeb, 2015; Mandina & Mugadza, 2014). Cr(III) is essential for the metabolism of carbohydrates, lipids, and proteins, serving as a cofactor for insulin (Pechová & Pavlata, 2018). Khodavirdipour et al. (2020) added that supplementation with chromium may enhance insulin sensitivity, and metabolism of lipids, and potentially reduce symptoms of depression. Nevertheless, an overabundance of Cr can be harmful to both humans and animals. Cr(VI) as a pollutant is a significant concern because of its high solubility and ability to contaminate the food chain (Sharma et al., 2020; Ukhurebor et al., 2020). Cr can penetrate the human body via ingestion, skin contact, and breathing, leading to health problems like liver and kidney damage, skin ulcers, bronchial asthma, and cancer (Andleeb, 2015; Pokhrel & Pokhre, 2022). The World Health Organization (2011) has defined a temporary standard set at 0.05 mg/L for chromium in drinking water, while the acceptable range for the chromium content in fish feed ranging from 0.05 to 0.15 mg per kg of body mass (Aslam et al., 2022).

Natural occurrence, farming, and urban runoff are the various sources of copper (Cu) in European rivers according to Comber et al. (2022). Cu is also exposed in developed nations through food and water, such as plumbing leaching (WHO, 2011). In human health, Cu acts as a cofactor in many metalloenzymes and physiological processes; electron transport, mitochondrial functions, and enzyme activities (Vetchý, 2019; Xiang & Liu, 2021). It is also involved in myelination, synaptic activity, and neurotrophic factor signalling within the nervous system (Gromadzka et al., 2020). In fish, Cu as well as zinc and selenium, play a role in improving growth, disease resistance, and specific immunity when used in the right amounts (Bagheri et al., 2024). The regulation of Cu levels in the body is crucial because imbalances can result in serious health problems. A study by Xiang and Liu (2021), associated Cu with the

growth of tumours and the functioning of the immune system. Dabi (2020) also emphasized that human-caused sources have the potential to elevate copper levels in aquatic systems, possibly resulting in harmful impacts on aquaculture organisms.

Iron (Fe) ranks as the fourth most abundant metal in the crust of the Earth. Fe is crucial for biological systems due to its unique coordination chemistry and redox properties (Hasnain & Kanwal, 2020; Sánchez et al., 2017). As mentioned by Teixeira et al. (2012), its flexibility can be seen in different oxidation levels and forms: ferrous oxide (FeO), magnetite (Fe_3O_4), and ferric oxide (Fe_2O_3). Fe is crucial for the well-being of humans, as it is involved in various important functions such as transporting oxygen, making DNA, transferring electrons, creating red blood cells, enabling haemoglobin to function, and aiding in energy metabolism (Abbaspour et al., 2014; Gupta, 2014). According to Utkurovich (2021), Fe deficiency can cause anaemia, decreased ability to work, and increased vulnerability to illnesses, especially in kids. On the contrary, excessive accumulation of iron, known as Fe overload, can lead to tissue damage because of its capacity to create free radicals, which can raise the likelihood of cancer (Abbaspour et al., 2014; Mehboob, 2021). Pinyopornpanish et al. (2023) and Mehboob (2021) also reported that Fe overload also affects the liver and other organs such as liver cirrhosis, hepatocellular carcinoma, and diabetes mellitus; where the primary reasons are hereditary hemochromatosis, iron-loading anaemias, chronic liver conditions, and recurrent blood transfusions.

Manganese (Mn) is an essential microelement important for various enzyme functions as well as promoting immune cell growth and enhancing cellular immunity (Kippler & Oskarsson, 2024; Qi et al., 2024). Although Mn deficiency is not common, excessive exposure to the metal can result in serious health issues. Research on rats has found a connection between exposure to Mn and

immune system performance, with the amount of exposure and the duration of exposure being significant factors (Qi et al., 2024). Recently, Mn has become of interest because of its ability to cause neurotoxicity. In the Czech Republic, they are utilising waste from pyrite mining near Chvaletice to create high-purity Mn for electric vehicle batteries, showing the growing industrial need for this metal (Holý, 2022). According to Michalke et al. (2007), there is growing concern over how humans are exposed to Mn through environmental and occupational sources, like using methylcyclopentadienyl manganese tricarbonyl (MMT) in fuel additives. Clarke-Lambert et al. (2014) reported that high levels of airborne manganese from occupations such as mining, welding, and steel manufacturing can cause manganism, a condition with symptoms akin to Parkinson's disease. Various health conditions, such as hepatic cirrhosis, polycythemia, hypermanganesemia, dystonia, and symptoms similar to parkinsonism, have been associated with Mn poisoning (Chen et al., 2018).

Lead (Pb) has a long history as an anthropogenic source contaminant in Europe dating back to the Bronze Age (Kern et al., 2021). Humans are exposed to lead (Pb) through inhalation, ingestion, and skin contact from sources like food, water, air, tobacco smoke, dust, and soil (Gundacker et al., 2021). Nowadays, there is a trend of Pb exposure based on the country and its economic status. Lead paint and industrial emissions are still rampant in rich countries, while poor and average-income countries face more challenges due to their relaxed regulations (Obeng-Gyasi, 2019). Common sources of Pb are electronic waste, batteries, glazed ceramics, cosmetics, and traditional medicines; while mining, burning fossil fuels, and industrial activities are major human-caused sources (Evans et al., 2018; Flegal et al., 2013; Obeng-Gyasi, 2019). Fishing and the use of ammunition can lead to Pb exposure in water, according to Goddard et al. (2008), and the strategies to reduce this risk are creating al-

ternatives to lead in fishing gear, conducting educational programs, and enforcing rules to restrict the use of lead. Despite many prohibitions on lead in numerous items due to pollution and water consumption, it remains a threat. Several body systems, such as neurological, skeletal, and cardiovascular functions, can be interfered with, as Pb can accumulate in the bones, liver, and kidney (Collin et al., 2022; Gundacker et al., 2021). Kids are more susceptible than adults to health problems caused by lead poisoning and it can impair cognitive development (Ahmed et al., 2019; Green & Pain, 2019). Regular monitoring of lead levels in fish and health evaluation is essential in safeguarding aquatic ecosystems and public health (Nevárez et al., 2015; Zolfaghari, 2018). Treatments such as taking micronutrients and antioxidants are suggested, with chelation therapy being advised for those with high blood lead levels (Collin et al., 2022).

Strontium (Sr) is a trace element found in groundwater, commonly at elevated levels in areas with poor drainage and mineral breakdown containing Sr (D. Li et al., 2021). Slag piles can seriously contaminate soil and groundwater; based on source identification studies by Ding et al. (2021), they found that Sr slag piles and industrial operations are significant sources of Sr pollution in the environment. Research done on rainbow trout by Klaczek et al. (2022) highlighted that the water calcium levels can impact Sr accumulation in fish, altering sodium concentrations and enzyme activities, where the buildup of Sr in both plasma and gills rises as the waterborne Sr concentration increases. While exposure of mussels to Sr causes oxidative stress, DNA damage, and dysfunction in cells (Z. Cheng et al., 2024). In human however, Sr is essential for maintaining and repairing bone health. It hinders the breakdown of bone tissue and encourages the formation of new bone, resulting in higher bone mineral density (Cheng et al., 2019; You et al., 2022).

Zinc (Zn) has numerous physiological roles, including cell division, metabolism, and immune responses in human (Kiouri et al., 2023). According to Hussain et al. (2022), approximately two billion people globally are zinc-deficient, and this causes oxidative and organ damage. Contrary to popular belief, it is zinc that has a special significance to cerebral functions and neurodevelopment; scarcity and abundance of zinc influence neurologic disturbances (Choi et al., 2020). Zinc deficiency has shown a correlation to autism spectrum disorder (ASD), determination of neural connections and neurotransmission (Khalil & Yazbek, 2021). Research by Kiouri et al. (2023) shows that Zn has overall therapeutic effects in various diseases, including cancer and COVID–19. Biofortified food is considered a better choice than chemical supplementation (Hussain et al., 2022). While, excessive zinc intake can cause toxicity, with symptoms including fever, difficulty breathing and nausea (Hussain et al., 2022). Environmental zinc toxicity, however, can similarly alter the diversity of microbial populations in soil and water, where some bacterial species present promise as suitable candidates for bioremediation (S. Hussain et al., 2022).

2.4.2. Bioaccumulation and Biomagnification of Heavy Metals

Bioaccumulation occurs when an organism accumulates chemicals from its environment and food by taking in more than it eliminates, resulting in higher levels in its tissues than in the surrounding medium (Daley et al., 2014; Popek, 2018; Schäfer et al., 2015). Bioaccumulation trends differ based on ecosystem and species. Wang and Rainbow (2008) highlighted that, in the case of different bivalve species and between freshwater and marine species, studies have shown variations in metal accumulation. Besides that, Bawuro et al. (2018) pointed out that accumulation patterns differ between species and are affected by factors like age, geographical range, and species-specific traits. Another study over an extended period of molluscs and fish in coastal areas in the Black

Sea (Romanian sector) shows inter- and intraspecific variations due to factors like diet, environment, age, season and location (Oros & Gomoiu, 2011). Moiseenko and Gashkina (2020) added that climatic factors and environmental factors (organic matter content, pH, salinity, and calcium concentrations) can also influence the bioavailability and buildup of metals. While the accumulation of substances can be different between pelagic and benthic routes (Mendoza-Carranza et al., 2016).

A process by which toxins accumulate progressively at higher concentration levels towards the top of a food chain is known as biomagnification, which indicates that the animals that are at the very apex of the food pyramid can have higher concentrations of toxins than those lower down the pyramid (Popek, 2018; Thanigaivel et al., 2022). The extent of biomagnification can be influenced by various factors like latitude, temperature, and the characteristics of the ecosystem (Córdoba-Tovar et al., 2021). For instance, mercury concentrations tend to increase as they travel through the food chain, especially in predatory fish (Moiseenko & Gashkina, 2020; Yang et al., 2023). On the other hand, metals like cadmium, copper, and lead often show lower concentrations at higher levels—a phenomenon called biodilution (Verstijnen et al., 2024; Yang et al., 2023). According to Bhup et al. (2011), they found that certain metals, including iron, zinc, and copper, are known to be present in large amounts, whereas cadmium and lead are typically present in smaller amounts. Mendoza-Carranza et al. (2016) stated that high levels of zinc are prevalent in various segments of the food chain, with crustaceans consuming plankton and other foods. While smaller plankton tend to have greater levels of metals in their bodies, except for cadmium, as noted by Chouvelon et al. (2019).

Heavy metals can seriously harm fish, ecosystems, and even pose risks to human health when they enter our food supply (Bashir et al., 2020). Some research has found that metal concentrations in water are within safe limits for

human consumption. However, in sediments, these concentrations often exceed acceptable levels, potentially leading to ecological hazards (Jitar et al., 2015; Mendoza-Carranza et al., 2016). While some studies report metal levels below safety thresholds, others have identified concentrations such as lead that surpass safe standards (Bawuro et al., 2018; Bhup et al., 2011). To address these environmental and health concerns, it is crucial to manage wastewater from households and industrial discharges effectively (Murtala et al., 2012).

2.4.3. Mechanisms of Bioaccumulation in Fish

Uptake pathways

Heavy metals build up in organisms through various mechanisms, such as active transport and ion exchange, which enable their movement along the food chain (Emon et al., 2023). Metals are primarily absorbed by fish through the gills, digestive system, and skin, with each metal showing a preference for certain tissues. In freshwater fish, these organs are particularly vital for managing ion levels and maintaining acid-base equilibrium. The gills, with their specialised cells, play a key role in absorbing ions and regulating pH, while the kidneys contribute to overall pH balance by controlling how much bicarbonate is reabsorbed (Perry et al., 2003). It is also a sensitive organ critical for breathing, osmoregulatory processes, acid-base regulation, and excretion of nitrogenous waste, and serves as the main entry point for toxic substances (Evans et al., 2005). Similarly, as the kidney filters blood, it encounters various waste products and toxins that the fish needs to expel (Zeitoun & Mehana, 2014). This combined action of the gills and kidneys helps the fish adapt to changes in their aquatic environment. The liver functions as a primary site of bioaccumulation, largely due to its detoxification role and extensive blood supply (Laurén & Wails, 2018).

Tissue-Specific Accumulation Patterns

Studies have shown that different fish species accumulate metals in their organs in unique ways, with the liver and gills often being primary areas of focus (Bawuro et al., 2018; Murtala et al., 2012). Higher concentrations tend to accumulate in active metabolic tissues, like the gills, liver and kidneys, while muscles show lower accumulation levels (Subotić, Spasić, et al., 2013b; Zeitoun & Mehana, 2014). Metals like arsenic, nickel, and zinc tend to accumulate in specific patterns across species, particularly in the liver and kidneys (Ferreira et al., 2019; Kousar & Javed, 2014). The gills, liver, and kidneys are critical sites where heavy metals tend to accumulate (Shahjahan et al., 2022b). Although muscle tissue accumulates fewer heavy metals than organs like the liver or gills, monitoring muscle concentrations remains essential given its significance as a food source for humans (Castro-González & Méndez-Armenta, 2008). In fish, metals do not distribute evenly across organs. For instance, mercury is primarily found in the liver and muscles, while cadmium tends to concentrate in the kidneys and gills. Lead, on the other hand, is often detected in both the kidneys and liver (Moiseenko & Gashkina, 2020). Generally, the liver shows the greatest accumulation of many toxins when examining fish body parts (Ferreira et al., 2019; Kousar & Javed, 2014; G. Wang et al., 2017). Although the bioaccumulation factor (BCF) differs among various tissues and types of substances, it is often highest in the digestive organs (Ferreira et al., 2019; Zhao et al., 2015). Studies from various regions have shown that metal accumulation in fish tissues often exceeds safe limits (Hussain et al., 2021; Perumal et al., 2023). Despite the lower metal concentrations in fish muscles than those in the liver or kidneys, they can still pose health risks to people who eat them (Al-Mayahi' et al., 2020; Hussain et al., 2021). Thus highlighting how essential it is to regularly monitor to ensure the safety of fish for human consumption.

2.4.4. Role of Fish as Bioindicator of Trace Element Contamination

As the final link in the aquatic food chain, fish can accumulate persistent synthetic chemicals and heavy metals, reflecting levels of environmental pollution and posing potential risks to humans who consume them (Tashla et al., 2018). When subjected to elevated levels of heavy metals, fish respond through complex molecular mechanisms, which include alteration in gene expression responsible for the absorption, distribution, storage, and release of these metals (Tripathi & Poluri, 2021). Utilising fish as bioindicators allows us to monitor stress responses at various biological levels, providing valuable insights into sublethal effects and the relationships between pollutants and their impacts on ecosystems (Adams et al., 1989). Studies have shown that fish often store metals like cadmium, chromium, copper, lead, mercury and zinc in their organs, with concentrations frequently exceeding those present in their surrounding water (Ali et al., 2020; Łuczyńska et al., 2018).

Consuming polluted fish can lead to serious health threats to humans, with studies linking metals like lead and chromium to both cancerous and non-cancerous effects (Panda et al., 2023). These metals can negatively impact fish growth, reproduction, and overall physiology (Emon et al., 2023; Shahjahan et al., 2022b). Changes in blood composition and biochemistry can be caused by trace element toxicity, which will lead to abnormal cell formation and result in specific organ diseases (Shahjahan et al., 2022b). Moreover, heavy metals can interfere with early developmental phases, impair reproductive functions, and lead to irregularities in reproductive systems (Shahjahan et al., 2022b). Researchers utilise bioindicators to evaluate indicators of DNA damage, enzyme function, lipid processing, and histopathological alterations, all of which reflect the overall health of aquatic ecosystems (Adams et al., 1989; Tashla et al., 2018). Fish play an essential role as bioindicators for assessing environmental risks and anticipating future patterns in water pollution. A thorough

understanding of these mechanisms is crucial for crafting effective bioremediation plans and safeguarding aquatic habitats.

