Short thesis for the degree of Doctor of Philosophy (PhD)

Fish scales and plant leaves as noninvasive tools for ecological and environmental monitoring

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1. INTORUDCTION

In pace with the continuous loss of biodiversity and widespread effects of anthropogenic contamination, it is important to comprehend the intricate ecosystem dynamics and rapid changes in the global environment. The development and application of effective, practical, and noninvasive assessment methods has become a top goal in ecological and environmental research towards sustainable management. Conventional approaches frequently entail invasive sampling such as removing tissue cores, uprooting plants, or collecting entire fish, which can injure organisms, disturb ecosystems, and restrict long-term research, emphasizing the need for noninvasive substitutes. In turn, researchers are increasingly interested in studying potential candidates to be used as noninvasive tools from the biological archives found in nature.

Using tissues like fish scales and plant leaves could be one of the prominent possible options. These easily accessible, naturally occurring biological matrices provide a ground-breaking, nonintrusive method of ecological and environmental study. This study investigates the possibilities of using fish scales and plant leaves as robust yet convenient noninvasive tools for ecology and ecosystem health research.

2. AIMS AND OBJECTIVES

The research is designed in two work packages (WP). Work package one (WP1) aimed to analyze the concentration of scale elements in different fish species in connection with their habitats and feeding habits. The hypotheses of WP1 were:

H1.1. We hypothesized that the concentration of scale elements has some sort of connection with the natural environment of fish habitat.

H1.2. We hypothesized that the concentration of scale elements has some sort of connection with fish feeding habits.

Work package two (WP2) aimed to analyze the concentration of microplastics (MPs) and elements in plant leaves along an urbanization gradient. The hypotheses of WP2 were:

H2.1. We hypothesized that the concentration of MPs and metals is higher in residential and industrial areas than in rural areas.

H2.2. We hypothesized that the study of the pollution index (PI) for metals indicates a low level of pollution in rural areas and a high pollution level in industrial and residential areas.

H2.3. We also hypothesized that the leaves of *Polyalthia longifolia* are useful indicators for assessing the level of air pollution.

3. MATERIALS AND METHODS

3.1. Analysis of fish scales

3.1.1. Species selection

The current research is carried out elemental analysis in different fish species from: (i) freshwater, (ii) coastal water, and (iii) freshwater-brackish water-marine water environments.

3.1.1.1. Freshwater fish

Two cyprinid (Cyprinidae) fish, grass carp, *Ctenopharyngodon idella* (Cuvier et Valenciennes, 1844) and mrigal, *Cirrhinus mrigala* (Hamilton, 1822), were used in the study analyzed by micro-X-ray fluorescence (μ-XRF). The number of fish used in the analysis for the freshwater study was 2 species x 1 individual. The characteristics of the fish species are presented in Table 1.

Table 1. The ecological characteristics of the freshwater fish species.

Species	Common name	Local name	Habitat	Migration pattern	Feeding habit	Reference
Ctenopharyngodon idella	Grass carp	Grass carp	Benthopelagic	Potamodromous	Herbivorous	(FAO, 2009a; Froese and Pauly, 2025)
Cirrhinus mrigala	Mrigal carp	Mrigel, Mirka	Demersal	N/A	Illiophagous	(FAO, 2009b; Froese and Pauly, 2025)

3.1.1.2. Coastal migratory fish

Five coastal fish, hilsha- *Tenualosa ilisha* (Hamilton, 1822), flathead sillago- *Sillaginopsis panijus* (Hamilton, 1822), Asian sea bass- *Lates calcarifer* (Bloch, 1790), pama croaker- *Otolithoides pama* (Hamilton, 1822), and corsula- *Rhinomugil corsula* (Hamilton, 1822), using inductively coupled plasma optical emission spectroscopy (ICP-OES). Table 2 lists the characteristics of the fish species. The number of fish used in the analysis for the coastal migratory fish scale study was 5 species x 2 individuals.

