



**Bioaccumulation of Trace and Macro Elements in Freshwater
Ecosystems: Using Aquatic Vertebrates as Bioindicators of Chronic
and Episodic Metal Exposure**

Thesis for the Degree of Doctor of Philosophy (PhD)

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Debrecen, 2025

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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Debrecen, 10 October 2025

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Signature of the supervisor

Bioaccumulation of Trace and Macro Elements in Freshwater Ecosystems: Using Aquatic Vertebrates as Bioindicators of Chronic and Episodic Metal Exposure

Dissertation submitted in partial fulfillment of the requirements for the doctoral (PhD) degree in
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Written by Haithem Aib

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DEDICATION

TO

To my beloved parents, whose unwavering support, sacrifices, and endless encouragement have made everything possible. Your love and belief in me have been the foundation of this journey, and I owe every step of my success to your tireless efforts.

To my dear siblings, for their constant companionship, laughter, and motivation. Your presence has been a source of strength and joy throughout this academic path.

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Table of Contents

CHAPTER 01 INTRODUCTION AND OBJECTIVES.....	12
1.1 INTRODUCTION	12
1.2. PROBLEM STATEMENT.....	13
1.3. OBJECTIVE OF THE STUDY	15
CHAPTER 02 LITERATURE REVIEW	16
2.1 WATER QUALITY IN FRESHWATER ECOSYSTEMS.....	17
2.1.1 <i>DEFINITION AND IMPORTANCE OF WATER QUALITY</i>	17
2.2 FACTORS INFLUENCING WATER QUALITY.....	18
2.2.1 <i>NATURAL FACTORS</i>	18
2.2.2 <i>ANTHROPOGENIC FACTORS</i>	19
2.3 IMPLICATIONS OF POOR WATER QUALITY	20
2.3.1 <i>ECOLOGICAL IMPACTS</i>	20
2.3.2 <i>HUMAN AND ECONOMIC CONSEQUENCES</i>	21
2.4 ROLE OF WATER QUALITY IN HEAVY METAL CONTAMINATION	22
2.5 HEAVY METALS IN AQUATIC ENVIRONMENTS.....	22
2.5.1 <i>OVERVIEW OF HEAVY METALS</i>	22
2.5.2 <i>ESSENTIAL VS. NON-ESSENTIAL METALS:</i>	23
2.5.3 <i>Sources of Heavy Metal Pollution</i>	24
2.6 FATE AND TRANSPORT IN AQUATIC SYSTEMS:	25
2.6.1 <i>Mechanisms of Dissolution and Transport</i>	25
2.6.2 <i>SEDIMENTATION AND BIOAVAILABILITY</i>	26
2.6.3 <i>Common Heavy Metals of Concern</i>	26
2.7 MECHANISMS OF TOXICITY AND BIOACCUMULATION.....	29
2.7.1 <i>TOXICOLOGICAL MECHANISMS</i>	29
2.7.2 <i>BIOACCUMULATION AND BIOMAGNIFICATION.....</i>	29
2.7.2.1 <i>Bioconcentration Factor (BCF).....</i>	29
2.7.2.2 <i>Bioaccumulation Coefficient (BAC)</i>	30
2.7.3 <i>BIOACCUMULATION OF HEAVY METALS IN BIOTA</i>	30
2.7.4 <i>BIOACCUMULATION OF HEAVY METALS IN FRESHWATER FISH</i>	31
2.7.5 <i>DIFFERENCES BETWEEN BIOACCUMULATION IN VARIOUS TISSUES</i>	32
• 2.7.5.1 <i>Muscle vs. Liver</i>	32
• 2.7.5.2 <i>Scales vs. Soft Tissues</i>	32
• 2.7.5.3 <i>Gills vs. Liver.....</i>	33
• 2.7.5.4 <i>Turtle Shells as Hard Tissue Accumulators.....</i>	33
• 2.7.5.5 <i>Importance of Carapace Tissue in Environmental Monitoring</i>	33
• 2.7.5.6 <i>Common Metal Accumulation Patterns in Shells</i>	33
• 2.7.5.7 <i>Scute–Tissue Element Relationships.....</i>	34
• 2.7.5.8 <i>Temporal Resolution Provided by Scute Growth</i>	34
2.8 FACTORS INFLUENCING BIOACCUMULATION	34

2.8.1 ENVIRONMENTAL AND BIOLOGICAL FACTORS INFLUENCING BIOACCUMULATION.....	34
2.8.2 SPECIES TRAITS AND LIFE STAGE	35
2.8.3 FEEDING HABITS	35
2.8.4 HABITAT TYPE	35
2.8.5 ENVIRONMENTAL CONDITIONS.....	36
2.9 USE OF AQUATIC ORGANISMS AS BIOINDICATORS	36
2.9.1 RELEVANCE TO FISH SCALES AND TURTLE SHELLS	36
2.9.2 FISH SCALES AS BIOINDICATORS OF HEAVY METAL ACCUMULATION	36
2.9.3 TURTLE SHELLS REFLECTING SEDIMENT-ASSOCIATED METAL EXPOSURE	36
2.9.4 INFLUENCE OF SPECIES TRAITS AND ENVIRONMENTAL FACTORS	37
2.9.5 SENSITIVITY TO ENVIRONMENTAL CHANGE	37
2.9.6 ECOLOGICAL ROLE AND FOOD WEB POSITION	37
2.9.7 CAPACITY TO INTEGRATE LONG-TERM STRESSOR EXPOSURE	38
2.9.8 NON-INVASIVE BIOMARKERS: FISH SCALES AND TURTLE SHELLS.....	38
2.9.9 ADVANTAGES OVER INTERNAL TISSUES (LIVER, MUSCLE, KIDNEY)	38
2.9.10 ANALYTICAL TECHNIQUES USED IN SCALE AND SHELL ANALYSIS	39
2.10 ECOLOGICAL AND HUMAN HEALTH IMPACTS OF HEAVY METALS	41
2.10.1 EFFECTS ON AQUATIC LIFE	41
2.10.2 EFFECTS ON HUMAN HEALTH	42
2.11 MONITORING STRATEGIES	43
2.11.1 REGULATORY FRAMEWORKS AND GUIDELINES.....	43
2.11.2 INTERNATIONAL STANDARDS (WHO, UNEP, EU).....	43
2.11.3 NATIONAL REGULATIONS AND LOCAL POLICIES	44
2.12 RESEARCH GAPS AND FUTURE DIRECTIONS.....	44
2.12.1 LONG-TERM MONITORING AND CUMULATIVE IMPACTS.....	44
2.12.2 SUBLETHAL AND COMBINED EFFECTS OF MULTIPLE STRESSORS	45
2.12.3 ROLE OF SEDIMENTS AND BIOAVAILABILITY IN HEAVY METAL DYNAMICS	46
2.12.4 CLIMATE CHANGE AND MOBILIZATION OF HEAVY METALS	47
2.12.5 NOVEL BIOINDICATORS AND ADVANCED ANALYTICAL APPROACHES.....	47
2.12.6 NANOTECHNOLOGY AND HEAVY METAL REMEDIATION	48
CHAPTER 03: MATERIAL AND METHODS.....	49
3.1 STUDY DESIGN OVERVIEW	49
3.2 STUDY AREA.....	49
3.2.1 RIVER MAROS.....	49
3.2.2 LAKE REGHAIA	50
3.3 SAMPLE COLLECTION.....	52
3.3.1 Fish scales in river Maros	52
3.3.2 Water, sediment and turtle shells in Raghai lake	54
3.3.3 Environmental Sample Preparation and Analysis of turtle shell.....	54
3.3.4 Turtle Shell Preparation and Analysis.....	55
3.3.5 Ecological Characteristics for studied species.....	56

3.4 ANALYTICAL MEASUREMENT.....	57
3.4.1 ANALYTICAL MEASUREMENT OF TRACE ELEMENTS IN FISH SCALES	57
3.4.1.1 Analytical Measurements XRF	57
3.4.1.2 SEM (Scanning Electron Microscope).....	58
3.4.1.3 Analytical Measurements ICP-OES.....	59
3.4.2 ANALYTICAL MEASUREMENT OF TURTLE SHELLS	59
3.5. DATA ANALYSIS.....	60
3.5.1 FISH SCALES	60
3.5.2 TURTLE SHELLS	61
CHAPTER 04. RESULTS.....	62
4.1. FISH SCALES RESULTS.....	62
4.1.1 ELEMENTAL CHARACTERIZATION AND BIOLOGICAL FEATURES.....	62
4.1.1.1 BIOLOGICAL CHARACTERISTICS OF THE STUDIED FISH AND TURTLES	62
4.1.2. TRACE ELEMENT CONCENTRATIONS IN RIVER WATER.....	63
4.1.3. MACRO- AND TRACE ELEMENT ACCUMULATION IN FISH SCALES	63
4.1.4. CORRELATION BETWEEN ELEMENTAL CONCENTRATIONS, FISH WEIGHT, AND TROPHIC LEVEL	65
4.1.5. XRF ANALYSIS OF ELEMENTAL DISTRIBUTION	67
4.1.6 SEM-EDS ANALYSIS OF MORPHOLOGICAL AND ELEMENTAL FEATURES	68
4.2 TURTLE SHELLS RESULTS	71
4.2.1 ELEMENTAL COMPOSITION AND SEX-BASED DIFFERENCES.....	71
4.2.2 MICRO-XRF ELEMENTAL MAPPING.....	73
4.2.3 CORRELATION PATTERNS AMONG SHELL ELEMENTS	74
4.2.4 SEASONAL VARIATION OF HEAVY METALS IN SEDIMENTS AND WATER	78
4.2.5 BIOINDICATOR RELATIONSHIPS BETWEEN SHELL AND ENVIRONMENTAL CONCENTRATIONS	79
A COMPARISON OF AVERAGE METAL CONCENTRATIONS ACROSS SHELLS, SEDIMENTS, AND WATER IS SHOWN IN (FIGURE 13). SHELLS CONSISTENTLY REFLECTED HIGHER LONG-TERM ACCUMULATION OF TRACE METALS (E.G., ZN, MN, Pb) COMPARED TO WATER, WITH SEDIMENT VALUES LYING IN BETWEEN.	79
CHAPTER 05 DISCUSSION.....	81
5.2 FISH SCALES AS BIOINDICATORS	82
5.2.1 SPECIES-SPECIFIC PATTERNS	82
5.2.2 INFLUENCE OF BODY SIZE AND TROPHIC STATUS.....	86
5.2.3 RELATIONSHIP WITH ENVIRONMENTAL MATRICES	87
5.2.4 METHODOLOGICAL CONSIDERATIONS.....	88
5.3 TURTLE SHELLS AS BIOINDICATORS	90
5.3.1 ELEMENTAL COMPOSITION AND ENVIRONMENTAL RELEVANCE	90
5.3.2 SEX-LINKED DIFFERENCES IN ACCUMULATION	91
5.3.3 ELEMENTAL MAPPING AND SPATIAL HETEROGENEITY	92
5.3.4 MORPHOMETRICS AND BIOACCUMULATION.....	94
5.3.5 LINKS WITH ENVIRONMENTAL MATRICES.....	95
5.3.6 CONSERVATION AND ECOLOGICAL IMPLICATIONS	97

5.4 COMPARATIVE INSIGHTS: FISH SCALES VS. TURTLE SHELLS.....	98
5.4.1 <i>COMPLEMENTARY BIOINDICATOR ROLES</i>	98
5.4.2 <i>DIFFERENCES IN EXPOSURE PATHWAYS</i>	98
5.4.3 <i>SPECIES-SPECIFIC AND SEX-SPECIFIC SIGNALS</i>	100
5.4.4 <i>METHODOLOGICAL CONSIDERATIONS</i>	101
5.5 METHODOLOGICAL REFLECTIONS AND FUTURE DIRECTIONS	101
5.5.1 <i>Strengths of the Approach</i>	101
5.5.2 <i>Future Research Directions</i>	102
CHAPTER 06 CONCLUSION	105
6. 1 KEY FINDINGS	105
6.2 CONTRIBUTIONS AND IMPLICATIONS.....	106
6.3 LIMITATIONS	107
6.4 SUMMARY	107
7. ACKNOWLEDGMENTS	108
8. REFERENCES	109

List of abbreviations

Acronym	Full Form
AF	Accumulation Factor
Al	Aluminium
AN	Atomic Number
As	Arsenic
ATOMKI	Institute for Nuclear Research (Hungary)
BAF	Bioaccumulation Factor
BAC	Bioaccumulation Coefficient
BCF	Bioconcentration Factor
BSAF	Biota–Sediment Accumulation Factor
Ca	Calcium
Cd	Cadmium
CH ₃ Hg ⁺	Methylmercury ion
Cl	Chloride
Co	Cobalt
COD	Chemical Oxygen Demand
Cr	Chromium
Cu	Copper
DOM	Dissolved Organic Matter
DO	Dissolved Oxygen
EDS	Energy Dispersive Spectroscopy
EPA	Environmental Protection Agency (U.S.)
EU	European Union
Fe	Iron
FAO	Food and Agriculture Organization
HNO ₃	Nitric Acid
H ₂ O ₂	Hydrogen Peroxide
Hg	Mercury
ICP-OES	Inductively Coupled Plasma – Optical Emission Spectrometry
ICP-MS	Inductively Coupled Plasma – Mass Spectrometry
K	Potassium
Mg	Magnesium
Mn	Manganese
MTF	Metal Transfer Factor
Ni	Nickel
NNK	Nemzeti Népegészségügyi Központ (Hungarian Public Health Center)
Pb	Lead
P	Phosphorus
PCA	Principal Component Analysis

ROS	Reactive Oxygen Species
Sb	Antimony
SEM	Scanning Electron Microscope
Sr	Strontium
TF	Transfer Factor
Ti	Titanium
UNEP	United Nations Environment Programme
UNESCO	United Nations Educational, Scientific and Cultural Organization
US EPA	United States Environmental Protection Agency
UV	Ultraviolet
WHO	World Health Organization
WDXRF	Wavelength Dispersive X-ray Fluorescence
XRF	X-ray Fluorescence
Zn	Zinc

Chapter 01 Introduction and Objectives

1.1 Introduction

Aquatic ecosystems are vital for sustaining biodiversity and ecosystem services; however, their ecological integrity is increasingly threatened by anthropogenic pressures such as urbanization, industrialization, wastewater discharge, and agricultural runoff (Malaj et al., 2014). Among the various pollutants affecting freshwater systems, heavy metals and trace elements are of particular concern due to their toxicity, persistence, and ability to bioaccumulate and biomagnify through aquatic food webs (Aib et al., 2025; H. Ali et al., 2013; Ebol et al., 2020; Jaishankar et al., 2014).

Heavy metals, commonly defined as elements with a density greater than 5 g/cm³ (Csuros & Csuros, 2016), can exert harmful effects even at low concentrations (Mahboob et al., 2014). Metals such as lead (Pb), cadmium (Cd), chromium (Cr), copper (Cu), nickel (Ni), and zinc (Zn) are non-biodegradable and tend to accumulate in sediments and biota (Alloway, 2013; Förstner & Wittmann, 1981; MacFarlane & Burchett, 2000). Their accumulation can lead to physiological, genetic, and reproductive impairments in aquatic organisms, ultimately destabilizing ecosystems and reducing biodiversity (S. Khan et al., 2010; Luo et al., 2011; Mitra et al., 2022; Zaynab et al., 2022).

These issues are especially critical in freshwater wetlands, rivers, and lakes. For example, Lake Reghaïa in northern Algeria is a Ramsar-designated wetland of high ecological importance, yet it faces severe degradation due to urban encroachment, industrial discharges, and untreated wastewater (Bouhezila & Hacene, 2020; MedWet Managers Network, 2020). In such vulnerable environments, effective and sensitive monitoring strategies are needed to detect and manage contamination. While water quality assessments have traditionally relied on physicochemical measurements, biomonitoring using aquatic organisms is increasingly recognized as a more robust approach because it reflects long-term exposure rather than short-term fluctuations (H. Ali & Khan, 2019b). Moreover, non-invasive biomonitoring methods are gaining emphasis to minimize ethical concerns and allow repeated sampling. In this context, reptiles such as the Mediterranean pond turtle (*Mauremys leprosa*) and freshwater fishes such as the chub (*Squalius cephalus*) and nase (*Chondrostoma nasus*) have demonstrated strong potential as bioindicators due to their

ecological roles, habitat fidelity, and capacity to accumulate contaminants (De Solla & Fernie, 2004; EL Hassani et al., 2019a; Le Gal et al., 2023; Mauremys leprosa: Luiselli, 2023).

Turtles are excellent candidates for long-term pollution assessment because of their long lifespan, high trophic level, and strong site fidelity. The keratin and bone that compose a turtle's shell allow heavy metals to accumulate over time, providing a non-lethal matrix for studying bioaccumulation (Meyers-Schöne et al., 1993; Sakai et al., 2000a). Likewise, the continuous growth of fish scales enables them to incorporate metals during their formation, making them indicative of both recent and historical exposure to waterborne contaminants (Jiwan & Ajay, 2011; Łuszczek-Trojnar & Nowacki, 2021). Scales can be collected repeatedly and non-destructively from live individuals—unlike internal organs or otoliths—making them suitable for life-history studies and long-term environmental monitoring (Burger et al., 2010; Łuszczek-Trojnar & Nowacki, 2021; Vašek et al., 2021). The elemental signatures preserved within scales reflect not only pollution levels but also ecological characteristics of fish, including habitat use, feeding strategies, migration patterns, and physiological stress responses (Vašek et al., 2021; Zaib-Un-Nisa et al., 2021). Additionally, advanced analytical techniques such as particle-induced X-ray emission (PIXE) and micro-X-ray fluorescence (μ -XRF) allow spatially resolved elemental mapping of both scales and shells, enabling detailed quantification of trace and macroelements (Meyers-Schöne et al., 1993; Soe Aye et al., 2018).

1.2. Problem Statement

*1.2.1. Fish Scales from Chub (*Squalius cephalus*) and Nase (*Chondrostoma nasus*) as Non-Invasive Bioindicators of Trace and Macroelements in the Maros/Mureş River*

Urbanization, industrial discharge, and agricultural runoff are placing increasing pressure on freshwater ecosystems, making effective monitoring essential. The Maros/Mureş River, a transboundary water body in Central Europe, is exposed to these anthropogenic stressors, which threaten its ecological integrity. Traditional environmental assessments often rely on water and sediment analyses or destructive sampling of aquatic organisms—approaches that can be both ethically and ecologically problematic. To address these limitations, this study explores the use of fish scales as a non-lethal biomonitoring tool. Two native fish species, the chub (*Squalius cephalus*) and the nase (*Chondrostoma nasus*), were selected due to their ecological relevance,

availability, and distinct trophic and habitat preferences. Fish scales—composed primarily of collagen and hydroxyapatite—serve as biological archives that progressively incorporate elements from the surrounding environment. Macroelements (e.g., Ca, K, Mg, Na, P, S) and trace elements (e.g., Al, Ba, Cu, Cr, Fe, Mn, Sr, Zn) were quantified using inductively coupled plasma optical emission spectrometry (ICP-OES) and X-ray fluorescence (XRF). Additionally, scanning electron microscopy (SEM) provided insight into scale microstructural variations. The results revealed clear species-specific differences in elemental accumulation, highlighting the influence of ecological behavior and habitat use on bioaccumulation patterns.

These findings support the use of fish scales as reliable, non-invasive bioindicators of aquatic pollution. This approach enables repeated monitoring over time with minimal ecological disturbance and aligns with modern environmental management strategies that prioritize sustainability, long-term ecosystem assessment, and animal welfare.

1.2.2. Turtle Shells as Bioindicators of Heavy Metal Contamination in Algerian Freshwater Wetlands

Uncontrolled urban expansion, untreated wastewater discharge, and industrial activities are increasingly threatening Algerian wetland ecosystems, including those recognized as Ramsar sites. These pressures have contributed to elevated heavy metal concentrations in surface waters and sediments, posing serious risks to aquatic biodiversity—particularly for long-lived, slow-reproducing species such as freshwater turtles.

The Mediterranean pond turtle (*Mauremys leprosa*), a native and vulnerable species commonly found in Algerian wetlands, has gained attention as a valuable bioindicator due to its omnivorous diet, long lifespan, and strong site fidelity. However, conventional biomonitoring approaches that rely on destructive tissue sampling are constrained by ethical and conservation concerns. To overcome these limitations, the present study examines the potential of turtle shells as non-invasive bioaccumulators of trace and heavy metals.

Turtle shells, composed of keratin overlaying bony plates (carapace and plastron), can gradually incorporate metals such as Pb, Cd, Zn, and Fe through both dietary uptake and direct environmental exposure. By analyzing shell samples collected from turtles in polluted and reference sites—

including the heavily impacted urban wetland of Reghaïa Lake—this study aims to assess spatial variation in contamination and evaluate associated ecological risks. Using the non-destructive micro X-ray fluorescence (μ XRF) analytical technique, the study contributes to the growing body of research supporting shell-based monitoring as a conservation-friendly alternative. The findings are expected to strengthen early warning systems, enable comparative assessments of wetland pollution, and support evidence-based policy measures for protecting aquatic habitats and the species that depend on them.

1.3. Objective of the Study

This dissertation aims to contribute to the advancement of non-invasive biomonitoring methodologies for assessing trace and heavy metal pollution in Algerian freshwater ecosystems. Specifically, the objectives are:

- In relation to environmental samples (water and sediment) in order to evaluate the seasonal and spatial variations of these elements in Reghaïa Lake and the River Maros.
- To determine how much trace and heavy metal bioaccumulation existed for instance: Cd, Pb, Zn, Cu, Ni, Mn, Al, Fe, Cr, Ba, Sr, the shells of *Mauremys leprosa*, and certain macroelements in freshwater fish scales.
- To investigate how elemental accumulation is impacted by environmental factors, feeding ecology, and habitat.
- To validate the use of fish scales and turtle shells as non-lethal, reliable bioindicators using advanced analytical techniques (XRF, ICP-OES).
- To provide baseline data on metal contamination in Algeria's ecologically delicate and poorly observed freshwater habitats in order to aid conservation and management initiatives.

Chapter 02 Literature Review

Freshwater ecosystems are essential to life on Earth, supporting high biodiversity, regulating nutrient cycles, and providing resources such as drinking water, irrigation, and fisheries. However, these systems are increasingly threatened by human activities, including industrialization, urbanization, and agricultural expansion. Among the various pollutants affecting freshwater environments, heavy metals are particularly concerning due to their toxicity, persistence, and long-term impacts on both ecosystems and human health (Dudgeon et al., 2006; Tchounwou et al., 2012; Vörösmarty et al., 2010).

Heavy metals are commonly defined as elements with a density greater than 5 g.cm^{-3} (Csuros & Csuros, 2016). The term is often used to describe metals and metalloids associated with environmental contamination and potential toxicity (Duffus, 2002), and has more recently been expanded to include naturally occurring metals with atomic numbers above 20 and densities exceeding 5 g.cm^{-3} (Nagajyoti et al., 2010). Metals such as lead (Pb), cadmium (Cd), mercury (Hg), and chromium (Cr) enter freshwater systems through wastewater discharge, mining, industrial activities, and agricultural runoff (H. Ali & Khan, 2018a, 2019b; He et al., 2005). Unlike many organic pollutants, heavy metals are non-biodegradable and persist in water, sediments, and living organisms. Through bioaccumulation and biomagnification, their concentrations increase along food webs, posing significant risks to aquatic life and human health.

Heavy metals are toxic because they interfere with essential biological processes, inducing oxidative stress and inhibiting enzymatic activity, which disrupts growth, reproduction, and survival in aquatic organisms (Eisler, 2000; Flora et al., 2012). Fish are particularly sensitive to such exposure and are therefore widely recognized as reliable bioindicators of freshwater ecosystem health. Accumulation of metals in fish tissues not only affects their physiological and ecological functions but also poses health risks to humans who consume contaminated fish.

Accurate understanding of heavy metal contamination dynamics is crucial for effective monitoring and management. Conventional assessments that measure metal concentrations in water and sediments often fail to capture long-term biological effects. Bioindicators, particularly fish, provide a more integrative perspective because they reflect cumulative exposure over time. Among biological matrices used for monitoring, fish scales offer a non-lethal and practical method for

assessing heavy metal bioaccumulation. Scales grow continuously and preserve chronological exposure records, allowing researchers to evaluate both past and current contamination. Moreover, their collection does not harm the organism, making them a sustainable and effective tool for environmental monitoring.

This literature review offers a thorough analysis of the contamination of freshwater ecosystems by heavy metals, with a focus on fish bioaccumulation. The significance of monitoring water quality is emphasized, along with the mechanisms of heavy metal toxicity and the function of fish and their scales as bioindicators. This chapter lays the groundwork for assessing the ecological and health effects of heavy metals by synthesizing recent research and highlights the necessity of creative and sustainable monitoring methods in freshwater environments.

2.1 Water Quality in Freshwater Ecosystems

2.1.1 Definition and Importance of Water Quality

Understanding and defining water quality is essential for promoting pro-environmental behavior and evaluating the effects of human interactions with freshwater systems (Votruba & Corman, 2020). Water quality encompasses the physical, chemical, and biological properties of water that determine its suitability for drinking, recreation, and ecological functions (Fulazzaky, 2010; WHO, 2021, 2022). Maintaining high water quality is vital for supporting aquatic ecosystems, sustaining biodiversity, and ensuring the health of organisms that depend on freshwater resources (Ahmed et al., 2022).

Key indicators of water quality include temperature, turbidity, pH, dissolved oxygen, nutrient concentrations, and the presence of pollutants such as organic contaminants and heavy metals. Freshwater ecosystems—such as lakes, rivers, and wetlands—play a fundamental role in regulating hydrological cycles, supporting diverse plant and animal communities, and acting as natural filters for pollutants (Gopal, 2020; L. K. Sharma & Naik, 2024). Preserving high water quality is therefore critical to maintaining ecological balance and preventing ecosystem degradation.

2.2 Factors Influencing Water Quality

2.2.1 Natural Factors

- *Geology and Hydrology*

The geological composition of an area plays a major role in determining the chemical characteristics of surface and groundwater. Water passing through mineral-rich formations such as limestone or dolomite typically contains elevated calcium and magnesium levels, contributing to increased water hardness (Chapelle, 2001; Hem, 1985). Likewise, trace metals including iron, manganese, and arsenic may naturally leach into water bodies from surrounding soils and bedrock, depending on local geochemical conditions (WHO, 2017). In addition to geology, hydrological processes strongly influence water quality. Factors such as water flow rates, groundwater recharge, and interactions between surface water and aquifers affect the concentration and distribution of dissolved substances. For instance, fast-flowing rivers are generally more oxygenated and less mineralized, whereas slow-moving groundwater can accumulate higher mineral content over time (Meybeck & Helmer, 1989; Winter et al., 1998). Streamflow-driven sediment transport also influences turbidity and the movement of particulate-bound nutrients and pollutants.

- *Climate and Seasonal Variability*

Climatic factors such as temperature, precipitation patterns, and seasonal cycles strongly influence water quality. Increased rainfall can elevate surface runoff, introducing pollutants, nutrients, and sediments into aquatic systems (Delpla et al., 2009). In contrast, reduced water volumes during dry seasons or droughts may concentrate existing contaminants, increasing toxicity risks for aquatic organisms (Whitehead et al., 2009). Temperature fluctuations also affect biochemical processes, including microbial decomposition, algal growth, and dissolved oxygen solubility (Moss, 2013).

Seasonal changes can further modify lake stratification patterns, altering oxygen distribution and nutrient cycling, particularly in temperate and tropical regions (Robert G. Wetzel, 2001). Therefore, understanding the interaction between hydrological regimes and climate variability is essential for accurately predicting and managing water quality under changing climatic conditions.