3. MATERIALS AND METHODS

3.1. Sampling Area and Fish Collection

The Szamos River, referred to as Someş in Romanian, is the second-biggest tributary of the Tisza River. Stretching a total distance of 415 km, most of the river is in Romania, while around 50 km flows through Hungary. Historically, Romania faced multiple pollution events, positioning the Szamos River among the most severely affected rivers in Europe by contamination (Kraft et al., 2003, 2006). During the beginning of the 2000s, cyanide and heavy metals were introduced into the sediments and water of the river from mining operations in Romania, where the primary contaminants, such as lead (Pb), cadmium (Cd), copper (Cu), and zinc (Zn), originate from extensive mining activities in the region also impacted the Tisza River (Antal et al., 2013; Simon et al., 2017a). Research revealed that the Szamos River contained significantly greater strontium levels than the Upper Tisza River, resulting in its consideration for this study (Málnás et al., 2014a; Simon et al., 2017a). The fish samples were collected in November 2013 (autumn), where age determination was based on growth assessment methods referenced in the literature (Epler et al., 2009; Vlach and Dusek, 2005). The sampling location was in the Csenger area, close to the border between Hungary and Romania (47°50'17.89"N, 22°41'37.48"E), as shown in Figure 1(A).

The Maros River is the largest tributary of the Tisza River. The 750km long river drains the Transylvanian basin westward and reaches the Tisza River at Szeged. It is one of the most important rivers in the Carpathian basin and covers approximately 30,332 km² of the drainage basin (ICPDR, 2019). Most of its drainage area is covered by mountains and hills, with only a tiny section being flat. The rocks of the terraces are made up of coarse sediments and clay surface erosion deposits. Maros River is also popular for recreational fishing among anglers. Following the previous studies (Kovács & Kiss, 2010; Nyeste

et al., 2019), there was evidence of recent pollution in the Maros River. Hence, it was selected as our study site. The sampling work was done during October 2020 (autumn) for two days along the Maros River near Makó, near the Romanian border (46°12'12.45"N, 20°27'14.11"E), as shown in Figure 1B.

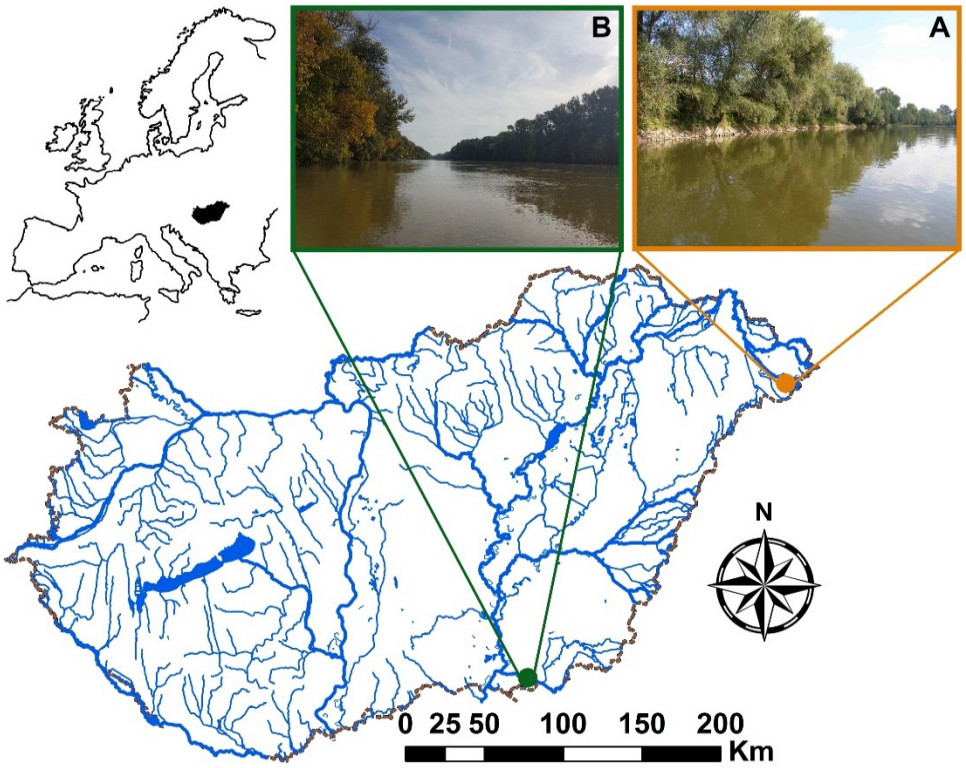

Figure 1. Map of the sampling sites. A: Szamos/Someş river near Csenger, B: Maros River near Makó.

Fish were captured using an electrofishing device (Hans Grassl IG200/2b, PDC, operating at 75–100 Hz, 350–650 V, with a maximum output of 10 kW; Hans Grassl GmbH, Germany). The captured fish were euthanised on-site immediately after capture using clove oil and brought to the laboratory, where the samples were refrigerated at -18 ºC. Ethical approval for the experimental protocol and endpoints was granted by the Workplace Animal Experiments Committee of the University of Debrecen (approval number: HBH/01/00971-

2/2013). All procedures were conducted following appropriate national and international protocols and regulations. The study adhered to the principles as detailed in the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines.

3.2. Fish Biological Characteristics

Adult fish (Maros River)

One hundred adult specimens were collected (n = 100), with ten individuals per species: asp, barbel, catfish, chub, common carp, common nase, silver carp, common bream, white-eye bream, and zander. The characteristics of sampled fishes are presented in Appendix 1.

Juvenile fish (Szamos River)

Fifteen cyprinid fish were examined (n = 15), with five individuals per species. The standard length and body mass (mean ± standard deviation) for the juveniles were determined as follows: barbel (52.1 ± 2.4 mm; 2.56 ± 0.32 g), chub (60.6 ± 5.1 mm; 4.06 ± 1.23 g), and nase (72.3 ± 3.8 mm; 5.57 ± 1.10 g). Based on these measurements, the fish were classified as belonging to the 0+ age group, indicating juveniles (Epler et al., 2009; Vegh et al., 2020).

3.3 Sample Processing and Element Analysis

Prior to dissection, the beakers are soaked overnight in 1% nitric acid, rinsed thoroughly with distilled water, dried, and labelled accordingly. It is a crucial step for precision testing and to avoid cross-contamination. The mass of the clear and dry beakers was measured and recorded. After that, the samples from the refrigerator were thawed to room temperature until the sample was manageable. The standard length (SL), total length (TL), and body mass (M) of each fish were recorded to the nearest 0.1 mm and 0.01 g, respectively. Following that, plastic instruments and gloves were used to extract the tissue samples

(muscle, gills, and liver) to prevent any contamination by metals. The left side of each fish was prepared for tissue sampling by carefully removing scales and skin along the dorsal region above the dorsal line. A cube-shaped section of muscle tissue (approximately 1–2 cm^3) was excised. Liver samples of similar dimensions (1–2 cm^3) were also collected. Gills were sampled by removing part of the branchial system (gill filament and gill arch) and extracting the second gill arch on the left side.

Before the weighing process, the dissected samples were rinsed with double-deionised water (Milli-Q) and then placed into glass beakers on an analytical balance (Precisa 240A). They were dried overnight at 105 °C until a constant mass was achieved, and the dried samples were then weighed to determine their dry mass. In the same container, samples were digested for 4 hours at 80 °C on an electric hot plate with 4.0 mL of 65% nitric acid (m/m, reagent grade, Merck) and 1.0 mL of 30% hydrogen peroxide (m/m, reagent grade, Merck). The samples, once digested, were transferred into volume-calibrated plastic centrifuge tubes and diluted to a final volume of 10 mL containing 1% nitric acid (m/m, reagent grade, Merck) and Milli-Q water (Braun et al., 2009). The quantitative evaluation of trace element levels in the samples was analyzed with an Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES 5100, Agilent Technologies). The ICP-OES measurement parameters and operating conditions are outlined in Appendix 2.

The macroelements (Ca, K, Mg, Na) were prepared from a 1000 mg L^{-1} mono-element spectroscopic standard (Scharlau), and the trace elements (Cd, Cr, Cu, Fe, Mn, Pb, Sr, Zn) from a 1000 mg L^{-1} multi-element spectroscopic standard solution (ICP IV, Merck) (Mester et al., 2022). A 5-point calibration process was employed in both cases, with standard solutions diluted using 0.1 M HNO3 prepared in ultrapure water (Mester et al., 2022). Limits of detection (μg L^{-1}) and the average detection limits (mg kg^{-1} wet weight) applied were as

follows: Ca (0.070; < 0.001), Cd (1.863; 0.009), Cr (0.489; 0.004), Cu (0.414; 0.003), Fe (0.856; 0.006), K (0.270; 0.002), Mg (0.080; 0.001), Mn (0.057; < 0.001), Na (0.484; 0.004), Pb (1.235; 0.009), Sr (0.051; < 0.001), and Zn (3.125; 0.023). The detection limit for each sample was established based on the instrumental detection limit using the diluted sample mass and volume.

The setup included an autosampler (SPS 3, Agilent Technologies), a Meinhard-type nebuliser, and a double-pass spray chamber (Herman et al., 2011). A five-point calibration approach was applied based on the ICP multi-element standard solution IV (Merck) (Herman et al., 2011; Nyeste et al., 2019). The measurement process involved the use of certified reference material (ERM-BB422, fish muscle) (Herman et al., 2011; Nyeste et al., 2019). The metal recoveries were found to be within 10% of the certified values, and the wavelengths and measuring parameters were selected according to the software of instrument (MP EXpert) guidelines. All element concentrations were presented as mg kg^{-1} wet weight (ww).

3.4. Statistical Analysis

The bioconcentration factor (BCF) refers to the ratio of the concentration of a chemical in an organism (or a specific tissue) compared to its concentration in the water or sediment (Ivanciuc et al., 2006; Nyeste et al., 2019, 2024):

$$BCF_{water\ or\ sediment} = C_{fish} / C_{water\ or\ sediment}$$

where C_{fish} refers to the concentration of the chemical in the organism's whole body or tissue (mg kg^{-1} wet weight), C_{water} is the concentration in the water (mg L^{-1}), and $C_{sediment}$ is the concentration of the trace element in the sediment (mg kg^{-1} dry weight). Data on trace element concentrations in sediments and water were sourced from the National Environmental Information System of Hungary (OKIR in Hungarian) database. The concentrations were evaluated against the

chronic concentration criteria (CCCs) for freshwater set by the National Recommended Water Quality Criteria (USEPA, 2017).

Metal pollution index (MPI) was assessed to compare the total content of trace elements excluding the macro elements (Cd, Cr, Cu, Fe, Mn, Pb, Sr, Zn) for the different tissues of each specimen. The following formula represents the MPI (Ju et al., 2017; Nyeste et al., 2019; Usero et al., 1997):

$$MPI = (C_1 \times C_2 \times C_3 \times \ldots C_n)^{1/n}$$

where C_n indicates the mean concentrations of trace element *n* in the examined tissue (mg kg^{-1} wet weight).

The statistical analysis was conducted using IBM SPSS Statistics (Version: 28.0.11 (14)) for Mac (*IBM*, 2011) and Past 3.03 (Hammer et al., 2001) software with the Shapiro-Wilk test applied to test for normal distribution. Levene's test (Levene, 1960) was used to assess the homogeneity of variances. Due to the lack of normality in the data (Shapiro-Wilk test, $p < 0.05$), the non-parametric Kruskal-Wallis test was applied to compare the concentrations and BCF values of elements in the muscle, gills, and liver of each juvenile species (Habib et al., 2022; Nyeste et al., 2019). As a post hoc test, the Mann-Whitney U test was used to investigate the significant differences between the groups (Habib et al., 2022). With the macroelements excluded, Principal Component Analysis (PCA) was used to examine the differences between species based on the concentration of trace elements (Cd, Cr, Cu, Fe, Mn, Pb, Sr, Zn) in the gill, liver and muscle of the juveniles and adults. The potential health risks of consuming these fish species were assessed, and the concentrations of trace elements in muscle tissue were compared with the maximum acceptable concentrations (MACs) established by the European Union (European Union, 2008) and the Food and Agriculture Organization of the United Nations (Nauen, 1983).

4. RESULTS

4.1. Bioaccumulation Patterns in Juvenile Fish (Szamos River)

4.1.1. Trace Element Levels in Sediments and Water

The trace element concentrations in sediment and water for 2013 were sourced from the National Environmental Information System of Hungary (OKIR) from previous studies and were summarised in Appendix 3 and Appendix 4 (Málnás et al., 2014; Simon et al., 2017b).

Following the National Recommended Water Quality Criteria guidelines (USEPA, 2017), it is noted that the average concentrations of Cu, Pb, and Zn exceeded the chronic criterion concentrations (CCCs) for freshwater. Although the annual average concentration of Cd remained below the threshold, the monthly average concentrations exceeded the CCCs multiple times throughout 2013. The trace element levels in sediments showed that the average concentration of Cr indicated moderate pollution in the sediments. Meanwhile, the average concentrations of Cu, Mn, and Zn classified the sediment as highly polluted based on sediment toxicity classifications (Baudo & Muntau, 2020).

4.1.2. Overview of Trace Element Concentrations in the Tissues of Juveniles

The concentration of macroelements (Ca, K, Mg, Na) and trace elements (Cd, Cr, Cu, Fe, Mn, Pb, Sr, Zn) in the muscle, gill and liver tissues is separately assessed due to their distinct physiological roles and bioaccumulation characteristics.

For the macroelements, the Kruskal-Wallis test revealed significant differences ($p < 0.05$) (Appendix 5) across the three species, and tissues: muscle (K, Mg, Na), gills (Ca and Mg), and liver (K, Mg and Na). For muscle, barbel consistently exhibited lower concentrations of essential macronutrients (K and Mg) compared to nase and chub, while chub showed the highest concentrations

(Ca and Na). Trace elements like Zn and Cr were significantly higher in barbel, while nase showed elevated levels of Sr concentrations. Among the gills, chub exhibited the highest concentrations of Ca, and barbel exhibited the lowest concentrations of Mg. The liver of nase contained significantly higher concentrations of K, Mg, and Na than that of chub.

In the case of essential and non-essential elements, across all tissues, the liver had the highest concentrations of Cd, Cr, and Pb, whereas the gills contained the most Fe, Mn, and Sr. The highest concentrations of Cu and Zn were recorded in the liver of nase and barbel, respectively, while chub showed the highest Zn levels in the gills. However, Cu concentrations in the gills of the nase were lower than those in the chub. Among all tissues, the lowest levels of trace elements were consistently noted in the muscle across all species analysed. The concentrations of Cd were below the detection limit in the muscle, gills, and liver of nase, as well as in the muscle and gills of barbel

Statistical analysis using the Kruskal–Wallis test revealed significant interspecific differences in trace element levels. Subsequent pairwise comparisons in muscle, significant variation ($p < 0.05$, Mann–Whitney U test) was observed for Cd, Cr, Cu, Fe, Mn, Sr, and Zn, while in gills, significant differences were noted for Cd, Cr, Cu, Mn, Sr, and Zn (Appendix 5). In the liver, Cd, Cr, Cu, Fe, and Pb concentrations varied significantly among species. In muscle, barbel exhibited the highest concentrations of Cr and Cu, nase showed the highest Sr concentrations, and chub demonstrated the highest Cd concentrations ($p < 0.05$, Mann–Whitney U test) (Appendix 5). The concentrations of Mn were the lowest in chub, and for Zn, the lowest values were recorded in nase. In gills, chub exhibited the highest levels of Cd and Zn, while the lowest concentrations of Cr were recorded in chub ($p < 0.05$, Mann–Whitney U test) (Appendix 5). Nase displayed the lowest levels of Cu and Zn, and barbel showed the lowest Sr concentrations in gills ($p < 0.05$, Mann–Whitney U test) (Appendix 5). In

liver tissue, the nase had the greatest concentrations of Cu, while nase also exhibited the lowest concentrations of Cd ($p < 0.05$, Mann–Whitney U test), with the lowest levels of Cr and Pb in the chub ($p < 0.05$) (Appendix 5).

