Metal bioavailability, bioaccumulation, phyto-remediation and microplastics as their adsorption site in the Mandovi Estuary, Goa

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DECLARATION BY STUDENT

I hereby declare that the data presented in this dissertation report entitled, "Metal bioavailability, bioaccumulation, phyto-remediation and microplastics as their adsorption site in the Mandovi Estuary, Goa" is based on the results of investigations carried out by me in the discipline of Marine Sciences at the School of Earth, Ocean and Atmospheric Sciences, Goa University under the supervision of Dr. M. R. Nasnodkar and the same has not been submitted elsewhere for the award of a degree or diploma by me. Further, I understand that Goa University or its authorities will not be responsible for the correctness of observations/experimental or other findings given in the dissertation.

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COMPLETION CERTIFICATE

This is to certify that the dissertation report "Metal bioavailability, bioaccumulation, phyto-remediation and microplastics as their adsorption site in the Mandovi Estuary, Goa" is a bonafide work carried out by Ms. Varsha Kumari under my supervision/mentorship in partial fulfilment of the requirements for the award of the degree of Master of Science in the discipline of Marine Sciences at the School of Earth, Ocean and Atmospheric Sciences, Goa University.

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CHAPTER - I

INTRODUCTION

1.1 Introduction

The transitional area between the marine and fluvial ecosystems is the estuary, one of the significant geomorphic landforms of the coastal zone that has been the subject of substantial research. According to **Pritchard (1967)** “an estuary is a semi-enclosed coastal body of water, which has a free connection with open sea, and within which sea water is measurably diluted with fresh water derived from land drainage”. Estuaries can be classified based on tidal range, geomorphology and circulation pattern (salinity distribution).

The tidal range can be used as a parameter to classify the estuary as microtidal, mesotidal and macrotidal. The microtidal estuaries are formed in areas where the tidal range is < 2m. There is dominance of freshwater discharge which leads to the formation of a highly stratified, salt-wedge type of an estuary. The mesotidal estuaries are formed in areas wherein the tidal range varies from 2m to 4m. Such estuaries exhibit meandering characteristics. The macrotidal estuaries are formed in areas where there is prevalence of strong tidal current with tidal range of 4m or more. There is formation of long linear sand bars parallel to the tidal flow near the mouth of the estuary.

An estuary is also classified as coastal plain, bar-built, fjord and tectonic type based on the geomorphology. The coastal plain estuaries, frequently referred to as drowned river valley estuaries, are typically found in temperate zones and are formed when rising sea level~~s~~ flood pre-existing river valleys. They have low sedimentation and shallow depths (rarely surpassing 30 metres). The bar-built or restricted-mouth estuaries are distinguished by barrier beaches or islands that are formed parallel to the shoreline and separate the estuary from the sea/ocean. The sediments deposited by sea/ocean waves accumulate to produce barrier beaches and islands. Lagoons are sheltered areas of calm water that exist between the coast and barrier beach or islands. The fjords are found in high latitudes, and they are often long, narrow valleys with steep sides formed by moving glaciers. The glaciers create deep channels into the Earth, leaving a shallow, narrow sill near the sea/ocean. When the glaciers retreat, seawater floods the deeply cut valleys, forming estuaries. The tectonic estuaries are formed by earthquakes or fissures in the

Earth's crust, resulting in faults in regions adjacent to the sea/ocean. The faults cause a portion of the crust to sink, creating a hollow basin and when the sea/ocean fills the basin, a tectonic estuary is formed.

The estuaries are also classified based on the water circulation or vertical structure of salinity as salt-wedge, slightly stratified or partially mixed, and vertically mixed or well mixed types. This classification takes into account the conflict between mixing from tidal force and buoyant forcing from river discharge. Tidal prism, another name for the volume of sea/oceanic water that enters the estuary during each tidal cycle, is the measure of mixing caused by tidal forcing. The salt-wedge estuaries are the most stratified, or least mixed, of all estuaries **(Geyer et al. 1989)**. They are also known as "highly stratified estuaries". They form when a fast-flowing river empties into the sea/ocean and tidal currents are weak. The water circulation in such estuaries is determined by the force of the river pushing fresh water out to sea, not by tidal currents moving seawater upstream. Floating above the seawater is fresh water because it is less dense than saltwater. The freshwater floats on top of the saline wedge of water at the bottom, forming a clear division between the water bodies. Whereas, in case of slightly stratified or partially mixed estuaries, freshwater and saltwater mix at all depths; the lower layers of water, however, usually stay saltier than the upper layers. The salinity drops upstream and reaches its maximum at the estuary's mouth. A vertically mixed or well-mixed estuary, forms when river flow is low and tidally generated currents are moderate to vigorous. The salinity of water in vertically mixed estuaries remains constant from the surface to the bottom. The strong tidal currents remove the vertical layering of freshwater floating above denser seawater, and salinity is governed by the daily tidal stage. An estuary's salinity is highest at the mouth towards the sea/ocean and decreases as it moves up the river. According to **Hayes (1975)**, who adopted the classification scheme put forth by **Davies (1964)**, "the classification of an estuary could be best based on the tidal range" and "the tidal range has the broadest effect in determining the large-scale differences in grain size accumulation. Based on seawater intrusion, an estuary is divided into lower, middle, and upper regions. The lower estuary is dominated by high energy tides and waves, and is subjected to marine processes being in proximity to sea/ocean. The upper estuary is highly influenced by freshwater discharge from river and its tributaries, while the intermediate or middle portion of the estuary represents strong mixing zone of saline water and freshwater. Consequently, the mixing of two water bodies of different densities in the middle estuary promotes flocculation of suspended particles and their deposition as bed load. The middle and lower estuarine regions give rise to inlets or sub-channels that are mostly governed by tidal action. The intertidal regions (intermittently submerged and exposed with the fall and rise of the tides respectively) within lower and middle portions of the estuary have development of sub-environments viz., mudflats, mangroves, etc., due to deposition of sediments from river and sea. They are the result of larger-scale sea level fluctuations, intermediate-scale channel and gully migration, and erosion and depositional processes. The mudflats are barren land without vegetation and are formed on the periphery of estuaries because of finer sediments being deposited on the landward side boarder of estuary or even in the middle of the estuarine channel. The flats that are developed or formed near the mouth region have rich composition of coarser (sand) particles and are often referred as "Tidal flats" **Bates and Jackson (1987)**. On the contrary, the mangrove sub-environment has dense patch of mangrove plants capable of tolerating stress of salinity. Both these sub-environments have rich deposition of finer (silt and clay) sediments. In fact, the deposition of finer sediments is higher at mangrove site owing to strong barrier from mangrove plants that aid in breakdown of energy of tides and waves. Subsequently, the deposition of organic matter is relatively more in mangrove sediments than the mudflats. The mudflat and mangrove sub-environments within estuaries provide vital habitats for a wide range of flora and fauna. These sub-environments being in proximity to coastal region receive significant proportion of nutrients and therefore, support rich biodiversity. Many of the marine fauna prefer estuarine mangrove ecosystem as suitable grounds for breeding of juvenile species as dense patch of mangrove ecosystem with intricate roots protect them from predators.

Estuaries serve as both transportation corridors and recreational areas for people. The construction of ports and harbours across estuaries has increased recently. Estuarine channels are also subject to human activities viz., dredging, sand mining, industrial waste disposal, agricultural and aquaculture waste, coastal rehabilitation, and other uses that include navigation, shipping, and transportation. The estuaries receive several pollutants from natural processes as well as anthropogenic activities, one of the important pollutants introduced in estuaries is metal which has ecological implications. The weathering of rocks containing minerals rich in metals from the catchment area is a vital natural source of metals in estuaries, in addition to contributions from aeolian transport. In recent years, the estuaries have witnessed tremendous enrichment of metals due to human-induced activities viz., ore mining and its transportation, industrial, agricultural waste, ship building and maintenance activities etc., The metals in their dissolved form once introduced in the estuary get adsorbed on suspended sediment particles and are eventually deposited with cohesive sediment on the estuarine bed. The metal as a pollutant is non-biodegradable, persistent and toxic in the marine environment.

The concentration of metal in sediment is regulated by various factors viz., salinity, pH, grain size, organic matter, Fe-Mn oxides or hydroxides, sulphides, salinity, pH, etc. The salinity of estuarine water has an impact on the partitioning of dissolved metal phases in sediments. An increase in salinity also increases the desorption of metals from the sediments and suspended sediment particles. This is mostly because soluble inorganic complexes are formed when metals like cadmium bind with sulphate and chloride. Furthermore, metals and cations, particularly calcium and sodium compete for the adsorption sites. They even displace metals that are mildly and weakly sorbed. The gradient of the salinity is a decisive factor for the metal bioavailability in an estuary. Also, pH can alter the metal chemistry of estuarine environment. The concentration of H^+ ions will increase with acidic pH. The H^+ ions compete with other metal cations for free binding sites on the sediment surface, particularly in the carbonate fraction. The metal cations in their carbonate form in sediments are prone to be desorbed by the H^+ ions and then replaced. As a result, a drop in pH makes it easier for metals to move from sediment into the water column, increasing the metal bioavailability.

The metals are generally associated with fine grain size particles as compare to coarser sediment such as sand. It is attributed to sorption, co-precipitation and complexation of metals with sediment particle surfaces and coatings. Smaller the grain size of particles, higher will be the surface area by volume ratios i.e., there will be ample of free binding surfaces for the adsorption of metals. Thus, the concentration of metals is relatively higher in finer sediments than coarser sediments. The specific surface area of sediment is dependent on granulometric parameters and mineral composition. In an estuarine environment, higher hydrodynamics near the mouth of an estuary aid in the retention of coarser sediments, whereas finer silt and clay particles are moved to the middle region of the estuary by wave and tide action. It promotes the deposition of finer sediments and the formation of sub-environments such as mudflats and mangroves in the middle estuary. As a result, the proportion of metals in sediments is significantly higher in the estuary's middle region than its mouth region.

The organic matter gets readily adsorbed on the surface of sediment particles in the marine environment~~s~~, where it is primarily abundant in finer sediments than coarser sediments. The abundance of unbound binding sites in finer sediments allows for the adsorption of organic material~~s~~. Thus, the organic matter is often deposited in higher concentration in sediments at the lower estuary than the middle estuary. The complexation of surface-metal-ligand enhances the sorption of metals onto finer sediment enriched in organic matter. Both mudflat and mangrove sediments have high organic matter content and support enrichment of metals.

The Fe and Mn are redox sensitive metals that too plays an important role in the deposition of metals in estuarine sediments. Both are present in numerous oxidation states in the estuary. Under oxic conditions, Fe and Mn readily precipitate as oxides or hydroxides that are effective scavengers of other trace metals in estuaries. The precipitated form of Fe and Mn are present as coatings on the surface of sediment particles which enhances adsorption of trace metals on sediment particles. However, these oxides or hydroxides of Fe and Mn are highly unstable under anoxic conditions. The lack of oxygen triggers their dissolution and release them in their dissolved form. It in turn, causes release/mobilization of adsorbed trace metals from the surface of Fe-Mn oxides or hydroxides to water column or interstitial water. Thus, the redox potential of estuarine water regulates the adsorption-desorption of metals in or from sediments and influences the overall chemistry of estuarine water and sediments.

The sulphide is yet another factor which governs the distribution of metals in estuaries. For instance, as the depth of the water column increases, the level of oxygen also diminishes. It leads to anoxic circumstances which favours the reduction of sulphate form of sulphur to sulphide. The reduction in oxygen level is accomplished through the oxidation and decomposition of organic materials in the marine environment through microbial mediated processes. Acetate, propionate, butyrate, and iso-butyrate are the main organic substrates that are oxidized during sulphate reduction, and they help to create anoxic conditions in sediments. The sulphides when encounter soluble forms of metals viz., Fe, Cr, etc., often precipitate as metal sulphides under anoxic conditions.

Further, the replenishment of oxygen oxidises sulphide to sulphate, and mobilizes adsorbed trace metals to water column or pore water.

In addition, microplastics are another persistent pollutant in estuaries and it offers a suitable adsorption site for metals, and regulates metal bioavailability and bioaccumulation processes. Due to their affordability, light weight, durability, and malleability, plastics are utilized extensively around the world **(Boucher and Friot 2017)**. The potential uses for plastics are essentially infinite. That being said, plastic garbage is a feature of the marine ecosystem due to its widespread use **(Chen 2020)**. According to **Jambeck et al. (2015)**, about 300 million tons of plastic items are generated worldwide each year, with over 10% ending up in the ocean. In the ocean, there are two types of plastics: big plastic wastes and tiny plastic particles called microplastics that are less than 5 mm in size **(Thevenon et al. 2014)**. The physico-chemical characteristics of microplastics augment their capacity to enrich specific contaminants, like metals and persistent organic pollutants, for which microplastics can serve as crucial vectors within the marine ecosystem. Furthermore, harmful compounds included in plastic itself, such as flame retardants, plasticizers, and antibacterial agents, are discharged into the water and contribute to pollution **(Law and Thompson 2014)**. The pollutants that are conveyed by microplastics or adhered to their surface have the potential to enter the global food chain, enrich organisms, and cause complicated toxicity to marine life **(Rios Mendoza and Jones 2015; Rochman et al. 2018)**. According to **Cole et al. (2011)**, microplastics are a major threat to both human health and the survival of marine life.

The metals in sediments can exist in various geochemical forms, and they often show distinct physical and chemical behaviours with regard to their mobility, chemical interactions, biological availability, and possible toxicity **(Almas et al. 2006)**. According to **Tessier et al. (1979)**, a given metal in sediments is present in five distinct geochemical fractions, namely, exchangeable, carbonate, Fe-Mn oxide, organic/sulphide bound, and residual. The first four fractions together are regarded as the bioavailable fraction, which is active, mobile, environmentally unstable, and reactive to changes in the chemical and physical parameters of estuarine water **(Pardo et al. 1990).** On the other hand, the minerals in sediments' have lattice structure holding strongly embedded metals in their residual form. The residual form of metals is highly stable, unreactive, and immobile in the environment. The metals that are released into estuaries as a result of human activity are regarded as the bioavailable form, whereas metals that are obtained from the natural weathering of the catchment area rocks are found in residual form in sediments **(Purushothaman et al. 2007)**. The metals are persistent in nature and its distribution in the sediment is influenced by a number of variables, including processes, pH and Eh levels, mineral content, and sediment size **(Wen and Allen 1999)**. The metals in their exchangeable form are susceptible to mobilization with changes in ionic composition, while carbonate bound metals can be mobilized with changes in hydrogen ion composition. The oxygen level can trigger metal adsorption-desorption processes in Fe-Mn oxide/hydroxide and sulphide fractions. The metals associated with organic matter are susceptible to mobilization with decomposition of organic matter by microorganisms. These physico-chemical factors make metals readily bioavailable and are easily assessable to flora and fauna in the estuaries. Metals in their bioavailable forms can be assimilated by the estuarine fauna from water, sediment, and other substrates. The net accumulation and retention of a metal in a tissue or entire organism due to exposure to metals in their accessible forms is known as metal bioaccumulation. The term "metal bioaccumulation" refers to the accumulation of metals in an organism that can affect a single tissue or the entire organism, including any metals that are absorbed by the organism or adsorbed to surfaces or to a specific tissue; which is usually expressed on a weight (dry or wet) adjusted basis. Metals can bioaccumulate from the air, water, solid phases (organic and inorganic phases in soil and sediment), and diet, among all environmental sources. The previous research **(Dessai et al. 2009)** has shown that metal contamination of the sediment from estuarine environments is significant.

The phyto-remediation is a technique that employs plants to remove or degrade organic contaminants and toxic metals from soils, sediments, and waterways. According to **Adriano et al. (1999)**, this process involves chemical buildup in plants, breakdown of organic molecules, microbial stimulation for decomposition, absorption and volatilization, and reduced mobility and bioavailability of pollutants in the environment. This could be acknowledged as a low-cost, environmentally beneficial way to immobilize, stabilize, degrade, transfer, remove, or detoxify organic and inorganic pollutants (such as hydrocarbons, heavy metals, and metalloids), as well as nutrients and organic matter. Plants such as mangrove, which flourish in marshy environments, have the potential for phyto-remediation. The intertidal zones of tropical and subtropical shorelines are home to the salt-tolerant plants. According to **Primavera et al. (2019)**, the mangrove community is able to thrive in a wide variety of extreme environmental circumstances and has developed special adaptations to deal with them, such as salt-excreting leaves, gas-exchanging pneumatophores, and the ability to produce viviparous propagules. The mangrove habitat function as buffers or sinks, drawing metals out of water by rendering them immobile. Metal retention is facilitated by their anaerobic, and sulphide and organic matter rich characteristics. Mangroves have the capacity to control metal intake at the root level and restrict translocation to the shoot, which makes them extremely resistant to metals even in the presence of metal-contaminated sediments **(Chakraborty et al. 2013)**. The urban development has led to significant anthropogenic impacts on mangrove forests, which protect coastal landforms and act as sediment traps. Mangroves are productive ecosystems that enrich coastal waters, yield commercial products, support coastal fisheries, and store endangered faunas. However, their unique ability to survive in high-salt and anoxic conditions and their tolerance to trace/heavy metal stress contribute to their potential use in preventing pollutant from entering food chains and web.

