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

Thesis for the Degree of Doctor of Philosophy (PhD)

by

Md. Sohel Parvez

Supervisors:

Dr. Herta Czédli and Prof. Dr. Edina Kundrát-Simon

UNIVERSITY OF DEBRECEN

Doctoral Council for Natural Sciences and Engineering

Juhász-Nagy Pál Doctoral School

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

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Written by Md. Sohel Parvez
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The official opponents of the dissertation:
Dr. ....
Dr. ....
The evaluation committee:
chairperson: Dr. ....
members: Dr. ....
Dr. ....
Dr. ....
Dr. ....

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DEDICATION

TO

My parents, who sacrificed a lot to pave my way to success throughout.

My children, who missed their father’s love and company for this long time, I was abroad.

My wife, who made significant sacrifices and took on dual responsibilities during my absence.

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Important acronyms and abbreviations

AN	Atomic Number
ANOVA	Analysis of Variance
CRM	Certified Reference Materials
CP	Considerable Pollution
HAP	Hydroxyapatite
ICP-AES	Inductively Coupled Plasma Atomic Emission Spectroscopy
ICP-MS	Inductively Coupled Plasma-Mass Spectroscopy
ICP-OES	Inductively Coupled Plasma–Optical Emission Spectrometry
LA-ICPMS	Laser Ablation-Inductively Coupled Plasma Mass Spectroscopy
LoD	Limit of Detection
LP	Low Pollution
MP	Moderate Pollution
MPs	Microplastics
NPs	Nanoplastics
PET	Polyethylene Terephthalate
PI	Pollution Index
SD	Standard Deviation
SEM	Scanning Electron Microscope
SPSS	Statistical Package for the Social Sciences
VHP	Very High Pollution
XRF	X-Ray Fluorescence
μ-XRF	Micro-X-Ray Fluorescence

Chapter 1: Introduction and objectives

1.1. Introduction

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 monitoring approaches frequently entail invasive sample procedures 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 among possible options. These easily accessible, naturally occurring biological matrices provide a ground-breaking, nonintrusive method of ecological and environmental study.

Fish scales, for example, which develop continually during a fish's life span, record chemical traces providing an intricate historical profile of the aquatic environment (Filipović Marijić et al., 2022; Pouilly et al., 2014). They incorporate different elements internally during formation and continuous development, derived primarily from food, as well as externally through adsorption and absorption from the surrounding water (Khawar et al., 2024; Pourang, 1995; Varol et al., 2022). A multifaceted interaction between the hydro-geochemistry of the surrounding environment, the trophic ecology of the fish, the unique chemical behavior of each element, and the habitat features within the environment, such as benthic and pelagic, drives the accumulation of elements in fish scales. Therefore, scale analysis offers a snapshot of migration trends, nutrition changes, and historical pollution exposures over time without compromising the fish. It is crucial in investigating the feasibility of scale analyses for ecological insights through studying fish having a life history with a broad geographical spectrum. Analysis of species spanning diverse ecological circumstances provides a better picture of the topic in different scenarios. Therefore, the research carried out elemental analysis in three separate case studies encompassing various fish species from: (i) fresh freshwater, (ii) coastal, and (iii) freshwater-brackish water-marine water environments. Using fish scales as noninvasive tools for ecological assessments in aquatic ecosystems could help to bridge the gap between old invasive approaches and current biomonitoring techniques. Besides staying noninvasive, it also offers numerous advantages over conventional intrusive methods, such as being quick, cheap, and simple.

Similarly, plant leaves have the ability to collect atmospheric contaminants, serve as incredibly effective passive air samplers, and provide a momentary view of the environmental circumstances (Molnár et al., 2020; Simon et al., 2021, 2020). They reflect the bioavailability of contaminants like metals in soil via root intake (Alsafran et al., 2022), and their vast leaf canopy effectively traps airborne contaminants such as particulate matter, microplastics, etc. Therefore, the deposition of microplastics and potentially toxic elements in plant leaves from industrial, residential, and rural areas was investigated, exploring the potential of plant leaves as noninvasive tools for environmental analysis. The simultaneous use of these two methods, aquatic signals trapped in fish scales and terrestrial impulses recorded by plant leaves, offers an exceptionally thorough and non-destructive lens for ecological and environmental monitoring. This approach is in line with the increasing focus on biomonitoring and sustainable science, providing a non-invasive, environmentally conscious means of evaluating environmental changes over time. This study investigates the possibilities of using fish scales and plant leaves as robust yet convenient noninvasive instruments for ecology and ecosystem health research, tracking the sources of pollution necessary for efficient conservation and repair plans in a world that is changing quickly.

1.2. Aims and hypotheses

The current research is designed in two work packages (WP) to study the potential of fish scales and plant leaves as noninvasive tools for ecological and environmental monitoring. Work package one (WP1) aimed to explore the potential of fish scales as noninvasive tools for ecological studies. The specific objective was 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 explore the potential of plant leaves as noninvasive tools for environmental studies. The specific objective was 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.

Chapter 2: Literature Review

2.1. Fish scales as a tool for ecological analysis

Fish scales are composed of both biological and inorganic substances. The biological portion, which comprises collagen, fat, lecithin, sclerotin, and vitamins, accounts for around 41–45% of the scale (Luo et al., 2020; Syandri et al., 2023). About 38–46 percent of it is inorganic and is made up of calcium phosphate, hydroxyapatite, and Ca, Mg, Fe, and Zn (Syandri et al., 2023). Fish take up various elements from both the food they consume and the surrounding waters that later could be accumulated into calcified matrices such as scales and otoliths (Khawar et al., 2024; Pourang, 1995; Varol et al., 2022). Such hard structures continue to grow throughout their lives, allowing the geochemical alterations to be retained in these tissues as long-term records of metal exposure across the life span (Filipović Marijić et al., 2022; Pouilly et al., 2014).

Fish scale elemental makeup is modulated by the broad natural environmental context (freshwater, brackish, marine) and specific habitat type within the environment (demersal, pelagic), as well as feeding behaviors through specific biochemical mechanisms. Fish incorporate different ions, like Sr, Ba, into their scales directly from ambient water during mineralization (Wells et al., 2000). For example, Sr higher (Sr/Ca ratio) in marine fish scales, while Ba (Ba/Ca ratio) is elevated in scales of fish from freshwater systems (Wang et al., 2016). Water pH and salinity affect ion solubility, bioavailability, and uptake of various ions such as acidic waters increase Al/Cd uptake, whereas salinity shifts Na/K ratios (Ghallab and Usman, 2007; Javed et al., 2022; Rostern N, 2017). The brackish water environment (estuaries and lagoons) for being a dynamic transition zone, is subject to frequent changes in salinity, pH, turbidity, DOC, and oxygen, all of which have a significant impact on metal speciation and bioavailability. Elemental deposition rates could be changed by faster metabolic processes brought on by elevated temperatures (Islam et al., 2019; Volkoff and Rønnestad, 2020). Demersal and bottom-feeder fish living on or near the bottom are exposed to sediment-associated metals (Chan et al., 2021). Pelagic fish living in the open water column acquire most of their elements from planktonic prey and primarily water-dissolved metals (Le Croizier et al., 2016). Higher trophic level (carnivorous) fish acquire elements such as Hg, As, and occasionally Se by biomagnification as they go up the food chain, opposite to their lower trophic counterparts (Al-Sulaiti et al., 2022). The isotopes $\delta^{15}N$ and $\delta^{13}C$ document the sources of food; for example, elevated $\delta^{15}N$ implies a higher trophic position (carnivorous vs. herbivorous), whereas $\delta^{13}C$ suggests carbon sources (benthic vs. pelagic) in fish (Gorbatenko et al., 2008; Nilsen et al., 2008).

Scale chemistry portrays the elemental makeup of the environment where fish reside (Wells et al., 2003). Especially, trace element composition reflects habitat use and environmental circumstances over time and is pertinent to be used in life history research (Tray et al., 2022). For being the outermost part of the body, fish scales have a high possibility of coming into contact with components in the water bodies, making them an intriguing potential tool to be utilized as a bioindicator (Hidayati et al., 2013). Scale elemental accumulation matches the levels of those elements in the environment (Moreau et al., 1983). Therefore, the composition of the scale's chemicals may be used to measure temporal trends in the environmental quality of the catchment water and to depict metals in the environment (Cobelo-García et al., 2017). The composition of calcified parts is recognized to be a helpful tool in studying many aspects of their biology because, as they develop, these parts keep a clue to the environment (Filipović Marijić et al., 2022). The fish life history information, especially migration and origin, could be inferred from the scale chemical features (Pouilly et al., 2014). However, fish scale samples can usually be analyzed by micro-X-ray Fluorescence-µ-XRF (Medaković et al., 2016; Schrøder et al., 2023), particle-induced x-ray emission-PIXE (Czedli et al., 2014), electron microprobe on a wavelength-dispersive X-ray scanning electron microscope (Courtemanche et al., 2006), scanning electron microscopy (SEM) and scanning electron microscopy with energy dispersive X-ray spectroscopy-SEM–EDX (Chuaychan et al., 2016), laser ablation inductively coupled plasma mass spectroscopy-LA-ICPMS (Tray et al., 2022), atomic absorption-AAS (Łuszczek-Trojnar and Nowacki, 2021) etc.

The potential of the application of fish scales in a variety of domains, such as ecological and environmental research, is attracting increasing interest. Elemental composition of scale has been studied by multiple researchers for a variety of objectives. Such as for the identification of fish stock. Among the earliest available reported works, Sr/Ca proportions in the scales have been utilized by Bagenal et al. (1973) to distinguish the marine trout species, *Salmo trutta,* from its freshwater-residing counterpart. Scale elemental analysis was utilized to distinguish between freshwater and saltwater striped bass, *Morone saxatilis,* by flame atomic absorption spectrophotometry (Belanger et al., 1987). On the basis of calcium and magnesium, they identified about 95% of freshwater and 95% of marine fish. Coutant and Chen, (1993) also analyzed the same fish using laser ablation mass spectrometry and noted that freshwater *M. saxatilis* had lower Sr levels, while estuarine *M. saxatilis* had greater Sr levels.

Additionally, scale and otolith chemistry of *Oncorhyncus clarki* were studied to assess their associations (Wells et al., 2003). They speculated that the element composition of fish scales and otoliths might be utilized to describe movement in freshwater using hard-part chemistry. Habitat and population structure of *Lates calcarifer* were investigated on the basis of scale barium and strontium levels (Pender and Griffin, 1996). Courtemanche et al. (2006) explored the migration patterns of anadromous *Salvelinus fontinalis* by wavelength-dispersive X-ray microscopy. They demonstrated that *S. fontinalis* possessed higher Sr/Ca proportions in the scale when exposed to a marine environment. Wild variant consistently possessed elevated Sr/Ca proportions than those of freshwater-dwelling species. Tray et al. (2022), characterizing fish life history and migration patterns from trace elements analysis in the scales of salmon (*Salmo salar*). The origin of different fishes was figured out by analyzing ^{87}Sr/^{86}Sr proportions on calcified matrices, including scales (Pouilly et al., 2014). They demonstrated the 87Sr/86Sr ratio as a precise biogeochemical tag to discriminate fish origin at the basin level, as well as at the sub-basin level.

Furthermore, the effectiveness of scales for tracking trace element concentrations in walleyed pike, *Sander vitreus* scales, was examined by Ofukany et al. (2015), who concluded that scales could be helpful in comprehending nutrient fluctuations, especially in aquatic systems. In order to determine their potential as a bioindicator of pollution, fish scales were examined in assessing heavy metals in water, besides general water quality monitoring (Sultana et al., 2016). They demonstrated that the scales displayed a variety of morphological abnormalities and distinct scale structures, including radii, annuli, and circuli in *Labeo rohita*, *Cirrhinus mrigala*, and *Catla catla*. Łuszczek-Trojnar et al. (2022) demonstrated that levels in scale match the length of time and dosages of elemental exposure to it, highlighting evidence that fish scale can be used as environmental bioindicators. Filipović Marijić et al. (2022) analyzed the patterns of elemental accumulation and distribution in different tissues, including scales of brown trout (*Salmo trutta*), to be used as metal exposure indicators. Fish scales can disclose ancient remnants of aquatic contamination, demonstrating that scales are an excellent biomarker for researching the environmental status of water bodies (Morán et al., 2018). The historical environmental alterations in aquatic environments have been reinstated by analyzing preserved fish scale samples (Vašek et al., 2021). They analyzed stable carbon (δ13C) and nitrogen (δ15N) isotopes in cyprinid fish roach and bream scales. This study indicated the functionality of scale samples to yield data on the surroundings.

Most of the cyprinid fish have significant socioeconomic importance all around the globe. Grass carp, *Ctenopharyngodon idella* (Cuvier et Valenciennes, 1844) is a native Chinese carp that has been introduced to many countries (FAO, 2009a), and mrigal, *Cirrhinus mrigala* (Hamilton, 1822) is endemic to Indo-Gangetic River catchments, found widely in the Southeast Asian region (FAO, 2009b). Fish from the Cyprinidae family, which is the second-largest vertebrate family on Earth, are frequently utilized as focal species in biological and ecological research (Nelson et al., 2016). Grass carp and mrigal have often been utilized in investigations relevant to the depiction of element accumulation. The buildup of different potentially toxic elements (heavy metals), Cd, As, Pb, and Zn, was reported in scales, together with other organs (Teodorof et al., 2009), and levels of Hg in scales along with the muscle of grass carp (*C. idella*) (Behrooz et al., 2012). Soe Aye et al. (2018) investigated Al, Cr, Cd, As, Ni, Hg, and Pb in the muscle of *C. mrigala* by an energy dispersive X-ray fluorescence experiment.

Hilsha, *Tenualosa ilisha* (F. Hamilton, 1822), is a coastal anadromous Clupeoid fish migrating upstream for spawning (Froese and Pauly, 2025). It has huge commercial and aesthetic value as the national fish of Bangladesh and is a significant part of the people and local culture. Flathead sillago or Gangetic whiting, *Sillaginopsis panijus* (Hamilton, 1822) is an amphidromous fish living in shallow, open muddy bays and estuaries (Froese and Pauly, 2025). Asian sea bass or barramundi, *Lates calcarifer* (Bloch, 1790) is a catadromous fish that inhabits rivers and migrates downstream to the estuaries for breeding. Pama croaker, *Otolithoides pama* (Hamilton, 1822), is an amphidromous Sciaenid that is very common in the Meghna River estuary (Froese and Pauly, 2025). Corsula, *Rhinomugil corsula* (Hamilton, 1822), is an anadromous Mullet living in coastal rivers and estuaries (Rahman, 1989).

The common carp, *Cyprinus carpio* (Linnaeus, 1758) is a commercially important, hardy, and adaptable fish species of Cyprinidae (Minnows or carps) family, which is found in a variety of freshwater habitats, like lakes, rivers, and ponds (Froese and Pauly, 2025) Its ubiquity makes it readily available for research in many regions. Common carp can grow to substantial sizes and have long lifespans (Froese and Pauly, 2025), allowing absorption of metals over time, providing a history of exposure to pollutants. Carp can tolerate a range of environmental conditions, including polluted water (Froese and Pauly, 2025). Their ability to thrive in a variety of aquatic ecosystems makes them useful for pollution assessment in different environmental settings by many authors around the globe (Mancera-Rodríguez et al., 2024; Uçkun and Uçkun, 2021). Common carp are mainly bottom dwellers but travel the middle to upper column for food (Froese and Pauly, 2025). Asian seabass or barramundi, *L. calcarifer* (Bloch 1790) is an euryhaline member of the family Latidae (Lates perches) which dwells in rivers prior to going to the estuary to breed (Fishbase, 2024). It grows to substantial sizes and has relatively long lifespans (Fishbase, 2024). They are proactive predators that primarily consume crustaceans and fish (FAO, 2024). Gold belly croaker, *Chrysochir aureus* (Richardson, 1846) is a commercially important warm temperate tropical-subtropical marine fish of the Sciaenidae family (Froese and Pauly, 2025). It is an offshore warm water demersal fish living mostly in the bottom water with a benthic feeding behavior (Xu et al., 2018; Zhang et al., 2019). *C. aureus* has been studied for heavy metal pollution (Azmi et al., 2019; H. Huang et al., 2022; Zhu et al., 2020), microplastics pollution (Wang et al., 2024; Zhang et al., 2019).

Fish life history and ecological insights, including habitat, depth, and migration patterns, are useful for sustainable management. Ecological and life cycle information could be utilized to comprehend the general ecology of fishes and the functional structure of fish populations (Frimpong and Angermeier, 2010). The importance of species traits in understanding the underlying mechanisms of community formation is becoming more well-recognized (Belmaker et al., 2013). Previous research demonstrated the link of scale elements to their feeding habit, migration, and environment, thereby showing the potential of using scale chemistry for fish life history and ecological insights (Pouilly et al., 2014; Tray et al., 2022). However, primarily otoliths (Filipović Marijić et al., 2022; Hüssy et al., 2024; Martinho et al., 2020; Moll et al., 2019; Pouilly et al., 2014; Tripp et al., 2020), and in certain cases, the vertebral column (Feitosa et al., 2020) and other skeletal material (Clarke, 2005) were employed in environmental and ecological studies that involve intrusive sampling.

Whereas using scales instead provides several distinct advantages, making them an attractive alternative to more disruptive sampling methods. Scales can be collected with almost no impairment on the fish, thus providing nonlethal and noninvasive sampling. In addition to providing nonlethal sampling, scalable for a wide range of systems from small ponds to oceans. Additionally, scales remain present throughout their lives, facilitating the records of longer periods (Łuszczek-Trojnar et al., 2022). Fish scale records metal exposure throughout their growth across the life span and analyzing these could reveal trophic shifts or migration patterns throughout their life span. The chemical signatures of elements accumulation and their fluctuations reflect environmental status and diet history. Given that fish scales are simple to collect without causing harm and precisely reflect the degree of environmental element composition, they seem like a fantastic environmental tool, enabling studies of especially rare and threatened species with minimal disturbance (Łuszczek-Trojnar et al., 2022; Tray et al., 2022).

Moreover, scale analysis would allow continuous data to be taken from the same fish with intervals to assess deposition over time, supporting catch-and-release studies and long-term monitoring. Furthermore, scale analysis would enable studies from archival collections to identify historical patterns. Thus, employing scales also reduces ethical concerns. Finally, such methods should be more cost-effective and field-friendly, easier to collect, requiring only simple tools (forceps or a scalpel) and no specialized training. Thus, fish scales are a Swiss Army knife for ecological and environmental studies, offering insights into growth, diet, stress, evolution, and pollution—all without harming the fish. Their integration with modern techniques (isotopes, genomics, imaging) could make them indispensable for aquatic research.

2.2. Plant leaves as a tool for environmental analysis

Similar to fish scales in aquatic systems, plant leaves can accumulate airborne pollutants by trapping them on their canopy (Simon et al., 2021; Sun et al., 2021; Zha et al., 2018). Air quality is a severe concern, particularly in cities and industrialized areas (Cetin and Jawed, 2022; Karacocuk et al., 2022; Serbula et al., 2013; Yadav and Rajamani, 2006). It has an impact on both the environment and human health (Morawska, 2024; Yadav and Rajamani, 2006, 2004). Even short-term exposure to some air contaminants can cause a variety of health problems, including lung diseases (Manisalidis et al., 2020). Various contaminants in the atmosphere, especially nanoplastics (NPs) or, in some situations, microplastics (MPs), could be inhaled, triggering manifestations like inflammation. Furthermore, plastic and its additives (plasticizers, colors) have the potential to be mutagenic, carcinogenic, and harmful to reproduction (Gasperi et al., 2018). MPs are continuously released into the atmosphere, where they are further transported by wind (Koutnik, 2022). The ability of MPs to travel vast distances has been demonstrated by evidence of their presence in the air far from their origin (Habibi et al., 2022; Zhang et al., 2020). These atmospheric MPs may deposit on plant foliage both wet and dry precipitation processes (Fujiwara et al., 2011; Liu et al., 2020). Several investigations have shown that terrestrial plant leaves are highly effective in trapping atmospheric MPs (Fujiwara et al., 2011; Liu et al., 2020; Roblin and Aherne, 2020). Liu et al. (2020) reported MPs to comprise up to 28% of all compounds deposited in plant leaves. Their presence and interactions in soil and aquatic environments have been thoroughly explored, but less attention has been paid to MPs in the air (Enyoh et al., 2019). Therefore, there is a dearth of studies on the presence and impacts of atmospheric MPs (Zhang et al., 2020).

Besides MPs, heavy metals are significant causes of air contamination. Because of the higher levels of industry and technology over the last several decades, the rate of emissions of these metals is increasing (Olajire and Ayodele, 2003). These elements, released into the atmosphere by numerous anthropogenic sources, pose a substantial hazard not only to the environment but also to public health (Varrica et al., 2022). Exposure to heavy metals can result in serious respiratory illnesses (Humairoh et al., 2020). Plants absorb the heavy metals from the polluted environment through their root systems, which are then accumulated in the body, including leaves, making them metal hyperaccumulators (Alsafran et al., 2022). Elemental levels in each particular area are substantially reflected by the nearby plant species (Molnár et al., 2020). Heavy metal levels in the air must be routinely checked to safeguard public health and the environment (Cetin and Jawed, 2022; Karacocuk et al., 2022). Therefore, studying contaminants like MPs and heavy metals in the atmosphere is essential.

Plants can efficiently capture tiny airborne particles on their leaf (Simon et al., 2021; Sun et al., 2021; Zha et al., 2018). Plant leaves can serve as a powerful sink for air pollutants (Chen et al., 2016; X. Liu et al., 2022). Pollutant accumulation in plant leaves is governed by a number of interconnected factors, including pollutant properties, plant traits, and the surrounding environment. Leaves on plants have a large surface area, extensive three-dimensional spaces, and irregular surfaces that can catch atmospheric particles like MPs and other hazardous materials (Huang et al., 2022; Zha et al., 2018). Pollutant adsorption is influenced by the morphology of the leaf surface, including cuticles, trichomes, and roughness (Li et al., 2017). Especially, leaves with high coverage act as an important sink for such airborne contaminants, and over time, such depositions turn into significant quantities (Bi et al., 2020). Hairy trichomes and roughness help retain particles. Hairy trichomes of leaves expand the space accessible for collecting pollutants from the air and inhibit their resuspension, which facilitates aerial fragment entrapment (Perera et al., 2024). Typically, plants with coarse and hairy leaves are better able to absorb larger amounts of contaminants compared to those with uniform, waxy leaves. Wax on the epidermis helps to absorb lipophilic pollutants on leaf and affects wettability (Li et al., 2017). Soil characteristics like pH, organic matter, and cation exchange capacity affect the solubility and the bioavailability of contaminants, especially metals, for root uptake (Cao et al., 2022) and consequently their deposition in leaves. The deposition of air pollutants by plant foliage is also determined by the particle sizes, wind direction, and velocity (Dadkhah-Aghdash et al., 2022). Environmental factors like precipitation and wind speed govern particle deposition and resuspension. Precipitation, primarily rainfall, is the major process of washing off surface-accumulated pollutants (Zhou et al., 2021).

The leaves of evergreen perennial species retain more pollutants because they have the capacity to capture pollutants over a longer period than those of their deciduous counterparts that seasonally shed leaves (Turan et al., 2011). Therefore, leaves of evergreen plants are usually preferred for leaf-based environmental pollution studies. Mast tree, *Polyalthia longifolia* (Sonn.), of the Annonaceae family is a tall and attractive, perennial ornamental tree (Shamshad et al., 2022). Planting these trees closely together makes a towering natural wall, and this is usually planted as a barrier against harmful substances because it is known to accumulate a lot of contaminants (Sultan et al., 2022). As a result, *P. longifolia* was previously investigated for pollution assessments in Bangladesh (Shahrukh et al., 2023; Sultan et al., 2022) and in other regions (Patel et al., 2023; Uka et al., 2021). Plant leaves samples can usually be analyzed by inductively coupled plasma mass spectrometry-ICP-MS (Pleijel et al., 2023; Turan et al., 2011), inductively coupled plasma optical emission spectrometry-ICP-OES (Ozyigit et al., 2022; Simon et al., 2021), Flame Atomic Absorption Spectrophotometry-AASF (Koucim et al., 2021), atomic absorption spectrometry-AAS (Uka et al., 2021) etc.

Anthropogenic activities provoke tricky changes in leaf elemental profiles via many mechanisms, impacting both necessary and non-essential elements and reflecting pollution, climate, and land-use effects (Molnár et al., 2020; Simon et al., 2021, 2014). Fossil fuel burning and agriculture boost airborne N and S compounds, which are absorbed by leaves, potentially leading to nutritional imbalances (Aneja et al., 2009). Industrial operations, automobile emissions, mining, waste disposal, and pesticide release different heavy metals in the environment. Plants absorb these and are then deposited on leaves, elevating leaf concentrations. Fertilizers increase N and P in the soil (Yang et al., 2024), but they may also alter micronutrients such as Zn and Fe (Singh et al., 2018). Pesticides may change the microbiology of the soil, which could have an indirect impact on nutrient intake, for example, decreased mycorrhizal symbiosis could limit the absorption of phosphorus (Steiner et al., 2024). The practice of irrigation with salty water elevates sodium (Na^+) and chloride (Cl^-) levels in leaves (Niu et al., 2008). Releases of industrial SO_2 and NOx reduce the pH of the soil, releasing manganese (Mn^{2+}) and aluminum (Al^{3+}), which build up in leaves and become hazardous (Aneja et al., 2009). Changes in land use, for example deforestation, decrease the canopy's ability to cycle nutrients, which lowers the amount of calcium and magnesium in regrowing leaves. These alterations impact plant health and overall ecological and environmental relationships, rendering leaf element makeup a crucial bioindicator for environmental monitoring.

Plant leaves are useful for biomonitoring environmental pollution (Simon et al., 2020). Jafarova et al. (2023) utilized urban tree leaflets as a bioindicator for analyzing atmospheric microplastic pollution in cities. Simon et al. (2014) used tree leaves as an indicator of air pollution through elemental analysis, showing their fascinating potential to be utilized as proxies of urban environmental status. Simon et al. (2020) performed an ecological investigation of particulate material in urban habitats using *Tilia europaea* tree leaves. They concluded that trees are especially useful biological indicators suited for biomonitoring studies of terrestrial pollution. Jafarova et al. (2023) used roadside higher plant, *Robinia pseudoacacia* leaflets as biomonitors for analyzing atmospheric microplastic deposition. They reported that urban parks had a much bigger number of plastic microfibers owing to their prominent city environments. Simon et al. (2014) used tree leaves as indicators of the accumulation of pollutants through elemental analysis. They analyzed the quantity of dust that has been deposited and the level of pollutants present in that dust on different tree leaves along an urbanization gradient. Simon et al. (2021) also demonstrated the usefulness of trees as urban health indicators from the elemental levels in leaves. The study depicted the sensitivity of plants to air contamination, showing their potential to be utilized as indicators of the state of the urban environment. The contaminated aerosol particles from the atmosphere settling on the leaves of plants were analyzed for an assessment of pollution along an industrial–urban–rural gradient (Molnár et al., 2020). The research demonstrated that the level of elements in each environment is substantially affected by the nearby plant species.