4.1.3. Correlational Analysis of Juvenile Fish Species

A notable decline ($p < 0.05$, Mann–Whitney U test) in certain macroelement concentrations was observed with increasing trophic levels (Appendix 6). Specifically, trophic levels were inversely associated with K in muscle, Na in liver, and Mg in muscle and gills.

Further analysis indicated that the concentrations of certain trace elements demonstrated a significant difference associated with trophic levels. Specifically, a notable pattern of the metal concentration was observed in muscle (Cr, Cu, Fe, and Zn), and gills (Cu and Zn) exhibited a positive correlation with increasing trophic levels. Conversely, the metal concentrations in the muscle and gills (Sr) and liver (Cu) decreased significantly with increasing trophic levels. These findings stress the influence of trophic levels on the concentration patterns of trace elements in fish tissues.

4.1.4. Principal Component Analysis (PCA) of Juvenile Fish Species

The PCA revealed distinct separations among the species based on trace element patterns in muscle and liver shown in Figure 2 and Figure 4, respectively. In muscle, PCA1 explained 58.85% of the data variation, and PCA2 explained 20.75% of the data variation (Figure 2). In the liver, PCA1 explained 44.92% of the data variation, and PCA2 accounted for 29.58% (Figure 4). In gills, PCA 1 explained 53.72% of the total variance, while PCA2 accounted for 20.61% (Figure 3). The trace element profiles in gills displayed clear species-specific separations, though nase showed slight overlap with barbel and

chub. The PCA plot with the associated Spearman's rho values is shown in each figure.

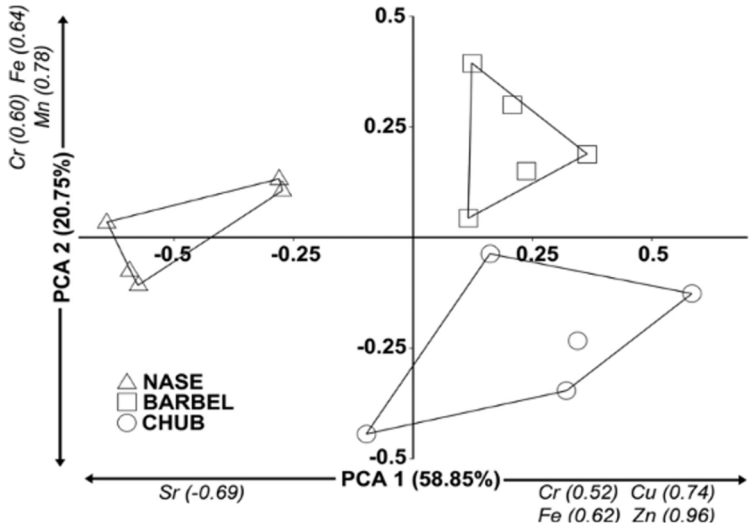

Figure 2. The PCA plot showing the trace element concentrations (mg kg^{-1}, wet weight) in muscle of the juveniles from the Szamos River.

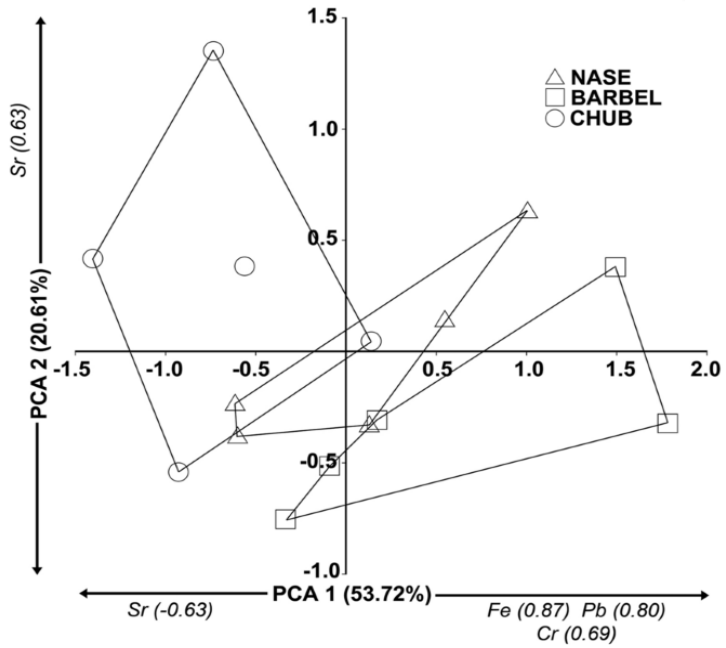

Figure 3. The PCA plot showing the trace element concentrations (mg kg^{-1}, wet weight) in gills of the juveniles from the Szamos River.

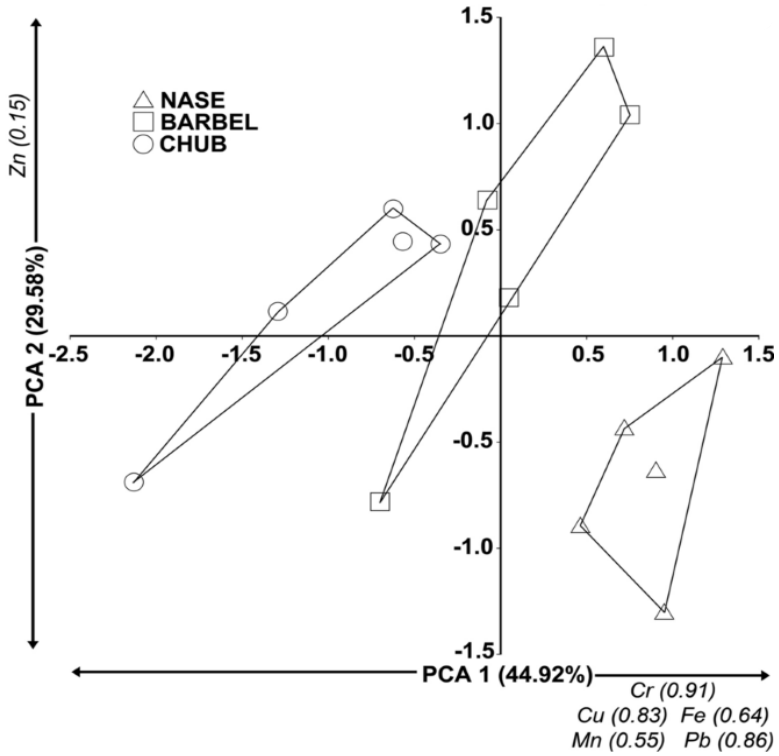

Figure 4. The PCA plot showing the trace element concentrations (mg kg^{-1}, wet weight) in liver of the juveniles from the Szamos River.

4.1.5. Bioconcentration Factors for Juvenile Fish Species

The bioconcentration factors for juveniles for sediments and water are summarised in Appendix 7. Based on the Mann-Whitney U test ($p < 0.05$) for the water, there are significant differences in the following elements with the most elevated BCF values in their respective tissues: liver (Cd, Cr, Cu, and Pb) and gills (Fe and Mn). The highest value of BCF in muscle for water is observed in chub (Cd) and barbel (Cr and Cu). Conversely, the Mann-Whitney U test ($p < 0.05$) shows significant differences for the lowest BCF values in the water of the muscle in chub (Mn), and nase (Zn). The most elevated BCF values in water for the gills are presented in chub (Cd and Zn) and barbel (Cr), while the lowest is found in nase (Cu and Zn). In the case of the liver, there are significant differences ($p < 0.05$, Mann-Whitney U test) shown in the highest BCF in

water was found in nase (Cu), while the lowest values were in nase (Cd) and chub (Cr). The same trends is observed in sediments based on the Mann-Whitney U test ($p < 0.05$) for the BCF values, where additionally, the gills have the highest BCF for sediments for Sr.

4.1.6 Metal Pollution Index (MPI) in the Tissues of Juvenile Species

The MPI values are the highest in the muscle and gills of barbel, and nase is the highest in the liver (Figure 5). Contrary to that, chub exhibited the lowest MPI values across all analysed tissues.

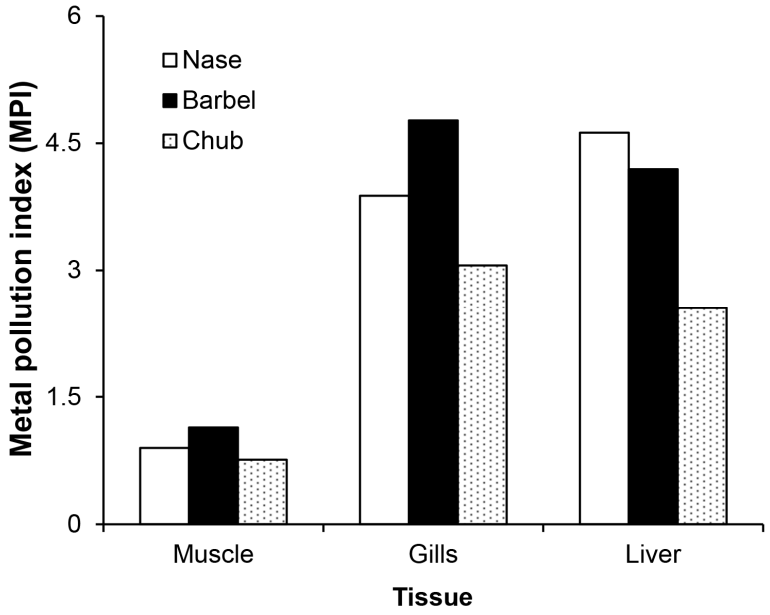

Figure 5. MPI in the different tissues of cyprinid juveniles from the Szamos River.

4.2. Bioaccumulation Patterns in Adult Fish (Maros River)

4.2.1. Trace Element Concentrations in Water

Summary statistics of the overall trace element concentrations in water samples from the Maros River are presented in Appendix 8. The data were obtained from OKIR and other previous studies (Málnás et al., 2014b; Simon et al.,

2017a). The threshold limits were obtained from the National Recommended Water Quality Criteria (EPA, 2018). Among the analysed elements, concentrations of iron (Fe) were notably elevated, with mean values reported to have exceeded the threshold by a substantial margin, indicating potential contamination. While the following trace elements are below the threshold values: Cd, Cr, Cu, Mn, Pb and Zn.

4.2.2. Overview of Trace Element Concentrations in the Tissues of Adults

The macronutrients (Ca, K, Mg, Na) and the micro trace elements (Cd, Cr, Cu, Fe, Mn, Pb, Sr, Zn) in the muscle, gill and liver are separately assessed due to their distinct physiological roles and bioaccumulation characteristics (Appendix 10). For muscle, barbel consistently exhibited lower concentrations of essential macronutrients (K and Mg) compared to nase and chub, while chub showed the highest concentrations (Ca and Na). Trace elements like Zn and Cr were significantly higher in barbel, while Sr concentrations were notably elevated in nase. In gills, chub exhibited the highest Ca concentrations and barbel the lowest Mg concentrations. In the liver, nase had significantly higher concentrations of K, Mg, and Na compared to chub.

The Mann-Whitney U test revealed significant interspecies variation ($p < 0.05$) for all of the elements in the muscle, gills and liver. Overall, the muscle exhibits the lowest concentrations for all the analysed elements. The highest concentrations in the gill were found in Cr (asp), Mn (white-eye bream), and Sr (asp). Meanwhile, in the liver, the concentrations are the highest in Cu (silver carp), Cd (bream), Fe (silver carp) and Zn (carp). Cd concentrations remained below the detection limit (BDL) in muscle and gill tissues, Pb was BDL across all sampled tissues. The white-eye bream has the highest concentration of most of the elements, while in contrast, the zander has the lowest concentrations in most of the studied elements.

4.2.3. Principal Component Analysis (PCA) of Adult Fish Species

(A) Muscle

The principal component analysis (PCA) of ten fish species for muscle yielded two components that explained 84.22% of the variation (Figure 6). PCA1 accounted for 59.68% of the variation and was primarily influenced by Fe and Zn concentrations, while PCA2 accounted for 24.54% of the variation, strongly influenced by Sr. The PCA revealed a clear separation of the two piscivorous species, catfish and zander, from the other species, with catfish associated with lower Fe and zander with lower Mn concentrations (Figure 6 and Appendix 10). Among the remaining eight species (asp, barbel, chub, common carp, common nase, common bream, silver carp, and white-eye bream), two main overlapping groups were observed. Chub, nase, and barbel clustered near the origin, indicating relatively similar metal profiles, whereas asp, bream, and silver carp were more widely dispersed, reflecting higher differentiation in trace element concentrations. White-eye bream and common carp formed two distinct clusters along the PCA2 axis, differentiated by high Fe concentrations, while variations along PCA1 were associated with high Sr (white-eye bream) and Zn (common carp) levels, respectively (Figure 6 and Appendix 10).

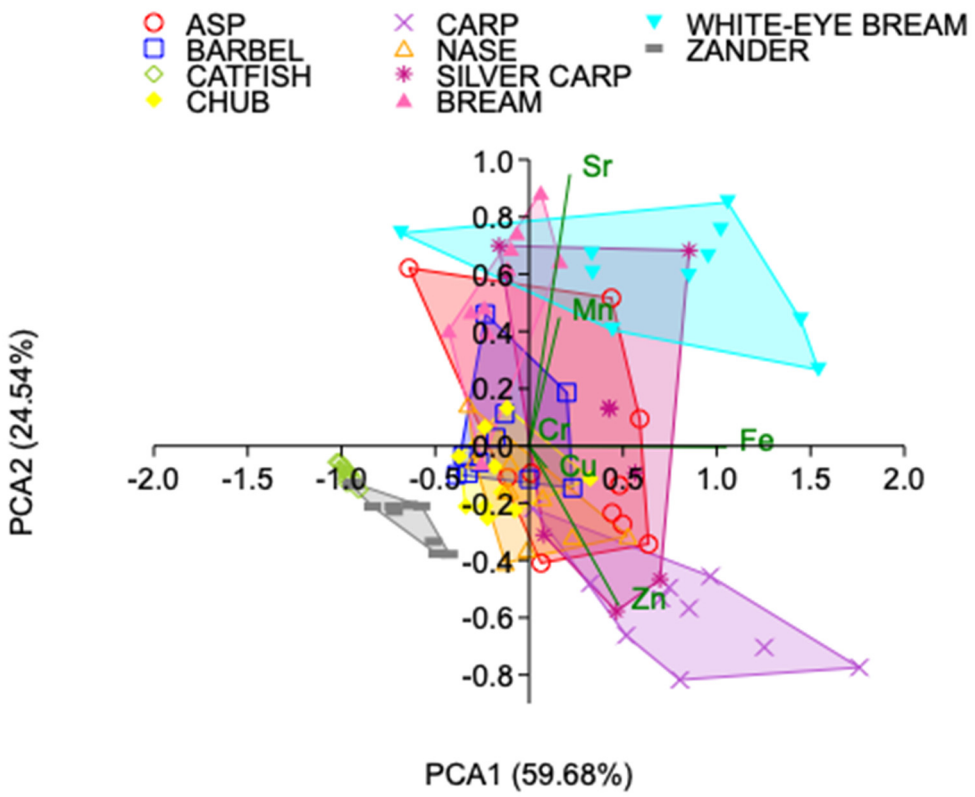

Figure 6. The PCA plot of the concentration of trace elements in the muscle (mg kg^{-1}, wet weight) of the adult species from the Maros River.