1.2 Study Area

Goa has a coastal stretch of about 105 km lying between Latitude 14°54’ N and 15°48’ N and Longitude 72°41’ E and 74°20’ E. The Mandovi River, one of the main rivers in Goa, originates in the Western Ghats at Bhimgad in the Belagavi District of Karnataka. It flows towards west and discharge the freshwater into the Arabian Sea. The annual precipitation received in the catchment area is 2932 mm (**Nasnodkar and Nayak 2015**) that leads to river runoff of ~ 258 m^3/s during monsoons (June to September). The river shows a drastic decrease in its runoff of ~ 6 m^3/s during the dry season (October to May; **Vijith et al. 2009**). The confluence of the Mandovi River and the Arabian Sea results in the formation of estuary which is around 70 km long and has a basin area of 1530 km^2 **(Gaonkar et al. 2021).** Estuary experiences a 2.3-m tidal range during spring tide which is reduced to 1.5 m at neap tide (Rao et al. 2011). The catchment area rocks consist of Dharwar Super Group of Archean-Proterozoic age composed of meta-conglomerate/breccias, quartzite, carbonate quartz-chlorite schist, quartz-chloritebiotite-amphibole schist, metagreywacke, banded iron and manganese (**Dessai et al. 2009, Kanetkar et al. 2022**). Rock types are capped with laterites and are rich in Fe-Mn ores. Around 27 mines were operational (past) in the catchment area (**Nayak 1999**) and they produced 1500-6000 tons of wastes/mine (**Fernandes and Nayak 2009**). The transportation of mined ore using barges through the Mandovi estuarine channel often spilled ore material in the water column **(Prajith et al. 2016).** Apart from ore mining activities, industrial, agricultural, domestic, municipal discharge, and fishing and recreational activities are additional contributors of metals in the Mandovi Estuary (**Kessarkar et al. 2013; Veerasingam et al. 2015**). Although, not enough research studies are carried out on MPs in the Mandovi Estuary there is high possibility of MPs addition in the estuary due to excessive usage of plastic material on land as well as during fishing activity.

1.3 Objectives

- To determine metal bioavailability and bioaccumulation in the Mandovi Estuary.
- To understand the potential of microplastics as adsorption site for metals.
- To understand the phyto-remediation potential of the mangrove plants.

1.4 Literature Review

Fernandes and Nayak (2014)	The concentration and distribution of the metals Fe, Mn, Cu, Co, Cr, Pb, and Zn in the intertidal sediments of Thane Creek and Ulhas Estuary were evaluated. The findings demonstrated the significant role of organic matter and grain size as metal carriers. Comparing the Ulhas Estuary to Thane Creek, the former showed higher levels of metal contamination due to proximity to industrial sites.
Pradit et al. (2016)	The sediment of Pattani Bay in Thailand and the Setiu Wetlands in Malaysia were studied to assess trace metal concentrations, sediment grain size, and organic matter. The results showed that Pattani Bay had higher mean concentrations of As, Cd, Cu, Pb and Zn compared to the Setiu Wetlands. High levels of As were detected in both seasons, but were classified as moderately polluted in the dry season. The Enrichment Factor showed significant anthropogenic activity as related input for As and Pb in both areas. Silt-clay content and organic matter were higher in Pattani Bay than in the Setiu Wetlands. The differences in sediment characteristics could be attributed to the sea, as Pattani Bay is comparatively enclosed by the Gulf of Thailand, compared to the Setiu Wetlands exposed to the South China Sea conditions.
Lai et al. (2013)	The study examined the impact of sediment grain size and land use on the distribution of heavy metals (Cr, Ni, Zn, Pb, Cd, Cu, As, and Hg) in sediments from 19 sub-basins of the Han River Basin, Korea. The findings revealed that all metals showed significant correlations with the fine silt fraction of the sediments. However, significant relationships with percent urban area were found only for Cd, Cu, Hg, and Pb. Most sediments were classified as "unpolluted to moderately polluted," except for a sub-basin with the highest urban area, which was highly polluted with Cu, Hg, and Pb. No relationship was found between agricultural land use and metal enrichment. The principal

	component analysis showed that grain size distribution and urban area contributed 55% of the metal distribution variance. The metals viz., Cd, Cu, Hg, and Pb were appeared to be influenced by grain size effects as well as land use.
Veerasingam et al. (2015)	The concentration of Co, Pb, Mn, Cu, Cr, and Zn in the sediments of the Mandavi Estuary was studied to comprehend the metal depositional trends and the degree of contamination. The findings showed that trace metal concentrations in the surface sediments were enriched, and metal concentration decreased with depth, indicating an excess of anthropogenic loading, particularly from mining activities in recent years.
Merhaby et al. (2018)	The metal pollution in surface sediments along the Lebanese coast, harbors and bays was investigated. The Beirut Port site was highly polluted with Cd, Pb, Zn and Cr whereas, Tripoli Port site showed moderate degree of pollution.
Noronha-D' Mello and Nayak (2015)	Metals viz., Fe, Mn, and Cr were found to be abundant in the mangrove sediments in the Zuari Estuary. The bioavailable concentration of Mn posed a concern to the biota.
Li et al. (2007)	The investigation on the distribution of metals in the coastal wetland of the Pearl River Estuary in China, revealed that the estuary was highly polluted with Cd, Zn, and Ni. Furthermore, an increased concentration of Cd and Zn in the exchangeable fraction indicated their ecological risk.
Dessai and Nayak (2009)	The metal speciation in the Zuari Estuary was investigated and discovered that Mn and Co concentrations were greater in the exchangeable fraction, indicating metal bioavailability in the estuarine system.

Kesavan et al. (2013)	The concentration of Cd, Co, Cu, Fe, Mg, Mn, Pb, and Zn in the sediments, shells, and tissues of molluscs, namely *Meretrix meretrix, Crassostrea madrasensis*, and *Cerithidea cingulate* was investigated in the Uppanar Estuary. The concentration of Mg was found to be higher in shells and tissues, while the concentration of Zn and Cu was below the concerning threshold level. The sediments had the highest Fe concentration, followed by shells and tissues.
Lakmapurkar et al. (2020)	The investigation was carried out on three bottom feeder species, *Mugil curema, Boleopthalmus dussumieri, and Scylla serrata*, from five different estuaries of Gujarat to evaluate metal concentrations (Cu, Zn, Pb, Cd, and Hg) in sediment and estuarine biota. The result indicated significant variations in metal concentration in South Gujarat, India. The concentrations varied in descending order, with Cu being predominant in *B. dussumieri* and Zn higher in *M. curema* and *S. serrata*. The Bio-Concentration Factor (BCF) revealed low total metal concentrations in all species, with *M. curema* having the highest concentration. The industrialization and anthropogenic activities led to higher Pb concentrations than the FAO/WHO limit suggesting metal accumulation in sediment and muscle tissue of benthic species as hazardous for human health.
Paz-Alberto et al. (2013)	The investigation was carried out in the mangrove ecosystems of Zambales, Philippines to disclose the phyto-remediation potential of mangrove plants. According to the study, mangroves have advantageous qualities that enable them to take Pb from contaminated sediments in locations that are directly impacted by coal-fired power plants. Thus, construed phyto-remediation potential of mangrove plants.
MacFarlane et al. (2003)	The accumulation and distribution of heavy metals in the grey mangrove, *Avicennia marina*, in Australia's estuary wetlands were

	investigated. The concentration of Zn, Cu, and Pb was monitored in sediments and in mangrove roots and leaf tissues. It was reported that, with temporal monitoring, *A. marina* roots might be used as biological indicators of Cu, Pb, and Zn and leaves for Zn.
Godoy et al. (2019)	The study demonstrated microplastics as effective carrier for metals in aquatic environment. Adsorption of metals, particularly Cr, Cu, Co, and Pb, was influenced by specific surface, porosity, and morphology of microplastics. The study also highlighted the influence of salinity and water chemistry in adsorption of metals on microplastics.
Munari et al. (2017)	The survey in Terra Nova Bay, Antarctica, found 1661 items of plastic debris, with fibers being the most common type. The microplastics accounted for 78.4% of the debris. The most common polymer was styrene-butadiene-styrene copolymer (94.13% by weight). The plastic debris concentration decreased with distance from the Mario Zucchelli Base.
Saha et al. (2021)	The study found MPs in water, sediment, and biota from the Sal estuary in Goa, India. The average concentrations in water column and sediment were 48 ± 19 MP particles/L and 3950 ± 930 MP particles/kg, respectively. The highest concentrations were found in finfish, with polymers were dominant in the gut and whole shellfish. The presence of MPs in commercially important shellfish and finfish samples highlighted potential health and ecological risks, as shellfish is a delicacy and a major player in the seafood industry. The study also found small-sized MPs with 37 different types, highlighting the impact of plastic pollution on the coastal marine biota.
Wang et al. (2020)	The study found a medium microplastic pollution level in the Gulf of Thailand's surface sediments, with small microplastics accounting for over 70% of total numbers. Fibrous microplastics, mainly from municipal sewage discharge, were the dominant pollutant. The study

	also found an inconspicuous correlation between grain size and microplastics, highlighting the environmental risks posed by microplastics to marine ecosystems.

CHAPTER - II

MATERIALS AND METHODS

2.1 Introduction

The biogeochemical processes that are predominant in the environment are represented by a sample of a particular amount. The samples are taken from the target site or sites, stored, and then examined for a number of parameters. For the study to yield reliable results that accurately reflect both the environment and the environmental challenges, the standard analytical procedure must be followed. Thus, the materials, procedures, and techniques must meet internationally recognized standards.

2.2 Field Sampling

The surface sediment samples were collected from eight sites representing mudflat (M1 and M2) and mangrove (M3-M8) intertidal regions along the Mandovi Estuary using a plastic scoop and transferred in pre-labelled polythene bags (Fig. 2.1). Also, duplicate samples from eight stations were collected in cloth bags for microplastic analysis. Additionally, edible benthic biota and mangrove (pneumatophores, stem and leaves) samples in proximity to sediment sampling stations were handpicked and stored in pre-labelled cloth bags. The sampling locations were noted down using the Global Positioning System (GPS) (Table 2.1). The sediment, edible biota and mangrove samples were transported to the laboratory in an ice-box. Upon reaching the laboratory, the sediment samples were dried in an oven at 60°C for sedimentological and geo-chemical analysis, and at 90°C for microplastic analysis. The biota and mangrove species were washed and cleaned with Milli Q water to remove dirt particles. Later, the samples were identified (Table 2.2) and preserved at -20°C and 4°C respectively until further analysis.

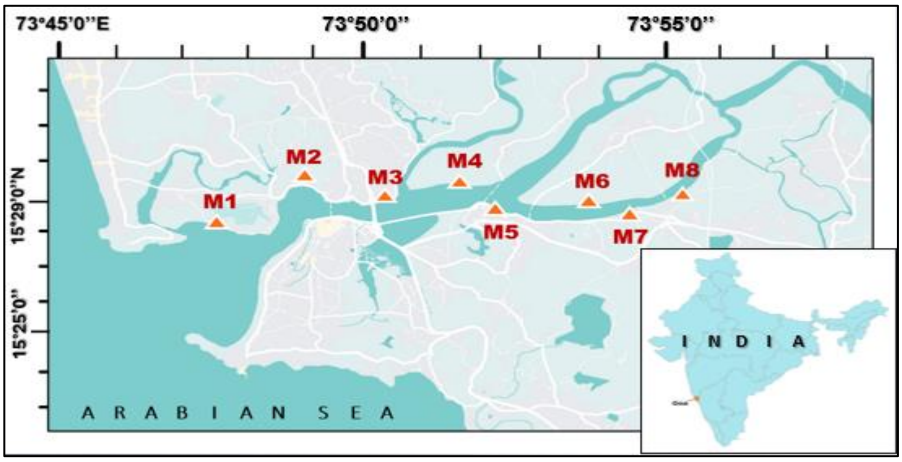


Fig. 2.1 Map showing sampling locations in Mandovi Estuary.

Table 2.1 The locations of sampling sites across the Mandovi Estuary.

Sampling stations	Latitude	Longitude
M1	15.499917°N	73.789042°E
M2	15.51029°N	73.814549°E
M3	15.509735°N	73.843242°E
M4	15.51251°N	73.85798°E
M5	15.505484°N	73.863645°E
M6	15.505125°N	73.887276°E
M7	15.504385°N	73.906154°E
M8	15.504385°N	73.91898°E

Table 2.2: The biota and mangrove species collected from the Mandovi Estuary.

Sampling stations	**Biota**	**Mangrove**
M1	• *Anadara antiquata* • *Meretrix meretrix* • *Crassostrea madrasensis*	-
M2	• *Saccostrea cucullata*	-
M3	• *Saccostrea cucullata*	• *Sonneratia alba*
M4	• *Saccostrea cucullata*	• *Rhizophora mucronata* • *Acanthus ilicifolius*
M5	• *Polymesoda erosa* • *Saccostrea cucullata* • *Crassostrea madrasensis*	• *Avicennia corniculatum*
M6	-	• *Avicennia officinalis* • *Rhizophora mucronata* • *Acanthus ilicifolius*
M7	• *Crassostrea madrasensis*	• *Avicennia marina*
M8	• *Crassostrea madrasensis* • *Polymesoda erosa* • *Saccostrea cucullata* • *Scylla serrata*	• *Rhizophora mucronata*

2.3 Metals in Sediment, Biota and Mangrove Plants

2.3.1 Sedimentological and Geo-Chemical Analysis

A portion of the dried (60°C) bulk sediment sample was used for the grain size analysis, while the remaining dried sediment sample was powdered using an agate mortar and pestle, and was utilized for the estimation of Total Organic Carbon (TOC), total metals and their speciation study.

2.3.1.1 Grain Size Analysis

The size of the sediment particles is determined by the hydrodynamic conditions and the depositional environment. The pipette method involving the settling velocity principle based on the Stoke's law was used to determine the Sand:Silt:Clay ratio **(Folk 1974).**

In this method, 10 g of oven-dried bulk sediment sample were added in a 1000 ml glass beaker. The glass beaker was filled with distilled water and the sediments were mixed with distilled water. The sediments were allowed to settle overnight. Next day, the water was decanted without disturbing the settled sediments to remove the salinity. The beaker was re-filled with distilled water and the sediments were allowed to settle. The water was again decanted next day and the process was repeated for five to six days and the removal of salinity was verified by adding $AgNO_3$ solution to the decanted water. Upon the removal of salinity, 10 ml of 10 % sodium hexametaphosphate was added to sediments to dissociate clay particles. Next day, 5 ml of 30 % hydrogen peroxide solution was added to oxidize organic matter completely and was kept undisturbed for a day. The treated sediments were sieved through a 63-micron (230 mesh size) sieve and the filter was collected in a 1000 ml measuring cylinder to separate sand component from silt and clay. The beaker was thoroughly washed until the solution became clear. The sand retained on sieve was transferred into the 100 ml pre-weighed beaker and was dried in an oven at 60°C. The filtrate in the glass cylinder was made up to 1000 ml mark using the distilled water and homogenized for about 2 minutes with the help of a stirrer. The stirring time was noted down and the solution was allowed to settle. The room temperature was determined using a thermometer and the extraction time was calculated at size 8 ϕ following the Table 2.3.

Table 2.3: Time schedule to be used for pipette analysis.

Size Ø	Depth at which pipette is to be inserted (cm)	Time at which water is to be pipetted out Hours: Minutes: Seconds				
		$28^{0}C$	$29^{0}C$	$30^{0}C$	$31^{0}C$	$32^{0}C$
4	20	00:00:48	00:00:46	00:00:46	00:00:44	00:00:44
5	10	00:01:36	00:01:34	00:01:32	00:01:29	00:01:28
6	10	00:06:25	00:06:15	00:06:06	00:06:57	00:05:52
7	10	00:25:40	00:25:02	00:24:25	00:24:49	00:23:27
8	10	01:42:45	01:40:13	01:37:42	01:37:15	01:33:51
9	10	06:30:00	06:40:40	06:32:50	06:32:10	06:11:30
10	10	27:06:00	26:30:00	-	-	-

According to the extraction time, 25 mL clay solution was pipetted out from the depths of 10 cm and was transferred into 100 mL pre-weighed beaker. The beaker was dried in an oven at 60°C. The weight of dried sand and clay particles in the beakers was measured and used for calculation of the percentage of sand, silt and clay using the following formulae:

% Sand = (Weight of sand / Total weight of sediments) ×100.
X = (Weight of clay × 1000/25) – 1.
% Clay = (X / Total weight of sediment) × 100.
% Silt = 100 - (% of Sand + % of Clay).

2.3.1.2 TOC

The modified Walkley-Black method **(Gaudette et al. 1974)** was utilized to ascertain the TOC in powdered sediment. It involves exothermic heating using potassium dichromate ($K_2Cr_2O_7$) and oxidation with concentrated sulphuric acid (H_2SO_4).

The glass-wares were cleaned with chromic acid prior to use. The powdered sediment of 0.5 g weight was taken in a 500 ml conical flask. The sediment was treated with 10 ml of 1 N standard potassium dichromate solution, and 20 ml of sulphuric acid and silver sulphate mixture. The conical flask was gently swirled for 1 minute and allowed to stand for 30 minutes. Later, 200 ml of Milli Q water was added to mixture in a conical flask, followed by 10 ml of 85 % orthophosphoric acid and 0.2 g of sodium fluoride, and was mixed gently. Further, few drops of diphenylamine indicator were added and the solution was titrated against 0.5 N ferrous ammonium sulphate till one drop brilliant green end point. The oxidation of chlorine ions was prevented by addition of silver sulphate. The standardization blank was performed following the same procedure without the sediment.

The percentage of TOC was calculated using the following formula:

$$\% \text{ of TOC} = 10\,(1 - T/S) \times F$$

Where, S = Standardization blank titration, ml of ferrous solution
T = Sample titration, ml of ferrous solution
F = (1.0 N) × 12/4000 × 100/Sample weight = 0.6, when the sample weight is exactly 0.5 g,
Where, 12/4000 = m. eq. wt. of carbon

2.3.1.3 Total Metal

The digestion of sediment sample for the estimation of total metals was carried out following the method proposed by **Jarvis and Jarvis (1985)** which was later modified by **Sholkovitz (1990).** The apparatus was washed with chromic acid prior to use.