2.2.2 Anthropogenic Factors

- *Urbanization and Industrialization*

Freshwater systems are greatly impacted by urbanization and industrialization because they introduce a variety of pollutants (Paul & Meyer, 2001; WHO, 2021). Heavy metals like lead, cadmium, and zinc are frequently found in urban runoff, which comes from industrial processes, building materials, and vehicle emissions (M. M. Ali et al., 2021). These metals can accumulate in aquatic environments, posing risks to aquatic life and human health (K. P. Singh et al., 2004). Water quality is further deteriorated by industrial discharges, which also contribute other contaminants like synthetic chemicals and nutrients (Islam et al., 2015). For instance, Urban areas have been linked to higher pollution levels in local water bodies, which can cause ecological imbalances and health issues, according to studies (Wu et al., 2023).

- *Agricultural Activities*

In freshwater systems, agricultural activities are the main cause of nutrient pollution (US EPA, 2025). Excess nitrogen and phosphorus are released into the environment through the use of pesticides and fertilizers (Carpenter et al., 1998). These nutrients have the potential to seep into bodies of water, encouraging algal blooms and causing eutrophication, which damages aquatic life and reduces oxygen levels in the water (V. H. Smith & Schindler, 2009). Furthermore, pesticides have the potential to introduce harmful substances that impact human health as well as aquatic life (Stehle & Schulz, 2015). Effective management strategies are necessary because research shows that agricultural runoff significantly contributes to nutrient loading in many regions (Wenjun et al., 2011).

- *Wastewater and Sewage*

A variety of contaminants, such as pathogens, nutrients, and heavy metals, are introduced into freshwater systems when untreated or insufficiently treated wastewater and sewage are released (WHO, 2022). Waterborne disease transmission, aquatic habitat deterioration, and the buildup of toxic compounds in the food chain can all result from this contamination (Edokpayi et al., 2017). For example, Studies have shown that water bodies receiving sewage effluents contain dangerous

chemicals and bacteria, underscoring the necessity of better wastewater treatment facilities to safeguard the environment and public health (Avelino Núñez-Delgado, 2023).

2.3 Implications of Poor Water Quality

2.3.1 Ecological Impacts

- *Biodiversity Loss*

The health of aquatic organisms is disturbed by contaminants like heavy metals, which lowers species diversity and abundance (H. Ali & Khan, 2019b). Industrialization, mining, agricultural runoff, and natural processes all contribute to the introduction of heavy metals like cadmium, lead, and mercury into aquatic systems (Jaishankar et al., 2014), like volcanic activity and the weathering of rocks. These metals have the potential to build up in aquatic environments, endangering both human health and aquatic life (Mitra et al., 2022). For instance, According to studies, heavy metals can have disastrous effects on aquatic biota, such as fish, benthos, and plankton, by lowering turbidity, dissolved oxygen concentrations, and water quality (Das et al., 2023; El-Sharkawy et al., 2025).

- *Ecosystem Imbalance*

Changes in water quality can destabilize aquatic ecosystems by altering nutrient cycling and food web dynamics (Heathwaite, 2010; Raimi et al., 2021; Tumanyan et al., 2020). Excessive inputs of nitrogen and phosphorus from agricultural runoff often lead to eutrophication, which reduces dissolved oxygen and harms aquatic organisms (Hashimi & Hashimi, 2020; M. N. Khan & Mohammad, 2014; Tiwari & Pal, 2022; Wurtsbaugh et al., 2019). Such nutrient enrichment increases phytoplankton biomass, promoting algal blooms that block sunlight, deplete oxygen, and shift species composition, ultimately reducing biodiversity (Kudela et al., 2010; Leibold, 1999; Makareviciute-Fichtner et al., 2024; Reynolds, 1998).

Altered water quality can also disrupt the transfer of nutrients and energy across trophic levels (de Guzman et al., 2022). Elevated nutrient concentrations may reduce the availability of high-quality food sources for aquatic organisms, impairing their growth and reproductive success. These

changes can modify species interactions and compromise overall ecosystem stability (Guo et al., 2016; Schreck, 2010; Schreck et al., 2001; Yan et al., 2024).

2.3.2 Human and Economic Consequences

- *Public Health Risks*

Significant risks to public health arise from contaminated water, which can spread waterborne illnesses like cholera, typhoid, hepatitis A, and other gastrointestinal infections (Lanrewaju et al., 2022). The presence of heavy metals in contaminated water sources, such as arsenic, lead, cadmium, and mercury, is linked to serious toxic consequences, such as cancer, kidney damage, neurological disorders, and reproductive problems (Jaishankar et al., 2014). Long-term exposure to even low concentrations of heavy metals can cause chronic health issues because of their bioaccumulative nature and ability to disrupt cellular and enzymatic functions (Munir et al., 2021; Rehman et al., 2018). In developing regions, Inadequate infrastructure for sanitation and water treatment increases these risks, leaving communities more susceptible to long-term health problems and disease outbreaks (UNESCO, 2020, 2022).

- *Economic Losses*

Economic sectors like agriculture, fishing, and tourism that depend on clean water can be severely impacted by poor water quality (OECD, 2012). For instance, Fish die-offs and a drop in commercial fishery yields are caused by eutrophication and toxic algal blooms, which are exacerbated by nutrient pollution from agricultural runoff (V. H. Smith & Schindler, 2009). Additionally, tourism suffers because contaminated waterways discourage leisure activities and make places less appealing (Dokulil, 2014; Ghulamrabbany et al., 2013). Governments and households are also financially strained by rising water treatment and healthcare expenses associated with waterborne illnesses (Malik et al., 2012). The World Bank has estimated that poor sanitation and water-related diseases cost some countries up to 4.3% of their GDP annually (World Bank Group, 2023).

2.4 Role of Water Quality in Heavy Metal Contamination

Heavy metals are major contributors to water quality degradation due to their high toxicity, persistence, and ability to bioaccumulate in aquatic organisms (H. Ali & Khan, 2019a). Unlike many organic pollutants, heavy metals such as lead (Pb), cadmium (Cd), mercury (Hg), and arsenic (As) do not degrade and can remain in aquatic systems for long periods, posing sustained ecological risks (Aib et al., 2025). Through bioaccumulation and biomagnification, they accumulate in sediments and move through the food chain, affecting fish, invertebrates, and ultimately humans who consume contaminated aquatic organisms (H. Ali et al., 2019; Sonone et al., 2021).

The environmental behavior of heavy metals is strongly influenced by water quality parameters such as pH, redox potential, dissolved oxygen, temperature, and salinity (Paloma de Almeida et al., 2023). For instance, metals become more soluble and mobile in acidic conditions, increasing their toxicity (Naseem & Tahir, 2001). In contrast, under alkaline conditions, metals may precipitate or form complexes, temporarily reducing their bioavailability but leading to long-term accumulation in sediments (Förstner, 1982). Because metal speciation and transformation determine their uptake and biological effects, understanding these processes is essential for evaluating ecological and toxicological risks in freshwater systems (Campbell, 2009; W. X. Wang & Rainbow, 2008). This knowledge is fundamental for developing accurate water quality models, conducting risk assessments, and implementing pollution management strategies that protect aquatic biodiversity and public health (H. Ali & Khan, 2018, 2019).

2.5 Heavy Metals in Aquatic Environments

2.5.1 Overview of Heavy Metals

Heavy metals are naturally occurring elements with high atomic weight and densities greater than 5 g/cm³ (H. Ali & Khan, 2018; Appenroth, 2010). They include non-essential metals such as lead (Pb), cadmium (Cd), mercury (Hg), and arsenic (As), which have no known biological function and are toxic even at low concentrations, as well as essential metals such as iron (Fe), copper (Cu), and zinc (Zn), which are required in trace amounts for physiological processes in aquatic organisms (Appenroth, 2010; Jaishankar et al., 2014; Jan et al., 2015; Rainbow, 1985).

Heavy metals enter aquatic environments from both natural sources, such as volcanic activity and rock weathering, and anthropogenic sources, including mining, industrial effluents, agricultural runoff, and domestic wastewater (Kapoor & Singh, 2021). Once introduced, these metals can accumulate in sediments and biomagnify through the food web, posing risks to aquatic life and human health via seafood consumption (Dey et al., 2024). Elevated concentrations disrupt physiological functions in aquatic organisms, including osmoregulation, enzyme activity, and reproduction (Duruibe et al., 2007). Moreover, metals such as cadmium and mercury induce oxidative stress, damage cellular structures, and interfere with DNA replication and repair in aquatic biota (Baby et al., 2010).

2.5.2 *Essential vs. Non-Essential Metals:*

- **Essential Metals**

Trace amounts of essential metals such as zinc (Zn), copper (Cu), iron (Fe), and manganese (Mn) are required for the normal growth, development, and physiological functioning of aquatic organisms. These elements play key roles in enzymatic activity, electron transport, photosynthesis, and cellular homeostasis (McComb et al., 2014; Rainbow, 2002). For example, iron is involved in oxygen transport and redox reactions, while manganese contributes to photosynthetic water-splitting and antioxidant defense mechanisms (Rajesh Kumar & Madhoolika, 2005). However, when present in excess, even essential metals can become toxic, leading to oxidative stress, membrane damage, and impaired nutrient uptake (Pandey et al., 2003).

- **Non-Essential Metals**

Non-essential metals such as lead (Pb), cadmium (Cd), mercury (Hg), arsenic (As), and chromium (Cr) have no known biological function and are toxic to most organisms even at low concentrations. Their primary sources in aquatic environments include mining, industrial effluents, atmospheric deposition, and agricultural activities (Farag et al., 1998; Khalidi-Idrissi et al., 2023; Timothy & Tagui Williams, 2019). Once introduced, these metals can bioaccumulate in organisms and persist in sediments and the water column. Through biomagnification, they move up the food web, increasing risks to higher trophic levels, including humans (Saidon et al., 2024). Non-

essential metals can cause endocrine disruption, neurotoxicity, genotoxicity, and inhibition of key enzymes (Ciosek et al., 2023; Jan et al., 2015; Maret & Moulis, 2013).

2.5.3 Sources of Heavy Metal Pollution

The distribution and persistence of heavy metals in freshwater ecosystems result from both natural and anthropogenic inputs. Heavy metals such as cadmium (Cd), zinc (Zn), mercury (Hg), arsenic (As), silver (Ag), chromium (Cr), copper (Cu), iron (Fe), and platinum (Pt) are high-density elements that can adversely affect environmental and human health (Y. G. Gu et al., 2018; N. Wang et al., 2021). Because they are non-biodegradable, heavy metals may remain hazardous even at low concentrations (Brodin et al., 2017; Ferrey et al., 2018).

Some heavy metals, including zinc, cobalt, copper, iron, and selenium, are essential micronutrients involved in various metabolic and biochemical processes, but can become toxic when present in elevated concentrations (Amra et al., 2019). Although natural biogeochemical processes such as rock weathering, volcanic activity, and forest fires release heavy metals into the environment (Dalziel, 1999; López-Maldonado, 2018), anthropogenic activities are the primary contributors to elevated levels in aquatic ecosystems. These include mining, industrial discharge, agricultural runoff, sewage contamination, traffic emissions, and improper waste management practices (Blaser et al., 2000; Muhammad et al., 2011; Sarkar, 2018; Srivastava et al., 2016, 2017; Srivastava et al., 2018).

2.5.4 Industrial Sources

Industrial processes represent one of the major sources of heavy metal contamination in aquatic environments. Wastewater from mining, metal smelting, electroplating, thermal power plants, tanneries, and various manufacturing industries often contains elevated concentrations of Pb, Hg, Cd, Cr, and As (Nouri et al., 2009). For example, mercury emissions from coal-fired power plants can enter water bodies through atmospheric deposition (Driscoll et al., 2013). Lead contamination may originate from battery production and the corrosion of old water pipes (Jaishankar et al., 2014; Kim & Herrera, 2010).

These metals can accumulate in benthic organisms and biomagnify through the food web, posing risks to higher trophic levels including humans (Sonone et al., 2021). Moreover, insufficiently treated wastewater from metal-processing industries may contain high levels of Cu, Ni, and Zn, further increasing ecological risk (Varol & Şen, 2012).

2.5.5 Agricultural Sources

Agricultural activities contribute to heavy metal pollution primarily through the use of agrochemicals. Fertilizers, manure, pesticides, and herbicides may contain trace amounts of Cd, As, Cu, and Zn, which can accumulate in soils and eventually leach into aquatic systems (Alengebawy et al., 2021). Older arsenic-based pesticides are particularly persistent, remaining in soils long after their application (Nicholson et al., 2003). Prolonged exposure to metal-contaminated waters can result in bioaccumulation among aquatic organisms and lead to chronic toxicity effects (Paasivirta, 2000).

2.5.6 Natural Sources

Although anthropogenic sources account for most heavy metal contamination in aquatic environments, natural processes also release metals into water bodies. Volcanic eruptions, forest fires, weathering of metal-rich geological formations, and soil leaching can introduce metals such as Hg, Cr, and Ni to surface and groundwater (Förstner & Wittmann, 1981; Garrett, 2000). For instance, mercury may be naturally released into rivers and lakes through erosion or seismic activity (Ebinghaus et al., 1999). Additionally, land-use changes and deforestation increase erosion, while dam construction can resuspend historically deposited metal-rich sediments into the water column (Alloway, 2013; H. Singh, 2001).

2.6 Fate and Transport in Aquatic Systems:

2.6.1 Mechanisms of Dissolution and Transport

In aquatic environments, heavy metals commonly occur in ionic forms such as Pb^{2+} , Cd^{2+} , Cu^{2+} , Zn^{2+} , and Hg^{2+} . Their behavior in water is strongly influenced by interactions with dissolved organic matter (DOM) and inorganic ligands, as well as with suspended particulate matter and

sediments (Diamond, 2012). The chemical speciation of metals determines their mobility, solubility, and bioavailability. For instance, complexation with organic ligands can reduce metal toxicity by decreasing the concentration of free ions, while simultaneously enhancing long-range transport in water bodies (Reeder et al., 2006; Vladimir N. et al., 2005).

Heavy metals frequently adsorb onto sediment particles or suspended solids, leading to their deposition in benthic zones (Dang et al., 2020; Linnik & Zubenko, 2000). However, environmental conditions such as pH play a critical role in determining whether metals remain bound or become mobile. Acidic conditions—often linked to acid rain or acid mine drainage—increase metal solubility, enhancing their bioavailability and toxicity (Haines, 1981). For example, a decrease in pH can enhance the dissolution of cadmium and lead, reducing their retention on sediment surfaces (Shrestha et al., 2003).

2.6.2 Sedimentation and Bioavailability

Sediments function as both reservoirs and secondary sources of heavy metals in aquatic ecosystems. While the association of metals with sediments may initially lower their toxicity, environmental fluctuations in pH, temperature, redox conditions, or hydrodynamic disturbance can trigger desorption, leading to their remobilization into the water column (Zhang et al., 2014). This process is especially common in shallow or eutrophic systems where frequent sediment disturbance occurs.

Microbial activity can further modify metal speciation and impact toxicity. Notably, sulfate-reducing bacteria in anoxic sediments can convert inorganic mercury into methylmercury (CH_3Hg^+), a highly toxic compound capable of biomagnification through aquatic food webs (Boening, 2000). Methylmercury accumulates predominantly in fish and poses significant neurotoxic risks to aquatic organisms and humans consuming contaminated seafood (Driscoll et al., 2013; Peng et al., 2016). The fate of heavy metals also depends on their affinity for organic and mineral components within sediments. Metals such as Cu and Zn tend to form stable complexes with organic matter, whereas Ni and Cr more commonly bind to mineral phases (Czikkely et al., 2018; Degryse et al., 2009).

2.6.3 Common Heavy Metals of Concern

- **Lead (Pb)**

Lead is a highly toxic, non-essential heavy metal that causes severe neurotoxic effects in aquatic organisms, particularly fish and invertebrates, by impairing growth, reproduction, neurological function, and enzyme activity (Farooq et al., 2021; C. Singh et al., 2023). In humans—especially children—chronic exposure to lead is associated with developmental and cognitive deficits. Major sources of lead contamination in aquatic systems include industrial discharges, urban runoff, deteriorating lead-based infrastructure, and legacy residues from leaded paints (WHO, 2017). In aquatic environments, the mobility and bioavailability of lead are influenced by pH, water hardness, and dissolved organic matter, which regulate the formation of soluble or insoluble lead complexes (Lead et al., 2018).

- **Cadmium (Cd)**

Cadmium is extremely toxic even at trace concentrations. In aquatic organisms, it disrupts reproduction, growth, renal function, and calcium metabolism, often resulting in skeletal deformities (H. Sharma et al., 2015). Cadmium contamination primarily originates from phosphate fertilizers, mining operations, fossil fuel combustion, and electroplating activities. Due to its persistence, cadmium bioaccumulates in aquatic organisms and biomagnifies through food webs, posing long-term ecological and human health risks (Uddin et al., 2024).

- **Mercury (Hg)**

Mercury poses severe ecological risks due to its transformation into methylmercury (CH_3Hg^+) by anaerobic microorganisms in aquatic sediments. Methylmercury biomagnifies through aquatic food chains, leading to elevated concentrations in top predators such as large fish and birds, thereby threatening both wildlife and human consumers (Driscoll et al., 2013; Ma et al., 2019). Major anthropogenic sources include artisanal gold mining, waste incineration, chlor-alkali production, and coal combustion. Systems characterized by high organic matter and low pH are particularly conducive to mercury methylation (Basu, 2023; Uddin, Khanom, & Islam, 2024).

- **Chromium (Cr)**

Chromium occurs primarily in two oxidation states: trivalent chromium (Cr(III)), which is an essential micronutrient in trace quantities, and hexavalent chromium (Cr(VI)), which is highly toxic and carcinogenic. Cr(VI) is highly soluble and mobile, and induces oxidative stress and tissue damage in fish and aquatic invertebrates, particularly in the liver, kidneys, and gills (Eisler, 1986; J. Liang et al., 2021). Major sources include textile dyeing, leather tanning, electroplating, and pigment manufacturing industries (Agrawal et al., 2006). Its toxicity is regulated by pH, redox potential, and interactions with organic matter (Spry & Wood, 1989; Z. Wang et al., 2016).

- **Zinc (Zn)**

Zinc is an essential trace element involved in numerous enzymatic and physiological functions. However, at elevated concentrations, it becomes toxic, particularly affecting fish growth, osmoregulation, and gill structure (Skidmore, 1970). Excess zinc commonly originates from mining, metal processing, galvanized materials, and agricultural runoff. Zinc bioavailability increases in soft water with low calcium concentrations, intensifying toxicity risks (Saravanan et al., 2024).

- **Copper (Cu)**

Copper is a necessary micronutrient but becomes toxic at elevated levels. Excess copper damages gill tissues, disrupts ion regulation, and affects reproductive and neurological processes in aquatic organisms (Grosell et al., 2007). Anthropogenic sources include wastewater discharge, urban stormwater, agricultural fungicides, and antifouling boat paints (Comber et al., 2023). Copper toxicity depends on metal speciation, water hardness, and complexation with organic ligands (Florence, 1982).

- **Nickel (Ni)**

Nickel may be required in trace amounts but is generally toxic to aquatic organisms at high concentrations, affecting gill permeability, enzyme activity, and reproduction in fish, algae, and daphnids (Muyssen et al., 2011; Pyle & Couture, 2011; Blewett et al., 2016). Major sources include fossil fuel combustion, mining operations, electroplating, and effluent from wastewater treatment

plants. Its toxicity varies depending on pH, water hardness, and the presence of chelating agents (Costa et al., 2022; Sabeen et al., 2019).

2.7 Mechanisms of Toxicity and Bioaccumulation

2.7.1 Toxicological Mechanisms

The toxicity of heavy metals in aquatic environments primarily stems from their ability to disrupt essential physiological and biochemical processes in exposed organisms. These metals can enter aquatic biota through direct absorption from water, ingestion of contaminated food or sediments, or dermal contact (Asuquo Isangedighi et al., 2019; Sonone et al., 2021). Once internalized, their toxic effects vary depending on chemical form, concentration, and organism physiology (Jaishankar et al., 2014; X. Wu et al., 2016). A major toxic mechanism involves oxidative stress—an imbalance between reactive oxygen species (ROS) generation and antioxidant defense. Metals such as cadmium (Cd), lead (Pb), and mercury (Hg) promote ROS formation by disrupting mitochondrial electron transport and redox cycling (Stohs & Bagchi, 1995; Valko et al., 2005).

2.7.2 Bioaccumulation and Biomagnification

2.7.2.1 Bioconcentration Factor (BCF)

The BCF shows how much a heavy metal is enriched in an organism compared to its environment. Its definition is "the ratio of a heavy metal's concentration in an organism's tissue to that of the abiotic medium (water and sediments)". It is computed using the following formula.

$$BCF = \frac{C_{\text{organism}}}{C_{\text{abiotic medium}}}$$

C_{organism} = concentration of the metal in the organism tissue (e.g., µg/g)

$C_{\text{abiotic medium}}$ = concentration of the metal in the surrounding medium (e.g., water or sediment, in µg/L or µg/g), (S. X. Liang et al., 2016).

Other terms like Accumulation Factor (AF), Transfer Factor (TF), Metal Transfer Factor (MTF), Bioaccumulation Factor (BAF), and Biota-Sediment Accumulation Factor (BSAF) are used by some authors and are calculated appropriately. Nonetheless, each of these indices shows how much of a heavy metal has accumulated in the organism in comparison to the environment in which it grows or lives.

2.7.2.2 Bioaccumulation Coefficient (BAC)

BAC is calculated by the following equation:

$$\text{BAC} = \frac{C_{\text{plant}}}{C_{\text{soil}}}$$

C_{plant} is the metal concentration in the plant tissue (e.g., $\mu\text{g/g}$ or mg/kg)

C_{soil} is the metal concentration in the surrounding soil (e.g., $\mu\text{g/g}$ or mg/kg). (S. X. Liang et al., 2016).

Indices such as the bioconcentration factor (BCF), bioaccumulation factor (BAF), and biota–sediment/soil accumulation coefficient (BAC) are essential for evaluating heavy metal contamination, as they depend on both the metal concentration in organisms and the surrounding environmental medium. However, these indices should be interpreted cautiously, since they are often inversely related to metal concentrations in soil, sediments, or water. For instance, a fish in less contaminated water may exhibit a higher BCF for a given metal than one from a more polluted environment, simply due to lower ambient metal levels. Similarly, increasing soil metal concentrations have been shown to cause an exponential decline in BCF values for metals in crop grains (S. Wang et al., 2017).

2.7.3 Bioaccumulation of Heavy Metals in Biota

Heavy metals persist in the environment due to their non-degradable nature and can accumulate within organisms over time. Their uptake and bioaccumulation are influenced by factors such as pH, organic matter content, and metal speciation, which determine their bioavailability in soil and water. Plants often accumulate metals that are more readily bioavailable, making them effective

indicators of environmental contamination (Shtangeeva, 1995). Due to their sensitivity to environmental changes, various plant species have therefore been widely used as bioindicators of heavy metal pollution. Likewise, certain animal species, such as the date mussel (*Lithophaga lithophaga*), are recognized as reliable bioindicators in marine systems (Miedico et al., 2016).

The bioaccumulation of heavy metals in biota poses significant ecological and public health risks, as contaminated food webs can transfer metals across trophic levels. Organisms may experience antagonistic, synergistic, or additive effects when exposed to multiple metals simultaneously (Álvarez & Ramírez, 2001). This discussion focuses specifically on the accumulation of heavy metals in freshwater fish and rice—both of which are important dietary staples—as well as in cigarette tobacco, which represents an additional exposure pathway for smokers.

2.7.4 Bioaccumulation of Heavy Metals in Freshwater Fish

Heavy metals enter aquatic organisms through the ingestion of contaminated food, uptake from sediments, and direct absorption from water (Youssef & Tayel, 2004). Freshwater fish are therefore highly vulnerable to toxic metals released from natural and anthropogenic activities, making heavy metal contamination a critical environmental issue with implications for fish health and human food safety (Rahman et al., 2012). Assessing heavy metal accumulation in fish from various aquatic systems is essential for environmental monitoring and risk evaluation (Yousafzai et al., 2017). Measuring metal concentrations in fish tissues is also vital for determining food safety standards and maintaining sustainable fisheries (Ariyae et al., 2015). Although fish provide high-quality protein, unsaturated fatty acids, and low cholesterol (Malakootian et al., 2016), the consumption of contaminated fish poses health risks, particularly for vulnerable populations such as children, pregnant women, and individuals with pre-existing health conditions.

The bioaccumulation of heavy metals in freshwater fish has wide-ranging ecological, social, and environmental consequences. Contaminated fish can transfer metals to humans through consumption, leading to adverse health outcomes (Dwivedi et al., 2015). Heavy metals also impair fish physiology and immune function; for example, exposure to metal-contaminated wastewater increased stress and disease susceptibility in *Channa punctatus* (Javed & Usmani, 2015). Moreover, heavy metal pollution contributes to declines in fish biodiversity and population

abundance. In Pakistan's River Indus, heavy metal contamination has been identified as a major factor reducing aquatic species diversity and abundance (Al-Ghanim et al., 2016). The persistence and biomagnification of heavy metals therefore represent ongoing threats to aquatic ecosystems and human health (H. Ali & Khan, 2018, 2019).

2.7.5 Differences Between Bioaccumulation in Various Tissues

The distribution of heavy metals among tissues in aquatic organisms is not uniform. Factors such as tissue function, metabolic activity, and the chemical characteristics of the metal influence accumulation patterns. Some tissues reflect recent or direct exposure through water and diet, while others act as primary sites for detoxification and long-term storage (Canli & Atli, 2003; Pourang et al., 2005).

- 2.7.5.1 Muscle vs. Liver

Muscle tissue, which is of primary concern for human consumption, typically exhibits lower metal concentrations compared to metabolically active organs. In contrast, the liver is a principal site for the accumulation of metals such as cadmium (Cd), lead (Pb), and mercury (Hg) due to its roles in detoxification, sequestration, and storage (A. B. Yilmaz, 2003). While muscle tissues accumulate metals mainly through dietary exposure, their relatively low metabolic activity limits retention (Abdulla & Chmielnicka, 1989). The liver, however, synthesizes metal-binding proteins, such as metallothioneins, which facilitate metal detoxification and storage (Heath, 2018).

- 2.7.5.2 Scales vs. Soft Tissues

Fish scales are an effective and non-invasive matrix for monitoring metal exposure (Aib et al., 2025; Couture & Pyle, 2011). Due to their minimal metabolic turnover, scales retain metals over extended periods, serving as archival indicators of long-term exposure (Mariet et al., 2017). Techniques such as X-ray fluorescence (XRF) and inductively coupled plasma optical emission spectrometry (ICP-OES) are commonly used to quantify metal concentrations in scales (Aib et al., 2025; S. R. Khan et al., 2022; Neiva et al., 2018; Parvez et al., 2025; West et al., 2014). This approach enables reliable environmental monitoring without harming the organism (Demirak et al., 2006).

- 2.7.5.3 Gills vs. Liver

Gills are particularly sensitive to dissolved metals due to their direct contact with the aquatic environment. Metals such as copper (Cu) and zinc (Zn) accumulate in gill tissues through active and passive absorption (Playle, 1998). Metals circulating in the bloodstream are subsequently stored in the liver, where detoxification occurs. Chronic exposure or elevated metal levels may impair hepatic function, induce oxidative stress, and cause cellular damage (Roméo et al., 1999).

- 2.7.5.4 Turtle Shells as Hard Tissue Accumulators

Turtle shells function as reliable biological matrices for assessing heavy metal accumulation due to their composite structure of an inner bony layer and an outer keratinized layer (dos Santos et al., 2021; Rieppel, 2013). Similar to fish scales, shells exhibit low metabolic turnover and therefore reflect cumulative exposure over long time periods (Dias et al., 2023; Jackson, 2011). Metals such as Pb, Cd, Hg, Cr, and As can bind to keratin or to hydroxyapatite in the bony layer (Chan, 2024; Day, 2003). In polluted environments, these metals are incorporated into the shell during growth (Martínez-López et al., 2017a). Surface scutes, which can be sampled non-lethally or collected after natural shedding, provide valuable contamination records (Mattei et al., 2015). Because of their longevity and ecological significance, turtles serve as effective sentinel species in wetland and riverine ecosystems (Tanabe et al., 2022a; Burger & Garber, 1995).