(B) Gills

PCA identified two principal components that summarized 74.73% of the variation in gills tissue (Figure 7). PCA1 accounted for 47.32% of the variation and was primarily influenced by Zn concentrations, whereas PCA2 accounted for 27.41%, mainly influenced by Mn, followed by Sr and Fe. The analysis revealed a clear separation of two omnivorous species, chub and common carp, from the rest, with chub associated with the highest Zn concentrations (Figure 7 and Appendix 10). Piscivorous species were generally clustered near the origin, although catfish (highest Fe) and zander (lowest Sr) formed distinct subgroups. Asp, characterized by high Cr and Sr but low Fe, overlapped partially with both catfish and zander. Among the omnivorous species, common

bream overlapped with zander and asp, while barbel (lowest Zn), white-eye bream (highest Cu), and the two herbivorous species, nase (lowest Cr) and silver carp, were positioned closely together, reflecting similar metal accumulation patterns (Figure 7 and Appendix 10).

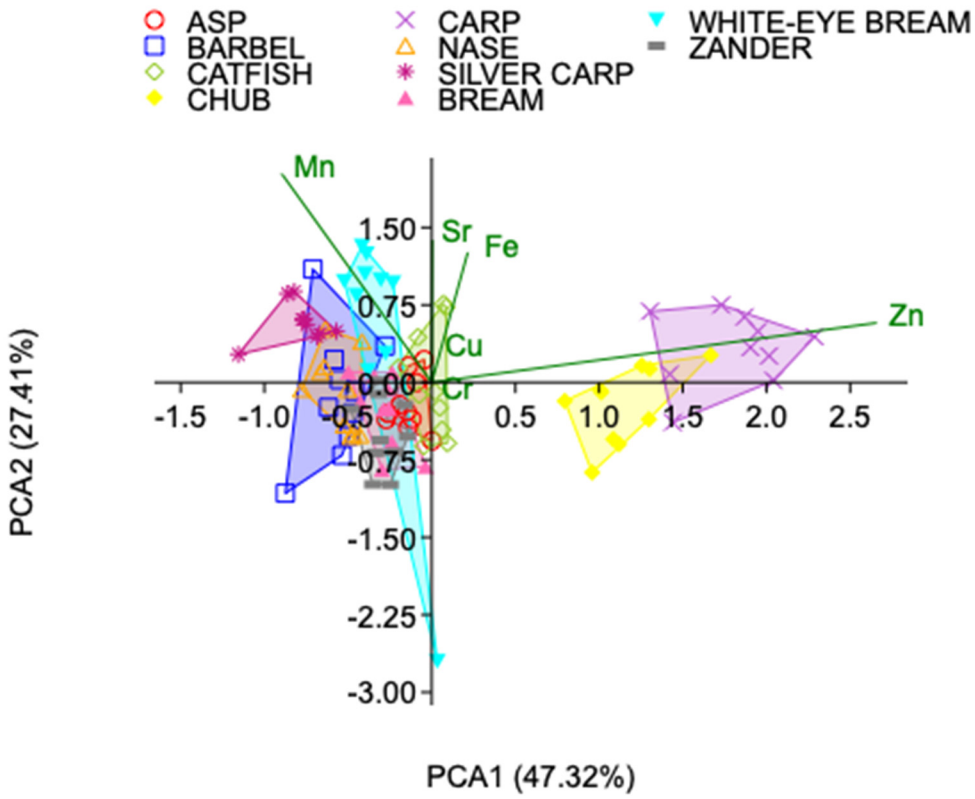

Figure 7. The PCA plot of the concentration of trace elements in the gills (mg kg^{-1}, wet weight) of the adult species from the Maros River.

(C) Liver

PCA identified two principal components in the liver that revealed the 74.82% of the variation (Figure 8). PCA of liver metal concentrations across ten fish species yielded two principal components explaining 74.82% of the total variation. PCA1 accounted for 52.92% of the variation and was primarily influenced by Fe, followed by Cu, while PCA2 explained 21.90% and was

mainly influenced by Zn, followed by Cu. The herbivorous silver carp exhibited a wide distribution along PCA1, reflecting substantial variation in its liver metal content, particularly the highest Fe and Cu concentrations (Figure 8, Appendix 10). Along PCA2, the omnivorous common carp displayed the highest Zn concentrations and a broad distribution, indicating considerable variability in Zn accumulation. The asp, the only piscivorous species in this dataset, overlapped predominantly with omnivorous species (bream, barbel, chub, carp, silver carp, and white-eye bream) and the herbivorous nase along PCA2, suggesting shared patterns in liver metal accumulation (Figure 8, Appendix 10).

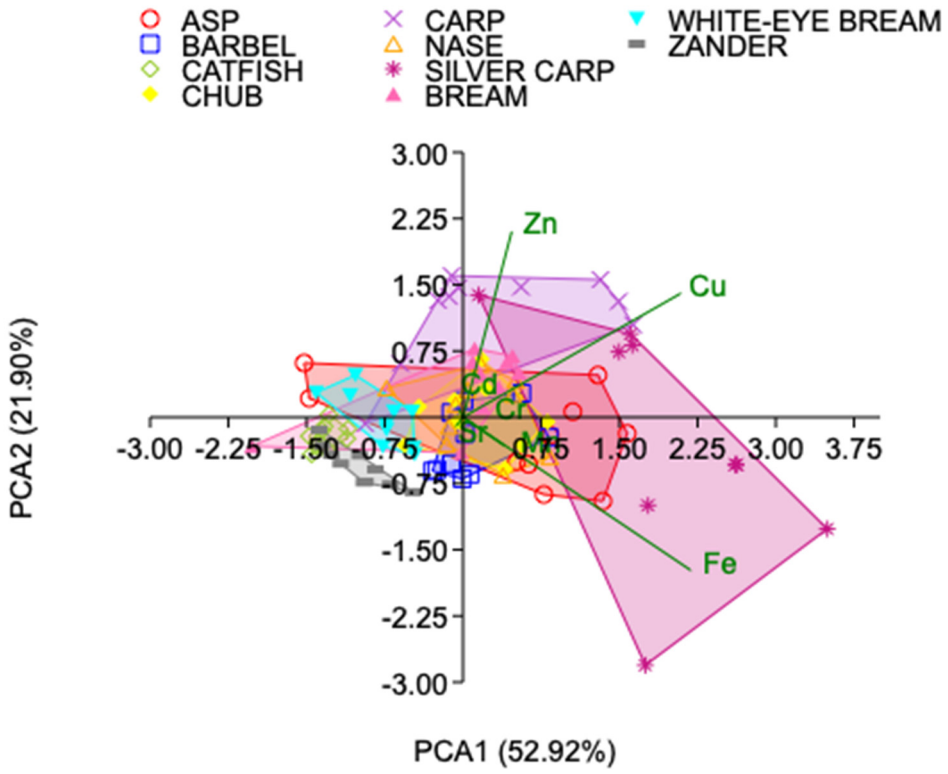

Figure 8. The PCA plot of the concentration of trace elements in the liver (mg kg^{-1}, wet weight) of the adult species from the Maros River.

4.2.4 Metal Pollution Index (MPI) in the Tissues of Adult Species

Overall, the MPI values were consistently high in the MPI of gills in all species, with common carp being the highest and zander the lowest (Figure 5). We observed the highest MPI in the liver of the silver carp and the lowest in catfish. Similar to the juvenile, the MPI of muscle were consistently low. The highest is noted in common nase and the lowest is by zander.

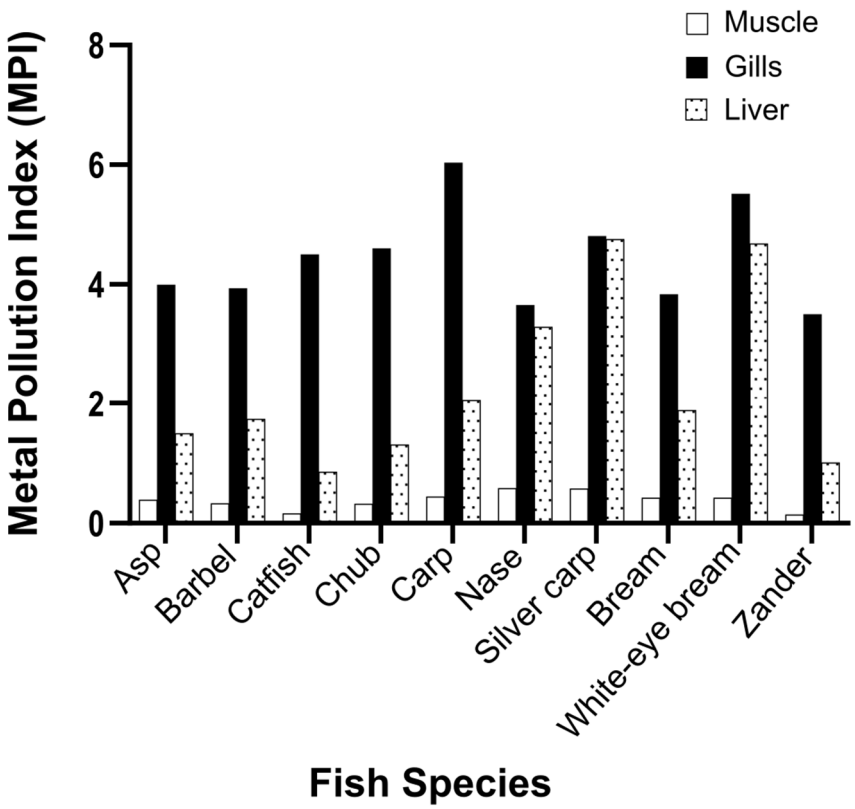

Figure 9. Metal pollution index (MPI) in the different tissues of adults from the Maros River detailed in Appendix 10.

5. DISCUSSION

5.1. Analysis of Trace Element Concentrations in Sediments and Water

Environmental matrices are important for assessing pollution levels in aquatic environments over time. Balogh et al. (2015) suggest that sediment trace element patterns are excellent long-term indicators, while water trace element patterns can indicate recent or ongoing contamination. The data of the sediments suggest persistent ongoing pollution in the Szamos River, with Cr exhibited moderate pollution levels and the other elements such as Cu, Mn, and Zn are denoted in heavily polluted conditions (Málnás et al., 2014b; Simon et al., 2017a). In the case of water, the trace elements levels in Cu, Cd, Pb, and Zn followed the same trend and surpassed the freshwater CCCs in the year 2013 (EPA, 2018). The chemical analysis of the water from OKIR in Szamos River showed an increase of about 2 to 10 times of these elements compared to the data from the former years. According to Hamad et al. (2012), trace metals, namely Pb, Zn, Cd, and Cu, are generally present in more reactive forms; either as dissolved ions or in labile states. The influx of these elements is largely attributed to anthropogenic sources like domestic sewage, agro-industrial processes, and agricultural inputs (Koh et al., 2015). Our findings aligned with previous studies on trace element deposition in sediment, chub and dragonfly larvae (*Gomphus flavipes*) that showed significant trace element contamination in the Szamos River in 2013, mainly linked to industrial work (mining) and agricultural activities (Málnás et al., 2014b; Nyeste et al., 2019; Simon et al., 2017c).

In the case of Maros River, the water chemistry data from OKIR in 2020 shows Fe have exceeded the threshold values by 10 times according to the CCCs specified by EPA (2018). Other elements, namely Cd, Cu, Mn, Pb, and Zn were recorded at concentrations below their threshold values. The Fe influx that had exceeded limits is a potential ecological concern. According to Koh et

al. (2015), Fe is widely recognised as a key sorbent in natural systems because it is prevalent in soils and rivers, where it frequently forms coatings on other particles. The excess Fe in water can result from agricultural runoff, including soil fertilisation and pesticide use (Kortei et al., 2020; Viana et al., 2021). There are studies in Northern Europe and North American freshwaters that observed a notable rise in Fe levels during autumn and winter, suggesting that the trend was attributed to warmer winter temperatures and increased rainfall, which created anoxic soil conditions and facilitated the release of Fe into nearby water bodies and increased runoff containing highly coloured and aromatic dissolved organic matter (Björnerås et al., 2017; Riise et al., 2023; Sarkkola et al., 2013). Although Fe is not essential for aquatic life, its elevated levels, along with high aluminium (Al) concentrations, can cause harmful effects, such as genetic mutations and bone disorders in vertebrates (Chandrapalan & Kwong, 2020; Rahmani et al., 2018; Viana et al., 2021). The elevated Fe levels in the water pose significant risks to aquatic organisms and humans who rely on it for drinking water, while the rising Fe concentrations in freshwater ecosystems over recent years underscore a growing environmental issue (Bianchi et al., 2019; Björnerås et al., 2017; Chandrapalan & Kwong, 2020; Sarkkola et al., 2013; Viana et al., 2021). There is a lack of studies on local lithology in both the Maros River and Szamos River, which limits the understanding of natural contributions to trace element concentrations in these rivers.

5.2. Assessment of Bioaccumulation in Juvenile Fish from the Szamos River

The patterns of macroelement accumulation (Ca, K, Mg, Na) in the juveniles reflect their distinct physiological roles. This result is anticipated because the essential roles of macroelements in fish physiology and survival have been well-documented in many studies (Khawar et al., 2024; Ustaoğlu et al., 2024). Specifically, the mean concentrations of macronutrients in the tissue samples

of the juveniles can be ranked based on their accumulation levels in the following sequence: K>Ca>Na>Mg in muscle, Ca>Na>K>Mg in gills and K>Na>Ca>Mg in the liver. The high concentrations of Ca and Mg were observed in the gills. This might be due to the reason that Ca serves an essential function in developing and supporting skeletal frameworks like arches and rakers while also being a primary site for calcium uptake, especially under hypoxic conditions for species such as hagfish (Borowiec, 2021; Playle, 1998). K concentration was the highest in the muscle of the nase. The liver of nase and barbel, as well as the gills of chub, exhibited significantly higher Na concentrations. This pattern underscores the fundamental role of sodium as an electrolyte, supporting liver functions and facilitating its absorption via the gills (Maetz, 1976; Nyeste et al., 2019). These observations are consistent with findings from both field research and laboratory studies on juvenile common carp (Harangi et al., 2017b; Nikolić et al., 2024; Nikolić, Skorić, et al., 2022; Subotić et al., 2021).

In terms of trophic position, the juveniles and their diets can be ranked as follows: omnivorous barbel > omnivorous chub > herbivorous nase. There is a direct relationship between the trace element concentrations and their trophic levels in juveniles. It is observed in the muscle tissues for Cr, Cu, Fe, and Zn, as well as the gills for Cu and Zn. A significant association between trophic level and trace element concentrations was identified in muscle tissues, irrespective of sediment and water contamination status. While Cu and Zn concentrations surpassed established pollution thresholds in both sediment and water, Fe levels remained within acceptable standards, and Cr was detected at only moderately polluted levels in sediments (Baudo & Muntau, 2020; EPA, 2018). These findings highlight the effect of dietary habits within the muscle tissue of juvenile trace element composition (Farkas et al., 2003; Nikolić et al.,

2021; Nyeste et al., 2019). Over the last decade, research has increasingly underscored the significance of muscle tissue in the processes of trace element bioaccumulation and biomagnification, particularly among juveniles (Jia et al., 2017a; Merciai et al., 2014; Ndimele et al., 2017; Nikolić et al., 2021; Nyeste et al., 2019).