0.2 g of powdered sediment sample was placed in a Teflon beaker. 10 ml of acid mixture involving HF, HNO_3, $HClO_4$ was added slowly to the sediment sample in the ratio of 7:3:1, respectively. Proper care was taken to avoid excessive frothing and the mixture was completely dried on a hot plate at 150°C. After drying, 5 ml of additional acid mixture was added to the beaker and the sample was dried for 1 hour. Later, 2 ml of the concentrated HCl was added and the sample was dried completely. The dried sample in a Teflon beaker was extracted with 10 ml of 1:1 HNO_3 and warmed for few minutes. The contents of the Teflon beaker were filtered through the Whatman filter paper in a 50 ml volumetric flask and the volume was made up to 50 ml with Milli Q water. The solution was then transferred to a pre-cleaned labelled polyethylene bottle.

The concentration of total metals viz., Fe, Mn, Zn, Cu, Co and Ni in sediments was determined using the flame Atomic Absorption Spectrophotometer (AAS), Thermo-Fischer, iCE 3000 Series. The concentration of total Hg in sediments was determined using the Direct Mercury Analyser, DMA-80.

2.3.1.4 Metal Speciation

The five-steps sequential extraction procedure proposed by **Tessier et al. (1979)** which was later modified by **Dessai and Nayak (2009)** was adopted in the present study to understand the speciation of metals in sediments.

The method determines the concentration of a metal in different species or forms (exchangeable, carbonate, Fe-Mn oxide, organic matter/sulphide, and residual fractions) present in the sediment. The procedure is very specific with respect to pH, Eh, temperature, storage of treated samples, etc. The metals in the residual form are present in the lattice structure of minerals in the sediment and are environmentally unreactive. On the other hand, exchangeable, carbonate, Fe-Mn oxide and organic matter/sulphide bound fractions are considered as bioavailable and are environmentally reactive. The sequential extraction of metals was carried out as follows:

2.3.1.4.1 Exchangeable Fraction (F1)

It is the most labile fraction in sediments and the metal in the exchangeable form is highly mobile. They are highly susceptible to desorption from sediment surface with small changes in the ionic composition of water. The exchangeable fraction was extracted as follows:

1 g of the dried and powdered sediment sample was taken into a centrifuge tube and was treated with 8 ml of 1 N $MgCl_2$ at pH 7. It was continuously agitated for 1 hour. Later, the mixture was centrifuged at 8000 rpm for 10 minutes. The supernatant was filtered into 25 ml volumetric flask and the volume was made up to 25 ml with the Milli Q water. The sample was stored in a pre-cleaned labelled polyethylene bottle at refrigerated temperature for further analysis. The sediment residue (I) was used for the next step.

2.3.1.4.2 Carbonate Fraction (F2)

The metal associated with the carbonate fraction of the sediment can be leached out by treatment with sodium acetate at pH 5.

The residue I was treated with 8 ml of 1 N sodium acetate at pH 5. The mixture was continuously agitated for 5 hours. Later, the mixture was centrifuged at 8000 rpm for 10 minutes. The supernatant was filtered into 25 ml volumetric flask and the volume was made up to 25 ml with the Milli Q water. The sample was stored in a pre-cleaned labelled polyethylene bottle at refrigerated temperature for further analysis. The sediment residue (II) was used for the next step.

2.3.1.4.3 Fe-Mn Oxides Fraction (F3)

The Fe and Mn are redox sensitive metals and are effective scavengers of trace metals in the marine environment viz., Zn, Cu, Co, Ni, etc.

The residue II was treated with 20 ml of 0.04 M hydroxylamine hydrochloride in 25 % acetic acid at 96±3°C in a water bath for 6 hours. The solution was gently shaken for mixing after certain time intervals. After 6 hours, the mixture was cooled and was centrifuged at 8000 rpm for 10 minutes. The supernatant was filtered into 25 ml volumetric flask and the volume was made up to 25 ml with the Milli Q water. The sample was stored in a pre-cleaned labelled polyethylene bottle at refrigerated temperature for further analysis. The sediment residue (III) was used for the next step.

2.3.1.4.4 Organic Matter/Sulphide Fraction (F4)

This fraction includes the form of metal bound to organic matter and/or sulphides. The detritus has inert properties which hold the metals in this fraction in sediments.

The residue III was treated with 3 ml of 0.02 M HNO_3 and 5 ml of 30 % H_2O_2 at pH 2. The mixture was heated at 85°C in a water bath for 2 hours. Later, additional 3 ml of 30 % H_2O_2 (pH 2) was added to the mixture and was heated at 85°C in a water bath for 3 hours. After 3 hours, the mixture was treated with 5 ml of 3.2 M ammonium acetate in 20 % HNO_3 and was subjected to agitation for 30 minutes. Thereafter, the mixture was centrifuged at 8000 rpm for 10 minutes. The supernatant was filtered into 25 ml volumetric flask and the volume was made up to 25 ml with the Milli Q water. The sample was stored in a pre-cleaned labelled polyethylene bottle at refrigerated temperature for further analysis. The sediment residue (IV) was used for the next step.

2.3.1.4.5 Residual Fraction (F5)

The metal in the residual form is present in the lattice structure of minerals in the sediment and is usually considered as cohesive part of the sediment. The residue IV was transferred to a pre-cleaned Teflon beaker and the metal was extracted with an intense acidic treatment following the same procedure as in case of total metal estimation in sediments. The extracted solution was then filtered through Whatman filter paper in 25 mL volumetric flask. The volume of filtrate (extracted sample) was made to 25 mL using Milli Q water. It was transferred into a pre-cleaned labelled polyethylene bottle and was stored at refrigerated temperature until further analysis.

All the sequentially extracted fractions were aspirated through the flame Atomic Adsorption Spectrophotometer (AAS), Thermo-Fischer, iCE 3000 Series to determine the concentration of metals viz., Fe, Mn, Zn, Cu, Co and Ni.

2.3.2 Biota Analysis

The accurate determination of metal content of marine species is an essential initial phase in monitoring ecological conditions, controlling pollution, and enforcing food safety regulations. The biota draws metals from water, sediment and food particles in the marine environment which get accumulated in their tissues and can be passed on to humans via the food chain. The samples of edible estuarine benthic biota (clams, oysters and crab) that had been stored at 90^0C were brought to room temperature. Later, the soft tissue inside the shells was extracted using a plastic knife. Further, transferred to a pre-cleaned petri dish and was dried for 72 hours at 60°C. A porcelain pestle and mortar were used to pulverize the dry material.

1 g of finely powdered biota sample was placed in a Teflon beaker and was digested with 2 ml of HNO_3 and 1 ml of $HClO_4$, at 120°C on a hot plate for 3 hours **(Yuzereroglu et al. 2010).** Later, the digested sample was filtered using Whatman filter paper into a 25 ml volumetric flask and was made up to the 25 ml mark using the Milli Q water. The sample was then stored in a polyethylene bottle at refrigerated temperature for metal (Fe, Mn, Zn, Cu, Co, and Ni) analysis using the flame AAS, while the concentration of Hg in biota was determined using the Direct Mercury Analyser, DMA-80.

2.3.3 Mangrove Analysis

The following method was used to determine the metal concentration in the mangrove pneumatophores, stems and leaves:

The mangrove pneumatophores, stems and leaves were cut into small pieces using a plastic knife and dried in an oven at 60°C. The dried mangrove sample was powdered using an agate mortar and pestle **(Nath et al. 2014).**

1g of the powdered mangrove sample was placed in a Teflon beaker and was acid digested with 10 mL of HNO_3 and 5 mL of H_2O_2 for two hours at 90°C on a hot plate **(MacFarlane and Burchett 2002)**. The digested mangrove sample was then filtered using Whatman filter paper and transferred into a 25 ml volumetric flask. The final volume in the volumetric flask was made to 25 ml using the Milli Q water. Later, the sample was transferred to a pre-cleaned polyethylene bottle and stored in a refrigerator until the metal (Fe, Mn, Zn, Cu, Co, and Ni) analysis using the flame AAS. Also, the concentration of Hg in mangrove samples was determined using the Direct Mercury Analyser, DMA-80.

2.3.4 Metal Pollution Indices

The following pollution indices were used to understand the contamination and pollution of metals in mudflat and mangrove sediments of the Mandovi Estuary:

2.3.4.1 Contamination Factor (CF)

To assess the level of contamination of metals in the sediments, the CF was calculated **(Barbieri 2016)** as follow:

$$CF = C_{metal} / C_{background}$$

Where, C_{metal} was the concentration of metal in the studied sediment sample and $C_{background}$ was the background value of the same metal taken as Upper Crustal Average (UCA) value **(Wedepohl 1995)**. The CF is classified into four categories **(Pekey et al. 2004):** CF value < 1 refers to low contamination; 1 ≤ CF value < 3 refers to moderate contamination; 3 ≤ CF value < 6 refers to high contamination; and CF value > 6 refers to very high contamination.

2.3.4.2 Geo-Accumulation Index (I_{geo})

The I_{geo} proposed by **Muller (1979)** was used to assess the metal pollution in the sediments of the Sal Estuary. The I_{geo} value was calculated using the following equation:

$$\mathrm{Igeo} = \log_2 \frac{\mathrm{Cn}}{1.5 \times \mathrm{Bn}}$$

Where, Cn represented the concentration of metal in studied sediment sample and Bn was the background value of the same metal from upper crust **(Wedephol 1995)**. To account for variances in background values caused by lithological differences, the factor 1.5 was used**.** The degree of metal pollution was evaluated using the classification given in the Table 2.4 **(Rubio et al. 2000; Praveena et al. 2008)**.

Table 2.4: Classification of Geo-accumulation Index (Igeo).

Geo-accumulation index	**Igeo class**	**Pollution intensity**
> 5	6	Very strongly polluted
> 4–5	5	Strong to very strongly polluted
> 3–4	4	Strongly polluted
> 2–3	3	Moderately to strongly polluted
> 1–2	2	Moderately polluted
> 0–1	1	Unpolluted to moderate polluted
< 0	0	Practically unpolluted

2.3.5 Metal Toxicity Assessment

The toxicity of metals in the mangrove sediments was assessed using the Risk Assessment Code (RAC), Screening Quick Reference Table (SQUIRT) and modified Biota Sediment Accumulation Factor (mBSAF). These indices provide with the indication of ecological state of the environment.

2.3.5.1 Risk Assessment Code (RAC)

The RAC was used to determine the risk of metals to the sediment associated biota. It is the sum of percentage of exchangeable and carbonate fractions of a metal in the sediment. The assessment of risk of metal to biota was carried out following the criteria proposed by **Perrin et al. (1985)**. The RAC value < 1 % indicates no risk, RAC value 1 – 10 % indicates low risk, RAC value 11 – 30 % indicates medium risk, RAC value 31 – 50 % indicates high risk, and RAC value > 50 % indicates very high risk and possibility of metal entering into the food chain.

2.3.5.2 Screening Quick Reference Table (SQUIRT)

The SQUIRT was developed by National Oceanic and Atmospheric Administration (NOAA). The toxicity of metal to sediment associated biota was assessed by comparing its bioavailable concentration (sum of exchangeable, carbonate, Fe-Mn oxides and organic matter/sulphide fractions) in the sediments with the Sediment Quality Values (SQV) as described in the Sediment Quality Guidelines (SQG). The terms used and the guidelines were categorized into five classes by **Buchmann (1999)**.

Table 2.5: Screening Quick Reference Table (SQUIRT).

Metals	**Threshold Effect Level (TEL)**	**Effect Range Low (ERL)**	**Probable Effect Low (PEL)**	**Effect Range Median (ERM)**	**Apparent Effect Threshold (AET)**
Fe (%)	-	-	-	-	22 (Neanthes)
Mn (ppm)	-	-	-	-	260 (Neanthes)
Zn (ppm)	124	150	271	410	410 (Infaunal community impacts)
Cu (ppm)	18.7	34	108	270	390 (Microtox & Oyster larvae)
Co (ppm)	-	-	-	-	10 (Neanthes)
Ni (ppm)	15.9	20.9	42.8	51.6	110 (Echinoderm Larvae)
Hg (ppm)	0.13	0.15	0.7	0.71	0.41 (Microtox)

2.3.5.3 modified Biota Sediment Accumulation Factor (mBSAF)

The accumulation of metals by biota from the associated sediments was evaluated through the determination of mBSAF proposed by **Dias and Nayak (2016).**

$$\text{mBSAF} = \frac{\text{Metal concentration in organisms tissue}}{\text{Bioavailable metal concentration in sediment}}$$

The mBSAF of < 1 suggest that the biota is de-concentrator of metal, whereas the value ranging from 1 to 2, and > 2 suggest biota as micro-concentrator and macro-concentrator of metal, respectively.

2.3.5.4 Human Health Risk Assessment

The edible bivalves collected from the Mandovi Estuary are part of dietary intake of humans. Therefore, it is crucial to assess the risk associated to humans on consumption of the edible bivalves. It was carried out by comparing the concentration of metal in the tissues of biota with the standard permissible limits (Table 2.6).

Table 2.6: Standard permissible limits of metals.

Metals	**Standard permissible limit**	**References**
Fe (%)	0.0034 − 0.0107	Charbonneau & Nash (1993)
Mn (ppm)	0.01	WHO (1989)
Zn (ppm)	40	WHO (1989)
Cu (ppm)	30	FAO/WHO (2004)
Co (ppm)	0.1− 5	FAO/WHO (2004)
Ni (ppm)	70 − 80	USFDA (1993)
Hg (ppm)	0.002	WHO (2016)

2.3.6 Assessment of Phyto-Remediation Potential

The phyto-remediation potential of mangrove species was evaluated based on the Translocation Factor **(Usman and Mohamed 2009).** The TF was calculated using the following formula:

$$\mathrm{TF} = \frac{\text{Concentration of metal in the aerial plant organ}}{\text{Concentration of metal in the stems/roots}}$$

TF value > 1, indicates that the plant is an accumulator of metals in the aerial parts which is a crucial criterion for phyto-extraction. TF value < 1, means the plant is an extruder of metals and/or higher accumulator of metals in the roots than aerial organs.

2.4 Microplastic Analysis

2.4.1 Microplastic Identification and Quantification

The analysis of microplastics in sediments was carried out following a slightly modified method by **Rivoira et al. (2020)** initially proposed by **Masura et al. (2015).** The method considers microplastics in the size range from 5 mm to 0.3 mm. The procedure involved the following steps:

2.4.1.1 Sieving

The dried 500 g of sediment sample was sieved through 5 mm and 0.3 mm sieves. The dry sieving was carried out for sandy sediment sample (M1 and M2) and the clayey sediment samples (M3, M4, M5, M6, M7 and M8) were subjected to wet sieving. The sediment fractions of size >5 mm and < 0.3 mm were discarded. The solids retained on the 0.3 mm sieve were dried at 90°C and was subjected to density separation process.

2.4.1.2 Density Separation I

The sieved and dried sediment sample was transferred in a 500 ml glass beaker and mixed vigorously with 300 ml of super-saturated NaCl solution using a glass rod to float out the microplastics. The floating solids were collected in a separate glass beaker and oven dried at 90°C till complete dryness.

2.4.1.3 Wet Peroxide Oxidation (WPO)

The dried sample from the density separation step was mixed with 20 ml of aqueous 0.05 M Fe (II) solution and 20 ml of 30 % hydrogen peroxide solution. The mixture was allowed to stand at room temperature for five minutes. Later, a stir bar was placed in a beaker and the beaker was covered with a watch glass. The sample in the beaker was heated on a hotplate at 75°C and as soon as the bubbles were observed at the surface, the beaker was removed from the hotplate and placed in the fume hood till the boiling subsided. The mixture was then heated to 75°C for additional 30 minutes. If any organic matter was visible, the sample was heated for an additional 30 minutes by adding an extra 20 ml of 30 % hydrogen peroxide. This process was repeated until no organic matter was visible. Approximately 6 g of NaCl was added per 20 ml of sample to increase the density of the aqueous solution (~5 M NaCl). The mixture was heated at 75°C until the dissolution of salt.

2.4.1.4 Density Separation II

Upon WPO, the solution from the beaker was loosely covered with an aluminium foil and left to settle overnight. The floating solids from the beaker were collected on a clean 0.3 mm sieve and the settled solids were discarded.

2.4.1.5 Identification and Quantification of Microplastics

An inverted light microscope (Nikon ECLIPSE T*i*-S) was used to examine the solids collected on the sieve at magnification of 40 X. The microplastics were then identified and classified using the microplastic identification guide (**Marine & Environmental Research Institute n.d.**). Furthermore, the microscope was used to quantify each type of microplastic.

2.4.2 Microplastic Pollution Assessment Indices

The indices viz., the Pollution Load Index (PLI) **(Kabir et al. 2021)** and the Coefficient of Microplastic Impact (CMPI) **(Rangel-Buitrago et al. 2021)** were used to evaluate pollution and effects of microplastics.

2.4.2.1 PLI

The PLI was determined to assess the level of microplastic pollution in the intertidal sediments of the Mandovi Estuary. If the PLI is > 1, then the station is classified as polluted with microplastics. The PLI was calculated using the following formula:

$$PLI = {}^{C_i}/_{C_o}$$

where $\boldsymbol{C_i}$ is the microplastic abundance at station $\boldsymbol{i}$ and $\boldsymbol{C_o}$ is the lowest microplastic abundance obtained in the study considered as the baseline concentration.