There are several advantages of the utilization of plant leaves as noninvasive tools over traditional invasive methods, like minimal disturbance, cost-effectiveness, over long-term monitoring. Plants could reflect air and soil pollution at the same time. Since plant root systems uptake heavy metals, for example, Pb, Cd, etc., which accumulate in leaves (Alsafran et al., 2022). On the other hand, the airborne contaminants like MPs can settle on plants through deposition (Fujiwara et al., 2011), serving as an interim sink for hazardous matters (Liu et al., 2020). Thus, plant leaves could serve as natural, low-tech pollution sensors, integrating data from air, soil, and water systems. Plant leaves are easily accessible and can be collected without destructive sampling, where no specialized equipment is required. Allows cost-effective lab analysis, which is more affordable compared to traditional methods of analyzing soil and air sampling together. Additionally, leaves from perennial trees could provide multi-year accumulation data from wide coverage distributed naturally across different areas. Plant leaves have been utilized as bioindicator for studying the environmental status of an area (Molnár et al., 2020; Simon et al., 2021; Zhang et al., 2022). Regardless of the species, plant leaves have the ability to gratuitously gather MPs in the air (Liu et al., 2020). Plant leaves have been shown to be highly effective at accumulating air pollutants and acting as a substantial dust-entrapping surface, particularly in urban areas (Chen et al., 2016; X. Liu et al., 2022). Previous research showed that by capturing contaminants on their leaves, such as foliar dust, plants may efficiently absorb and store particles in the air (Simon et al., 2021; Sun et al., 2021; Zha et al., 2018). Dust on leaves can sometimes be a more useful tool for environmental assessment than using soil or other plant parts, such as in urban settings (Liu et al., 2022). The current research intended to analyze MPs in the dust deposited on plant leaves and leaves elemental analysis.

Chapter 3: Materials and Methods

3.1. Analysis of fish scales

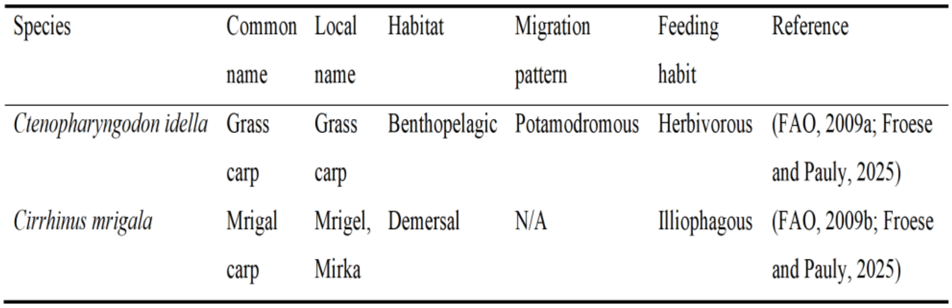
3.1.1. Species selection

When investigating the potential of scales to provide ecological insights, it is crucial to select fish species with a wide range of life histories, spanning diverse ecological circumstances and a broad geographical spectrum. Therefore, the current research 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). Previous studies indicated the usefulness of scale trace element analysis of scale samples of cyprinid fish (Vašek et al., 2021). The number of fish used in the analysis for the freshwater study was 2 species x 1 individual. The characteristics of the freshwater 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

Coastal environment or coastal water occurs in coastal areas where seawater meets the landmass, for instance, in estuaries. Coastal waters occupy about 7 % of the entire ocean surface and are the most biogeochemically active zones of the ocean, accounting for over half of the ocean's overall ecosystem services (Lønborg et al., 2021). Being a very active and distinct environment of the Earth, it is important to study the scale elemental analysis in the coastal fish species. Especially selecting species having migratory movement encompassing the different compartments of the coastal areas, like coastal rivers to sea or vice versa, offers a record of accumulation from different water systems in the body. Thus, migratory coastal species provide a metal history across a broad spectrum of environmental settings, which is crucial in investigating the feasibility of scales for element analyses. The study employs five economically significant coastal fish: hilsha- *Tenualosa ilisha* (F. Hamilton, 1822), flathead sillago or Gangetic whiting- *Sillaginopsis panijus* (Hamilton, 1822), Asian sea bass or barramundi- *Lates calcarifer* (Bloch, 1790), pama croaker- *Otolithoides pama* (Hamilton, 1822), and corsula or corsula mullet- *Rhinomugil corsula* (Hamilton, 1822), using inductively coupled plasma optical emission spectroscopy (ICP-OES). Table 2 lists the characteristics of the fish species under study. The number of fish used in the analysis for the coastal migratory fish scale study was 5 species x 2 individuals. The fish were obtained from the artisanal fishery on the Bangladesh coast of the Bay of Bengal. Artisanal fishing operations in coastal waterways of Bangladesh encompass sections of rivers, estuaries, and the marine environment in the Bay of Bengal (Mustafa et al., 2023).

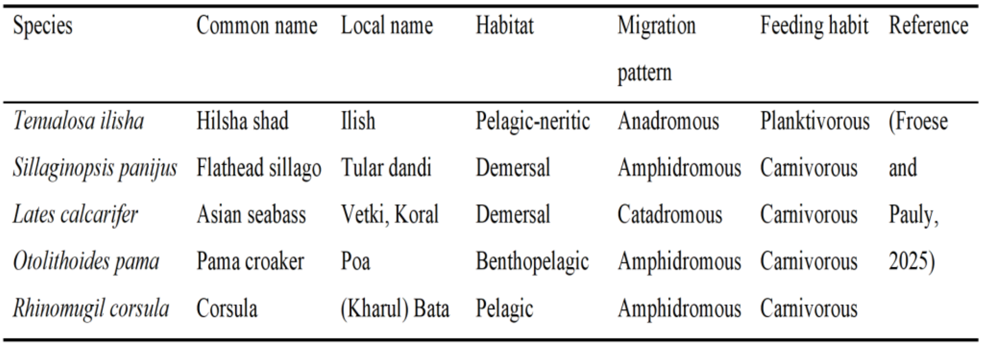
Table 2. The ecological characteristics of the coastal migratory fish species.

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

3.1.1.3. Freshwater-brackish water-marine fish

Freshwater available in the inland lakes, streams, and rivers greatly differs based on the variety of soil and rock with which the water comes into contact. Seawater, however, is normally almost similar in terms of its composition anywhere in the Earth's basins (Sverdrup et al., 1942). Brackish water features the intermediate characteristics found in the transitional zones where fresh and seawater come together in a natural environment (Rich and Maier, 2015). One important distinction between these sorts of habitats is salt content, or water salinity, with seawater having the most, freshwater having the lowest, and brackish water falling somewhere in the middle. Few investigations have been conducted to analyze fish scale elements on species spanning diverse ecological circumstances from different environments. The research carried out elemental analysis on the fish scales from freshwater-brackish water-marine water environments together. Fish samples were collected from freshwater, brackish water or estuarine, and marine water for scale microchemistry analysis during the current study. The selection of species was made depending on factors such as their economic significance, tolerance to contaminants, availability, and wide dispersion, and prior study. Common carp- *Cyprinus carpio* (Linnaeus, 1758), Asian seabass or barramundi- *Lates calcarifer* (Bloch 1790), and gold belly croaker- *Chrysochir aureus* (Richardson, 1846), were collected and 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 analysis for the freshwater-brackish water-marine study was 3 species (one from each environment) x 4 individuals. Sample collection was performed from the fishermen fishing in the respective water bodies. 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 and Pauly, 2025)
Sillaginopsis panijus	Flathead sillago	Tular dandi	Demersal	Amphidromous	Carnivorous	
Lates calcarifer	Asian seabass	Vetki, Koral	Demersal	Catadromous	Carnivorous	
Otolithoides pama	Pama croaker	Poa	Benthopelagic	Amphidromous	Carnivorous	
Rhinomugil corsula	Corsula	(Kharul) Bata	Pelagic	Amphidromous	Carnivorous	

3.1.2. Preparation of the fish scale samples

After being gathered, the fish specimens were frozen at -20°C and then allowed to defrost at room temperature before the scales were collected (Chuaychan et al., 2016). Then the fish specimens were carefully washed with both tap water and distilled water to eliminate any adhering contaminants. Measurements of total length and body weight were taken for each fish. 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. Dirt and mucus were removed from the scales using distilled water, and any remaining mucus was thoroughly cleansed using a soft bristle brush. Prior to the investigation, the scales were allowed to air dry at room temperature.

3.1.3. Chemical analysis with ICP-OES

In atmospheric wet digestion, 0.2 g sample were digested by the mixture of 5.0 ml 65% (m/m) HNO_3 and 1.0 ml 30% (m/m) H_2O_2. Without loss, digested samples were shifted into volume-calibrated plastic centrifuge tubes and diluted up to 15.00 mL with ultrapure water. Until the next process, the solutions were left at room temperature. 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). Autosampler (Agilent SPS4), Meinhard® type nebulizer, and double-pass spray chamber, and a five-point calibration technique were undertaken (ICP VI, Merck). From the mono-element spectroscopic standard of 1000 mg/l (Scharlau), standard solutions of the macroelements have been generated, whereas those of the microelements have been prepared from the multi-element spectroscopic standard solution of 1000 mg/l (ICP IV, Merck). A point calibration approach was practiced, with standard solutions diluted with 0.1 M HNO_3 produced by ultrapure water.

3.1.4. Chemical analysis with ICP-MS

Prior to analysis, the samples underwent microwave-assisted acid digestion following the procedure outlined in Application Note HPR-FO-17 (Milestone Start D, Italy). 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 the contents were gently mixed by hand for 1–2 minutes to ensure thorough interaction with the acids. The vessels were sealed, loaded into the rotor segment, and digested in the microwave system for 30 minutes at 200°C. After digestion, the rotor was allowed to cool to room temperature, and the digested solutions were transferred into pre-marked volumetric flasks. The digested samples were analyzed using the inductively coupled plasma mass spectrometry (ICP-MS; PerkinElmer NexION 2000) method (Jovičić et al., 2023; Parvin et al., 2023). Approximately 0.4 mL/min of each sample was nebulized into the core of the argon plasma. At high plasma temperatures, the sample aerosols were vaporized, atomized, and subsequently ionized. The resulting ions were introduced into a quadrupole mass spectrometer, which separated them based on their mass-to-charge ratio (m/z). Elemental concentrations were quantified in mg/kg using external calibration with ICP multielement standards. To ensure analytical accuracy and precision, digestion recoveries were assessed using certified reference materials (SRM 1946 and SRM 1947, specifically designed for fish matrices). Recovery rates for all target elements exceeded 94%, demonstrating the credibility of the procedure. The QC was also checked and maintained by using the internal standard solution for ICP-MS. The limits of detection (LOD), which the instrument calculated automatically and were found between 0.1 and 0.5 parts per trillion (ppt). All measurements represent the mean of three replicate analyses, with standard deviations below 3%. Reported metal concentrations were blank corrected using field blanks to account for any potential contamination.

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 (Medaković et al., 2016; 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. The M-Quant software that came with the device was used to evaluate assessment data. For the quantification, the fundamental parameter (FP) approach was applied.

3.1.6. Statistical analysis

Statistical Package for the Social Sciences (SPSS) version 26.0 (IBM, 2019), and PAleontological Statistics (PAST) version 5.2.1 (Hammer, 2025; NHM, 2025) were used for data analysis. Firstly, data were checked for normality (Shapiro–Wilk test) and homogeneity (Levene's test). When data met the assumptions, one-way analysis of variance (ANOVA) was performed, followed by the post-hoc DMRT (Duncan Multiple Range Test), otherwise, Kruskal-Wallis non-parametric test was applied, followed by Dunn's multiple pairwise comparison test with Bonferroni corrections to check the mean differences at $p < 0.05$ (Sultana et al., 2016). For statistical analysis, when Heavy metal levels were below the detection limits of the test (<LoD), a value of 0 was assigned.

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. The industrial and residential regions were selected in Dhaka, while the rural site was a rural village. The industrial site was in Savar (Hemayatpur), Dhaka. Different types of enterprises operate here because of the closeness to the country's main economic and administrative center and capital, Dhaka city. The residential site was located in Gulshan, Dhaka City, characterized by anthropogenic activity and excessive traffic. There are roughly 10.2 million people living in the city, making it the most densely inhabited megacity in the nation (BBS, 2022). In contrast, the rural location was in a village in (Lohagora) Narail district within the Khulna division, having 788,673 people, having little human activity or traffic, emphasizing the area's clean, untouched nature (BBS, 2022).

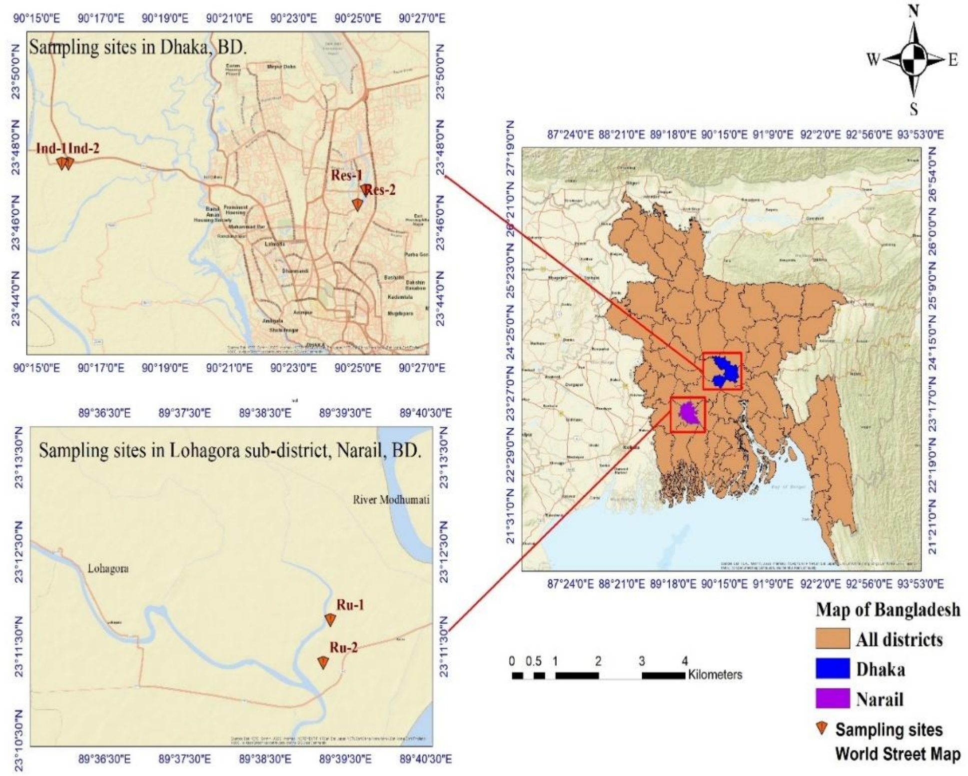


Figure 1. Studied areas in Bangladesh along an urbanization gradient (Note: Ind = industrial area, Res = residential area, and Ru = rural area).

An evergreen tree species, *Polyalthia longifolia* (Sonn.), commonly known as mast tree, locally called "Debdaru" (দেবদারু) in Bangla, was selected for leaf sample collection. It is commonly planted in Bangladesh and other Southeast Asian regions as an ornamental plant along roadsides due to its compact, attractive, and slender shape as well as its beautiful green leaves (Figure 2). Two sites were selected from each area, trees were randomly selected at each site, and a total of 180 leaves were collected for analysis. Paper bags were used to collect the samples, which were then kept at room temperature in a dark location before investigation.

(**a**) (**b**)

Figure 2. *P. longifolia* on street dividers in Dhaka (**a**), dust accumulated on leaves is apparent by the dark coloring (**b**).

3.2.2. Analysis of microplastics

The collected *P. 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 were used to separate the dust samples from washed-out waters through the filtration process. After placing the leaves in a 250 mL glass beaker, 150 mL of deionized water was added and mixed well by stirring with a stirrer for 10 min. 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. Following that, the samples were moved to 100 mL beakers that had been previously weighed and completely evaporated. The beakers containing the samples were reweighed in order to calculate the dry weight of the collected leaf dust samples. After that, the samples were moved to glass tubes, 25 mL of filtered, deionized water was added, and MPs were further analyzed. 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) using a pyrolator (GA/PY-3030D) was used to determine the MPs content following the methods described in previous studies (Bouzid et al., 2022; Santos et al., 2023). The program of GC-MS was as follows: 40 °C for 2 min, 280 °C for 10 min, and 320 °C for 10 min. For the pyrolysis, 600 °C was used, and the time duration was 12 s. A mixture of 11 polymers (Frontier Laboratories) was utilized for calibration.

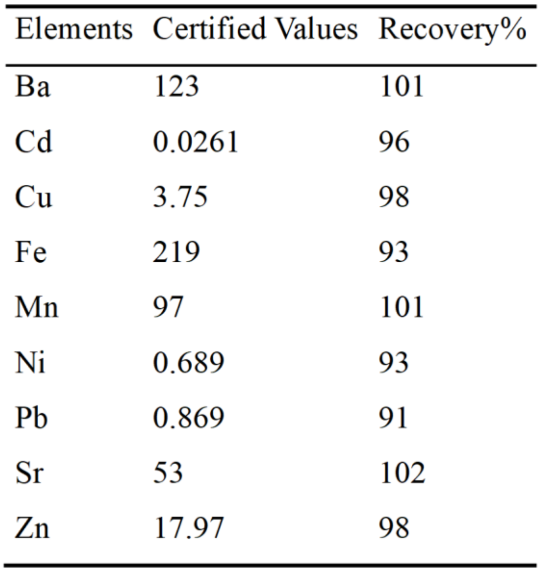
3.2.3. Quality assurance and quality control (QA/QC)

The likelihood of contamination makes quality assurance and control (QA/QC) especially important for microplastic analyses; thus, established quality control procedures were strictly followed during sampling and analysis (Belontz and Corcoran, 2021; Munno et al., 2023). Lab coats and clothing made of natural fabrics, like cotton, were put on while conducting sampling, processing, and analysis for the MP study instead of synthetic fabrics. Natural rubber latex gloves were used, and they were regularly changed. The laboratory's air circulation was kept as regulated as feasible to lessen the contamination and spread of fibers. All glassware, stainless-steel filter meshes, scissors, and tweezers were thoroughly cleaned with laboratory detergent and hot water, followed by three rinses with filtered deionized water. Processing glassware was stored in secured cabinets or inverted when not in use after being dried at a high temperature to eliminate any remaining plastic residues. The entire sample preparation and analysis procedure used a blank.

3.2.4. Measurements of element concentration

P. longifolia leaves were cleaned with tap water because distilled water could significantly alter the elements' levels in the leaves as a result of the osmosis process. Leaf samples were dried for 24 hours at 60 °C, and then they were homogenized with an electrical mixing device. Utilizing an open digestion system, the 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 and (Simon et al., 2021). Inductively coupled plasma–optical emission spectrometry (ICP-OES 5110, Agilent Technologies, Santa Clara, CA, USA) was employed to analyze the leaf samples' elements following the methods described in previous studies (Simon et al., 2021, 2014, 2011). The recoveries were less than 10% of the endorsed levels of elements, as peach leaves CRM (NIST1547) was utilized (Table 4).

Table 4. Certified mass fraction values for elements (mg/kg) in SRM 1547.



Elements	Certified Values	Recovery%
Ba	123	101
Cd	0.0261	96
Cu	3.75	98
Fe	219	93
Mn	97	101
Ni	0.689	93
Pb	0.869	91
Sr	53	102
Zn	17.97	98

3.2.5. Analysis of pollution index

The ratio of elements found in the leaves, and the background concentration was used to estimate the pollution index (PI), which provided a broad overview of the level of pollution at every location. The classes of degrees of pollution were modified from Faiz et al. (Faiz et al., 2009) and Steindor et al. (Steindor et al., 2016). The following formula was utilized to determine the PI of the tree leaves:

$$PI = C_{sample}/C_n, \tag{1}$$

Where, C_{sample} represents the target element's measured levels in the analyzed leaves and C_n represents the World Health Organization-recommended background or reference levels of that element in plants (WHO, 1996).

Pollution-degree classes on the basis of PI values: $PI < 1$ = 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).

3.2.6. Statistical analysis

Statistical Package for the Social Sciences (SPSS)-26.0 software was used to perform the statistical analysis of the data (IBM, 2019). Normality was checked with Shapiro-Wilk test and homogeneity of data was analyzed with Levene's test. The differences between the variables were examined using one-way analysis of variance (ANOVA), and then the Tukey's HSD (Honestly Significant Difference) post hoc test ($p < 0.05$) was used (Simon et al., 2021).

Chapter 4: Results

4.1. Fish scales

4.1.1. Freshwater fish scales

4.1.1.1. Scale chemistry and composition

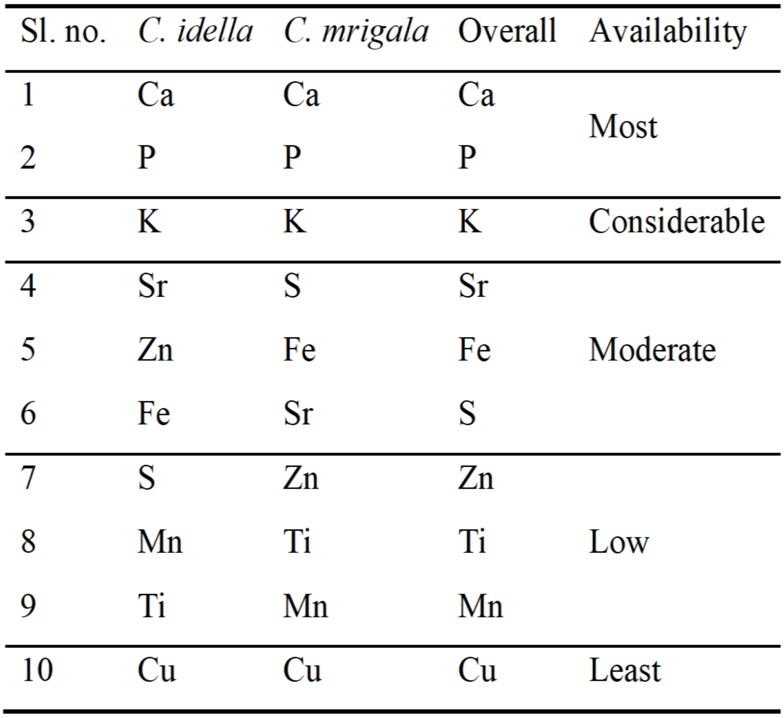
Different elements, namely calcium (Ca), copper (Cu), iron (Fe), potassium (K), manganese (Mn), phosphorus (P), sulfur (S), strontium (Sr), titanium (Ti), and zinc (Zn), were found in *C. idella* and *C. mrigala* scales by μ-XRF analysis. The quantitative results of element composition detected in the present investigation are shown in Table 5. Ca was the most abundant, followed by P, and Cu was the least abundant in the fish scales analyzed during the current study. The compositional analysis unequivocally delineates distinct proportions for each constituent element, as revealed by both count and weight percentages. 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*. The current investigation detected different elements accumulated in the scales. The distribution map of the elements on the scales of *C. idella* and *C. mrigala* is presented in appendix 1 and 2 respectively.

Table 5. 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

The hierarchy of the elements obtained in the current study was Ca>P>K>Sr>Zn>Fe>S>Mn>Ti>Cu in *C. idella* and Ca >P>K>S>Fe>Sr>Zn>Ti>Mn>Cu in *C. mrigala* (Table 6). The determined elements were classified into five categories based on their overall availability in the scales: most, considerable, moderate, low, and least (Table 6). These groupings are also depicted in the network analysis (Figure 5A-C) and classical cluster analysis, dendrogram (Figure 5D).

Table 6. The hierarchy (largest to smallest, starting at the top) of the elements in the freshwater fish scales.



Sl. no.	*C. idella*	*C. mrigala*	Overall	Availability
1	Ca	Ca	Ca	Most
2	P	P	P	
3	K	K	K	Considerable
4	Sr	S	Sr	Moderate
5	Zn	Fe	Fe	
6	Fe	Sr	S	
7	S	Zn	Zn	Low
8	Mn	Ti	Ti	
9	Ti	Mn	Mn	
10	Cu	Cu	Cu	Least

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). The overall weight percent of the elements in the first group ranges from 2.3166 to 8.9116, while that of the second group is 0.0108. The third category consists of Sr, Fe, and S, which are moderately available elements in the scales studied (Table 5; Figure 5A). The overall weight percent of the elements in the third group ranges from 0.0051 to 0.0063. The fourth class includes Zn, Ti, and Mn, the availability (overall weight percent is between 0.0013 and 0.0038) of which is low in scales (Table 5; Figure 5B). Cu is the least available, with an overall weight percentage of 0.0004, forming the last group. The network analysis (Figure 5A-C) and classical cluster analysis, dendrogram (Figure 5D), portray these element groups.