Table 2. The ecological characteristics of the coastal migratory fish species.

Species	Common name	Local name	Habitat	Migration pattern	Feeding habit	Reference
Tenualosa ilisha	Hilsha shad	Ilish	Pelagic-neritic	Anadromous	Planktivorous	(Froese
Sillaginopsis panijus	Flathead sillago	Tular dandi	Demersal	Amphidromous	Carnivorous	and
Lates calcarifer	Asian seabass	Vetki, Koral	Demersal	Catadromous	Carnivorous	Pauly,
Otolithoides pama	Pama croaker	Poa	Benthopelagic	Amphidromous	Carnivorous	2025)
Rhinomugil corsula	Corsula	(Kharul) Bata	Pelagic	Anadromous	Carnivorous	

3.1.1.3. Freshwater-brackish water-marine fish

Common carp- *Cyprinus carpio* (Linnaeus, 1758), Asian seabass- *Lates calcarifer* (Bloch 1790), and gold belly croaker- *Chrysochir aureus* (Richardson, 1846), were studied as representatives of freshwater, brackish water, and marine water habitats, respectively, using inductively coupled plasma mass spectrometry (ICP-MS). The number of fish used in the study was 3 species (one from each environment) x 4 individuals. The characteristics of the fish are shown in Table 3.

Table 3. The ecological characteristics of freshwater-brackish water, or estuarine-marine fish species.

Species	Common name	Local name	Habitat	Migration pattern	Feeding habit	Reference
Tenualosa ilisha	Hilsha shad	Ilish	Pelagic-neritic	Anadromous	Planktivorous	(Froese
Sillaginopsis panijus	Flathead sillago	Tular dandi	Demersal	Amphidromous	Carnivorous	and
Lates calcarifer	Asian seabass	Vetki, Koral	Demersal	Catadromous	Carnivorous	Pauly,
Otolithoides pama	Pama croaker	Poa	Benthopelagic	Amphidromous	Carnivorous	2025)
Rhinomugil corsula	Corsula	(Kharul) Bata	Pelagic	Amphidromous	Carnivorous	

3.1.2. Preparation of the fish scale samples

After being gathered, the fish were frozen at -20°C and then allowed to defrost at room temperature before the scales were collected. The fish specimens were carefully washed and using delicate forceps, scales were collected from both sides of each fish-fifteen from the left and fifteen from the right-above the lateral line and beneath the dorsal fin.

3.1.3. Chemical analysis with ICP-OES

About 0.2 g sample were digested by 5.0 ml 65% (m/m) HNO_3 and 1.0 ml 30% (m/m) H_2O_2. Without digested samples were shifted into volume-calibrated plastic centrifuge tubes and diluted up to 15.00 mL with ultrapure water. By inductively coupled plasma optical emission spectrometry (ICP-OES, 5110 Vertical Dual View, Agilent Technologies), the chemical analysis of the digested samples was performed (Simon et al., 2011).

3.1.4. Chemical analysis with ICP-MS

Around 0.50 g sample has been weighed into individual TFM vessels (modified PTFE, copolymerized PTFE), which were then placed inside a safety shield. To each vessel, 7 mL of ultrapure HNO_3 and 1 mL of H_2O_2 have been introduced and digested in the microwave system for 30 minutes at 200°C. The digested samples were analyzed using the inductively coupled plasma mass spectrometry (ICP-MS; PerkinElmer NexION 2000) method (Parvin et al., 2023).

3.1.5. Micro-XRF analysis

The dry and clean scales were examined with a micro-X-ray Fluorescence (µ-XRF; Bruker M4 TORNADO) equipment (Schrøder et al., 2023). A Rh X-ray tube was used for the investigation, with an accelerating voltage of 50 kV and a current of 400 µA. An area of around 1.2 mm by 1 mm was chosen for each scale. A spot size was focused at 20 µm using the default polycapillary lens. The air velocity was 100 ms per pixel, and the step size was 100 µm. Distinctive X-ray lines with a 30 mm^2 active area were obtained by two energy-dispersive detectors.

