

**Short Thesis for the degree of Doctor of
Philosophy (PhD)**

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

By Haithem Aib

Supervisor:
Dr. Herta Czédli



UNIVERSITY OF DEBRECEN
Juhász-Nagy Pál Doctoral School
Debrecen, 2025

1. INTRODUCTION

Freshwater ecosystems are among the most critical components of the biosphere, supporting biodiversity, nutrient cycling, and essential ecosystem services. They serve as habitats for a variety of organisms and supply water for industrial, agricultural, and residential purposes (Bashir et al., 2020). However, human-induced stresses like urbanization, industrialization, agricultural runoff, and untreated wastewater discharges are posing a growing threat to their ecological balance. These activities lead to the buildup of contaminants, particularly trace elements and heavy metals, which are hazardous to human health and ecological integrity because they linger in aquatic environments (Aib et al., 2025; Ali et al., 2013; Ebol et al., 2020; Jaishankar et al., 2014).

Heavy metals, generally defined as elements with a density greater than 5 g/cm^3 (Csuros & Csuros, 2016), are of particular concern due to their toxicity, persistence, and potential for bioaccumulation in aquatic food webs (Alloway, 2013; Förstner & Wittmann, 1981; MacFarlane & Burchett, 2000). Common metals such as lead (Pb), cadmium (Cd), chromium (Cr), copper (Cu), nickel (Ni), and zinc (Zn) accumulate in sediments and biota, disrupting physiological, reproductive, and ecological processes in aquatic organisms (Khan et al., 2010; Luo et al., 2011; Mitra et al., 2022; Zaynab et al., 2022). Their

persistence and biomagnification make them long-term stressors, often resulting in ecosystem instability and biodiversity loss (MacFarlane & Burchett, 2000; Mahboob et al., 2014; Bouhezila & Hacene, 2020; MedWet Managers Network, 2020).

Transboundary rivers, such as the Maros/Mureş in Central Europe, are particularly vulnerable to ecological pressures from agricultural and urban runoff, emphasizing the need for effective monitoring to identify and mitigate contamination. Conventional water quality assessments often rely on physicochemical measurements, which provide only snapshots of contamination. In contrast, biomonitoring with aquatic organisms offers integrated information on long-term pollutant exposure (Ali & Khan, 2019). Ethical and conservation considerations further highlight the value of non-invasive methods that allow repeated sampling without harming organisms. Vertebrates such as fish and freshwater turtles are particularly promising bioindicators, given their ecological relevance and capacity to accumulate pollutants in their tissues (De Solla & Fernie, 2004; EL Hassani et al., 2019; Le Gal et al., 2023; Luiselli, 2023).

Particularly useful as non-lethal bioaccumulation matrices are turtle shells and fish scales. Collagen and hydroxyapatite make up fish scales, which are constantly growing and incorporate metals during formation to

reflect both recent and past exposure (Jiwan & Ajay, 2011; Łuszczek-Trojnar & Nowacki, 2021). Their collection is simple and non-destructive, making them sustainable for long-term monitoring (Burger et al., 2010; Łuszczek-Trojnar & Nowacki, 2021; Vašek et al., 2021). Turtle shells, which are composed of bone and keratin, also incorporate metal pollutants over time, indicating both episodic and chronic exposure (Meyers-Schöne et al., 1993; Soe Aye et al., 2018). These tissues not only reduce ethical concerns but also align with conservation strategies for threatened species such as *Mauremys leprosa*.

Advanced analytical techniques increase the usefulness of these biomarkers. X-ray fluorescence (XRF) and inductively coupled plasma optical emission spectrometry (ICP-OES) offer sensitive elemental quantification, whereas the structural effects of contamination are revealed by scanning electron microscopy (SEM). These techniques allow for both temporal and spatial evaluations of metal exposure when used on biomaterials like scales and shells.

The pressing need for long-term, non-invasive biomonitoring techniques is discussed in this dissertation. It assesses the use of turtle shells from the Mediterranean pond turtle (*Mauremys leprosa*) in Algerian wetlands and fish scales from chub (*Squalius cephalus*) and nase

(*Chondrostoma nasus*) in the Maros/Mureş River to measure heavy metal and trace metal contamination. Comparative insights into regional pollution dynamics are provided by the study's focus on both Central European and North African ecosystems, which also advances the field of aquatic ecotoxicology.

2. MATERIALS AND METHODS

2.1 Study Areas

2.1.1 Maros/Mureş River (Hungary/Romania)

A significant transboundary waterway in Central Europe, the Maros/Mureş River flows through Hungary and Romania before joining the Tisza River. Its ecological health is impacted by a number of anthropogenic stresses, such as industrial operations, urban wastewater discharge, and agricultural runoff. It is ecologically significant for biodiversity and regional water management due to its status as a major tributary of the Tisza. Because of the river's ecological significance, accessibility, and known pollution pressures, it was selected for this study.



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

2.1.2 Reghaïa Lake (Algeria)

The Ramsar-designated wetland and biodiversity hotspot is Reghaïa Lake in northern Algeria. The lake is experiencing severe anthropogenic stress despite its conservation status, especially from untreated sewage, urbanization, and industrial discharge. It offers a chance to investigate the effects of heavy metal pollution in ecosystems where freshwater biodiversity is threatened because it is a representative wetland in North Africa.

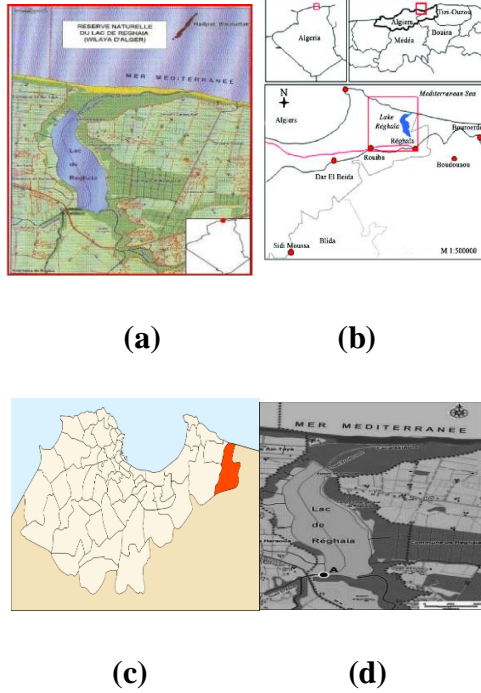


Figure 2. (a) Regional context of Lake Reghaïa ($36^{\circ}45'–36^{\circ}48' N$, $3^{\circ}19'–3^{\circ}21' E$), a 75 ha freshwater Ramsar wetland located 29 km east of Algiers in the Mitidja Plain. (b) Delineated wetland boundary with indicated sampling stations for water, sediment, and turtle shells.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

2.2 Target Species

2.2.1 Fish Species

Two freshwater fish species were selected:

- **Chub** (*Squalius cephalus*) – an omnivorous species widely distributed in European rivers, known for its ecological importance and sensitivity to pollution.
- **Nase** (*Chondrostoma nasus*) – a benthic species with distinct ecological traits, feeding mainly on algae and detritus, making it a useful indicator of sediment-associated pollutants.