- 2.7.5.5 Importance of Carapace Tissue in Environmental Monitoring

Turtle shells accumulate trace elements over time, enabling reconstruction of long-term exposure histories. They allow ethical, non-lethal sampling by shaving or clipping small scute segments. Studies have demonstrated that for several elements, scute concentrations correlate with levels in internal tissues, allowing scutes to serve as proxies for chronic exposure (Bezerra et al., 2013; Shaw et al., 2021).

- 2.7.5.6 Common Metal Accumulation Patterns in Shells

Turtle shells commonly contain both essential metals (Zn, Cu, Fe, Mn, Sr) and toxic metals (Hg, Pb, Cd, As, Cr). Pb and Cd tend to be elevated near industrial and mining sites, while Hg often indicates biomagnification risk. Patterns vary by species, trophic behavior, and contamination sources (Barraza et al., 2019; Bruno et al., 2021).

- 2.7.5.7 Scute–Tissue Element Relationships

The relationship between metal concentrations in scutes and internal tissues is element-specific. Mercury (Hg) often shows strong correlations with liver and muscle levels, while cadmium (Cd) accumulates more readily in kidney tissue. Lead (Pb) and arsenic (As) may display more variable correlations due to differing uptake and storage pathways (Sakai et al., 2000).

- 2.7.5.8 Temporal Resolution Provided by Scute Growth

Turtle scutes grow by incremental keratin deposition, allowing sampling across growth layers to reconstruct temporal exposure histories from seasonal to multi-year scales. The effective temporal resolution depends on species growth rate and the specific scute sampled (Peharda et al., 2021; Shaw et al., 2021, 2023). While internal tissues primarily reflect recent exposure, scutes preserve long-term contamination signatures.

2.8 Factors Influencing Bioaccumulation

Depending on the dose and duration of exposure, certain heavy metals—although essential in small quantities for biological systems—can become toxic to living organisms (Zoghi et al., 2014). Heavy metal toxicity has been associated with mutagenesis, teratogenic effects, and carcinogenesis. These metals induce the generation of reactive oxygen species (ROS), leading to oxidative stress (Dasharathy et al., 2022; Kocadal et al., 2020). Oxidative stress is implicated in numerous diseases and physiological disorders. Moreover, heavy metals function as metabolic toxins (Jomova & Valko, 2011). Their toxicity is primarily attributed to their strong affinity for sulfhydryl (SH) groups, resulting in the inhibition of key enzymatic systems involved in cellular energy synthesis (Csuros & Csuros, 2016).

2.8.1 Environmental and Biological Factors Influencing Bioaccumulation

A variety of biological and environmental factors combine to influence the multifactorial process of heavy metal accumulation in aquatic organisms. Determining the efficacy of bioindicators like fish scales and turtle shells in tracking metal pollution in freshwater ecosystems and interpreting bioaccumulation patterns require an understanding of these factors.

2.8.2 Species Traits and Life Stage

Species-specific physiological traits strongly influence metal uptake, distribution, and storage in aquatic organisms. Variations in metabolic rate, lipid content, osmoregulatory efficiency, and tissue composition result in differing accumulation capacities among species (Vargas-Chacoff et al., 2015). Benthic and long-lived species often accumulate higher metal levels due to prolonged exposure to contaminated sediments (Jezierska & Witeska, 2006a). Life stage also plays a key role: juveniles, with higher metabolic and growth rates, exhibit greater metal uptake relative to body weight (Azad, 2013; Jugo, 1977), while adults may accumulate more metals over time through chronic exposure. Despite lower accumulation, juveniles remain more susceptible to metal toxicity (Muyssen & Janssen, 2007).

2.8.3 Feeding Habits

Feeding practices have a direct impact on the organs' metal exposure pathways. Metals that are susceptible to biomagnification, like mercury (Hg) and cadmium (Cd), are more likely to be transferred trophically to carnivorous fish and omnivores that consume contaminated prey (H. Ali & Khan, 2019b; Oros, 2025). Predators like wolves and wild dogs may scavenge turtles, causing them to accumulate metals through exposure to the environment and tainted food sources in the ecosystem (Steven R. Sheffield et al., 2020). Particularly in contaminated freshwater systems, filter feeders and detritivores which ingest particles from sediment or water columns are also highly susceptible to accumulating sediment-bound metals (Usero et al., 2004). Hence, distinct patterns arising among herbivorous, omnivorous, and carnivorous species may be indicative of dietary habits, as evidenced by the bioaccumulation seen in fish scales and turtle shells.

2.8.4 Habitat Type

Heavy metal exposure and accumulation in aquatic organisms are closely linked to their ecological roles and trophic positions. Predators at higher trophic levels experience biomagnification, leading to elevated pollutant concentrations in their tissues (Danovaro et al., 2023). Marine fish, which bioaccumulate metals and occupy multiple trophic levels, serve as key bioindicators of heavy metal contamination (Khushbu et al., 2022; Oros, 2025). Similarly, turtles ingest metals from sediments through their benthic feeding habits and sediment-associated habitats (Amiard, 1992).

Their ecological roles and food-chain positions thus make them effective indicators of sediment-related metal pollution.

2.8.5 Environmental Conditions

The mobility, solubility, and speciation of heavy metals in aquatic environments are influenced by physicochemical factors like temperature, salinity, dissolved oxygen, pH, and redox potential (Patrick et al., 1977). For example, a lower pH makes metals more soluble, which improves their bioavailability and absorption (Reeder et al., 2006). Similarly, in ectothermic organisms, higher temperatures may increase uptake rates and metabolic activity (Rubalcaba et al., 2020). The amount of organic matter also affects metal availability by chelating or binding metals and lowering the concentrations of free ions in water (Luoma & Rainbow, 2005).

2.9 Use of Aquatic Organisms as Bioindicators

2.9.1 Relevance to Fish Scales and Turtle Shells

Hard tissues that can accumulate and retain metals over long periods of time make up turtle shells and fish scales, providing a time-integrated indicator of environmental exposure (Aib et al., 2025; dos Santos et al., 2021; Yu et al., 2011a).

2.9.2 Fish Scales as Bioindicators of Heavy Metal Accumulation

A study by (Jangu et al., 2021) examined the buildup of heavy metals in Labeo calbasu scales from India's Harike Wetland. Fish scales were found to contain notable concentrations of heavy metals, such as Pb, Cr, and Zn, indicating their potential as bioindicators of environmental contamination (Shakir et al., 2020). Similarly, another study showed that fish scales, which have higher concentrations of metals like Pb and Zn than soft tissues, may be a reflection of exposure to heavy metals over time (Parvez et al., 2025).

2.9.3 Turtle Shells Reflecting Sediment-Associated Metal Exposure

According to studies done on loggerhead turtles (*Caretta caretta*) in Tunisia's Gulf of Gabès, the turtles' tissues accumulate high levels of heavy metals like Pb, Cr, and Zn. Turtles living in sediment-rich benthic zones may be more exposed to and accumulate these metals, according to

the study (Hrizi et al., 2024). Furthermore, a study conducted on freshwater turtles in wetlands in Lake Michigan discovered that the concentrations of heavy metals that the turtles accumulate in their bodies correspond to the benthic habitats' levels of contamination (D. L. Smith et al., 2016).

2.9.4 Influence of Species Traits and Environmental Factors

Turtles also accumulate heavy metals in their bodies, with concentrations reflecting the levels of contamination in their benthic habitats, according to a study conducted on freshwater turtles in wetlands in Lake Michigan (Jamil Emon et al., 2023; W. X. Wang & Rainbow, 2008). Furthermore, a study by Gnanasekaran and Raj (2023) highlighted how environmental factors and sediment properties affect the bioavailability and buildup of heavy metals in benthic organisms (Gnanasekaran et al., 2023).

2.9.5 Sensitivity to Environmental Change

The sensitivity of aquatic organisms to environmental changes varies, especially when it comes to heavy metal contamination. Their sensitivity to contaminants is influenced by physiological and behavioral characteristics unique to each species. For instance, Since the levels of cortisol in fish scales do not fluctuate over long periods of time, they have been used to measure chronic stress responses (Laberge et al., 2019; O'toole et al., 2024). Freshwater turtles are especially vulnerable to heavy metal accumulation because of their benthic lifestyle and extended exposure to sediments (Becker et al., 2025; Yu et al., 2011). Turtles living in contaminated wetlands have been found to have higher levels of metals such as lead (Pb) and cadmium (Cd) in their tissues, which suggests that they are sensitive to environmental contaminants (D. L. Smith et al., 2016; Yu et al., 2011).

2.9.6 Ecological Role and Food Web Position

Heavy metal exposure and accumulation in aquatic organisms are strongly influenced by their ecological roles and positions within food webs. Higher trophic level predators are more likely to experience biomagnification, which raises the levels of pollutants in their tissues (Danovaro et al., 2023). For example, the ability of marine fish to bioaccumulate and transfer trophic levels, as well as their role in food webs, has made them important bioindicators of heavy metal pollution (Khushbu et al., 2022; Oros, 2025). In a similar vein, turtles are exposed to metals that have settled

in sediments because they frequently eat benthic organisms and live in environments higher in sediment (Amiard, 1992). Their ecological habits and place in the food chain make them useful markers of metal contamination linked to sediments.

2.9.7 Capacity to Integrate Long-Term Stressor Exposure

Certain biological tissues can integrate and reflect long-term exposure to environmental stressors. For example, fish scales retain cortisol levels that indicate chronic stress and prolonged contaminant exposure (Aerts et al., 2015; Samaras & Pavlidis, 2022). Likewise, turtle shells, composed of keratin and bone, accumulate heavy metals over time and function as historical records of environmental contamination (Faust et al., 2014). For instance, in *Trachemys dorbigni* from rural and urban areas in southern Brazil, multiple metals—including Al, As, Cd, Cr, Cu, Fe, Hg, Mn, Ni, Pb, and Zn—were detected in blood, water, and carapace samples (Stark et al., 2024). Similarly, a study of *Caretta caretta* from the southeastern Mediterranean coast of Egypt reported Hg, Cd, Pb, and As across various tissues, with Pb highest in muscle and As and Cd highest in kidneys (Abdallah, 2023). Reviews further indicate that while arsenic is often more concentrated in muscle tissues, cadmium, copper, and zinc tend to accumulate primarily in the liver and kidneys (Dias et al., 2023; Storelli & Marcotrigiano, 2003). These patterns vary by species and growth stage, demonstrating that tissue-specific analyses offer valuable insight into long-term exposure and contaminant dynamics in turtles.

2.9.8 Non-Invasive Biomarkers: Fish Scales and Turtle Shells

In aquatic ecotoxicology, non-invasive and non-lethal biomonitoring methods have gained popularity in recent years. One promising method for tracking heavy metal contamination in freshwater ecosystems is the use of hard external tissues, like turtle shells and fish scales. When compared to conventional biomonitoring that uses internal organs like the liver, muscles, or kidneys, these tissues provide a number of ethical and practical benefits.

2.9.9 Advantages Over Internal Tissues (liver, muscle, kidney)

Turtle shells and fish scales are valuable non-invasive bioindicators of environmental contamination for several reasons (Aib et al., 2025; Ortiz-Santaliestra et al., 2019; Parvez et al.,

2025). Their collection is typically non-destructive or entirely non-lethal, allowing for repeated sampling and long-term monitoring of the same individuals and populations (J. Keller et al., 2014; Potgieter, 2022; Thorstensen et al., 2022). Unlike soft tissues, scales and shells exhibit slow metabolic turnover, enabling them to act as long-term accumulators of heavy metals and trace elements. Hard tissues therefore provide more stable and cumulative contamination profiles, while internal tissues may reflect short-term physiological processes such as detoxification and excretion (Álvarez & Ramírez, 2000; Hogstrand & Haux, 1991). Metals such as Pb, Cd, and Zn have been shown to bioaccumulate in both fish scales and turtle shells in association with environmental pollution gradients (Adel et al., 2017; Salvarani, 2019). Furthermore, these external tissues can preserve chronological exposure information: fish scales contain stratified growth rings that record temporal variations in metal accumulation (Jayaseelan et al., 2014), while keratinized turtle scutes reflect prolonged contaminant incorporation and persistent environmental stress (Adel et al., 2017).

In contrast, internal tissues such as the liver and kidneys, although often exhibiting high metal concentrations, may be influenced by detoxification, dietary intake, and metabolic regulation, complicating interpretations of environmental exposure (Kojadinovic et al., 2007). Additionally, sampling internal organs may require sacrificing the organism, which is ethically problematic, particularly for protected or threatened species. Non-destructive analytical techniques such as energy-dispersive X-ray spectroscopy (EDS), inductively coupled plasma optical emission spectrometry (ICP-OES), and X-ray fluorescence (XRF) therefore provide accurate and sensitive quantification of metal concentrations in scales and shells while preserving the integrity of the organism (Tuzen & Soylak, 2007).

2.9.10 Analytical Techniques Used in Scale and Shell Analysis

- **X-ray Fluorescence (XRF)**

X-ray fluorescence (XRF) is a non-destructive analytical technique that detects and quantifies elemental compositions through secondary X-rays emitted by atoms excited by a primary source (Acquafredda, 2019; V. K. Singh et al., 2022). It is particularly advantageous for analyzing hard tissues like turtle shells and fish scales, requiring minimal preparation and enabling multi-element

detection. XRF has been effectively applied to measure metals such as Fe, Mn, Zn, and Cu in fish scales exposed to acidic mine drainage, demonstrating its reliability for both laboratory and field studies (Mendoza et al., 2023; Vives et al., 2006). Its non-destructive nature also permits repeated measurements and long-term specimen preservation.

- **Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES)**

Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) is a robust and widely applied technique for quantifying elements in biological and environmental samples. It detects photons emitted at element-specific wavelengths after atom excitation in high-temperature plasma (Hou et al., 2000). In bioindicator studies, ICP-OES has been effectively used to measure heavy metal concentrations in fish scales from polluted freshwater systems, demonstrating its high sensitivity and reproducibility (Sen et al., 2011). Owing to its low detection limits and multi-element capabilities, ICP-OES is ideal for routine monitoring of contaminated aquatic environments (Farahani et al., 2015; Pridhvi Krishna & Sundararajan, 2022).

- **Inductively Coupled Plasma Mass Spectrometry (ICP-MS)**

Inductively Coupled Plasma Mass Spectrometry (ICP-MS) is an ultra-sensitive technique capable of detecting trace metals at parts-per-trillion levels, combining plasma atomization with mass spectrometric detection (Nisianakis et al., 2009). Its application to green sea turtle scutes highlights its precision in aquatic ecotoxicology studies (Shaw et al., 2021). When used with certified reference materials, ICP-MS ensures high analytical accuracy and also enables isotopic analysis to trace metal sources and pathways (Balaram et al., 2023).

- **Scanning Electron Microscopy with Energy-Dispersive X-ray Spectroscopy (SEM-EDS)**

Scanning Electron Microscopy coupled with Energy Dispersive Spectroscopy (SEM-EDS) combines surface imaging with elemental analysis, enabling both visual and quantitative assessment of contaminants (Carter et al., 2022). It is particularly effective for examining microstructural alterations in calcified tissues exposed to pollutants. Using SEM-EDS, Jangu et al. (2021) detected Pb, Cr, Zn, Ni, Cu, and Hg in *Labeo rohita* scales, linking metal accumulation to

surface damage and deposition. The technique helps identify localized contamination and metal distribution across scale or shell layers (Acharya, 2023).

2.10 Ecological and Human Health Impacts of Heavy Metals

2.10.1 Effects on Aquatic Life

Heavy metals are highly persistent, toxic even in trace amounts, and resistant to microbial degradation, leading to long-term ecological risks (Woo et al., 2009). Once introduced into aquatic environments, they bind to suspended particles and accumulate in sediments, forming major contamination reservoirs (KeJian & ChunLing, 2008; J.T. Gurrieri, 1998). These sediment-bound metals can be assimilated by macrophytes and benthic organisms, disrupting aquatic food webs and decreasing biodiversity (Tolulope et al., 2009). Elevated metal concentrations also alter diatom assemblages and overall river ecological balance (De Jonge et al., 2008; Morin et al., 2007). Bioaccumulation and biomagnification through the food chain expose higher trophic-level organisms, including humans, to heightened toxicological risks (Tolulope et al., 2009). In fish, metals enter via gills, skin, diet, or direct absorption, bind to blood proteins, and accumulate in organs such as the liver, kidneys, muscle, bone, or fat (Zienab, 2006; Tolulope et al., 2009). Metal exposure also induces reactive oxygen species (ROS) and oxidative stress, impairing physiological function (Woo et al., 2009). Mercury (Hg), largely present as methylmercury formed in sediments, poses severe neurotoxic and bioaccumulative hazards to aquatic organisms and humans (Zienab, 2006). Cadmium (Cd) accumulates mainly in the liver, kidneys, lungs, and bones, and is associated with Itai-itai disease, osteoporosis, emphysema, and cardiovascular disorders (Sobha et al., 2007; Duruibe et al., 2007; Lalor, 2008). Zinc (Zn), though essential, can cause anemia, renal and hepatic failure, and reproductive impairment at high levels (Duruibe et al., 2007). Similarly, excessive copper (Cu) exposure leads to gastrointestinal disturbance, hepatic and renal damage, and neurological dysfunction (Stern et al., 2007).

Benthic macroinvertebrates—key contributors to nutrient cycling and aquatic food webs—are widely used as bioindicators of metal pollution due to their sensitivity to environmental contamination (Iwasaki et al., 2009). Their contamination can adversely affect higher trophic

levels, including fish populations that rely on them as prey. Likewise, plants grown in contaminated soils may transfer heavy metals into the food chain, creating risks for human health (Jordão et al., 2006). Chronic exposure to heavy metals results in their gradual accumulation in soft tissues, often producing toxic effects that may only become apparent after long periods (S. Khan et al., 2008).

Nickel (Ni) exposure can lead to skin irritation and adverse effects on the nervous system, respiratory tract, and mucous membranes (Argun et al., 2007). Lead (Pb) is a potent neurotoxin that damages the kidneys, liver, brain, and reproductive system even at low concentrations, causing anemia, cardiovascular dysfunction, developmental disorders, and cognitive deficits (Kazemipour et al., 2008; Odum, 2016). Children are particularly vulnerable due to Pb's impact on brain development, with zinc and calcium deficiencies further increasing Pb absorption (Duruibe et al., 2007). Chromium (Cr) occurs mainly in the forms Cr(III) and the more toxic Cr(VI), the latter being a carcinogenic oxidizing agent that induces oxidative stress and cellular damage (Huang et al., 2009; Jeyasingh & Philip, 2005; Shaffer et al., 2001). Mercury (Hg), toxic in all its forms and biologically non-essential, can cause gastrointestinal damage, infertility, and severe neurological impairment; methylmercury, in particular, leads to irreversible central nervous system damage (Duruibe et al., 2007). The toxicity of arsenic (As) depends on its chemical form, but it commonly disrupts ATP synthesis and protein function, resulting in energy depletion and potential carcinogenic outcomes. Chronic exposure can also lead to neurological symptoms resembling Guillain-Barré syndrome (Duruibe et al., 2007).

2.10.2 Effects on Human Health

Heavy metals such as lead (Pb), cadmium (Cd), mercury (Hg), chromium (Cr), and arsenic (As) pose major health concerns due to their toxicity, environmental persistence, and capacity to bioaccumulate through food webs. Human exposure primarily occurs through contaminated water and the consumption of aquatic organisms, particularly fish, which can accumulate these metals in their tissues (Jaishankar et al., 2014; Järup, 2003). Lead (Pb) severely impairs neurological development in children, resulting in cognitive and behavioral deficits, while in adults it contributes to kidney dysfunction, reproductive impairments, and hypertension (Needleman, 2004; Tchounwou et al., 2012). Cadmium (Cd), a nephrotoxic and carcinogenic metal, is associated with

bone demineralization (Itai-Itai disease), renal failure, and long-term accumulation in the liver and kidneys (Satarug et al., 2010). Mercury (Hg), particularly in the form of methylmercury, is a potent neurotoxin linked to fetal developmental disorders and the well-documented Minamata disease in Japan (Harada, 1995; Grandjean et al., 1997). Chromium (Cr) exists as the essential Cr(III) and the highly toxic Cr(VI), the latter inducing oxidative stress, DNA damage, and carcinogenic effects in the digestive and respiratory systems (Zhitkovich, 2011). Inorganic arsenic (As) is associated with cardiovascular disorders and several cancers, including those of the skin, bladder, and lungs (Naujokas et al., 2013). Widespread arsenic contamination in groundwater, especially in Bangladesh, India, Southeast Asia, and in surface waters near mining and industrial zones, poses severe public health challenges. The bioaccumulation of heavy metals in turtle shells and fish scales provides a valuable indicator of both ecological contamination and potential human exposure risks. Studies have demonstrated strong correlations between metal concentrations in aquatic organisms and those detected in nearby human populations consuming them (Burger et al., 2002; Storelli, 2008).

2.11 Monitoring Strategies

2.11.1 Regulatory Frameworks and Guidelines

A framework of national and international regulations that specify acceptable limits, environmental quality targets, and enforcement procedures is necessary for the efficient monitoring of heavy metal contamination in freshwater systems. These frameworks guarantee data comparability across regions and direct sampling activities, including those that use non-invasive bioindicators like fish scales and turtle shells.

2.11.2 International Standards (WHO, UNEP, EU)

The World Health Organization's Guidelines for Drinking-water Quality (GDWQ) establish health-based limits for metal concentrations, including lead (Pb) at 10 µg/L, nickel (Ni) at 70 µg/L, and copper (Cu) at 2 mg/L (AHMED A. SHARKAWY et al., 2019; Freire-Gibb et al., 2014; Prasad et al., 2022; Richards et al., 1997). Although these values are designed for drinking water, they are widely adopted by national authorities as reference points for both human health protection and broader ecological monitoring (WHO, 2018). Within the European Union, the Water Framework

Directive (WFD, 2000/60/EC) sets the objective of achieving “good ecological and chemical status” across all surface and groundwater bodies (ECHA, 2000; Kaika, 2003). It enforces Environmental Quality Standards (EQS) for priority metals such as cadmium (Cd), nickel (Ni), lead (Pb), and mercury (Hg), as specified in the Priority Substances Directive (2008/105/EC; amended 2013) (FMAFCEP, 2013). Complementary legislation, including the Drinking Water Directive (98/83/EC) and its recast (2020/2184), strengthens monitoring requirements and applies precautionary limits that are in some cases stricter than WHO guidelines (Wikipedia ““Drinking Water Directive,”” 2025). In addition, UNEP’s Minamata Convention on Mercury targets the reduction of anthropogenic mercury emissions into aquatic environments. Together, these international and regional policies reinforce national efforts to regulate, monitor, and mitigate the impacts of persistent and bioaccumulative heavy metals.

2.11.3 National Regulations and Local Policies

Hungary’s environmental legal framework is grounded in Act LIII of 1995 on Environmental Protection, which governs pollution control and water resource management (Évi környezetvédelmi törvény, 1995). Complementary legislation, including Act LVII of 1995 on Water Management (FAO, 1995), details the monitoring and maintenance of river and lake chemical quality. These laws align with EU directives and are supported by local municipal regulations. Under Hungary’s implementation of the Drinking Water Directive, routine testing for heavy metals such as Pb, Ni, and Cr is mandatory. Following WHO and EU standards, the National Public Health Center (NNK) publishes periodic water quality reports (Évi környezetvédelmi törvény, 1995). Additionally, local risk maps—such as those highlighting lead contamination in older Budapest neighborhoods—underscore the need for targeted interventions and systematic monitoring, including practices like tap flushing and filter use (Nébih, 2019; NNK, 2018).

2.12 Research Gaps and Future Directions

2.12.1 Long-term Monitoring and Cumulative Impacts

Long-term environmental monitoring is essential for understanding the cumulative effects of heavy metal contamination in freshwater ecosystems (Edo et al., 2024). Hard tissues such as fish scales offer a valuable means of assessing chronic exposure because, unlike internal organs that

primarily reflect recent or acute contamination, scales accumulate metals gradually over time (A. Hadi & S. F. Alwan, 2012). Their slow metabolic turnover and incremental growth allow scales to retain historical records of metal deposition along growth rings, similar to the chronological information stored in otoliths or tree rings (Campana, 1999). Metals such as lead (Pb), cadmium (Cd), and zinc (Zn) have been shown to accumulate in scales proportionally to ambient concentrations in water and sediment, making scales reliable indicators of long-term environmental contamination.

Integrating non-invasive tissue analyses such as scale monitoring into long-term biomonitoring programs can address key gaps in current aquatic pollution assessment strategies. When paired with seasonal hydrological data, these methods can help distinguish cumulative or delayed ecological responses to anthropogenic stressors, including wastewater effluents, agricultural runoff, and industrial discharges (B. Moss, 2007; S. M. Adams, 2002). Furthermore, consistent annual sampling or multi-year analyses of archived scales can reveal environmental thresholds or ecological tipping points, thereby improving risk assessment frameworks and supporting more informed environmental management and policy decisions (Campana, 1999b; Peterson et al., 2003; Schindler & Hilborn, 2015).

2.12.2 Sublethal and Combined Effects of Multiple Stressors

Although the acute toxicity thresholds of heavy metals are well established, increasing attention is being directed toward their sublethal effects, particularly under conditions where multiple environmental stressors interact (Crain et al., 2008; Folt et al., 1999; More et al., 2019). In freshwater ecosystems, fish and turtles are frequently exposed to complex mixtures of pollutants alongside additional stressors such as endocrine disruptors, pesticides, hypoxia, pathogens, and thermal fluctuations (Heugens et al., 2001; Walker et al., 2005). Nevertheless, most ecotoxicological research has examined single-contaminant exposures, overlooking potential synergistic, additive, or antagonistic interactions among co-occurring stressors (Altenburger et al., 2003; T. Brodin et al., 2014).

Sublethal effects—those that impair physiological performance, reproductive success, or survival without causing immediate mortality—are of particular concern. Even low concentrations of

copper (Cu) and cadmium (Cd) have been associated with immune suppression, reduced feeding efficiency, reproductive impairment, and altered swimming behavior in fish (Handy et al., 2003; Pane et al., 2004). Similarly, turtles exposed to metal mixtures may experience developmental abnormalities, shell malformations, and neurological impairment, although empirical studies remain comparatively limited (Burger & Gochfeld, 2000). Interactions with additional stressors, such as elevated temperatures or endocrine-disrupting compounds, can further intensify physiological disturbances (Jezierska & Witeska, 2006b; Noyes et al., 2009a). For instance, increased temperatures elevate metabolic rates in ectotherms, leading to greater metal uptake and enhanced oxidative stress (Niyogi & Wood, 2004).

From a biomonitoring perspective, there is a growing need to understand how such combined stressors are recorded in non-invasive indicators, including turtle shells and fish scales. Although metal accumulation patterns in these hard tissues are well documented, their potential to reflect biochemical stress markers—such as oxidative damage, microstructural degradation, or alterations in trace element composition—remains insufficiently explored (B. Sharma et al., 2014; Zhou et al., 2008). This represents a key research gap in developing effective long-term monitoring strategies for freshwater ecosystem

2.12.3 Role of Sediments and Bioavailability in Heavy Metal Dynamics

In freshwater ecosystems, sediments play a pivotal role in controlling the fate and behavior of heavy metals, functioning both as long-term sinks and as secondary sources of contamination. Metals deposited from the water column can later be reintroduced into the aquatic environment through changes in temperature, redox potential, or pH, as well as through physical processes such as desorption, diffusion, and resuspension (Eggleton & Thomas, 2004). These mechanisms enhance metal bioavailability to benthic organisms, including demersal fish and turtles that interact closely with sediment substrates (Luoma & Rainbow, 2011).

Both dissolved metals and those remobilized from sediments can accumulate in fish scales and turtle shells, providing indicators of chronic exposure. Turtles that forage in benthic zones frequently accumulate lead (Pb) and chromium (Cr) through direct sediment contact or consumption of contaminated prey (Meyers-Schöne & Walton, 1994), while fish residing or feeding near sediment interfaces often exhibit elevated metal concentrations in their scales (Canli

& Atli, 2003b). These patterns highlight the need for integrated sediment–water–organism monitoring approaches, as traditional water-based assessments may underestimate the ecological relevance and bioavailability of sediment-associated metals (George W. Ware, 1994).