3.2. Analysis of plant leaves
3.2.1. Studied species sampling areas

Three sampling areas were selected along an urbanization (Industrial-Residential-Rural) gradient (Figure 1) in Bangladesh. An evergreen tree species, *Polyalthia longifolia* (Sonn.), was selected for leaf sample collection. Two sites were selected from each area, trees were randomly selected at each site, and a total of 180 leaves were collected for analysis.

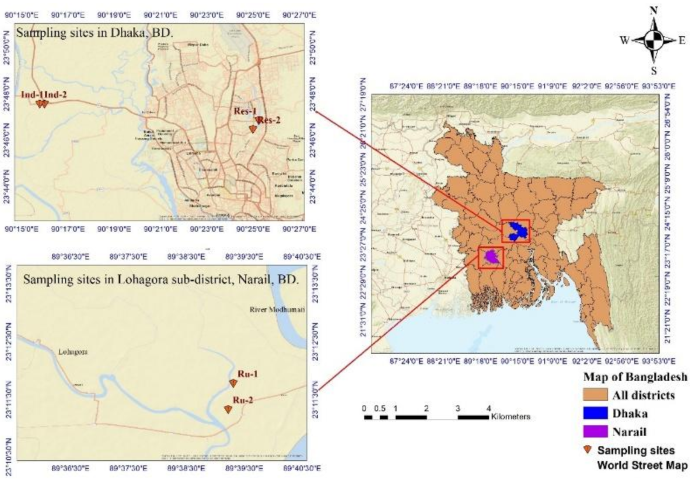

Figure 1. Studied areas in Bangladesh along an urbanization gradient (Note: Ind = industrial area, Res = residential area, and Ru = rural area).

3.2.2. Analysis of microplastics

Polyalthia longifolia leaves were washed off with filtered, deionized water to collect the dust deposited on the leaves. A vacuum pump and 47 mm Whatman glass microfiber filter was used to separate the dust samples. 50 mL of deionized water was added and mixed well. Then the dust samples were sieved with a 150 µm pore size metal sieve. The leaves were rinsed once more with 50 mL of filtered deionized water, which was filtered and introduced to the samples. Subsequently, the 200 mL of dust-containing suspension was placed on a hotplate, it was evaporated at 105 °C to reduce its volume to 25 mL. After that, 25 mL of filtered, deionized water was added. Following overnight treatment with 100 mL of 30% (m/m) hydrogen peroxide, the samples were filtered through a 25 mm-diameter sieve fitted with a 0.7 µm glass filter. Gas Chromatography–Mass Spectrometry (GC-MS, Trace 1610 with ISQ7610) with a pyrolator (GA/PY-3030D) was used to determine MPs (Bouzid et al., 2022).

3.2.3. Measurements of element concentration

Leaf samples were dried for 24 hours at 60 °C and homogenized with an electrical mixer. Samples were digested for four hours at 80 °C with 5 mL of 65% (m/m) nitric acid and 2 mL of 30% (m/m) hydrogen peroxide. Inductively coupled plasma–optical emission spectrometry (ICP-OES 5110, Agilent Technologies, Santa Clara, CA, USA) was employed for elemental analysis (Simon et al., 2011).

3.2.4. Analysis of pollution index

The pollution index (PI) is estimated using the following formula:

$$PI = C_{sample}/C_n, \quad (1)$$

Where, C_{sample} is the target element's measured levels in the leaves and C_n is the World Health Organization-recommended background or reference levels of that element in plants (WHO, 1996). Pollution-degree classes were $PI < 1$ = low pollution (LP), $1 \leq PI < 3$ = moderate pollution (MP), $3 \leq PI < 6$ = considerable pollution (CP), and $PI \geq 6$ = very high pollution (VHP).