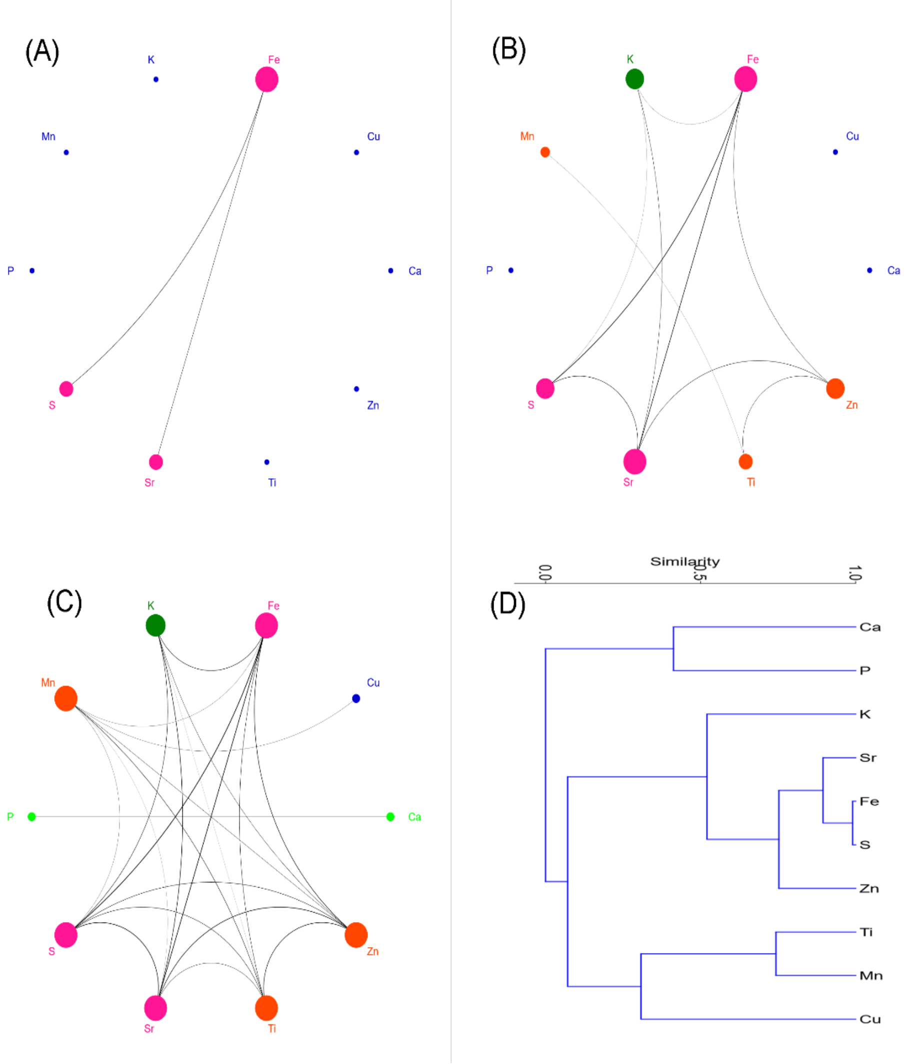


Figure 5. Network plot of elements in the freshwater fish scales (A-C) and a dendrogram of elements used by hierarchical clustering analysis (D).

4.1.1.2. Elements across habitats

Scales of benthopelagic fish dwelling in the water column (*C. idella*) were observed to possess higher Ca, K, Mn, and Zn than those in demersal fish (*C. mrigala*) (Figure 6). On the other side, the demersal fish (*C. mrigala*) have higher Cu, Fe, P, S, Sr, and Ti in the scales.

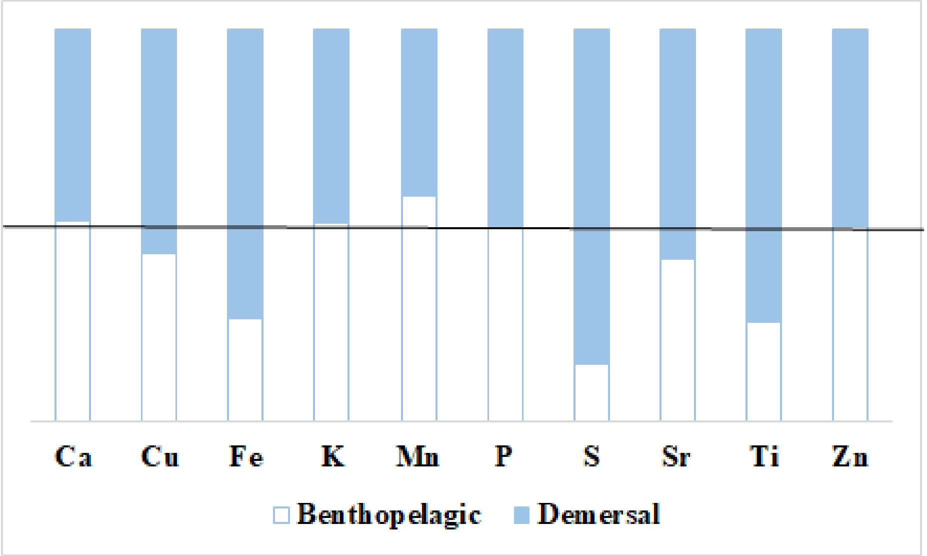


Figure 6. Element concentration in the freshwater fish scales across habitats. The 100% stacked bar shows the % of the average value of the mean concentrations of fish types (Benthopelagic and Demersal) shared by each element's sum. The entire bar stands for 100%, and the horizontal line in the center of the bars indicates 50% of the bar for comparing the types (Benthopelagic and Demersal).

4.1.1.3. Elements across feeding habits

Scales of herbivorous fish, *C. idella* were observed to possess higher Ca, K, Mn, and Zn than those in illiophagous fish (*C. mrigala*) (Figure 7). The illiophagous fish (*C. mrigala*), on the other hand, had higher Cu, Fe, P, S, Sr, and Ti in their scales.

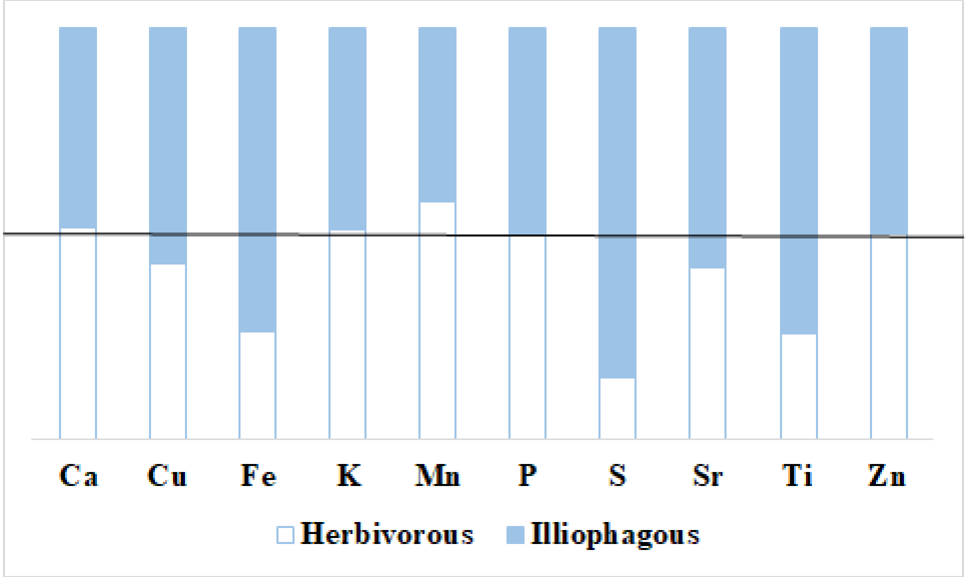
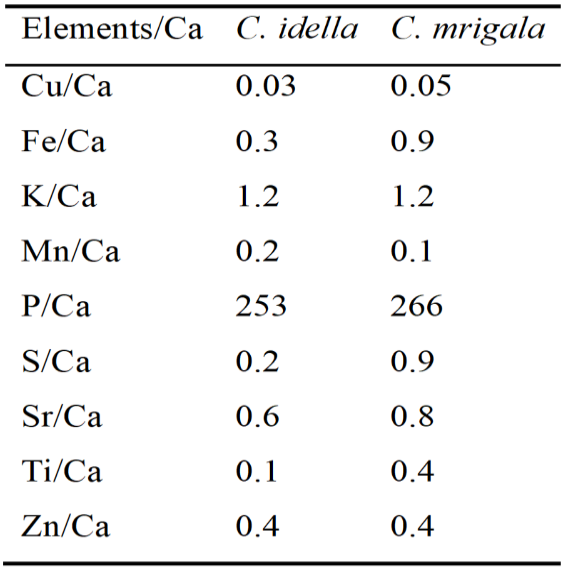


Figure 7. Element concentration in the freshwater fish scales across feeding habits. The 100% stacked bar shows the % of the average value of the mean concentrations of fish types (Herbivorous and Illiophagous) shared by each element's sum. The entire bar stands for 100%, and the horizontal line in the center of the bars indicates 50% of the bar for comparing the types (Herbivorous and Illiophagous).

4.1.1.4. Element: calcium ratios

The element: Ca ratios obtained in the study are presented in Table 7. In the current study, the Mn/Ca ratio was found to be higher in the scale of *C. idella*, while the other element/Ca ratios were lower than that of *C. mrigala*.

Table 7 Element/Ca ratios in the freshwater fish scales (Note: Dividing the values by 10^3 yields actual values).



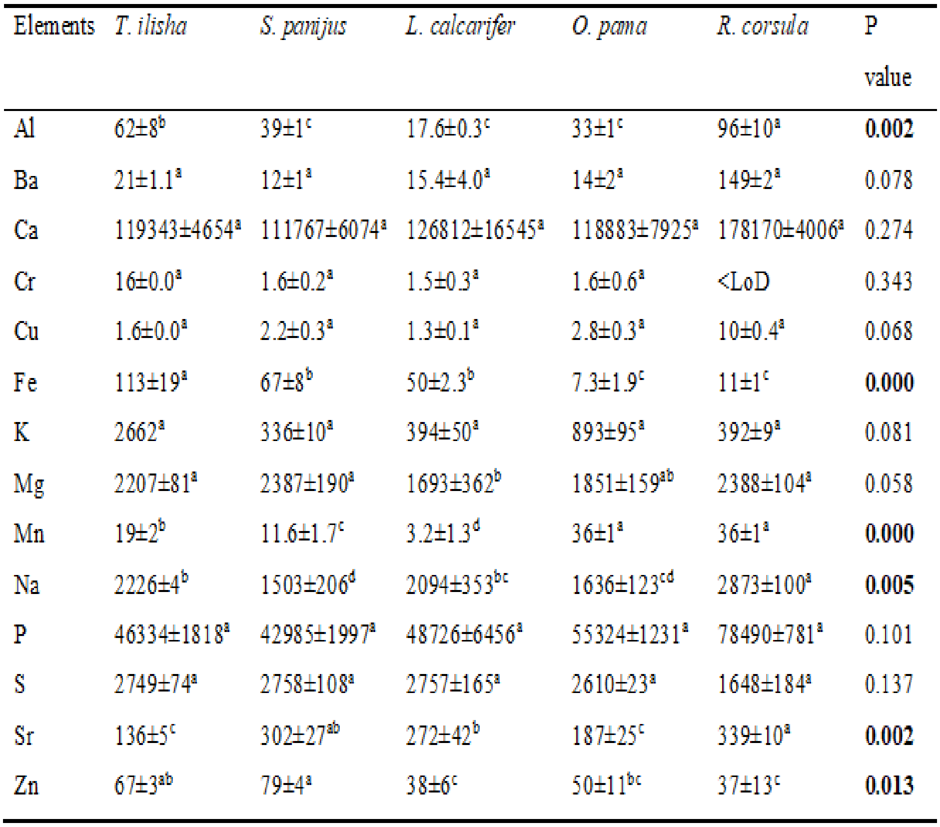
Elements/Ca	*C. idella*	*C. mrigala*
Cu/Ca	0.03	0.05
Fe/Ca	0.3	0.9
K/Ca	1.2	1.2
Mn/Ca	0.2	0.1
P/Ca	253	266
S/Ca	0.2	0.9
Sr/Ca	0.6	0.8
Ti/Ca	0.1	0.4
Zn/Ca	0.4	0.4

4.1.2. Coastal migratory fish scale

4.1.2.1. Scale chemistry and composition

This study examines the elements Aluminium (Al), Barium (Ba), Calcium (Ca), Chromium (Cr), Copper (Cu), Iron (Fe), Potassium (K), Magnesium (Mg), Manganese (Mn), Sodium (Na), Phosphorus (P), Sulphur (S), Strontium (Sr), and Zinc (Zn) in five marine fish species namely, *T. ilisha*, *S. panijus*, *L. calcarifer*, *O. pama*, and *R. corsula* by ICP-OES. Element concentrations (mg/kg) obtained are given in Table 8. The total length and weight of the fish samples 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). One-way ANOVA reveals significant differences ($p<0.05$) in the concentrations of Al, Fe, Mn, Na, Sr, and Zn (Table 8).

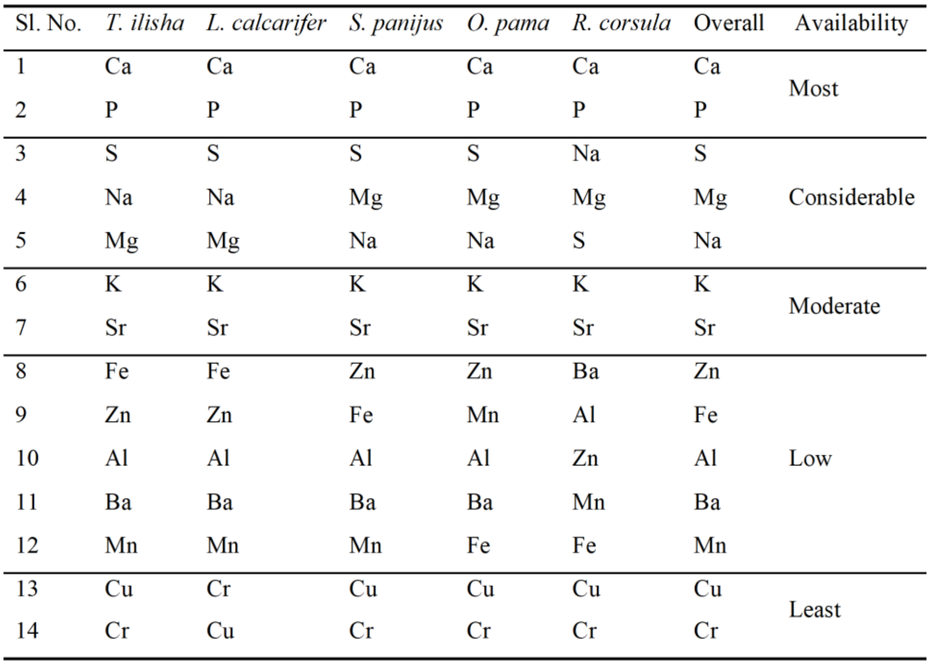
Table 8 Element concentrations (mean ± SD; mg/kg) in the coastal migratory fish scales. Superscript letters in the same row denote significant differences ($p < 0.05$) at Duncan's test. Bold fonts depict significant p-values. LoD: Limit of detection.



Elements	*T. ilisha*	*S. panijus*	*L. calcarifer*	*O. pama*	*R. corsula*	P value
Al	62 ± 8^{b}	39 ± 1^{c}	17.6 ± 0.3^{c}	33 ± 1^{c}	96 ± 10^{a}	**0.002**
Ba	21 ± 1.1^{a}	12 ± 1^{a}	15.4 ± 4.0^{a}	14 ± 2^{a}	149 ± 2^{a}	0.078
Ca	119343 ± 4654^{a}	111767 ± 6074^{a}	126812 ± 16545^{a}	118883 ± 7925^{a}	178170 ± 4006^{a}	0.274
Cr	16 ± 0.0^{a}	1.6 ± 0.2^{a}	1.5 ± 0.3^{a}	1.6 ± 0.6^{a}	<LoD	0.343
Cu	1.6 ± 0.0^{a}	2.2 ± 0.3^{a}	1.3 ± 0.1^{a}	2.8 ± 0.3^{a}	10 ± 0.4^{a}	0.068
Fe	113 ± 19^{a}	67 ± 8^{b}	50 ± 2.3^{b}	7.3 ± 1.9^{c}	11 ± 1^{c}	**0.000**
K	2662^{a}	336 ± 10^{a}	394 ± 50^{a}	893 ± 95^{a}	392 ± 9^{a}	0.081
Mg	2207 ± 81^{a}	2387 ± 190^{a}	1693 ± 362^{b}	1851 ± 159^{ab}	2388 ± 104^{a}	0.058
Mn	19 ± 2^{b}	11.6 ± 1.7^{c}	3.2 ± 1.3^{d}	36 ± 1^{a}	36 ± 1^{a}	**0.000**
Na	2226 ± 4^{b}	1503 ± 206^{d}	2094 ± 353^{bc}	1636 ± 123^{cd}	2873 ± 100^{a}	**0.005**
P	46334 ± 1818^{a}	42985 ± 1997^{a}	48726 ± 6456^{a}	55324 ± 1231^{a}	78490 ± 781^{a}	0.101
S	2749 ± 74^{a}	2758 ± 108^{a}	2757 ± 165^{a}	2610 ± 23^{a}	1648 ± 184^{a}	0.137
Sr	136 ± 5^{c}	302 ± 27^{ab}	272 ± 42^{b}	187 ± 25^{c}	339 ± 10^{a}	**0.002**
Zn	67 ± 3^{ab}	79 ± 4^{a}	38 ± 6^{c}	50 ± 11^{bc}	37 ± 13^{c}	**0.013**

The elements identified in the fish scales during the present study include both essential elements and certain heavy metals, such as Cu, Cr, Zn, and Fe, that may be dangerous at levels above a threshold. Nevertheless, of all the components found, their concentration was low (Table 8). The distribution of elements in the coastal migratory fish scales is presented in appendix 3-7 and scanning electron microscope (SEM) images in appendix 8-12. Calcium has the highest average concentration of any element across all species, followed by P, S, Mg, Na, K, Sr, Zn, Fe, Al, Ba, Mn, Cu, and Cr, respectively. The hierarchy of the overall mean levels of elements was Ca>P>S>Mg>Na>K>Sr>Zn>Fe>Al>Ba>Mn>Cu>Cr. The hierarchy of mean concentrations (mg/kg) of the identified elements in the fish is shown in Table 9.

Table 9. The hierarchy (largest to smallest order: top to bottom) of the elements in the coastal migratory fish scales.



Sl. No.	*T. ilisha*	*L. calcarifer*	*S. panijus*	*O. pama*	*R. corsula*	Overall	Availability
1	Ca	Ca	Ca	Ca	Ca	Ca	Most
2	P	P	P	P	P	P	
3	S	S	S	S	Na	S	Considerable
4	Na	Na	Mg	Mg	Mg	Mg	
5	Mg	Mg	Na	Na	S	Na	
6	K	K	K	K	K	K	Moderate
7	Sr	Sr	Sr	Sr	Sr	Sr	
8	Fe	Fe	Zn	Zn	Ba	Zn	Low
9	Zn	Zn	Fe	Mn	Al	Fe	
10	Al	Al	Al	Al	Zn	Al	
11	Ba	Ba	Ba	Ba	Mn	Ba	
12	Mn	Mn	Mn	Fe	Fe	Mn	
13	Cu	Cr	Cu	Cu	Cu	Cu	Least
14	Cr	Cu	Cr	Cr	Cr	Cr	

The detected elements were grouped into five categories depending on their overall concentrations as most, moderate, low, and least available. In the analysis, we formed a network displaying similarities (Bray-Curtis similarity index) based on the concentration elements detected in this research. This elemental categorization is also apparent in the network analysis diagram (Figure 13) and the standard cluster analysis, dendrogram (Figure 14).

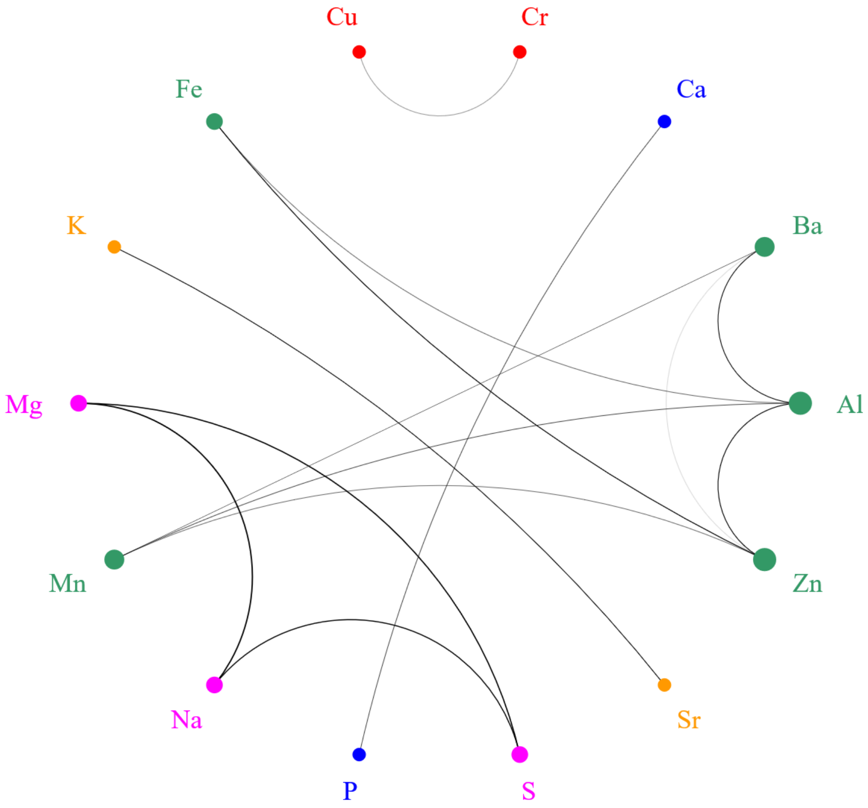


Figure 13. Network plot of elements in the coastal migratory fish scales.

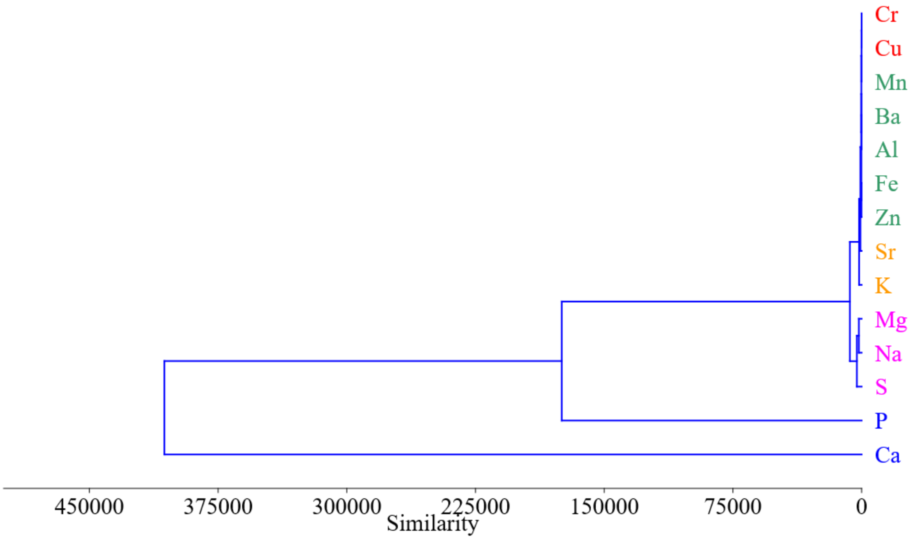
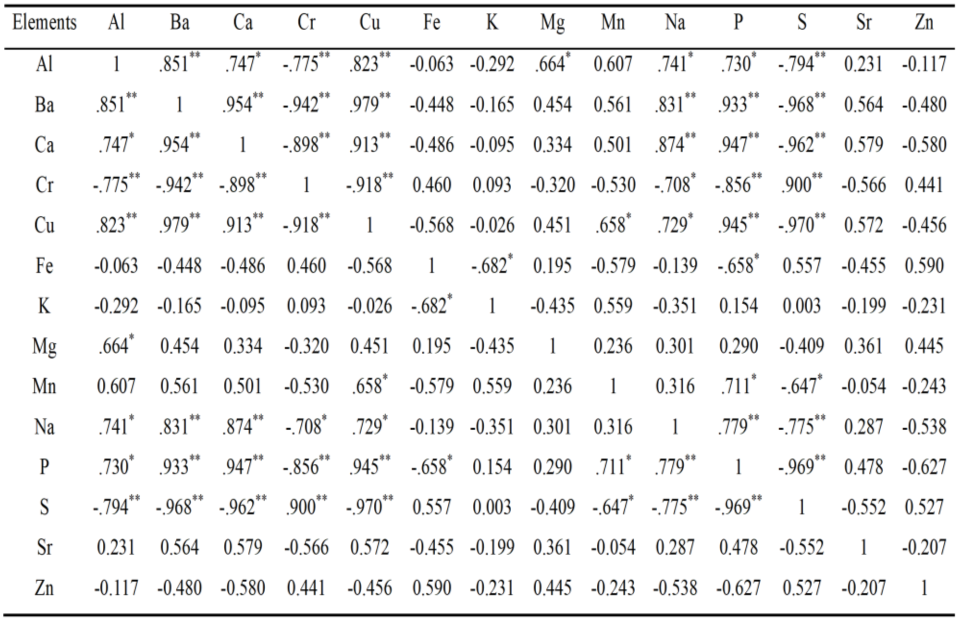


Figure 14. Dendrogram of elements in the coastal migratory fish scales.

The Pearson correlation coefficients represent the correlation among the elements detected in the scales of fish from the coastal environment (Table 10).

Table 10. The Pearson correlation coefficients of elements in the coastal migratory fish scales. "**" indicates correlation is significant at the 0.01 level (2-tailed) while "*" denotes correlation is significant at the 0.05 level (2-tailed).



Elements	Al	Ba	Ca	Cr	Cu	Fe	K	Mg	Mn	Na	P	S	Sr	Zn
Al	1	.851**	.747*	-.775**	.823**	-0.063	-0.292	.664*	0.607	.741*	.730*	-.794**	0.231	-0.117
Ba	.851**	1	.954**	-.942**	.979**	-0.448	-0.165	0.454	0.561	.831**	.933**	-.968**	0.564	-0.480
Ca	.747*	.954**	1	-.898**	.913**	-0.486	-0.095	0.334	0.501	.874**	.947**	-.962**	0.579	-0.580
Cr	-.775**	-.942**	-.898**	1	-.918**	0.460	0.093	-0.320	-0.530	-.708*	-.856**	.900**	-0.566	0.441
Cu	.823**	.979**	.913**	-.918**	1	-0.568	-0.026	0.451	.658*	.729*	.945**	-.970**	0.572	-0.456
Fe	-0.063	-0.448	-0.486	0.460	-0.568	1	-.682*	0.195	-0.579	-0.139	-.658*	0.557	-0.455	0.590
K	-0.292	-0.165	-0.095	0.093	-0.026	-.682*	1	-0.435	0.559	-0.351	0.154	0.003	-0.199	-0.231
Mg	.664*	0.454	0.334	-0.320	0.451	0.195	-0.435	1	0.236	0.301	0.290	-0.409	0.361	0.445
Mn	0.607	0.561	0.501	-0.530	.658*	-0.579	0.559	0.236	1	0.316	.711*	-.647*	-0.054	-0.243
Na	.741*	.831**	.874**	-.708*	.729*	-0.139	-0.351	0.301	0.316	1	.779**	-.775**	0.287	-0.538
P	.730*	.933**	.947**	-.856**	.945**	-.658*	0.154	0.290	.711*	.779**	1	-.969**	0.478	-0.627
S	-.794**	-.968**	-.962**	.900**	-.970**	0.557	0.003	-0.409	-.647*	-.775**	-.969**	1	-0.552	0.527
Sr	0.231	0.564	0.579	-0.566	0.572	-0.455	-0.199	0.361	-0.054	0.287	0.478	-0.552	1	-0.207
Zn	-0.117	-0.480	-0.580	0.441	-0.456	0.590	-0.231	0.445	-0.243	-0.538	-0.627	0.527	-0.207	1

4.1.2.2. Elements across habitats

Out of the five fish investigated in this research, three (*T. ilisha*, *O. pama*, and *R. corsula*) were pelagic, and the others (*S. panijus* and *L. calcarifer*) were demersal fish. The scales of demersal fish had greater levels of Cr, Fe, S, Sr, and Zn (Figure 15). On the other hand, it was found that pelagic fish scales included greater amounts of Al, Ba, Ca, Cu, K, Mg, Mn, Na, and P. However, the variations in the concentrations of Al, Mn, and P were statistically significant ($P \leq 0.05$) between the habitats (Table 11).