2.4.2.2 CMPI

The CMPI was calculated to evaluate the impact of microplastics based on their shape.

$$CMPI = \frac{Specific\ MPs'\ Shape}{Total\ MPs'}$$

The impact of microplastic pollution was categorized by **Rangel-Buitrago et al. (2021)** as minimum (CMPI = 0.0001–0.1), average (CMPI = 0.11–0.5), maximum (CMPI = 0.51–0.8) and extreme (CMPI = 0.81–1).

2.5. Microplastics as Carriers of Metals

2.5.1 Modified Aqua Regia Extraction

The metals viz., Fe, Zn, Cu, Co and Ni adsorbed on the surface of microplastics were extracted using the modified Aqua Regia extraction procedure (**Holmes et al. 2012**). The microplastics were weighed prior to chemical analysis. A known weight of the microplastic from each station was placed in a sterile 15 ml polypropylene centrifuge tubes. 10 ml of 20 % (v/v) Aqua Regia (HCl:HNO_3; 3:1) was added to the microplastics. The centrifuge tubes were agitated for a period of 24 hours at room temperature at 150 rpm **(Holmes et al. 2012; Brennecke et al. 2016; Goh et al. 2022)**.

Following the extraction process, the mixture was filtered through a Whatman filter paper (number 41 having a pore size 20 µm). The filtrate was collected in a glass vial and the concentration of metals was determined using the Flame Atomic Absorption Spectrophotometer (Thermo Scientific-iCE 3000 Series AAS model) **(Brennecke et al. 2016).**

CHAPTER - III

RESULTS AND DISCUSSION

3.1 Metals in sediment and biota.

3.1.1 Grain size and TOC

The concentration of sand varied from 14.71 – 79.34 % in the surface sediments of the Mandovi Estuary (Table 3.1 and Fig. 3.1). The clay showed the variations from 2.56 – 38.88 %, while silt was in the range of 17.02 – 50.87 %. The TOC varied from 1.78 – 5.87 % in the Mandovi Estuary.

Table 3.1 The range and average of sand, silt, clay and TOC in sediment.

Stations	Sand %	Silt %	Clay %	TOC %
M1	79.34	17.02	3.64	1.78
M2	77.41	20.03	2.56	1.92
M3	14.71	46.41	38.88	5.84
M4	23.33	44.99	31.68	5.87
M5	35.13	50.87	14.00	5.53
M6	15.30	47.42	37.28	5.85
M7	26.38	44.74	28.88	5.84
M8	21.48	48.12	30.40	5.79
Range	14.71 - 79.34	17.02 - 50.87	2.56 - 38.88	1.78 - 5.87
Average	36.64	39.95	23.42	4.80

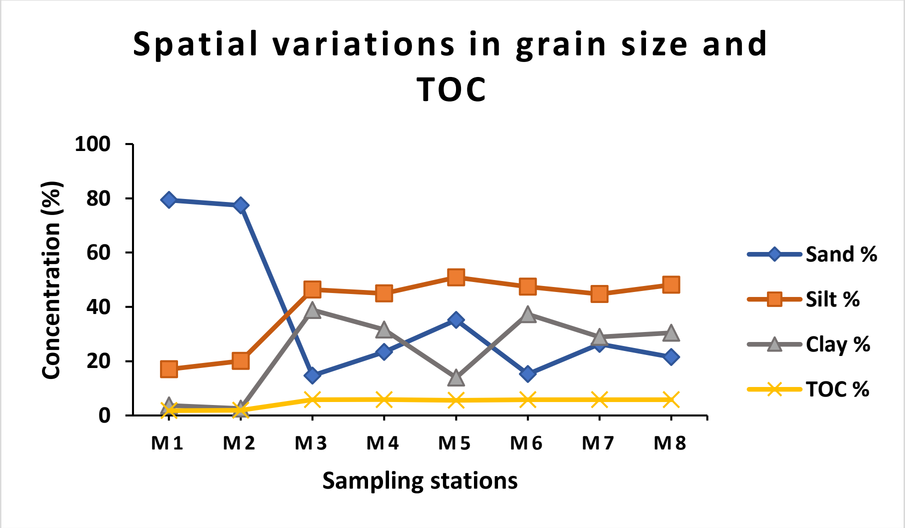


Fig. 3.1 The grain size and TOC distribution in surface sediments of the Mandovi Estuary.

The concentration of sand was more than 70 % in the surface sediments at sampling stations M1 and M2. At stations M4, M5, M7 and M8, it reduced to less than 40 %, and at stations M3 and M6, it reduced to less than 20 %. The decrease in sand at stations M3, M4, M6, M7 and M8 was well compensated by silt which was more than 40 %. The clay was above 20 % at stations M3, M4, M6, M7 and M8 while, it was less than 15 % at rest of the stations. The relative enrichment of coarser particles at stations M1 and M2 was attributed to higher hydrodynamics at these stations induced by waves and tidal action. These stations represented the lower region of the Mandovi Estuary subjected to high wave and tidal action. It facilitates retention of coarser sediments, and the finer (silt and clay) sediments are transported to middle estuarine region (**Nasnodkar and Nayak 2015**). The estuarine dynamics might have governed the sorting of sediment grain size from lower (coarser) to middle (finer) region of the Mandovi Estuary. The marine sediments carried by tidal currents, play a crucial role in regulating sediment deposition **(Sundermann and Feng 2004; Dessai et al. 2009).** The TOC values were > 5 % at stations M3, M4, M5, M6, M7 and M8, relatively higher than the stations M1 and M2. Therefore, the variations in TOC were governed by the distribution of finer sediments. The finer sediments have larger surface area by volume ratio than coarser sediments **(Nasnodkar and Nayak 2018)** and thus, provided ample of free binding surfaces for TOC.

3.1.2 Total metals

The concentration of total metals (Fe, Mn, Zn, Cu, Co, Ni and Hg) from lower to middle region of the Mandovi Estuary are presented in the Table 3.2 and Fig. 3.2a and b. The Fe varied from 4.18 – 8.61 % while Mn ranged from 750 – 2618 ppm. The metals viz., Zn, Cu, Co, Ni and Hg ranged from 91 – 155, 36 – 106, 22 – 31, 50 – 98 ppm and 20 – 148 ppb respectively. The Fe was highest at station M5, while Mn was more at station M8. The metals viz., Zn, Cu and Co were highest at station M7 and Ni was more at station M6. The concentration of Hg was highest at stations M8. The metals exhibited highest concentration at stations M5 - M8 representing the middle estuarine region. The relatively lower hydrodynamics in the middle estuary offer calm depositional environment with enrichment of silt and clay particles**.** The presence of high concentration of finer sediments in the middle of the Mandovi Estuary might have helped in the retention of relatively more metals than at sampling stations representing the lower estuarine region. The percentage of Fe in sediments was relatively more in the middle region (stations M3 -M8) than the lower estuary (stations M1 and M2). Fe readily precipitates at low salinity which might have regulated its spatial distribution in the Mandovi Estuary. Although, Mn precipitates at slightly higher salinity conditions, the relatively higher concentration of Mn in the middle estuary than the lower region could be due to anthropogenic input.

Table 3.2 Range and average of total metals in sediment

Stations	**Fe (%)**	**Mn (ppm)**	**Zn (ppm)**	**Cu (ppm)**	**Co (ppm)**	**Ni (ppm)**	**Hg (ppb)**
M1	4.18	1246	91	38	24	67	20
M2	5.65	750	115	36	22	50	59
M3	7.28	2114	132	72	26	94	67
M4	5.81	1764	120	62	23	84	66
M5	8.61	1613	133	64	24	76	84
M6	7.59	866	123	71	29	98	125
M7	7.07	2604	155	106	31	94	85
M8	6.91	2618	139	78	30	89	148
Range	4.18 – 8.61	750 – 2618	91 – 155	36 – 106	22 – 31	50 – 98	20 – 148
Average	6.64	1697	126	66	26	81	82
Upper crustal average **(Wedepohl 1995)**	3.09	527	52	14.3	11.6	18.6	56

The concentration of Hg was higher in the middle region of the Mandovi Estuary, with significant variation based on sediment type. Sandy sediments exhibited lower Hg levels compared to mangrove (soft/clay) sediments, likely due to increased surface area and decreased grain size, enhancing affinity with metal ions, including Hg in mangrove sediments **(Kwasigroch et al. 2021)**.

The concentration of all metals (Fe, Mn, Zn, Cu, Co, Ni and Hg) in the surface sediments of the Mandovi Estuary exceeded the upper crustal average value. Thus, indicated their enrichment in the sediments of the Mandovi Estuary mainly through human-induced activities. The catchment area of the Mandovi Estuary hosts several mining belts and the ore was transported in an open system via barges through estuarine channels for last several decades **(Dessai and Nayak 2009)**. The previous studies have revealed an enrichment of metals (Fe, Mn, Zn, Cu, Co and Ni) in the Mandovi Estuary from open cast ferromanganese mining activities **(Siraswar et al. 2021; Kanetkar et al. 2022)**. Although, there was a halt in mining operations in the last few years, but the transportation of ores previously stored at mining sites might have increased metal concentration in estuarine sediments. Even, the barge maintenance units established along the estuarine bank act as sources of metallic waste. Additionally, an input from industrial, domestic sewage discharge and agricultural waste might have enhanced the level of metals in sediments **(Gaonkar et al. 2021)**. The previous study conducted by **Bhat et al. (2014)** reported 70 ppb of Hg in the sediments of the Mandovi Estuary. It was attributed to non-point sources viz., sewage, mining and port activities. Therefore, the present study revealed a slight increase (10 ppb) in the average concentration of Hg in sediments of the Mandovi Estuary.

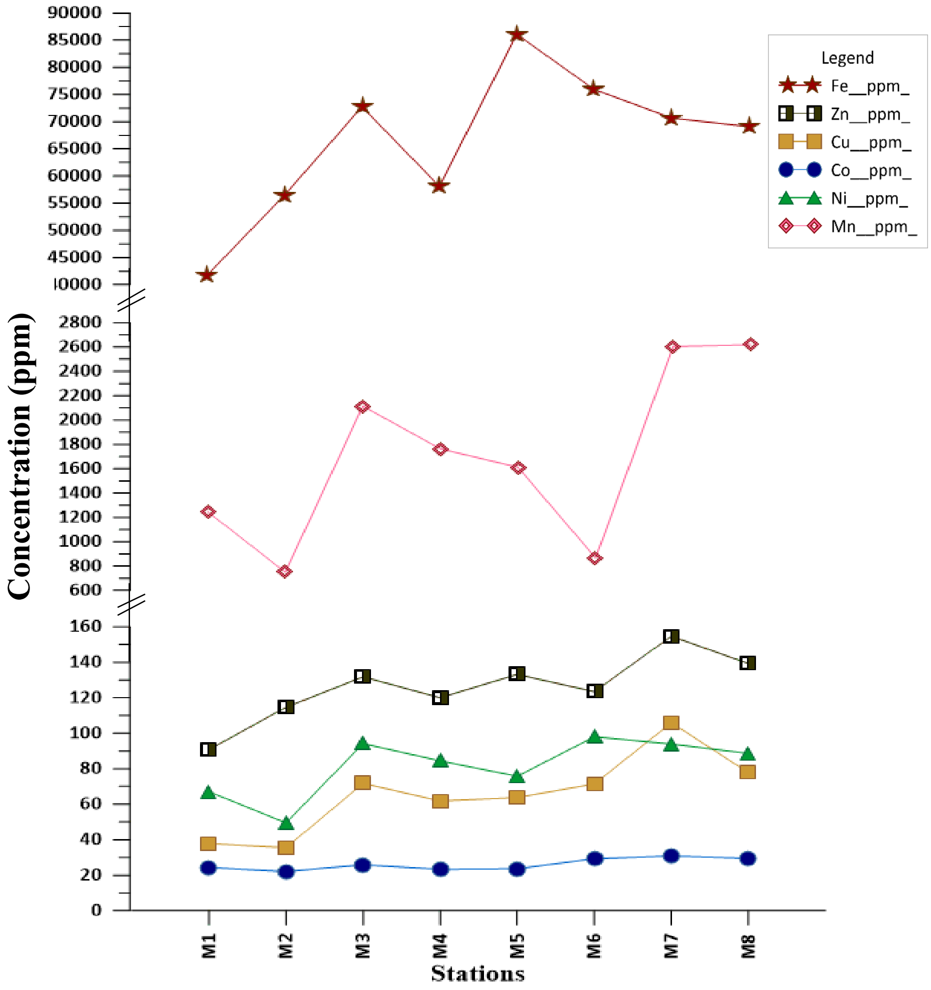


Fig. 3.2a. The concentration of total metals (Fe, Mn, Zn, Cu, Co and Ni) in the surface sediments of the Mandovi Estuary.

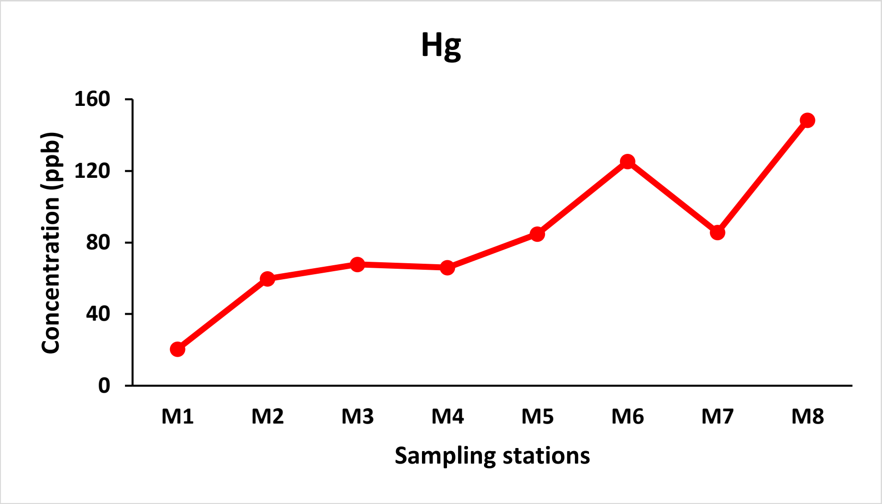


Fig. 3.2b. The concentration of Hg in the surface sediments of the Mandovi Estuary.

3.1.3 Metal pollution indices

The Contamination Factor (CF) was calculated to evaluate the degree of metal contamination in sediments of the Mandovi Estuary.

Table 3.3a. The Contamination Factor (CF) of metals in sediments.

Stations	**CF**						
	Fe	**Mn**	**Zn**	**Cu**	**Co**	**Ni**	**Hg**
M1	1.35	2.36	1.75	2.64	2.10	3.61	0.37
M2	1.83	1.42	2.21	2.48	1.90	2.66	1.07
M3	2.36	4.01	2.53	5.02	2.23	5.06	1.21
M4	1.88	3.35	2.31	4.32	2.01	4.54	1.18
M5	2.79	3.06	2.56	4.46	2.04	4.07	1.51
M6	2.46	1.64	2.37	4.99	2.52	5.27	2.24
M7	2.29	4.94	2.98	7.41	2.66	5.05	1.53
M8	2.24	4.97	2.68	5.45	2.55	4.77	2.65
Criteria	**CF value**	**Inference**					
	CF < 1	Low contamination					
	1 ≤ CF < 3	Moderate contamination					
	3 ≤ CF < 6	Considerable contamination					
	CF ≥ 6	Very high contamination					

According to the CF, Fe exhibited moderate contamination (stations M1 - M8) in the Mandovi Estuary (Table 3.3a). Mn indicated a considerable level of contamination at stations M3, M4, M5, M7 and M8, while showed moderate contamination at stations M1, M2 and M6. Zn and Co displayed moderate contamination in the sediments of the Mandovi Estuary. The CF revealed a very high level of Cu contamination at station M7, and a considerable level of contamination at stations M3, M4, M5, M6 and M8. It was moderately contaminated at stations M1 and M2. Ni showed moderate contamination at station M2, while displayed considerable contamination levels at stations M1, M3, M4, M5, M6, M7 and M8. Hg showed low contamination at station M1, while displayed moderate contamination at stations M2 – M8. Therefore, the computation of CF unveiled moderate to considerable to very high contamination of metals in the sediments of the Mandovi Estuary, primarily attributed to anthropogenic activities.

Table 3.3b. Geo-accumulation Index (I_{geo}) considering upper crustal values as background concentration.

Stations	**Igeo**						
	Fe	**Mn**	**Zn**	**Cu**	**Co**	**Ni**	**Hg**
M1	-0.15	0.66	0.22	0.82	0.48	1.27	-2.03
M2	0.29	-0.08	0.56	0.73	0.34	0.83	-0.49
M3	0.65	1.42	0.76	1.74	0.58	1.76	-0.31
M4	0.33	1.16	0.62	1.53	0.42	1.60	-0.35
M5	0.89	1.03	0.77	1.57	0.44	1.44	0.01
M6	0.71	0.13	0.66	1.74	0.75	1.81	0.58
M7	0.61	1.72	0.99	2.30	0.83	1.75	0.03
M8	0.58	1.73	0.84	1.86	0.76	1.67	0.82

The Fe and Mn concentration at stations M1 and M2, respectively, was practically unpolluted in the sediment (Table 3.3b). However, Fe at stations M2 – M8, and Mn at stations M1 and M6 ranged from unpolluted to moderately polluted class. Mn was moderately polluted at stations M3, M4, M5, M7 and M8. The Zn and Co fell under unpolluted to moderately polluted class. The pollution intensity of Cu ranged from unpolluted to moderately polluted class at stations M1 and M2. It was moderately polluted at stations M3, M4, M5, M6 and M8, and ranged from moderately to strongly polluted class at station M7. Ni was moderately polluted overall, except for station M2, which was unpolluted to moderately polluted in the Mandovi Estuary. The Hg in sediments of the Mandovi Estuary (stations M1 – M8) was practically unpolluted. The Igeo values indicated that the sediments in the Mandovi Estuary were unpolluted to moderately polluted with metals viz., Fe, Zn and Co. Conversely, the sediments were moderately polluted with Mn and Ni, while Cu indicated a level of pollution ranging from moderately polluted class to moderately - strongly polluted class. Thus, construed anthropogenic input of metals (mainly Mn, Cu and Ni) in the Mandovi Estuary.