3.3. Statistical analysis

Statistical Package for the Social Sciences (SPSS) version 26.0 and PAleontological Statistics (PAST) version 5.2.1 were used for data analysis.

4. RESULTS AND DISCUSSION
4.1. Fish scales
4.1.1. Freshwater fish scales
4.1.1.1. Scale chemistry and composition

The quantitative results of element composition detected in the freshwater fish scales are shown in Table 4. The total length and weight (mean ± standard deviation) of was 43.77 ± 3.77 cm; 1161.67 ± 67.14 g for *C. idella*, and (35.77 ± 2.97 cm; 910 ± 101 69.54 g) for *C. mrigala*.

Table 4. Element composition in the freshwater fish scales (Note: AN: Atomic Number; Wt.%: Weight percentage).

Elements	AN	Series	*C. idella*		*C. mrigala*	
			Net	Wt.%	Net	Wt.%
Ca	20	K series	33556091	9.1262	31295648	8.6969
Cu	29	K series	8894	0.0003	11887	0.0004
Fe	26	K series	46553	0.0027	130469	0.0076
K	19	K series	22562	0.0110	21162	0.0106
Mn	25	K series	20059	0.0015	14187	0.0011
P	15	K series	585516	2.3132	575189	2.3200
S	16	K series	978	0.0015	5338	0.0086
Sr	38	K series	186581	0.0052	258783	0.0073
Ti	22	K series	5437	0.0011	15772	0.0032
Zn	30	K series	123104	0.0038	119498	0.0038

Regardless of species, Ca and P are the most plentiful in fish scales in the current study, ranking first (most availability), followed by K in second place (considerable availability). Ca and P were also the most prevalent in the scales of *Labeo rohita* (Brraich and Jangu, 2013). The network analysis (Figure 2A-C) and dendrogram (Figure 2D), portray these element groups.

4.1.1.2. Elements across habitats

Scales of benthopelagic fish (*C. idella*) possessed higher Ca, K, Mn, and Zn than those in demersal fish (*C. mrigala*). Demersal fish (*C. mrigala*) had higher Cu, Fe, P, S, Sr, and Ti. Demersal fish (*C. mrigala*) possessed higher values of more elements, six out of ten, hence are more likely for the species that live on the bottom to have acquired more elements with higher values than their counterparts.

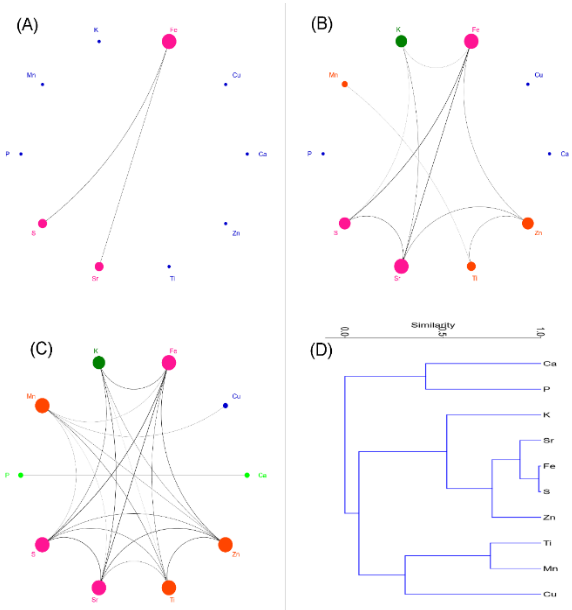

Figure 2. Network plot of elements (A-C) and dendrogram (D)

4.1.1.3. Elements across feeding habits

Scales of herbivorous fish, *C. idella* possessed higher Ca, K, Mn, and Zn. The illiophagous fish (*C. mrigala*), on the other hand, had higher Cu, Fe, P, S, Sr, and Ti in their scales. Herbivorous *C. idella*, feed mainly on aquatic vegetation which uptake Ca from water and are naturally rich in K and may concentrate Mn and Zn. But sediments may store these metals in forms less accessible to illiophagous or filter feeders. Illiophagous, *C. mrigala* lives on decayed vegetation and decomposing organic materials that might have lost their minerals as they decompose.