2.12.4 Climate Change and Mobilization of Heavy Metals

Climate change is increasingly recognized as a key driver influencing the mobilization, distribution, and bioavailability of heavy metals in aquatic ecosystems. Variations in temperature, precipitation, and extreme weather can alter geochemical conditions, affecting metal solubility and transport (Unep et al., 2007). Elevated temperatures enhance biological metabolism and chemical reaction rates, increasing metal uptake by aquatic organisms, while heavy rainfall and flooding intensify runoff from polluted soils and mining areas, introducing more metals into water bodies (Di Guardo et al., 2018).

Additionally, acidification and redox potential shifts—common under warming and eutrophication—can remobilize sediment-bound metals, restoring the bioavailability of previously sequestered contaminants (Noyes et al., 2009). This poses heightened risks for benthic and long-lived species, such as fish and turtles, that accumulate metals like arsenic (As), lead (Pb), and cadmium (Cd) (Simpson et al., 2004).

2.12.5 Novel Bioindicators and Advanced Analytical Approaches

Identifying new bioindicators that improve the sensitivity, specificity, and non-invasiveness of heavy metal pollution monitoring remains an important research priority. While soft tissues such as muscle and liver are commonly used, fish scales and turtle shells are increasingly recognized for their ability to record long-term contamination without harming the organism (Burger, 2006; Jabeen & Chaudhry, 2010; Smith et al., 2019). Due to their slow metabolic turnover and incremental growth, these hard tissues provide chronologically layered evidence of metal exposure, making them particularly valuable for assessing chronic environmental contamination (Rainbow, 2002; W. X. Wang & Rainbow, 2008).

Advances in analytical techniques, including ICP-MS, ICP-OES, and XRF, have greatly improved multi-element detection and quantification, enabling detailed spatial profiling of metals across growth structures such as scale annuli or shell stratified keratin layers (Jenkins, 1995; López-

Botella et al., 2023; Yoseif Makonnen et al., 2016). Additionally, SEM-EDS imaging allows microscale visualization of elemental distribution and the detection of morphological or structural alterations linked to contaminant exposure (De Oliveira-Filho et al., 2004).

Emerging omics-based and computational approaches—such as proteomics, metabolomics, and machine learning—present further opportunities to integrate biochemical, physiological, and environmental datasets for improved pollution assessment (Dakal et al., 2024; Kayode-Edwards et al., 2024; P. Sharma et al., 2022). However, their application to fish scales and turtle shells remains limited, indicating a notable research gap and an important direction for future work.

2.12.6 Nanotechnology and Heavy Metal Remediation

Recent advances in nanotechnology offer promising approaches for remediating heavy metal pollution in freshwater ecosystems. Due to their high surface area, reactivity, and adjustable physicochemical characteristics, nanomaterials such as nanoparticles, nanocomposites, and functionalized nano-adsorbents are highly effective in binding and removing metals including Pb, Cd, Cr, and Hg from aqueous environments (Y. Zhou et al., 2019). Although the application of nano-remediation in studies involving non-invasive bioindicators like fish scales and turtle shells remains in its early stages (Khin et al., 2012), emerging research highlights the dual potential of nanomaterials for both detection and treatment. For example, biosensors incorporating metal-oxide nanostructures have demonstrated real-time monitoring capacities for trace metal concentrations, enabling more responsive pollution management (He et al., 2021). However, concerns regarding the ecological risks of engineered nanomaterials remain significant. Their small size and high mobility can facilitate entry into food webs, where they may accumulate in organisms and contribute to cellular stress or toxicity (Nel et al., 2006). As such, the development of nanotechnological solutions must prioritize green synthesis routes, biodegradability, and rigorous environmental risk assessment to ensure safe implementation (Rama Rao Karri et al., 2018).

Integrating non-invasive biomonitoring tools, such as scale and shell analyses, with nanomaterial-enhanced detection and remediation strategies may provide a sustainable and highly sensitive framework for addressing heavy metal contamination in freshwater environments. Ultimately, progress in freshwater biomonitoring will require interdisciplinary collaboration across chemistry,

ecology, and environmental policy, particularly in understanding sediment dynamics, cumulative stressors, and climate-driven shifts in metal mobility.

Chapter 03: Material And Methods

3.1 Study Design Overview

In this thesis, heavy metal contamination in freshwater ecosystems is assessed using biological matrices and a comparative ecotoxicological approach. Two different bioindicator organisms were used: the pond turtle *Mauremys leprosa* from Reghaïa Lake, Algeria, and the European chub and Common nase (*Chondrostoma nasus* and *Squalius cephalus*) from the Maros River in Hungary. Hard tissue (shells and scales) morphometric, chemical, and spectroscopic analyses are combined with physicochemical analysis of the environmental matrices (water and sediment) in this study.

3.2 Study Area

3.2.1 River Maros

The Hășmașu Mare Range of the Eastern Carpathian Mountains in Romania is the source of the River Maros (Mureș), one of the Tisza River's largest tributaries. It has a drainage basin of 30,332 km² and measures roughly 789 km in length as it flows westward across Romania and southeast Hungary. Geocoordinates for the sampling for this study were N46.20276, E20.45203, close to the Hungarian town of Makó. This river segment is renowned for its ecological diversity and is impacted by several anthropogenic pressures, including urbanization, industrial discharges, and agricultural runoff. (Figure 1) illustrates the geographical location of the sampling site near Makó, as well as the course of the Maros River across the Hungarian landscape. The left panel provides a regional map of southeastern Hungary, while the right panel shows the river's longitudinal pathway from the Romanian border to the sampling area. These visual references support the spatial context of the study and highlight the transboundary nature of the river system.

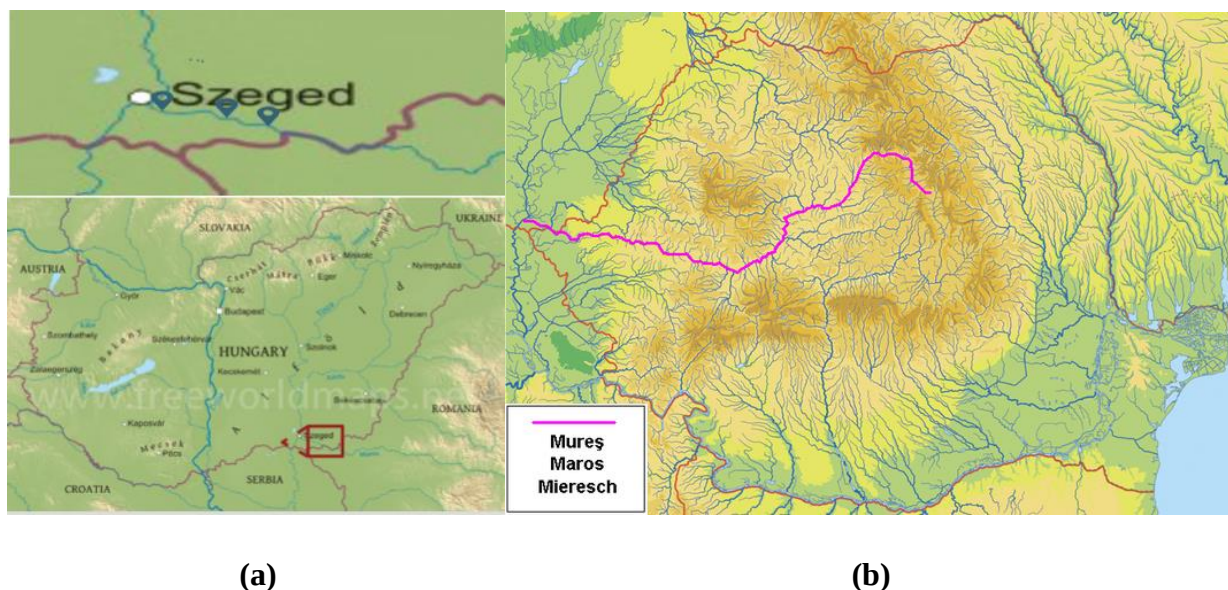
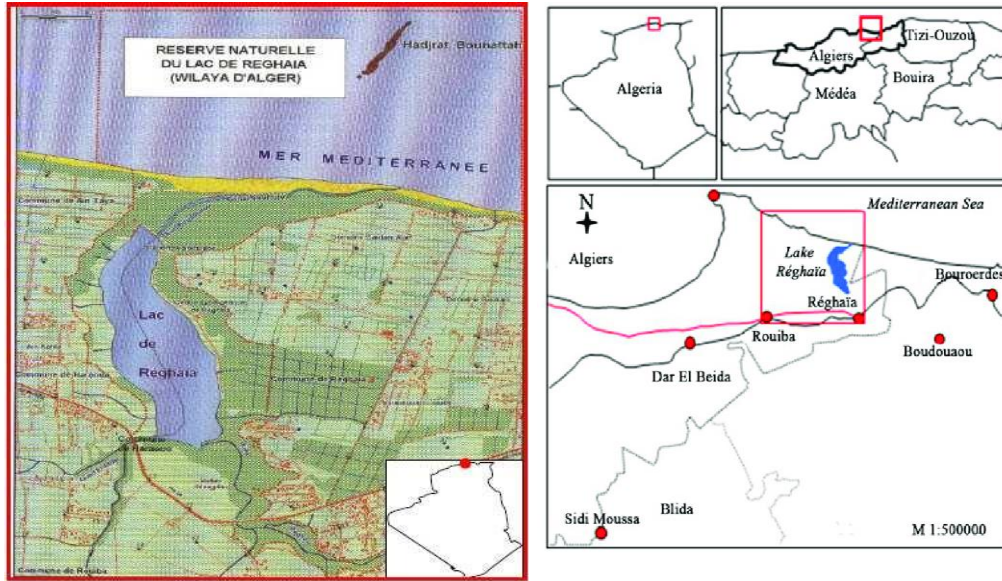


Figure 1. (a) Detailed view of southeastern Hungary including the Makó sampling site near the Hungarian–Romanian border. **(b)** Topographic course of the Maros River through Hungary from Romanian border to Makó. (Source: Wikimedia)

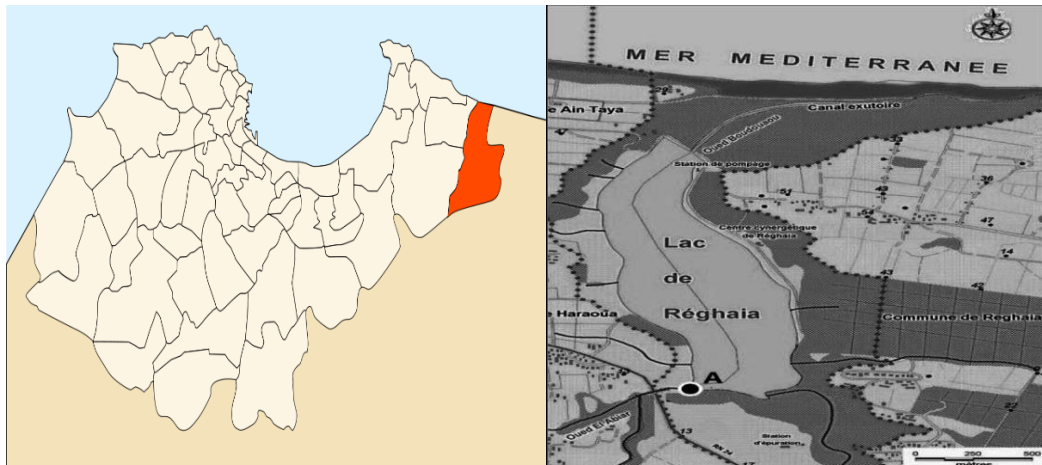
3.2.2 Lake Reghaïa

Lake Reghaïa is a coastal wetland located in the northern Algerian province of Tipaza. Owing to its ecological importance—particularly as a habitat for migratory birds and diverse aquatic species—it has been designated as a Ramsar site. However, Lake Reghaïa is currently subjected to considerable anthropogenic pressures, including industrial effluents, expanding urban development, and agricultural runoff, all of which contribute to environmental degradation and potential heavy metal contamination. In this study, the shells of the Mediterranean pond turtle (*Mauremys leprosa*), a resident and ecologically significant species in the lake, were used as bioindicators to evaluate long-term exposure to heavy metals due to their capacity to accumulate contaminants in keratin–bone matrices. (Figure 2) provides a geographical and environmental context of the study site. (Figure 3) illustrates the geographical location of Lake Reghaïa along the Mediterranean coastline and presents a Google Earth satellite view highlighting its shoreline, surrounding land use, and the broader landscape setting.



(a)

(b)



(c)

(d)

Figure 2. (a) Regional context of Lake Réghaïa ($36^{\circ}45' - 36^{\circ}48' \text{ N}$, $3^{\circ}19' - 3^{\circ}21' \text{ E}$), a 75 ha freshwater Ramsar wetland located 29 km east of Algiers in the Mitidja Plain (Rougeot, 1978). (b) Delineated wetland boundary with indicated sampling stations for water, sediment, and turtle shells (Metna et al., 2015). (c) Local drainage network spanning tributaries Oued Réghaïa, El Biar, Berraba, and Gasbia (Source: Wikimedia). (d) Administrative map showing lake location within Reghaïa commune, Algiers Province (Khelif et al., 2016).

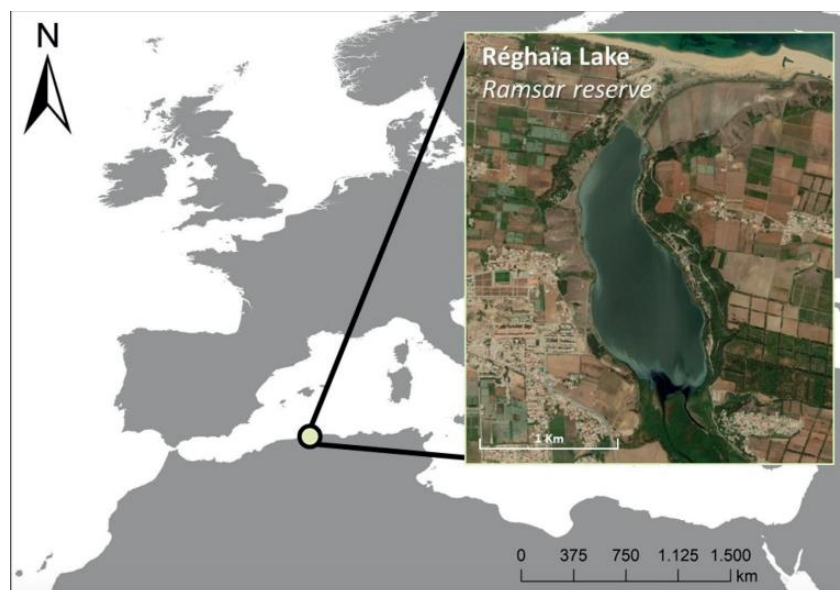


Figure 3. Geographical location and «Google Earth» satellite image of the Reghaia Lake (36° 46.363' N; 3° 20.129' E) (Djitli et al., 2021).

3.3 Sample Collection

3.3.1 Fish scales in river Maros

In December 2022, eight *Squalius cephalus* (chub) and six *Chondrostoma nasus* (nase) were collected from the Maros River at Makó using an electric fishing device (Hans Grassl EL64 II GI, DC, 300/600 V max, 7 kW; Hans Grassl GmbH, Schöna am Königssee, Germany) under permit HaGF/68/2021. The sampling location was at coordinates N46.20276, E20.45203. Fish were transported to the laboratory in aerated river water.

Standard length (SL) and total weight (W) were measured to the nearest 0.1 mm and 0.01 g, respectively, and individuals representing different size classes were included (Figures 4a–c, 5). Fish were euthanized by spinal severing in accordance with institutional ethical guidelines to obtain the full set of tissues used in the broader research program. However, it is important to note that the use of scales as a bioindicator itself is inherently non-lethal, and future studies can fully avoid sacrificing fish by applying established non-invasive scale sampling procedures. Six scales were collected from each side of each fish along an imaginary line from the anterior base of the

anal fin to the posterior base of the dorsal fin, specifically within 2–3 rows above the lateral line (B.C., 1997). Scales were rinsed with distilled water and stored at -18°C until processing.

Trace element data for the Maros River water were obtained from the Hungarian National Environmental Information System (OKIR). The values used correspond to the Makó monitoring station, located at the same river section where biological sampling occurred, and represent monthly measurements collected throughout 2022. (**OKIR, National Environmental Monitoring Database, Makó Station, 2022**).



Figure 4. (a) Measurement of fish specimen weight using a digital balance. (b) Collected fish specimens representing different size classes used in the study. (c) Fish specimen prepared for tissue and scale sampling in the laboratory.



Figure 5. Field trip and lab work

3.3.2 Water, sediment and turtle shells in Raghai lake

Sampling in Lake Reghaia (Ramsar Site, Algeria) was conducted between **January and April 2020, with two sampling campaigns per month**. Results were averaged to represent winter (January–February) and spring (March–April) conditions. Water samples (collected at ~20 cm depth) and surface sediments (~0–5 cm) were taken from multiple points distributed around the lake shoreline to capture spatial environmental variation.

Additionally, approximately thirty *Mauremys leprosa* shell samples were collected. These were obtained non-lethally, originating from naturally shed scutes, carcasses of predated or scavenged individuals, and remains found near the lake shoreline. No live turtles were disturbed or handled. The use of keratinized scutes as a bioindicator is particularly advantageous because they are durable, easy to preserve, and integrate environmental exposure over time. Given the ecological significance of Lake Reghaia and the anthropogenic pressures from nearby recreational and urban activity, these shell samples provide a valuable and ethically sustainable means of assessing heavy metal contamination in the ecosystem.

3.3.3 Environmental Sample Preparation and Analysis of turtle shell

Water samples were collected in pre-cleaned, acid-washed 500 mL polyethylene bottles. For total recoverable metals, samples were collected unfiltered and acidified on-site to $\text{pH} < 2$ using ultrapure HNO_3 (trace-metal grade), with acid volume and pH recorded. Samples were kept at 4

°C and transported to the laboratory within 12 h. Field blanks (ultrapure water handled identically at the sampling site) were included in each sampling campaign.

Surface sediments were collected using a stainless-steel corer, transferred to acid-washed polyethylene bags, kept cool, and transported to the laboratory. In the laboratory, sediments were homogenized, air-dried at ≤ 40 °C, sieved to < 2 mm, and stored until analysis. Moisture content and dry weight were recorded. For analysis, 50–100 mL aliquots of acidified water samples were digested with concentrated HNO_3 . Sediment subsamples (0.2–0.5 g dry weight) were digested following EPA 3051A (microwave-assisted $\text{HNO}_3 \pm \text{HCl}$) or, where applicable, EPA 3050B ($\text{HNO}_3 + \text{H}_2\text{O}_2$ followed by HCl). Digestion conditions followed EPA and instrument manufacturer specifications. Turtle carapaces used for bioindicator analysis were collected from the study site and transported to the laboratory, where they were cleaned, dried, and prepared for elemental analysis, as shown in (Figure 6).



Figure 6. Turtle carapaces collected around the lake and transferred to the laboratory for further examination.

3.3.4 Turtle Shell Preparation and Analysis

Collected turtle shells were first cleaned of debris, rinsed with deionized water, and air-dried. Morphometric measurements were conducted to assess the physical dimensions of the turtle shells. (Table 1) presents the morphometric data including the straight-line carapace length (L) and other relevant parameters. The samples were categorized by sex for analysis.

Table 1. Morphometric Parameters of Turtle Shells

n	SEX	LA (mm)	Lp(mm)	L
1	M	124,4	NA	NA
2	M	110,1	NA	NA
3	M	83	NA	NA
4	F	96	NA	NA
5	M	116,1	134	193
6	M	139,2	158,1	200
7	M	111	NA	189
8	M	127	131	195
9	M	116	132	199
10	F	126	NA	NA
11	M	115	129	193
12	M	112	126	189
13	M	112	130	189
14	F	124	144	207
15	M	112	128	186
16	F	NA	149	NA
17	F	NA	121	NA
18	F	118	129	195,5
19	F	122,9	134,9	187
20	F	114,2	126	195,5
21	F	111	NA	NA
22	F	110	128	184
23	F	111	124	179
24	F	122	144	216
25	F	118,9	140	207,5

NA = Not Available; measurements were not recorded for these individuals due to damage

3.3.5 Ecological Characteristics for studied species

Suitable bioindicators for evaluating aquatic environmental quality, the chosen species are significant elements of freshwater ecosystems. Common nase (*Chondrostoma nasus*) and European chub (*Squalius cephalus*), the fish species under study, are cyprinids that are extensively found in rivers and streams throughout Europe. While the chub is an omnivorous and opportunistic feeder that inhabits a variety of habitats, the nase is a benthic grazer that specializes in scraping algae and periphyton in fast-moving environments (Kawamura, 2008). Their ecological roles complement one another, reflecting different trophic niches within riverine systems (Table 2).

Table 2. Ecological characteristics fish species

Species	Common Name	Family	Habitat Preference	Feeding Habit	Ecological Role
<i>Chondrostoma nasus</i>	Common nase	Cyprinidae	Fast-flowing rivers with gravelly bottoms	Herbivorous, scrapes algae and periphyton	Algae grazer, maintains periphyton balance
<i>Squalius cephalus</i>	European chub	Cyprinidae	Slow- and fast-flowing rivers, lakes, ponds	Omnivorous, feeds on plants, insects, crustaceans, small fish	Opportunistic predator, regulates invertebrate and small fish populations

This study also included the Mediterranean pond turtle (*Mauremys leprosa*), a freshwater chelonian that is widespread in the Mediterranean basin and North Africa. In addition to serving as a sentinel of habitat quality, this omnivorous species, which lives in rivers, ponds, and reservoirs, is essential in controlling invertebrate populations and nutrient cycling (Danilov, 2005; Le Gal et al., 2024) (Table 3).

Table 3. Ecological characteristics of turtle species

Species	Common Name	Family	Habitat Preference	Feeding Habit	Ecological Role
<i>Mauremys leprosa</i>	Mediterranean pond turtle	Geoemydidae	Freshwater habitats such as rivers, streams, ponds, and reservoirs with vegetation	Omnivorous, consumes aquatic plants, invertebrates, fish, carrion	Key species in freshwater ecosystems, controls invertebrate populations and contributes to nutrient cycling

3.4 Analytical measurement

3.4.1 Analytical Measurement of Trace Elements in Fish Scales

3.4.1.1 Analytical Measurements XRF

Micro-XRF analysis was performed using a Bruker M4 TORNADO instrument (Bruker, Billerica, MA, USA), equipped with a rhodium (Rh) X-ray tube operating at 50 kV accelerating voltage and 200 μ A current. For each fish scale, an area of approximately 1.2 mm $\times$ 1 mm was selected for measurement. The spot size was focused to 20 μ m using the built-in polycapillary lens, and measurements were conducted in ambient air with a scanning velocity of x ms per pixel and a step size of y. Two energy-dispersive detectors with a 30 mm² active area recorded the characteristic X-ray emissions. Data evaluation was carried out using the M-Quant software (version 1.6.621.0), and elemental quantification was based on the fundamental parameter (FP) method.

3.4.1.2 SEM (Scanning Electron Microscope)

A scanning electron microscope (SEM) uses an electron beam that is focused on a very small area to image a sample's surface point by point rather than all at once. The sample's composition and topography are revealed by the different signals that are produced when the electrons interact with the material. Our goal was to use high-resolution electron imaging to identify the surface differences between the two species. The morphology of fish scales can affect their capacity to accumulate and retain trace and macroelements, which makes this analysis essential. Scale thickness, surface roughness, and porosity are examples of structural variations that may affect how scales interact with environmental pollutants and reveal whether or not they are suitable for monitoring contamination. The scales were cleaned and then dried using ethanol in ascending order. Following the addition of 70% ethanol, each scale was positioned between two microscope slides for two to three days, in accordance with literature recommendations to prevent surface rippling (Jordanova et al., 2018). After treatment, the scales were consistently dried on filter paper to ensure uniform preparation (Esmaeili & Gholami, 2011).

Scanning electron microscopy (SEM) measurements were conducted at the Laboratory for Heritage Science of HUN-REN ATOMKI using a JEOL JSM-IT500HR instrument (JEOL, Tokyo, Japan). This SEM system supports elemental composition analysis through EDS detectors, as well as imaging capabilities via cathodoluminescence, Raman spectroscopy, backscattered electron imaging, and secondary electron imaging. All imaging and measurements were performed in low vacuum mode, enabling the examination of non-conductive materials under an adjustable nitrogen

atmosphere. Operating conditions included an accelerating voltage of 20 kV and a probe current of 0.05 nA.

3.4.1.3 Analytical Measurements ICP-OES

Approximately 0.2 g of sample material—equivalent to 25 scales per fish, was weighed using an analytical balance (Precisa ES 225SM-DR). Atmospheric wet digestion was performed using a mixture of 5 mL of 65% (m/m) nitric acid (HNO₃; reagent grade, Merck, Darmstadt, Hesse, Germany) and 1 mL of 30% (m/m) hydrogen peroxide (H₂O₂; reagent grade, Merck). Following digestion, samples were diluted with ultrapure water (Synergy UV, MilliporeSigma, Burlington, MA, USA) to a final volume of 15–20 mL and transferred without loss into volume-calibrated plastic centrifuge tubes. Until additional elemental analysis, the solutions were stored at room temperature. Agilent Technologies' ICP-OES 5110 Vertical Dual View inductively coupled plasma optical emission spectrometry was used to measure the samples' elemental concentration. A Meinhard® type nebulizer, double-pass spray chamber, and autosampler (Agilent SPS4) were utilized in conjunction with a five-point calibration protocol (ICP VI, Merck, Darmstadt, Hesse, Germany). Standard solutions for trace elements (Ag, Al, B, Ba, Bi, Cd, Co, Cu, Cr, Fe, Li, Mn, Na, Ni, Pb, Sr, Zn) and macroelements (Ca, K, Mg, Na, P, S) were prepared using a multi-element spectroscopic standard solution at 1000 mg L⁻¹ (ICP IV, Merck) and mono-element spectroscopic standards at 1000 mg L⁻¹ (Scharlau, Sentmenat, Barcelona, Spain). In both cases, a 5-point calibration process was used for which standard solutions were diluted with 0.1 M HNO₃ prepared in ultrapure water. The following wavelength lines of the MP-AES (Microwave Plasma-Atomic Emission Spectroscopy) analysis were used: Ca 317.933 nm, K 766.491 nm, Mg 285.213 nm, Na 589.592 nm, Cd 214.439 nm, Cr 267.716 nm, Cu 324.754 nm, Fe 238.204 nm, Mn 257.610 nm, Pb 220.353 nm, Sr 407.771 nm, and Zn 213.857 nm.

3.4.2 Analytical Measurement of Turtle shells

Micro-XRF mapping was conducted using a Bruker M4 TORNADO instrument (Bruker, Billerica, MA, USA), equipped with a rhodium (Rh) X-ray tube source. The system operated without a filter at an accelerating voltage of 50 kV and a current of 400 µA. The analyses were carried out in 20 mbar vacuum on an approximately 1 cm² area of the flat surface of each sample. The beam was focused to a spot size of 20 µm using the built-in polycapillary lens, with a mapping step size of

40 μm and a scanning velocity of 1 mm/s. Characteristic X-ray lines were collected by two energy-dispersive detectors, each with a 30 mm² active area. Since hydrogen, carbon, nitrogen, and oxygen cannot be detected with this instrument, the results are considered semi-quantitative. Data evaluation was performed using the M-Quant software (Bruker M4 TORNADO) based on the fundamental parameter (FP) approach. Spectrometer calibration was verified prior to measurements with certified Bruker standards. Additional certified reference standards (707 and 744 gold standards from Fluxana, Bedburg-Hau, Germany; 133X AGA3 A and 133 AGA2 silver from ARMI MBH, Manchester, NH, USA; and IARM 90C, MBH 31X78735.8 B, MBH 32X SN6 B bronze from ARMI MBH, Manchester, NH, USA) were used as self-checks to ensure measurement accuracy.