Figure 3. Field trip and lab work

The selection of both species was based on their accessibility, abundance in the Maros/Mureş River, and unique habitat use and feeding habits that affect metal uptake and accumulation in scales.

2.2.2 Turtle Species

A vulnerable species in Algerian wetlands, the **Mediterranean pond turtle (*Mauremys leprosa*)**, was chosen for shell examination. Because of its long lifespan, omnivorous diet, and high site fidelity, it is a perfect bioindicator of metal accumulation over the long term. Because of conservation concerns, only non-invasive shell sampling was done.



Figure 4. Turtle carapaces collected around the lake and transferred to the laboratory for further examination.

2.3 Sampling Strategy

Under authorized ethical permits, fish were seasonally collected using seine nets and electrofishing at various locations along the Maros/Mureş River. After non-lethally removing the scales from the dorsal and lateral

lines, they were rinsed in distilled water and placed in sterile containers for storage.

Samples of turtle shells were collected opportunistically from individuals found in the surrounding wetlands and Reghaïa Lake. To ensure the animals were not harmed, tiny pieces from the carapace scutes were carefully clipped. Before being analyzed in a lab, the samples were labeled, transported under strict supervision, and stored.

In order to establish environmental baselines for metal concentrations and evaluate correlations with bioindicator data, water and sediment samples were also gathered from both study areas.

2.4 Analytical Techniques

2.4.1 Elemental Analysis

- *X-ray Fluorescence (XRF)*: utilized for the non-destructive measurement of macroelements and traces in turtle shells and fish scales.
- *Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES)*: used to accurately detect metals like Al, Ba, Cu, Cr, Fe, Mn, Sr, Zn, Pb, Cd, and Ni as well as elements like Ca, K, Mg, Na, P, and S.
- *Micro-X-ray Fluorescence (μ XRF)*: used specifically to assess the spatial distribution of heavy metals across scute surfaces in turtle shells.

2.4.2 Structural Analysis

- *Scanning Electron Microscopy (SEM)*: used to examine the surface morphology and mineralization patterns of fish scales exposed to varying pollution levels in order to determine microstructural variations.

2.5 Statistical Analysis

Before analysis, the data underwent homogeneity and normality tests. Since non-normal distribution patterns exist, elemental concentrations across sites, seasons, and species were compared using non-parametric tests, such as the Mann-Whitney U and Kruskal-Wallis tests. Dunn's tests performed after the fact revealed particular group differences. Correlation analyses were conducted to investigate the connections between biological (scales, shells) and environmental (water and sediment) concentrations. We employed Principal Component Analysis (PCA) to determine the main factors affecting patterns of elemental accumulation. Bioindicator responses to pollution gradients were predicted using regression models.

2.6 Ethical Considerations

Every procedure complied with the moral standards for the conservation and study of animals. Non-invasive sampling guaranteed that the species under study, especially the protected *Mauremys leprosa*, would not

suffer any harm. Before beginning fieldwork, the required permits were acquired from academic and environmental authorities in Algeria and Hungary.

3. RESULTS

3.1 Elemental Concentrations in Fish Scales

3.1.1 Macroelements

According to their mineralized collagen-hydroxyapatite structure, fish scales from *Squalius cephalus* and *Chondrostoma nasus* had high concentrations of calcium (Ca) and phosphorus (P). Magnesium (Mg), potassium (K), sodium (Na), and sulfur (S) were among the other macroelements found. Along the Maros/Mureş River, there was spatial variation, with upstream locations showing higher Ca and P concentrations and downstream sections impacted by industrial and agricultural runoff showing comparatively lower values.

3.1.2 Trace and Heavy Metals

Iron (Fe), manganese (Mn), strontium (Sr), copper (Cu), chromium (Cr), aluminum (Al), barium (Ba), and zinc (Zn) were among the trace elements that were consistently detected. Importantly, *Squalius cephalus* displayed higher levels of Zn and Cu, whereas *Chondrostoma nasus* accumulated more Fe and Mn, indicating species-specific ecological traits.

Particularly in downstream locations, detectable concentrations of heavy metals such as cadmium (Cd), lead (Pb), and nickel (Ni) were detected. There were clear seasonal variations, with higher levels of Pb and Cd during times of lower flow, indicating less dilution and more retention of pollutants.

3.1.3 Species-Specific Differences

Significant variations in elemental accumulation between the two fish species were found by comparative analysis. Higher concentrations of sediment-associated metals (Fe, Mn) were found in the scales of *Chondrostoma nasus*, whereas *Squalius cephalus* displayed higher concentrations of waterborne elements like Zn and Cu. This distinction emphasizes how habitat use and feeding ecology affect patterns of bioaccumulation.

Table 1. Descriptive statistics (mean \pm SD) of morphometric parameters in *Mauremys leprosa*.

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

Table 2. 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.

Table 3. 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	Element	Chub
Ca	95,445 \pm 14,151	106,815 \pm 13,627	Ca	95,445 \pm 14,151
K	3388 \pm 758	3205 \pm 651	K	3388 \pm 758
Mg	1771 \pm 234	2222 \pm 516	Mg	1771 \pm 234
Na	1194 \pm 760	254 \pm 570	Na	1194 \pm 760
P	36,810 \pm 6724	44,160 \pm 8207	P	36,810 \pm 6724
S	3486 \pm 466	3135 \pm 86	S	3486 \pm 466
Al	19.10 \pm 9.62	21.60 \pm 7.83	Al	19.10 \pm 9.62
Ba	15.90 \pm 11.20 *	53.70 \pm 14.80 *	Ba	15.90 \pm 11.20 *
Cr	1.19 \pm 0.24	1.49 \pm 0.31	Cr	1.19 \pm 0.24

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

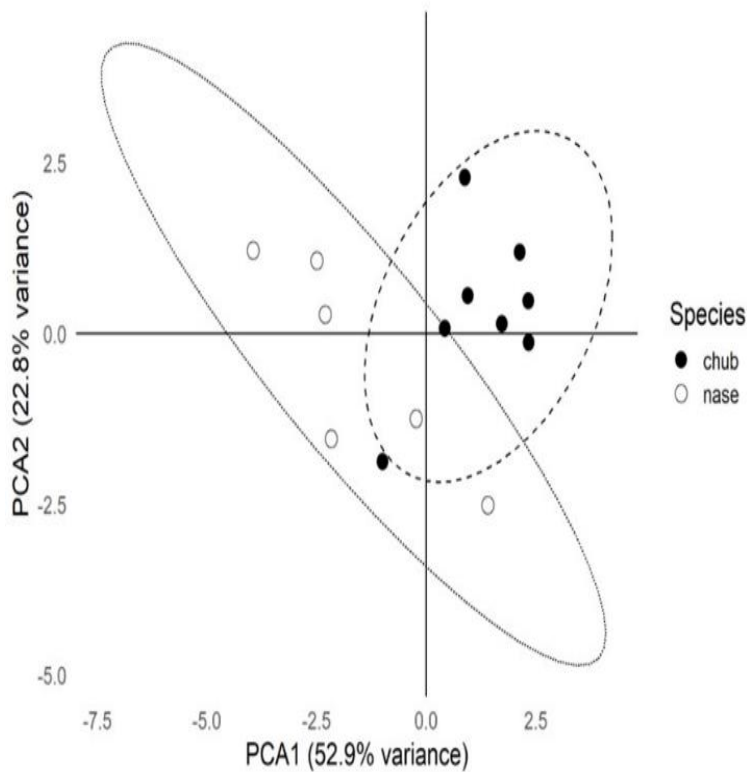


Figure 5. Principal component analysis (PCA) of trace element concentrations in fish scales. Ellipses represent 95% confidence intervals for each species.