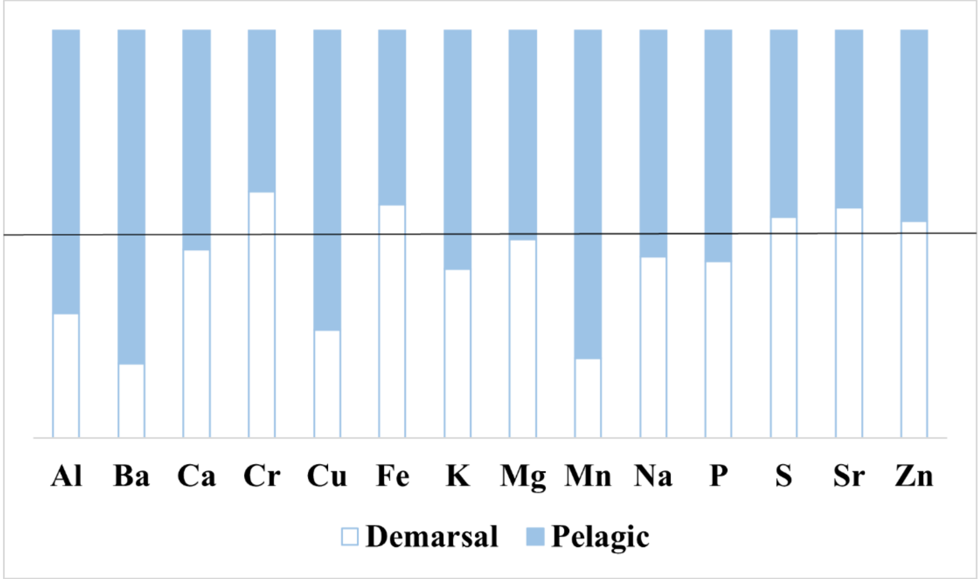
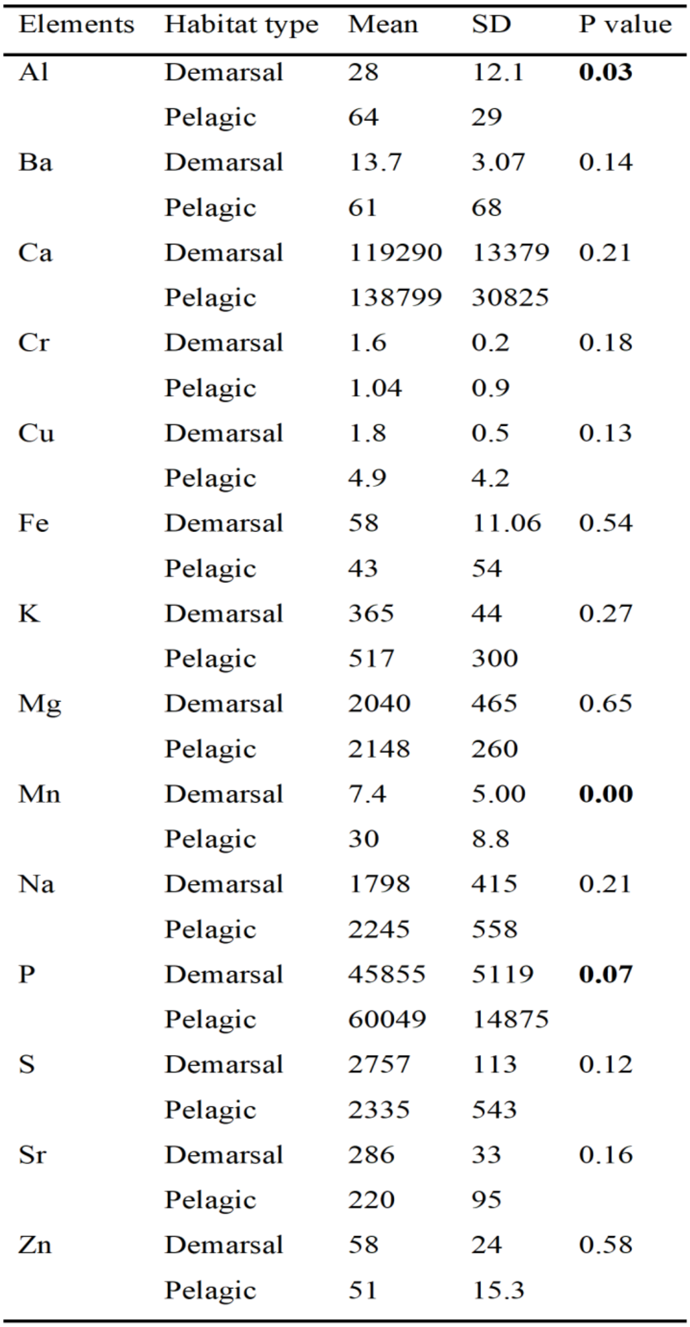


Figure 15. Element concentrations in the coastal migratory fish scales across habitats. The 100% stacked bar shows the % of the average value of the mean concentrations of fish types (Demersal and Pelagic) shared by each element's sum. The entire bar stands for 100%, and the horizontal line in the center of the bars indicates 50% of the bar for comparing the types (Demersal and Pelagic).

The pelagic fish *O. pama* possesses the maximum levels of K (893±95.4 mg/kg), Mn (36±0.9 mg/kg), and the lowest level of Fe (7.3±1.9 mg/kg). The greatest amounts of Al (96±9.5 mg/kg), Ba (149±2.02 mg/kg), Ca (178170±4006 mg/kg), Cu (10.3±0.4 mg/kg), Mg (2387±104.1 mg/kg), Na (2873±99 mg/kg), P (78489±780 mg/kg), and Sr (339±9.8 mg/kg) are found in *R. corsula*, a different pelagic species whereas, the lowest concentrations are found for Cr (<LoD), S (1648±183 mg/kg), and Zn (36±12.6 mg/kg).

Table 11. Statistical t-test results of the element concentrations in the coastal migratory fish scales across habitats.



Elements	Habitat type	Mean	SD	P value
Al	Demarsal	28	12.1	**0.03**
	Pelagic	64	29	
Ba	Demarsal	13.7	3.07	0.14
	Pelagic	61	68	
Ca	Demarsal	119290	13379	0.21
	Pelagic	138799	30825	
Cr	Demarsal	1.6	0.2	0.18
	Pelagic	1.04	0.9	
Cu	Demarsal	1.8	0.5	0.13
	Pelagic	4.9	4.2	
Fe	Demarsal	58	11.06	0.54
	Pelagic	43	54	
K	Demarsal	365	44	0.27
	Pelagic	517	300	
Mg	Demarsal	2040	465	0.65
	Pelagic	2148	260	
Mn	Demarsal	7.4	5.00	**0.00**
	Pelagic	30	8.8	
Na	Demarsal	1798	415	0.21
	Pelagic	2245	558	
P	Demarsal	45855	5119	**0.07**
	Pelagic	60049	14875	
S	Demarsal	2757	113	0.12
	Pelagic	2335	543	
Sr	Demarsal	286	33	0.16
	Pelagic	220	95	
Zn	Demarsal	58	24	0.58
	Pelagic	51	15.3	

4.1.2.3. Elements across feeding habits

There are two distinct feeding patterns among the fish species examined in this study: *T. ilisha* is planktivorous, whereas the other species are carnivorous. Carnivorous species had comparatively higher mean levels of Ba, Ca, Cu, K, Mn, P, and Sr, where the difference of Sr was significant (Figure 16, Table 12). 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 (113±19 mg/kg) among all the elements and the lowest levels of K (265±1.9 mg/kg) and Sr (136±4.9 mg/kg).

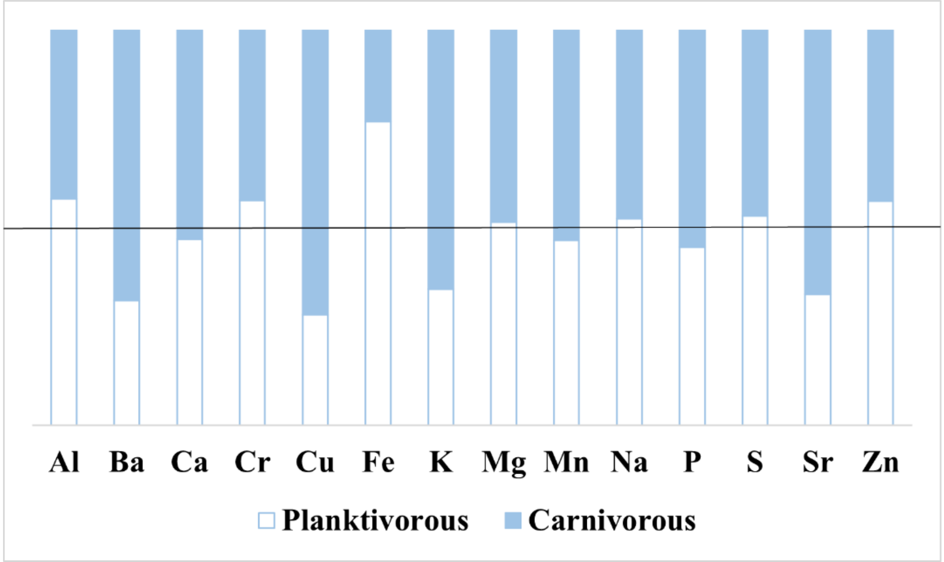
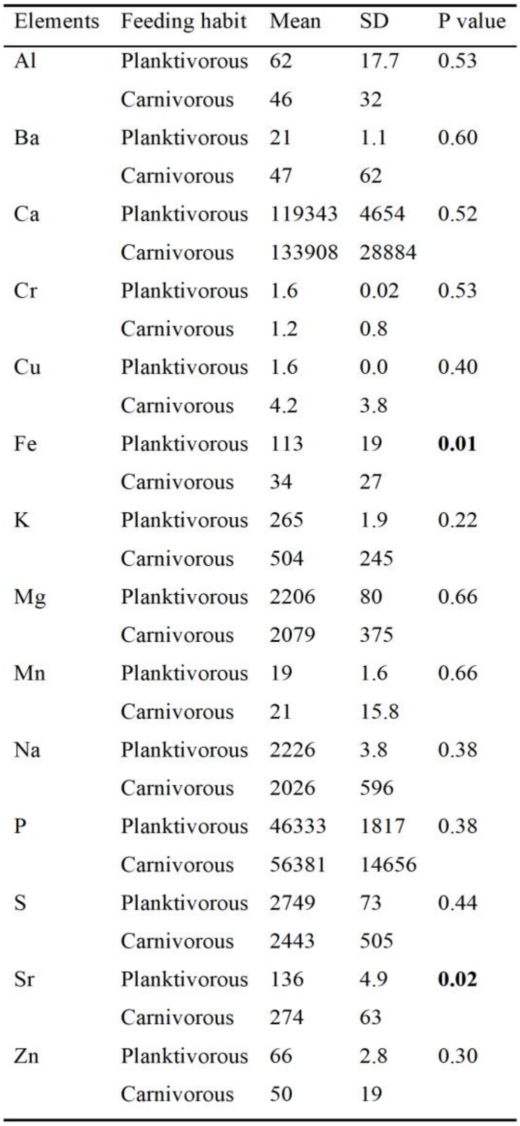


Figure 16. Element concentrations in the coastal migratory fish scales across feeding habits. The 100% stacked bar shows the % of the average value of the mean concentrations of fish types (Planktivorous and Carnivorous) shared by

each element's sum. The entire bar stands for 100%, and the horizontal line in the center of the bars indicates 50% of the bar for comparing the types (Planktivorous and Carnivorous).

Table 12. Statistical t-test results of the element concentrations in the coastal migratory fish scales across feeding habits.

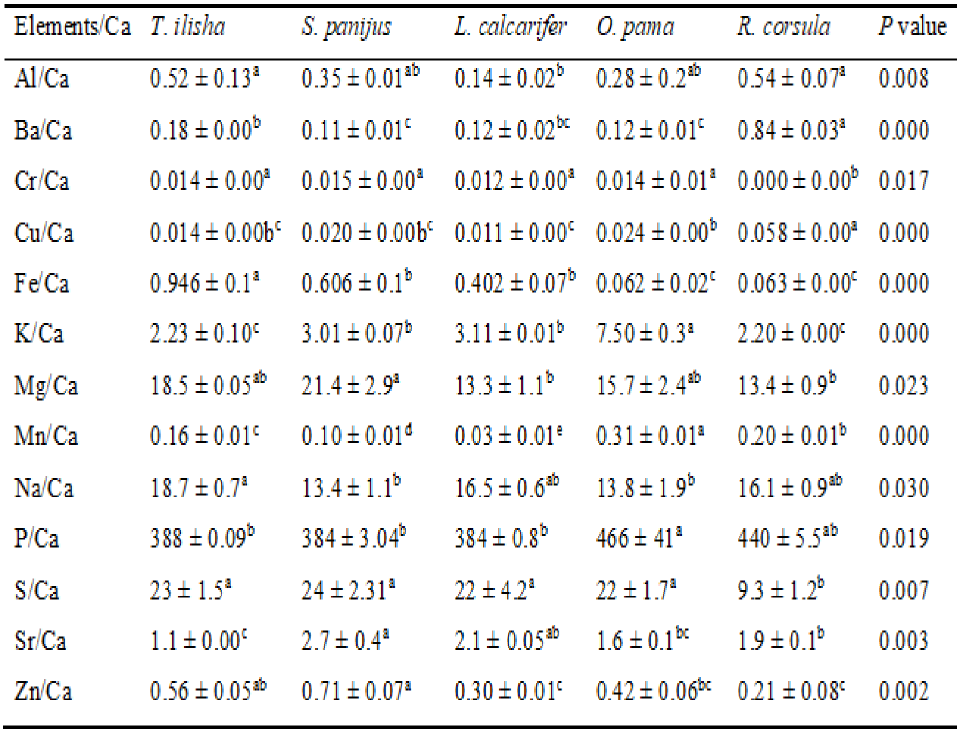


Elements	Feeding habit	Mean	SD	P value
Al	Planktivorous	62	17.7	0.53
	Carnivorous	46	32	
Ba	Planktivorous	21	1.1	0.60
	Carnivorous	47	62	
Ca	Planktivorous	119343	4654	0.52
	Carnivorous	133908	28884	
Cr	Planktivorous	1.6	0.02	0.53
	Carnivorous	1.2	0.8	
Cu	Planktivorous	1.6	0.0	0.40
	Carnivorous	4.2	3.8	
Fe	Planktivorous	113	19	**0.01**
	Carnivorous	34	27	
K	Planktivorous	265	1.9	0.22
	Carnivorous	504	245	
Mg	Planktivorous	2206	80	0.66
	Carnivorous	2079	375	
Mn	Planktivorous	19	1.6	0.66
	Carnivorous	21	15.8	
Na	Planktivorous	2226	3.8	0.38
	Carnivorous	2026	596	
P	Planktivorous	46333	1817	0.38
	Carnivorous	56381	14656	
S	Planktivorous	2749	73	0.44
	Carnivorous	2443	505	
Sr	Planktivorous	136	4.9	**0.02**
	Carnivorous	274	63	
Zn	Planktivorous	66	2.8	0.30
	Carnivorous	50	19	

4.1.2.4. Element: calcium ratios

The element: Ca ratios obtained in the coastal migratory fish scales are shown in Table 13. In the present investigation, *T. ilisha* had the maximum Fe/Ca, Na/Ca, and the minimum Sr/Ca ratios. Whereas *S. panijus* exhibited the highest Cr/Ca, Mg/Ca, S/Ca, Sr/Ca, Zn/Ca, and the lowest Ba/Ca, Na/Ca proportions. The current investigation revealed that *L. calcarifer* possessed the least Al/Ca, Cu/Ca, Mg/Ca, Mn/Ca, and P/Ca proportions. The *O. pama* exhibited the highest K/Ca, Mn/Ca, and P/Ca, while the lowest Fe/Ca ratios. The maximum proportions of Al/Ca, Ba/Ca, Cu/Ca, and the minimum proportions of Cr/Ca, K/Ca, S/Ca, and Zn/Ca are possessed by *R. corsula*.

Table 13. Element/Ca ratios (mean ± SD) in the coastal migratory fish scales. Superscript letters in the same row denote significant ($p < 0.05$) difference by Tukey's HSD test. (Note: Dividing the values by 10^3 yields actual values).



Elements/Ca	*T. ilisha*	*S. panijus*	*L. calcarifer*	*O. pama*	*R. corsula*	*P* value
Al/Ca	0.52 ± 0.13^{a}	0.35 ± 0.01^{ab}	0.14 ± 0.02^{b}	0.28 ± 0.2^{ab}	0.54 ± 0.07^{a}	0.008
Ba/Ca	0.18 ± 0.00^{b}	0.11 ± 0.01^{c}	0.12 ± 0.02^{bc}	0.12 ± 0.01^{c}	0.84 ± 0.03^{a}	0.000
Cr/Ca	0.014 ± 0.00^{a}	0.015 ± 0.00^{a}	0.012 ± 0.00^{a}	0.014 ± 0.01^{a}	0.000 ± 0.00^{b}	0.017
Cu/Ca	$0.014 \pm 0.00b^{c}$	$0.020 \pm 0.00b^{c}$	0.011 ± 0.00^{c}	0.024 ± 0.00^{b}	0.058 ± 0.00^{a}	0.000
Fe/Ca	0.946 ± 0.1^{a}	0.606 ± 0.1^{b}	0.402 ± 0.07^{b}	0.062 ± 0.02^{c}	0.063 ± 0.00^{c}	0.000
K/Ca	2.23 ± 0.10^{c}	3.01 ± 0.07^{b}	3.11 ± 0.01^{b}	7.50 ± 0.3^{a}	2.20 ± 0.00^{c}	0.000
Mg/Ca	18.5 ± 0.05^{ab}	21.4 ± 2.9^{a}	13.3 ± 1.1^{b}	15.7 ± 2.4^{ab}	13.4 ± 0.9^{b}	0.023
Mn/Ca	0.16 ± 0.01^{c}	0.10 ± 0.01^{d}	0.03 ± 0.01^{e}	0.31 ± 0.01^{a}	0.20 ± 0.01^{b}	0.000
Na/Ca	18.7 ± 0.7^{a}	13.4 ± 1.1^{b}	16.5 ± 0.6^{ab}	13.8 ± 1.9^{b}	16.1 ± 0.9^{ab}	0.030
P/Ca	388 ± 0.09^{b}	384 ± 3.04^{b}	384 ± 0.8^{b}	466 ± 41^{a}	440 ± 5.5^{ab}	0.019
S/Ca	23 ± 1.5^{a}	24 ± 2.31^{a}	22 ± 4.2^{a}	22 ± 1.7^{a}	9.3 ± 1.2^{b}	0.007
Sr/Ca	1.1 ± 0.00^{c}	2.7 ± 0.4^{a}	2.1 ± 0.05^{ab}	1.6 ± 0.1^{bc}	1.9 ± 0.1^{b}	0.003
Zn/Ca	0.56 ± 0.05^{ab}	0.71 ± 0.07^{a}	0.30 ± 0.01^{c}	0.42 ± 0.06^{bc}	0.21 ± 0.08^{c}	0.002

4.1.3. Freshwater-brackish water-marine fish scales

4.1.3.1. Scale chemistry and composition

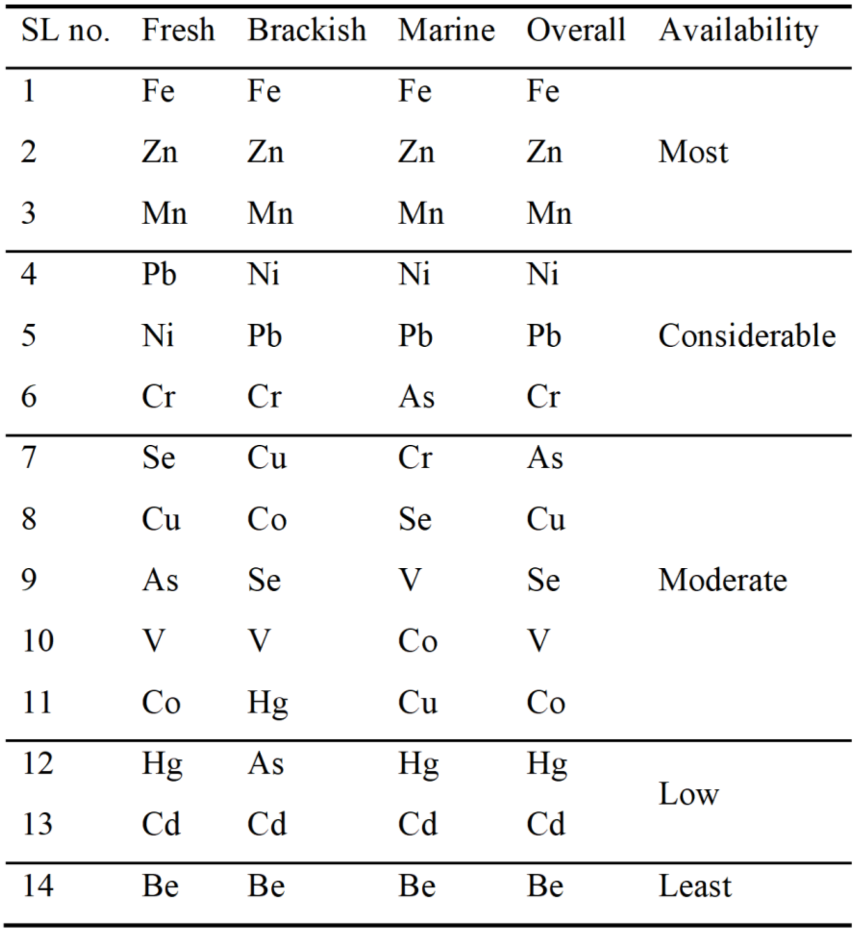
Different micro- and trace elements such as metals: Beryllium (Be), Cobalt (Co), Iron (Fe), Manganese (Mn), Nickel (Ni); transition metal Vanadium (V); metalloids: Arsenic (As), Selenium (Se), and potentially toxic elements Cadmium (Cd), Chromium (Cr), Copper (Cu), Mercury (Hg), Lead (Pb), Zinc (Zn) were investigated by ICP-MS in the study. The mean concentrations of the elements are presented in Table 14. The total length and weight (Mean ± SD) of the fish were 37±1.7 cm and 1083±105 g for *C. carpio*, 42±2.6 cm and 1040±169 g for *L. calcarifer*, and 41±0.7 cm and 881±58 g for *C. aureus*.

Table 14. Element concentrations (mean ± SD) in the freshwater-brackish water-marine fish scales. Asterisks (*) denote Dunn's pairwise multiple comparison post hoc test with Bonferroni correction, and significant P values ($P \leq 0.05$) are in bold fonts.

Elements	Observed mean concentrations (mg/kg)			
	Freshwater	Brackish water	Marine	P value
As	0.4±0.06	0.06±0.06*	1.6±0.9*	**0.007**
Be	0.01±0.01*	0.001±0.00*	0.005±0.01	**0.032**
Cd	0.06±0.04	0.01±0.01	0.07±0.05	0.087
Co	0.3±0.03a	0.2±0.03b	0.3±0.03a	**0.005**
Cr	2.3±0.8	1.5±0.7	1.3±0.7	0.218
Cu	0.7±0.2	0.9±1.3	0.3±0.2	0.092
Fe	403±73	482±70.6	799±470	0.098
Hg	0.1±0.1	0.09±0.13	0.09±0.08	0.666
Mn	24±2.3	5.3±1.05*	25±6.6*	**0.021**
Ni	2.8±0.3b	3.6±0.8ab	4.3±0.5a	**0.014**
Pb	3.2±2.2	1.5±1.4	2.7±2.8	0.500
Se	0.7±0.1a	0.2±0.1b	0.9±0.1a	**0.000**
V	0.4±0.2	0.2±0.02	0.5±0.5	0.292
Zn	141±29*	25±7.3*	32±2.8	**0.015**

The highest concentrations of Be, Cr, Hg, Pb, and Zn were observed in the scales of freshwater fish (*C. carpio*). Estuarine or brackish water fish (*L. calcarifer*) had the highest Cu in their scales. However, the maximum concentrations of As, Cd, Fe, Mn, Ni, Se, and V were found in marine fish (*C. aureus*) scales. The variations in the levels of As, Be, Co, Mn, Ni, Se, and Zn were statistically significant ($P \leq 0.05$) among all the elements detected.

Table 15. The hierarchy (largest to smallest order: top to bottom) of the elements in the freshwater-brackish water-marine fish scales.



SL no.	Fresh	Brackish	Marine	Overall	Availability
1	Fe	Fe	Fe	Fe	Most
2	Zn	Zn	Zn	Zn	
3	Mn	Mn	Mn	Mn	
4	Pb	Ni	Ni	Ni	Considerable
5	Ni	Pb	Pb	Pb	
6	Cr	Cr	As	Cr	
7	Se	Cu	Cr	As	Moderate
8	Cu	Co	Se	Cu	
9	As	Se	V	Se	
10	V	V	Co	V	
11	Co	Hg	Cu	Co	
12	Hg	As	Hg	Hg	Low
13	Cd	Cd	Cd	Cd	
14	Be	Be	Be	Be	Least

The hierarchy of the elements obtained in the current study was Fe>Zn>Mn>Pb>Ni>Cr>Se>Cu>As>V>Co>Hg>Cd>Be in freshwater fish. While in the scales of brackish water fish, the hierarchy of the elements obtained in the current study was Fe>Zn>Mn>Ni>Pb>Cr>Cu>Co>Se>V>Hg>As>Cd>Be. The hierarchy of the elements obtained in the current study was Fe>Zn>Mn>Ni>Pb>As>Cr>Se>V>Co>Cu>Hg>Cd>Be in marine fish. However, the overall hierarchy of the elements in the scales of all fish obtained in the current study was Fe>Zn>Mn>Ni>Pb>Cr>As>Cu>Se>V>Co>Hg>Cd>Be (Table 15).