However, certain trace elements showed no clear relationship with trophic levels despite significant variations in their concentrations, particularly in the gills and liver. This suggests that factors beyond trophic position may play a role in trace element accumulation. Current studies have highlighted that habitat selection may have a greater impact than their trophic levels on the trace element profiles of juveniles (Jiang et al., 2022; Llamazares Vegh et al., 2022; Nikolić et al., 2021). This perspective aligns with our results, as the concentrations of most trace elements showed no significant variation in both benthivorous species such as herbivorous nase and the omnivorous barbel (except for Cu) but displayed marked differences when compared to the omnivorous chub that prefers the pelagic habitat. The high Cu concentrations in benthic herbivorous nase in our studies went against the assumption of our hypothesis, as it has the lowest trophic position from all the species studied for juveniles. However, it is aligned with other previous investigations in the rivers and reservoirs in Serbia waters, where the liver of nase had exhibited the highest Cu levels. (Djikanović et al., 2016; Nikolić et al., 2024; Subotić et al., 2019). This finding may be attributed to the dietary preference of nase for benthic algae, as green algae and diatoms are known to bioaccumulate significant levels of Cu (González-Dávila et al., 2000; Novák et al., 2020). From this reasoning, the downward trend between Cu levels in the liver and the trophic level of the juveniles studied is likely linked to the species-specific feeding habits of nase. The limitations of this are trophic indices were obtained from FishBase, which provides estimated trophic positions based on taxonomic relationships, body

size, and available ecological data. These estimates offer a useful approximation of trophic position; however, direct measurement of trophic levels (e.g., via stable isotope analysis, SIA) was not performed. Consequently, trophic level assignments for juvenile individuals may not fully reflect ontogenetic dietary shifts and should be interpreted as species-level estimates rather than exact individual-level values.

In 2013, Cd concentrations in the water of Szamos River were approximately ten times higher than in previous years, surpassing CCCs for several months, based on water chemistry data from OKIR. This finding is reflected by the elevated Cd concentrations detected in the muscle, gills and liver of chub. The case is not the same for the other juveniles, the nase and barbel had Cd levels that were below the detection limit in the gills and the liver. Singh et al. (2011) state that Cd and Pb adversely affect health, even at low concentrations. This increase in Cd concentration was evident in the trace element profile of pelagic species, such as chub, with less pronounced effects on benthic juveniles. Similar trends have been observed in multispecies studies, where higher Cd concentrations in water predominantly accumulated in pelagic species (Llamazares Vegh et al., 2022; Milošković et al., 2016).

Pb concentrations, on the other hand, were highest in the benthic juveniles, particularly in the liver. This might be due to the different pathways of exposure to Pb and Cd in fish. This reasoning is supported by previous studies, where despite the elevated Cd and Pb concentrations in the water, the Pb are deposited in fish primarily through food consumption, while the absorption of Cd by the fish was through respiration that takes place in the gills (Jia et al., 2017b; Subotić et al., 2015). Generally, habitat selection plays a significant role in the trace element profile of gills (Nyeste et al., 2019; Subotić, Spasić, et al., 2013b). The exposure route of trace elements via gills in fish had a more pronounced effect on the deposition of trace element patterns in pelagic species

than the benthic species (Jiang et al., 2018; Milošković et al., 2016; Subotić, Spasić, et al., 2013b). These findings support the second assumption of our hypothesis that habitat preference influences trace element patterns in fish.

Previous studies have documented the effectiveness of fish juveniles as trace element pollution bioindicators, which is attributed to their faster growth and metabolic rates, increased intake of food, greater water flow through gills in respiration, and underdeveloped toxin elimination systems compared to adults (Jia et al., 2017b; Llamazares Vegh et al., 2021; Nikolić et al., 2021; Nyeste et al., 2019). The assessment of the juvenile as a bioindicator in contaminated water bodies was done in this study by examining the impact of habitat preference and feeding diet on the bioaccumulation trend. We found that the Cu and Zn concentrations were elevated in both sediments and the water of the Szamos River. The species which inhabit the same contaminated microenvironment will have bioaccumulation patterns that rely heavily on their diet. However, previous studies show higher Zn concentration in the gills of pelagic chub, instead of the liver which supposedly accumulated more Zn in bioaacumulation process (Djikanović et al., 2016; Jia et al., 2017b; Machala et al., 2001; Podrug & Raspor, 2009; Subotić et al., 2015). Other studies highlighted that ongoing Zn pollution might be the cause of more elevated Zn pollution in pelagic species, specifically in the gills (Harkabusová et al., 2012; Nikolić, Poleksić, et al., 2022; Subotić, Višnjić Jeftić, et al., 2013).

From the sediment data, Cr showed moderate contamination, while Mn exhibited high levels of enrichment relative to background concentrations. However, for water, both concentrations are below the CCCs. This is reflected in the juveniles that were studied, where nase and barbel in the benthic environment have elevated concentration levels of Cr and Mn compared to the chub that favours the pelagic habitat. Contrary to the characteristics of Cd accumulation in our findings, despite exceeding the CCCs in water for a while, this

toxic metal was not deposited in the sediments, resulting in higher Cd concentrations in the gills of pelagic chub in comparison to benthic species like barbel and nase. These findings suggest that, in juveniles, habitat use may exert a stronger influence on trace element accumulation than diet or trophic position. This is supported by other studies that highlighted that habitat selection plays a bigger role than the diet that influences the biomonitoring potential in fish juveniles (Jia et al., 2017b; Llamazares Vegh et al., 2022). In addition, Kottelat & Freyhof (2007) reported that the diets of the studied larvae and cyprinid juveniles mostly rely on similar prey, which reduced the importance of their trophic position. Fish larvae begin exogenous feeding once yolk reserves are depleted and after they hatch, young juveniles primarily consume zooplankton before gradually transitioning to prey that is specifically for the species over the following months (Heming & Buddington, 1988; Yúfera & Darias, 2007). The trend is similar in other studies where diet have a weaker effect on bioaccumulation patterns in juveniles compared to adults (Djikanović et al., 2016; Farkas et al., 2003; Nyeste et al., 2019).

The juveniles in this study were subjected to various environmental trace element stressors despite belonging to the same stretch of the Szamos River, which might be due to the foraging habit of the fish species in the given habitat. The trace element in the sediments can be released into the water by benthic species known as bioturbators (Adámek & Maršálek, 2013). As a result, the pelagic and bottom-dwelling species in the same aquatic systems may have different bioaccumulation patterns in their microenvironments. Additionally, the trace elements interaction and competition by the uptake pathways (i.e. gill uptake or dietary uptake) would also influence the bioavailability of trace elements in the fish. This is supported by recent physiology studies on the gills and intestinal absorption in fish (Einhorn et al., 2024; Kwong, 2024). In early

life stages, habitat preference emerges before dietary specialisation, further influencing trace element profiles in juveniles. These findings highlight the significant role of habitat preference in juvenile bioaccumulation of trace elements, where it contributes to the enhancement of the juveniles as bioindicators of polluted environments through their specific habitat selection. The use of juveniles emphasising pelagic and benthic habitats is especially valuable in implementing a monitoring framework to assess ongoing or prolonged sediment and water pollution levels.

5.3. Assessment of Bioaccumulation in Adult Fish from the Maros River

Calcium and phosphorus are among the most extensively studied macroelements in fish. In contrast, considerably less attention has been given to sodium, potassium, and magnesium concentrations (Stoyanova, 2018). Based on the mean concentrations of the macroelements in the studied adult fish, the analysis revealed significant differences ($p < 0.05$) in muscle, gills and liver. The general rank of the mean elemental concentration is as follows: Ca > K > Na > Mg. The mean concentrations of macronutrients in the tissue samples of the tested fish can be ranked based on their accumulation levels in the following sequence: K > Ca > Na > Mg in muscle, Ca > Na > K > Mg in gills and K > Na > Ca > Mg in the liver. The highest K accumulation was found in the muscle (omnivorous bream). Whereas the concentrations of Ca (piscivorous asp), Na (piscivorous catfish) and Mg (piscivorous asp) were the most elevated in the gills. The measured concentrations of the macroelements in the adult fishes were found to be aligned with their physiological roles. The gills of fish serve as the primary organ for osmoregulation, regulating the balance of ions (Na+, K+, and Mg+), which is crucial for maintaining the internal environment, particularly in fluctuating aquatic environments of the fish. The gills are particu-

larly sensitive to environmental factors like pollution, salinity, pH, and biochemical oxygen demand (BOD), which can affect their osmoregulatory function (Abduljabbar Rasmi & Majdy Faisal, 2024; Uchida et al., 2000). The chloride cells in the gill epithelium play a critical role in this process, using enzymes like sodium-potassium adenosinetriphosphatase to transport ions efficiently (Marshall, 1995; S. D. McCormick, 1995; Uchida et al., 2000). The presence of elevated concentrations of Ca, Mg, and K in the gills is consistent with their role in ion transport and osmoregulation, as these ions are essential for maintaining proper cellular and physiological functions (Hootman & Philpott, 1980). Additionally, these high concentrations of minerals are likely linked to the involvement of gills in both ion secretion and ion uptake, which is critical for the overall health and osmoregulatory efficiency of the fish (Perry, 1997; Zadunaisky, 1997). Furthermore, the relatively high concentrations of K and Mg in the gills reflect their role in facilitating essential biochemical processes, such as enzyme function and cellular communication, which are vital for the survival of fish in diverse environmental conditions (Steffens, 2006).

Based on the mean concentrations of the non-essential and trace elements (Cd, Cr, Cu, Fe, Fe, Mn, Sr and Zn) in the studied adult fish, significant differences ($p < 0.05$) were observed in all the studied tissues (muscle, gills and liver). The general rank of the mean elemental concentration is as follows: Fe > Zn > Cu > Sr > Mn > Cd. The mean concentrations in the tissue samples of the tested fish can be ranked based on their accumulation levels in the subsequent order: Zn > Fe > Sr > Mn > Cu > Cr in muscle, Zn > Fe > Sr > Mn > Cu > Cr in gills and Fe > Zn > Cu > Mn > Sr > Cd> Cr in the liver. These findings, where Fe and Zn are mostly the highest in the tissues studied, are aligned with other studies such as in the Nile River in Egypt and Dukan Lake in Iraq (Ghannam, 2021; Kareem et al., 2022). Based on the Mann-Whitney test ($p < 0.05$),

the highest Cr and Sr concentration levels were detected in the gills of the piscivorous asp, while the highest Mn concentration levels were detected in the gills of the omnivorous white-eye bream. In the liver, planktivorous silver carp dominated the Cu and Fe concentrations. The highest concentrations of Cd and Zn were found in omnivorous bream and omnivorous carp, respectively. The muscle tissue consistently exhibited the least accumulation in all of the studied trace elements. Metal accumulation in fish varies by organ, with the liver and gills typically showing the highest concentrations, particularly for Cu, Zn, Fe, and Sr in previous studies (Dikanović et al., 2016; Subotić, Višnjić Jeftić, et al., 2013). This is due to the crucial role of the liver in the toxin removal process, biotransformation, and excretion of pollutants, as well as factors like environmental metal availability, fish age, species-specific differences, and the presence of metal-binding ligands in liver tissues (Khaled et al., 2016; Shinn et al., 2009). Species differences affect accumulation patterns, as seen in Prussian carp, which accumulate more metals in the gills, while species like the common nase show higher levels in the liver (Dikanović et al., 2016). These variations are influenced by membrane permeability and species-specific traits, leading to greater bioaccumulation in the gills and liver than in the muscle tissue (Kousar & Javed, 2014).

Asp is the highest in the trophic level of all the adult species that were studied. The trend aligned with our hypothesis for asp following the highest Cr and Sr concentration in the gills. Cr has the tendency to accumulate in the organs like the liver, gills, and kidneys of fish and often exceeds the safe limit in polluted waters, causing oxidative stress, immune dysfunction, and tissue damage that affects the survival of the fish (Das, 2024; Hu et al., 2024; Panda et al., 2023; Qadir & Malik, 2011). Similar to our findings, a higher accumulation of Cr and Hg in the gills and liver is recorded for piscivores compared to polyphagous species (Subotić et al., 2013b). The main reason might be attributed to

the feeding diet and habitat preference of the asp. Asp is a benthopelagic piscivorous species with opportunistic foraging habits. In the biodiverse Danube waters near Belgrade, asp is identified as non-selective predator, as evidenced by a diet dominated by abundant cyprinids such as bleak (*Alburnus alburnus*), goldfish (*Carassius auratus*), and roach (*Rutilus rutilus*) (Krpo-Cetkovic et al., 2010; VaŠek et al., 2013). Being the only predator species in the Cyprinidae family, asp was found to outgrow all other cyprinid species in the waters of Poland (Krpo-Cetkovic et al., 2010). During its lifetime, asp passes critical dietary stages, starting from early zooplanktivory followed by a short transition into surface arthropod feeding during summer and ending with facultative or obligate piscivory (Adámek et al., 2019; Specziár & Rezsu, 2009). These transitions underline the adaptive foraging strategy during ontogeny as a warranty for survival and ecological flexibility that could be accounted for in the bioaccumulation of Cr and Sr.

Our findings show that the active pelagic asp has the capability of bioaccumulating more waterborne trace elements compared to sediment. The diet of the asp, which is mostly benthic organisms and benthivorous fish, could suggest potential biomagnification of the Cr and Sr. This finding is similar to a study by Das Pinkey et al. (2024) on the pelagic olive barb (*Systomus sarana*), which consumes benthic organisms and has a higher metal pollution index than other benthic fish species. The high trace element concentration in suspended sediment has been suggested to facilitate their release into the water column and enhance their bioavailability and uptake through respiration via gills (Pinkey et al., 2024). Similarly, the Liuyang River, Szamos River, Yangtze River and Yellow River have shown identical trends of pelagic species being among the most sensitive species to waterborne metal contamination (Das Pinkey et al., 2024; Jia et al., 2018; D. Li et al., 2023; Nyeste et al., 2024; S. Zhao et al., 2012).

Trace element accumulation in fish gills is well-documented in many studies, it is because the gills serve as an active site through which pollutants are taken in and affect the receptors for entry (Makarenko et al., 2021). The extensiveness of the surface area, the thinness of the diffusion barrier, and constant exposure to large volumes of water create a scenario where dissolved contaminants are in contact with gill surfaces and facilitate the entry of metals through processes of respiration and osmoregulation (Fernandes & Moron, 2019; Yancheva et al., 2022). High Mn concentration in the gills of white-eye bream is aligned with the results obtained in common bream and carp from Poland and barramundi from Bangladesh, suggesting that gills are the main organs of Mn accumulation (Niemiec & Wiśniowska-Kielian, 2015; Rajkowska & Protasowicki, 2012; Tahity et al., 2022). Since Mn concentrations in fish gills change rapidly depending on environmental changes, they act as an important indicator of aquatic pollution, which was also reported in previous studies (Niemiec & Wiśniowska-Kielian, 2015; Pereira et al., 2010; Vieira et al., 2012). Although our study is limited without the sediment data for Mn concentration, this finding might show a prolonged Mn contamination in the sediment, a possibility supported in some early studies (Faria et al., 2008; Niemiec & Wiśniowska-Kielian, 2015). This might be due to the nature of white-eye bream as a benthopelagic species that prefers the deeper section of the river, near the vegetation and substrates. It also has an opportunistic foraging habit, and omnivorous diet and feeds on zooplankton, insect larvae and detritus.