Table 4. Spearman correlation coefficients between trace element concentrations in scales and trophic levels of chub and nase.

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.

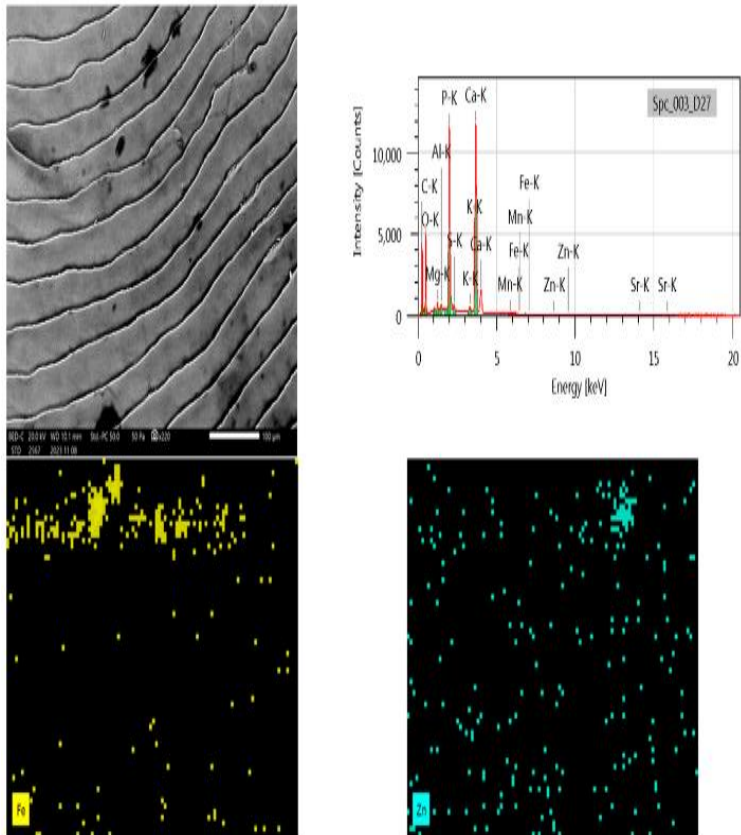


Figure 6. Scanning electron microscopy image with X-ray fluorescence spectra and elemental mapping of iron and zinc in chub scale samples.

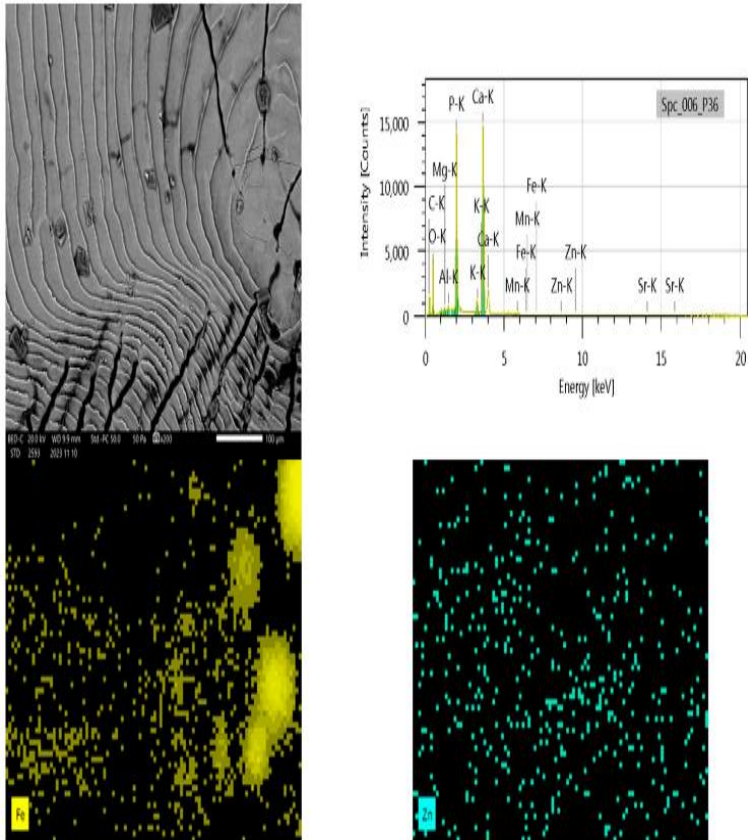


Figure 7. Scanning electron microscopy image with X-ray fluorescence spectra and elemental mapping of iron and zinc in nase scale samples

Table 5. Elemental concentrations in fish scales of chub and nase across varying body weights. The table presents the net values and weight percentages ([wt.%]) for elements detected using XRF analysis, including P, S, Cl, Ca, Mn, Fe, Ni, Zn, and Sr. Data are grouped by species (chub and nase) and individual fish weights, providing detailed elemental profiles helpful for comparative qualitative analysis.

Elements	Net	[wt. %]	Net	[wt. %]	Net	[wt. %]	Net	[wt. %]	Net	[wt. %]
P	876,6	2.8	1,038,	3.2	1,091,	3.4	1,163,	3.6	1,088,	3.3
	42	02	717	68	565	37	305	27	533	67
P	1,211,	3.8	1,103,	3.5	1,091,	3.4	1,071,	3.3	1,157,	3.5
	275	00	236	05	683	00	610	03	732	45
S	40,88	0.0	55,71	0.0	40,35	0.0	19,33	0.0	38,87	0.0
		66	1	87	7	62		29		58
S	33,85	0.0		0.0	21,19	0.0	29,00	0.0	26,70	0.0
	2	53	30,68	49	8	32	9	43	8	39
Cl	5291	0.0	15,96	0.0	5360	0.0	28,91	0.0	13,25	0.0
		06	8	17		05	6	29	7	13
Cl	11,65	0.0	18,45	0.0		0.0	23,87	0.0		0.0
	3	12	9	20	8914	09	6	23	397	00
K	350,9	0.2	544,7	0.3	1,000,	0.5	1,284,	0.7	905,7	0.5
	02	10	26	16	170	74	764	34	71	11
K	1,301,	0.7	1,253,	0.7	665,3	0.3	847,5	0.4	748,6	0.4
	317	66	031	58	54	72	32	69	42	05
Ca	32,81	15.	44,02	20.	50,02	23.	54,34	25.	51,04	23.
	5,220	338	8,122	362	9,439	222	7,832	304	6,833	351
Ca	49,91	23.	41,69	20.	53,38	24.	53,53	24.	61,72	27.
	7,523	660	3,283	146	1,183	203	4,851	137	7,312	379