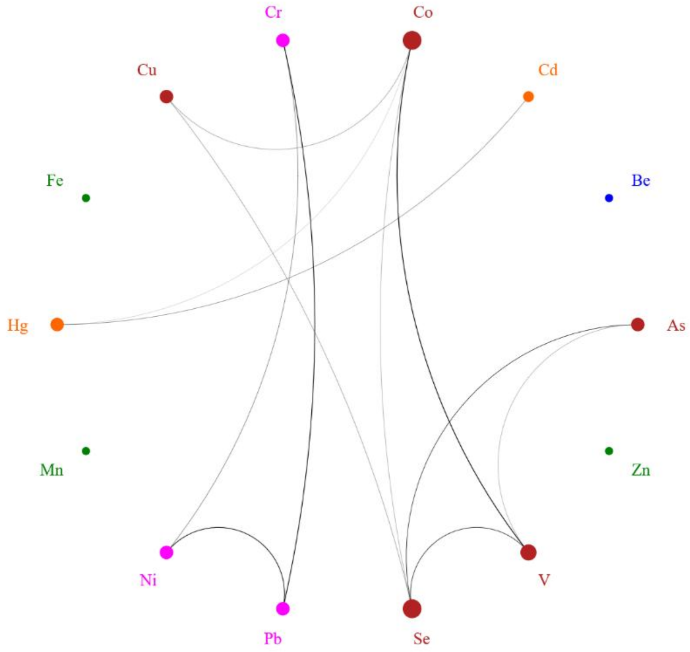


Figure 17. Network plot of elements in the freshwater-brackish water-marine fish scales.

The determined elements were classified into five categories based on their overall availability in the scales of all fish from fresh water, brackish water, and marine environment: most, considerable, moderate, low, and least available. These groupings are also depicted in the network analysis (Figure 17) and classical cluster analysis, dendrogram (Figure 18). Regardless of the environment and species, Fe, Zn, and P, respectively, were the most plentiful, hence are in the most available group (first) of elements in the fish scales of freshwater-brackish water-marine environments. The overall mean concentrations of the elements in the group varied from 18.4 to 562 mg/kg. The second group (considerable availability) consists of Ni, Pb, and Cr, and the overall mean concentrations of the group ranged from 1.7 to 3.6 mg/kg. However, As, Cu, Se, V, and Co form the third group of elements having considerable availability. Their overall mean levels ranged from 0.3 to 0.7 mg/kg. The elements, Hg and Cd, have low availability (fourth group), and their overall mean concentrations fluctuate between 0.05 and 0.1 mg/kg. While Be was found to have the least availability, with an overall mean concentration of 0.01 mg/kg only, it is therefore in the last group.

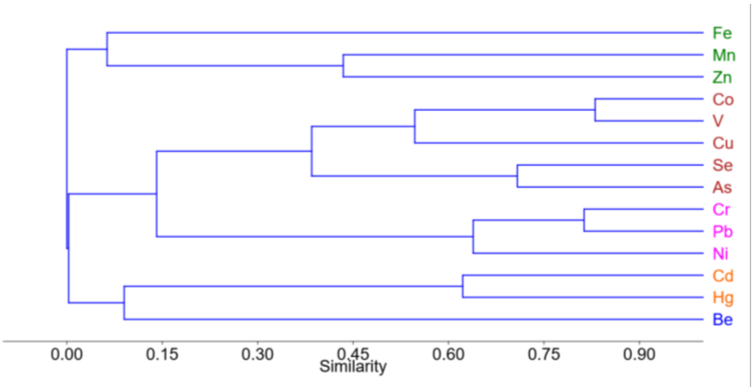
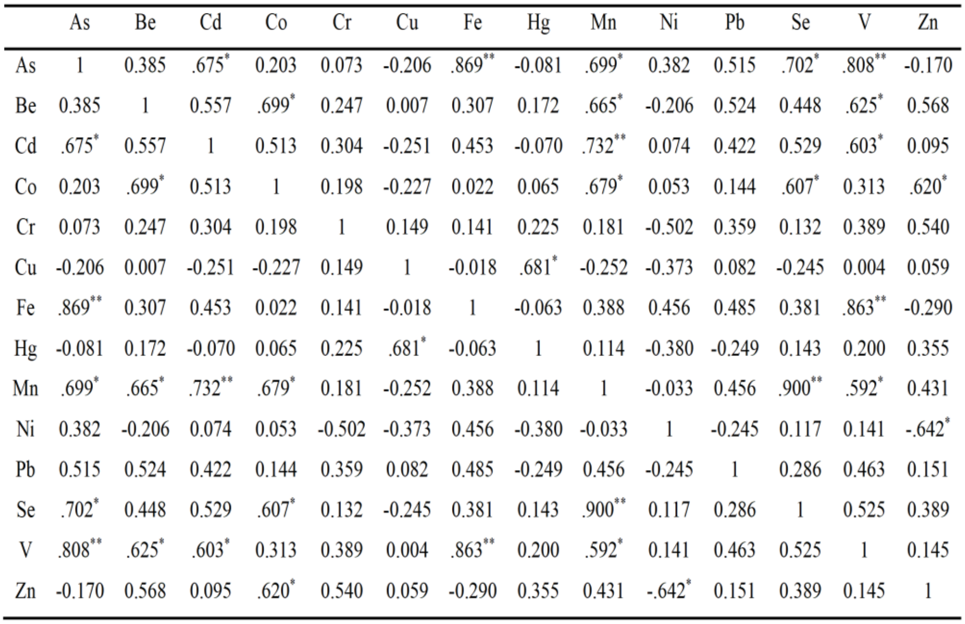


Figure 18. Dendrogram of elements in the freshwater-brackish water-marine fish scales.

The Pearson correlation coefficients represent the correlation among the elements detected in the freshwater-brackish water-marine fish scales (Table 16).

Table 16. The Pearson correlation coefficients of elements in the freshwater-brackish water-marine fish scales. “**” indicates correlation is significant at the 0.01 level (2-tailed) while “*” denotes correlation is significant at the 0.05 level (2-tailed).



	As	Be	Cd	Co	Cr	Cu	Fe	Hg	Mn	Ni	Pb	Se	V	Zn
As	1	0.385	.675*	0.203	0.073	-0.206	.869**	-0.081	.699*	0.382	0.515	.702*	.808**	-0.170
Be	0.385	1	0.557	.699*	0.247	0.007	0.307	0.172	.665*	-0.206	0.524	0.448	.625*	0.568
Cd	.675*	0.557	1	0.513	0.304	-0.251	0.453	-0.070	.732**	0.074	0.422	0.529	.603*	0.095
Co	0.203	.699*	0.513	1	0.198	-0.227	0.022	0.065	.679*	0.053	0.144	.607*	0.313	.620*
Cr	0.073	0.247	0.304	0.198	1	0.149	0.141	0.225	0.181	-0.502	0.359	0.132	0.389	0.540
Cu	-0.206	0.007	-0.251	-0.227	0.149	1	-0.018	.681*	-0.252	-0.373	0.082	-0.245	0.004	0.059
Fe	.869**	0.307	0.453	0.022	0.141	-0.018	1	-0.063	0.388	0.456	0.485	0.381	.863**	-0.290
Hg	-0.081	0.172	-0.070	0.065	0.225	.681*	-0.063	1	0.114	-0.380	-0.249	0.143	0.200	0.355
Mn	.699*	.665*	.732**	.679*	0.181	-0.252	0.388	0.114	1	-0.033	0.456	.900**	.592*	0.431
Ni	0.382	-0.206	0.074	0.053	-0.502	-0.373	0.456	-0.380	-0.033	1	-0.245	0.117	0.141	-.642*
Pb	0.515	0.524	0.422	0.144	0.359	0.082	0.485	-0.249	0.456	-0.245	1	0.286	0.463	0.151
Se	.702*	0.448	0.529	.607*	0.132	-0.245	0.381	0.143	.900**	0.117	0.286	1	0.525	0.389
V	.808**	.625*	.603*	0.313	0.389	0.004	.863**	0.200	.592*	0.141	0.463	0.525	1	0.145
Zn	-0.170	0.568	0.095	.620*	0.540	0.059	-0.290	0.355	0.431	-.642*	0.151	0.389	0.145	1

4.1.3.2. Elements across environments

The highest concentrations of Be, Co, Cr, Hg, Pb, and Zn were in the scales of freshwater fish (*C. carpio*). Among these, the levels of Be, Co, and Zn were significantly higher ($p \leq 0.05$) than their marine and brackish water counterparts. Brackish water or estuarine fish (*L. calcarifer*) had the highest Cu in their scales. Marine fish (*C. aureus*) had the highest levels of As, Cd, Fe, Mn, Ni, Se, and V. Among these, the levels of As, Mn, Ni, and Se were significantly higher ($p \leq 0.05$) than their fresh and brackish water counterparts.

4.1.3.3. 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*) (Figure 19). 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*) (Table 17). In general, benthopelagic species (*C. carpio* and *C. aureus*) demonstrated accumulation of more elements at higher levels compared to their counterpart.

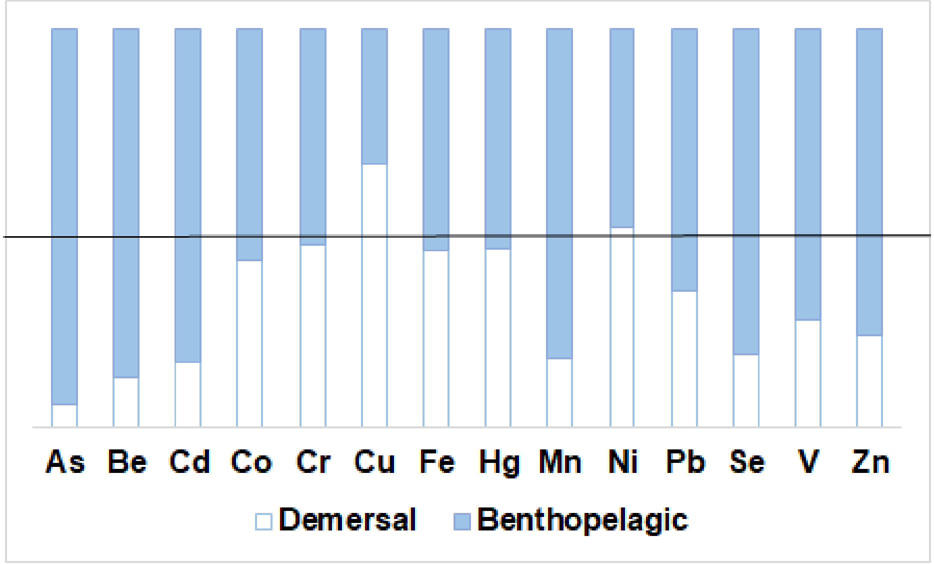
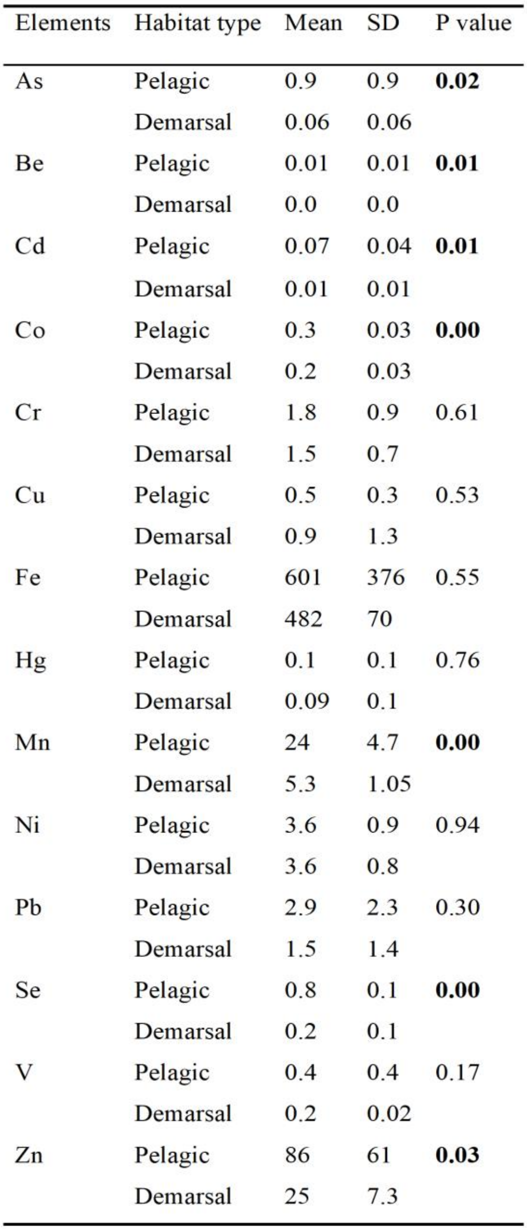


Figure 19. Element concentrations in the freshwater-brackish water-marine fish scales across habitats. The 100% stacked bar shows the % of the average value of the mean concentrations of fish types (Demersal and Benthopelagic) shared by each element's sum. The entire bar stands for 100%, and the horizontal line in the center of the bars indicates 50% of the bar for comparing the types (Demersal and Benthopelagic).

Table 17. Statistical t-test results of the element concentrations in the freshwater-brackish water-marine fish scales across habitats.



Elements	Habitat type	Mean	SD	P value
As	Pelagic	0.9	0.9	**0.02**
	Demarsal	0.06	0.06	
Be	Pelagic	0.01	0.01	**0.01**
	Demarsal	0.0	0.0	
Cd	Pelagic	0.07	0.04	**0.01**
	Demarsal	0.01	0.01	
Co	Pelagic	0.3	0.03	**0.00**
	Demarsal	0.2	0.03	
Cr	Pelagic	1.8	0.9	0.61
	Demarsal	1.5	0.7	
Cu	Pelagic	0.5	0.3	0.53
	Demarsal	0.9	1.3	
Fe	Pelagic	601	376	0.55
	Demarsal	482	70	
Hg	Pelagic	0.1	0.1	0.76
	Demarsal	0.09	0.1	
Mn	Pelagic	24	4.7	**0.00**
	Demarsal	5.3	1.05	
Ni	Pelagic	3.6	0.9	0.94
	Demarsal	3.6	0.8	
Pb	Pelagic	2.9	2.3	0.30
	Demarsal	1.5	1.4	
Se	Pelagic	0.8	0.1	**0.00**
	Demarsal	0.2	0.1	
V	Pelagic	0.4	0.4	0.17
	Demarsal	0.2	0.02	
Zn	Pelagic	86	61	**0.03**
	Demarsal	25	7.3	

4.1.3.4. Elements across feeding habits

The present study's findings demonstrated that element concentrations varied across the fish species examined, as a function of their feeding habit (Figure 20). There are two different feeding habits among the fish species investigated in this research, with *C. carpio* being omnivorous while *C. aureus* and *L. calcarifer* possess carnivorous feeding habits. The elements were found in an order of Fe>Zn>Mn>Pb>Ni>Cr>Se>Cu>As>V>Co>Hg>Cd>Be for omnivorous fish while Fe>Zn>Mn>Ni>Pb>Cr>Cu>Se>V>As>Co>Hg>Cd>Be for the carnivorous fish. The higher concentrations of As, Fe, and Ni were found in carnivorous fishes (*C. aureus* and *L. calcarifer*), where the amount of Ni is statistically significant ($P \leq 0.05$) (Table 18).

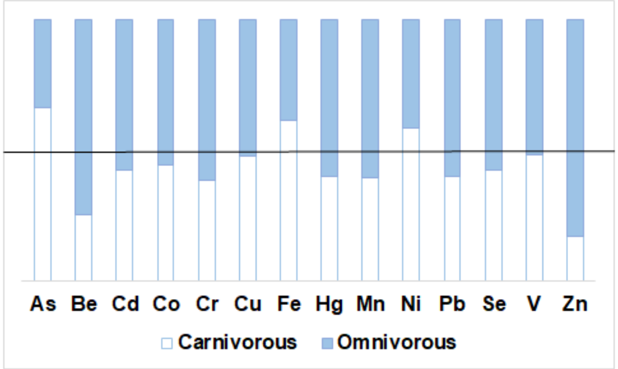
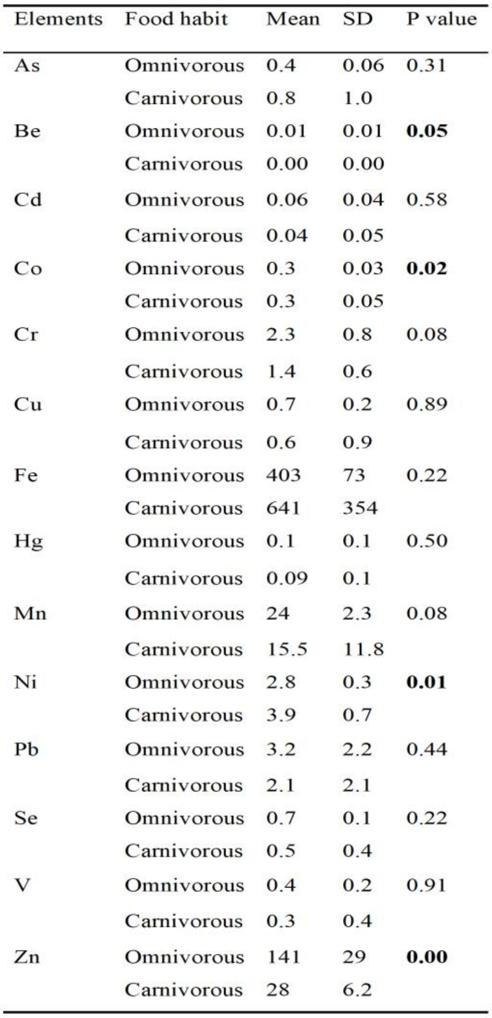


Figure 20. Element concentrations in the freshwater-brackish water-marine fish scales across feeding habits. The 100% stacked bar shows the % of the average value of the mean concentrations of fish types (Carnivorous and Omnivorous) shared by each element's sum. The entire bar stands for 100%, and the horizontal line in the center of the bars indicates 50% of the bar for comparing the types (Carnivorous and Omnivorous).

Omnivorous fish (*C. carpio*), on the other hand, observed to have 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 ($P \leq 0.05$) Table 18.

Table 18. Statistical t-test results of the element concentrations in the freshwater-brackish water-marine fish scales across the feeding habits.



Elements	Food habit	Mean	SD	P value
As	Omnivorous	0.4	0.06	0.31
	Carnivorous	0.8	1.0	
Be	Omnivorous	0.01	0.01	**0.05**
	Carnivorous	0.00	0.00	
Cd	Omnivorous	0.06	0.04	0.58
	Carnivorous	0.04	0.05	
Co	Omnivorous	0.3	0.03	**0.02**
	Carnivorous	0.3	0.05	
Cr	Omnivorous	2.3	0.8	0.08
	Carnivorous	1.4	0.6	
Cu	Omnivorous	0.7	0.2	0.89
	Carnivorous	0.6	0.9	
Fe	Omnivorous	403	73	0.22
	Carnivorous	641	354	
Hg	Omnivorous	0.1	0.1	0.50
	Carnivorous	0.09	0.1	
Mn	Omnivorous	24	2.3	0.08
	Carnivorous	15.5	11.8	
Ni	Omnivorous	2.8	0.3	**0.01**
	Carnivorous	3.9	0.7	
Pb	Omnivorous	3.2	2.2	0.44
	Carnivorous	2.1	2.1	
Se	Omnivorous	0.7	0.1	0.22
	Carnivorous	0.5	0.4	
V	Omnivorous	0.4	0.2	0.91
	Carnivorous	0.3	0.4	
Zn	Omnivorous	141	29	**0.00**
	Carnivorous	28	6.2	

4.2. Plant leaves

4.2.1. Deposition of microplastics

We found MPs of the polyethylene terephthalate (PET) category in *P. longifolia* leaves dust in both industrial and residential locations. The levels of other types of MPs were below the limit of detection. PET concentrations ranged from 789 µg/g to 1243 µg/g, in industrial regions and from 591 µg/g to 672 µg/g in residential locations in Dhaka (Figure 21).

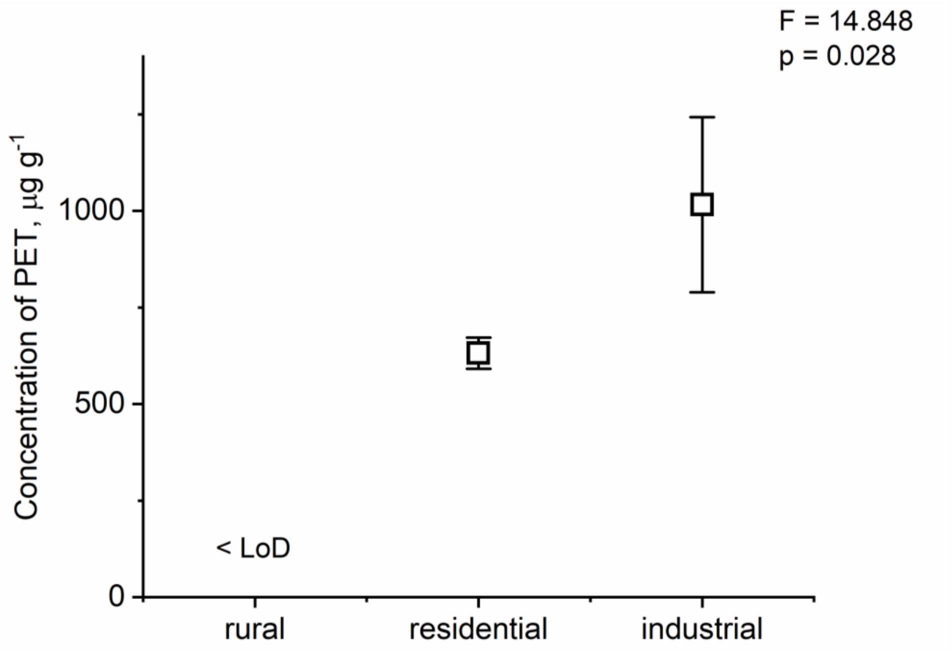
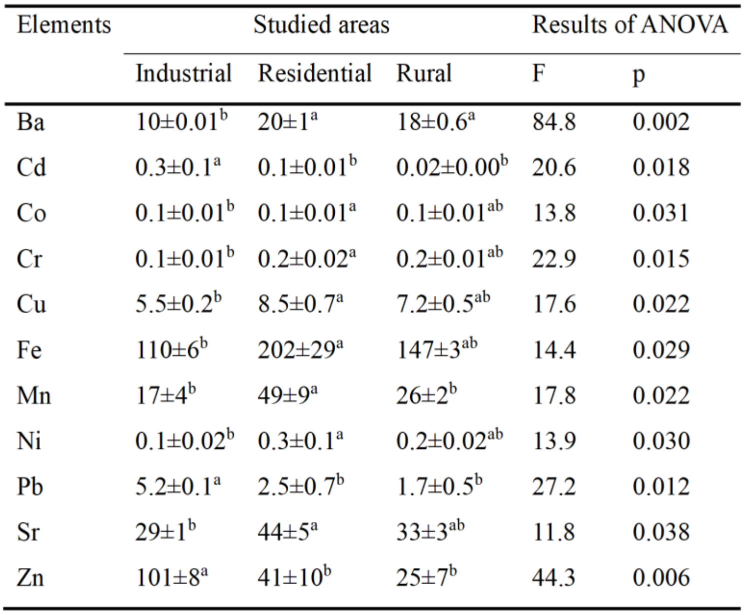


Figure 21. PET concentrations (mean ± SD) in the deposited dust on leaves. LoD: limit of detection.

4.2.2. Elements in leaves

The concentrations of elements, Ba, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Sr, and Zn were determined in the leaves of *P. longifolia* (Table 19). The hierarchy of the levels of elements in the rural area: Fe>Sr>Mn>Zn>Ba>Cu>Pb>Cr>Ni>Co>Cd; in the residential area: Fe>Mn Sr>Zn>Ba>Cu>Pb>Ni>Cr>Co>Cd; and in the industrial area: Fe>Zn>Sr>Mn>Ba>Cu>Pb>Cd>Cr>Ni>Co.

Table 19 Element concentrations in leaves (mean ± SD, mg/kg). Superscript letters in the same row denote significant ($p < 0.05$) differences by Tukey's HSD test.



Elements	Studied areas			Results of ANOVA	
	Industrial	Residential	Rural	F	p
Ba	10±0.01^{b}	20±1^{a}	18±0.6^{a}	84.8	0.002
Cd	0.3±0.1^{a}	0.1±0.01^{b}	0.02±0.00^{b}	20.6	0.018
Co	0.1±0.01^{b}	0.1±0.01^{a}	0.1±0.01^{ab}	13.8	0.031
Cr	0.1±0.01^{b}	0.2±0.02^{a}	0.2±0.01^{ab}	22.9	0.015
Cu	5.5±0.2^{b}	8.5±0.7^{a}	7.2±0.5^{ab}	17.6	0.022
Fe	110±6^{b}	202±29^{a}	147±3^{ab}	14.4	0.029
Mn	17±4^{b}	49±9^{a}	26±2^{b}	17.8	0.022
Ni	0.1±0.02^{b}	0.3±0.1^{a}	0.2±0.02^{ab}	13.9	0.030
Pb	5.2±0.1^{a}	2.5±0.7^{b}	1.7±0.5^{b}	27.2	0.012
Sr	29±1^{b}	44±5^{a}	33±3^{ab}	11.8	0.038
Zn	101±8^{a}	41±10^{b}	25±7^{b}	44.3	0.006

4.2.3. Pollution index

The PI values obtained in the study are shown in Table 20. The PI for Cd was higher than 1 in rural regions, suggesting a moderate degree of pollution; it was higher than 3 in residential sites, indicating considerable pollution; and higher than 6 in industrial areas, indicating very high pollution in Dhaka City's industrial activity (Figure 22). The PI for Pb was less than 1 in rural regions, indicating low pollution, and ranged from 1 to 3 in residential and industrial sites, indicating moderate contamination (Figure 23).