4.1.2. Coastal migratory fish scale

4.1.2.1. Scale chemistry and composition

The total length and weight of the fish were *T. ilisha* (40.7±0.16 cm; 910.7±14.04 g), *S. panijus* (38.6±0.6 cm; 183.3±11.2 g), *L. calcarifer* (46.6±2.9 cm; 1217.7±151.2 g), *O. pama* (33.5±0.6 cm; 159.6±12.4 g), and *R. corsula* (36.2±0.9 cm; 161.5±15.7 g). The detected elements were grouped into five categories depending on their overall concentrations which is apparent in the network analysis chart (Figure 3).

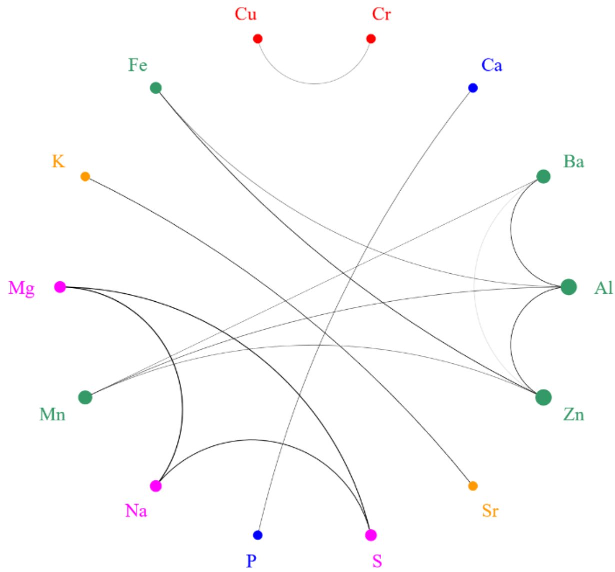

Figure 3. Network plot of elements in the coastal migratory fish scales.

4.1.2.2. Elements across habitats

Demersal fish had higher Cr, Fe, S, Sr, and Zn. Pelagic fish had higher Al, Ba, Ca, Cu, K, Mg, Mn, Na, and P. The variations of Al, Mn, and P were statistically significant ($P \leq 0.05$). Demersal fish live in close to sediments that are rich in elements like Cr, Fe, S, Sr, and Zn (El-Sorogy et al., 2021).

4.1.2.3. Elements across feeding habits

Carnivorous fish had higher mean levels of Ba, Ca, Cu, K, Mn, P, and Sr, where the difference of Sr was significant. Planktivorous fish had higher mean levels of Al, Cr, Fe, Mg, Na, S, and Zn, with a significant difference in the case of Fe. Carnivorous species possessed the highest amounts of Al, Ba, Ca, Cr, Cu, K, Mg, Mn, Na, P, S, Sr, and Zn. In contrast, planktivorous fish have the maximum levels of only Fe among all the elements. This suggests that carnivorous animals have a relatively higher propensity for accumulating metals than their planktivorous fish via biological magnification.

4.1.3. Freshwater-brackish water-marine fish scales

4.1.3.1. Scale chemistry and composition

The total length and weight (Mean ± SD) of the fish were 37±1.7 cm and 1083±105 g for *Cyprinus carpio*, 42±2.6 cm and 1040±169 g for *Lates calcarifer*, and 41±0.7 cm and 881±58 g for *Chrysochir aureus*. The determined elements were classified into five categories based on their overall availability in the scales. These groupings are also depicted in the network analysis.