Mn	8714	0.0 02	16,1	0.0 04	95,78 8	0.0 25	55,15 1	0.0 15	95,52 1	0.0 25
	31,19	0.0 08	83,14	0.0 22	79,09 2	0.0 21	103,4 15	0.0 27	122,3 2	0.0 32
Fe	54,89 9	0.0 11	61,03 6	0.0 13	14,45 8	0.0 03	34,54 2	0.0 07	10,64	0.0 02
	60,78 3	0.0 12	21,37 1	0.0 04	42,68 7	0.0 09	19,32 1	0.0 04	26,36 9	0.0 05
Ni	0	0.0 00	0	0.0 00	0	0.0 00	0	0.0 00	0	0.0 00
	0	0.0 00	0	0.0 00	0	0.0 00	0	0.0 00	0	0.0 00
Zn	199,3 01	0.0 23	220,9 16	0.0 26	196,2	0.0 23	220,8 39	0.0 26	250,4 31	0.0 29
	251,1 43	0.0 29	210,9 03	0.0 24	223,3 68	0.0 26	433,6 6	0.0 50	263,7 51	0.0 31
Sr	179,2 87	0.0 19	195,4 73	0.0 21	255,3 44	0.0 28	288,4 63	0.0 32	272,8 7	0.0 30
	231,3 36	0.0 25	221,3 65	0.0 24	258,4 7	0.0 28	276,2 79	0.0 30	342,7 32	0.0 37

3.2 Elemental Concentrations in Turtle Shells

Turtle shells from *Mauremys leprosa* in Reghaia Lake exhibited substantial accumulation of both essential and toxic elements.

3.2.1 Macroelements

Ca and P were dominant, consistent with the shell's keratinized bone composition. Elevated Sr was also detected, likely linked to sediment interactions in polluted sites.

3.2.2 Heavy Metals

Turtles collected from Reghaïa Lake had higher levels of Pb, Cd, and Zn than those found in reference wetlands. Turtles living in areas receiving untreated wastewater and industrial discharge had especially high Pb levels. Cd accumulation was also significant, reflecting chronic contamination pressures.

3.2.3 Spatial Variability

Site-specific contamination patterns were confirmed by the significantly lower heavy metal levels found in turtle shell samples from less disturbed wetlands. Turtle shells' function as long-term integrators of environmental pollution is highlighted by the variation in metal concentrations.

Table 6. Descriptive statistics (mean \pm SD) of shell element concentrations (mg/kg) in *Mauremys leprosa*.

Element	Overall	Male	Female	P-value	Sig.
Mg	3621.73 \pm 791.35	4026.09 \pm 847.20	3275.14 \pm 536.21	0.04	*
P	101460.50 \pm 11594.13	100014.50 \pm 11915.58	102699.93 \pm 11163.24	0.55	n.s.
S	3126.23 \pm 1157.49	3493.00 \pm 1230.92	2806.08 \pm 961.03	0.09	n.s.
K	1096.38 \pm 612.22	1268.33 \pm 662.49	951.92 \pm 527.94	0.07	n.s.
Ca	415697.81 \pm 17696.79	414450.44 \pm 18058.77	416798.88 \pm 17606.67	0.48	n.s.
Ti	345.69 \pm 242.96	388.79 \pm 302.54	306.86 \pm 166.71	0.29	n.s.
Mn	194.96 \pm 221.47	277.38 \pm 255.78	121.50 \pm 162.69	0.05	*
Fe	2150.73 \pm 1492.58	2397.18 \pm 1635.71	1942.21 \pm 1330.71	0.31	n.s.
Zn	598.27 \pm 238.41	632.37 \pm 240.93	568.68 \pm 237.19	0.42	n.s.
Sr	1621.65 \pm 627.06	1689.65 \pm 719.93	1560.29 \pm 538.31	0.66	n.s.

Al	3975.15 ± 3106.92	4813.00 ± 3800.02	3215.65 ± 1989.02	0.03	*
Sb	1490.69 ± 1081.60	2109.06 ± 1156.39	904.42 ± 704.05	0.02	*
Cu	4.62 ± 9.68	7.27 ± 12.41	2.29 ± 6.44	0.08	n.s.
Pb	9.62 ± 20.42	14.27 ± 24.30	5.35 ± 15.76	0.04	*
Ni	0.23 ± 0.78	0.00 ± 0.00	0.42 ± 0.99	0.15	n.s.
Si	8942.92 ± 6056.17	11066.00 ± 7079.62	6938.25 ± 4242.44	0.06	n.s.
As	1.58 ± 7.86	0.00 ± 0.00	2.96 ± 10.90	—	—

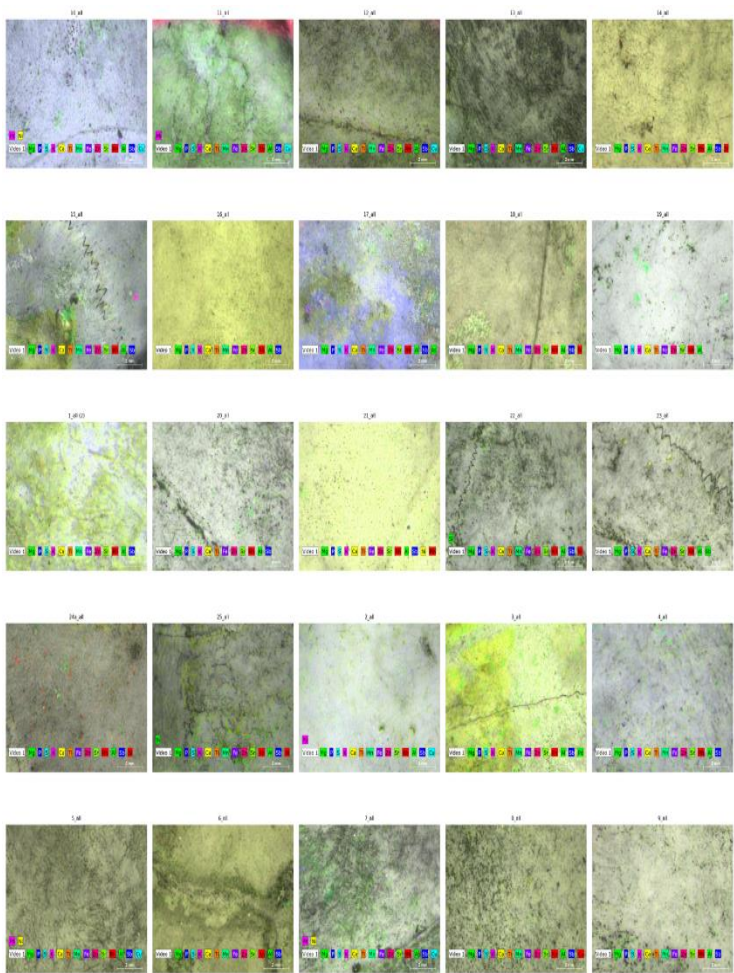


Figure 8. Elemental distribution patterns in freshwater turtle shells (n=25) obtained using micro-XRF mapping.