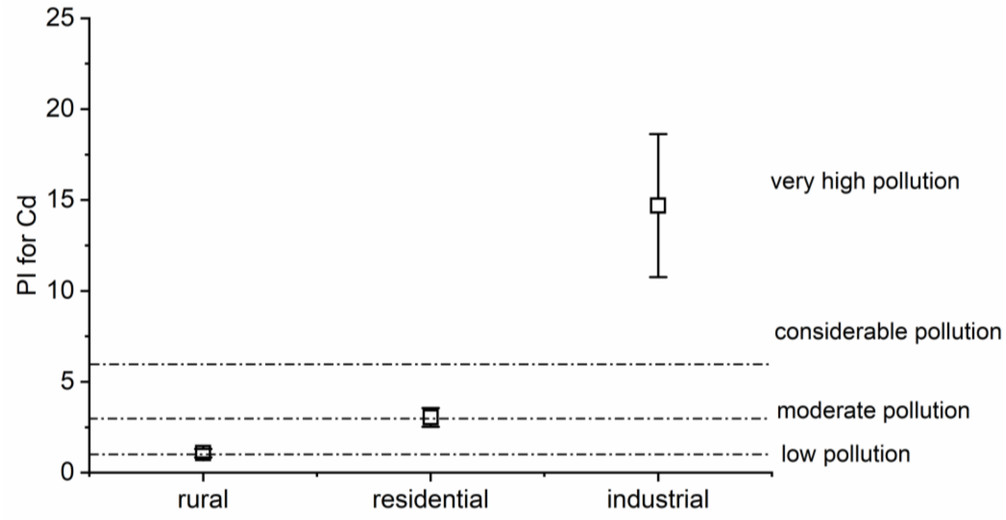


Figure 22. The PI of Cd in the study areas (mean ± SD).

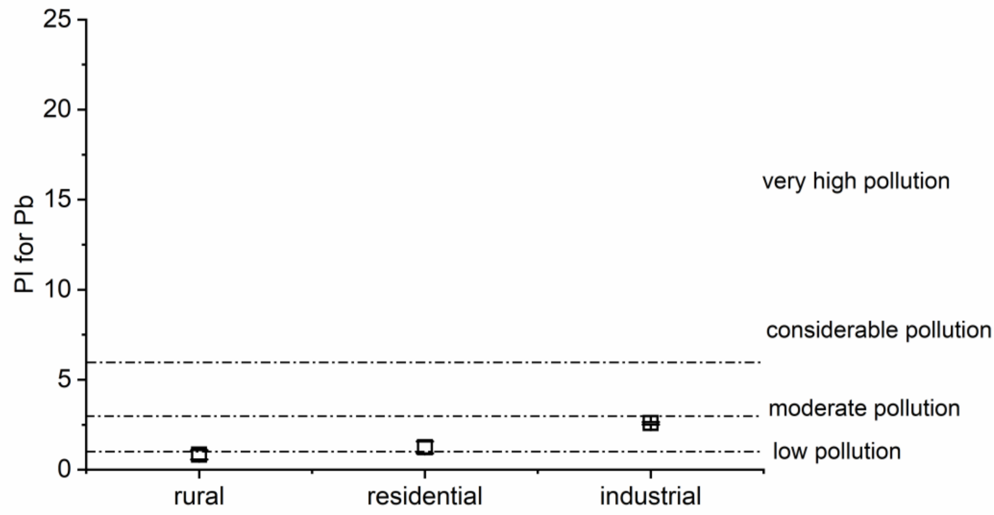


Figure 23. The PI of Pb in the study areas (mean ± SD).

Table 20. Pollution index values (mean ± SD) in the study areas and the results of ANOVA analysis. Superscript letters in the same row indicate significant differences ($p < 0.05$) by Tukey's HSD test.

Metals	Studied areas			Results of ANOVA	
	Rural	Residential	Industrial	F value	P value
Cd	1.07 ± 0.2^{b}	3.04 ± 0.5^{b}	14 ± 3.9^{a}	20.6	0.018
Cr	0.1 ± 0.01^{ab}	0.2 ± 0.01^{a}	0.1 ± 0.01^{b}	21.8	0.016
Cu	0.7 ± 0.05^{ab}	0.9 ± 0.07^{a}	0.6 ± 0.01^{b}	19.4	0.019
Ni	0.02 ± 0.01^{ab}	0.03 ± 0.01^{a}	0.01 ± 0.01^{b}	10.5	0.044
Pb	0.8 ± 0.3^{b}	1.2 ± 0.3^{b}	2.6 ± 0.07^{a}	27.9	0.012

Chapter 5: Discussion

5.1. Fish scales

5.1.1. Freshwater fish

5.1.1.1. Scale chemistry and composition

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). The overall weight percent of the elements in the first group ranges from 2.3166 to 8.9116, while that of the second group is 0.0108. Ca and P were also the most prevalent in the scales of *Labeo rohita* (Brraich and Jangu, 2013). Usually, Ca and P are the most abundant in scales since these molecules are important for scale structure and strength (Chuaychan et al., 2016).

The network plot analysis depicts the relationships among the elements, indicating their similarity in general type and possible sources. This is represented by the interconnecting network. The thickness of the lines and node size indicate the strength of the similarity and bonding in the groups (Hammer, 2024). The hierarchical clustering analysis also shows connections and similarities in the element groups. From these, we can infer that there is a relationship between the elements within the groups and some dissimilarity in the elements in other groups. In our study, Ca and P are the most abundant and essential elements forming the scales and are also found at the highest level, followed by K and the other element groups. The elements in the last group include Cu, which is among the heavy metals, and although it has important roles, but could be harmful when accumulated in excess.

The elements include heavy metal, for example, Cu. Although these are important for physiology but could be detrimental when present in excess. The accumulation of different heavy metals, such as Cd and Hg, was also reported in the tissue of *C. mrigala* by XRF analysis in previous research (Soe Aye et al., 2018). Behrooz et al. (2012) also reported mercury in the scales and muscles of *C. idella*. Teodorof et al. (2009) analyzed Cd, As, Pb, and Zn in the scales, muscle, gill, and liver of *C. idella* and obtained the highest metal content in the scales. The presence of titanium in fish, even at a low level, is alarming due to its potential toxicity. After acute exposure, Ti was found to accumulate in the liver, muscle, and brain, as well as reduce muscular acetylcholinesterase activity, indicating neurotoxic potential in *Prochilodus lineatus* (Carmo et al., 2019). TiO2 nanoparticles were accumulated in body tissues and were found to be genotoxic and potentially cytotoxic to *Trachinotus carolinus* (Vignardi et al., 2015). However, the observed heavy metals in the scales of *C. idella* and *C. mrigala* during the current study could have originated from natural sources as well as from direct and indirect anthropogenic activities. Both natural and man-made sources emit heavy metals in the aquatic ecosystem. Various point and nonpoint sources, including industrial discharge and agricultural runoff, could be possible anthropogenic sources. Different elements detected in the current study by the μ-XRF technique were analyzed in the study. It was demonstrated that this approach was a great one, especially for analyzing fish scales.

5.1.1.2. Elements across habitats

Scales of benthopelagic fish dwelling in the water column (*C. idella*) were observed to possess higher Ca, K, Mn, and Zn than those in demersal fish (*C. mrigala*). Being a benthopelagic fish, *C. idella* lives in the mid-lower layer of the water column. They can nevertheless acquire nutrients from water and from consuming primary producers as well. On the other side, the demersal fish (*C. mrigala*) have higher Cu, Fe, P, S, Sr, and Ti in the scales. Bottom-living (*C. mrigala*) possesses higher values of more elements, six out of ten accumulated in the scales, than their counterpart in the current study. It is more likely for the species that live on the bottom to have acquired more components with higher values than their counterparts. Rajar et al., (2025) also found that the tissues of the bottom-feeder fish had higher levels of metals than those of the other fish species. Living in close proximity to the sediment, illiophagous *C. mrigala* is subjected to a variety of geochemical circumstances. Sediments act as a sink for elements such as Cu, Fe, and Ti that settle out of the water column, originating from geological or terrestrial sources (El-Sorogy et al., 2021; Gülşen-Rothmund et al., 2023; Larson et al., 2015).

The decomposition of organic detritus is a significant source of P in sediments. Sr and Ti are frequently linked with calcium in sedimentary mineral formations (e.g., carbonates, titanium dioxide). Ti, originating from volcanic minerals or terrestrial runoff, is a prevalent lithogenic element in sediments (Larson et al., 2015; Liu et al., 2024). As a result, water deposition, organic matter decomposition, minerals like iron oxides, and anthropogenic activities can all contribute to the metal richness of bottom sediments. Decomposition of organic matter, volcanic activity, mineral weathering, and sulfide oxidation are natural sources of dissolved sulfur oxides in freshwater environments (Zak et al., 2021). Detritus and decaying organic matter in sediments are rich in amino acids having sulfur, such as cysteine and methionine (Brock, 1978; Kiene et al., 1990), resulting in increased S incorporation into structural proteins such as collagen in scales. Demersal *C. mrigala* may assimilate more Sr because it is more readily available in benthic sediments (e.g., from groundwater or carbonate dissolution) than herbivorous *C. idella* scales. Additionally, Sr levels could rise above Ca by replacing Ca with Sr in biomineralized structures, including scales (Courtemanche et al., 2006).

5.1.1.3. Elements across feeding habits

Fish species can gain various elements in their body as a result of their varied feeding habits (Ling et al., 2013; Pourang, 1995). Being a herbivorous fish *C. idella*, feed mainly on aquatic vegetation (Li et al., 2023). Aquatic plants uptake Ca from water and are naturally rich in K (Fox et al., 2018). Aquatic macrovegetation may concentrate Mn and Zn, but sediments may store these metals in forms less accessible to illiophagous or filter feeders. Even while herbivores often eat lower trophic levels, they can nevertheless acquire nutrients from primary producers. On the contrary, *C. mrigala* is an illiophagous fish that lives on decayed vegetation and decomposing organic materials and might have lost their mineralization as they decompose. Herbivorous *C. idella* living in the water column and taking primarily aquatic vegetation has a chance to obtain more Ca, K, Mn, and Zn through eating aquatic vegetation since the hydrophytes are capable of absorbing these minerals into their body from the surrounding water medium, they live (Fox et al., 2018), and absorption from the surrounding water (Khawar et al., 2024; Pourang, 1995; Varol et al., 2022). The illiophagous fish (*C. mrigala*), on the other hand, could get most of their minerals, like had higher Cu, Fe, P, S, Sr, and Ti in their scales, from taking food close to the sediment and sediment itself to get incorporated into their scales. On the other hand, herbivorous *C. idella* feeding on phytoplankton or macrophytes with lower metal levels are less likely to acquire these elements. Sulfur may be high in the illiophagous *C. mrigala* due to the ingestion of sulfate-reducing bacteria or sulfur-containing substances in the anoxic sediment layers. Therefore, the findings suggest that it concerns their feeding habits and the source of their food, which to be accumulated in the fish scales. Various studies pointed out that fish feeding patterns and living environments have profound effects on the accumulation of minerals in their body (Wu et al., 2023).

5.1.2. Coastal migratory fish

5.1.2.1. Scale chemistry and composition

The detected elements have been organized into five categories based on their overall concentration as most, moderate, low, and least available. The first category includes Ca and P, and they are the most prevalent in the examined scales, regardless of the fish. Ca is the most abundant element, followed by P, in all species investigated. The present investigation is in conformity with the observation of Brraich and Jangu, (2013) where Ca (38 %) was the most abundant element, followed by P (31 %), found in the *Labeo rohita* scale. Scales generally include a large number of Ca phosphate (Chuaychan et al., 2016). In the hierarchy of fishes, the second group includes the considerably available elements S, Mg, and Na, which rank third to fifth. Ca, P, S, Mg, and Na may therefore be regarded as macroelements. The third category, which is moderately available, is made up of K and Sr. Regardless of species, K is ranked sixth in the hierarchy while Sr is ranked seventh. The fourth group, which includes the most elements - Zn, Fe, Al, Ba, and Mn - has low availability and is ranked eighth to twelfth. K, Sr, Zn, Fe, Al, Ba, and Mn can therefore be thought of as microelements. Since Cu and Cr are the last and least available group, they could be referred to as trace elements because they are ranked either thirteenth or fourteenth in the concentration hierarchy. This elemental assortment is apparent in the network analysis chart (Figure 13) and the standard cluster analysis, dendrogram (Figure 14). In the analysis, we formed a network displaying similarities (Bray-Curtis similarity index) based on the concentration elements detected in this research.

The elements identified in the present study include both essential elements and certain Heavy metal, such as Cu, Cr, Zn, and Fe, that may be dangerous at levels above a threshold. Nevertheless, of all the components found, their concentration was the lowest (Tables 7 and 8). Jovičić et al. (2023) found Heavy metal in five different fish species' scales at varied levels. Rahman et al. (2018) also reported higher Heavy metal, Cu (19±0.05 µg/g), and Zn (110±0.27 µg/g) concentrations in the *L. calcarifer* scale than in the present study, which suggests that the current study region is somewhat less polluted. However, the Heavy metal might have been sourced because of human actions such as sewage and industrial waste, which entered coastal waterways through river outflows. Rivers that discharge into the coastal areas of Bangladesh include large areas of aquaculture and agricultural operations that employ a variety of pesticides and fertilizers.

5.1.2.2. Elements across habitats

Demersal fish living in proximity to benthic sediments that are rich in elements like Cr, Fe, S, Sr, and Zn, originating from natural weathering or anthropogenic sources (e.g., industrial runoff) could cause elevated levels of these elements in their body (El-Sorogy et al., 2021; Gülşen-Rothmund et al., 2023; Larson et al., 2015). Sr levels could rise above Ca by replacing Ca with Sr (Courtemanche et al., 2006). Sulfur may come from sulfate-reducing bacteria that break down sulfate in sediments (Jørgensen et al., 2019). Whereas Sr and Zn may build up through sediment ingestion and the consumption of benthic organisms, like macroinvertebrates. Continuous exposure to dissolved elements and suspended particles in coastal waters could be the reason for the elevated concentrations of Al, Ba, Ca, Cu, K, Mg, Mn, Na, and P in pelagic fish. For example, Al from urban runoff, industrial discharges, acid sulfate soils, and clay minerals (Angel et al., 2015); Ba from biogenic mineral barite (BaSO4) in plankton (Jacquet et al., 2007). The Ganges-Brahmaputra-Meghna river system contributes substantial amounts of Al, Mn, and P in the coastal waters of Bangladesh, particularly in surface waters where pelagic species dwell. While demersal fish are more exposed to sediment-bound metals, they are less impacted by these inputs. Al has a greater direct impact on pelagic zones due to its abundance in riverine runoff. While Ca and P are major components of biomineralized structures like scales, reflecting the consumption of more plankton in the pelagic habitat. Higher Ca and P levels may indicate the need for more scale strength for quick swimming in pelagic species. Pelagic fish, *Tenualosa ilisha*, for instance, a migratory fish that needs effective osmoregulation to drive uptake of Na, K, and Mg. Mn is redox-sensitive (Hummer et al., 2022); pelagic oxic environments may enhance dissolved Mn intake, while demersal sediments can sequester Mn under reducing circumstances.

Compared to pelagic fishes, which inhabit the water column, demersal fishes tend to have a greater likelihood of incorporating a higher quantity of metals because of their proximity to sediment (Garnero et al., 2018). *S.*

panijus, a demersal fish, had the maximum level of Zn (78±3.6 mg/kg), followed by Cr (1.6±0.2 mg/kg), and S (2757±107 mg/kg), but the lowest concentrations of Ba (11.9±0.7), Ca (111767±6074 mg/kg), Na (1502±206 mg/kg), and P (42984±1997 mg/kg). Contrary to the present finding, Gull et al. (2024) showed lower amounts of zinc in demersal species in contrast to their pelagic counterparts. *L. calcarifer*, another demersal fish, had the lowest level of Cu (1.3±0.1 mg/kg), followed by Al (17.6±0.3 mg/kg), Mg (1693±362 mg/kg), and Mn (3.2±1.3 mg/kg) in this study. Nevertheless, a prior study found that Zn (79 to 84 mg/kg) was elevated in the pelagic fish, while Fe (541 to 649 mg/kg) and Cu (3.09 to 3.6 mg/kg) were raised in the demersal fish, which is incompatible with the current results (Rejomon et al., 2010).

However, these variations can be attributed to differences in the analysis methodology (e.g. the use of different samples and equipment) and in the availability and accessibility of the elements across different locations owing to different biogeochemical features. The level of elements that are assimilated in a fish's body depends on the species, trophic position, food intake, and environmental characteristics of the ecosystem (Garnero et al., 2018). Teleost scale metal levels fluctuate with species, between 16% and 59% (Seshaya et al., 1963). The water chemistry plays an important role in the fish scale metal composition (Wells, 2000). The scale's elemental composition is closely related to the chemicals in the environment in which they live (Brraich and Jangu, 2013). Thus, variations in water chemistry, resulting from geographical location or other elements, such as temperature fluctuations, can affect scale composition (Kerr and Campana, 2014). Therefore, the quantity of elements absorbed in fish scales might vary according to the species, flow seasons, and the water bodies (Shakir et al., 2020).

5.1.2.3. Elements across feeding habits

Carnivores had higher levels of K and Sr, while planktivorous fishes had higher levels of Fe. It is observed that carnivorous fish had the highest quantity 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 (113±19 mg/kg) out of the elements detected and the lowest levels of K (265±1.9 mg/kg) and Sr (136±4.9 mg/kg). This suggests that carnivorous animals have a relatively higher propensity for accumulating metals than their planktivorous fish. These findings are consistent with Jabeen et al. (2011), who found that carnivorous and herbivorous fish were shown to accumulate metals in their organs differently; carnivorous species had significantly ($p<0.05$) greater amounts of Al, As, Cr, Ni, and Zn. Ling et al. (2013), also obtained that planktivorous species typically have lower body metal accumulation rates. Carnivorous fish that eat smaller fish, crustaceans, and zooplankton have the potential to amass higher levels of metals via biological magnification. Intriguingly, carnivorous fish are more popular among consumers overall, despite the fact that eating them has increased the likelihood of ingestion, indicating a risk to public health (Silva et al., 2014).

5.1.3. Freshwater-brackish water-marine fish

5.1.3.1. Elements across freshwater-brackish water-marine environments

The scales of freshwater fish (*C. carpio*) were found to have the greatest quantities of Be, Co, Cr, Hg, Pb, and Zn. Among these, the levels of Be, Co, and Zn were significantly higher than their marine and brackish water counterparts. The weathering of the Earth's surface increases the presence of metals such as beryllium (Be), cobalt (Co), chromium (Cr), lead (Pb), and zinc

(Zn) in freshwater systems (Ali et al., 2019). These could also come from anthropogenic activities, like runoff from industry and agriculture. Freshwater bodies are likely to receive metals from urban and industrial sources, such as Pb from the previous use of leaded gasoline, batteries, and paints; Zn from galvanizing and tires; and Cr from tanneries and electroplating. Coal combustion releases Hg, which can deposit onto terrestrial systems and finally wash into waterways (Streets et al., 2018). Mercury (Hg), especially methylmercury, transforms into methylmercury in aquatic systems and then bio-accumulates in the food chain (Al-Sulaiti et al., 2022), reaching high levels in predatory species. Freshwater sediments tend to store elements like Cr, Pb, Hg, and Zn (Soetan et al., 2024). With its voracious feeding nature, *C. carpio* seems to ingest sediment (Wahab et al., 2002). Consequently, the elements stored therein could be taken up by the fish during sediment ingestion. It was reported that, given its function as a major metal sink in the water bodies, sediment plays a significant role in the uptake of metal by *C. carpio* (Gwimbi et al., 2020). Furthermore, the dearth of chloride ions in freshwater reduces the precipitation of metals, resulting in enhanced bioavailability of metals for uptake by fish (Magalhães et al., 2015). Additionally, smaller water volumes in freshwater systems compared to oceans result in less dilution, allowing pollutants to accumulate more easily.

Brackish water or estuarine fish (*L. calcarifer*) had the highest Cu in their scales. Because the salinity can fluctuate significantly over a relatively short distance (10–32 m) and throughout the day due to tidal cycles, estuaries are extremely changeable habitats (Rich and Maier, 2015). In estuaries, organic ions could perform as metal ligands, creating coordination compounds that reduce free metal cations, thus lessening their bioavailability (Sánchez-Marín et al., 2007). Coordination complexes are often not capable of crossing the barrier, for example, membranes, due to their greater molecular weights

(Richards et al., 1999). Estuarine brackish water environments are dynamic habitats that produce a diverse range of organic and inorganic substances when freshwater and seawater combine. Organic ions could originate from man-made sources such as complex compounds used in detergents, industrial processes and exist naturally as well, like humic and fulvic acids (Reeve, 2022). Because of the region's dense population, agricultural and industrial discharges, estuaries in Bangladesh are more likely to have abundant organic ions produced in combination with natural and man-made processes. For example, coastal mangrove plants possess tannins and lignins (Irman et al., 2022) that could be exuded into the estuarine waters. Thus, the organic compounds from Sundarbans, the world’s largest mangrove forest, along with other sources acting as metal ligands, could potentially hinder common metal cations from being absorbed into fish scales at higher levels.

Estuaries often lie near urban and industrial discharge sites, resulting in elevated copper contamination from antifouling coatings, wastewater, and stormwater drainage. Copper-based fungicides (e.g., Bordeaux) and fertilizers used in agriculture can leach into rivers and accumulate in estuaries (Pesce et al., 2024). Estuaries experience heavy vessel traffic, leading to possible Cu leaching from antifouling paints used in ships and boats. The shipbreaking industry on the Chittagong coast releases heavy metals, including Cu from antifouling paints, wiring, and hull corrosion, into the coastal and estuarine waters (Hossain and Islam, 2006). Besides receiving inland discharges, estuaries are also exposed to marine inputs, contributing to the further rise of Cu levels. However, being a dynamic zone where freshwater meets seawater, estuaries create a special physicochemical environment, causing specific metal ions like Cu to bind to sediments (Skrabal et al., 1997). Cu has the highest affinity for organic binding phases in estuarine sediments than other metals such as Ni and Pb, because of its rapid water exchange rate, increased stability due to the Jahn-Teller phenomenon, and high ionic potential (Chakraborty et al., 2016). These make Cu more available in estuarine brackish water. Since *L. calcarifer* is a bottom-feeding predatory fish, it may accumulate Cu by consuming smaller contaminated prey and ingesting contaminated sediments.

Marine fish (*C. aureus*) had the highest levels of As, Cd, Fe, Mn, Ni, Se, and V. Among these, As, Mn, Ni, and Se levels were significantly higher than their fresh and brackish water counterparts. Marine environments in the Bay of Bengal may have higher baseline levels of As, Cd, Fe, Mn, Ni, Se, and V from natural geological and anthropogenic sources (Khan et al., 2017). In water, H+ competes with metal ions, which are reduced in higher pH, allowing metals to be more readily bioavailable for fish to absorb (Magalhães et al., 2015). Sea water has a higher pH than freshwater; hence, it could be a reason for the accumulation of comparatively more metals, such as Fe, Mn, Ni, and V, in sea water. Davis and Gatlin (1996) reported, instead of from consumed food, the majority of marine fish obtain a great portion of their micronutrients from the surrounding water. Some metals, like Fe and Mn, are often higher in marine systems due to natural sedimentation and redox conditions coexisting in a range of surface and near-surface environments in different forms (J. Liu et al., 2022). Mn and Fe are crucial for the growth and photosynthesis process of phytoplankton (Balaguer et al., 2022), thus entering the food chain at the base, which are then transferred into the fish's body.

Anthropogenic inputs from coastal industries, for instance, shipbreaking activities on the Chittagong coast, contribute to the release of different heavy metals into marine waters (Hossain and Islam, 2006). Vanadium is a key trace component in fossil fuels (Hope, 2008), which could be released from the thousands of vessels plying on the seas using such fuels, and also from land-based sources. Se is essential for marine fish and often biomagnifies in the food chain. It was established by many researchers that Se has an antagonistic action with Hg; therefore, the concentration of Hg decreases with an increase of Se in the fish body (Chen et al., 2001; Okati et al., 2021). The accumulation of lower Hg and significantly ($P < 0.05$) higher Se at the same time in the marine fish during the current study agrees with the established Se-Hg antagonistic effect. The concentration of trace elements As and Cd, along with other elements, in the body of *C. aureus* was observed from the East China Sea coast (H. Huang et al., 2022). Similarly, the accumulation of Cd, Mn, and Se, together with some other elements, in the tissues of *C. aureus* was reported from peninsular Malaysia (Azmi et al., 2019).

5.1.3.2. Elements across habitats

Fish incorporate various substances from the living environment and their diet into the scales. Demersal fish have different trends in feeding habits compared to pelagic ones because they live at the bottom of the water bodies and hence depend largely on organisms living on the basin floor. But demersal fish, *L. calcarifer*, have stronger sediment contact because of living close to the floor, where Cu and Ni often accumulate due to industrial and geological sources. In Bangladesh, Cu and Ni are prevalent industrial pollutants found in shipbreaking in Chittagong (Hossain and Islam, 2006) and tannery, leather, and textile effluents(Hossain et al., 2021). After draining out into aquatic systems, these metals bind to estuarine organic matter and fine sediments due to their high binding affinity with organic matter and sulfides under anoxic conditions (Chakraborty et al., 2016). The estuarine demersal fish *L. calcarifer*, which lives in contact with contaminated sediments, frequently scrapes the seabed, resulting in scale exposure to these metals. As exterior structures, scales can directly absorb these metals from the surroundings, besides assimilating them from food.

Compared to demersal fish, benthopelagic fish inhabit both the water column and near-bottom environments, where dissolved metals such as Cd, Pb, and Hg are more abundant. Their broader vertical range may expose them to a wider array of contaminants in the water column, including As, Cd, Pb, and Zn, that frequently have a connection to agricultural runoff, sewage, or industrial effluents. These might result in greater As, Cd, Pb, Hg, and Zn levels in benthopelagic fish *C. carpio* and *C. aureus*, while lower levels in demersal fish *L. calcarifer*. Rejomon et al. (2010) found that *Cyanoglossus macrostomus*, a benthopelagic species, has a higher concentration of Co than other species. Demersal fish were reported to accumulate lower levels of Zn than pelagic ones, and this conforms to the present research (Gull et al., 2024). Benthopelagic fish *C. carpio*, on the other hand, is recognized for its resilience to polluted surroundings and may store more metals in tissues, including scales, without causing rapid harm (Mancera-Rodríguez et al., 2024; Uçkun and Uçkun, 2021). As is associated with groundwater leaching, more bioavailable in the water column (Anawar et al., 2003). Metals like Cd, Pb, Hg often originate from urban runoff, pesticides (Parvin et al., 2022), and coal combustion (Munawer, 2018) which are more likely to be dissolved and suspended in the water column and are more likely to be absorbed by benthopelagic species.