3.5. Data analysis

3.5.1 Fish Scales

The R version 4.4 was used for all statistical analysis. Zero. The Shapiro-Wilk test was used to evaluate the data's normality in order to guarantee the suitability of statistical techniques. The homogeneity of variances, on the other hand, was assessed using Levene's test. For comparisons, non-parametric tests were used because of deviations from normality or heterogeneity of variances. Macro- and trace element concentrations in fish scales were compared between chub and nase using the Kruskal–Wallis test, followed by post hoc pairwise comparisons with the Mann–Whitney U test to identify species-specific differences. The mean body weights of nase and chub were compared using Welch's test to examine variations caused by unequal variances. To compare the weight distributions between chub and nase, the Kolmogorov–Smirnov test was applied. Principal Component Analysis (PCA) was conducted on the trace element concentrations (mg kg⁻¹, wet weight) measured in fish scales to explore multivariate patterns among elements and species. Prior to analysis, all variables were $\ln(x + 1)$ -transformed to reduce skewness, stabilize variances, and improve adherence to PCA assumptions. Because the elements differed in magnitude and units, the PCA was performed on a correlation matrix, ensuring that each variable contributed equally to the analysis. No rotation (e.g., varimax or oblimin) was applied, and the results therefore represent unrotated, orthogonal principal components.

Given the small sample size ($N = 14$), standard factor-analytic suitability diagnostics such as the Kaiser–Meyer–Olkin (KMO) index, Bartlett's test of sphericity, and RMSR were not computed,

as they would not provide reliable or interpretable values for this dataset. Instead, PCA was applied strictly as an exploratory tool to visualize structure in the dataset. The first two principal components explained 52.9% and 22.8% of the variance, respectively (75.7% cumulatively), and their scores were used to illustrate species-level clustering, with 95% confidence ellipses delineating group separation. In addition, Spearman correlation analysis was performed to examine associations between trophic level and trace element concentrations, revealing species-specific relationships among elements.

All statistical analyses were conducted using a significance threshold of $p < 0.05$. Results are reported as test statistics—H for the Kruskal–Wallis test, W for the Mann–Whitney U test, and D for the Kolmogorov–Smirnov test—accompanied by their respective p-values.

3.5.2 Turtle Shells

Normality was primarily assessed using the Shapiro–Wilk test. When clarification was needed, additional omnibus tests (D’Agostino–Pearson and Jarque–Bera) were consulted to confirm distributional patterns. The outcomes of all normality tests are reported in the Results section. Variables that met the assumptions of normality (P, S, Ca, Sb) were analyzed using parametric tests: independent *t*-tests for two-group comparisons and one-way ANOVA for comparisons among multiple groups. Non-normally distributed variables (Mg, K, Ti, Mn, Fe, Zn, Sr, Al, Si) were evaluated using non-parametric alternatives, including the Mann–Whitney U test and the Kruskal–Wallis test. Metals with more than 30% missing values (Cu, Pb, Ni, As) were excluded from inferential analyses. Descriptive statistics (mean $\pm$ standard deviation) were calculated for morphometric parameters and shell element concentrations. Sex-based differences were tested using appropriate parametric or non-parametric methods. Correlation analyses were performed to assess relationships between shell elements, morphometric traits, and sex, using Spearman rank correlation. To further examine the links between turtle shell chemistry and environmental compartments (sediment and water), element-specific Spearman correlations were computed across seasonal datasets. All statistical tests were two-tailed, with significance set at $p \leq 0.05$. Graphical outputs, including heatmaps, bar charts, and network diagrams, were generated to visualize statistical relationships and trends.

Chapter 04. Results

4.1. Fish scales Results

4.1.1 Elemental Characterization and Biological Features

4.1.1.1 Biological Characteristics of the Studied Fish and turtles

- **Fish**

A total of 14 individual fish were analyzed, comprising eight specimens of chub (*Squalius cephalus*) and six specimens of nase (*Chondrostoma nasus*). The mean body weight of chub was 118.88 ± 91.62 g, while that of nase was 59.65 ± 44.79 g. Statistical comparison using Welch's t-test revealed no significant difference in the mean body weights between the two species ($t = 1.59$, $df = 10.66$, $p = 0.1405$). Despite this, the high variability in individual weights suggests differences in age, which may serve as a confounding factor due to age-related differences in bioaccumulation of trace elements.

In addition, a Kolmogorov–Smirnov test confirmed that the distribution of body weights did not significantly differ between species ($D = 0.417$, $p = 0.4602$). The trophic levels of the two species, based on values obtained from the FishBase database, were 2.0 for chub and 2.7 for nase.

- **Shell Morphometric Analysis**

A total of 25 turtle shells from *Mauremys leprosa* were investigated. The mean Carapace Length (LA), Plastron Length (Lp), and Total Shell Length (L) were **115.30 ± 11.01 mm**, **133.78 ± 9.67 mm**, and **194.38 ± 9.40 mm**, respectively. (Table 4) presents descriptive statistics for morphometric measurements of *Mauremys leprosa*. No significant differences were observed between males and females in maximum shell length (LA), axial length, or effective length (Lp) ($p > 0.05$).

Table 4. Descriptive statistics (mean $\pm$ SD) of morphometric parameters in *Mauremys leprosa*.

Variable	Overall (mm)	Male (mm)	Female (mm)
LA	115.45 ± 10.56	114.82 ± 12.62	116.08 ± 7.95
Axial length	134.11 ± 9.25	133.51 ± 9.57	134.54 ± 8.99
Lp	195.11 ± 9.36	192.56 ± 4.52	197.67 ± 11.90

4.1.2. Trace Element Concentrations in River Water

The water quality data were obtained from the Hungarian OKIR database. For the analysis, the monthly data from the year 2022 were used, and an annual mean was calculated for each parameter. Descriptive statistics are presented in (Table 5) Among the measured elements, only iron (Fe) exhibited mean concentrations exceeding the chronic criterion concentrations (CCC) for freshwater ecosystems as established by the United States Environmental Protection Agency (USEPA).

Table 5. Descriptive statistics of total trace element concentrations in water from the Maros River obtained from the National Environmental Information System of Hungary (OKIR in Hungarian) database for 2022.

Trace Element Concentration ($\mu\text{g L}^{-1}$) in Water				
Element	Min	Max	Mean $\pm$ SD	Threshold Values ($\mu\text{g L}^{-1}$) ^a
Al	1.05	13	4.68 $\pm$ 3.68	–
Cd	BDL	BDL	BDL	0.72
Cr	0.005	0.042	0.016 $\pm$ 0.011	74
Cu	0.008	0.051	0.019 $\pm$ 0.012	3.1
Fe	729	12,500 *	4725 $\pm$ 3769 *	1000
Mn	48	446	176 $\pm$ 138	–
Ni	0.006	0.073	0.026 $\pm$ 0.027	52
Pb	0	0.013	0.00475 $\pm$ 0.00411	2.5
Zn	0.006	0.084	0.033 $\pm$ 0.025	120

^a Criterion chronic concentrations (CCCs) for the freshwater of National Recommended Water Quality Criteria (EPA, 2018). * The concentration of trace elements in the water was higher than the threshold value.

4.1.3. Macro- and Trace Element Accumulation in Fish Scales

Macro-elements (Ca, K, Na, Mg, P, and S) and trace elements (Ag, Al, B, Ba, Bi, Cd, Co, Cr, Cu, Fe, Li, Mn, Ni, Pb, Sr, and Zn) were quantified in fish scales to assess patterns of bioaccumulation (Table 6).

Table 6. Mean concentrations ($\pm$ standard deviation) of macro- and trace elements in the scales of chub and nase from the Maros River.

(A) Trace Element Concentrations in Scales		
Element	Chub	Nase
Ca	95,445 $\pm$ 14,151	106,815 $\pm$ 13,627
K	3388 $\pm$ 758	3205 $\pm$ 651
Mg	1771 $\pm$ 234	2222 $\pm$ 516
Na	1194 $\pm$ 760	254 $\pm$ 570
P	36,810 $\pm$ 6724	44,160 $\pm$ 8207
S	3486 $\pm$ 466	3135 $\pm$ 86
Al	19.10 $\pm$ 9.62	21.60 $\pm$ 7.83
Ba	15.90 $\pm$ 11.20 *	53.70 $\pm$ 14.80 *
Cr	1.19 $\pm$ 0.24	1.49 $\pm$ 0.31
Cu	1.19 $\pm$ 0.23	1.37 $\pm$ 0.83
Fe	3.29 $\pm$ 0.83 *	8.39 $\pm$ 5.19 *
Mn	12.63 $\pm$ 18.29 *	24.15 $\pm$ 15.81 *
Sr	143.88 $\pm$ 34.72 *	175.63 $\pm$ 23.23 *
Zn	116.31 $\pm$ 25.07	124.93 $\pm$ 35.96

* The values in the same row are significantly different (Kruskal–Wallis test, $p < 0.05$).

- **Macroelements**

Among macroelements, only sulfur (S) showed a statistically significant interspecies difference ($H = 8.07$, $df = 1$, $p < 0.005$). No significant differences were found for calcium ($H = 1.67$, $p = 0.197$), potassium ($H = 0.15$, $p = 0.699$), sodium ($H = 3.27$, $p = 0.071$), magnesium ($H = 2.82$, $p = 0.093$), or phosphorus ($H = 3.75$, $p = 0.053$).

- **Trace Elements**

Several trace elements namely Ag, B, Bi, Cd, Co, Li, Ni, and Pb, were below detection limits across all samples. Significant differences in accumulation between species were identified for barium ($H = 8.07$, $p < 0.01$), iron ($H = 3.75$, $p < 0.05$), manganese ($H = 6.67$, $p < 0.01$), and strontium ($H = 4.82$, $p < 0.05$). Conversely, aluminum ($H = 0.82$, $p = 0.366$), chromium ($H = 3.05$,

$p = 0.081$), copper ($H = 0.02$, $p = 0.897$), and zinc ($H = 1.21$, $p = 0.272$) did not exhibit statistically significant differences.

- **Principal Component Analysis (PCA)**

PCA indicated clear separation between chub and nase based on elemental concentrations in scales. The first principal component (PC1) explained 52.9% of total variance, while the second component (PC2) accounted for 22.8% (Figure 7). This analysis underscores species-specific differences in elemental accumulation.

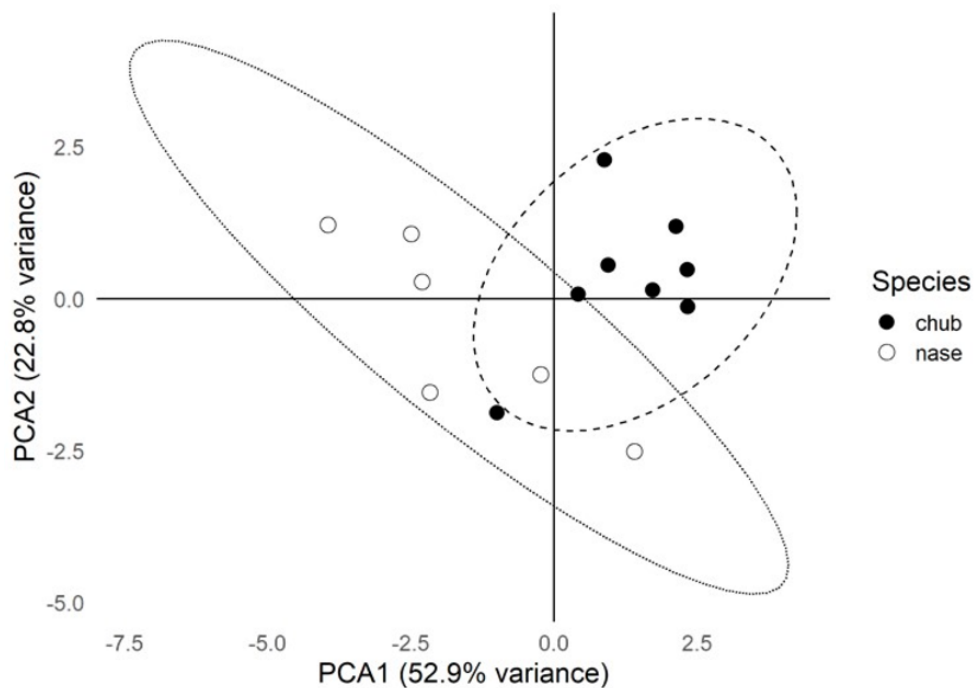


Figure 7. Principal component analysis (PCA) of trace element concentrations in fish scales. Ellipses represent 95% confidence intervals for each species.

4.1.4. Correlation Between Elemental Concentrations, Fish Weight, and Trophic Level

- **Trophic Level**

Spearman correlation analysis showed in (Table 7), between trophic level and element concentrations revealed a strong positive association with sulfur ($\rho = 0.79$, $p < 0.001$), suggesting potential biomagnification. Negative correlations were observed for phosphorus ($\rho = -0.54$, $p < 0.05$), barium ($\rho = -0.79$, $p < 0.001$), iron ($\rho = -0.54$, $p = 0.05$), manganese ($\rho = -0.72$, $p < 0.01$), and strontium ($\rho = -0.61$, $p = 0.05$).

Table 7. Spearman correlation coefficients between trace element concentrations in scales and trophic levels of chub and nase.

Only correlations significant at $p < 0.05$ are reported; all others are indicated as n.s.

Elements	
Ca	n.s.
K	n.s.
Mg	n.s.
Na	n.s.
P	-0.537
S	0.788
Al	n.s.
Ba	-0.788
Cr	n.s.
Cu	n.s.
Fe	-0.537
Mn	-0.716
Sr	-0.608
Zn	n.s.

n.s.: not significant at $p \geq 0.05$.

- **Fish Weight**

All correlations reported below are significant at $p < 0.05$. In chub, body weight showed significant positive correlations with sodium ($p < 0.001$) and phosphorus ($p < 0.05$), whereas zinc displayed a significant negative correlation ($p < 0.01$). In nase, body weight was significantly negatively correlated with aluminum ($p < 0.05$), chromium ($p < 0.01$), copper ($p < 0.05$), and iron ($p < 0.05$) (Table 8).

Table 8. Spearman correlation coefficients between element concentrations and body weight in chub and nase.

All correlations shown in bold are significant at $p < 0.05$; non-significant correlations are shown as n.s.

Elements	Chub	Nase
Ca	n.s.	n.s.
K	n.s.	n.s.
Mg	n.s.	n.s.
Na	0.976	n.s.
P	0.738	n.s.
S	n.s.	n.s.
Al	n.s.	-0.886
Ba	n.s.	n.s.
Cr	n.s.	-0.928
Cu	n.s.	-0.943
Fe	n.s.	-0.943
Mn	n.s.	n.s.
Sr	n.s.	n.s.
Zn	-0.905	n.s.

n.s.: not significant at $p \geq 0.05$.

4.1.5. XRF Analysis of Elemental Distribution

XRF was employed for the preliminary, qualitative analysis of elemental distribution in fish scales (Table 9). This non-destructive method enables rapid screening and sample selection based on elemental presence. The results demonstrate that XRF is suitable for distinguishing samples with higher elemental concentrations and offers substantial advantages in terms of efficiency and cost-effectiveness.

Table 9. Elemental Concentrations in Chub and Nase Fish Scales Across Varying Body Weights.

Chub	27g		44g		91g		136g		144g	
Nase	28g		45g		91g		136g		214g	
Element	Net	[wt.%]	Net	[wt.%]	Net	[wt.%]	Net	[wt.%]	Net	[wt.%]
P	876,642	2.802	1,038,717	3.268	1,091,565	3.437	1,163,305	3.627	1,088,533	3.367
	1,211,275	3.800	1,103,236	3.505	1,091,683	3.400	1,071,610	3.303	1,157,732	3.545
S	40,88	0.066	55,711	0.087	40,357	0.062	19,33	0.029	38,87	0.058
	33,852	0.053	30,68	0.049	21,198	0.032	29,009	0.043	26,708	0.039
Cl	5291	0.006	15,968	0.017	5360	0.005	28,916	0.029	13,257	0.013
	11,653	0.012	18,459	0.020	8914	0.009	23,876	0.023	397	0.000
K	350,902	0.210	544,726	0.316	1,000,170	0.574	1,284,764	0.734	905,771	0.511
	1,301,317	0.766	1,253,031	0.758	665,354	0.372	847,532	0.469	748,642	0.405
Ca	32,815,22	15.338	44,028,12	20.362	50,029,43	23.222	54,347,83	25.304	51,046,83	23.351
	0		2		9		2		3	
	49,917,52	23.660	41,693,28	20.146	53,381,18	24.203	53,534,85	24.137	61,727,31	27.379
Mn	8714	0.002	16,1	0.004	95,788	0.025	55,151	0.015	95,521	0.025
	31,19	0.008	83,14	0.022	79,092	0.021	103,415	0.027	122,32	0.032
Fe	54,899	0.011	61,036	0.013	14,458	0.003	34,542	0.007	10,64	0.002
	60,783	0.012	21,371	0.004	42,687	0.009	19,321	0.004	26,369	0.005
Ni	0	0.000	0	0.000	0	0.000	0	0.000	0	0.000
	0	0.000	0	0.000	0	0.000	0	0.000	0	0.000
Zn	199,301	0.023	220,916	0.026	196,2	0.023	220,839	0.026	250,431	0.029
	251,143	0.029	210,903	0.024	223,368	0.026	433,66	0.050	263,751	0.031
Sr	179,287	0.019	195,473	0.021	255,344	0.028	288,463	0.032	272,87	0.030
	231,336	0.025	221,365	0.024	258,47	0.028	276,279	0.030	342,732	0.037

4.1.6 SEM-EDS Analysis of Morphological and Elemental Features

SEM imaging was conducted to examine the surface morphology of fish scales and to complement elemental data. Morphological features such as focus, circuli, radii, and chromatophores were examined. Clear structural differences were observed between chub and nase scales (Figures 8 and 9), consistent with species-specific characteristics. Elemental mapping confirmed the presence of elements such as phosphorus (P), sulfur (S), chlorine (Cl), calcium (Ca), potassium (K),

magnesium (Mg), manganese (Mn), iron (Fe), nickel (Ni), zinc (Zn), and strontium (Sr). Notably, manganese and strontium displayed more heterogeneity across the samples.

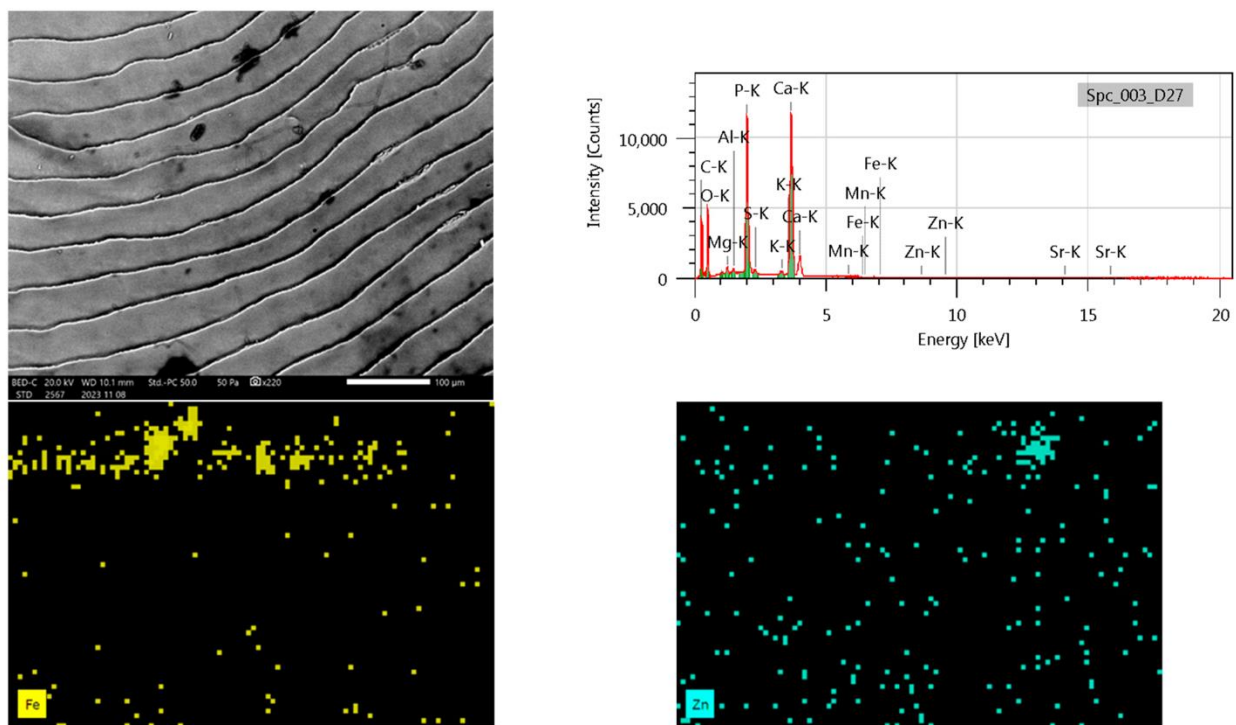


Figure 8. Scanning Electron Microscopy (SEM) Image with X-Ray Fluorescence (XRF) Spectra and Elemental Mapping of Iron (Fe) and Zinc (Zn) in Chub Fish Scale Samples

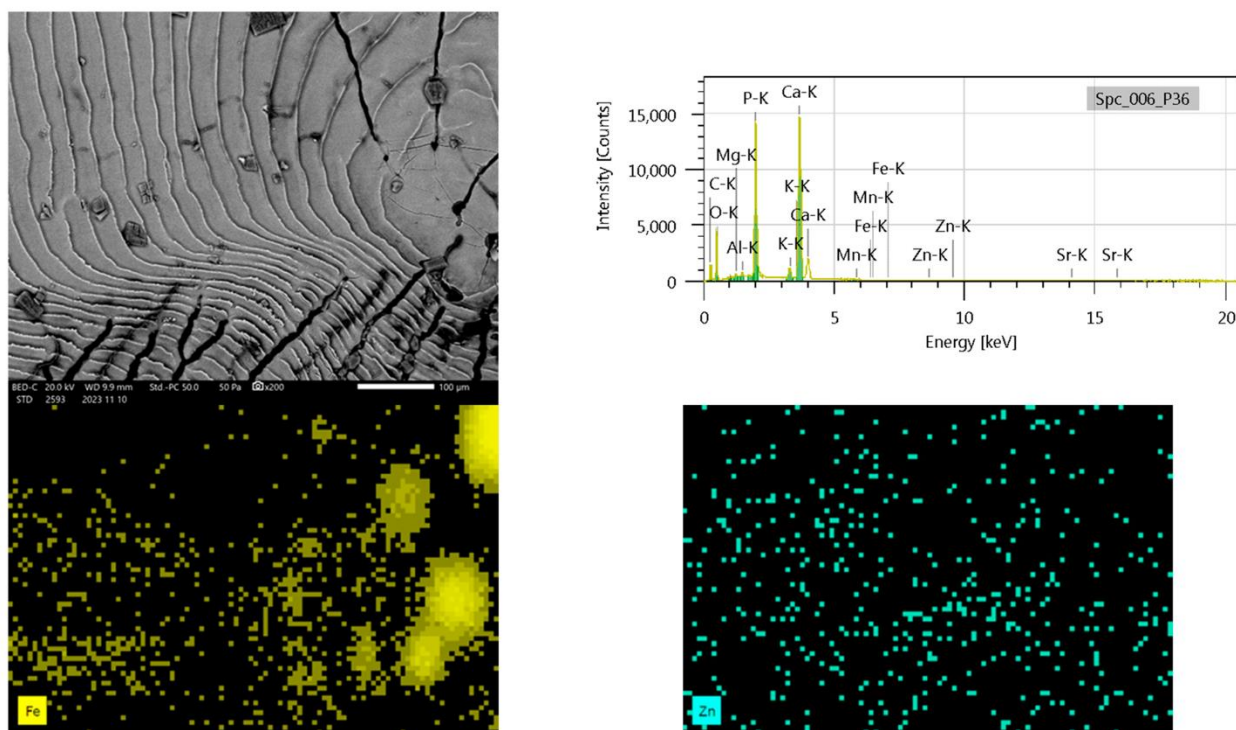


Figure 9. Scanning Electron Microscopy (SEM) Image with X-Ray Fluorescence (XRF) Spectra and Elemental Mapping of Iron (Fe) and Zinc (Zn) in Nase Fish Scale Samples.

In chub scales, elevated concentrations of calcium and phosphorus were observed, with consistent presence of sulfur and manganese. In contrast, the elemental composition of nase scales was more variable, with many scales showing low or undetectable levels of manganese and iron. This heterogeneity likely reflects both environmental conditions and biological differences between species.

4.2 Turtle shells Results

4.2.1 Elemental Composition and Sex-Based Differences

The mean elemental concentrations in turtle shells are shown in (Table 10). The main constituents were calcium and phosphorus, which reflected the keratin and bone's structural makeup. At lesser but noticeable concentrations, trace metals like Fe, Zn, Mn, and Sr were also found. Significant differences were observed between sexes (Barraza et al., 2023; Simsek et al., 2025). Males accumulated higher concentrations of Mg, Mn, Sb, and Pb, whereas females showed elevated Al levels ($p < 0.05$). According to normality testing, the remaining elements showed skewed patterns, but P, S, Ca, and Sb had normal distributions. Therefore, for normally distributed elements, parametric tests were employed, while for the remaining elements, non-parametric tests were employed. Although they are reported descriptively, elements with significant missing data (Cu, Pb, Ni, and As) were not included in the inferential analysis.

Table 10. Descriptive statistics (mean $\pm$ SD) of shell element concentrations (mg/kg) in *Mauremys leprosa*.

Element	Overall	Male	Female	Test	p-value	Sig.
Mg	3621.73 $\pm$ 791.35	4026.09 $\pm$ 847.20	3275.14 $\pm$ 536.21	Mann–Whitney U	0.04	*
P	101460.50 $\pm$ 11594.13	100014.50 $\pm$ 11915.58	102699.93 $\pm$ 11163.24	t-test	0.55	n.s.
S	3126.23 $\pm$ 1157.49	3493.00 $\pm$ 1230.92	2806.08 $\pm$ 961.03	t-test	0.09	n.s.
K	1096.38 $\pm$ 612.22	1268.33 $\pm$ 662.49	951.92 $\pm$ 527.94	Mann–Whitney U	0.07	n.s.
Ca	415697.81 $\pm$ 17696.79	414450.44 $\pm$ 18058.77	416798.88 $\pm$ 17606.67	t-test	0.48	n.s.
Ti	345.69 $\pm$ 242.96	388.79 $\pm$ 302.54	306.86 $\pm$ 166.71	Mann–Whitney U	0.29	n.s.
Mn	194.96 $\pm$ 221.47	277.38 $\pm$ 255.78	121.50 $\pm$ 162.69	Mann–Whitney U	0.05	*
Fe	2150.73 $\pm$ 1492.58	2397.18 $\pm$ 1635.71	1942.21 $\pm$ 1330.71	Mann–Whitney U	0.31	n.s.
Zn	598.27 $\pm$ 238.41	632.37 $\pm$ 240.93	568.68 $\pm$ 237.19	Mann–Whitney U	0.42	n.s.
Sr	1621.65 $\pm$ 627.06	1689.65 $\pm$ 719.93	1560.29 $\pm$ 538.31	Mann–Whitney U	0.66	n.s.
Al	3975.15 $\pm$ 3106.92	4813.00 $\pm$ 3800.02	3215.65 $\pm$ 1989.02	Mann–Whitney U	0.03	*
Sb	1490.69 $\pm$ 1081.60	2109.06 $\pm$ 1156.39	904.42 $\pm$ 704.05	t-test	0.02	*
Cu	4.62 $\pm$ 9.68	7.27 $\pm$ 12.41	2.29 $\pm$ 6.44	Mann–Whitney U	0.08	n.s.
Pb	9.62 $\pm$ 20.42	14.27 $\pm$ 24.30	5.35 $\pm$ 15.76	Mann–Whitney U	0.04	*
Ni	0.23 $\pm$ 0.78	0.00 $\pm$ 0.00	0.42 $\pm$ 0.99	Mann–Whitney U	0.15	n.s.
Si	8942.92 $\pm$ 6056.17	11066.00 $\pm$ 7079.62	6938.25 $\pm$ 4242.44	Mann–Whitney U	0.06	n.s.
As	1.58 $\pm$ 7.86	0.00 $\pm$ 0.00	2.96 $\pm$ 10.90	Excluded (>30% missing)	—	—

*Note: p -values ≤ 0.05 considered statistically significant. Sig.: * $p \leq 0.05$; n.s., not significant.*

4.2.2 Micro-XRF elemental mapping

Micro-XRF elemental mapping revealed that the major and trace element distributions in the 25 turtle shell samples were heterogeneous (Figure 10). Calcium and phosphorus were consistently the most abundant elements, forming a homogenous matrix, in accordance with the structural makeup of keratinized bone tissue. Localized hotspots of trace metals such as Fe, Zn, Mn, and Sr were often found along the growth lines and microstructural characteristics of the shell. On the other hand, potentially dangerous elements like Pb, Sb, and Al showed uneven and irregular deposition, with higher intensities in some people, suggesting different environmental exposure levels. These maps demonstrate that the elemental accumulation distribution is not uniform; rather, it reflects the shell's microstructure and the external contamination sources.