Table 7. Spearman rank correlations between shell element concentrations (mg/kg), morphometric parameters (LA, Axial length, Lp, Effective length), and sex in tortoises

	Se	LA	Ax	Lp	L	Mg	P	S	K	Ca	Ti	M	Fe	Zn	Sr	Al	Sb	Cu	Pb	Ni	Si	As
Se	1.0																					
LA		1.0																				
Ax			1.0																			
Lp				1.0																		
L					1.0																	
Mg						1.0																
P							1.0															
S								1.0														
K									1.0													
Ca										1.0												
Ti											1.0											
M												1.0										
Fe													1.0									
Zn														1.0								
Sr															1.0							
Al																1.0						
Sb																	1.0					

Cu	1.0	N.	N.	N.
Pb	1.0	N.	N.	N.
Ni	1.0	N.	N.	N.
Si		1.0	N.	
As			1.0	

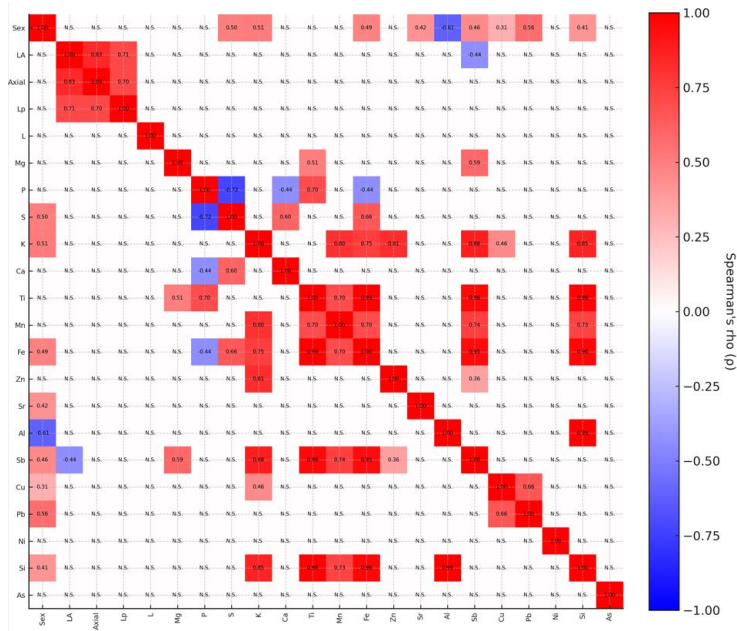


Figure 9. Spearman's rank correlation matrix of elemental concentrations and morphometric variables in *Mauremys leprosa* shells (final dataset, n = 25). Color intensity indicates the strength and direction of ρ . Non-significant correlations (p > 0.05, FDR-adjusted) are marked as "N.S.".

Network of Strong Correlations Between Shell Elements

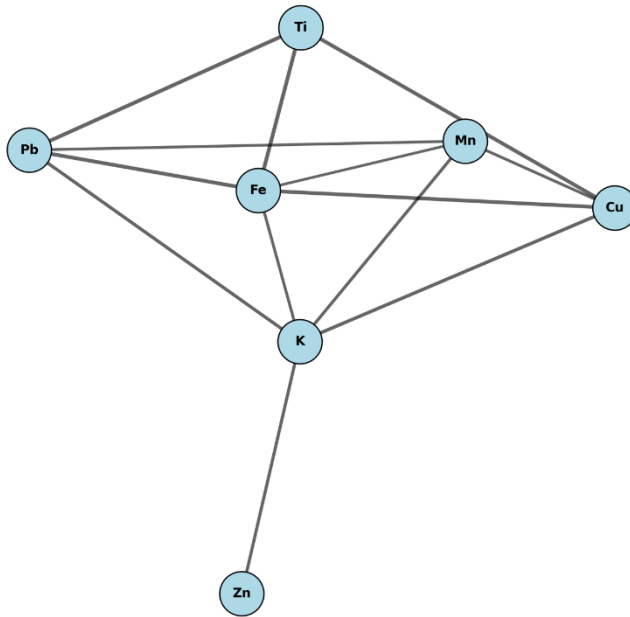


Figure 10. Elemental Correlation Network Revealing Shared Sources and Incorporation Pathways.

Table 8. 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

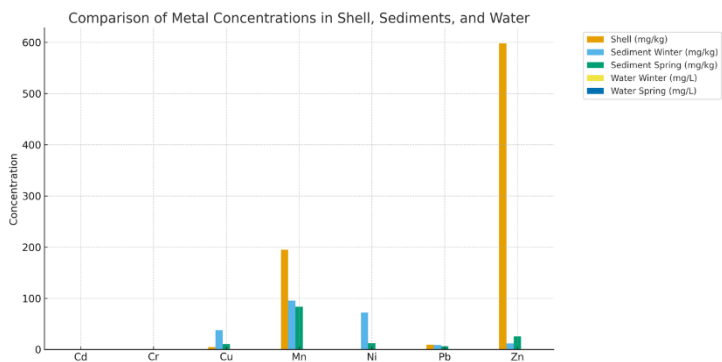


Figure 11. Bar chart comparing average metal concentrations in turtle shells, sediments (winter and spring), and water (winter and spring).

Table 9. 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

3.3 Scanning Electron Microscopy (SEM) Findings

Fish scale SEM imaging showed clear microstructural differences between the reference and polluted sites. Scales from contaminated areas showed surface erosion, irregular mineralization, and disruptions in the organization of collagen fibers. Greater concentrations of heavy metals like Pb and Cd were associated with these morphological changes.

The use of scale microstructure as an extra indicator of environmental stress is supported by the fact that scales

from comparatively unpolluted sites maintained smoother surfaces and distinct growth rings.

3.4 Statistical Analysis

3.4.1 Comparative Tests

- Mann–Whitney U and Kruskal–Wallis tests confirmed significant differences ($p < 0.05$) in elemental accumulation across sites and species.
- Dunn’s post hoc analysis indicated that downstream Maros/Mureş sites and Reghaïa Lake had significantly higher heavy metal burdens compared to reference locations.

3.4.2 Correlations

Strong positive correlations were observed between environmental concentrations (water and sediment) and biological matrices (scales and shells), particularly for Pb, Cd, and Zn. This confirmed the reliability of both fish scales and turtle shells as bioindicators of pollution levels.

3.4.3 Principal Component Analysis (PCA)

PCA highlighted distinct clustering of sites based on metal contamination profiles. In fish, Zn, Cu, and Sr contributed most strongly to variation, whereas in turtles, Pb and Cd were the primary drivers. These results

emphasize species- and habitat-specific responses to environmental contamination.