Demersal fish (*L. calcarifer*) is more predatory, prey on benthic organisms that might have higher Cu and Ni from their own exposure, such as bioaccumulation through diet. Benthopelagic *C. carpio* and *C. aureus* feed more on detritus, benthic invertebrates, and sediments, expanding their exposure to a broader spectrum of metals, including As, Cd, Hg, Pb, and others that are more frequent in contaminated sediments.

5.1.3.3. Elements across feeding habits

Higher quantities of Cd, Pb, Hg, and Mn were found in omnivorous species, which might be attributed to diet and mineral bioaccumulation. Sauliutė et al. (2020) demonstrated that omnivorous fish *Rutilus rutilus* accumulated greater amounts of Cr, Cu, Ni, and Zn. In an experimental exposure to Cu, omnivorous fish (*Astyanax altiparanae*) presented greater sensitivity to Cu and accumulated the metal in their tissues, while the carnivorous fish (*Hoplias malabaricus*) did not absorb it (de Paula et al., 2021). The current results agree with these previous findings. Being omnivorous, these fish eat a wider range of food items from both plant and animal communities, whereas carnivorous fish eat animal foods only. Such broad-spectrum feeding habits enable omnivorous fish to acquire more elements from various food items consumed than their carnivorous counterparts. On the other side, omnivorous fish (*C. carpio*), being a benthopelagic fish, have prolonged mid-water stay, expanding their exposure to dissolved and suspended metals in the water column besides dietary intake.

Although (Eisler, 1981) greater levels of Hg are observed in predatory (carnivorous) fish owing to their trophic status; this metal exhibits biomagnification and follows an accumulation pattern, which varies by species and region. However, in the present research, Hg levels were more substantial in the omnivorous fish as they can occasionally choose to feed on animals or carnivores rather than plants, resulting in higher metal levels (Serviere-Zaragoza et al., 2021). Also, Zn was found with a higher concentration in omnivores than in carnivores, which is supported by the other finding reported in (Serviere-Zaragoza et al., 2021). Elements in the seawater (whether delivered by runoff or from localized sources) are absorbed by macroalgae, which are then absorbed by omnivores. Thus, the highest Zn concentrations were recorded in omnivorous fish, as Zn tends to accumulate in regions where significant volumes of organic waste are discharged and is found in higher levels in fish associated with or feeding on the substrata (Junior et al., 2002). However, among the elements, Fe, Zn, and Mn were found excessively high both for omnivorous and carnivorous fishes. Khoshnood et al. (2012) reported similar findings, with Fe and Zn being the highest recorded elements.

Several studies have demonstrated that sediment in water bodies serves as a key sink for metal contamination and plays a crucial role in their intake by fish (Yi et al., 2011). Fe, for example, may be found in higher abundance in demersal fish due to their eating habits, since this metal accumulates rapidly in sediment (Hatje et al., 1998). The occurrence of Cr, Pb, Ni, Hg, and Zn was reported to be found in greater levels in clayey deposits and organic matter, suggesting a higher affinity of such metals to demersal fishes (de Souza-Araujo et al., 2022). Cr, for instance, is a supplementary element used in leather, inks, and steel production (Junior et al., 2002). It frequently occurs in substantial quantities in coastal regions as a result of surface runoff, which might explain why omnivorous fish have greater Cr concentrations than carnivorous fish. Cd and Pb are hazardous metals that may be ingested, retained, and incorporated by species throughout the food chain, eventually accumulating in marine bodies from agricultural wastewater and urban residential waste (Kosanovic et al., 2007). The present research demonstrated higher concentrations of Cd and Pb in omnivores, which might be attributed to their detritivore tendencies (Arain et al., 2008).

5.1.4. Scale element ratios as potential habitat signature

5.1.4.1. Freshwater fish scales

The elemental composition and their relative proportions in hard components record information on where a creature inhabits and interacts with its surroundings throughout its life phases (Adey et al., 2009). Wang et al. (2016) demonstrated that the fish scale element: Ca ratios of essential elements, notably Sr/Ca, Ba/Ca, and Mn/Ca, are intimately related to the biogeochemical aspects of the ecosystem in which they dwell. In the present investigation, the Mn/Ca proportion was found to be higher in the scale of *C. idella*, while the other element/Ca ratios were lower than that of *C. mrigala*. The concentrations of Mn fluctuate with the depth of the water column and are typically highest at the surface (Klinkhammer and Bender, 1980). Thus, the herbivore *C. idella* living in the upper column than *C. mrigala* has the potential to acquire elevated levels of Mn through the consumption of aquatic vegetation rich in Mn, and absorption from the surrounding water. At the same time, an increased uptake of Mn in *C. idella* might competitively prevent other elements from being incorporated, lowering their ratios to Ca. Additionally, *C. mrigala* may have lower element/Ca ratios than *C. idella* because it regulates Ca more tightly or sequesters other trace metals more effectively. However, these require further research to confirm.

Essential minerals play significant roles in maintaining proper skeletal structures and physiological functions. Therefore, the proportions of important elements such as P/Ca are crucial for organisms. In contrast to terrestrial organisms, which have to rely only on their diet for calcium absorption, fish have the opportunity of an almost limitless supply of calcium from water (Metz et al., 2014). Thus, the measure of P/Ca in the fish's calcified structures could essentially reflect their association with their food intake and habitat. Fish residing in calcium-poor water or after migrating from high to low Ca water can draw Ca and P from their scales (Metz et al., 2014). Adey et al. (2009) demonstrated that the variations of element: Ca ratios are distinctly linked to farmed and wild *Salmo salar*. Analysis of Ca and Mg was used to distinguish between freshwater and saltwater striped bass, *Morone saxatilis* (Belanger et al., 1987). A thorough image of the aquatic ecosystems in which the fish reside can be obtained by integrating scale element proportions with other data, like site geology, water chemistry, and species-specific traits.

5.1.4.2 Coastal migratory fish scales

The elemental composition of hard components provides information about how a creature inhabits its surroundings throughout its life. Fish scale elemental makeup is inferred as a good biogeochemical indicator for assessing their environment (Wang et al., 2016). These ratios could be utilized as potential indicators of habitat signature since Ca is the most common metal found in fish scales (Feitosa et al., 2020; Wang et al., 2016). The hard part levels of some metals like Sr, Ba, Mn, and Mg were considered to match ambient levels, making them useful markers of habitat occupancy (McMillan et al., 2017; Moll et al., 2019). Salinity specifically affects Sr and Ba, with the former being more prevalent in freshwater and the latter in marine environments (McMillan et al., 2017; Tripp et al., 2020). Thus, variations in salinity have a positive correlation with the proportions of Sr/Ca and Ba/Ca in fish hard structures (Martinho et al., 2020). It became evident that the fluctuations of Ba/Ca, Cd/Ca, and Sr/Ca ratios were favorably correlated with environmental levels (Wells, 2000).

Since marine water has quantities of Ca, Mg, and Sr that are at least a single order of degree higher than those of freshwater, and as the amounts of Ba in sea water are roughly two degree of order smaller than those in freshwater, fish scales containing elevated Sr/Ca and Mn/Ca proportions or reduced Ba/Ca proportions could be indicative of marine water (Wang et al., 2016). Adey et al. (2009) observed that the change from freshwater to seawater was associated with a drop in Ba concentration in salmon scales. Sr, especially ^{90}Sr, a long-lived radioisotope, is deposited in hard structures (Mironyuk et al., 2022). Compared to the majority of freshwater sources, seawater has higher strontium levels. Scales absorb Sr when exposed to seawater because it can replace calcium, and since seawater contains a higher percentage of it than freshwater, it makes it possible to identify patterns of immigration in freshwater and seawater (Courtemanche et al., 2006). *Salmo salar* displayed elevated Sr concentrations in the marine part and an increase of Sr content, while the river to sea transfer (Tray et al., 2022). Compared to freshwater residents of the same species, *Salmo trutta* had 1.5 times as much strontium in their scales (Bagenal et al., 1973). *Salvelinus fontinalis* scales were shown to have higher Sr/Ca proportions during marine exposure, and wild anadromous fish scales consistently had higher Sr/Ca ratios than those of freshwater-dwelling species (Courtemanche et al., 2006). Additionally, Coutant and Chen, (1993) noted that freshwater *Morone saxatilis* had lower Sr concentration, while estuarine *M. saxatilis* had higher Sr levels.

In the present investigation, *O. pama* exhibited the lowest Ba/Ca and the highest Mn/Ca proportions. *O. pama* is an amphidromous species, meaning that it migrates from rivers to the ocean and vice versa. Therefore, the *O. pama* specimens examined in this investigation have an attachment to seawater. In conjunction with possessing amphidromous movement patterns and the greatest Sr/Ca and Zn: Ca ratios, *S. panijus* samples also showed an affinity for seawater, similar to that of *O. pama*. The Sr/Ca ratio alone can be emphasized here because the P and Zn concentrations in the river and sea are very close. The current investigation revealed that *L. calcarifer* possessed the least Al/Ca, Cu/Ca, and Mn/Ca proportions, whereas *T. ilisha* had the maximum Fe/Ca, K/Ca, Mg/Ca, Na/Ca, P/Ca, S/Ca, and the lowest Sr/Ca proportions. Possessing the lowest Sr/Ca and Mn/Ca ratios in *T. ilisha* and *L. calcarifer*, respectively, reflecting their affinity for freshwater. As migratory fishes, *T. ilisha* is an anadromous species, and *L. calcarifer* is a catadromous species; both species have an aptitude to migrate to freshwater habitats at a particular period of their lives.

5.2. Plant leaves

5.2.1. Deposition of microplastics

The concentration of MPs in the residential and industrial sites was significantly higher than those in the rural sites ($p = 0.028$). In Narayanganj, Bangladesh, soils from industrial areas had higher mean concentrations of MPs (4.6 ± 4.4 particles/kg) than soils close to cities (1.9 ± 1.8 particles/kg) and urban farmlands (0.6 ± 0.8 particles/kg) (Tajwar et al., 2023). In Dhaka, (Rabin et al., 2023) similarly discovered that the street dust in industrial regions had the highest concentration of MPs (17 particles/g), while the residential areas had the lowest concentration (13.9 particles/g), which aligns with the present results. In the environment, PET-type MPs and NPs are common in airborne forms (Ortega and Cortés-Arriagada, 2023). PET was shown to be the most common microplastic deposited on leaves among other atmospheric MPs (Perera et al., 2024). PET, along with other MPs, was observed by Jafarova et al. (2023) in the leaves of *Robinia pseudoacacia*. PET and polyamide were also identified by Peng et al. (2023) among the two MPs most frequently found materials in the interior dust of urbanized areas.

PET and polyvinyl chloride MPs had a higher likelihood of settling due to their higher density compared to other plastics (Koutnik, 2022). Because of its excellent chemical and physical characteristics, PET is used in various sectors, including textiles, food packaging, beverages, water and juice bottles, vehicles, electronics, and building materials (Joseph et al., 2024; Perera et al., 2024; Tamoor et al., 2022; Ungureanu et al., 2020). Additionally, PET is utilized in blends with other thermoplastics and thermosets, making it a widely used plastic worldwide (Tamoor et al., 2022). 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 (Ungureanu et al., 2020; Welle, 2021). Approximately 92% of the PET-MPs detected in urban dust are thought to originate from the clothing sector (Peng et al., 2023). Over time, plastics released into the environment may break down, spread, and move through the atmosphere. The area surrounding Dhaka is home to a vast variety of industries, primarily clothing-related ones. Thus, it's possible that the PET-MPs discovered in the study came from different anthropogenic activities, including industrial operations. The lipophilic or lipid-loving nature of MPs and NPs causes them to be drawn to the leaf wax layer, where they are subsequently absorbed and deposited on the leaf surface (Liu et al., 2023; Perera et al., 2024; Zhou and Xia, 2024). Tao et al. (2023) demonstrated that fragments could utilize leaf wax to create C–H·π-type hydrogen bonds. The PET particles deposited on the leaves might have been adsorbed by this mechanism. Nevertheless, the quantity of MPs on tree leaves differs according to the kind of leaf, and surface characteristics such as texture and hydrophilic properties (Leonard et al., 2023).

MPs adhered to leaves could be harmful to plants in different ways. Sun et al. (2021) demonstrated that polystyrene MPs in leaves cause oxidative stress. Additionally, MPs stuck to the surface of leaves may block light and prevent the photosynthetic process (Bi et al., 2020). Besides being harmful to plants, MPs affixed to the surface of leaves can also function as an absorbent layer for additional substances like volatile organic matters on the surface of leaves, which is similarly harmful to the wellness of plants (Bi et al., 2020). PET-MPs have the potential to act as carriers for various airborne contaminants that exist in the air (Ortega and Cortés-Arriagada, 2023). The deposition of plastic fragments by plants could serve as a pathway for agricultural vegetation to adsorb pollutants, putting the health of humans at risk (Sun et al., 2021). Furthermore, the leaves of terrestrial flora may serve as a temporary trap and a possible source of further microplastic pollution (Liu et al., 2020).

5.2.2. Elements in leaves

In comparison to leaves in residential or rural areas, leaves in the industrial region had significantly ($p < 0.05$) higher concentration of Cd (0.29 ± 0.08 mg/kg), Pb (5.2 ± 0.14 mg/kg), and Zn (101 ± 7.9 mg/kg), demonstrating the impact of industrial operations. This result affirms the notion that industrial processes are the primary source of significant air-contaminating elements such as Cd and Pb (WHO, 2007). The industrial site was observed to have elevated amounts of Cd (0.3 to 2.2 µg/g), Pb (5.4 to 34 µg/g), and Zn (11.0 to 30 µg/g) in various tree foliage, while urban areas had raised Cu (1.06 to 7.4 µg/g) and Al (12.01 to 38 µg/g) (Doğanlar and Atmaca, 2011).

In comparison to their residential counterparts, industrial particulate matter had elevated amounts of Cd (5 ± 3 ng/m^3), Pb (118 ± 144 ng/m^3), and Zn (529 ± 330 ng/m^3) (Karar et al., 2006). Likewise, Voutsa et al. (1996) demonstrated that the atmospheric particles in industrialized locations were considerably rich in Zn (610–4700 ng/m^3), Pb (70–1020 ng/m^3), and Cd (0.8–7.8 ng/m^3). High levels of Pb (206 ± 111 to 554 ± 352 ng/m^3), Zn (790 ± 579 to 1112 ± 815 ng/m^3), and Cd (6 ± 4 to 16 ± 14 ng/m^3) in the air were also detected by Kang et al. (2018) in industrial locations. Pb (57 ± 36), Zn (187 ± 84), and Cu (58 ± 17) were reported in the dust of Dhaka (Rahman et al., 2021). Shahrukh et al. (2023) found Cd, Pb, Cr, and Ni in tree leaves and speculated that Pb concentrations were high in Dhaka's atmosphere. Several other researchers also reported findings that were somewhat comparable to ours (Aissa and Kéloufi, 2012; Cetin et al., 2007; Hassan and Basahi, 2013; Jalali and Khanlari, 2008; Molnár et al., 2020; Woszczyk et al., 2018).

It was observed that the levels of Ba, Co, Cr, Cu, Fe, Mn, Ni, and Sr were higher in the rural and residential areas and lower in the industrial sites. The levels of Ba were significantly greater in residential and rural areas compared to industrialized areas. Molnár et al. (2020) also discovered that rural regions have a greater level of Ba than industrial areas. The industries in the investigated region are not as closely related to these metals as other heavy sectors, which could be the reason for the lower levels of these components in industrial regions. Despite the lack of significant industrial activity, there is a great deal of traffic in the rural and residential regions, particularly in the residential areas. Additionally, a number of large-scale development projects are now underway in the vicinity of the residential sites in the current study, which may be another cause causing the rising amounts of certain potential contaminants (Kabir et al., 2021). For example, increases in Cr content are linked to high traffic loads because of the presence of chromium trioxide (CrO_3) in rubber employed in car tires and catalytic converters, which may contain 25 ppm of Cr (Shahrukh et al., 2023).

Urban and industrial zones contribute to a variety of pollutants since they are centers of human activity (Ciarkowska et al., 2019). The industrial area for this investigation was in Savar, Dhaka. By hosting the Dhaka Export Processing Zone, as well as several other industries, including textiles, textile washing, printing and dyeing, pharmaceuticals, brickfields, welding, galvanizing, electroplating, etc., it is one of the most significant industrial zones in Bangladesh (Anny et al., 2017; Hossain et al., 2024; Rahman and Mallick, 2010). The high amounts of Cd, Pb, and Zn in the area may be caused by the emissions and effluents released by these enterprises. There have been reports of several metals from clothing pollutants, such as Cd, Pb, and Zn

(Manzoor et al., 2006; Odipe et al., 2019), dyeing-house sludge (Islam et al., 2009; Liang et al., 2013), pharmaceutical-factory wastewaters (Bibi et al., 2023; Islam et al., 2010), and electroplating industries (Kirichenko et al., 2020; Sultan et al., 2022).

Nevertheless, vehicles may also discharge these into the air (Aksu, 2015; Viard, 2004). Traces of harmful elements like As, Cd, Cr, Cu, Zn, Pb, and Ni are commonly detected as coming from vehicle exhaust (Hao et al., 2018). In Dhaka, it was found that the main sources of Pb, Zn, Mn, and Cr in dust as well as Pb, Zn, Ni, and Mn in plant leaves were emissions from traffic, while the main sources of Cd, Cu, and Ni in dust as well as Cd and Cr in leaves were industries (Sultan et al., 2022). Elevated Heavy metal levels in industrial areas may be mostly caused by the automobile and manufacturing industries (Hassan and Basahi, 2013). In addition to the Dhaka Aricha expressway passing through the industrial sampling site, the Gabtoli bus station serves as a gateway from the capital for people from the northwestern and northern parts of Bangladesh.

Consequently, the area is quite contaminated due to the emissions of numerous cars and the various industrial activities that are dispersed throughout it. Pb and Cd are among the dangerous trace elements found in leaded fuel used in autos (Olowoyo et al., 2022). Even though they are currently prohibited and have been replaced with unleaded fuels, it has been observed that these still contain a trace amount of lead, since gasoline may not remove lead from the environment (Yaro et al., 2015). The extensive utilization of lead-containing fuel prior to the ban, however, may have left behind contamination of soil and other environmental areas. Remobilization and resuspension may also act as a sink for persistent lead contamination, releasing lead into the air constantly when disturbed.

Lead poisoning may also result from the smelting of lead and the processing and recycling of discarded lead-acid batteries (Majumder et al., 2021; Rahman, 2022). The high results, long lifespan, low maintenance requirements, and exceptional resistance to physical and electrical damage make cadmium a common supplementary or rechargeable power source in Ni/Cd batteries (Olowoyo et al., 2022). Batteries are used extensively in solar systems, rickshaws, simple bikes, and immediate power supplies; most of these are either improperly recycled or discarded, which causes PB and Cd to leak into the environment (Khan et al., 2018; Majumder et al., 2021; Rahman, 2022).

The majority of zinc emissions come from automobiles, where lubricant and gasoline are combined and burned in the piston chambers, since zinc is a component in lubricating oil, which is emitted during combustion (Begum et al., 2006). Additionally, wearing tires, producing rubber using zinc compounds, and producing galvanized objects can all emit zinc (Begum et al., 2004). Furthermore, there are several brick fields surrounding the industrial region of the current study, which use primarily coal. Coal contains traces of several heavy metals, such as Pb, Cd, etc (Munawer, 2018). Previous research concluded that coal fly ash was a source of Cd, Pb, and Zn in the air in Dhaka (Islam et al., 2015; Salam et al., 2003).

The industrial sampling site geology includes the uplifted Madhupur region, which is blanketed in Pleistocene-aged dark-reddish-brown to brownish-red Madhupur Clay Residuum, supported by the Plio-Pleistocene Dupi Tila sandstone formation (Ahmed et al., 2016; Maitra and Akhter, 2011). Earlier studies demonstrated that the makeup of the parent rock affects the soil's accumulation of hazardous materials, and that reddish or red soil typically contains significant quantities of different metals (Yu et al., 2022). Heavy metals from the soil can be taken up by the plant root system and then transferred to the leaves. Thus, it is possible that multiple of the previously mentioned factors contributed to the elevated concentrations of these elements in the industrial sites instead of coming from one origin.

5.2.3. Pollution index

The pollution index (PI) represents the influence of each element at the chosen site, which aids in the assessment or continuation of actions aimed at improving environmental conditions by evaluating the level of environmental degradation (Sultan et al., 2022). The elevated PI level for the heavy metals can be attributed to the extensive use of batteries in electric bicycles, rickshaws, immediate power supplies, and solar power systems, most of which are insufficiently recycled and maintained, resulting in the leakage of heavy metals, including Pb and Cd, into the environment (Khan et al., 2018; Majumder et al., 2021; Rahman, 2022). A significant number of battery-operated electric vehicles are utilized in Dhaka City; nonetheless, waste batteries are inadequately gathered for recycling or are indiscriminately disposed of (Khan et al., 2018). The elevated Pb PI value found in residential and industrial areas may be attributed to substantial Pb levels present in Dhaka's air (Shahrukh et al., 2023). As the world continues its digital transformation, Bangladesh is also experiencing an increase in the bulk of electronic garbage, or e-waste. Mowla et al. (2021) found that in Dhaka, heavy metals, including Pb, originate from improper treatment of such material and illegitimate e-waste outlets. Increased Cd concentrations in the environment could result from the presence of tannery businesses nearby (Rahman et al., 2014). According to Rahman et al. (2019), heavy industrial operations are a major factor in Dhaka City's status as a hotspot for Pb, Ni, Cd, and As pollution. Nevertheless, low contamination (PI < 1) was found for other heavy metals (Cr, Cu, and Ni).

All the heavy metals showed significant ($p < 0.05$) differences in PI values between the rural, residential, and industrial regions. The ability of a plant to absorb any metal from its environment is often determined by the type of plant, the element's characteristics, and the combined effects of all biotic and abiotic factors (Molnár et al., 2020). The capacity of plants to build up heavy metals depends on plant tissue, species, metal types, and levels (Eben et al., 2024). Jashim et al. (2021) determined that several plant leaves in Dhaka City were vulnerable to air pollution after analyzing their air pollution-tolerance index. Sultan et al. (2022) additionally, looked into the PI values for heavy metals in other Bangladeshi cities, and the conclusions align with the present ones. Amadi and Chuku, (2023) found that *P. longifolia* had both a translocation factor and a bioaccumulation factor larger than 1, indicating that metals were more concentrated in plants and that various plant components had the capacity to accumulate metals, particularly cadmium, lead, and zinc. Our results are consistent with those of Kabata-Pendias, (2010) and Turer et al. (2001), who showed that plants may absorb metals from the air via leaf.