3.1.4 Speciation of metals

The range and the average concentration of different geochemical forms of metals in sediments of the Mandovi Estuary is presented in Table 3.4 and Fig. 3.3.

Table 3.4 Range and average of metals in different sediment fractions in the Mandovi Estuary. ND: Not detected as the concentration was below the detection limit.

Metal - Station	**Exchangeable (%)**	**Carbonate (%)**	**Fe-Mn oxide (%)**	**Organic matter/Sulphide (%)**	**Residual (%)**
Fe – M1	ND	3.02	8.49	1.01	87.47
Fe – M2	ND	1.60	6.21	0.36	91.83
Fe – M3	0.87	1.10	5.82	5.33	86.88
Fe – M4	0.81	1.42	6.97	9.71	81.09
Fe – M5	0.89	1.03	5.69	4.06	88.34
Fe – M6	0.61	1.39	4.28	4.92	88.80
Fe – M7	1.29	1.21	5.78	7.23	84.49
Fe – M8	0.90	1.42	6.12	4.72	86.84
Metal - Station	**Exchangeable (%)**	**Carbonate (%)**	**Fe-Mn oxide (%)**	**Organic matter/Sulphide (%)**	**Residual (%)**
Mn – M1	6.96	13.84	31.42	36.81	10.97
Mn – M2	16.91	7.51	13.12	30.65	31.81
Mn – M3	13.61	19.59	40.60	9.21	16.99
Mn – M4	6.18	13.44	17.09	55.77	7.51
Mn – M5	5.77	9.48	20.12	50.18	14.45
Mn – M6	9.81	24.46	37.55	10.42	17.76
Mn – M7	16.56	39.98	30.77	7.10	5.58
Mn – M8	11.82	40.07	33.00	5.90	9.21
Metal - Station	**Exchangeable (%)**	**Carbonate (%)**	**Fe-Mn oxide (%)**	**Organic matter/Sulphide (%)**	**Residual (%)**

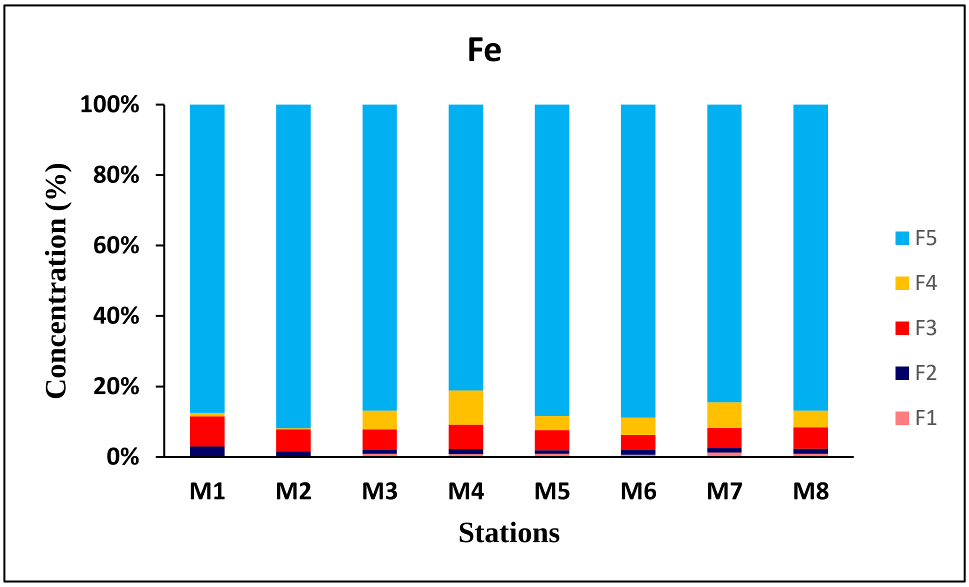
Zn – M1	0.32	0.47	1.74	0.92	96.55
Zn – M2	1.17	1.64	8.35	2.97	85.87
Zn – M3	0.95	1.64	8.56	6.26	82.59
Zn – M4	1.51	1.71	8.33	7.50	80.96
Zn – M5	1.78	2.51	11.81	5.90	77.99
Zn – M6	1.12	1.82	8.26	6.53	82.27
Zn – M7	0.99	3.08	15.51	9.64	70.78
Zn – M8	1.05	2.79	11.87	8.49	75.80
Metal - Station	**Exchangeable (%)**	**Carbonate (%)**	**Fe-Mn oxide (%)**	**Organic matter/Sulphide (%)**	**Residual (%)**
Cu – M1	1.77	5.57	7.95	5.95	78.76
Cu – M2	1.70	6.16	9.39	7.87	74.89
Cu – M3	0.48	2.20	3.40	37.02	56.89
Cu – M4	0.96	2.24	2.00	38.47	56.33
Cu – M5	0.89	2.61	5.25	40.91	50.34
Cu – M6	0.65	2.03	4.46	46.77	2.03
Cu – M7	0.51	1.74	4.06	59.41	34.28
Cu – M8	0.69	2.34	3.98	46.35	46.64
Metal - Station	**Exchangeable (%)**	**Carbonate (%)**	**Fe-Mn oxide (%)**	**Organic matter/Sulphide (%)**	**Residual (%)**
Co – M1	3.91	3.28	23.54	11.54	57.73
Co – M2	2.86	ND	29.69	8.92	58.53
Co – M3	3.71	5.63	28.89	14.37	47.39
Co – M4	5.47	6.90	27.32	19.91	40.40
Co – M5	4.71	5.72	28.25	12.71	48.61
Co – M6	2.00	5.35	31.83	18.95	41.88
Co – M7	2.47	5.43	31.44	20.36	40.30
Co – M8	ND	6.96	27.82	20.07	45.16
Metal - Station	**Exchangeable (%)**	**Carbonate (%)**	**Fe-Mn oxide (%)**	**Organic matter/Sulphide (%)**	**Residual (%)**

Ni – M1	5.64	8.98	11.04	5.25	69.08
Ni – M2	7.63	9.14	11.31	5.01	66.90
Ni – M3	4.49	5.74	9.94	11.17	68.66
Ni – M4	6.08	5.82	9.09	12.07	66.94
Ni – M5	6.94	6.95	9.65	9.29	67.17
Ni – M6	4.94	5.78	10.56	11.84	66.88
Ni – M7	6.02	6.31	10.75	11.70	65.22
Ni – M8	5.86	6.51	9.71	10.97	66.94

In the Mandovi Estuary, Fe was abundant and was more than 80 % in the residual fraction in sediments (Table 3.4; Fig. 3.3). The concentration of Fe in any individual bioavailable fractions was less than 10 %. The Mn was highest in the organic matter/sulphide fraction at stations M1 (36.81 %), M4 (55.77 %) and M5 (50.18 %). It was highest in the residual fraction at station M2 (31.81 %) and was also nearly equal in the organic matter/sulphide fraction (30.65 %). It displayed highest concentration in the Fe-Mn oxide fraction at stations M3 (40.60 %) and M6 (37.55 %). The carbonate fraction held highest proportion of Mn at stations M7 (39.98 %) and M8 (40.07 %). A significant (> 25 %) proportion of Mn was observed in Fe-Mn oxide (stations M1 and M7) and organic matter/sulphide (M2) fractions. Also, it was considerably (> 10 %) bound to exchangeable (stations M2, M3, M7 and M8), carbonate (stations M1, M3, M4 and M6), Fe-Mn oxide (stations M2, M4 and M5) and organic matter/sulphide (M6) fractions. Furthermore, the concentration of Mn in the labile (exchangeable and carbonate) fraction was > 10 % at all stations (M1-M8). The Zn was abundant (>70 %) in the residual fraction in the Mandovi Estuary. Additionally, Zn was in considerable amount (>10 %) in the Fe-Mn oxide fraction at stations M5, M7 and M8. The level of Cu was highest (> 50 %) in the residual fraction at stations M1 (78.76%), M2 (74.89 %), M3 (56.89 %), M4 (56.33 %), M5 (50.34 %) and M8 (46.64 %). It showed highest concnetration in the organic matter/sulphide fractions at staions M6 (46.77 %) and M7 (59.41 %). Also, reported significant (>25 %) concentration in organic matter/sulphide (stations M3, M4, M5 and M8) and residual (M7) fractions. The concentration of Co was > 40 % in the residual fraction at all stations (M1-M8). It was bound to the Fe-Mn oxide fraction in significant amount at stations from M2-M8. Also, it was available in considerable concentration in Fe-Mn oxide (station M1) and organic matter/sulphide (stations M1, M3, M4, M5, M6, M7 and M8) fractions. The concentration of Co in the labile fraction was > 10 % at stations M4 and M5. The level of Ni was > 65 % in the residual fraction at all stations (M1-M8). Also, it was present in considerable amount (>10 %) in Fe-Mn oxide (M1, M2, M6 and M7) and organic matter/sulphide (M3, M4, M6, M7 and M8) fractions. The labile fraction constituted > 10 % of Ni at all stations (M1-M8).

The speciation of metals in sediments of the Mandovi Estuary revealed highest concentration of Fe (stations M1-M8), Mn (satation M2), Zn (stations M1-M8), Cu (stations M1, M2, M3, M4, M5 and M8), Co (stations M1-M8) and Ni (stations M1-M8) in the residual fraction. The residual fraction, as described by **Ratuzny et al. (2009)**, is also known as the environmentally unreactive, inert, or immobile fraction in sediments. The metals found in this fraction originate naturally from the weathering of catchment area rocks **(Kanetkar et al. 2022)**. They are tightly bound within the mineral lattice structure of sediments and remain uninvolved in both geochemical and biological processes **(Zimmerman et al. 2010).** The metals viz., Mn (Fe-Mn oxide and organic matter/sulphide fractions), Cu (organic matter/sulphide fraction), Co (Fe-Mn oxide and organic matter/sulphide fractions) and Ni (Fe-Mn oxide and organic matter/sulphide fractions) were significantly bound to bioavailable fractions as well. The surface sediments on the estuarine bed are well oxygenated by ambient water column. The presence of oxygen facilitates precipitation of Fe and Mn as oxides or hydroxides **(Gadkar et al. 2019)**. These forms of Fe and Mn are effective scavangers of metals viz., Zn, Cu, Co, Ni, etc., in the estuarine environment **(Bhatkhande and Nasnodkar 2022)**. Thus, the metals get readily adsorbed onto precipated Fe and Mn oxides/hydroxides coatings available on the sediment surface. Furthermore, the presnece of significant proportion of metals in Fe-Mn oxide and organic matter/sulphide fractions was indicative of their anthropogenic origin and bioavilability in the estuary. The metals present in the bioavailable fractions primarily stem from human-induced activities and are loosely adhered to the surface of sediment particles **(Nasnodkar et al. 2021)**. Consequently, the bioavailable forms of metals signify their accessibility to sediment-associated biota. They are weakly bound and can easily transition from sediment into the water column, as they are highly sensitive to changes in the physico-chemical characteristics of estuarine water **(Cruz et al. 2020)**. Considering the frequent fluctuations in oxygen level (Eh) and microbial degradation of organic matter within dynamic marine ecosystems viz., estuaries, there is possibility of release or mobilization of metals from Fe-Mn oxide and organic matter/sulphide fractions in sediment into the water column in the Mandovi Estuary.

The metals viz., Mn, Ni and to certain extent Co were present in considerable (> 10 %) proportion in the labile form in sediments. The metals present in the labile fraction are most loosely held with weak forces on the sediment surface **(Pereira et al. 2023)**. A slight change in ionic composition of estuarine water and alteration in pH level can ignite desorption of metals from exchangeable and carbonate fractions, respectively **(Deep and Nasnodkar 2023)**. Thus, enhancing the bioavailability of metals to the sediment dwelling organisms. Therefore, the speciation of metals in sediments construed potential bioavailability of metals in the Mandovi Estuary.


Fe
Concentration (%)
100%
80%
60%
40%
20%
0%
M1
M2
M3
M4
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Stations
F5
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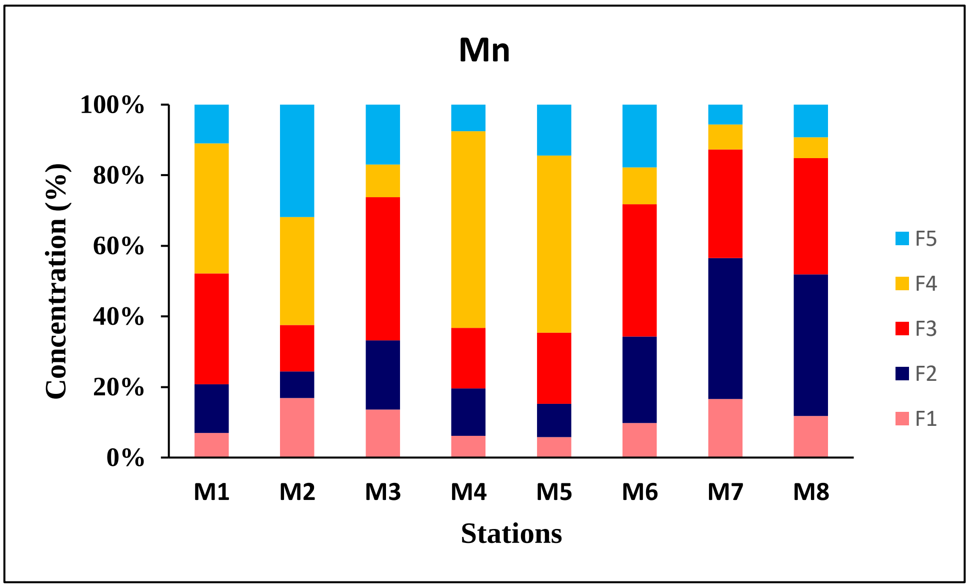
Mn
Concentration (%)
100%
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M1
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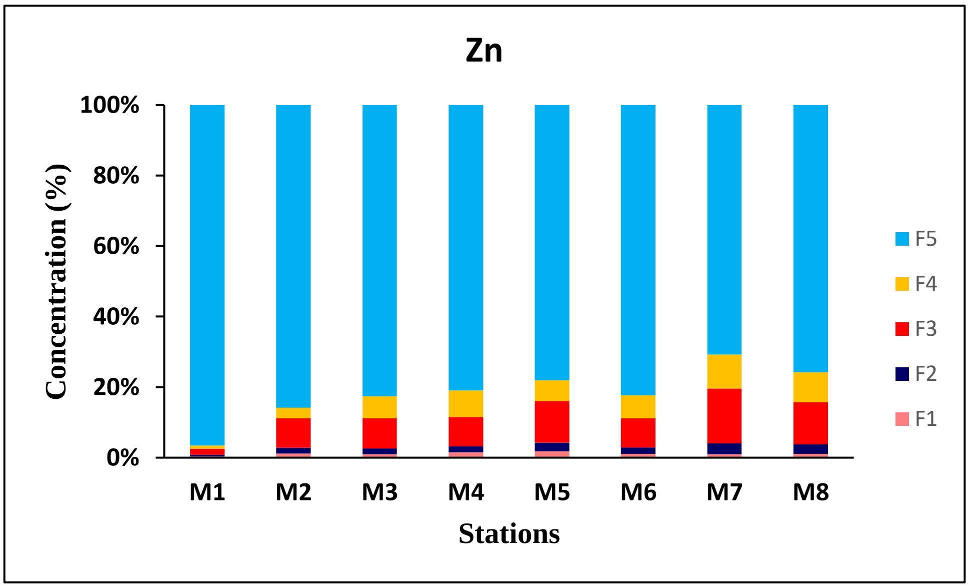
Zn
Concentration (%)
100%
80%
60%
40%
20%
0%
M1
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M4
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Stations
F5
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F3
F2
F1

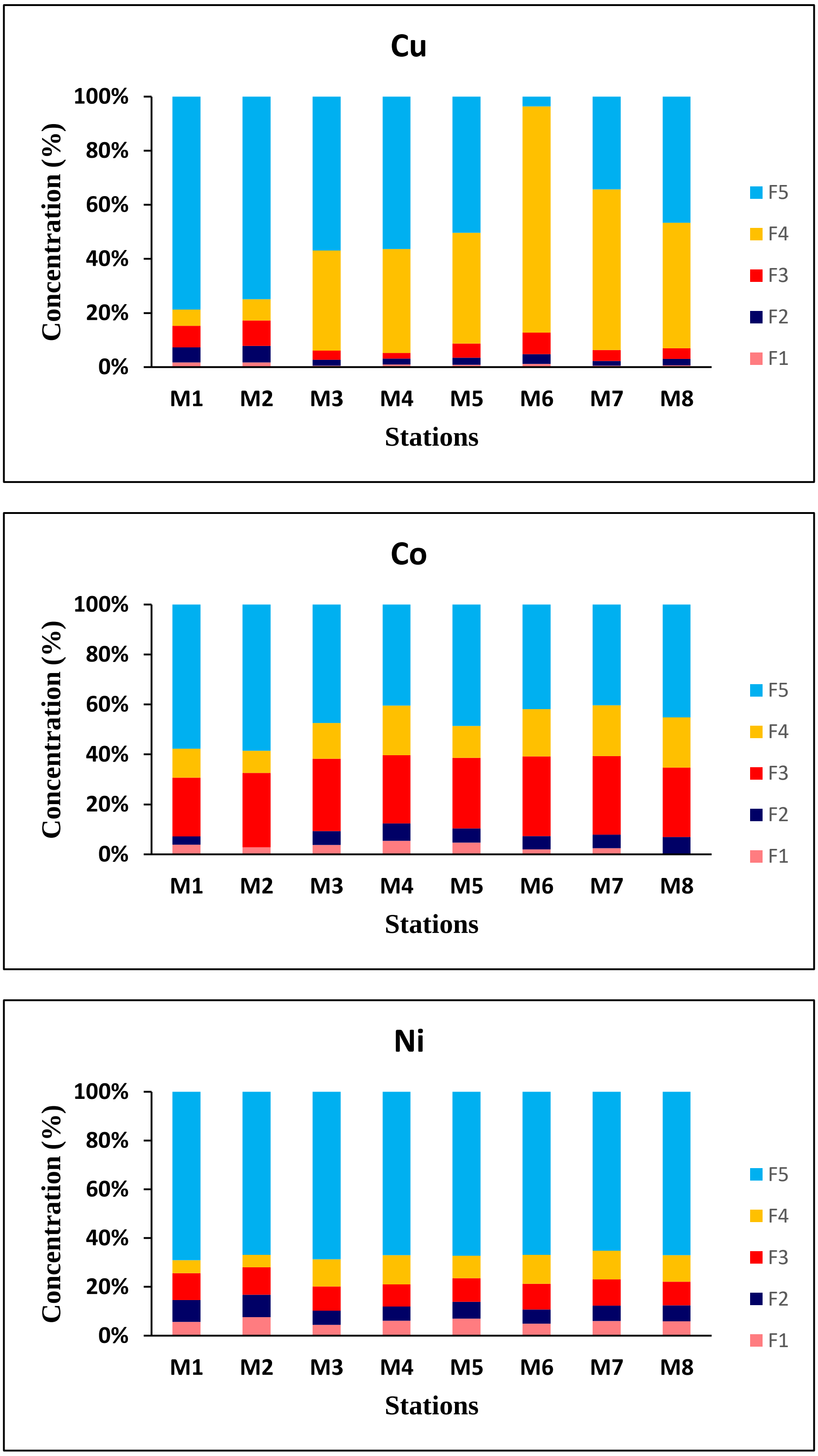


Fig. 3.3 The speciation of metals in the surface sediments of the Mandovi Estuary. F1: Exchangeable, F2: Carbonate, F3: Fe-Mn oxide, F4: Organic/ Sulphide and F5: Residual fraction.