3.5 Summary of Key Findings

- Turtle shells and fish scales were able to accurately depict the heavy metal contamination and macroelement composition.
- *Squalius cephalus* demonstrated a greater uptake of waterborne elements, whereas *Chondrostoma nasus* accumulated more sediment-associated metals.
- *Mauremys leprosa* shells demonstrated a clear distinction between less disturbed and polluted wetlands.
- Scales from contaminated sites showed microstructural damage, according to SEM analysis, which is consistent with higher heavy metal burdens.
- The strong correlation between environmental pollution and bioaccumulation in non-invasive tissues was validated by statistical analyses.

4. DISCUSSION

4.1 Non-Invasive Biomonitoring in Freshwater Ecosystems

The results of this study show that turtle shells and fish scales are useful, non-lethal matrices for determining the level of heavy metal and trace element contamination in freshwater environments. Conventional biomonitoring techniques frequently depend on direct water/sediment analyses or invasive internal tissue sampling, which may not account for cumulative exposure and present ethical issues (Burger et al., 2010; Łuszczek-Trojnar & Nowacki, 2021; Vašek et al., 2021). Scales and shells, on the other hand, allow for repeated sampling without endangering organisms and can record both episodic and chronic exposure to pollutants. This strategy is in line with conservation goals, especially in areas where freshwater biodiversity is threatened.

4.2 Bioaccumulation Patterns in Fish Scales

Fish scales serve as non-invasive bioindicators of both macro- and trace element contamination, providing ethical and effective means for monitoring aquatic ecosystems (B.C., 1997; Nagajyoti et al., 2010; Yilmaz et al., 2007). In this study, scales from chub (*Squalius cephalus*) and nase (*Chondrostoma nasus*) collected from

the Maros River revealed species-specific accumulation patterns influenced by ecological traits such as feeding habits, habitat use, and trophic position (Wright & Welbourn, 2011; Dikanović et al., 2016; Parvez et al., 2025). Chub, with its omnivorous diet including benthic invertebrates and detritus, exhibited higher uptake of sediment-associated trace metals (Fe, Cu, Mn), whereas the herbivorous–detritivorous nase accumulated higher levels of macroelements like Sr and Ba (Łuszczek-Trojnar & Nowacki, 2021; Aib et al., 2025).

Fish scales function as biological archives, recording both recent and historical exposure to contaminants, and can reflect variations in environmental and biogeochemical conditions (Coello & Khan, 1996). Analytical approaches such as X-ray fluorescence (XRF) effectively quantify these patterns, although factors such as scale morphology, elemental concentration, and structural heterogeneity may influence measurement accuracy (Yilmaz et al., 2007).

Overall, fish scales provide a robust, non-lethal tool for assessing both spatial and species-level differences in elemental uptake, emphasizing the need to integrate ecological traits and environmental context for accurate interpretation of bioaccumulation patterns (Coello & Khan, 1996; Wright & Welbourn, 2011; Bernal-Alviz et al., 2025; Łuszczek-Trojnar & Nowacki, 2021).

4.3 Turtle Shells as Long-Term Indicators

The usefulness of turtle shells as long-term integrators of heavy metal contamination is confirmed by the findings from *Mauremys leprosa* in Reghaïa Lake. When turtle shells from contaminated wetlands were sampled, the levels of Pb, Cd, and Zn were noticeably higher than those from reference locations. These results show cumulative exposure and reflect the ongoing contamination pressures in Algerian wetlands, which is not surprising considering the long lifespan and site fidelity of *M. leprosa*. According to earlier studies on turtles, the underlying bone and keratinized scutes are dependable stores of both toxic and necessary elements (dos Santos et al., 2021; Sakai et al., 2000). Because Pb and Cd are ecological hazards and can affect turtle health, reproduction, and survival, the elevated levels in Reghaïa Lake turtles are especially alarming (Bezerra et al., 2013). Furthermore, these results provide important baseline information for an area with little freshwater monitoring.

4.4 Structural Alterations as Indicators of Stress

Individuals from polluted sites showed microstructural changes, such as irregular mineralization, surface erosion, and disrupted collagen fiber organization, according to SEM analysis of fish scales. Elevated Pb and Cd levels were linked to these morphological changes, indicating that exposure to heavy metals has direct structural effects.

Other studies that link contamination to scale and shell integrity have reported similar findings (Łuszczek-Trojnar & Nowacki, 2021). These structural indicators offer a supplementary method of evaluating pollutant stress by adding another layer of evidence beyond elemental concentrations. The argument for using scales and shells as comprehensive bioindicators is strengthened by the combination of structural and chemical analyses.

4.5 Statistical Evidence for Bioindicator Reliability

The reliability of scales and shells as bioindicators was validated by the statistical analyses. Non-parametric tests revealed significant differences in metal concentrations between reference and polluted sites, and their representativeness of ambient conditions was confirmed by strong correlations with environmental concentrations. With Pb and Cd predominating in turtles and Zn, Cu, and Sr driving variation in fish, PCA further highlighted the role of particular elements in differentiating sites and species. These trends show different pollution sources at different study locations as well as ecological variations among organisms. Multivariate analyses' capacity to pinpoint the causes of contamination highlights this method's potential for use in larger environmental monitoring systems.

4.6 Ecological and Conservation Implications

The reliability of scales and shells as bioindicators was validated by the statistical analyses. Non-parametric tests revealed significant differences in metal concentrations between reference and polluted sites, and their representativeness of ambient conditions was confirmed by strong correlations with environmental concentrations. With Pb and Cd predominating in turtles and Zn, Cu, and Sr driving variation in fish, PCA further highlighted the role of particular elements in differentiating sites and species. These trends show different pollution sources at different study locations as well as ecological variations among organisms. Multivariate analyses' capacity to pinpoint the causes of contamination highlights this method's potential for use in larger environmental monitoring systems.

5. CONCLUSION

5.1 SUMMARY

This study investigated the bioaccumulation of trace and heavy metals in freshwater ecosystems utilizing non-invasive tissues, such as turtle shells from *Mauremys leprosa* in Algerian wetlands and fish scales from *Squalius cephalus* and *Chondrostoma nasus* in the Maros/Mureş River. The study offered strong proof that these external hard tissues accurately represent both chronic and episodic pollution exposure through the use

of sophisticated analytical techniques (XRF, ICP-OES, μ XRF, and SEM).

Key conclusions include:

1. Fish Scales as Bioindicators:

- Scales incorporated both macroelements (Ca, P, Mg, Na, K, S) and trace/heavy metals (Zn, Cu, Fe, Mn, Pb, Cd, Ni, Sr, Ba).
- *S. cephalus* scales accumulated higher levels of waterborne elements (Zn, Cu), while *C. nasus* scales showed stronger association with sediment-related metals (Fe, Mn).
- SEM revealed microstructural alterations in polluted sites, further validating scales as sensitive indicators of environmental stress.

2. Turtle Shells as Long-Term Archives:

- *M. leprosa* shells from polluted Algerian wetlands showed elevated Pb, Cd, and Zn compared to reference sites.
- Shells reflected long-term contamination trends due to the species' longevity, site fidelity, and keratin-bone structure.
- The approach demonstrated a conservation-friendly method for

monitoring pollution in protected and vulnerable species.