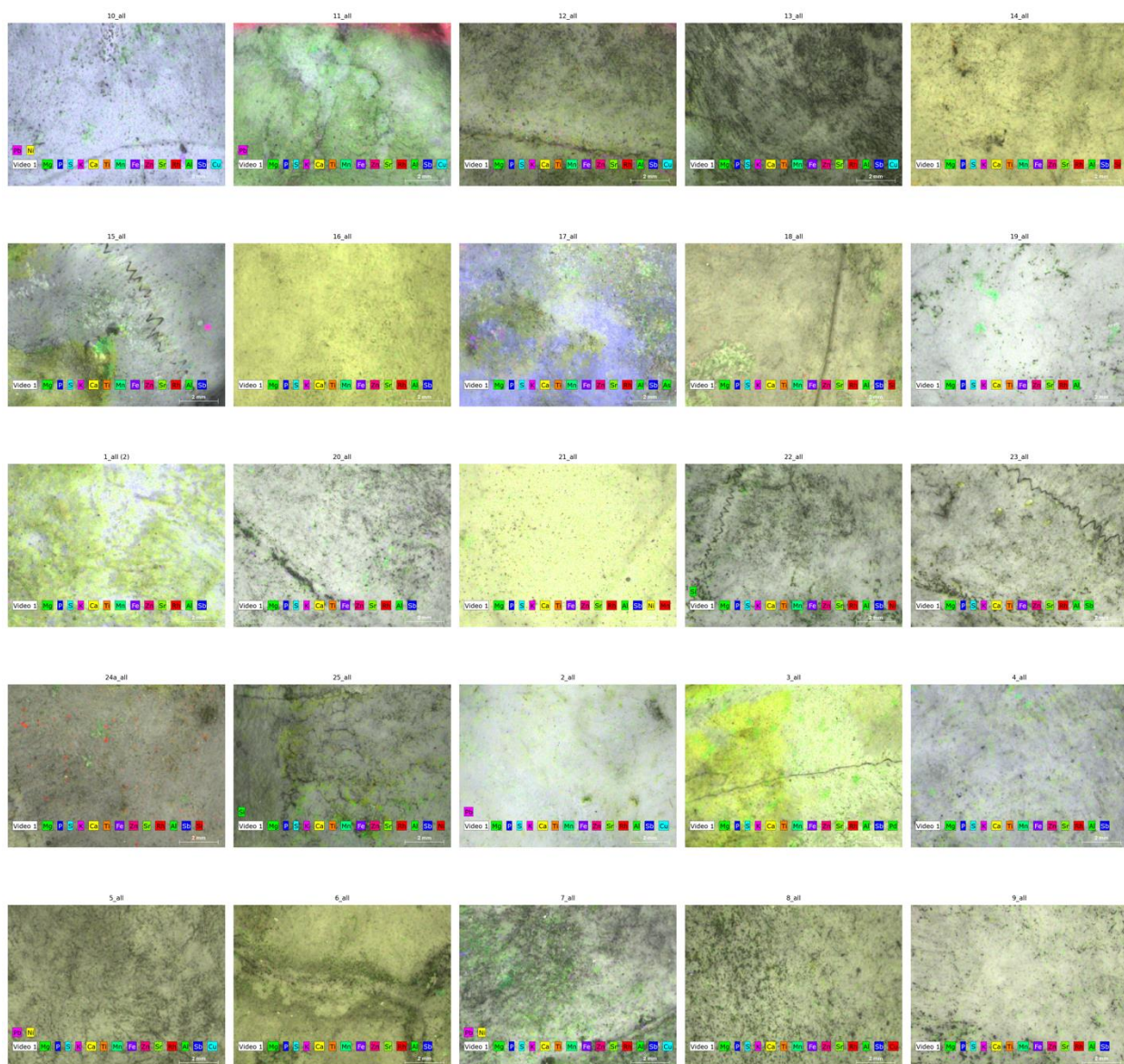


Figure 10. Elemental distribution patterns in freshwater turtle shells (n=25) obtained using micro-XRF mapping

4.2.3 Correlation Patterns Among Shell Elements

Spearman correlation analysis highlighted strong clustering among heavy metals (Ti, Mn, Fe, Zn, Cu, Pb), suggesting common environmental sources or shared incorporation pathways (Figure 12, Table 11). Calcium and phosphorus were positively correlated, consistent with their structural role, whereas phosphorus showed negative correlations with K (Figure 12).

Table 11. Spearman rank correlations between shell element concentrations (mg/kg), morphometric parameters (LA, Axial length, Lp, Effective length), and sex in Turtles

	Sex	LA	Axial length	Lp	L	Mg	P	S	K	Ca	Ti	Mn	Fe	Zn	Sr	Al	Sb	Cu	Pb	Ni	Si	As
Sex	1.0																					
LA	0.0	1.0																				
Axial length	0.0	0.0	1.0																			
Lp	0.0	0.0	0.0	1.0																		
L	0.0	0.0	0.0	0.0	1.0																	
Mg	0.0	0.0	0.0	0.0	0.0	1.0																
P	0.0	0.0	0.0	0.0	0.0	0.0	1.0															
S	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0														
K	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0													
Ca	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0												
Ti	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0											
Mn	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0										
Fe	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0									
Zn	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0								
Sr	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0							
Al	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0						
Sb	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0					
Cu	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0				
Pb	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0			
Ni	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0		
Si	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0	
As	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0

Only significant correlations are shown ($p \leq 0.05$); non-significant values are indicated as N.S. Sex is coded as female = 0, male = 1. Morphometric parameters: LA – maximum shell length (mm); Axial length – maximum straight-line carapace length (mm); Lp – effective length (mm);

L – overall length (mm). A Spearman's correlation analysis was conducted to assess the relationships between elemental concentrations and morphometric variables in *Mauremys leprosa* shells. The correlation matrix (Figure 11) revealed several notable associations. Positive correlations indicate that certain elements tend to accumulate together in the shell matrix, suggesting shared environmental sources or similar biochemical incorporation pathways. Conversely, negative correlations suggest differing uptake mechanisms or competitive interactions during deposition. Morphometric variables showed weak or inconsistent correlations with most elements, indicating that shell size had limited influence on metal accumulation patterns. Non-significant relationships ($p > 0.05$, FDR-adjusted) were marked as "N.S." to emphasize only robust associations. (Figure 12) presents a correlation network highlighting the elements that showed moderate to strong positive associations, based on the Spearman correlation analysis.

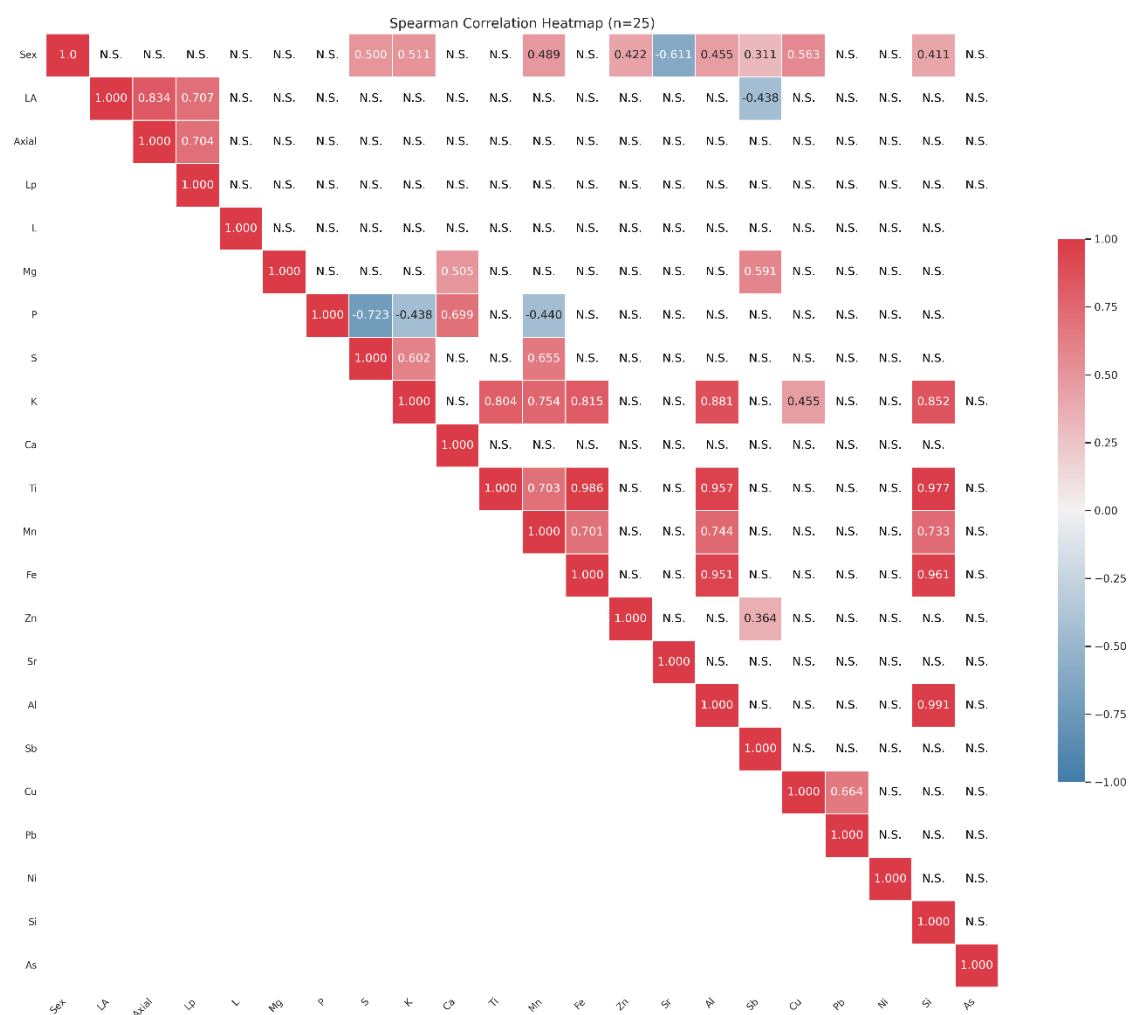


Figure 11. Spearman’s rank correlation matrix of elemental concentrations and morphometric variables in *Mauremys leprosa* shells (final dataset. n = 25). Color intensity indicates the strength and direction of ρ . Non-significant correlations ($p > 0.05$, FDR-adjusted) are marked as “N.S.”.

Network of Strong Correlations Between Shell Elements

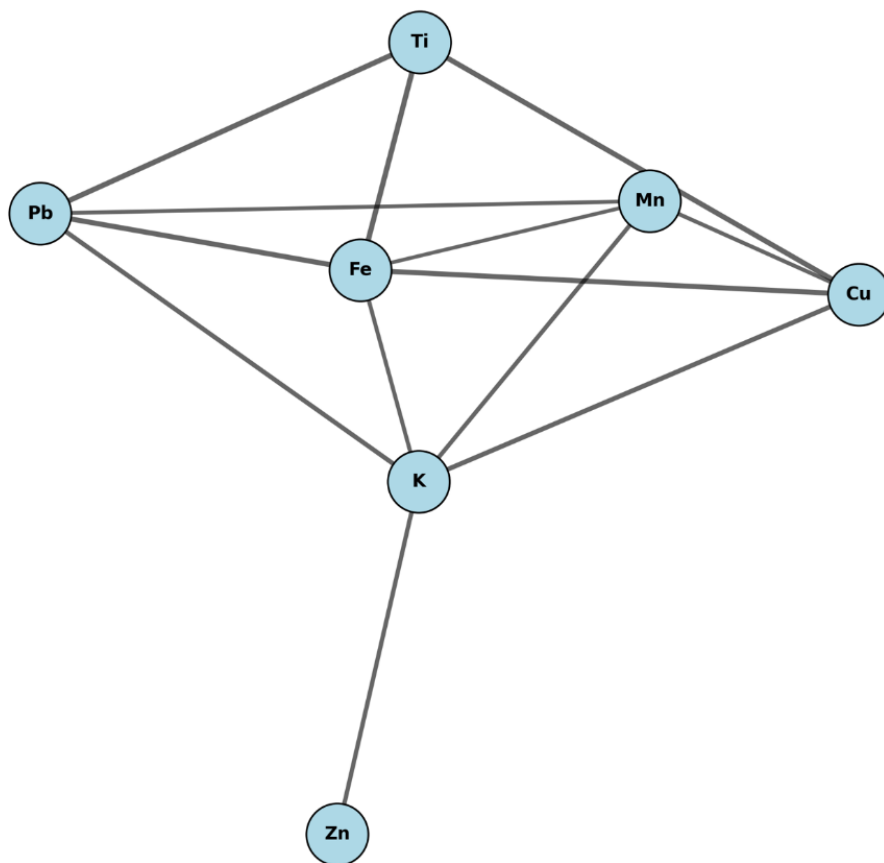


Figure 12. Elemental Correlation Network Revealing Shared Sources and Incorporation Pathways

4.2.4 Seasonal Variation of Heavy Metals in Sediments and Water

The seasonal levels of specific metals in water and sediments are shown in (Table 12). In contrast to water, where the majority of the metals were found at trace or undetectable levels, sediments had higher concentrations of Cu, Mn, Ni, Pb, and Zn. There was clear seasonal variation, with Zn rising from 12.14 to 25.58 mg/kg and sediment Cu falling from winter (37.51 mg/kg) to spring (10.55 mg/kg).

Table 12. Seasonal Variation of Heavy Metal Concentrations in Sediments and Water Samples

		Cadmium	Chromium	Copper	Manganese	Nickel	Lead	Zinc
SEDIMENTS (mg/kg)	Winter	0	0	37,51	95,47	72,04	8,73	12,14
	spring	0	0	10,55	83,79	12,33	6,26	25,58
WATER (mg/l)	Winter	0	0	0,04	0,07	0,04	0	0
	spring	0	0	0	0,03	0,02	0,04	0

4.2.5 Bioindicator Relationships Between Shell and Environmental Concentrations

A comparison of average metal concentrations across shells, sediments, and water is shown in (Figure 13). Shells consistently reflected higher long-term accumulation of trace metals (e.g., Zn, Mn, Pb) compared to water, with sediment values lying in between.

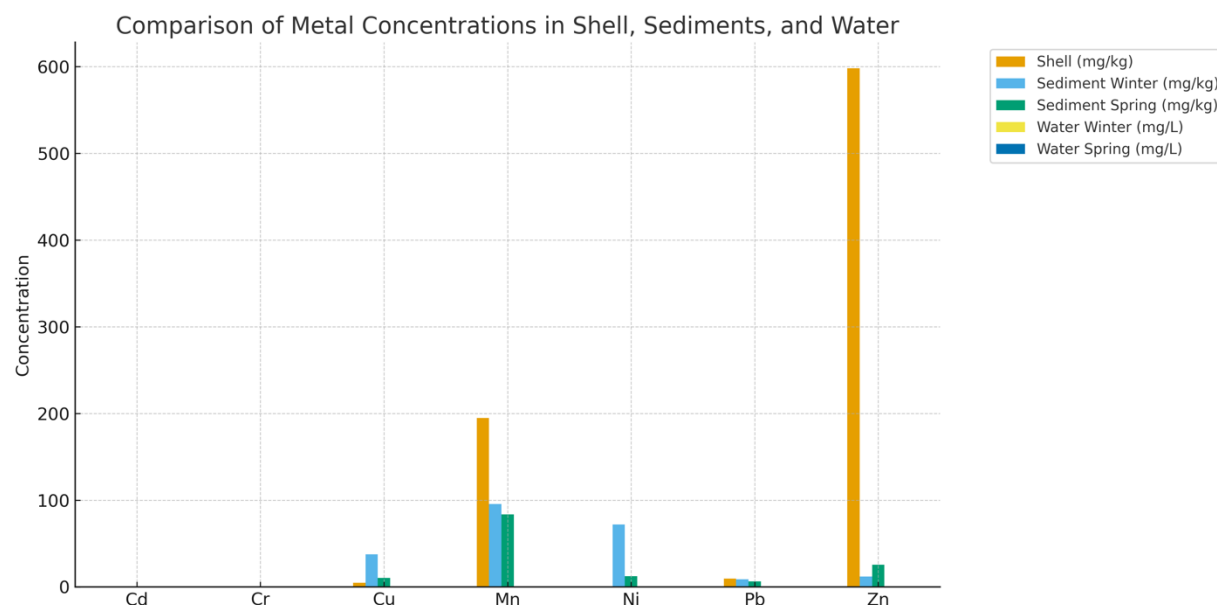


Figure 13. Bar chart comparing average metal concentrations in turtle shells, sediments (winter and spring), and water (winter and spring).

Spearman correlation analysis between shell and environmental concentrations showed the highest association with spring sediments ($\rho = 0.50$), though not statistically significant ($p > 0.05$) (Table 13). Correlations with winter sediments and water (both seasons) were weak.

Table 13. Spearman correlations between turtle shell and environmental (sediment, water) metal concentrations.

Comparison	Spearman rho	p-value
Sediment Winter	-0.20	0.747
Sediment Spring	0.50	0.391
Water Winter	-0.26	0.668
Water Spring	-0.05	0.935

Chapter 05 Discussion

The central aim of this dissertation is to demonstrate how pollution in freshwater ecosystems can be monitored without harming the organisms that inhabit them. The research focuses on fish scales and turtle shells as natural, non-invasive tissues capable of recording the presence of heavy metals in lakes and rivers. By examining two fish species (chub and nase) and one freshwater turtle species (*Mauremys leprosa*), and comparing their elemental signatures with those found in water and sediment, the study pursued two objectives: (1) to investigate how metal accumulation varies across species, locations, and seasons, and (2) to evaluate whether these hard tissues can serve as reliable and ethical indicators of both short-term and long-term contamination.

The results, presented in Chapters 3 and 4, lead to four key conclusions:

1. Scales and shells capture different timescales of exposure:

Fish scales reflect more recent and diet-influenced exposure, whereas turtle shells represent longer-term accumulation influenced by both sediment and trophic pathways.

2. Biological traits influence accumulation:

Species identity, sex, body size, and feeding behavior shape metal uptake patterns, meaning that biomonitoring programs must account for ecological and life-history factors.

3. Sediments act as long-term reservoirs of contamination:

Elements such as Cu, Mn, Ni, Pb, and Zn are retained in sediments, serving as persistent sources of exposure. This pattern is especially evident in turtle shells, which record contamination more consistently than water measurements.

4. The analytical framework is robust and practical:

By integrating XRF screening, ICP-OES quantification, micro-XRF spatial mapping, and SEM-EDS imaging, the study achieved a balance between cost, accuracy, and spatial detail in assessing metal incorporation.

This chapter situates the findings within broader scientific discussions on non-lethal biomonitoring, bioaccumulation mechanisms, and sediment–organism interactions. It considers how the outcomes support or challenge previous research and discusses their relevance for

freshwater conservation in Algeria and similar Mediterranean regions. The chapter concludes by offering recommendations for future monitoring strategies, highlighting fish scales and turtle shells as complementary bioindicator tools, and proposing a unified, conservation-oriented framework for ecological assessment and management.

5.2 Fish Scales as Bioindicators

5.2.1 Species-Specific Patterns

This study utilized scales from chub and nase collected from the Maros River as non-invasive bioindicators to monitor macroelement and trace element pollution, providing an ethical and effective approach to environmental assessment. Previous research has demonstrated that fish scales are reliable indicators of metal exposure (B.C., 1997; Nagajyoti et al., 2010). For example, studies on common carp have shown that scales can accurately reflect environmental concentrations of heavy metals (Łuszczek-Trojnar & Nowacki, 2021). Likewise, Coello and Khan (1996) reported that fish scales can begin accumulating metals earlier than internal tissues, making them sensitive early indicators of contamination. This aligns with our findings, which showed notable variations in the concentrations of elements such as Ba, Mn, Sr, and Zn in the fish scales.

Fish scales therefore function not only as protective external structures but also as biological archives, recording exposure over time (Coello & Khan, 1996). The significant species-specific differences observed between chub and nase suggest that elemental accumulation is influenced by ecological traits such as feeding habits and habitat use. However, it is important to note that bioaccumulation pathways are complex, as metals may enter scale tissues through both environmental exposure (e.g., dissolved or sediment-associated metals) and dietary intake (Aib et al., 2025; Parvez et al., 2025). Thus, while environmental contamination likely played a key role in shaping the macro- and microelement patterns observed, biological factors also contribute to the distinct elemental signatures. Overall, chub and nase represent effective bioindicator species because their scales can capture and reflect spatial and ecological variations in trace element availability, offering a non-destructive tool for ongoing freshwater monitoring programs.

This study highlights the promising role of fish scales as non-invasive tools for environmental monitoring, enabling the reconstruction of historical pollution patterns and the assessment of long-term anthropogenic impacts while maintaining ethical standards in ecologically sensitive regions (Yilmaz et al., 2007). A key advantage of fish scales is their capacity to function as archival tissues,

preserving elemental signatures that reflect both recent and past contamination events. Nevertheless, the accuracy of X-ray fluorescence (XRF) analysis may be influenced by factors such as elemental composition, sample morphology (e.g., thickness and structural heterogeneity), concentration levels, and the presence of interfering elements. Importantly, quantitative XRF measurements can also contribute to risk assessment concerning human consumption of contaminated fish, providing a useful dimension to ecotoxicological evaluation.

The fish scales analyzed in this study provided valuable insights into trace and macroelement accumulation in two cyprinid species with distinct ecological traits: chub (*Squalius cephalus*) and nase (*Chondrostoma nasus*). Principal component analysis (PCA) and correlation matrices demonstrated clear species-level separation, indicating that scale chemistry reflects both ecological behavior and environmental exposure. While nase scales showed relatively higher levels of Sr and Ba, chub exhibited elevated concentrations of Fe, Cu, and Mn. These differences align with their feeding ecologies (Wright & Welbourn, 2011; Dikanović et al., 2016; Parvez et al., 2025). The chub's omnivorous diet—which includes benthic invertebrates, detritus, and small prey—facilitates greater uptake of sediment-associated trace metals. In contrast, the nase's herbivorous–detritivorous feeding strategy favors macroelement accumulation associated with periphytic algae and detrital sources in the water column.

Comparable ecological influences on bioaccumulation have been observed elsewhere. For example, a comprehensive study on mercury in fish populations from the Atrato River basin in Colombia identified habitat use, body size, and feeding strategies as primary predictors of mercury accumulation, explaining more than 60% of variation in tissue concentrations. Benthic and pelagic feeders showed distinct enrichment patterns, reflecting their different resource-use strategies (Bernal-Alviz et al., 2025). Similarly, the species-specific divergence observed in the present study supports the conclusion that omnivory in *S. cephalus* promotes higher trace metal uptake, whereas herbivory–detritivory in *C. nasus* is associated with higher macroelement enrichment.

Meta-analyses of mercury biomagnification in freshwater environments provide additional evidence for the trophic and ecological regulation of metal accumulation. Two comparative studies summarized in (Table 14) showed that trophic magnification factors (TMFs) for mercury rise in colder, low-production systems with higher levels of dissolved organic carbon (DOC) and fall in response to atmospheric mercury deposition and phosphorus enrichment. These results

demonstrate how the biogeochemical context and ecosystem productivity control the degree of trophic transfer. These revelations support the idea that species-specific accumulation patterns are influenced by environmental factors (nutrient regimes, DOC availability) as well as ecological traits (diet, trophic level) (Lavoie et al., 2013; Li et al., 2019). The observed ecological structuring of metal accumulation is similar to these worldwide trends, despite the fact that our study concentrated on trace and macroelements rather than mercury. Interestingly, not all elements conform to the same trophic transfer rules. A study of rare earth elements (REEs) in the Po River (Italy) found that feeding ecology had no measurable influence on REE accumulation in fish tissues. Instead, a negative relationship with trophic level was observed, indicating trophic dilution rather than biomagnification (Pastorino et al., 2024). In that system, sediments functioned as the primary sink for REEs, and fish exhibited generally low bioaccumulation factors, with only a few elements (e.g., Sc, Y) showing notable bioconcentration. These findings contrast sharply with the patterns observed in the present study, where feeding mode clearly influenced the uptake of Fe, Cu, Mn, Sr, and Ba. Taken together, these contrasting outcomes illustrate that each group of elements (e.g., Hg, REEs, trace and macroelements) is governed by distinct biogeochemical behaviors and environmental pathways. As a result, the influence of ecological traits such as diet, habitat use, and trophic position must always be interpreted within the specific geochemical context and the mechanisms governing an element's transfer within food webs.

This leads to an important ecological principle underscored by our findings and those from Hg and REE-based research: functional ecology does shape contaminant bioaccumulation, but the strength and direction of this influence vary according to both the chemical properties of the element and the environmental conditions of the system. Therefore, beyond demonstrating that fish scales are reliable non-lethal bioindicators, our results emphasize the need to integrate ecological characteristics with geochemical behavior in order to accurately interpret interspecific and spatial variability in elemental accumulation.

Table 14. Comparative patterns of element accumulation across studies and their ecological drivers

Study/System	Element(s) Examined	Key Ecological Drivers	Observed Patterns	Relevance to Present Study
Maros River (Present study) – Fish scales of <i>Squalius cephalus</i> (chub) and <i>Chondrostoma nasus</i> (nase)	Trace & macroelements (Fe, Cu, Mn, Sr, Ba)	Feeding ecology (omnivory vs herbivory–detritivory), trophic level, habitat use	Chub enriched in Fe, Cu, Mn (linked to benthic/detrital diet); nase enriched in Sr, Ba (linked to algal/periphytic intake); clear PCA-based species separation	Confirms strong species-specific and ecological influences on accumulation; supports use of scales as bioindicators
Atrato River, Colombia (2019, Hg)	Total mercury (T-Hg)	Feeding habits, body size, habitat type, trophic level	Feeding ecology > body size > trophic level explained >60% variability; habitat stratified subpopulations	Strongly parallels species-level separation in Maros study; highlights importance of functional ecology
Hg Trophic Magnification Meta-analyses (Lavoie et al., 2013; Zhang et al., 2019)	Mercury biomagnification	Ecosystem productivity (DOC, P), climate (temperature), deposition	TMF highest in cold, low-productivity, DOC-rich waters; reduced with higher P or atmospheric deposition	Emphasizes role of environmental context in shaping trophic transfer, complementing species-specific ecological drivers observed in Maros study
Po River, Italy (2019, REEs)	Rare earth elements (Σ REEs, Sc, Y)	Trophic level, feeding ecology, sediment interaction	Feeding ecology had no influence; negative correlation with trophic level (trophic dilution); sediments main REE sink	Contrasts with Maros study: not all elements biomagnify; highlights element-specific behavior and need for context-dependent interpretation

5.2.2 Influence of Body Size and Trophic Status

The physiological traits, trophic positions, and habitat use of chub and nase likely contributed to the observed differences in elemental bioaccumulation in their scales. According to FishBase, the trophic level of chub is higher (≈ 3.0) compared to that of nase (≈ 2.7), reflecting their respective omnivorous versus herbivorous–detritivorous feeding strategies. Trophic position provides important insight into how ecological behavior can influence trace element uptake. For example, the comparatively elevated concentrations of manganese (Mn) and barium (Ba) detected in nase scales may be linked to its feeding preference for periphyton and detrital materials, which are directly influenced by substrate and sediment-associated sources. These findings are consistent with ecological frameworks emphasizing that feeding behavior and habitat niche strongly shape the pathways of metal incorporation (Aib et al., 2025; Wright & Welbourn, 2011).