3.1.5 Assessment of metal toxicity

3.1.5.1 Screening Quick Reference Table (SQUIRT)

The bioavailable concentration of metals in the sediments of the Mandovi Estuary were compared with the SQUIRT to evaluate their potential toxicity to sediment-associated biota (Table 3.5a). The bioavailable Fe, Cu and Ni in sediments of the Mandovi Estuary was below the AET value. Thus, indicating no adverse effects of these metals on the sediment-associated biota. However, the bioavailable Mn and Co concentrations exceeded/equalled the AET value at all stations and suggested their toxicity to sediment-associated biota. Also, Zn at station M1 was more than the AET level, while was below AET at stations from M2-M8. The application of SQUIRT revealed that the bioavailable concentration of Mn and Co was toxic to sediment-dwelling biota in the Mandovi Estuary. Additionally, toxicity from Zn was evident at station M1. The Mn toxicity induces oxidative stress in gills, kidney, liver and brain of aquatic organisms. It also exerts chronic, long-term effects on biota, viz., neurotoxicity, which impairs swimming ability. Mn toxicity can suppress immunity and impact fertility in aquatic biota **(Rodrigues et al. 2019)**. The elevated concentration of Co in biota can result in circulatory and nervous system disorders as well as bone diseases. Additional symptoms of Co toxicity include reduced weight gain, and its carcinogenic properties hinder DNA repair **(Kosiorek and Wsyzkowski 2019).**

Table 3.5a. Screening Quick Reference Table (SQUIRT) for metals in marine sediments **(Buchman 1999)** and bioavailable (F1+F2+F3+F4) concentration of metals in sediment.

Metals	**Threshold Effect Level (TEL)**	**Effect Range Low (ERL)**	**Probable Effect Low (PEL)**	**Effect Range Median (ERM)**	**Apparent Effect Threshold (AET)**	**Bioavailable concentration**							
						M1	M2	M3	M4	M5	M6	M7	M8
Fe (%)	-	-	-	-	22 (Neanthes)	12.53	8.17	13.12	18.91	11.66	11.20	15.51	13.16
Mn (ppm)	-	-	-	-	260 (Neanthes)	2878	1197	2096	3399	2363	1849	5332	3208
Zn (ppm)	124	150	271	410	410 (Infaunal community impacts)	549	168	194	175	197	190	226	194
Cu (ppm)	18.7	34	108	270	390 (Microtox & Oyster larvae)	29	22	56	47	53	69	111	68
Co (ppm)	-	-	-	-	10 (Neanthes)	14	10	18	16	13	17	20	17
Ni (ppm)	15.9	20.9	42.8	51.6	110 (Echinoderm Larvae)	54	38	69	65	54	74	72	72

3.1.5.2 Risk Assessment Code (RAC)

The RAC values for the metals (Fe, Mn, Zn, Cu, Co and Ni) in the sediments of the Mandovi Estuary are presented in the Table 3.5b.

Table 3.5b. Risk Assessment Code (RAC) for metals in sediment samples.

Metals	**RAC**							
	M1	M2	M3	M4	M5	M6	M7	M8
Fe	3.02	1.60	1.97	2.23	1.92	2.00	2.50	2.32
Mn	20.80	24.42	33.20	19.63	15.25	34.28	56.55	51.89
Zn	0.79	2.81	2.60	3.21	4.29	2.93	4.07	3.84
Cu	7.34	7.86	2.69	3.20	3.50	2.68	2.25	3.03
Co	7.19	2.86	9.34	12.37	10.42	7.35	7.90	6.96
Ni	14.62	16.77	10.23	11.90	13.89	10.72	12.33	12.38
Criteria	**RAC Value**		**Inference**					
	RAC < 1%		No potential adverse effect					
	RAC = 1-10%		Low potential adverse effect					
	RAC = 11-30%		Medium potential adverse effect					
	RAC = 31-50%		High potential adverse effect					
	RAC > 50%		Very high adverse effect					

The RAC demonstrated low potential adverse effects of Fe on biota in the Mandovi Estuary. Mn exhibited medium to high potential adverse effects on biota at stations from M1-M6, whereas it indicated very high potential adverse effects at stations M7 and M8. Zn and Cu exhibited low potential adverse effects with concentration below 10 %. Co indicated low potential for adverse effects at stations M1, M2, M3, M5, M6, M7 and M8, whereas it demonstrated moderate potential adverse effects at station M4. Ni suggested medium potential adverse effects on biota at stations M3 and M6, while medium potential adverse effects on biota at stations M1, M2, M4, M5, M7 and M8. Thus, the RAC analysis indicated the potential toxicity risk posed by metals viz., Mn, Zn, Cu, Co, and Ni to the biota in the Mandovi Estuary. Metals linked with the labile fraction are loosely attached to the sediment surface and are sensitive to alterations in ionic composition and hydrogen ion concentration of estuarine water **(Nasnodkar et al. 2021)**. Consequently, these metals can induce bioavailability and toxicity to estuarine biota.

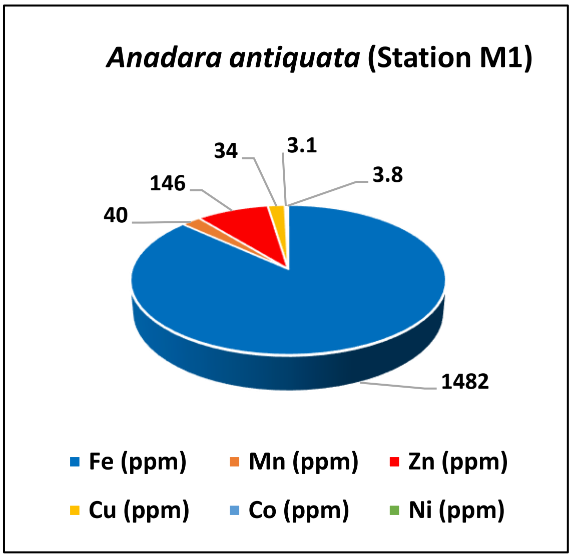
3.1.6 Metal bioaccumulation

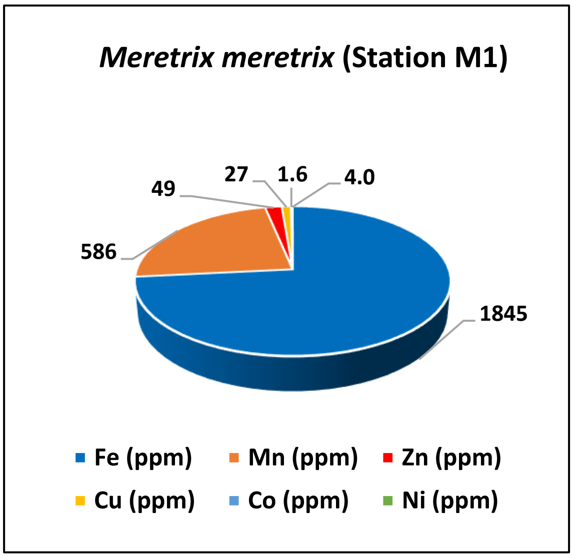
The concentration of metals in the soft tissues of the edible estuarine biota is presented in the Table 3.6a and Fig. 3.4. At station M1, *Anadara antiquata*, *Meretrix meretrix* and *Crassostrea madrasensis* exhibited highest concentration of Fe in soft tissues. Even, *Saccostrea cucullata* (station M2) and *Polymesoda erosa* (stations M5 and M8) accumulated high concentration of Fe than other metals. Further, *Saccostrea cucullata* had high level of Cu and Zn at Stations M3 and M4 respectively. The Zn was accumulated at greater extent than other metals in *Saccostrea cucullata* (stations M4, M5 and M8) and *Crassostrea madrasensis* (stations M5, M7 and M8). Also, Cu was highly accumulated in *Scylla serrata* at station M8. The concentration of Hg was lowest than other metals in soft tissues of biota in the Mandovi Estuary.

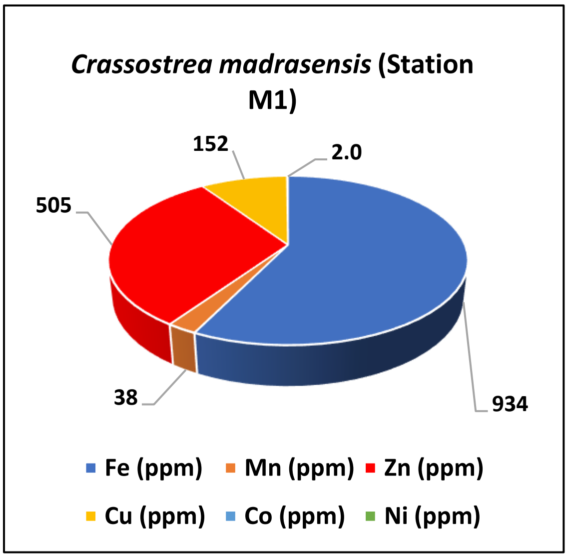
Table 3.6a. The concentration of metals in soft tissues of edible biota. ND = not detected as the concentration was below the detection limit.

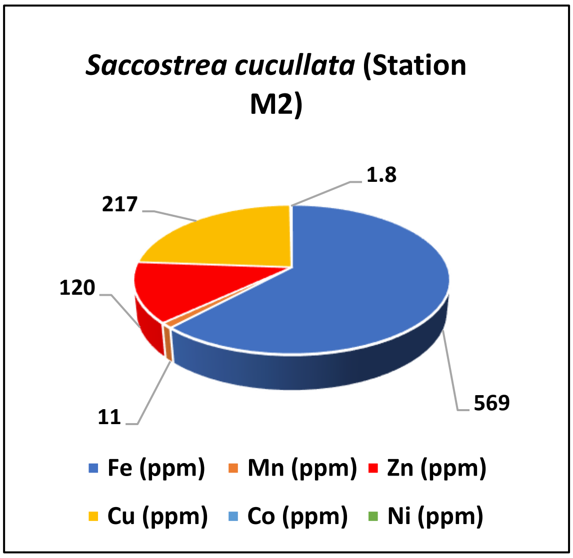
Stations	**Biota species**	**Metals concentration (ppm)**						
		Fe	**Mn**	**Zn**	**Cu**	**Co**	**Ni**	**Hg (ppb)**
M1	*Anadara antiquata*	1482	40	146	34	3	4	ND
	Meretrix meretrix	1845	586	49	27	2	4	45
	Crassostrea madrasensis	934	38	505	152	ND	2	ND
M2	*Saccostrea cucullata*	569	11	120	217	ND	2	35
M3	*Saccostrea cucullata*	200	10	199	335	ND	ND	57
M4	*Saccostrea cucullata*	463	49	954	217	ND	26	31
M5	*Polymesoda erosa*	1332	55	184	78	1	4	60
	Saccostrea cucullata	386	20	1484	196	ND	2	ND
	Crassostrea madrasensis	595	76	1716	259	1	3	60
M7	*Crassostrea madrasensis*	451	43	1486	311	ND	3	37
M8	*Crassostrea madrasensis*	424	41	878	224	ND	3	ND
	Polymesoda erosa	954	157	158	27	1	5	62
	Saccostrea cucullata	542	63	990	271	1	3	40
	Scylla serrata	190	90	178	255	0	3	100

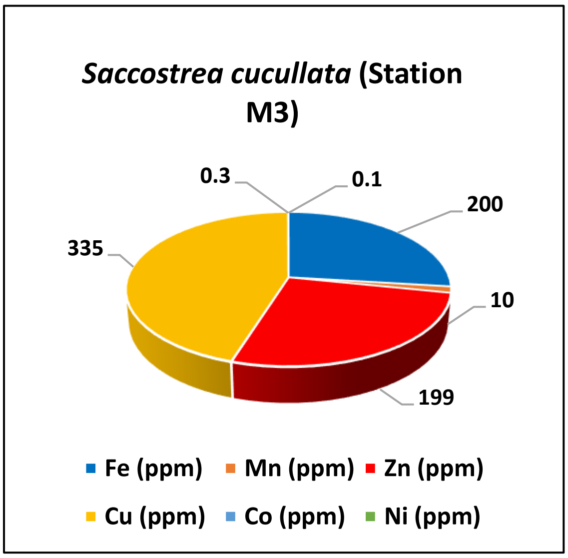
Most of the bivalve and crustacean species displayed strong preference for Fe, Zn and Cu in the Mandovi Estuary than other metals. These metals are referred as essential metals in the marine environment having role in growth and metabolic activities **(Jakimsha et al. 2011)**. Thus, the study of bioaccumulation of metals in edible bivalves and crustacean underscored the significance and their affinity to essential metals. The low level of Hg in the estuarine biota was attributed to its high toxicity even at trace level and non-essentiality in body functions. The concentration of metals varied in a given bivalve species collected from different stations, and also displayed variability among different species at a given sampling site. The discrepancies in the level of metals in bivalves was attributed to intrinsic and extrinsic factors that regulate bioaccumulation of metals. The physico-chemical properties of water viz., salinity, pH, Eh, ionic composition, etc., alters the bioavailability of metals in an estuarine environment. Also, genotype, phenotype, sex, age, size, species' feeding behaviours, trophic levels, etc., are other factors that decides the rate of accumulation and retention of metal in soft tissues of biota **(Khaled 2004)**. Moreover, the contamination gradients of metal sources are also a decisive factor in the bioaccumulation process **(Jayaprakash et al. 2015)**. The combination of all these factors might have resulted in the observed variations in metal level in estuarine biota.