4.1.3.2. Elements across habitats

The highest concentrations of Cu and Ni were found in the scales of demersal fish (*L. calcarifer*). While the highest amounts of As, Be, Cd, Co, Cr, Fe, Hg, Mn, Pb, Se, V, and Zn were in benthopelagic fish (*C. carpio* and *C. aureus*). Among all the elements, the quantity of As, Be, Cd, Co, Mn, Se, and Zn were significantly ($p \leq 0.05$) higher in the scales of benthopelagic fish (*C. carpio* and *C. aureus*). Benthopelagic species demonstrated accumulation of more elements at higher levels. Their broader vertical range in both the water column and near-bottom environments may expose them to a wider array of contaminants in the water column.

4.1.3.3. Elements across feeding habits

Carnivorous fishes (*C. aureus* and *L. calcarifer*) had higher As, Fe, and Ni, where the amount of Ni is statistically significant ($P \leq 0.05$). Omnivorous fish (*C. carpio*) possessed higher concentrations of the other 11 elements (Be, Cd, Co, Cr, Cu, Hg, Mn, Pb, Se, V, Zn), where the levels of Be, Co, Zn are statistically significant ($P \leq 0.05$). Omnivorous fish eat a wider range of food items from both plant and animal communities. Such broad-spectrum feeding habits enable omnivorous fish to acquire more elements from various food items consumed than their carnivorous counterparts.

4.2. Plant leaves

4.2.1. Deposition of microplastics

Significantly higher ($p = 0.028$) levels of polyethylene terephthalate (PET) type MPs were found in *P. longifolia* leaves dust in industrial and residential locations than those in the rural sites (Figure 4). PET is the prevalent packaging material, especially for the millions of water bottles and beverage containers drunk daily worldwide, releasing enormous amounts of PET into the environment. Over time, plastics released into the environment may break down, spread, and move through the atmosphere. It's possible that the PET-MPs discovered in the study came from different anthropogenic activities, including textiles and other industrial operations.

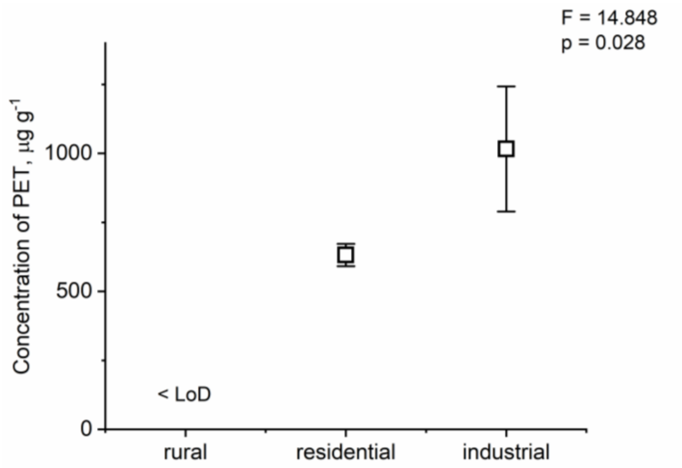

Figure 4. PET concentrations (mean ± SD) in the deposited dust on leaves. LoD: limit of detection.

4.2.2. Elements in leaves

Industrial region had significantly ($p < 0.05$) greater amounts of Cd, Pb, and Zn. This may be caused by different anthropogenic activities like industrial operations, vehicles, discarded lead-acid batteries, brick fields.

4.2.3. Pollution index

The PI for Cd was higher than 1 in rural regions, suggesting moderate pollution; it was higher than 3 in residential sites, indicating considerable pollution; and higher than 6 in industrial areas, indicating very high pollution. The PI for Pb was less than 1 in rural regions, indicating low pollution, and ranged from 1-3 in residential and industrial sites, indicating moderate contamination. The elevated PI of heavy metals can be attributed to different anthropogenic activities including industrial operations and traffic congestion.