Chapter 6: Conclusion

Fish incorporate various elements into their scales both from the surrounding environment and the food they eat. The study investigated the elements in the scales of different fish species from different environmental settings, such as freshwater, brackish water, and marine environments. Fish scale elements were found to have an association with and vary in relation to the natural environment of their habitat and feeding ecology. Element ratios, particularly Sr/Ca, Mn/Ca, and Ba/Ca ratios, were shown to have potential implications as habitat signatures. Therefore, it concerns their dwelling habitat and the source of their food, which is to be incorporated into the fish scales. These indicated that fish scales can be employed as a noninvasive tool for deciphering fish life history and ecological insights without endangering the fish. Integration of fish scale microchemistry with other modern techniques in both inter-species and inter-environmental contexts would make them indispensable for ecological research and sustainable management of fisheries resources. Similarly, higher concentrations of both microplastics and heavy metals were found in plant leaves with an increasing degree of urbanization, mirroring the impact of anthropogenic activities in different areas. It reflects the potential of the plant leaves to be utilized as an excellent tool in environmental biomonitoring. Overall, the findings of the study 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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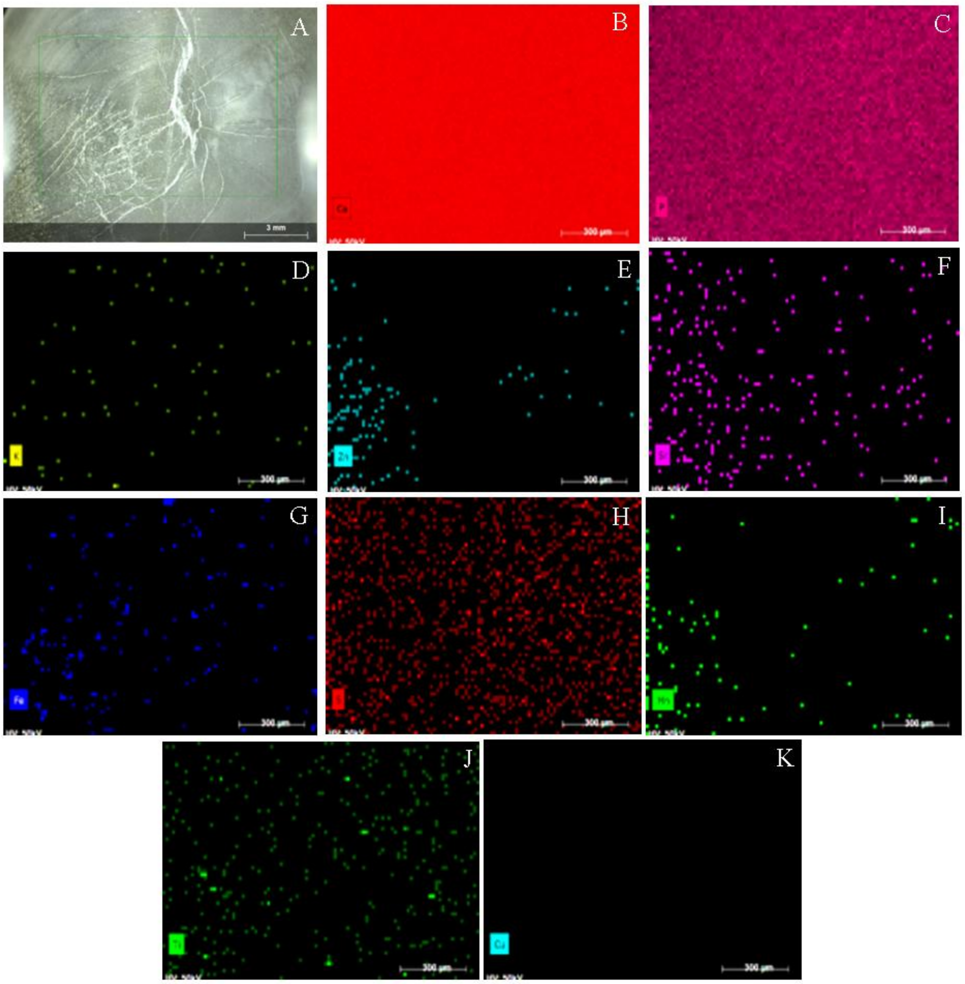
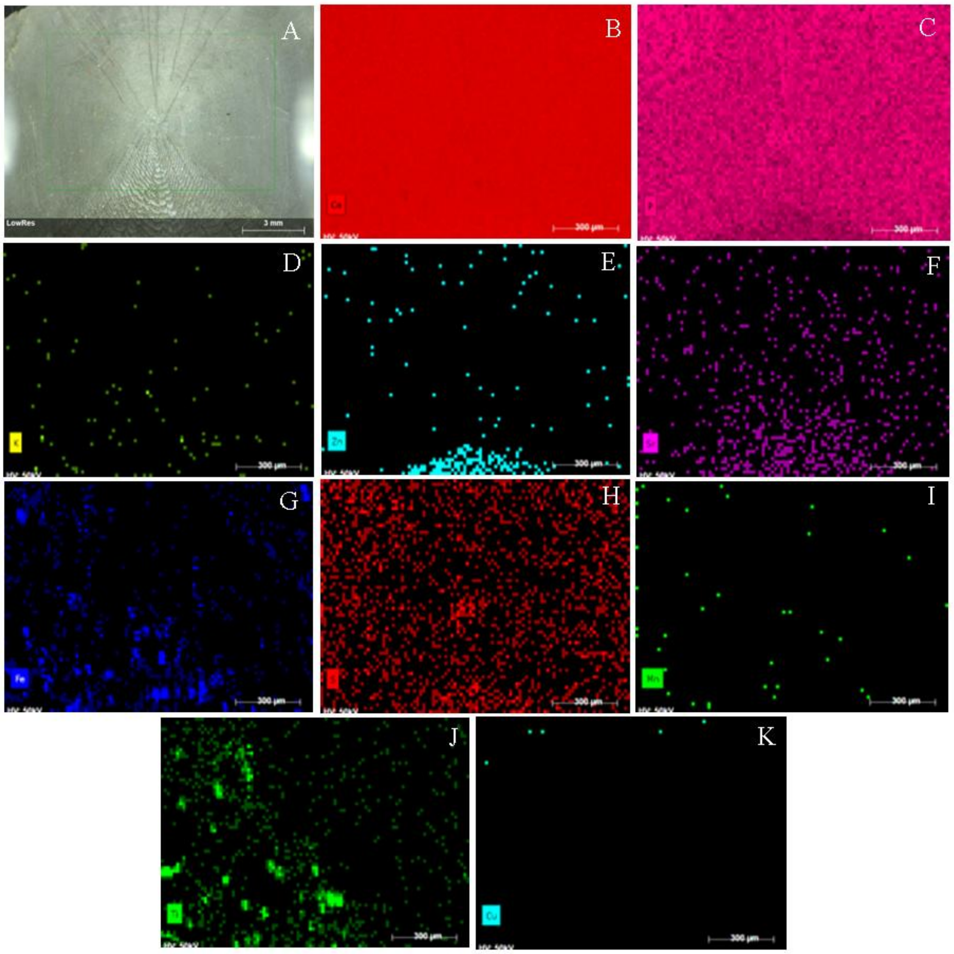
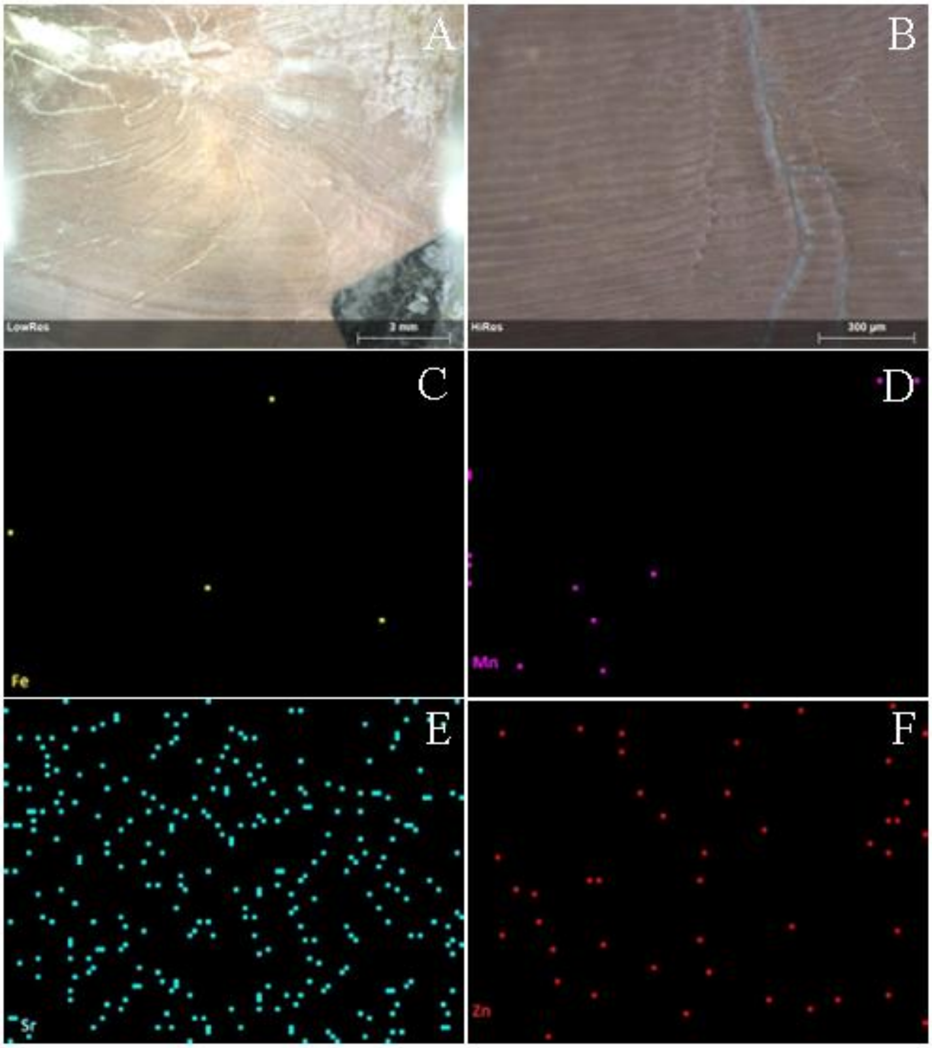
Appendix



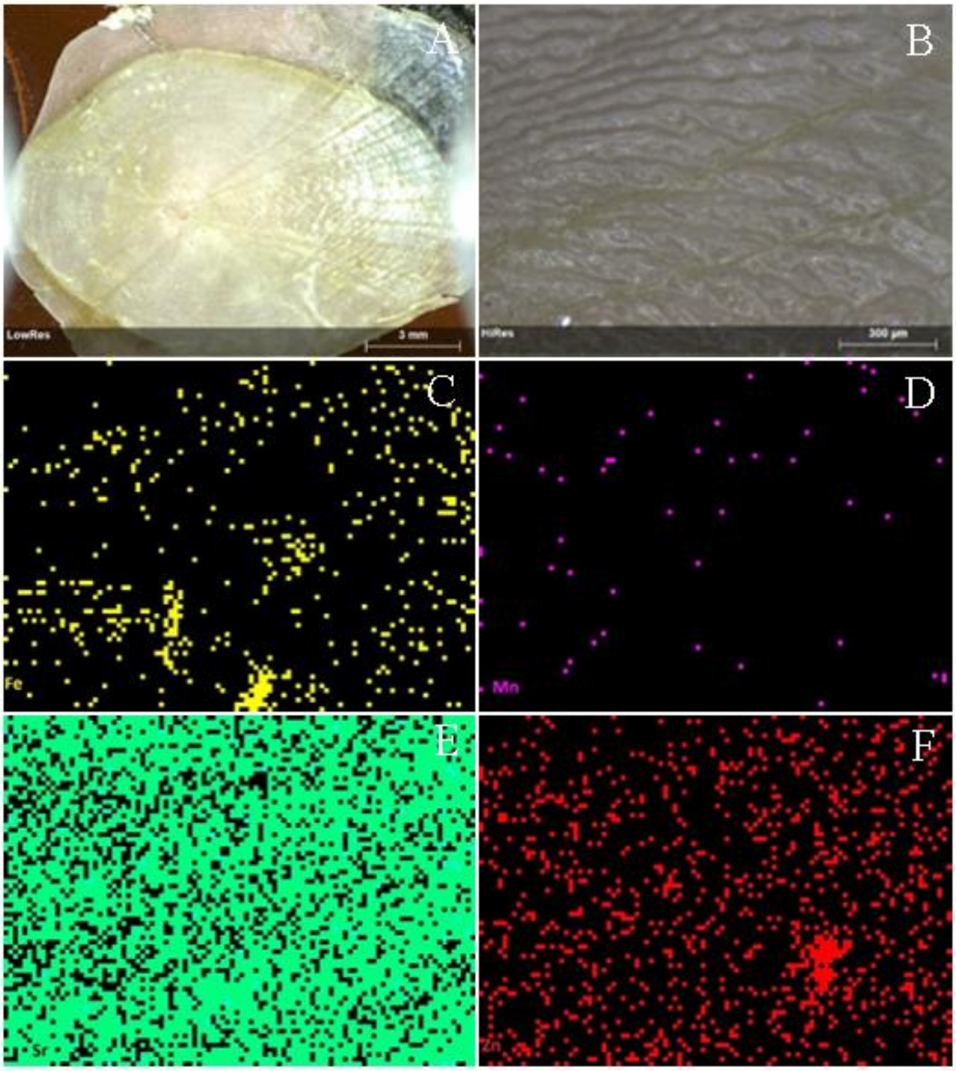
Appendix 1. Element distribution maps in the freshwater fish, *C. idella* scales. A. Scale specimen, B. Map of Ca, C. Map of P, D. Map of K, E. Map of Zn, F. Map of Sr, G. Map of Fe, H. Map of S, I. Map of Mn, J. Map of Ti, and K. Map of Cu.



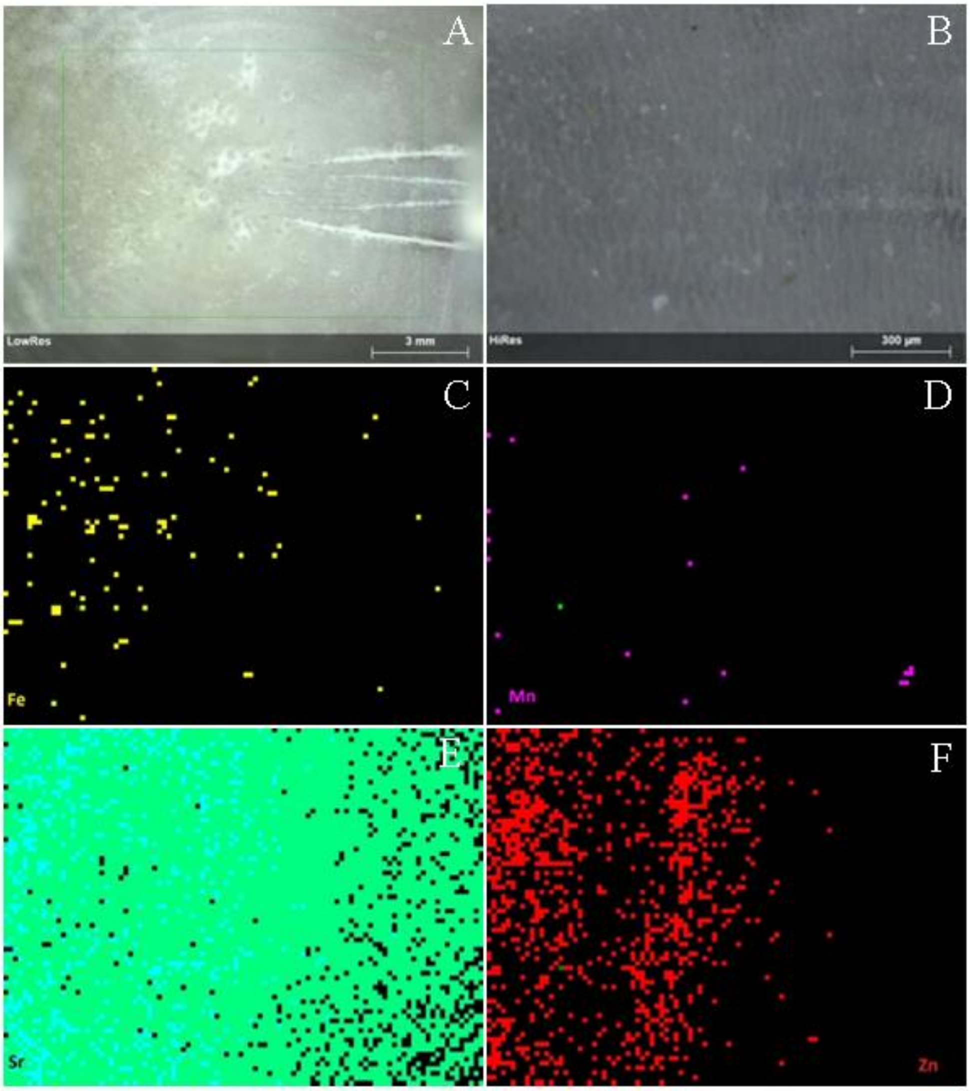
Appendix 2. Element distribution maps in the freshwater fish, *C. mrigala* scales. A. Scale specimen, B. Map of Ca, C. Map of P, D. Map of K, E. Map of Zn, F. Map of Sr, G. Map of Fe, H. Map of S, I. Map of Mn, J. Map of Ti, and K. Map of Cu.



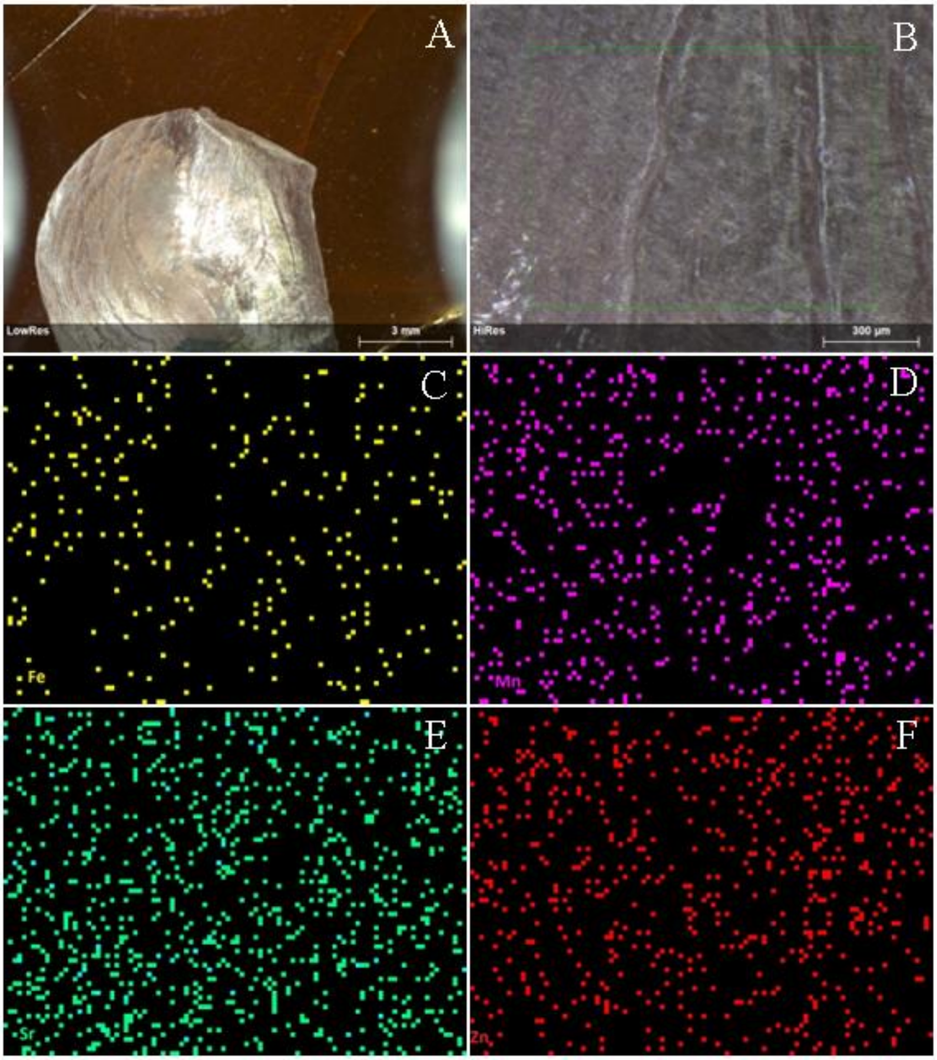
Appendix 3. The scale of *Tenualosa ilisha*. The scale in normal view (A), in high resolution (B), distribution map of Fe (C), distribution map of Mn (D), distribution map of Sr (E), distribution map of Zn (F).



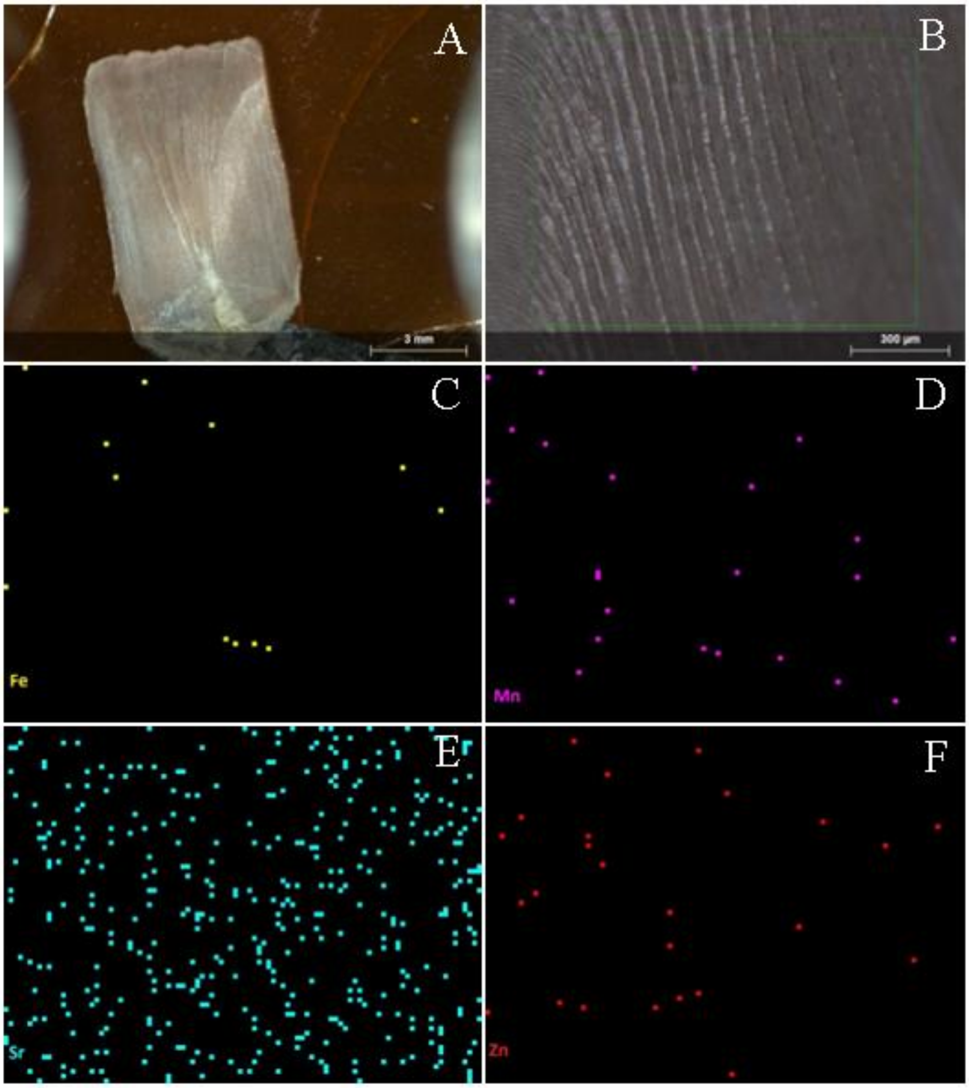
Appendix 4. The scale of *Sillaginopsis panijus*. The scale in normal view (A), in high resolution (B), distribution map of Fe (C), distribution map of Mn (D), distribution map of Sr (E), distribution map of Zn (F).



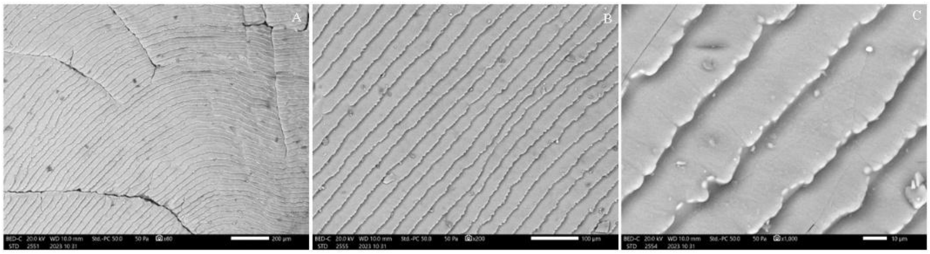
Appendix 5. The scale of *Lates calcarifer*. The scale in normal view (A), in high resolution (B), distribution map of Fe (C), distribution map of Mn (D), distribution map of Sr (E), distribution map of Zn (F).



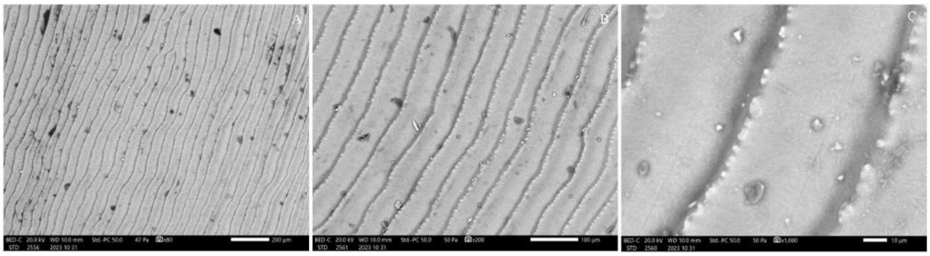
Appendix 6. The scale of *Otolithoides pama*. The scale in normal view (A), in high resolution (B), distribution map of Fe (C), distribution map of Mn (D), distribution map of Sr (E), distribution map of Zn (F).



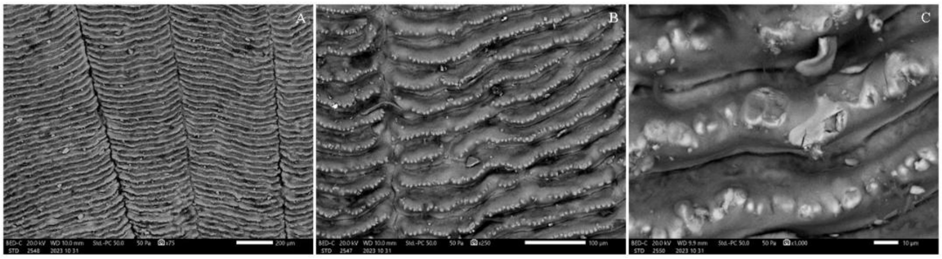
Appendix 7. The scale of *Rhinomugil corsula*. The scale in normal view (A), in high resolution (B), distribution map of Fe (C), distribution map of Mn (D), distribution map of Sr (E), distribution map of Zn (F).



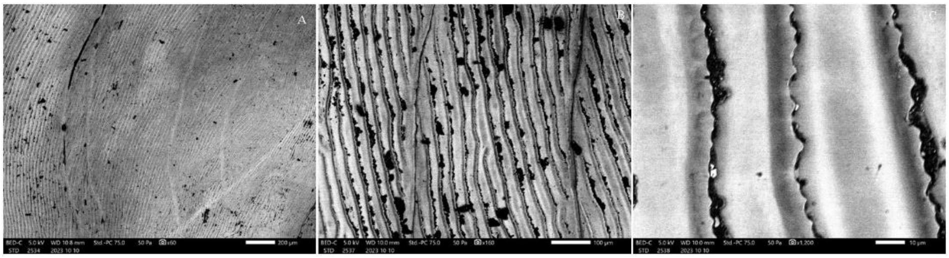
Appendix 8. Scanning electron microscope (SEM) images of the scale of *Tenualosa ilisha*. A. Scale at 80x, B. Scale at 200x, C. Scale at 1000x.



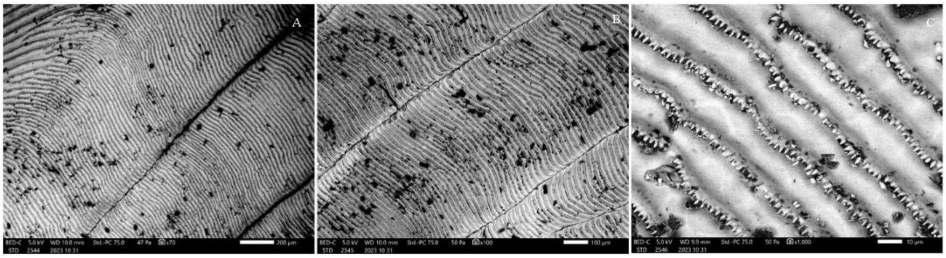
Appendix 9. Scanning electron microscope (SEM) images of the scale of *Sillaginopsis panijus*. A. Scale at 80x, B. Scale at 200x, C. Scale at 1000x.



Appendix 10. Scanning electron microscope (SEM) images of the scale of *Lates calcarifer*. A. Scale at 75x, B. Scale at 250x, C. Scale at 1000x.



Appendix 11. Scanning electron microscope (SEM) images of the scale of *Otolithoides pama*. A. Scale at 60x, B. Scale at 160x, C. Scale at 1200x.



Appendix 12. Scanning electron microscope (SEM) images of the scale of *Rhinomugil corsula*. A. Scale at 70x, B. Scale at 100x, C. Scale at 1000x.