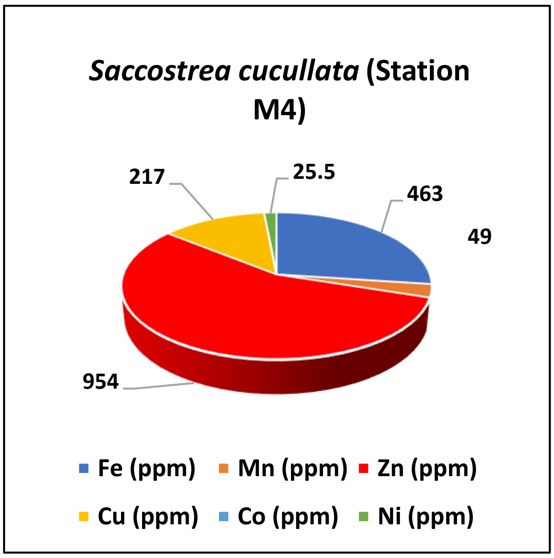

Anadara antiquata (Station M1)
34
3.1
146
3.8
40
1482
Fe (ppm)
Mn (ppm)
Zn (ppm)
Cu (ppm)
Co (ppm)
Ni (ppm)

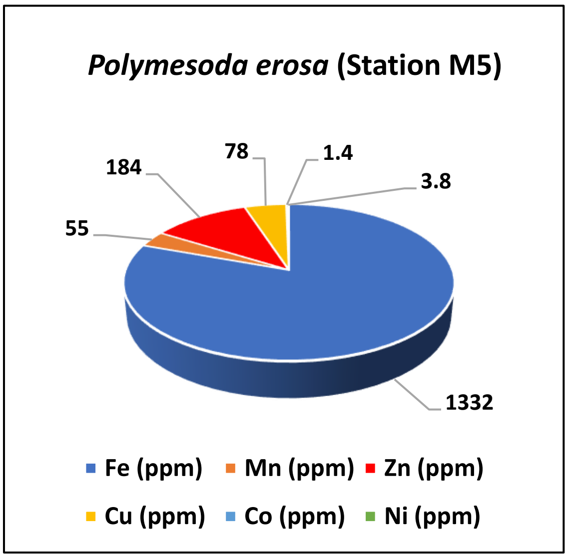

Meretrix meretrix (Station M1)
27
1.6
4.0
49
586
1845
Fe (ppm)
Mn (ppm)
Zn (ppm)
Cu (ppm)
Co (ppm)
Ni (ppm)

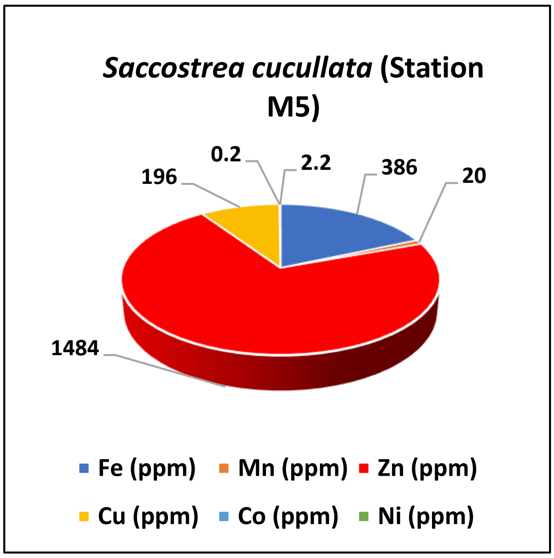

Crassostrea madrasensis (Station M1)
152
2.0
505
38
934
Fe (ppm)
Mn (ppm)
Zn (ppm)
Cu (ppm)
Co (ppm)
Ni (ppm)

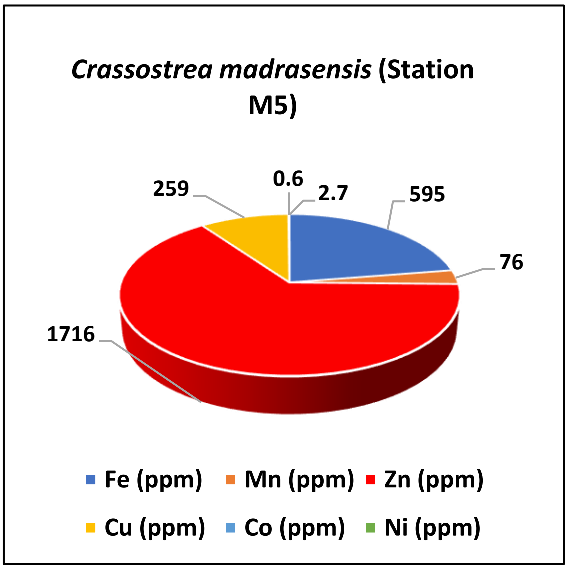

Saccostrea cucullata (Station M2)
1.8
217
120
11
569
Fe (ppm)
Mn (ppm)
Zn (ppm)
Cu (ppm)
Co (ppm)
Ni (ppm)

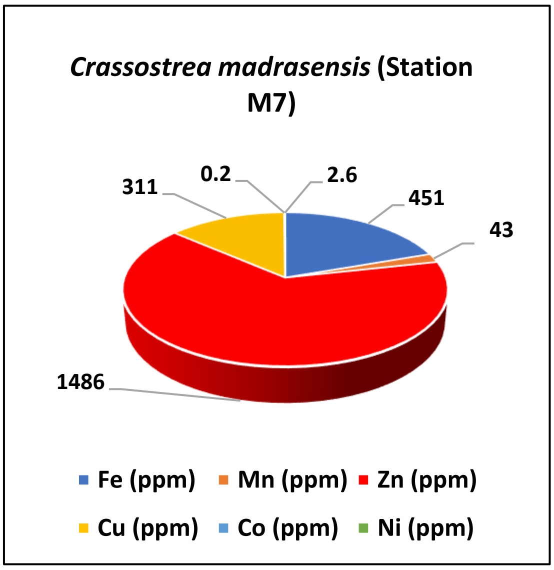

Saccostrea cucullata (Station M3)
0.3
0.1
200
335
10
199
Fe (ppm)
Mn (ppm)
Zn (ppm)
Cu (ppm)
Co (ppm)
Ni (ppm)

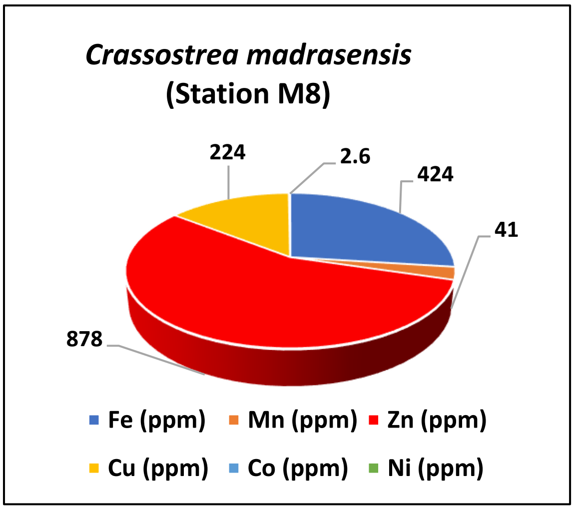

Saccostrea cucullata (Station M4)
217
25.5
463
49
954
Fe (ppm)
Mn (ppm)
Zn (ppm)
Cu (ppm)
Co (ppm)
Ni (ppm)

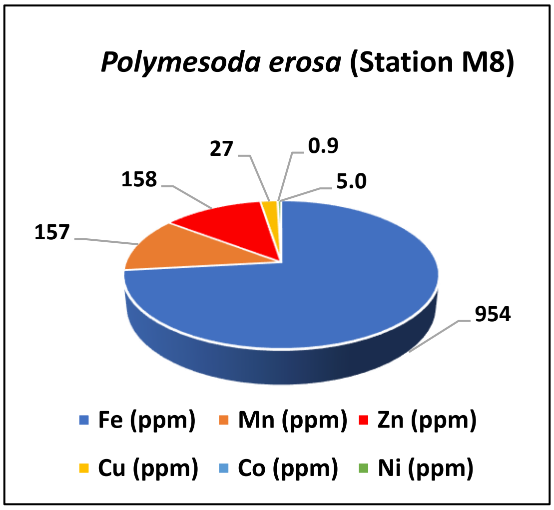

Polymesoda erosa (Station M5)
184
78
1.4
3.8
55
1332
Fe (ppm)
Mn (ppm)
Zn (ppm)
Cu (ppm)
Co (ppm)
Ni (ppm)


Saccostrea cucullata (Station M5)
196
0.2
2.2
386
20
1484
Fe (ppm)
Mn (ppm)
Zn (ppm)
Cu (ppm)
Co (ppm)
Ni (ppm)


Crassostrea madrasensis (Station M5)
259
0.6
2.7
595
76
1716
Fe (ppm)
Mn (ppm)
Zn (ppm)
Cu (ppm)
Co (ppm)
Ni (ppm)


Crassostrea madrasensis (Station M7)
311
0.2
2.6
451
43
1486
Fe (ppm)
Mn (ppm)
Zn (ppm)
Cu (ppm)
Co (ppm)
Ni (ppm)


Crassostrea madrasensis (Station M8)
224
2.6
424
41
878
Fe (ppm)
Mn (ppm)
Zn (ppm)
Cu (ppm)
Co (ppm)
Ni (ppm)


Polymesoda erosa (Station M8)
27
0.9
158
5.0
157
954
Fe (ppm)
Mn (ppm)
Zn (ppm)
Cu (ppm)
Co (ppm)
Ni (ppm)

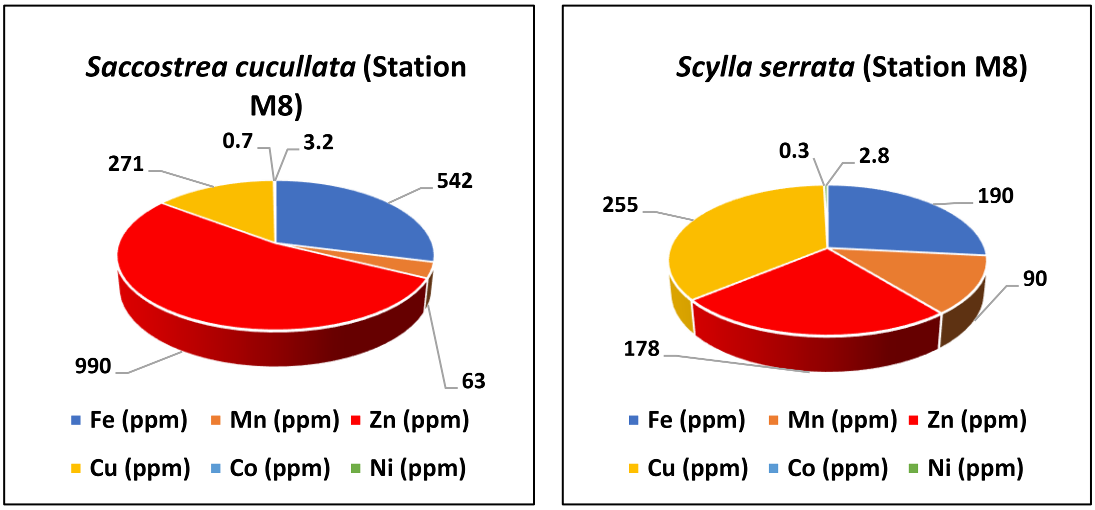


Fig. 3.4a. The concentration of metals (Fe, Mn, Zn, Cu, Co and Ni) in the soft tissues of the edible estuarine biota.

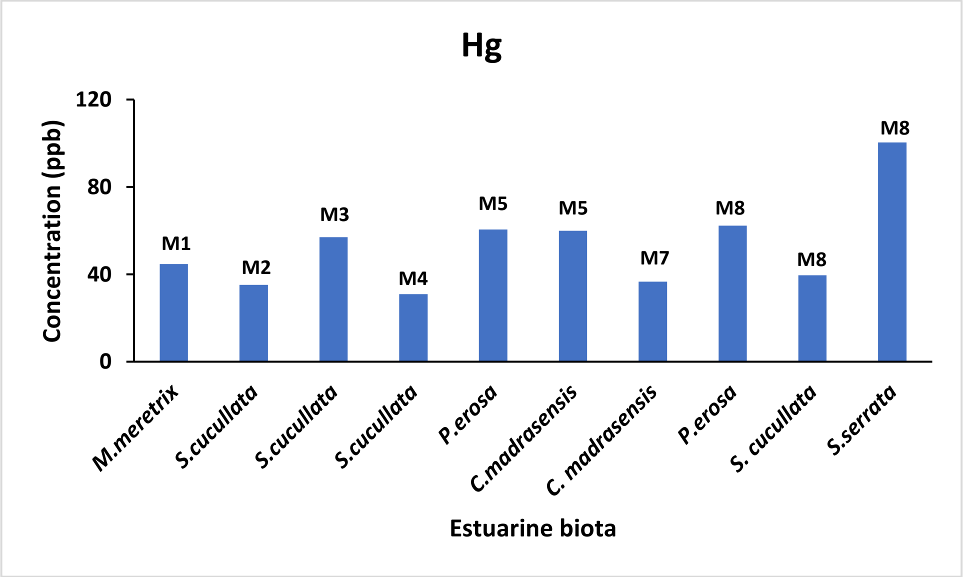


Fig. 3.4b. The concentration of Hg in the soft tissues of the edible estuarine biota.

The metal concentrations provided in Table 3.6b represent the permissible limits for marine biota and are deemed safe for human consumption. However, exceeding these permissible limits can pose toxicity risks to both biota and their consumers. The concentrations of metals viz., Fe, Mn, Zn and Cu (excluding *M. meretrix* – station M1 and *P. erosa* – station M8) in estuarine biota exceeded the permissible limits and thus, indicated potential toxicity to biota. In contrast, the concentrations of Co and Ni remained below the permissible limits. Moreover, the level of Hg in the estuarine biota was more than the safety limit at stations M1 (*M. meretix*), M2, M3, M4, M5 (*P. erosa* and *C. madrasensis*), M7 and M8 (*P. erosa*, *S. cucullata* and *S. serrata*). The excessive accumulation of metals (Fe, Mn, Zn, Cu and Hg) in the soft tissues of biota posed risks to both biota and humans through consumption. Hence, the investigation of metal bioaccumulation in edible bivalves and crustacean indicated their unsuitability for human consumption. The toxicity of metals viz., Fe, Mn, Zn, Cu, Co and Ni in humans can lead to various health issues including reduced energy levels and damage to vital organs such as the brain, lungs, kidneys, liver, and blood composition. Prolonged exposure to these metals may result in gradually progressing physical, muscular, and neurological degenerative conditions resembling diseases viz., multiple sclerosis, Parkinson’s disease, Alzheimer’s disease, and muscular dystrophy. Moreover, repeated long-term exposure to certain metals may even increase the risk of cancer **(Jarup 2003)**. The exposure to Hg can damage the nervous system, kidneys, liver and immune system **(Bernhoft 2012)**.

Table 3.6b Standard permissible limits of metals.

Metals	**Standard permissible limit**	**References**
Fe (%)	0.0034 − 0.0107	Charbonneau & Nash (1993)
Mn (ppm)	0.01	WHO (1989)
Zn (ppm)	40	WHO (1989)
Cu (ppm)	30	FAO/WHO (2004)
Co (ppm)	0.1 – 5	FAO/WHO (2004)
Ni (ppm)	70 – 80	USFDA (1993)
Hg (ppb)	0.002	WHO (2016)

3.1.7. modified Biota Sediment Accumulation Factor (mBSAF)

To assess the extent of metal bioaccumulation in edible biota and crustacean, the mBSAF was computed, and the findings are detailed in the Table 3.7.

Table 3.7 modified Biota Sediment Accumulation Factor (mBSAF)

Stations	**Biota species**	**Metals concentration (ppm)**					
		Fe	**Mn**	**Zn**	**Cu**	**Co**	**Ni**
		mBSAF	**mBSAF**	**mBSAF**	**mBSAF**	**mBSAF**	**mBSAF**
M1	*Anadara antiquata*	0.32	0.02	7.71	5.60	0.53	0.23
	Meretrix meretrix	0.40	0.23	2.60	4.46	0.27	0.24
	Crassostrea madrasensis	0.20	0.01	26.65	24.84	ND	0.12
M2	*Saccostrea cucullata*	0.14	0.01	5.05	38.67	ND	0.14
M3	*Saccostrea cucullata*	0.08	0.02	9.45	170.60	ND	0.09
M4	*Saccostrea cucullata*	0.15	0.06	10.32	20.23	0.56	0.18
M5	*Polymesoda erosa*	0.02	0.01	5.89	13.83	0.03	0.003
	Saccostrea cucullata	0.03	0.02	28.54	10.66	ND	1.19
	Crassostrea madrasensis	0.15	0.03	4.24	2.98	0.21	0.22
M7	*Crassostrea madrasensis*	0.04	0.01	34.21	7.44	0.03	0.12
M8	*Crassostrea madrasensis*	0.07	0.04	39.54	9.84	0.09	0.15
	Polymesoda erosa	0.04	0.01	22.49	4.27	0.02	0.10
	Saccostrea cucullata	0.04	0.01	18.71	6.16	ND	0.11
	Scylla serrata	0.09	0.05	3.37	0.74	0.10	0.21
Criteria	**BSAF/mBSAF**	**Inference**					
	mBSAF > 2	Organism referred as macro-concentrator of metal					
	mBSAF 2 – 1	Organism referred as micro-concentrator of metal					
	mBSAF < 1	Organism referred as de-concentrator of metal					

All bivalve species retrieved from the Mandovi Estuary were de-concentrator of Fe, Mn, Co and Ni (except at station M4). The *Saccostrea cucullata* was micro-concentrator of Ni at station M4. The bivalve species were macro-concentrators of Zn and Cu (except at station M8). The *Polymesoda erosa* was de-concentrator of Cu at station M8. The bivalve and crustacean species collected from the Mandovi Estuary displayed characteristics of being macro-accumulators of Zn and Cu. It suggested a pronounced inclination towards the assimilation and accumulation of Zn and Cu. These metals are recognized as essential elements in the marine environment and are frequently absorbed due to their significance in promoting growth and biological functions in biota **(Jakimsha et al. 2011)**.

3.2 Microplastics in sediments

3.2.1 Types, abundance and distribution of microplastics in sediments

In the Mandovi Estuary, there was dominance of fiber type of microplastics in sediments (Table 3.8). The concentration of fiber type of microplastic ranged from 53.02 (Station M1) to 94.61 (Station M7). Additionally, there was significant (> 25 %) concentration of film (Station M5) and fragment (Stations M1 and M6). The practice of fishing using gill nets and cast nets, along with net repair and mending activities contributes to microfiber load in the surrounding waters **(Saha et al. 2021)**. Also, clothing is one of the important sources of fiber type microplastics in the environment **(Napper and Thompson 2016)**. The discharge of washing machine effluents from household washing machines and laundry outlets might have even contributed to the enhancement of fiber type of microplastics in the beach sediments of Goa. The dumping of polythene bags into the Mandovi River/ Estuarine channel might have contributed to films, while fragments might be added due to further breakdown of fiber and film type of microplastics in the estuarine environment over the period of time **(Kalangutkar et al. 2024)**.

Table 3.8 The distribution of microplastic morphotypes (fibers, films and fragments) in the sediment.