However, while fish scales effectively retain elemental signatures, fish mobility can limit their utility for identifying highly localized contamination. Due to its broader migratory movements, the nase may integrate exposure from multiple reaches of the river, which can mask fine-scale spatial pollution gradients. In contrast, species such as chub that occupy more restricted home ranges may better reflect local environmental conditions. Furthermore, to maintain analytical consistency, scale sampling should be performed at standardized anatomical locations, as scale loss and subsequent regrowth can introduce variability in elemental accumulation over time.

The multivariate analysis also showed that different species had different pollution profiles, highlighting the accounting for interspecies ecological variability is essential when interpreting bioindicator data. Species-specific differences in elemental accumulation; such as those observed between chub and nase are often linked to variations in ecological roles and feeding behaviors. Previous research has demonstrated that heavy metal accumulation in fish is significantly influenced by dietary patterns and habitat preferences, underscoring the need to consider ecological context when evaluating contaminant levels. (Dikanović et al., 2016; Parvez et al., 2025). The observed differences in elemental accumulation between nase and chub are consistent with the findings of Järv et al. (2013), who demonstrated that species-specific ecological traits, including feeding adaptations and habitat use, strongly influence metal uptake patterns. In this study, higher Mn and Ba levels in nase likely reflect localized dietary sources and environmental

exposure associated with its herbivorous–detritivorous feeding mode. Morphometric variables also showed significant relationships with scale chemistry: Zn and Cu, which are linked to metabolic activity and growth, were more concentrated in larger individuals, whereas Pb and Cd—typically driven more by external environmental exposure—showed no clear correlation with body size. These dynamics parallel patterns reported in Mediterranean fish, where tissue-specific metal accumulation and generally weak or negative correlations with fish size indicate complex interactions among physiology, growth, and contaminant assimilation (Canli & Atli, 2003b). Furthermore, trophic level and feeding strategy strongly influenced the elemental fingerprints observed: nase exhibited more stable macroelement profiles consistent with its narrower dietary niche, while the omnivorous chub showed greater variability in trace metal accumulation. These results are also in agreement with findings from polyculture pond systems, where bottom-feeding species—analogue to chub’s benthic foraging—accumulated higher levels of sediment-associated heavy metals compared to species feeding higher in the water column (Rajar et al., 2025).

Together, these results emphasize that both biological traits (size, metabolism, trophic level) and ecological factors (feeding behavior, habitat exposure) jointly influence the accumulation of trace and macroelements in fish, reflecting the interplay between biological and environmental factors. Essential metals such as Zn and Cu tend to scale with metabolic turnover, while non-essential or toxic metals like Pb and Cd are more influenced by local environmental contamination, producing species-specific and site-dependent accumulation patterns.

5.2.3 Relationship with Environmental Matrices

The concentrations of metals in water and fish scales were not directly correlated in this study. However, previous research has shown that, under certain circumstances, scales can accurately reflect environmental metal levels (Wells et al., 2003). Future investigations could focus on establishing quantitative relationships between scale composition and water chemistry to strengthen these observations. In this study, principal component analysis (PCA) was conducted exclusively on trace elements due to their greater variability across environmental gradients and stronger association with anthropogenic pollution sources. Macroelements, in contrast, are more evenly distributed in aquatic environments and provide limited insight into regional contamination patterns. By focusing on trace elements, we were able to more effectively explore differences in

pollution among species and sampling sites. Notably, a strong correlation between iron (Fe) concentrations in water and fish scales indicates that scales can serve as reliable bioindicators for monitoring dissolved metals in aquatic systems. Fish scales accumulate metals through direct contact with water, and advanced analytical techniques allow precise measurement of these concentrations. Therefore, analyzing metal levels in fish scales provides a non-invasive approach for assessing pollution dynamics and long-term metal exposure in freshwater ecosystems (Aib et al., 2025).

High concentrations of iron (Fe) in the River Maros, exceeding freshwater safety criteria, indicate a significant ecological risk to aquatic life. High Fe levels can negatively impact aquatic ecosystems, harming organisms and contributing to environmental degradation, which necessitates a better understanding of Fe transport and effective river basin management strategies (Ahamad et al., 2024; Heikkinen et al., 2022). In contrast to Cd and Cr, which are found in water and sediments but have poor representation on scales, Pb and Ni exhibit weaker positive correlations. This implies that certain metals are more bioavailable or preferentially integrated into particular tissues than others. Nevertheless, metals cannot remain permanently fixed in sediment. When the physical-chemical properties of water change, some of these fixed metals will return to the water above and be accessible to living things (Sunjog et al., 2016).

5.2.4 Methodological Considerations

This study highlights the value of using fish scales as a humane and effective alternative to traditional lethal sampling methods, which often require extracting internal tissues such as muscle or liver. Fish scales are particularly effective at capturing trace element variability over time, as they preserve elemental signatures throughout the fish's lifespan, providing a continuous and non-invasive record of environmental exposure and physiological changes (Jovičić et al., 2023; Wells et al., 2000). Our findings strongly corroborate these observations, reinforcing the reliability of previous research (Wright & Welbourn, 2011).

This study advances prior work by employing sophisticated analytical techniques such as XRF and ICP-OES, which together provide both qualitative and quantitative evaluations. ICP-OES, with its high sensitivity and broad elemental coverage, excels at trace-level elemental analysis across a wide range of sample types, making it a versatile and powerful tool. XRF, in contrast, is particularly suited for qualitative and semi-quantitative analyses of minimally prepared samples

and is ideal for non-destructive examination of fish scales, especially for elements with concentrations above 1 ppm ($\mu\text{g/g}$). XRF also enables the generation of elemental maps, which allow for visual comparison of scales and spatial assessment of element distribution. Furthermore, scanning electron microscopy (SEM) provides additional insights into microstructural variations and elemental localization. Using both XRF and ICP-OES together achieves the highest level of analytical accuracy.

The study has limitations, including a modest sample size of eight chub and six nase, which may constrain the generalizability of the findings. Future studies should include larger sample sizes across broader spatial and temporal scales to confirm the observed patterns of elemental accumulation. Observed relationships between fish weight and trace element concentrations suggest that biological factors such as age and growth rate play a significant role in metal accumulation (Donadt et al., 2021; Järv et al., 2013). Variations in elemental concentrations likely reflect differences in age, growth rate, and environmental exposure. Because bioaccumulation fluctuates with age and related factors such as metabolic activity and duration of pollutant exposure, future research could reduce variability by standardizing the size or age of fish sampled. These results are in line with research suggesting that due to variations in exposure times and metabolic rates, younger fish may display different accumulation patterns than older ones (Jordanova et al., 2018). This investigation focused on the macroelements and trace elements present in the Maros River, with particular emphasis on their accumulation in fish scales. While toxic metals such as lead (Pb) and mercury (Hg) are critical indicators in ecotoxicological assessments, their concentrations in the sampled scales remained below detectable limits, possibly reflecting the specific industrial and anthropogenic activities in the study area. Although these findings are encouraging, it is important to acknowledge the study's limitations. Future research should aim to address the constraints posed by the relatively small sample size and incorporate a more comprehensive spatial and temporal dataset to enhance the reliability and applicability of the results. A broader understanding of pollution dynamics could be achieved by expanding the focus to multiple aquatic environments and integrating fish scale data with sediment and water quality analyses.

Fish scales offer several advantages as non-lethal markers of environmental contamination. Their ability to preserve elemental signatures over time makes them valuable for reconstructing past

pollution events (E. Lippitsch, 1990). However, interpreting these results requires consideration of potential limitations, including fish mobility, interspecies variability, and the possibility of scale regrowth. Advances in analytical techniques, such as particle-induced X-ray emission (PIXE) and synchrotron radiation, may further improve the accuracy of future biomonitoring studies. Additionally, quantitative data obtained from XRF analysis can contribute to assessing potential human health risks associated with consuming contaminated fish.

5.3 Turtle Shells as Bioindicators

5.3.1 Elemental Composition and Environmental Relevance

The predominance of calcium (Ca) and phosphorus (P) in *Mauremys leprosa* shells highlights their essential structural role in the mineralized matrices of bone and keratin (Dee H. et al., 2021; Rani et al., 2025; Rothschild et al., 2013). This observation is consistent with studies on chelonians and other vertebrates, where skeletal integrity and shell rigidity are primarily attributed to Ca–P complexes (Becker et al., 2025). These macronutrients are crucial for egg formation, skeletal maintenance, and shell strength (Kausar & Naureen, 2021; Sinclair-Black et al., 2023; Taylor, 1970). In addition to Ca and P, trace metals such as iron (Fe), zinc (Zn), manganese (Mn), and strontium (Sr) were detected, reflecting their physiological roles in mineral substitution, enzymatic activity, and bone metabolism (Becker et al., 2025). The detection of non-essential or potentially toxic elements—including aluminum (Al), antimony (Sb), and lead (Pb)—suggests environmental contamination, emphasizing the shell’s function as a passive reservoir for exogenous pollutants. Similar accumulation patterns have been reported in other keratinous structures, such as fish scales, bird feathers, and reptilian skin, further supporting the role of shells as indicators of environmental metal exposure (Becker et al., 2025; Naghilou et al., 2025).

In freshwater systems, the shells of *Mauremys leprosa* demonstrate strong potential as integrative bioindicators of metal exposure. As expected, their composition is dominated by calcium (Ca) and phosphorus (P), reflecting their essential roles in skeletal and keratinous matrices. The shells’ capacity to mirror regional geochemical baselines is further evidenced by notable enrichments of Fe, Mn, Zn, and Sr, which parallel concentrations observed in nearby sediments and water. Importantly, the presence of potentially harmful and non-essential elements such as Pb, Sb, and

Al points to anthropogenic contamination, consistent with pressures from agriculture and urbanization in Mediterranean wetlands.

Recent studies on chelonians highlight the integrative, long-term monitoring capacity of turtle shells. Due to the layered structure of scute keratin, shells preserve a time-stamped chemical record of environmental exposures, including metals and radionuclides. For instance, uranium signatures associated with historical nuclear weapons testing were retained in turtle shells from the Marshall Islands, providing valuable insights into the persistent ecological effects of such activities (Celia, 2023). Similarly, uranium incorporation into shell keratin at sites impacted by nuclear activities underscores the shells' function as a stable, inert reservoir of long-term environmental information (Tim Newcomb, 2023).

5.3.2 Sex-Linked Differences in Accumulation

Sexual dimorphism in trace element accumulation was evident, with males exhibiting higher Mg, Mn, Sb, and Pb, while females showed elevated Al levels. These differences likely arise from a combination of behavioral and physiological factors (Cox et al., 2007; Katona et al., 2021). Male turtles often have greater mobility and wider home ranges, increasing their exposure to contaminated microhabitats (Gibbons & Lovich, 1990; Keller et al., 2006). Additionally, female reproductive physiology, including higher metabolic rates and calcium mobilization for egg production, may influence element partitioning, leading to differential sequestration of metals between the sexes (Hopkins et al., 2012, 2013; Stewart & Eca, 2010). Elevated Pb and Sb in males are particularly concerning, as both elements are strongly associated with anthropogenic sources such as vehicular emissions, industrial discharge, and contaminated soils (Becker et al., 2025b; Bishop et al., 2010). Comparable patterns have been reported in freshwater turtles inhabiting urban and industrial wetlands, where males accumulate higher metal concentrations due to wider home ranges and greater environmental contact (Becker et al., 2025). Though less frequently studied in reptiles, Sb has also been linked to industrial activity and may act synergistically with Pb to exacerbate toxic effects (Naghilou et al., 2025).

In contrast, higher Al concentrations in females may reflect differences in dietary intake or sex-dependent detoxification pathways. Most aquatic and semi-aquatic turtles are omnivorous, consuming variable proportions of fresh vegetables and animal protein depending on the species (Maccolini, 2024). Females also employ energy-intensive detoxifying strategies to preserve

reproductive fitness and safeguard genetic material when exposed to elevated levels of metals and organic pollutants (Wilczek et al., 2013, 2014). Similar sex-related differences in trace element burdens have been documented in other chelonian species, where reproductive demands and calcium mobilization affected metal partitioning (Bishop et al., 2010). Collectively, these results align with previous findings and emphasize the importance of considering sex as a biological variable when evaluating turtles as bioindicators of metal contamination.

Elemental profiles exhibited clear sex-dependent differences: females accumulated more Al, whereas males showed higher levels of Pb, Sb, and Mn. These differences likely arise from a combination of physiological and behavioral factors. Females allocate significant energy to reproduction, which can alter elemental assimilation and deposition pathways, while males typically occupy larger home ranges, increasing their contact with contaminated substrates and water (Gibbons & Lovich, 1990). These patterns illustrate how life-history strategies mediate both exposure and bioaccumulation. Similar trends have been observed in other calcified structures. For example, studies on invertebrate shells from the Gulf of Gdańsk revealed that elemental concentrations (Ca, Na, Sr, Mg, Mn, Ba, Cu, Pb, V, Y, U, and Cd) were influenced not only by crystal type (calcitic vs. aragonitic) but also by organismal traits such as size and feeding habits, with smaller individuals often exhibiting higher concentrations than larger ones (Piwoni-Piórewicz et al., 2021). These findings support the view that environmental factors and biological characteristics—such as size, growth rate, feeding behavior, and sex—interact to shape the elemental fingerprints of biomineralized tissues. Consequently, the sex-related differences observed in *Mauremys leprosa* shells reflect a broader ecological principle: elemental incorporation is not solely determined by environmental exposure but is filtered through species- and sex-specific biological processes. This reinforces the value of turtle shells as bioarchives, capable of integrating both environmental contamination signals and life-history variation.

5.3.3 Elemental Mapping and Spatial Heterogeneity

The Micro-XRF elemental maps provide spatially resolved confirmation of the compositional trends observed in bulk analyses. Homogeneous distributions of calcium and phosphorus are consistent with their established roles in turtle shell mineralization, as reported in multiple studies of chelonian hard tissues (Martínez-López et al., 2017; Parolini et al., 2020). In contrast, the hotspot-like deposition of Fe, Zn, and Mn reflects environmental exposure rather than intrinsic

shell structure, paralleling patterns observed in other keratinous and calcified tissues (Parolini et al., 2020; Pushie et al., 2014). Patchy localization of Pb and Sb in certain individuals aligns with prior research documenting elevated and spatially variable lead accumulation in turtle shells from contaminated or urbanized sites, suggesting episodic or point-source inputs rather than uniform chronic uptake (Bishop et al., 2010; Martínez-López et al., 2017). Sex-dependent differences were also evident, with males exhibiting higher Pb and Sb and females showing higher Al. These differences likely result from variations in foraging behavior, physiology—including maternal transfer during vitellogenesis—and habitat use. Overall, these findings are consistent with previous reports of sex-specific metal burdens in *Mauremys* and other turtle species (Martínez-López et al., 2017; Parolini et al., 2020).

Our results demonstrate the complementary value of Micro-XRF and bulk assays: Micro-XRF provides spatial context, distinguishing structural from environmental signals, while bulk analyses quantify element concentrations for statistical evaluation. Methodological reviews increasingly recommend such integrative approaches for X-ray fluorescence imaging of biological tissues (Pushie et al., 2022). The observed sex-linked patterns and spatial heterogeneity reinforce the use of freshwater turtle shells, such as *Mauremys leprosa*, as integrative, non-invasive bioindicators of trace element contamination, while highlighting the importance of accounting for biological factors—sex, age, and growth lines—when interpreting exposure signals.

Micro-XRF elemental maps revealed the diverse range of contaminants in turtle shells. Homogeneous deposition of calcium (Ca) and phosphorus (P) aligns with their structural roles in shell integrity and biomineralization. In contrast, lead (Pb) and antimony (Sb) appeared in sporadic, hotspot-like clusters, likely reflecting localized or episodic contamination events such as point-source discharges, agricultural runoff, or stormwater inflows. This spatial heterogeneity underscores the value of combining bulk and imaging techniques: Micro-XRF visualizes element distributions to separate environmental contamination from structural signals, whereas ICP-OES provides precise quantitative concentrations (Pushie et al., 2022).

Recalibration studies have shown strong correlations between XRF and ICP-OES results, particularly for metals such as Cu, Mn, and Zn (e.g., r^2 up to 0.9998 for Cu) (Chojnacka et al., 2018). These findings illustrate that while ICP-OES remains the gold standard for accuracy and detection limits, XRF offers rapid and cost-effective data acquisition that can be reliably integrated

for environmental biomonitoring. ICP-based approaches, although accurate, are often time-consuming and may create workflow bottlenecks, whereas XRF expedites data collection and decision-making without compromising reliability (Lieven, 2025). By combining high-resolution Micro-XRF imaging with bulk ICP-OES measurements, our study not only validates shell chemistry as a quantitative biomonitoring tool but also provides detailed spatial insight into pollutant deposition. Capturing both elemental loads and their heterogeneity enhances the accuracy of turtle shells as bioindicators and delivers a more comprehensive understanding of pollution dynamics in freshwater systems.

5.3.4 Morphometrics and Bioaccumulation

Significant correlations between shell morphometric parameters and elemental concentrations suggest that growth influences bioaccumulation in *Mauremys leprosa*. Larger or longer shells tended to exhibit elevated levels of selected metals, reflecting the cumulative nature of contaminant integration over time. This supports the use of shell chemistry as a non-invasive, long-term biomarker of environmental exposure and ecosystem quality. However, not all morphometric traits showed strong associations with elemental burdens, indicating that metal incorporation is not solely driven by growth. External factors such as diet, habitat quality, trophic pathways, and seasonal variation likely play substantial roles in regulating trace element accumulation. This dual influence underscores the importance of interpreting bioaccumulation data within the broader context of both organismal biology and environmental conditions.

Our results demonstrate that *M. leprosa* shells are effective integrators of exposure to both trace and macroelements. Morphometric correlations indicate that growth contributes to cumulative bioaccumulation of metals like Fe, Zn, and Sr, with elevated concentrations observed in larger shells. This finding supports the concept of shell chemistry as a long-term repository of environmental contamination. The imperfect relationship between elemental burdens and size, however, highlights that ecological and environmental factors also modulate metal accumulation.

This interpretation aligns with recent ecological research demonstrating the strong influence of turtle habitat and behavior on shell-associated traits. For example, studies of carapace microbiomes reveal that microbial diversity on shells is closely linked to species-specific ecology and habitat use (Parks et al., 2024). These results emphasize the role of turtle shells as ecological “recorders,”

integrating both biological traits and environmental pressures. Environmental factors further modulate elemental signatures: in a mixed-use watershed in West Virginia, painted (*Chrysemys picta*) and snapping (*Chelydra serpentina*) turtles exhibited correlations between bioaccumulated metals, soil contamination, and land use. Pb levels in nails reflected local soil burdens, while Se levels were associated with mining proximity, illustrating the vulnerability of non-destructive keratinous tissues to human impacts (Becker et al., 2025). These findings support our observation of Pb, Sb, and Al enrichment in *M. leprosa* shells from impacted wetlands, demonstrating that agriculture and urbanization produce detectable contaminant signals that are recorded in shells.

Finally, by verifying turtles' ability to accumulate metals across tissues and species, marine studies broaden this interpretation. Significant relationships between elements were found in research on loggerhead (*Caretta caretta*) and green turtle (*Chelonia mydas*) in Northern Cyprus. Essential metals predominated over non-essential ones, and species-specific variations were clearly visible (Çelik et al., 2023). These findings demonstrate that chelonians, whether freshwater or marine, consistently build up and reflect environmental pollutants, making their tissues including shells useful bioindicators in a variety of environments. These comparative findings highlight how reliable turtle shells are as biomonitoring instruments. Their layered structure offers exposure records over time, and both extrinsic (land use, habitat quality, and sources of contamination) and intrinsic (growth, ecology) factors influence their chemistry. Evidence from freshwater, marine turtle, and microbiome studies converges to support the use of *M. leprosa* shells as integrative markers of ecosystem health as well as trace metal pollution indicators.

5.3.5 Links with Environmental Matrices

The positive correlations between metal concentrations in turtle shells and those measured in sediments and water highlight the potential of *Mauremys leprosa* shells as reliable bioindicators of local contamination. Elements such as Fe, Mn, and Zn consistently mirrored environmental matrices, reflecting patterns observed in fish scales and mollusk shells (Jian et al., 2022; Wilkinson et al., 2023). Due to their long lifespan, site fidelity, and direct exposure to both aquatic and terrestrial inputs, turtles provide an integrative record of chronic exposure, with shells acting as durable archives. The detection of heavy metals in shell tissues that parallel sediment and water levels underscores their value as sentinels for ecosystem health monitoring (Jian et al., 2021, 2022).

Our findings demonstrated positive correlations between metal concentrations in surrounding water and sediments—particularly Fe, Mn, and Zn—and the shell chemistry of *M. leprosa*. Their longevity and limited mobility enable turtles to integrate exposure over prolonged periods, reinforcing their suitability as local bioindicators. Importantly, non-invasive sampling of shells offers a sustainable approach to biomonitoring without threatening species of conservation concern. Previous research supports this notion: in a population of *M. leprosa* from a former mining district in southeastern Spain, blood analyses revealed elevated Zn, Cu, Pb, Hg, and Cd, with males generally exhibiting higher concentrations than females, likely due to maternal offloading during egg production (Martínez-López et al., 2017). Correlations between Cu–Cd and Cu–Hg in this population suggest activation of detoxification mechanisms such as metallothionein binding, emphasizing that turtles can physiologically cope with metal exposure while maintaining detectable contamination signals in shells.

The high adsorption capacity of shells is further confirmed by studies on *Mauremys caspica* from Zarivar International Wetland, Iran, where Zn, Pb, and Cd concentrations were elevated in both shell and blood samples (Moradi et al., 2022). These findings corroborate our results and indicate that shell tissues accurately reflect heavy metal burdens, serving as strong environmental archives. Across freshwater systems, the consistency of results in *M. leprosa* and *M. caspica* emphasizes the broad applicability of shell-based monitoring for diverse turtle taxa. Beyond freshwater habitats, marine turtles also act as persistent bioindicators of pollution; land-based contaminants can alter toxicokinetics and toxicodynamics, affecting health and population viability, as observed in *Chelonia mydas* (Dogruer, 2022). These mechanistic insights demonstrate the causal links between environmental pollution, physiological impacts, and ecological consequences.

Collectively, these studies reinforce that turtle tissues—whether shells, blood, or internal organs—are effective matrices for tracking pollutant exposure in aquatic environments. Our findings align with prior research, confirming the dual role of turtles as sentinels of ecological health and indicators of environmental quality. To bridge the gap between contaminant exposure and biological outcomes, future research should integrate shell chemistry with toxicokinetic modeling and health assessments, providing a more comprehensive understanding of the threats posed by pollutants to turtles and their ecosystems.

5.3.6 Conservation and Ecological Implications

The accumulation of toxic metals such as lead (Pb) and antimony (Sb) carries important ecological and physiological implications for chelonians. Lead, in particular, is known to disrupt calcium metabolism, impair immune function, and contribute to pathological conditions such as shell disease in emydid turtles (Bishop et al., 2010; Pounds, 1984). These effects are especially concerning given the long lifespan and slow detoxification rates of turtles, which increase their vulnerability to chronic exposure and bioaccumulation. The detection of Pb and Sb in shell tissue not only reflects environmental contamination but also signals potential health risks for individual organisms and broader ecosystem integrity (Al-Rawahy et al., 2007; Bishop et al., 2010; Santhosh et al., 2024; Steinhardt et al., 2016). While sequestration of Pb in the shell may mitigate systemic toxicity, it nonetheless indicates persistent environmental exposure with potential long-term consequences for health and reproduction (Collin et al., 2022; Kumar et al., 2020). Sb, though less studied in reptiles, is associated with industrial emissions and may act synergistically with other metals to exacerbate oxidative stress (Collin et al., 2022; L. Wang et al., 2024; Zhong et al., 2021).

The presence of heavy metals in the shells of the Mediterranean pond turtle (*Mauremys leprosa*) serves as an early-warning indicator of ecosystem degradation. This species inhabits Mediterranean wetlands that are increasingly impacted by pollution from agricultural runoff, industrial effluents, and urban expansion. *M. leprosa* bioaccumulates these metals in its tissues, making it a reliable bioindicator of water quality and environmental stress—even in individuals that appear physiologically healthy. Elevated concentrations of metals such as Pb, zinc (Zn), and copper (Cu) in *M. leprosa* reflect contamination from surrounding aquatic environments, reinforcing the species' value as a sentinel for monitoring ecosystem health (EL Hassani et al., 2019b; Martínez-López et al., 2017). All comparisons of heavy metal accumulation in *Mauremys leprosa* shells to 'less disturbed' or reference wetlands are based solely on previously published studies and not on additional field sampling. These literature-based references were integrated to contextualize our results and highlight the relative degree of contamination observed in the study sites. By referencing multiple studies from Mediterranean wetlands and other regions, we provide a broader ecological perspective while emphasizing that our data reflect the conditions of the sampled wetlands only.

5.4 Comparative Insights: Fish Scales vs. Turtle Shells

5.4.1 Complementary Bioindicator Roles

The complementary use of turtle shells and fish scales as bioindicators underscores the value of integrating biomarkers that operate across different ecological and temporal scales. Fish scales, with their relatively rapid turnover, provide short- to medium-term information reflecting seasonal variation and species-specific metabolic dynamics. In contrast, the slow-growing, permanent turtle shell functions as a long-term archive, integrating exposures at the habitat level and chronic contamination over extended periods. Together, these matrices offer a more complete picture of pollutant dynamics, capturing both acute environmental fluctuations and enduring ecological pressures.

This dual approach is particularly pertinent for turtles, whose life histories are characterized by slow growth and delayed maturation. As noted by Stubbs et al. (2019), the longevity of wild sea turtles and the logistical challenges of long-term recapture studies make it difficult to accurately assess growth rates. Shell chemistry provides a valuable proxy, incorporating exposure histories over multiple years and compensating for the absence of direct longitudinal growth data in natural populations.

The interpretive power of the turtle shell is further enhanced by its ontogenetic development. Cordero et al. (2018) highlighted that delayed tissue differentiation and developmental repatterning influence the late emergence of key shell traits, such as kinesis. This slow, constraint-driven morphogenesis indicates that the shell is a dynamic structure, whose ecological and functional significance evolves over time, rather than serving merely as a passive recorder of environmental pollutants. By linking pollutant accumulation with morphometric and developmental characteristics, shell-based bioindication captures both intrinsic biological processes and extrinsic environmental stressors, making it a robust tool for long-term biomonitoring.

5.4.2 Differences in Exposure Pathways

The comparison of turtle shells and fish scales highlights notable differences in how aquatic organisms integrate and reflect environmental contamination. Fish scales, as mineralized

structures primarily composed of calcium, magnesium, and other dissolved elements, serve as sensitive indicators of the surrounding water's chemical composition. Because they are in constant contact with the water column, scales are particularly effective at capturing dissolved-phase metals and minerals, making them reliable markers of waterborne pollution, including changes in water hardness and ionic content. Their dynamic exposure renders them especially responsive to short- to medium-term fluctuations in aquatic chemistry.

In contrast, turtle shells are biologically active, long-lived structures that function as integrative bioaccumulators of both dissolved and sediment-associated pollutants. Shells incorporate elements from nutrients ingested during feeding as well as contaminants directly contacted from the surrounding environment. Due to the semi-aquatic behavior of turtles, including feeding, burrowing, and basking, shells show a stronger correlation with sediment-bound metals such as Fe, Mn, and Pb (EPA, 2025). Importantly, turtle shells provide a reliable record of prolonged exposure to environmental stressors because they can adsorb pollutants from sediments and the water column while integrating contaminants over time, as summarized in Table 15.

