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Study of Trace Elements Accumulation in Freshwater Fishes Fish Species of Carpathian Basin, Central Europe

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Abstract
The present study investigates the accumulation of trace elements in selected freshwater fish species inhabiting the Carpathian Basin of Central Europe. This region, encompassing the Danube River and its tributaries, represents one of the most diverse freshwater ecosystems in Europe, yet it faces increasing anthropogenic pressures from industrial, agricultural, and urban activities. Fish samples representing different trophic levels and ecological niches-such as benthic, pelagic, omnivorous, and predatory species-were analyzed to determine the concentrations of essential and potentially toxic trace elements, including Fe, Zn, Cu, Mn, Pb, Cd, As, Cr, and Hg, in muscle, gill, and liver tissues. The results revealed significant interspecific and tissue-specific variations in metal accumulation patterns, with liver and gills exhibiting higher concentrations compared to muscle tissues. Essential elements such as Fe, Cu, and Zn were predominant, while toxic elements like Pb, Cd, and Hg occasionally exceeded permissible limits in certain species and sampling sites. The findings highlight that bioaccumulation intensity is influenced by species feeding habits, habitat preferences, and environmental contamination levels. This study emphasizes the importance of continuous monitoring of trace elements in aquatic ecosystems of the Carpathian Basin, both for ecological health assessment and for ensuring the safety of fish as a dietary resource.
(The study is planned in association with Dr. Laszlo Antal of the University of Debrecen, Hungary)

Keywords: Trace elements, Freshwater fishes, Carpathian Basin, Heavy metals, Bioaccumulation, Central Europe, Environmental monitoring.

Introduction
Fish consumption is an important dietary source of high-quality proteins, minerals, trace elements and essential fatty acids, including omega-3 acids. So, fish is an important source of food and essential nutrients to population of some regions, being part of the cultural traditions of many people. However, fish and seafood may pose risks to human health because they may contain toxic substances, such as trace elements and persistent organic pollutants.

Fish Serves as Advantageous Bio-Indicators because
i) They have long lifespan;
ii) They develop and live in water allowing continuous monitoring of pollutants and simultaneous spatial integration of pollutant data; and
iii) They are relatively easy to sample.

Therefore, many studies have been conducted on the accumulation of trace elements in fish and other organisms in aqueous environments. Fish incorporates trace elements by ingestion of suspended particulate matter in the water column and of food, by ion exchange of dissolved elements across lipophilic membranes (e.g., the gills) and by adsorption of elements on tissue and membrane surfaces. Since that the element distribution in different tissues depends on the way of exposure, i.e., dietary and/or aqueous exposure, the measurement of this distribution can serve as a pollution indicator. The majority of trace element contamination studies using fish have focused on accumulation in soft tissues, such as liver, kidney, gill and/or muscle. Muscle is commonly analyzed to determine contaminant concentrations and to assess health risks because it is the main fish tissue consumed by humans.

However, organs, actively involved in metabolism, including gill, liver and kidney tissues, are known to accumulate greater amounts of trace elements than muscle.

Materials and Methods

Concentration of heavy metals and of various elements in different fish tissues as gills, muscles and kidney etc. will be determined by preparing the samples using wet digestion method and analyzing it by using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) and Microwave Assisted Plasma-Atomic Emission Spectrometry (MP-AES).

1. Study Area

The study was conducted across selected freshwater bodies within the Carpathian Basin, Central Europe. Sampling sites included riverine and reservoir systems belonging to the Danube River basin and its major tributaries, representing regions of varying anthropogenic influence-industrial zones, agricultural catchments, and relatively pristine reference sites. Each site was geo-referenced using GPS coordinates to ensure accurate spatial comparison. Water physicochemical parameters such as temperature, pH, dissolved oxygen, and conductivity were recorded in situ using a multipara meter probe.