Contrary to our hypothesis, despite being the lowest in the trophic position of the species studied, planktivorous silver carp have accumulated the highest Cu and Fe concentrations in the liver. As a filter-feeding species, it ingests large volumes of water containing suspended particles, often including phytoplankton (mainly Chlorophyta), zooplankton and detritus, which exposed it to waterborne metal pollutants (Su et al., 2020; Xia et al., 2022). Their specialised

gill rakers and mucus-producing suprabranchial organ enable efficient filtration of fine particulate matter, increasing their exposure to dissolved contaminants (Calkins et al., 2012; Teodorof et al., 2009). Environmental factors such as temperature and nutrient levels influence prey availability, which can affect metal bioaccumulation through dietary intake (Xia et al., 2022). Additionally, their ability to thrive in slow-flowing or stagnant waters, where metal-laden sediments and organic suspensions are more concentrated, may further contribute to their trace element burden (Jawdhari et al., 2022b). There is a recent study indicated that algal blooms promote the uptake and retention of heavy metals such as cadmium and zinc in silver carp through digestion, absorption, and skin mucosal contact, leading to an increased metal concentration in various organs (X. Zhao et al., 2024). These factors highlight the vulnerability of silver carp to metal contamination despite their low trophic position. Studies have shown that metal accumulation varies across fish organs, with certain tissues exhibiting a greater tendency to retain specific metals (Canli & Atli, 2003; Kareem et al., 2022; Usero et al., 2004). While the liver is generally recognized as the primary site for metal deposition, some exceptions exist, such as iron accumulating more in the gills than the liver in Atlantic saury, *Scomberesox saurus* (Canli & Atli, 2003). This variation highlights species-specific differences in metal uptake and detoxification, reinforcing the observed high Mn concentration in the liver of silver carp.

The highest Cd accumulation in the liver was observed in omnivorous common bream. Common bream is a benthopelagic species with opportunistic foraging habits. Lacerda et al. (2020) stated that certain benthopelagic fish species exhibit greater metal accumulation and toxicogenetic responses, making them suitable bioindicators for contamination studies. Since mature common bream primarily consumes chironomid larvae and other benthic invertebrates, their Cd burden likely reflects sediment contamination rather than water pollution

(Farkas et al., 2002). The dietary flexibility of bream, including the ability to shift to particle or filter feeding under high zooplankton abundance, contributes to their increased Cd uptake (Froese & D. Pauly, 2022; Noël et al., 2013). Regional studies also confirm significant Cd accumulation in bream, such as in Lake Balaton in Hungary and the Seine River in France (Burada et al., 2014). Unlike mercury, Cd does not biomagnified along the food chain, making zooplankton and zoobenthos more reliable bioindicators of contamination than predatory fish (Burada, 2014). Some studies even report higher Cd levels in nonpredatory fish compared to piscivorous species, with the highest concentrations found in Wels catfish and the lowest in carp (Kenšová et al., 2010). This pattern is likely due to Cd being primarily accumulated through zooplankton and benthic organisms, which form the dietary basis of bream (Farkas et al., 2002). The high bioconcentration factor (BCF) of Cd in plankton further supports this trend, as planktonic organisms readily absorb and retain heavy metals from the surrounding environment (Burada et al., 2014). Additionally, in Lake Balaton, bream with higher growth rates exhibited elevated trace element levels, suggesting uptake through biomagnification (Farkas et al., 2002). The dietary dependence on invertebrates and their habitat preferences and adaptability further explain why bream accumulates more Cd than piscivorous species.

The high Zn accumulation in the liver of omnivorous common carp can be attributed to many factors. Our findings aligned with Teodorof et al. (2009) and Yousafzai et al. (2012), where it was reported that Zn has the highest biological accumulation in fish and was commonly the most abundant in the common carp. Some studies highlight that trace element pollution poses serious risks to common carp, leading to bioaccumulation primarily in the kidneys and liver, while muscles retain the lowest concentrations (Ambreen et al., 2015;

Kakade et al., 2020). Prolonged exposure causes oxidative stress, tissue damage in gills and kidneys, and gut microbiota alterations, with Co-Cd mixtures being the most toxic (Ambreen et al., 2015; Kakade et al., 2020). Contrary to that, others noted that accumulation varies according to species, as reported by Has-Schön et al. (2015), where the concentrations of Zn were greater in catfish than in carp in most tissues. This variation may depend on age, body weight, and habitat, as larger older individuals tend to accumulate metals in their bodies with time (Has-Schön et al., 2015). Zn also tops the list in silver carp, followed by Pb, Cd, and Hg, which accumulate according to species and environmental niches (Aski et al., 2022). Besides that, the high accumulation of Zn in common carp can be explained by the foraging habit of this species, which makes it a well-known bioturbator. According to X. Zhang et al. (2016), their suction-feeding behaviour disturbs sediments, resuspending nutrients and pollutants, which can enhance the bioavailability of metals in their diet. In addition, the carp species seriously alter the water quality and biomass of benthic algae while it mobilises the nutrients from sediment into the water column through its bioturbation process (X. Zhang et al., 2016). As omnivores with opportunistic foraging habits, they have a wide range of diets and primarily feed on benthic organisms, particularly molluscs such as the invasive *Dreissena* clams, which are abundant in the Danube River, contributing to their trace element intake (Teodorof et al., 2009). An experiment conducted in earlier studies shows that the higher Zn concentrations in common carp compared to silver carp are not influenced by diet or feeding habits but are instead attributed to a higher Zn absorption rate in common carp (Jeng & Sun, 1981). This further emphasises the species-specific physiology of common carp beyond their feeding habits and habitat preferences.

5.4. Human Health Implications

According to the Food and Agriculture Organization of the United Nations (FAO) and Commission Regulation (EC), the Maximum Allowable Concentration (MAC) in fish muscle for public health were set as follows: Cd (0.05 mg kg^{-1} ww), Cr (1.0 mg kg^{-1} ww), Cu (30.0 mg kg^{-1} ww), Fe (43.0 mg kg^{-1} ww), Mn (1.0 mg kg^{-1} ww), Pb (0.30 mg kg^{-1} ww), and Zn (40.0 mg kg^{-1} ww) (European Union, 2008; Nauen, 1983). For the juvenile species studied in Szamos River, the mean concentration of Cd (0.10 ± 0.17 mg kg^{-1} ww) and Pb (0.36 ± 0.31 mg kg^{-1} ww) in the muscle of the chub exceeded the MAC, while others (Cr, Cu, Fe, Mn, and Zn) remained below the MAC, and the fish meat is safe for consumption. Notably, the Cd and Pb concentrations in water were also reflected as polluted, while the sediments remained unpolluted by these toxic elements. This further highlights that the pelagic chub is more vulnerable to ongoing waterborne pollution than benthic species such as barbel and nase. It is also important to highlight that the mean elemental concentrations in the gills (Cu, Fe, Mn, Pb, Zn) and liver (Cd, Cr, Fe, Mn, Pb, Zn) exceeded the MAC. Thus, the consumption of the gills and liver is deemed unsafe and not recommended for humans. While Hungary enforces size limits for the cyprinids investigated, restricting anglers to harvesting adults, the alarming levels of Cd and Pb concentrations in juvenile chub pose risks to the potential of biomagnification in the ecosystem and hazards for human health in the case of unauthorised fishing. Thus, additional comprehensive studies on trace element patterns in adult fish from the Szamos River are essential for evaluating potential human health hazards.

For the muscle of adult species studied in Maros River, all of the mean elemental concentrations are below the MAC. Thus, consumption of the flesh of the fish is deemed safe. However, certain mean element concentrations in most

of the species surpassed the MAC in the gills (Mn, Zn) and liver (Cd, Fe, Mn, Zn). Thus, the consumption of the gills and liver is not recommended. Interestingly, we found that the MPI of the catfish and zander are the lowest in the muscle despite being on top of the trophic position, with common carp having the highest MPI in gills and silver carp in the liver. It is crucial to highlight that the average mean concentration of trace elements studied of the juveniles in Szamos River is substantially higher than the adult fish species in Maros River. While direct comparisons between juveniles and adults cannot be made due to site differences, the observed accumulation patterns raise the hypothesis that juvenile fish may exhibit heightened sensitivity to trace element exposure, which should be tested under controlled or site-matched conditions. However, further studies need to be done to fully understand the mechanism underlying their sensitivity and to establish them as reliable bioindicators. In a nutshell, regular monitoring is essential to ensure the continued safety of fish consumption from these two important tributaries of the Tisza River.

5.5. Recommendations for Future Studies

Our findings on the trace element accumulation studies of fish in the Szamos River and Maros River suggested research gaps that need a lot of attention in future research. Bioaccumulation studies in fish are complex, tedious and time-consuming; preliminary studies should be done to examine important factors which can also affect the fish bioaccumulation trend. Luoma & Rainbow (2005) stated that trace element bioaccumulation in fish is influenced by multiple factors, including species-specific physiology, environmental conditions, and metal properties. Besides that, seasonal variations also play a crucial role, with higher metal levels generally recorded during warmer months, particularly in the dry season, due to reduced dilution effects (Hassan et al., 2024;

Jolaosho et al., 2023; Ngo et al., 2022). This pattern has been observed in multiple freshwater and coastal ecosystems, such as Dukan Lake, Iraq, where metals like As, Cd, and Cu showed higher concentrations in summer, while Cr and Fe were more prevalent in winter (Kareem et al., 2022). Seasonal and hydrological effects should also be analyzed by comparing metal concentrations between wet and dry seasons to assess variability in contamination levels and potential flooding impacts on metal transport (Hassan et al., 2024; Jolaosho et al., 2023). Since our studies were done in autumn in both the Szamos River and Maros River, it would be beneficial to see the outcome in different seasons and the impact of flooding in the long term.

The elevated Cu and Fe concentrations observed in the liver of silver carp warrant further investigation. Both Fe and Cu are redox-active transition metals that can catalyse the formation of reactive oxygen species (ROS) through Fenton and Fenton-like reactions when present at elevated concentrations, potentially leading to oxidative stress if cellular antioxidant defences are overwhelmed (Lushchak, 2011; Valko et al., 2005). Experimental studies have shown that copper exposure can disrupt antioxidant defence systems in fish, altering enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), while increasing lipid peroxidation and causing tissue damage (Atli & Canli, 2010; Lushchak, 2011). More recently, copper-induced oxidative stress has also been linked to transcriptomic changes, intestinal microbiota alterations, and histopathological damage in common carp (*Cyprinus carpio*), indicating that elevated Cu burdens may have systemic physiological consequences beyond simple storage (L. Zhang et al., 2022).

Similarly, excessive iron accumulation has been shown to induce hematological disturbances, oxidative imbalance, and histological alterations in freshwa-

ter fish such as *Labeo rohita*, demonstrating that Fe overload can result in sublethal toxic effects (M. Singh et al., 2019). Taken together, these findings suggest that the elevated hepatic Fe and Cu concentrations observed in silver carp in the present study may reflect not only physiological regulation but also the potential for oxidative and tissue-level stress. Therefore, further assessment of antioxidant biomarkers, haematological parameters, and histopathological endpoints would help clarify whether the observed metal burdens represent adaptive detoxification or early toxicological effects.

There are limitations in our studies due to the lack of data in our studied areas. For instance, a biodynamic model can explain how bioaccumulation results from the balance between dietary intake, dissolved metal uptake, and elimination rates. Species-specific accumulation patterns should be investigated to identify key fish species that are more vulnerable to metal uptake based on their ecological niche and physiological sensitivity (Lacerda et al., 2020). Water quality parameters, such as pH, dissolved oxygen, and water temperature, should be monitored, as these factors influence metal bioavailability and toxicity (Hamada et al., 2024). Finally, sex-based differences in metal accumulation should be examined, as previous studies have shown that female fish may accumulate higher concentrations than males due to metabolic differences (Al-Yousuf et al., 2000; Canli & Atli, 2003). These recommendations will enhance the understanding of trace element distribution, bioaccumulation patterns, and ecological risks in the Maros River and Szamos River ecosystems.

6. CONCLUSIONS

This study concludes that the Szamos and Maros Rivers are contaminated with various trace elements in both water and sediments. By utilising fish at different life stages as bioindicators, we effectively assessed the extent of aquatic ecosystem pollution. Our findings highlight that feeding habits and habitat preference play a crucial role in trace element accumulation, with significant differences observed in both juveniles and adults in detecting metal pollution. Our findings suggest that trophic level plays a greater role in trace element accumulation in adults due to their specialised diet. In particular, asp, common carp, common bream, and white-eye bream showed a strong affinity for trace elements in the gills and liver, while silver carp exhibited a negative correlation, suggesting reduced uptake under the same conditions. In contrast, juveniles showed limited correlations, primarily in muscle tissue.

Notable species-specific differences in habitat preference were observed in both juvenile and adult fish. Pelagic species, including juvenile chub, adult asp, and adult silver carp, exhibited a higher tendency to bioaccumulate trace elements, highlighting the alarming pollution levels in the water. In contrast, benthic species such as juvenile barbel, juvenile nase, and adult common carp showed a stronger affinity for trace elements in sediments. Due to the lack of sediment data for the Maros River, potential Zn pollution is suggested from findings in adult common carp. Similarly, elevated Cd and Mn levels in adult common bream and adult white-eye bream suggest contamination. These differences reflect distinct exposure pathways between juveniles and adults, shaped by microhabitat characteristics and bioturbation.

We have also identified that the juvenile fish species exhibited heightened vulnerability and were highly sensitive in terms of bioaccumulation of heavy metals compared to adult species, which may suggests juveniles as a good bioindicators of metal contamination in the aquatic ecosystem. We identified

chub as the key bioindicator for juveniles, whereas in adults, asp, silver carp, common carp, common bream, and white-eye bream serve as critical bioindicators due to their strong affinity for different heavy metals. Given their ecological significance and widespread distribution in European rivers, these species are essential for monitoring aquatic pollution and assessing risks to both ecosystems and human health, particularly in commercial fishing and aquaculture. We also conclude that the muscle tissue of the juvenile chub is not safe for consumption due to the Cd and Pb concentrations that surpassed the MACs. While all analysed elements in adult muscle tissue remained below MACs thresholds. Despite this, continuous monitoring of adult fish from the Szamos River is crucial to assess potential health risks associated with human consumption, while regular surveillance in the Maros River is necessary to ensure long-term food safety.

7. SUMMARY OF NEW SCIENTIFIC FINDINGS

Szamos River (Juvenile)

- Elevated waterborne Cd, Pb, and Zn were linked to higher accumulation in pelagic juvenile chub, especially in muscle and gills, indicating dominant waterborne exposure.
- Sediment-associated Cr, Cu, and Mn accumulated more in benthic juveniles such as nase and barbel, reflecting habitat-driven uptake.
- Juveniles showed higher bioconcentration of Cd, Cr, Cu, and Pb in the liver and of Fe, Mn, and Sr in the gills, demonstrating clear tissue-specific accumulation at early life stages.
- Cd and Pb in juvenile chub muscle exceeded maximum allowable concentrations, thus, juvenile chub meat is unsafe for consumption, and it is identified as a reliable bioindicator of pollution in the Szamos River.

Maros River (Adult)

- Fe and Zn were the most abundant trace elements with feeding strategy and habitat preference influenced metal accumulation more strongly than trophic level.
- Piscivorous asp showed the highest Cr and Sr in gills, likely linked to pelagic exposure. Planktivorous silver carp accumulated the most Cu and Fe in the liver due to filter-feeding and high water intake, warranting further investigations. Omnivorous common bream had the highest Cd in the liver, indicating sediment-related accumulation. Common carp showed elevated Zn in the liver, associated with benthic feeding and physiological demand.
- Adult fish muscle concentrations remained below maximum allowable limits, indicating safe consumption, while gills and liver are not recommended for consumption .

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بِسْـــــــــــــمِ اللهِ الرَّحْمَنِ الرَّحِيْمِ

In the Name of Allah, the Most Merciful, The Most Compassionate.