3. Environmental Comparisons:

- Central Europe (Maros/Mureş River) exhibited diffuse contamination linked to agriculture and urbanization.
- North Africa (Reghaïa Lake) faced higher pollution from untreated wastewater and industrial discharges.
- Despite different contamination pressures, both scales and shells proved effective in detecting and differentiating pollution levels.

The usefulness of fish scales and turtle shells as non-invasive bioindicators is supported by this study's unique comparative data. Through the provision of morally sound, economically viable, and environmentally sound instruments, it advances biomonitoring practices in freshwater environments.

5.2 RECOMMENDATION

Based on the findings, several recommendations emerge for environmental monitoring and management:

1. Integration into Monitoring Programs:

- Include turtle shells and fish scales in regular freshwater monitoring programs in

addition to conventional physicochemical analyses.

- Complement long-term ecological assessments with these non-invasive techniques, especially in transboundary rivers and wetlands that are listed by Ramsar.

2. **Policy and Conservation Implications:**

- Reduce untreated wastewater discharges in Algeria and lessen agricultural runoff in Central Europe to improve pollution control in both regions under study.
- Encourage conservation laws that acknowledge non-invasive biomonitoring as an effective means of preserving endangered species, such as *Mauremys leprosa*.

3. **Research Expansion:**

- Expand biomonitoring activities to include more freshwater species (e.g. A. frogs, invertebrates) to expand the ecological scope of evaluations.
- To investigate the combined effects of pollutants, use elemental analysis in conjunction with isotopic and molecular approaches. Use scale and shell growth increments to reconstruct historical

contamination patterns through temporal studies.

4. **Capacity Building:**

- Increase the capacity of laboratories in developing nations (e.g. North Africa) to use cutting-edge analytical methods locally, like XRF and SEM.
- Give policymakers and conservationists instruction on how to use non-invasive biomonitoring.

6. REFERENCES

Aib, H., Czédli, H., Baranyai, E., Sajtos, Z., Döncz, B., Sohail Parvez, M., Berta, C., Varga, Z., Benhizia, R., & Nyeste, K. (2025). Fish Scales as a Non-Invasive Method for Monitoring Trace and Macroelement Pollution. *Biology*, 14(4), 344. <https://doi.org/10.3390/BIOLOGY14040344>

B.C. (1997). British Columbia Ministry of Forests and Range; Resources Information Standards Committee (RISC). Fish Collection Methods and Standards. B.C. Ministry of Environment, Lands and Parks.

Bashir, I., Lone, F. A., Bhat, R. A., Mir, S. A., Dar, Z. A., & Dar, S. A. (2020). Concerns and Threats of Contamination on Aquatic Ecosystems. *Bioremediation*

and Biotechnology, 1. https://doi.org/10.1007/978-3-030-35691-0_1

Bezerra, M. F., Lacerda, L. D., Lima, E. H. S. M., & Melo, M. T. D. (2013). Monitoring mercury in green sea turtles using keratinized carapace fragments (scutes). *Marine Pollution Bulletin*, 77(1–2), 424–427. <https://doi.org/10.1016/J.MARPOLBUL.2013.09.020>

Bernal-Alviz, J., Córdoba-Tovar, L., Pastrana-Durango, D., Molina-Polo, C., Buelvas-Soto, J., Cruz-Esquivel, Á., Marrugo-Negrete, J., & Díez, S. (2025). Influence of environmental and biological factors on mercury accumulation in fish from the Atrato River Basin, Colombia. *Environmental Pollution*, 366, 125345. <https://doi.org/10.1016/J.ENVPOL.2024.125345>

Burger, J., Jeitner, C., Schneider, L., Vogt, R., & Gochfeld, M. (2010). Arsenic, cadmium, chromium, lead, mercury, and selenium levels in blood of four species of turtles from the Amazon in Brazil. *Journal of Toxicology and Environmental Health - Part A: Current Issues*, 73(1), 33–40. <https://doi.org/10.1080/15287390903248877>

Coello, W. F., & Khan, M. A. Q. (1996). Protection against heavy metal toxicity by mucus and scales in fish. *Archives of Environmental Contamination and Toxicology*, 30(3), 319–326. <https://doi.org/10.1007/BF00212289/METRICS>

Dikanović, V., Skorić, S., & Gačić, Z. (2016). Concentrations of metals and trace elements in different

tissues of nine fish species from the Meduvršje Reservoir (West Morava River Basin, Serbia). *Archives of Biological Sciences*, 68(4), 811–819. <https://doi.org/10.2298/ABS151104069D>

dos Santos, R. L., de Sousa Correia, J. M., & dos Santos, E. M. (2021). Freshwater aquatic reptiles (Testudines and Crocodylia) as biomonitor models in assessing environmental contamination by inorganic elements and the main analytical techniques used: a review. *Environmental Monitoring and Assessment*, 193(8), 1–23. <https://doi.org/10.1007/S10661-021-09212-W/TABLES/2>

Łuszczek-Trojnar, E., & Nowacki, P. (2021). Common carp (*Cyprinus carpio* L.) scales as a bioindicator reflecting its exposure to heavy metals throughout life. *Journal of Applied Ichthyology*, 37(2), 235–245. <https://doi.org/10.1111/JAI.14154>

Metna, F., Lardjane-Hamiti, A., Boukhemza-Zemmouri, N., Boukhemza, M., Merabet, S., & Abba, R. (2015). Diet of the Coot *Fulica atra* (Aves, Rallidae) in the nature reserve of Lake Réghaïa (Algiers, Algeria). *Zoology and Ecology*, 25(1), 34–45. <https://doi.org/10.1080/21658005.2014.994363>

Meyers-Schöne, L., & Walton, B. T. (1994). Turtles as Monitors of Chemical Contaminants in the Environment. In *Reviews of Environmental Contamination and Toxicology* (pp. 93–153). Springer, New York, NY. https://doi.org/10.1007/978-1-4612-2634-5_3

Muysen, B. T. A., Brix, K. V., DeForest, D. K., & Janssen, C. R. (2011). Nickel essentiality and homeostasis in aquatic organisms. *Canadian Journal of Fisheries and Aquatic Sciences*, 12(2), 113–131. <https://doi.org/10.1139/A04-004>

Nagajyoti, P. C., Lee, K. D., & Sreekanth, T. V. M. (2010). Heavy metals, occurrence and toxicity for plants: a review. *Environmental Chemistry Letters*, 8(3), 199–216. <https://doi.org/10.1007/S10311-010-0297-8>

Parvez, M. S., Hoque, M. I., Siddique, M. N. M., Rahman, M. M., Baranyai, E., Sajtos, Z., Dönczö, B., Aib, H., Kader, M. A., Simon, E., & Czédli, H. (2025). Elemental analysis in the scales of commercially important coastal fishes and their connections with fish feeding habits and habitats. *Frontiers in Marine Science*, 12, 1546313. <https://doi.org/10.3389/FMARS.2025.1546313/BIBTEX>