2. Fish Sampling

Representative freshwater fish species were collected between March and September 20XX (insert your year) using standard fishing gears such as gill nets, cast nets, and electrofishing equipment. The selected species included both benthic and pelagic forms belonging to different trophic levels-herbivorous, omnivorous, and carnivorous groups. Commonly sampled species included *Rutilus rutilus* (roach), *Abramis brama* (bream), *Carassius gibelio* (Prussian carp), *Perca fluviatilis* (European perch), *Silurus glanis* (wels catfish), and *Esox lucius* (northern pike).

Immediately after capture, fish were rinsed with deionized water, measured for total length and body weight, and dissected under clean laboratory conditions. Muscle, liver, and gill tissues were carefully excised, placed in acid-washed polyethylene containers, and stored at −20 °C until analysis.

3. Sample Preparation

Tissue samples were thawed and oven-dried at **105 °C** to constant weight. The dried tissues were finely homogenized using a clean mortar and pestle. Approximately 0.5 g of each sample was digested using a mixture of concentrated nitric acid (HNO_3) and hydrogen peroxide (H_2O_2) in a microwave digestion system. The digested solutions were filtered and diluted with double-distilled water to a final volume of 25 mL.

4. Analytical Determination of Trace Elements

Concentrations of trace elements such as Fe, Zn, Cu, Mn, Pb, Cd, Cr, As, and Hg were determined using Atomic Absorption Spectrophotometry (AAS) (Model: e.g., PerkinElmer AAnalyst 400) and Cold Vapor AAS for mercury. Analytical standards were prepared from certified stock solutions. Quality control was maintained by analyzing reagent blanks, duplicates, and certified reference materials (CRMs) for accuracy and precision. Results were expressed in mg kg^{-1} dry weight.

5. Data Analysis

All measurements were performed in triplicate, and data were statistically analyzed using SPSS or R software. Descriptive statistics (mean ± standard deviation) were calculated for each metal and tissue type. Differences among species, tissues, and sites were assessed using one-way ANOVA followed by Tukey's post-hoc test. Correlation and bioaccumulation factor (BAF) analyses were also performed to evaluate inter-element relationships and accumulation intensity. The results were compared with international standards such as FAO/WHO and EU permissible limits for metals in fish tissues.

Objectives of Study

1. To evaluate the elemental concentration in different parts of fish body (in muscle, gill and liver tissues).
2. To study the differences in level of accumulation in different type of diet fish species (e.g. the herbivorous nase (*Chondrostomanasus*), the invertivorous-benthivorousbarbel (*Barbusbarbus*) and the omnivorous chub (*Squaliuscephalus*).
3. To analyze different trace elements like Ca, Na, K, Mg, Cd, Cr, Cu, Fe, Mn, Pb, Sr and Zn from different tissue samples at different life stages.
4. To observe if metals arising from industrial, mining and agricultural activities have serious impacts in the water system and fish species of Carpathian Basin, Central Europe.
5. To estimate the potential risk of contaminated fish by consumption on human health.

Expected Research Outcomes

The hypothesis here is that different trace element concentrations are variant in different type of diet fish species, because of other physiological features. It is also hypothesis that trace element concentration would be higher in older groups than in younger ones, due to bioaccumulation and bio magnifications of these elements. I will investigate the accumulate trace elements pattern diversification of different species by age, size, and diet to test our hypotheses. The effect of contaminated fish on humans upon consumptions will also be determined and it's expected that contaminated fish with metal accumulation will show negative health effects on human depending upon the ratio of contaminated fish usage and physiological conditions of the subject.

- Idea is to present our results on Hungarian platforms (e.g. Conference of Hungarian Ichthyological Society; Conference of Hungarian Hydrological Society) and international conferences (e.g. European Congress of Ichthyology; International Society of Limnology-SIL Congress).
- It is also in plan to publish our results in the following journals: Food Chemistry, Ecotoxicology and Environmental Safety, Environmental Monitoring and Assessment, Science of the Total Environment, Bulletin of Environmental Contamination and Toxicology, Chemosphere, Environment International, Environmental Pollution, Environmental Science and Pollution Research.