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٥ يُسْرًا ٱلْعُسْرِ مَعَ إِنَّ ٦ يُسْرًا ٱلْعُسْرِ مَعَ فَإِنَّ ۞

"(5) So, surely with hardship comes ease. (6) Surely with that hardship comes more ease." [Al-Inshirah, 94: 5-6]

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10. LIST OF SCIENTIFIC ACTIVITIES

Scientific activity in the topic of the dissertation

Scientific publications in international journals:

Nyeste, K.*, **Zulkipli, N.***, Uzochukwu, I. E., Somogyi, D., Nagy, L., Czeglédi, I., Harangi, S., Baranyai, E., Simon, E., Nagy, S. A., Velcheva, I., Yancheva, V., & Antal, L. (2024). Assessment of trace and macroelement accumulation in cyprinid juveniles as bioindicators of aquatic pollution: effects of diets and habitat preferences. *Scientific Reports*, *14*(1), 11288. **(D1; IF: 3.8)** https://doi.org/10.1038/s41598-024-61986-4

Yancheva, V.S., Georgieva, E., Velcheva, I.G., Iliev, I., Stoyanova, S.G., Vasileva, T.A., Bivolarski, V., Todorova-Bambaldokova, D., **Zulkipli, N.**, Antal, L., & Nyeste, K. (2022). Assessment of the exposure of two pesticides on common carp (*Cyprinus carpio* Linnaeus, 1758): Are the prolonged biomarker responses adaptive or destructive? *Comparative biochemistry and physiology. Toxicology & pharmacology: CBP*, 109446. **(Q1; IF: 2.24)** https://doi.org/10.1016/j.cbpc.2022.109446

Scientific presentations related to the topic of the dissertation:

Zulkipli, Nurfatin (2022). "*Trace Element Bioaccumulation in Target Tissues of Ten Edible Fishes from The Maros River, Hungary*". Paper presented at International Conference of Students and PhD Students "NATURE AND SCIENCE" at Stana de Vale, Romania

Scientific activity unrelated to the dissertation topic

Scientific publications in international journals:

Nurfatin, Zulkipli., Saiyidah, Nafisah, Hashim., N., N., Rodzali., Siti, Nur, Azmu'i, Abdullah., N., Muhammad., Tay, Chia-Chay., Wan, Rozianoor, Mohd, Hassan. (2023). Acetylcholinesterase inhibition by *Azadirachta indica* crude extract on *Pomacea canaliculata. Nucleation and Atmospheric Aerosols.* https://doi.org/10.1063/5.0115288

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Sharir, S., **Zulkipli, N.**, Mohamad, A., Farinordin, F.A., Zakeyuddin, S., Samat, A.A., Md Sah, A.S., & Md Nor, S. (2021). Habitat Use and Movement Activity of *Neolissochilus soroides* and *Channa lucius* during Post Inundation of Tembat Reservoir, Hulu Terengganu. Pertanika Journal of Tropical Agricultural Science. **(Q3; IF: 0.57)** https://doi.org/10.47836/pjtas.44.3.01

Scientific publications in Hungarian journals:

Nyeste, K. J., **Zulkipli, N.**, Uzochukwu, I. E., Somogyi, D., Nagy, L., Czeglédi, I., Harangi, S., Baranyai, E., Simon, E., Nagy, S. A., Velcheva, I., Yancheva, V., Antal, L.: Eltérő táplálkozású és habitatpreferencájú halivadékok indikátorszerepe a fémszennyezés kimutatásában. Halászatfejlesztés. 40, 30-31, 2023.

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Scientific presentations:

Zulkipli, Nurfatin (2021). "Acetylcholinesterase inhibition by Azadirachta indica crude extract on Pomacea canaliculata". Paper presented online at 3rd Symposium On Industrial Science And Technology (SISTEC) organized by Universiti Malaysia Pahang.

11. APPENDICES

Appendix 1. Characteristics of the sampled adult fish species (n=10)

Species	Minimum adult size (cm)	Total length (cm)	Mass (g)	Feeding group	Trophic level*
Asp	> 40	48.0 ± 7.7	1056.8 ± 507.4	piscivore	4.5
Barbel	> 40	50.2 ± 14.3	1478.7 ± 410.2	invertivore	3.1
Catfish	> 60	29.7 ± 5.2	139.1 ± 40.3	piscivore	4.4
Chub	> 25	39.2 ± 7.0	920.0 ± 506.1	omnivore	2.7
Carp	> 30	55.1 ± 12.6	3760.0 ± 2418.1	invertivore	3.1
Nase	> 20	24.8 ± 9.2	274.1 ± 380.6	herbivore	2.0
Silver carp	> 30	42.4 ± 23.4	2251.1 ± 2523.5	herbivore	2.0
Bream	> 15	31.1 ± 3.3	440.3 ± 152.8	omnivore	3.1
White-eye Bream	> 15	14.9 ± 9.1	110.3 ± 69.9	omnivore	2.9
Zander	> 40	60.7 ± 34.4	1572.0 ± 1371.9	piscivore	4.0

Note: * Data according to www.fishbase.org (Froese R. & D. Pauly, 2022)

Appendix 2. The ICP-OES measurement parameters and operating conditions

ICP-OES conditions I.

Common conditions	
Replicates	3
Pump speed	15 rpm
Uptake time	15 sec
Rinse time	30 sec
Read time	3 sec
RF power	1.20 kW
Nebulizer flow	0.70 L min^{-1} LLLmin^{-1} L min^{-1}
Plasma flow	12.0 L min^{-1}
Aux flow	1.0 L min^{-1}
Viewing height	8 mm

ICP-OES conditions II.

Element	Wavelength (nm)	Read time (sec)	Stabilization time (sec)	Viewing mode
Ca	317.933	3	3	radial
Cd	226.502	10	10	axial
Cr	267.716	10	10	axial
Cu	324.754	10	10	axial
Fe	238.204	10	10	axial
K	776.491	3	3	radial
Mg	285.213	3	3	radial
Mn	257.610	10	10	axial
Na	589.592	3	3	radial
Pb	220.353	10	10	axial
Sr	421.552	10	10	axial
Zn	213.857	10	10	axial

Appendix 3. Descriptive tables of total trace element concentrations in water samples from the Maros River (mean ± standard deviation) in 2019. Data are from the National Environmental Information System (OKIR) database.

Trace Metal	Mean ± SD (μg L^{-1})
Cadmium (Cd)	0.00002 ± 0.00003
Chromium (Cr)	0.01475 ± 0.00829
Zinc (Zn)	0.02625 ± 0.02007
Nickel (Ni)	0.02417 ± 0.02873
Copper (Cu)	0.01583 ± 0.01086
Lead (Pb)	0.00366 ± 0.00429
Aluminium (Al)	3.06358 ± 2.82717
Iron (Fe)	3262.75 ± 3031.42
Manganese (Mn)	163.25 ± 126.17

Appendix 4. Overview of the statistical summary for the total trace element concentrations in sediment and water samples from the Szamos River.

Element	Trace element concentration (mg kg^{-1} dry weight) in sediment			Toxicity classification of sediment[b]
	Min	Max	Mean ± SD	
Cr	18	57	32 ± 21	moderately polluted
Cu	32	111	58 ± 40	heavily polluted
Fe	12773	25997	15323 ± 6656	non-polluted
Mn	632	1961	1009 ± 667	heavily polluted
Pb	40	87	49 ± 24	non-polluted
Sr	11	35	17 ± 12	–
Zn	537	1073	646 ± 283	heavily polluted
	Trace element concentration (µg l^{-1}) in water			
Element	**Min**	**Max**	**Mean ± SD**	**Threshold values (µg l^{-1})[a]**
Cd	0.20	1.4*	0.58 ± 0.44	0.72
Cr	1	12	3.2 ± 3.7	74
Cu	5*	20*	9.5 ± 5.7*	3.1
Fe	45	658	366 ± 227	1000
Mn	1.8	388	146 ± 111	–
Pb	3*	20*	6.9 ± 6.3*	2.5
Zn	1	270*	136 ± 94*	120

Note: * Concentration of trace elements in the water was higher than the threshold value.

Appendix 5. Trace element concentrations (mg kg^{-1} wet weight) in the studied tissues of juveniles in the Szamos River (A) (mean ± SD).

(A) Trace element concentrations in the tissues of the juveniles				
Tissue	**Element**	**Nase**	**Barbel**	**Chub**
Muscle	**Ca**	354 ± 7.6^{a}	337 ± 67^{a}	377 ± 98^{a}
	K	2517 ± 46^{a}	1728 ± 299^{b}	2495 ± 28^{a}
	Mg	189 ± 11^{a}	141 ± 17^{b}	181 ± 17^{a}
	Na	387 ± 31^{a}	336 ± 58^{a}	445 ± 33^{b}
	Cd	BDL	BDL	0.10 ± 0.17
	Cr	0.12 ± 0.02^{a}	0.18 ± 0.04^{b}	0.12 ± 0.03^{a}
	Cu	0.33 ± 0.08^{a}	0.55 ± 0.07^{b}	0.43 ± 0.08^{a}
	Fe	7.4 ± 1.7^{a}	11 ± 1.6^{b}	$9.1 \pm 2.2^{a,b}$
	Mn	0.68 ± 0.06^{a}	0.87 ± 0.13^{a}	0.33 ± 0.08^{b}
	Pb	0.16 ± 0.06^{a}	0.19 ± 0.15^{a}	0.36 ± 0.31^{a}
	Sr	2.1 ± 0.22^{a}	1.3 ± 0.22^{b}	1.5 ± 0.31^{b}
	Zn	6.3 ± 0.81^{a}	11 ± 1.5^{b}	14 ± 3.4^{b}
Gills	**Ca**	3878 ± 178^{a}	3059 ± 279^{b}	6761 ± 3935^{c}
	K	1401 ± 85^{a}	1266 ± 737^{a}	1395 ± 346^{a}
	Mg	264 ± 21^{a}	178 ± 52^{b}	262 ± 50^{a}
	Na	369 ± 24^{a}	275 ± 83^{a}	452 ± 166^{a}
	Cd	BDL	BDL	0.07 ± 0.13
	Cr	0.39 ± 0.09^{a}	0.53 ± 0.10^{a}	0.26 ± 0.06^{b}
	Cu	0.42 ± 0.08^{a}	1.1 ± 0.54^{b}	0.84 ± 0.12^{b}
	Fe	72 ± 60^{a}	109 ± 97^{a}	39 ± 19^{a}
	Mn	13 ± 0.47^{a}	$11 \pm 3.5^{a,b}$	7.4 ± 2.2^{b}
	Pb	0.60 ± 0.20^{a}	0.68 ± 0.46^{a}	0.53 ± 0.68^{a}
	Sr	29 ± 5.2^{a}	14 ± 4.1^{b}	55 ± 40^{a}
	Zn	19 ± 1.1^{a}	39 ± 11^{b}	58 ± 8.9^{c}
Liver	**Ca**	611 ± 213^{a}	1192 ± 1002^{a}	682 ± 362^{a}
	K	2196 ± 120^{a}	$1626 \pm 1601^{a,b}$	842 ± 402^{b}
	Mg	256 ± 121^{a}	$131 \pm 93^{a,b}$	57 ± 24^{b}
	Na	572 ± 63^{a}	$411 \pm 141^{a,b}$	329 ± 13^{b}
	Cd	BDL	0.24 ± 0.53^{a}	0.29 ± 0.42^{a}
	Cr	2.5 ± 1.4^{a}	1.4 ± 0.57^{a}	0.67 ± 0.15^{b}
	Cu	14 ± 4.5^{a}	3.9 ± 0.77^{b}	2.2 ± 1.3^{b}
	Fe	$61 \pm 14^{a,b}$	71 ± 18^{a}	36 ± 17^{b}
	Mn	3.1 ± 1.3^{a}	5.2 ± 3.8^{a}	1.8 ± 0.70^{a}
	Pb	3.2 ± 1.2^{a}	2.1 ± 2.0^{a}	0.93 ± 0.52^{b}
	Sr	1.7 ± 0.53^{a}	1.3 ± 0.69^{a}	1.4 ± 0.48^{a}
	Zn	30 ± 11^{a}	74 ± 55^{a}	51 ± 27^{a}

Note: Below Detection Limit (BDL); The values with different superscript letters (^{a,b,c}) in the same row are significantly different based on the non-parametric Mann-Whitney U test ($p < 0.05$).

Appendix 6. Correlation analysis of trace element concentrations in fish tissues (n = 15) and trophic levels in the Szamos River (significant at, $p < 0.05$).

Elements	Tissues		
	Muscle	**Gills**	**Liver**
K	-0.794	n.s.	n.s.
Mg	-0.794	-0.567	n.s.
Na	n.s.	n.s.	-0.416
Cr	0.624	n.s.	n.s.
Cu	0.813	0.756	-0.549
Fe	0.699	n.s.	n.s.
Sr	-0.813	-0.643	n.s.
Zn	0.605	0.472	n.s.

Note: Non-significant (n.s.)

Appendix 7. Bioconcentration factor (BCF) values (presented as mean ± SD) represent the ratio of the mean trace element concentration in specific fish tissues (mg kg^{-1} wet weight) to their respective mean concentrations in (A) sediment (mg kg^{-1} dry weight) and (B) water (mg L^{-1})

(A) Bioconcentration factors in sediments				
Elements	**Tissue**	**Barbel**	**Chub**	**Nase**
Cr	**Muscle**	0.57 ± 0.11^{b}	0.38 ± 0.09^{a}	0.37 ± 0.07^{a}
	Gills	1.6 ± 0.31^{a}	0.80 ± 0.18^{b}	1.2 ± 0.27^{a}
	Liver	4.4 ± 1.8^{a}	2.1 ± 0.47^{b}	7.7 ± 4.5^{a}
Cu	**Muscle**	0.96 ± 0.12^{b}	0.75 ± 0.14^{a}	0.58 ± 0.15^{a}
	Gills	2.0 ± 0.94^{b}	1.5 ± 0.21^{b}	0.73 ± 0.15^{a}
	Liver	6.7 ± 1.3^{b}	3.8 ± 2.2^{b}	24 ± 7.8^{a}
Fe	**Muscle**	0.07 ± 0.01^{b}	$0.06 \pm 0.01^{a,b}$	0.05 ± 0.01^{a}
	Gills	0.71 ± 0.63^{a}	0.25 ± 0.12^{a}	0.47 ± 0.39^{a}
	Liver	0.46 ± 0.12^{a}	0.23 ± 0.11^{b}	$0.40 \pm 0.09^{a,b}$
Mn	**Muscle**	0.09 ± 0.01^{a}	0.03 ± 0.01^{b}	0.07 ± 0.01^{a}
	Gills	$1.1 \pm 0.34^{a,b}$	0.74 ± 0.22^{b}	1.3 ± 0.05^{a}
	Liver	0.51 ± 0.38^{a}	0.18 ± 0.07^{a}	0.31 ± 0.13^{a}
Pb	**Muscle**	0.40 ± 0.30^{a}	0.73 ± 0.63^{a}	0.32 ± 0.13^{a}
	Gills	1.4 ± 0.95^{a}	1.1 ± 1.4^{a}	1.2 ± 0.41^{a}
	Liver	$4.4 \pm 4.0^{a,b}$	1.9 ± 1.1^{b}	6.5 ± 2.5^{a}
Sr	**Muscle**	7.6 ± 1.3^{a}	8.8 ± 1.8^{a}	12 ± 1.3^{a}
	Gills	83 ± 24^{a}	315 ± 234^{a}	169 ± 30^{a}
	Liver	$7.4 \pm 4.0^{a,b}$	7.9 ± 2.8^{b}	8.4 ± 3.1^{a}
Zn	**Muscle**	0.57 ± 0.11^{b}	0.38 ± 0.09^{a}	0.37 ± 0.07^{a}
	Gills	1.6 ± 0.31^{a}	0.80 ± 0.18^{b}	1.2 ± 0.27^{a}
	Liver	4.4 ± 1.8^{a}	2.1 ± 0.47^{b}	7.7 ± 4.5^{a}

(B) Bioconcentration factors in water				
Elements	**Tissue**	**Barbel**	**Chub**	**Nase**
Cd	**Muscle**	–	168 ± 301	–
	Gills	–	126 ± 219	–
	Liver	415 ± 927[a]	511 ± 726[a]	–
Cr	**Muscle**	58 ± 12[b]	38 ± 9.3[a]	38 ± 6.7[a]
	Gills	166 ± 31[b]	81 ± 18[a]	122 ± 27[a]
	Liver	443 ± 179[a]	210 ± 47[b]	778 ± 454[a]
Cu	**Muscle**	59 ± 7.3[b]	46 ± 8.3[a]	35 ± 8.9[a]
	Gills	119 ± 57[b]	88 ± 13[b]	44 ± 8.9[a]
	Liver	409 ± 80[b]	229 ± 134[b]	1445 ± 473[a]
Fe	**Muscle**	31 ± 4.4[b]	25 ± 6.0[a,b]	20 ±4.7[a]
	Gills	296 ± 265[a]	106 ± 51[a]	196 ± 165[a]
	Liver	193 ± 48[a]	98 ± 46[b]	166 ± 37[a,b]
Mn	**Muscle**	6.0 ± 0.87[a]	2.3 ± 0.52[b]	4.6 ± 0.38[a]
	Gills	75 ± 24[a,b]	51 ± 15[b]	90 ± 3.2[a]
	Liver	36 ± 26[a]	12 ± 4.8[a]	21 ± 8.8[a]
Pb	**Muscle**	28 ± 21[a]	51 ± 44[a]	23 ± 8.8[a]
	Gills	98 ± 67[a]	77 ± 99[a]	87 ± 29[a]
	Liver	306 ± 281[a,b]	134 ± 75[b]	458 ± 175[a]
Zn	**Muscle**	80 ± 11[b]	99 ± 25[b]	46 ± 6.0[a]
	Gills	284 ± 83[b]	425 ± 65[c]	139 ± 8.3[a]
	Liver	540 ± 403[a]	376 ± 197[a]	220 ± 83[a]

Note: The values with different superscript letters ([a,b,c]) in the same row are significantly different based on the non-parametric Mann-Whitney U test ($p < 0.05$).