6. Conclusion

Scale elements have an association with the environment of fish habitat and feeding ecology. It concerns their dwelling habitat and the source of food, which is to be incorporated into the fish scales. It indicated that fish scales can be employed as a non-invasive tool for deciphering fish life history and ecology without endangering the fish. Similarly, higher concentrations of microplastics and metals were found in plant leaves with an increasing degree of urbanization, mirroring anthropogenic impacts in different areas. It reflects the potential of the plant leaves to be utilized as an excellent tool in environmental biomonitoring. The findings demonstrate the fascinating potential of fish scales and plant leaves as promising noninvasive tools for ecological and environmental monitoring.

New scientific results

- The study revealed that the bottom dwelling fish (i.e. demersal) accumulate sediment-associated elements such as Cu, Fe, S, Sr etc. in higher concentrations.
- Fish living in the water column (i.e. pelagic) acquire primarily water-dissolved elements and metals from their planktonic prey like Ca, K, Mn, Zn etc.
- Higher trophic, predatory fish (i.e. carnivorous) accumulate elements including As, Cu, Hg, Ni etc. through biomagnification, opposite to their lower trophic counterparts (herbivorous).
- Additionally, the scale element ratios, particularly the Sr/Ca, Mn/Ca, and Ba/Ca ratios, have been found to show potential signatory indications of fish habitat.
- Scale elemental makeup reflects the environment of the habitat and their feeding pattern thus fish scale is pertinent to be used in ecological studies.
- Leaves of *Polyalthia longifolia* were found to accumulate high concentration of PTE type microplastics along an industrial–residential–rural gradient in Bangladesh.
- *P. longifolia* can accumulate Cd, Pb, and Zn in high concentrations in its leaf tissues.
- The pollution index for Cd indicated a moderate level of pollution in the rural area, considerable pollution in residential area, and very high pollution in industrial area.
- *P. longifolia* plant leaves are a good bioindicator and suitable to be used for biomonitoring studies.

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Doctoral School: Pál Juhász-Nagy Doctoral School of Biology and Environmental Sciences
MTMT ID: 10083390

List of publications related to the dissertation

Foreign language scientific articles in international journals (3)

1. **Parvez, M. S.**, Hoque, I., Siddique, N. M., Rahman, M. M., Baranyai, E., Sajtos, Z., Döncző, 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.
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   DOI: http://dx.doi.org/10.3389/fmars.2025.1546313
   IF: 2.8 (2023)

2. **Parvez, M. S.**, Czédli, H., Hoque, I., Rahman, M. M., Anwar, A., Uddin, A. H. M. M., Hasan, S., Bibi, D., Tóthmérész, B., Magura, T., Simon, E.: Accumulation of Microplastics and Potentially Toxic Elements in Plant Leaves Along an Urbanization Gradient in Bangladesh.
   Toxics. 12 (12), 1-18, 2024. EISSN: 2305-6304.
   DOI: http://dx.doi.org/10.3390/toxics12120848
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List of other publications

Foreign language scientific articles in international journals (3)

4. Anwar, A., Mezbah Uddin, A. H. M., Hasan, S., **Parvez, M. S.**, Sipos, B., Bibi, D., Sajtos, Z., Tóthmérész, B., Magura, T., Simon, E.: Assessment of anthropogenic activities impact based on metals in soil and tree leaves along roadside in Bangladesh.
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5. Aib, H., Czédli, H., Baranyai, E., Sajtos, Z., Döncző, B., **Parvez, M. S.**, Berta, C., Varga, Z., Benhizia, R., Nyeste, K. J.: Fish Scales as a Non-Invasive Method for Monitoring Trace andMacroelement Pollution.
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   Int. J. Environ. Res. Public Health. 22 (5), 1-35, 2025. ISSN: 1661-7827.
   DOI: http://dx.doi.org/10.3390/ijerph22050799

Total IF of journals (all publications): 17,9
Total IF of journals (publications related to the dissertation): 10,5

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.

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