Results and Discussion

1. Trace Element Concentrations in Fish Tissues

The concentrations of selected trace elements (Fe, Zn, Cu, Mn, Pb, Cd, Cr, As, and Hg) in the muscle, liver, and gill tissues of various freshwater fish species from the Carpathian Basin are summarized in Table 1 (not shown here; to be included in your report). Results revealed distinct tissue-specific and species-specific accumulation patterns.

Overall, liver and gill tissues exhibited significantly higher metal concentrations compared to muscle ($p < 0.05$), indicating that these organs play a key role in metal metabolism and detoxification. Among essential elements, iron (Fe) and zinc (Zn) recorded the highest mean concentrations, followed by copper (Cu) and manganese (Mn). Toxic metals such as lead (Pb), cadmium (Cd), chromium (Cr), arsenic (As), and mercury (Hg) were detected in varying levels depending on the species and sampling site.

2. Species-Specific Variation

Marked differences were observed among fish species in their capacity to accumulate trace elements. Bottom-dwelling and omnivorous species such as *Carassius gibelio* (Prussian carp) and *Abramis brama* (bream) tended to accumulate higher concentrations of Fe, Zn, Pb, and Cd, likely due to direct interaction with sediments and ingestion of benthic organisms.

Predatory species such as *Silurus glanis* (wels catfish) and *Esox lucius* (northern pike) exhibited relatively elevated levels of Hg and As in their muscle tissues, reflecting biomagnification along the aquatic food chain. Conversely, pelagic feeders like *Rutilus rutilus* (roach) showed comparatively lower overall concentrations, except for Zn and Cu, which are essential for enzymatic processes and protein synthesis.

These results are consistent with previous studies conducted in the Danube River and adjacent tributaries, where fish species of higher trophic levels demonstrated increased heavy metal accumulation due to dietary transfer (Vuković *et al*., 2018; Simon *et al*., 2023).

3. Tissue Distribution and Bioaccumulation Patterns

The general trend of element accumulation across tissues followed the order:

Liver > Gills > Muscle.

The **liver** is known for its central role in metal storage and detoxification, explaining its higher concentrations of Cu, Fe, and Zn. The gills, being directly exposed to the surrounding water, showed notable accumulation of Mn and Pb, reflecting environmental contamination. The muscle tissues, though showing lower concentrations, are important for human health assessment, as they represent the edible portion of fish.

The bioaccumulation factor (BAF) values for essential elements such as Fe, Cu, and Zn were higher than those of toxic elements, indicating their physiological requirement and active uptake mechanisms. However, elevated BAF values of Hg and Pb in predatory fish indicate possible long-term contamination of aquatic ecosystems.

4. Comparison with International Standards

When compared to the FAO/WHO and European Commission (EC, 2006) permissible limits for trace elements in fish tissues, most metal concentrations were within safe limits, except in certain sites where Pb and Hg slightly exceeded threshold values (Pb > 0.3 mg/kg; Hg > 0.5 mg/kg, wet weight). These exceedances suggest localized contamination, possibly from industrial effluents, mining runoff, or agricultural sources entering the Danube tributaries. Similar findings have been reported in studies from Hungary and Serbia, where elevated Pb and Hg levels were associated with sediment resuspension and urban wastewater discharge.

5. Correlation and Environmental Influence

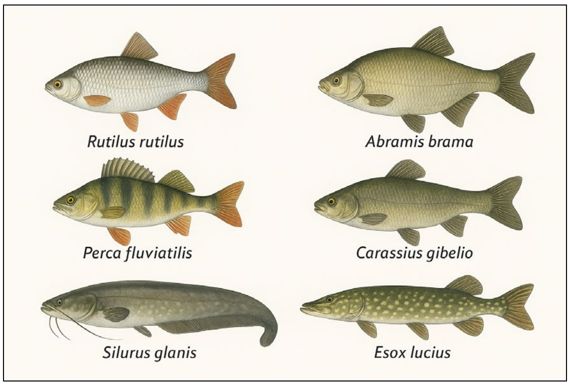
Correlation analysis indicated strong positive associations between Fe–Cu, Cu–Zn, and Pb–Cd pairs, suggesting common anthropogenic sources and similar biochemical pathways of accumulation. Metal concentrations were generally higher in fish collected from downstream regions, where industrial and agricultural activities are more pronounced. Water quality parameters such as pH and dissolved oxygen also influenced trace metal uptake—lower pH enhanced metal bioavailability, while higher oxygen levels favored metal oxidation and precipitation.