Table 15. Contamination Pathways and Indicators: Fish Scales and Turtle shells

	Fish scales	Turtle shells (Scutes)	References
Exposure method	The fish mainly absorb pollutants from the surrounding water through direct contact with their skin and gills. The calcareous structure of the fish's scales continuously absorbs nutrients and contaminants from the water as it grows. The growth rings of the scale store these pollutants indefinitely.	Primarily through their diet and direct contact with water and sediment, which leads to the buildup of contaminants in turtles and tortoises. When they forage in the sediment, chemicals that have settled on the bottom of a water body are released into the water. The hard shell plates (scutes) of the keratin contain trace elements that bind to them over time, incorporating and storing contaminants.	(Esposito et al., 2023; Jian et al., 2021b; Miguel et al., 2022; Shaw et al., 2021)
Examples of contaminants	According to studies, heavy metals like cadmium (Cd), chromium (Cr), iron (Fe), mercury (Hg), and zinc (Zn) accumulate in fish scales. Additionally, persistent organic pollutants (POPs) like dioxins and polychlorinated biphenyls (PCBs) can be absorbed from the water. When these elements accumulate, they can lead to deformities and alterations in the scale's morphology.	Heavy metals such as lead (Pb), mercury (Hg), and cadmium (Cd) have been found to accumulate in turtle shells. Additionally, research has identified legacy and emerging persistent organic pollutants in sea turtle tissue, including those associated with plastic debris. The composition of nest substrate and wetland soil can also influence the accumulation of heavy metals in turtles.	

5.4.3 Species-Specific and Sex-Specific Signals

In fish, the interspecific variations (nase vs. chub) and the sex-related variations in turtles emphasize how important it is to take biological context into consideration when analyzing bioindicator data. In fish, species-specific ecology has a significant influence on accumulation patterns. (H. Gu et al., 2022) demonstrated how temperature and water column position affect the number of scales and skin structure, with benthic species showing more scale adaptations and thicker skin. This bolsters our conclusion that ecological variability associated with diet and habitat use is captured by fish scales.

Accumulation in turtles is a result of both internal and external factors. Males have flatter, mating-adapted shells, while females develop domed, mechanically stronger shells, a dimorphism that may also affect contaminant integration, as shown by (Vega & Stayton, 2011). Additionally, (Foth et al., 2019) demonstrated that internal constraints shape some turtle morphology, rather than all

of it being driven by ecological factors. These findings imply that turtle shells record both environmental exposure and sex-linked physiological reactions.

5.4.4 Methodological Considerations

Fish scales have several obvious practical benefits, including ease of collection, low animal stress, and repeatable sampling. They are therefore an effective tool for monitoring contamination over extended periods of time and over wide areas. We can create reliable datasets that show actual environmental trends rather than isolated occurrences by compiling a large number of scales. Although the information provided by turtle shells about an individual's exposure history is fascinating, their use is much more restricted due to opportunistic sampling. It's like contrasting a detailed satellite map of the entire area (fish scales) with a single high-resolution photograph of a landmark (the turtle shell) both are valuable, but they have quite different uses.

Turtle shell analysis's greatest strength its rich, site-specific detail is also its greatest drawback when it comes to more general evaluations. A thorough analysis of a single shell provides valuable information about a single hotspot, but it is not scalable to provide a picture of the entire region or ecosystem. We cannot be sure that the results accurately reflect the population as a whole because turtle sampling is opportunistic. On the other hand, fish scales can be gathered in large quantities from numerous locations, providing a statistically sound image of the health of the ecosystem. Although methods such as SEM or Micro-XRF can identify the location of a contaminant accumulation in a single turtle, only extensive studies at the fish scale can demonstrate how contamination is shifting throughout the larger environment.

5.5 Methodological Reflections and Future Directions

5.5.1 Strengths of the Approach

The use of turtle shells and fish scales as non-lethal bioindicators for tracking heavy metal contamination in Algerian freshwater systems is strongly supported by this study. By utilizing both short-term (fish scales) and long-term (turtle shells) biomonitoring data, the combined approach offers a strong framework. A thorough grasp of elemental concentration and localization within

tissues is ensured by the application of sophisticated, multi-technique analysis (ICP-OES, XRF, SEM-EDS) (Chojnacka et al., 2018; Conrad et al., 2023; H. Gu et al., 2022; Tanabe et al., 2022).

Given the documented heavy metal contamination in Algerian waters from human-caused sources such as industrial and agricultural runoff, these findings are crucial. This framework provides a low-cost, non-invasive method for routine environmental evaluations. Establishing baseline metal concentration data for local species and validating this strategy across additional Algerian ecosystems should be the main goals of future research. Better trend analysis and more focused conservation tactics will be made possible by this.

5.5.2 Future Research Directions

1) Longitudinal and Multi-Temporal Studies

Although turtle shells and fish scales offer long-term contamination archives and short- to medium-term exposure data, respectively, integrated longitudinal studies monitoring the same populations over several seasons and years would enable a more accurate understanding of the temporal dynamics of metal bioaccumulation. Such research could shed light on the ways that age, reproductive cycles, and seasonal variations affect accumulation rates.

2) Mechanistic Studies of Bioaccumulation

Current findings show relationships among metal accumulation, feeding ecology, and morphometric traits. Future studies should look into the mechanistic processes that fish and turtles use for metal uptake, detoxification, and partitioning. Identification of metal-binding proteins like metallothioneins, metabolic profiling, and tissue-specific analyses may all be part of this. tissue-specific analyses, metabolic profiling, and identification of metal-binding proteins such as metallothioneins.

3) Expansion to Additional Species and Ecosystems

Studies should incorporate a greater variety of freshwater fish and turtle species from various ecological niches, wetland types, and geographic locations in order to generalize bioindicator

frameworks. Understanding species- and habitat-specific accumulation patterns and their predictive value for ecosystem monitoring would be enhanced by comparative studies.

4) Integration of Microbial and Elemental Analyses

According to recent data, the microbial communities found on turtle shells can serve as indicators of environmental factors and ecological characteristics. Future studies could examine whether microbial communities affect or interact with metal accumulation by combining elemental mapping and microbiome analysis, which could improve the usefulness of bioindicators.

5) Assessment of Emerging Contaminants

One category of pollutants is metals. Future research should also look at how pharmaceuticals, microplastics, and other newly discovered pollutants build up in fish scales and turtle shells, evaluating any possible negative or positive effects on organismal health when combined with metals.

6) Refinement of Analytical Techniques

While Micro-XRF and ICP-OES provide complementary insights, future work could explore high-resolution spatial mapping, synchrotron-based imaging, and isotopic fingerprinting to improve sensitivity and resolution of contaminant detection. Calibration studies could also refine cross-method quantification for long-term monitoring programs.

7) Ecotoxicological and Health Risk Assessment

Future studies should relate metal bioaccumulation to physiological and reproductive health outcomes in fish and turtles, given the patterns of accumulation that have been observed. This could help with risk assessments for both human consumption and wildlife, especially in contaminated rivers and wetlands close to cities or industrial areas.

8) Climate Change and Anthropogenic Stressors

Changing water temperatures, altered flow regimes, and increasing anthropogenic pressures may affect bioavailability and uptake of metals. Long-term studies should investigate how climate and land-use changes influence bioaccumulation trends and sentinel species effectiveness.

9) Development of Predictive Bioindicator Models

Predictive models of metal pollution in freshwater ecosystems may result from the integration of ecological traits, morphometrics, multi-species data, and environmental factors. These models may help with remediation and management plans for rivers, reservoirs, and wetlands.

Chapter 06 Conclusion

This dissertation shows that turtle shells and fish scales can accurately record multi-element contamination patterns in two different freshwater systems (a lake and a river), advancing non-invasive biomonitoring. The study produced a number of unique and practically significant findings using environmental sampling (water, sediments) and a complementary analytical suite (ICP-OES, micro-XRF mapping, XRF screening, SEM-EDS). These findings validate non-lethal indicators and provide baseline data for Algerian freshwater habitats that have received little attention.

6. 1 Key findings

1. Long-term exposure and intricate accumulation patterns are reflected in turtle shells (*Mauremys leprosa*). Although structural elements (Ca, P) make up the majority of the shell's composition, it also includes detectable trace metals (Fe, Zn, Mn, Sr) and anthropogenic pollutants (Pb, Sb, Al). Micro-XRF maps show irregular deposits of Pb, Sb, and Al as well as heterogeneous, microstructure-related "hotspots" of trace metals, suggesting localized incorporation pathways and variable individual exposure.
2. Sex, physiology and morphology matter. Statistically significant sex differences were observed (males: higher Mg, Mn, Sb, Pb; females: higher Al). Morphometric variables showed limited direct relationships to most elements, but the correlation network among elements points to shared sources or incorporation routes (strong clustering of Ti–Fe–Mn–Cu–Zn–Pb).
3. Bioaccumulation is influenced by environmental matrices, though these effects are not always strongly correlated in the short term. Because shells tended to lie above water but frequently around or above sediment values, and because sediments had significantly higher metal concentrations than water (Cu, Mn, Ni, Pb, Zn), shells were supported as integrative, long-term samplers. Although it was not statistically significant, the strongest correlation ($\rho = 0.50$) was found with spring sediments, indicating that direct shell–environment correlations may be impacted by temporal lags, variable bioavailability, or small sample sizes.
4. Fish scales are effective, species-sensitive bioindicators in riverine contexts. Scales of chub and nase capture species-specific accumulation: Ba, Fe, Mn and Sr differed significantly

between species, and PCA clearly separated species by elemental fingerprints (PC1 = 52.9% variance). SEM-EDS and XRF confirm heterogeneous, element-specific distributions on scales.

5. Patterns of accumulation are shaped by trophic ecology and body condition. Trophic level had a negative correlation with several elements (P, Ba, Fe, Mn, and Sr) and a positive correlation with sulfur, indicating that scale element signatures are influenced by feeding ecology and food-web position. There were species-specific relationships between body weight and specific elements for instance, Chub weight had a positive correlation with Na and P, while nase weight had a negative correlation with Al, Cr, Cu, and Fe. This suggests that life history and ontogeny must be taken into consideration when interpreting biomonitoring data.
6. Environmental risk signal in river water: Routine monitoring data for the Maros River showed iron concentrations well above chronic criterion concentrations (mean $\approx 4,725 \mu\text{g L}^{-1}$ vs. CCC = $1,000 \mu\text{g L}^{-1}$), highlighting an immediate water-quality concern that is also reflected, variably, in scale chemistry.
7. Methodological validation: SEM-EDS and micro-XRF offered spatially resolved context that supports biological interpretations, while XRF demonstrated itself as a potent, non-destructive screening tool to rank samples for additional ICP-OES analysis. The combined strategy produces high-quality quantitative data as well as economical screening.

6.2 Contributions and implications

1. This study offers new baseline elemental datasets for Algerian freshwater biota and sediments, which are essential for pollution assessment and conservation planning but are currently in short supply.
2. It validates fish scales and turtle shells as non-lethal, practical bioindicators suitable for long-term monitoring programs, with clear advantages for ethical sampling, repeat measures, and integration of exposure over time.
3. According to the findings, multi-indicator, multi-matrix monitoring (scales/shells + sediments + targeted water sampling) is necessary to capture both acute and chronic contamination signals across different ecosystem types (lake vs. river).

4. From a management point of view, the increased iron in the Maros River and the presence of Pb, Sb and Al in the individual shellfish indicate a localised pressure which justifies follow-up (source tracing, speciation studies and targeted remediation, if necessary).

6.3 Limitations

1. Several elements (Cu, Pb, Ni, As in shells and Pb, Co, Bi, Li, Cd, B, Ag in scales) were at or lower detection limits or had substantial missing data, limiting the power of some inferential analyses.
2. Sample sizes (e.g., turtle shells $n = 25$) and the use of available river monitoring databases introduce constraints on temporal resolution and statistical power for some environmental correlations.
3. Non-significant correlations between shells and some environmental matrices likely reflect time-integration different

6.4 Summary

This dissertation shows that fish scales and turtle shells, which are non-invasive hard tissues, are solid, morally acceptable, and informative matrices for trace and heavy metal biomonitoring in various freshwater environments.. Both basic knowledge (species- and sex-specific accumulation, element clustering and incorporation pathways, trophic and size effects) and useful results (baseline datasets, validated methodologies, a monitoring blueprint) are provided by the study, which can be used immediately to conserve and manage Algerian freshwater systems as well as those in the Mediterranean and Europe more generally. These results represent an original contribution to the science and practice of non-lethal biomonitoring, provide a solid basis for ongoing biomonitoring programs, and focused ecological risk assessments.

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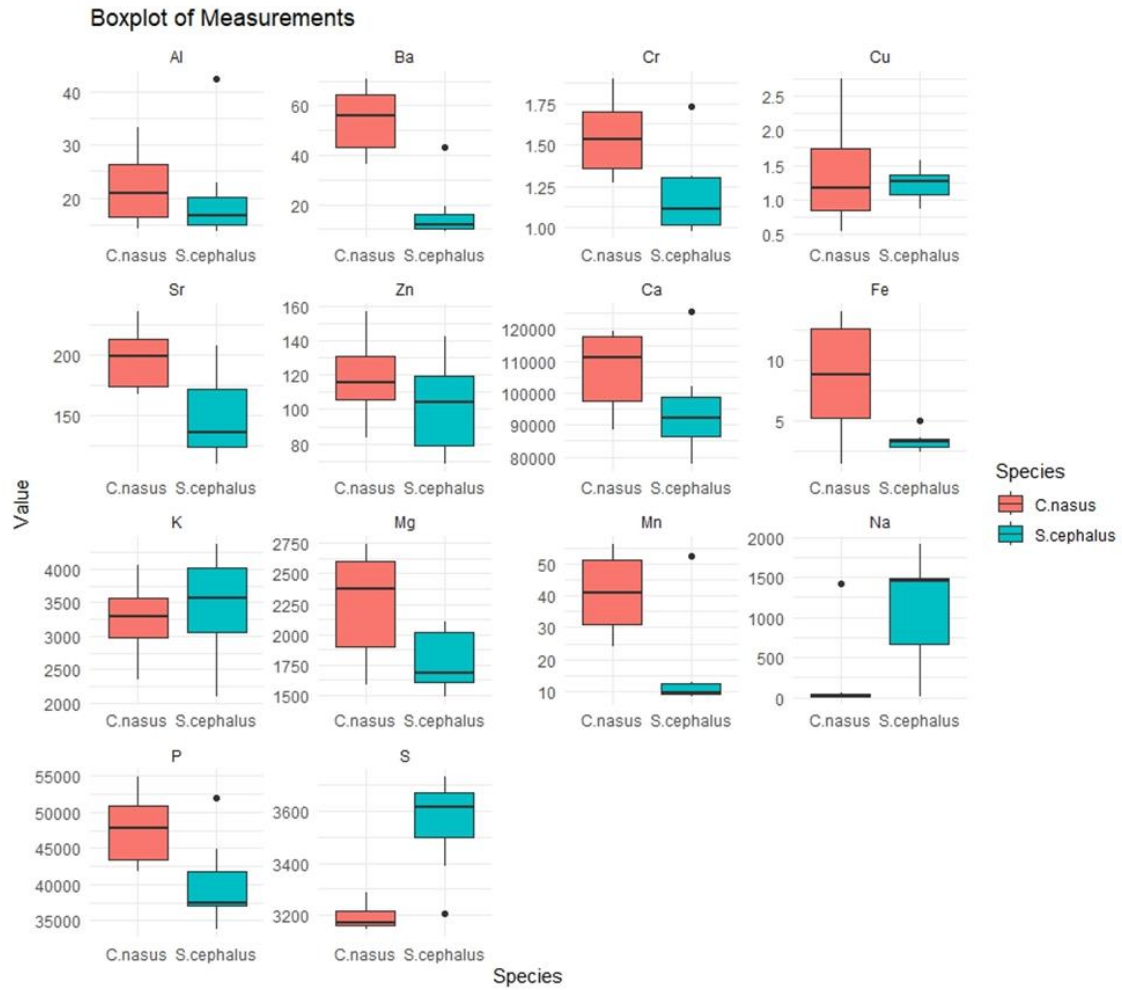
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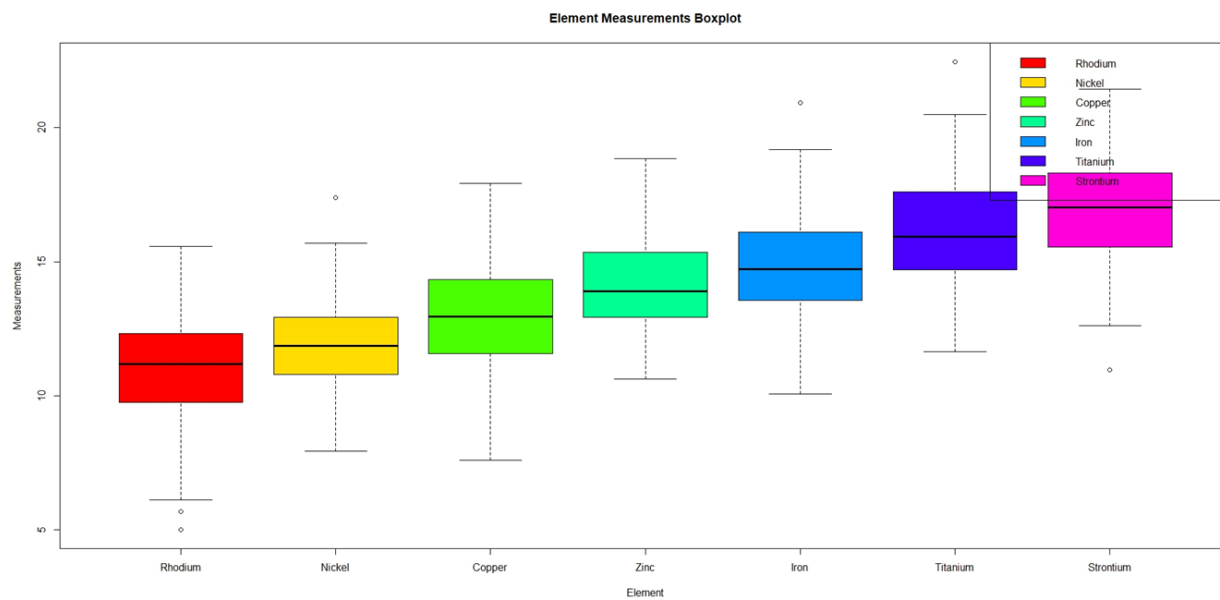
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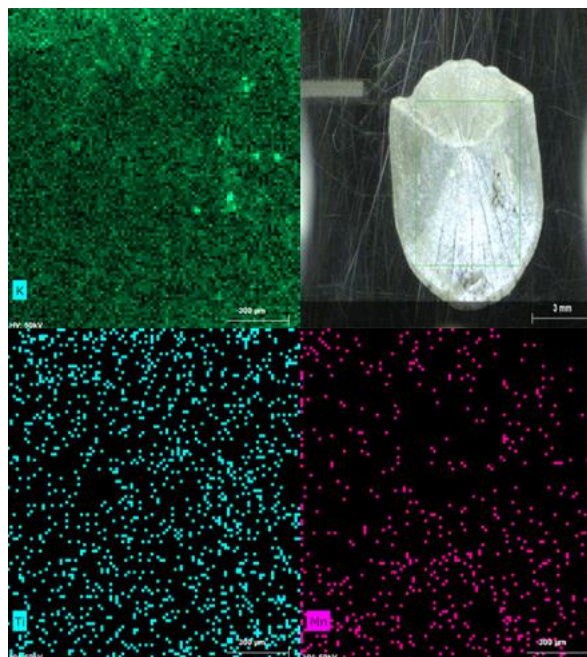
9. Appendix



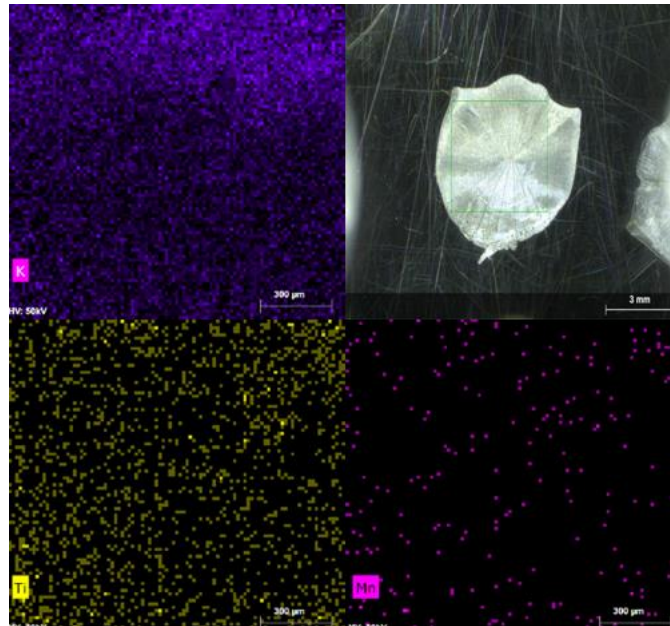
Appendix 1. Boxplot of Measurement with ICP Method



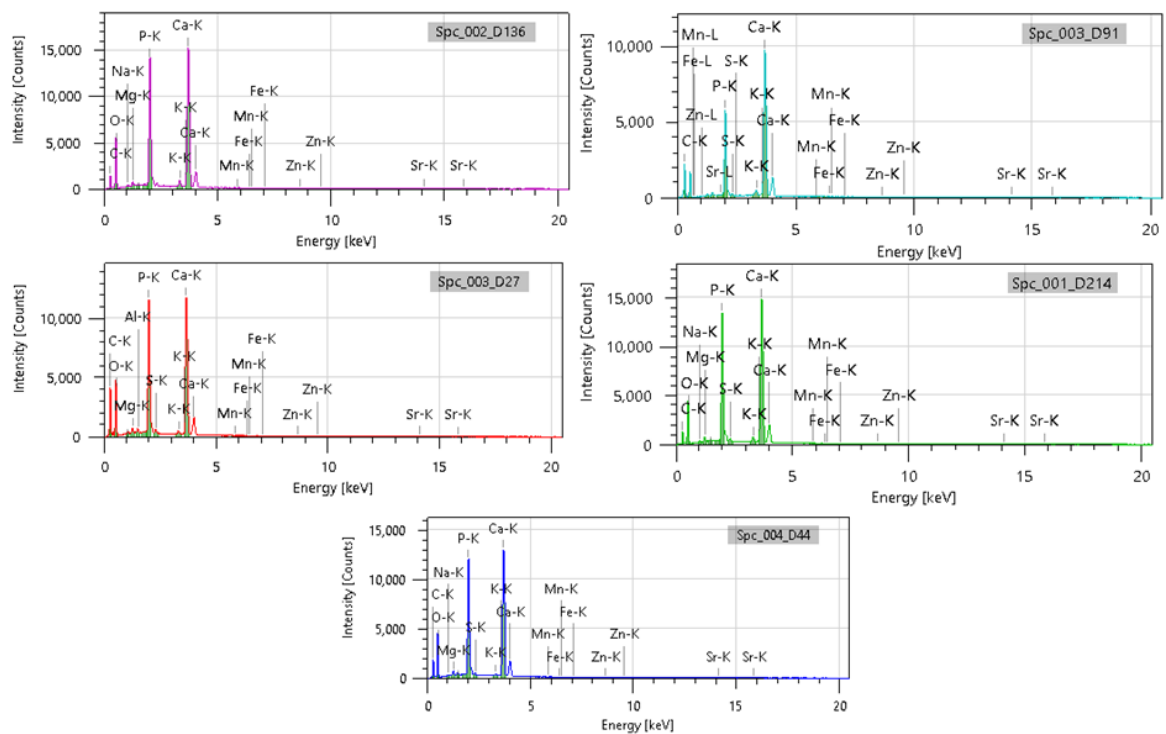
Appendix 2. XRF Analysis Results



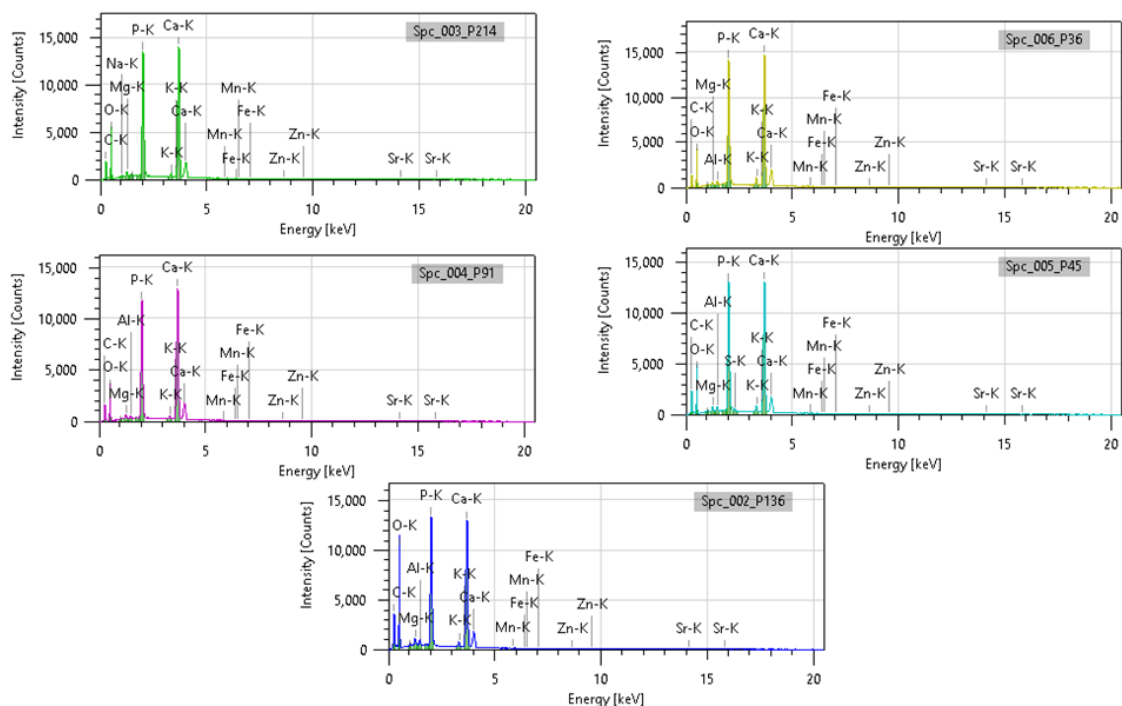
Appendix 3. Elemental Maps of *Chondrostoma nasus*



Appendix 4. SEM Scales Analysis of *Squalius cephalus*



Appendix 5. X-Ray Elemental Analysis of *Squalius cephalus* Scales



Appendix 6. X-Ray Elemental Analysis of *Chondrostoma nasus* Scales

Appendix 7. Elemental Composition of *Squalius Cephalus*

Element	AN	series	Net	[wt.%]
Phosphorus	15	K-series	809221	2,033245
Potassium	19	K-series	610331	0,193385
Calcium	20	K-series	40507138	7,130038
Titanium	22	K-series	8583	0,001033
Manganese	25	K-series	60900	0,002779
Iron	26	K-series	6474	0,000227
Nickel	29	K-series	23084	0,000488
Copper	30	K-series	147990	0,002804
Zinc	44	K-series	4553288	0,30201

Appendix 8. Elemental Composition of *Chondrostoma nasus*

Element	AN	series	Net	[wt.%]
Phosphorus	15	K-series	617447	1,8506
Potassium	19	K-series	333538	0,1205
Calcium	20	K-series	25180607	4,8689
Titanium	22	K-series	1161	0,0001
Manganese	25	K-series	9247	0,0004
Iron	26	K-series	7835	0,0003
Nickel	28	K-series	0	0,0000
Copper	29	K-series	19593	0,0004
Zinc	30	K-series	136399	0,0025