Appendix 8. Summary statistics of the total trace element concentrations in water samples from the Maros River.

Element	Concentration of trace element in water (µg l^{-1})			Threshold values (µg l^{-1})[a]
	Min	Max	Mean ± SD	
Cd	0	0.00009	0.00002 ± 3.13^{-5}	0.72
Cr	0.005	0.029	0.01 ± 0.0082	74
Cu	0.003	0.035	0.02 ± 0.01	3.1
Fe	347	10000*	3263 ± 3031*	1000
Mn	28	449	163 ± 126	–
Pb	0.0003	0.014	0.004 ± 0.004	2.5
Zn	0.06	0.082	0.03 ± 0.02	120

Note:
[a] Criterion chronic concentration (CCCs) for freshwater (EPA, 2018)
***** The concentration level of trace elements in the water that exceeded the threshold value.

Appendix 9. Descriptive tables of Metal Pollution Index (MPI) in the adult fish species from the Maros River.

Species	Muscle	Gills	Liver
Asp	0.39	4.00	1.50
Barbel	0.33	3.94	1.73
Catfish	0.16	4.50	0.86
Chub	0.32	4.60	1.31
Carp	0.44	6.04	2.05
Nase	0.59	3.66	3.29
Silver carp	0.57	4.81	4.75
Bream	0.42	3.84	1.88
White-eye bream	0.42	5.52	4.68
Zander	0.14	3.50	1.01

Appendix 10. Trace element concentrations (mg kg^{-1} wet weight) in the studied tissues of adults in the Maros River (B) (mean ± SD).

(B) Trace element concentrations in the tissues of the adults											
Tissue	**Element**	**Asp**	**Barbel**	**Catfish**	**Chub**	**Carp**	**Nase**	**Silver carp**	**Bream**	**White-eye bream**	**Zander**
Muscle	**Ca**	308.67 ± 289.23^{abc}	248.76 ± 189.35^{abc}	164.15 ± 16.3^{ab}	275.79 ± 178.56^{ac}	165.34 ± 107.22^{bf}	240.02 ± 96.64^{ab}	588.21 ± 372.29^{cd}	753.2 ± 323.48^{de}	890.25 ± 198.27^{e}	120.95 ± 40^{f}
	K	3644.1 ± 188.78^{ac}	3566.57 ± 168.19^{a}	3130.33 ± 151.32^{b}	3802.8 ± 179.2^{cd}	3182.9 ± 278.62^{b}	3644.19 ± 235.92^{acd}	3539.07 ± 249.11^{a}	3817.27 ± 128.11^{d}	2843.11 ± 572.56^{b}	3765.15 ± 227.06^{ad}
	Mg	273.63 ± 10.04^{a}	277.37 ± 15.21^{a}	209.82 ± 10.58^{b}	278.37 ± 16.86^{a}	223.25 ± 31.11^{bc}	286.82 ± 22.84^{a}	243.42 ± 23.23^{c}	309.85 ± 21.04^{d}	307.75 ± 9.77^{d}	269.32 ± 18.96^{a}
	Na	508.51 ± 104.89^{abe}	518.56 ± 31.14^{b}	781.58 ± 89.56^{c}	430.98 ± 89.75^{af}	522.3 ± 44.3^{b}	256.32 ± 48.72^{d}	432.5 ± 197.41^{abef}	294.6 ± 37.1^{d}	579.04 ± 119.84^{e}	385.48 ± 117.18^{f}
	Cd	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
	Cr	0.03 ± 0.01^{ab}	0.03 ± 0.01^{a}	0.02 ± 0^{bd}	0.04 ± 0.01^{ac}	0.03 ± 0.01^{ac}	BDL	0.03 ± 0.01^{a}	0.04 ± 0.01^{c}	0.02 ± 0.01^{bd}	0.02 ± 0.01^{d}
	Cu	0.23 ± 0.1^{ac}	0.19 ± 0.04^{ac}	0.09 ± 0.02^{b}	0.17 ± 0.06^{ad}	0.3 ± 0.13^{c}	0.26 ± 0.14^{ac}	0.18 ± 0.07^{ad}	0.14 ± 0.02^{d}	0.14 ± 0.05^{d}	0.06 ± 0.02^{e}
	Fe	2.78 ± 1.31^{a}	1.75 ± 0.68^{bc}	BDL	1.31 ± 0.37^{b}	4.44 ± 3.16^{a}	1.62 ± 0.7^{b}	2.41 ± 0.91^{ac}	1.48 ± 0.28^{b}	4.68 ± 3.27^{a}	0.48 ± 0.19^{d}
	Mn	0.18 ± 0.07^{a}	0.19 ± 0.08^{a}	0.11 ± 0.02^{b}	0.1 ± 0.04^{b}	0.1 ± 0.04^{b}	0.18 ± 0.07^{a}	0.52 ± 0.36^{a}	0.23 ± 0.16^{a}	0.98 ± 0.42^{c}	0.03 ± 0.01^{d}
	Pb	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
	Sr	0.48 ± 0.75^{ab}	0.23 ± 0.27^{a}	0.17 ± 0.02^{ab}	0.3 ± 0.26^{ab}	0.17 ± 0.15^{a}	0.2 ± 0.16^{ab}	0.79 ± 0.66^{bc}	1.21 ± 0.62^{cd}	1.55 ± 0.36^{d}	BDL
	Zn	4.14 ± 1.55^{acdf}	2.64 ± 0.18^{b}	3.3 ± 0.33^{c}	4.05 ± 0.9^{df}	10.57 ± 3.59^{e}	4.66 ± 1.46^{ad}	5.85 ± 1.93^{a}	2.36 ± 0.48^{b}	4.71 ± 1.36^{ad}	3.3 ± 0.86^{cf}

Gills	**Ca**	17771.9 ± 4744.74[a]	9887.41 ± 2147.58[ab]	14466.18 ± 5467.41[b]	12652.96 ± 1680[ab]	8566.64 ± 2765.74[ab]	7106.87 ± 1441.98[abcd]	11887.5 ± 2301.52[cd]	9160.05 ± 1635.36[c]	11466.14 ± 5205.51[d]	17663.55 ± 6670.3[e]
	K	1384.62 ± 275.32[ac]	1272.41 ± 351.04[af]	2328.89 ± 110.42[b]	1602.01 ± 320.16[cd]	1090.37 ± 257.73[d]	1185.77 ± 211.59[a]	1184.94 ± 320.7[af]	1743.62 ± 204.99[d]	1986.68 ± 60.51[e]	974.04 ± 217.44[f]
	Mg	509.73 ± 84.8[a]	439.73 ± 123.53[abc]	372.68 ± 67.1[bc]	463.88 ± 57.4[a]	240.26 ± 62.41[ab]	365.11 ± 103.66[c]	465.56 ± 64.6[a]	457.8 ± 109.51[abc]	479.81 ± 92.08[a]	356.5 ± 57.77[a]
	Na	1344.68 ± 214.1[ab]	1111.56 ± 278.71[bde]	1517.51 ± 238.93[a]	1420.26 ± 280.3[af]	864.25 ± 303.46[af]	761.76 ± 269.98[c]	1383.4 ± 95.47[a]	1135.68 ± 129.91[d]	1048.23 ± 86.5[e]	1197.28 ± 176.85[bdf]
	Cd	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
	Cr	0.31 ± 0.09[a]	0.22 ± 0.07[bc]	0.25 ± 0.08[ac]	0.21 ± 0.04[ce]	0.15 ± 0.07[ac]	0.14 ± 0.06[d]	0.28 ± 0.04[a]	0.19 ± 0.02[de]	0.2 ± 0.07[cd]	0.29 ± 0.08[ab]
	Cu	0.33 ± 0.06[ad]	0.37 ± 0.12[abd]	0.42 ± 0.08[b]	0.47 ± 0.1[b]	0.27 ± 0.19[abd]	0.42 ± 0.07[b]	0.38 ± 0.1[ab]	0.52 ± 0.19[bc]	0.66 ± 0.21[c]	0.29 ± 0.05[d]
	Fe	20.85 ± 8.79[a]	37.1 ± 17.7[bc]	41.69 ± 16.9[bc]	28.01 ± 13.58[ab]	25.05 ± 24.23[c]	27.3 ± 13.57[ab]	32.56 ± 9.28[bc]	29.56 ± 6.42[b]	35.13 ± 10.63[bc]	34.09 ± 22.39[abc]
	Mn	3.53 ± 0.78[a]	5.18 ± 2.38[ac]	5.31 ± 2.49[ac]	2.45 ± 0.52[b]	2.69 ± 1.19[a]	6.71 ± 1.86[c]	12.93 ± 0.62[d]	3.47 ± 1.34[ab]	13.82 ± 7.24[d]	4.09 ± 1.03[a]
	Pb	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
	Sr	35.96 ± 6.97[a]	23.42 ± 12.96[bcdf]	18.99 ± 7.72[bc]	24.59 ± 5.15[bdf]	14.11 ± 3.72[cd]	19.42 ± 5.32[c]	25.79 ± 5.97[df]	25.8 ± 4.63[f]	26.63 ± 11.7[ab]	12.25 ± 2.25[e]
	Zn	14.92 ± 1.28[a]	9.93 ± 2.13[a]	18.33 ± 2.86[c]	56.49 ± 15.75[d]	23.36 ± 39.16[e]	11.04 ± 0.93[b]	10.54 ± 1.84[b]	12.01 ± 1.12[f]	16.68 ± 2.44[ac]	12.81 ± 1.88[f]
Liver	**Ca**	92.73 ± 90.76[ac]	61.48 ± 21.13[a]	167.3 ± 49.37[b]	40.56 ± 16.19[c]	72.4 ± 23.41[a]	80.66 ± 43.63[a]	70.61 ± 45.92[ac]	77.81 ± 58.1[a]	546.13 ± 205.89[d]	89.86 ± 46.51[a]
	K	2687.36 ± 309.2[a]	2060.05 ± 281.01[e]	3016.35 ± 152.03[f]	2560.85 ± 146.3[ab]	2518.09 ± 242.55[a]	2139.62 ± 148.99[de]	2126.87 ± 376.89[de]	2726.9 ± 387.29[bc]	2846.76 ± 176.51[cf]	2390.27 ± 246.93[ad]
	Mg	153.56 ± 10.43[a]	144.3 ± 20.79[ac]	175.22 ± 9.11[b]	149.08 ± 21.65[a]	142.12 ± 18.91[ac]	162.46 ± 36.61[ab]	125.04 ± 16.66[c]	178.01 ± 31.55[b]	197.27 ± 46.15[b]	153.83 ± 30.14[ab]

Na	1173.55 ± 174.59^{ab}	1210.3 ± 277.09^{abd}	1082.88 ± 124.7^{a}	1223.94 ± 163.87^{abd}	1151.28 ± 171.99^{ab}	1091.48 ± 136.95^{a}	1402.23 ± 380.9^{bd}	915.63 ± 156.92^{c}	911.8 ± 155.79^{c}	1317.19 ± 104.96^{d}
Cd	0.06 ± 0.03^{a}	0.23 ± 0.14^{bcd}	0.11 ± 0.05^{ab}	0.17 ± 0.12^{abc}	0.25 ± 0.17^{cd}	BDL	BDL	0.26 ± 0.07^{d}	BDL	0.25 ± 0.13^{cd}
Cr	0.05 ± 0.02^{acd}	0.09 ± 0.04^{b}	0.03 ± 0.01^{c}	0.07 ± 0.04^{ab}	0.05 ± 0.02^{ad}	0.09 ± 0.07^{abd}	0.04 ± 0.01^{cd}	0.05 ± 0.02^{acd}	BDL	0.04 ± 0.02^{acd}
Cu	8.78 ± 6.23^{ad}	6.08 ± 2.8^{a}	1.47 ± 0.34^{b}	6.4 ± 3.03^{a}	8.23 ± 6.88^{ad}	8.94 ± 4.1^{ad}	26.45 ± 13.95^{c}	10.51 ± 4.58^{d}	2.42 ± 0.79^{e}	1.21 ± 0.23^{b}
Fe	183.3 ± 119.67^{ae}	90.56 ± 24.53^{b}	35.22 ± 3.46^{c}	93.51 ± 41.19^{abd}	88.06 ± 50.88^{abd}	67.82 ± 38.8^{df}	700.11 ± 734.01^{e}	58.26 ± 17.89^{df}	48.44 ± 18.64^{cf}	60.9 ± 25.79^{df}
Mn	0.93 ± 0.08^{a}	1.5 ± 0.61^{bd}	0.41 ± 0.1^{c}	0.84 ± 0.21^{a}	1.11 ± 0.37^{ab}	5.55 ± 4.74^{d}	2.53 ± 2.12^{abd}	1.03 ± 0.47^{ab}	2.38 ± 2.12^{abcd}	1.14 ± 0.45^{ab}
Pb	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
Sr	0.15 ± 0.17^{acdf}	0.17 ± 0.10^{ad}	0.31 ± 0.04^{b}	0.05 ± 0.02^{c}	0.11 ± 0.05^{af}	0.24 ± 0.17^{abcdef}	0.24 ± 0.15^{de}	0.12 ± 0.10^{adf}	0.36 ± 0.11^{be}	0.08 ± 0.06^{cf}
Zn	22.12 ± 6.63^{ac}	15.50 ± 4.12^{bef}	14.50 ± 1.30^{b}	21.96 ± 4.59^{c}	128.92 ± 56.18^{d}	16.96 ± 2.62^{ae}	24.82 ± 7.10^{c}	20.87 ± 6.56^{afc}	22.52 ± 4.36^{c}	14.66 ± 0.47^{b}

Note: Values marked with different superscript letters (^{a,b,c,d,e,f}) in the same row indicate significant differences (Mann-Whitney U test, $p < 0.05$).