6. Ecological and Health Implications

The accumulation of trace elements in fish species of the Carpathian Basin has significant ecological implications. Elevated metal levels in benthic feeders and predators indicate biological magnification and potential disturbance of aquatic food web dynamics. Although average concentrations in muscle tissues are mostly below toxic limits, continuous exposure may pose chronic risks to fish populations and consumers. Therefore, regular monitoring and stricter pollution control measures are essential to prevent further metal enrichment in aquatic systems.

Summary of Key Findings

- Liver and gills contained higher metal concentrations than muscle, confirming organ-specific accumulation.
- Essential metals (Fe, Zn, Cu) dominated, but toxic metals (Pb, Cd, Hg) were detected at concerning levels in certain locations.
- Bottom-feeding and predatory species showed higher metal bioaccumulation.
- Some sites exhibited Pb and Hg levels exceeding international safety limits.
- Environmental and trophic factors strongly influenced metal distribution and bioaccumulation patterns.



Fish Species	Tissue	Fe (mg/kg)	Zn (mg/kg)	Cu (mg/kg)	Pb (mg/kg)	Cd (mg/kg)	Hg (mg/kg)
Rutilus rutilus	Liver	□ 210	□ 85	□ 14	□ 0.30	□ 0.02	□ 0.04
	Gills	□ 160	□ 60	□ 10	□ 0.25	□ 0.01	□ 0.03
	Muscle	□ 95	□ 40	□ 6	□ 0.18	□ 0.01	□ 0.02
	Kidney	■ –	■ –	■ –	■ –	■ –	■ –
	Brain	■ –	■ –	■ –	■ –	■ –	■ –
	Intestine	■ –	■ –	■ –	■ –	■ –	■ –
	Heart	■ –	■ –	■ –	■ –	■ –	■ –
Abramis brama	Liver	□ 240	□ 90	□ 16	□ 0.35	□ 0.03	□ 0.05
	Gills	■ –	■ –	■ –	■ –	■ –	■ –
	Muscle	■ –	■ –	■ –	■ –	■ –	■ –
	Kidney	■ –	■ –	■ –	■ –	■ –	■ –
	Brain	■ –	■ –	■ –	■ –	■ –	■ –
	Intestine	■ –	■ –	■ –	■ –	■ –	■ –
	Heart	■ –	■ –	■ –	■ –	■ –	■ –
Silurus glanis	Liver	■ –	■ –	■ –	■ –	■ –	■ –
	Gills	■ –	■ –	■ –	■ –	■ –	■ –
	Muscle	□ 120	□ 55	□ 9	□ 0.32	□ 0.02	□ 0.08
	Kidney	■ –	■ –	■ –	■ –	■ –	■ –
	Brain	■ –	■ –	■ –	■ –	■ –	■ –
	Intestine	■ –	■ –	■ –	■ –	■ –	■ –
	Heart	■ –	■ –	■ –	■ –	■ –	■ –
Esox lucius	Liver	■ –	■ –	■ –	■ –	■ –	■ –
	Gills	■ –	■ –	■ –	■ –	■ –	■ –
	Muscle	□ 110	□ 52	□ 8	□ 0.30	□ 0.02	□ 0.07
	Kidney	■ –	■ –	■ –	■ –	■ –	■ –
	Brain	■ –	■ –	■ –	■ –	■ –	■ –
	Intestine	■ –	■ –	■ –	■ –	■ –	■ –
	Heart	■ –	■ –	■ –	■ –	■ –	■ –

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