Pyle, G., & Couture, P. (2011). Nickel. *Fish Physiology*, 31(PART A), 253–289. [https://doi.org/10.1016/S1546-5098\(11\)31005-9](https://doi.org/10.1016/S1546-5098(11)31005-9)

Sakai, H., Saeki, K., Ichihashi, H., Suganuma, H., Tanabe, S., & Tatsukawa, R. (2000). Species-specific distribution of heavy metals in tissues and organs of loggerhead turtle (*Caretta caretta*) and green turtle (*Chelonia mydas*) from Japanese coastal waters. *Marine Pollution Bulletin*, 40(8), 701–709. [https://doi.org/10.1016/S0025-326X\(00\)00008-4](https://doi.org/10.1016/S0025-326X(00)00008-4)

Vašek, M., Souza, A. T., Říha, M., Kubečka, J., Znachor, P., & Hejzlar, J. (2021). Stable isotope evidence from archived fish scales indicates carbon cycle changes over the four-decade history of the Římov Reservoir (Czechia). *Science of The Total Environment*, 755, 142550.

<https://doi.org/10.1016/J.SCITOTENV.2020.142550>

Wright, D. A., & Welbourn, P. M. (2011). Cadmium in the aquatic environment: a review of ecological, physiological, and toxicological effects on biota. *Canadian Journal of Fisheries and Aquatic Sciences*, 2(2), 187–214. <https://doi.org/10.1139/A94-012>

Yilmaz, F., Özdemir, N., Demirak, A., & Tuna, A. L. (2007). Heavy metal levels in two fish species *Leuciscus cephalus* and *Lepomis gibbosus*. *Food Chemistry*, 100(2), 830–835.

<https://doi.org/10.1016/J.FOODCHEM.2005.09.02>

7. PUBLICATIONS



UNIVERSITY of
DEBRECEN

UNIVERSITY AND NATIONAL LIBRARY
UNIVERSITY OF DEBRECEN

H-4002 Egyetem tér 1, Debrecen

Phone: +3652/410-443, email: publikaciok@lib.unideb.hu

Registry number: DEENK/610/2025.PL
Subject: PhD Publication List

Candidate: Haithem Aib

Doctoral School: Pál Juhász-Nagy Doctoral School of Biology and Environmental Sciences

List of publications related to the dissertation

Foreign language scientific articles in international journals (4)

- Aib, H., Czédli, H., Baranyai, E., Sajtos, Z., Dönczö, B., Parvez, M. S., Berta, C., Varga, Z., Benhizia, R., Nyeste, K. J.:** Fish Scales as a Non-Invasive Method for Monitoring Trace and Macroelement Pollution.
Biology. 14 (4), 1-16, 2025. EISSN: 2079-7737.
DOI: <http://dx.doi.org/10.3390/biology14040344>
IF: 3.5 (2024)
- Aib, H., Bakhouché, B., Nyeste, K. J., Dönczö, B., Chabani, S., Saadi, A., Varga, Z., Czédli, H.:** Hard Evidence from Turtle Shells: Tracing Metal and Non-Metallic Elements Bioaccumulation in Freshwater Ecosystems.
Environments. 12 (11), 1-20, 2025. EISSN: 2076-3298.
DOI: <http://dx.doi.org/10.3390/environments12110445>
IF: 3.7 (2024)
- Aib, H., Parvez, M. S., Czédli, H.:** Pharmaceuticals and Microplastics in Aquatic Environments: A Comprehensive Review of Pathways and Distribution, Toxicological and Ecological Effects.
Int. J. Environ. Res. Public Health. 22 (5), 1-35, 2025. ISSN: 1661-7827.
DOI: <http://dx.doi.org/10.3390/ijerph22050799>
- Aib, H., Czégény, I., Benhizia, R., Czédli, H.:** Evaluating the Efficiency of Wastewater Treatment Plants in the Northern Hungarian Plains Using Physicochemical and Microbiological Parameters.
Water. 16 (24), 1-17, 2024. EISSN: 2073-4441.
DOI: <http://dx.doi.org/10.3390/w16243590>
IF: 3





List of other publications

Foreign language scientific articles in international journals (4)

5. Parvez, M. S., Hoque, I., Siddique, N. M., Rahman, M. M., Baranyai, E., Sajtos, Z., Dönczö, B., **Aib, H.**, Kader, M. A., Simon, E., Czédli, H.: Elemental analysis in the scales of commercially important coastal fishes and their connections with fish feeding habits and habitats. *Front. Mar. Sci.* 12, 1-13, 2025. EISSN: 2296-7745.
DOI: <http://dx.doi.org/10.3389/fmars.2025.1546313>
IF: 3 (2024)
6. Megrerouche, R., Bouaguel, L., Houhamdi, I., **Aib, H.**, Redaouia, A., Boucherit, K., Houhamdi, M.: Wintering Diurnal Behavior of the Aquatic Bird: The Northern Shoveler *Spatula clypeata* (Anatidae) at Lac Ayata (El-Méghair, Algerian Sahara). *Egypt. J. of Aquatic Biolo. and Fish.* 29 (4), 5347-5366, 2025. EISSN: 2536-9814.
DOI: <http://dx.doi.org/10.21608/ejabf.2025.448208>
7. Redaouia, A., Bouaguel, L., Houhamdi, I., **Aib, H.**, Megrerouche, R., Boucherit, K., Houhamdi, M.: Wintering Water Birds in Lac Ayata (Oued Righ Valley, Algerian Sahara). *Egypt. J. of Aquatic Biolo. and Fish.* 29 (4), 2677-2696, 2025. EISSN: 2536-9814.
DOI: <http://dx.doi.org/10.21608/ejabf.2025.448447>
8. Benhizia, R., Phinzi, K., Hateffard, F., **Aib, H.**, Szabó, G.: Drought Monitoring Using Moderate Resolution Imaging Spectroradiometer-Derived NDVI Anomalies in Northern Algeria from 2011 to 2022. *Environments.* 11 (5), 1-17, 2024. EISSN: 2076-3298.
DOI: <http://dx.doi.org/10.3390/environments11050095>
IF: 3.7

Foreign language conference proceedings (1)

9. Benhizia, R., Saidi, F. A., **Aib, H.**, Ata, B.: Spatiotemporal Dynamics of Drought Conditions in Constantine, Algeria (2002-2022): Insights from MODIS-Derived Indices. In: Az elmélet és a gyakorlat találkozása a térinformatikában = Theory meets practice in GIS : Debreceni Egyetem Térinformatikai Konferencia és Szakkiállítás/ szerk. Abrisha-Molnár Vanda Éva, Debreceni Egyetemi Kiadó, Debrecen, 309-317, 2024. ISBN: 9789634906193

Total IF of journals (all publications): 16,9

Total IF of journals (publications related to the dissertation): 10,2

The Candidate's publication data submitted to the Tudóstér have been validated by DEENK on the basis of the Journal Citation Report (Impact Factor) database.

24 November, 2025