Stations	Type of microplastics in the sediments (%)		
	Fiber	Film	Fragment
M1	53.02	1.78	45.20
M2	90.68	3.73	5.59
M3	86.58	11.04	2.39
M4	92.79	6.34	0.86
M5	56.32	34.81	8.86
M6	62.87	9.09	28.03
M7	94.61	4.49	0.90
M8	91.74	7.97	0.27

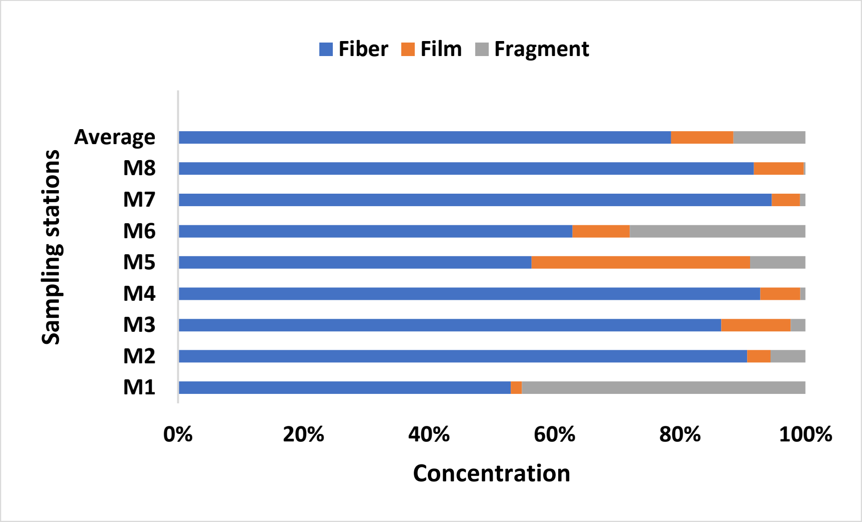


Fig. 3.5 The type of microplastics based on shape in sediments.

The fiber, film and fragment type of microplastics showed fluctuating trend in their spatial distribution from lower to middle portion of the Mandovi Estuary (Fig. 3.5). The fluctuations in the type of microplastics in sediments might be due to differential sources of microplastics in the estuary.

Table 3.9 The distribution of microplastics based on colour in the sediments.

Stations	Colour of microplastics in the sediments (%)									
	Blue	**Red**	**Black**	**Pink**	**Green**	**Yellow**	**White**	**Colourless**	**Orange**	**Purple**
M1	-	-	1.42	-	-	-	-	98.57	-	-
M2	3.10	0.93	-	-	-	-	0.31	95.65	-	-
M3	7.61	0.15	1.04	-	0.15	-	-	88.06	0.15	-
M4	2.30	-	1.15	0.29		0.29	2.30	93.66	-	-
M5	7.32	5.10	3.55	-	0.66	-	5.54	74.72	1.77	0.88
M6	0.38	0.38	0.76	-	-	-	1.14	97.35	-	-
M7	0.60	0.30	0.60	-	-	-	1.50	97.01	-	-
M8	1.24	0.41	0.83	-	-	-	0.69	96.84	-	-

Furthermore, the sediments of the Mandovi Estuary were enriched with colourless type of microplastics (Table 3.9). They ranged from 74.72 (Station M5) to 98.57 % (Station M1). Coloured microplastics were < 10 % in the sediments. The dominance of colourless microplastics in sediments can be attributed to greywater sources, as polyethylene is common in clothing, while polypropylene clear fibers can be from commercial and/or recreational fishing materials **(Govender et al. 2020)**. However, the degradation from bleaching and erosion processes can change the colour of the microplastic particles in the environment **(Martin et al. 2017)**.

3.2.2 Microplastics pollution indices

The PLI was determined to assess the level of pollution in the sediments of the Mandovi Estuary (Table 3.10a). The PLI was > 1 for all stations (M1 – M8) and therefore, revealed microplastic pollution in the Mandovi Estuary. The weathered fishing gear, when abandoned or damaged, can degrade into microplastics and enter the estuarine environments **(Padmachandran et al. 2023)**. Additionally, microplastics are transported from land to the marine environment primarily through river discharge. Urban areas surrounding estuaries also significantly contribute to microplastic pollution, as stormwater runoff carries microplastic particles from roads, buildings, and industrial sites. Moreover, both treated and untreated sewage effluents introduce microplastics originating from various sources viz., synthetic textiles and personal care products into aquatic ecosystems **(Wang et al. 2016)**.

Table 3.10a. The Pollution Load Index (PLI) for microplastics in the sediments.

Stations	**M1**	**M2**	**M3**	**M4**	**M5**	**M6**	**M7**	**M8**
PLI	1.06	1.22	2.54	1.31	1.71	1	1.27	2.75

Table 3.10b. The Coefficient of Microplastic Impact (CMPI) for microplastics in the sediments.

Stations	**M1**	**M2**	**M3**	**M4**	**M5**	**M6**	**M7**	**M8**
Fiber	0.53	0.91	0.87	0.93	0.56	0.63	0.95	0.92
Film	0.02	0.04	0.11	0.06	0.35	0.09	0.04	0.08
Fragment	0.45	0.06	0.02	0.01	0.09	0.28	0.01	0.003
Criteria	Minimum level of pollution (CMPI = 0.0001–0.1)							
	Average level of pollution (CMPI = 0.11–0.5)							
	Maximum level of pollution (CMPI = 0.51–0.8)							
	Extreme level of pollution (CMPI = 0.81–1)							

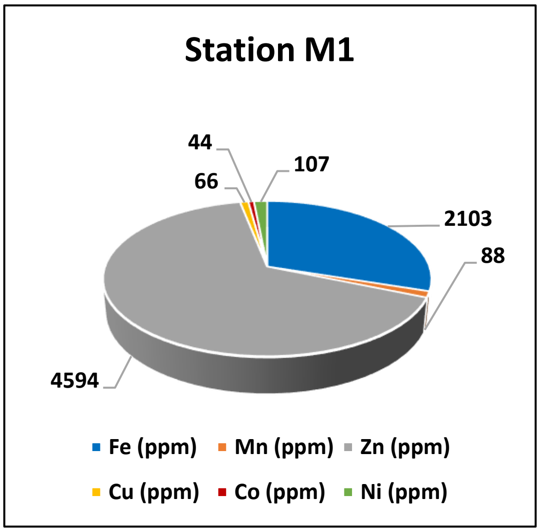
The CMPI was calculated to evaluate the impact of microplastics based on their shape (Table 3.10b). The stations M2, M3, M4, M7 and M8 showed an exceptionally extreme level of pollution, predominantly characterized by the presence of fibers. Conversely, stations M1, M5 and M6 displayed the maximum degree of pollution, with fibers. The stations M3 and M5 demonstrated an average level of pollution, whereas the remaining stations (M1, M2, M4, M6, M7 and M8) displayed minimum pollution levels in case of films. Thes fragment (except for stations M1 and M6) exhibited average level of pollution, while other stations M2, M3, M4, M5, M7 and M8 showed minimum level of pollution. The Mandovi Estuary displayed maximum to extreme level of pollution from fibre type of microplastics.

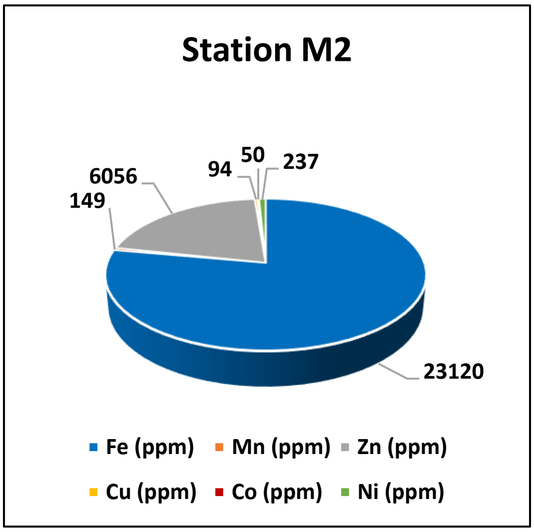
3.2.3 Metals on microplastics

The concentration of metals on the surface of microplastics in the Mandovi Estuary is presented in Table 3.11 and Fig. 3.6. Among the metals, the microplastics in the sediments exhibited highest concentration of Zn at stations, M1, M3, M4, M5, M6 and M8, whereas had more of Fe at stations M2 and M7. The concentration of Co was the lowest in comparison to other metals.

Table 3.11 The concentration of metals on the surface of microplastics.

Stations	Fe (ppm)	Mn (ppm)	Zn (ppm)	Cu (ppm)	Co (ppm)	Ni (ppm)
M1	2103	88	4594	66	44	107
M2	23120	149	6056	94	50	237
M3	4982	115	5442	56	35	191
M4	8304	318	13176	135	105	350
M5	2752	135	4830	55	39	100
M6	10308	552	21684	224	208	741
M7	18794	161	3150	61	31	212
M8	6178	269	8510	149	85	169





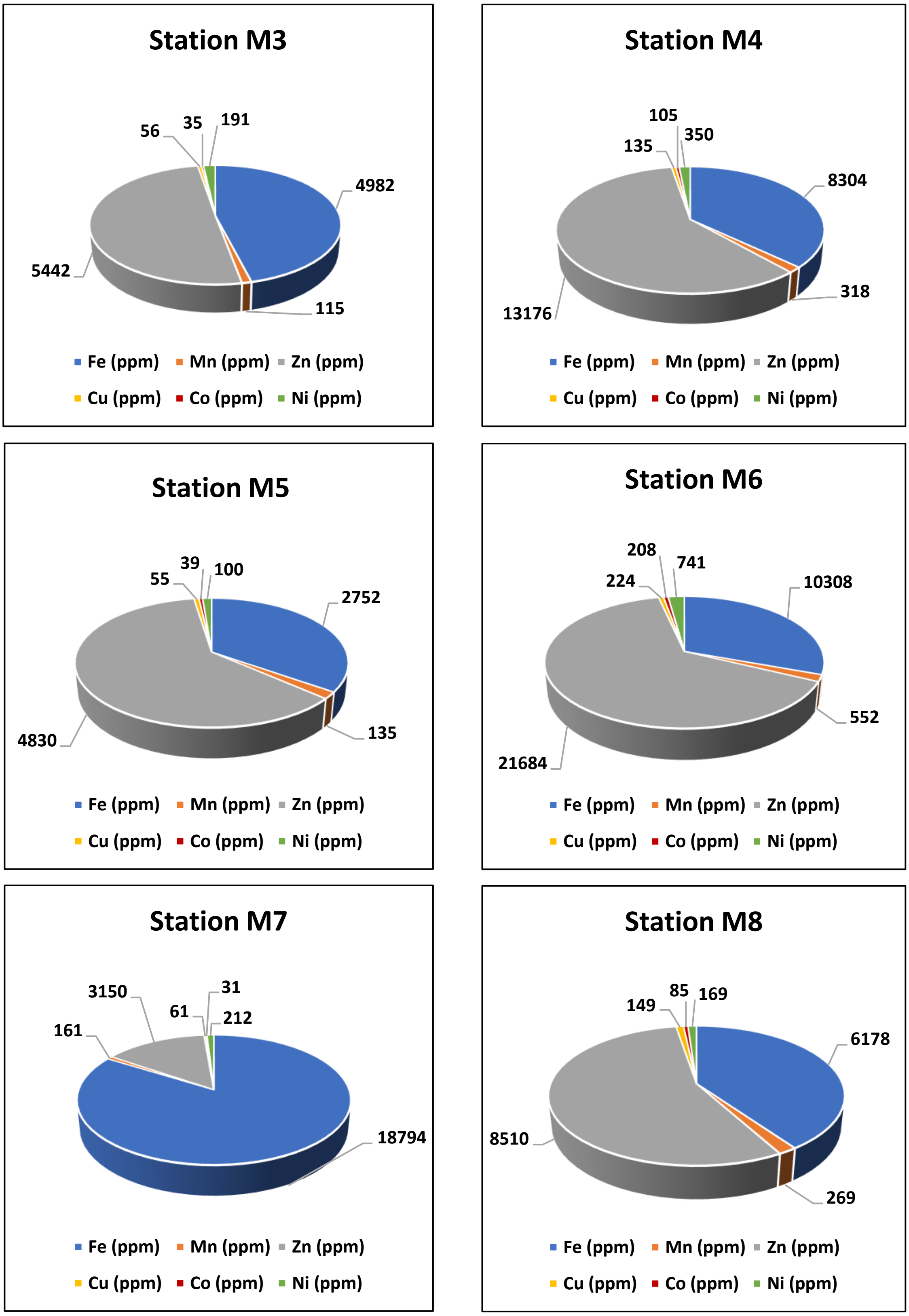


Fig. 3.6 The concentration of metals on the surface of microplastics along the Mandovi Estuary.

The investigation into the chemistry of microplastics revealed a significant affinity for metals viz., Fe, Mn, Zn, Cu, Co and Ni extracted from the Mandovi Estuary. This association underscores the capacity of microplastics to act as effective carriers of metals within the marine environment. Multiple studies have documented the substantial presence of metals adhering to the surface of microplastics **(Holmes et al. 2012; Brennecke et al. 2016; Hodson et al. 2017; Saeedi et al. 2018; Godoy et al. 2019; Amelia et al. 2021)**, particularly in fiber form (abundant in the present study), as opposed to films and fragments. The fiber-based microplastics offer a higher surface area-to-volume ratio, potentially enhancing the adsorption and retention of metals on their surface **(Khatmullina and Isachenko 2017; Zhou et al. 2019)**.

In microplastics (Stations M1 – M8), the concentration of Zn stood out significantly compared to other metals. Industrial activities, sewage effluents, land runoff, and transportation operations have been identified as key contributors to the influx of Zn in the Mandovi Estuary **(Alagarsamy 2006)**. Additionally, inputs from agrochemicals and composite materials likely contributed to the presence of bioavailable Zn in the estuary **(Krishna and Govil 2004)**. Given its extensive use in industries viz., pharmaceuticals and paints, Zn released from these anthropogenic sources might have boosted its concentration on microplastics **(Sharma and Chatterjee 2017; Wani et al. 2019)**. Moreover, Fe was found to be enriched in microplastics at stations M2 and M7, emerging as the second most abundant metal after Zn. The elevated level of Fe in the surface sediments of the Mandovi Estuary was construed through total and bioavailable metal analysis which was attributed to human-induced input, particularly open-cast mining of ferromanganese ores in recent years. The transportation of Fe ore minerals through the Mandovi estuary via barges, especially in the estuarine channels of Mandovi and Zuari, contributes significantly to this influx in an open system **(Kessarkar et al. 2015; Siraswar et al. 2021)**. Moreover, the differences in the levels of Zn, Fe, and other metals (Mn, Cu, Co and Ni) adhered to the microplastics across various stations were linked to factors viz., deposition sites, mineral stability, density, grain size, hydrodynamics, and transporting agents etc., **(Chaudhri and Grewal 1985; Nayak 1999).**

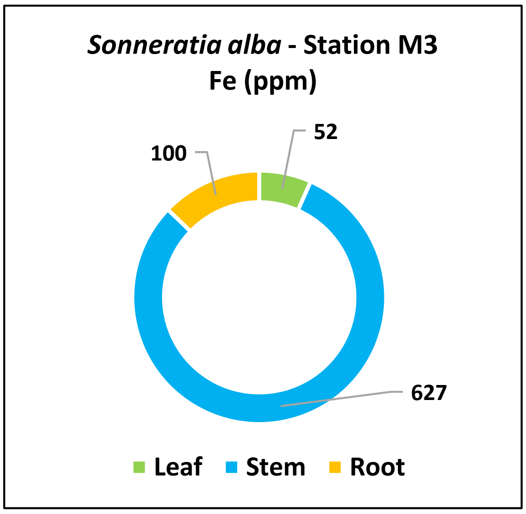
3.3 Accumulation of metals in mangroves

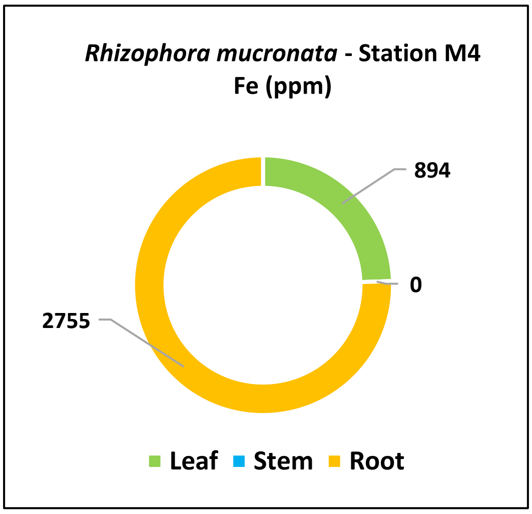
The concentration of metals in different parts viz., root, stem and leaves of the mangrove species are presented in Table 3.12 and Fig. 3.7.

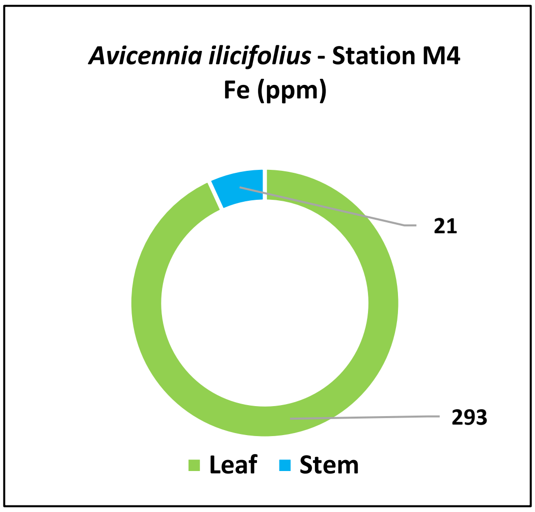
Table 3.12 The concentration of metals in mangrove species.

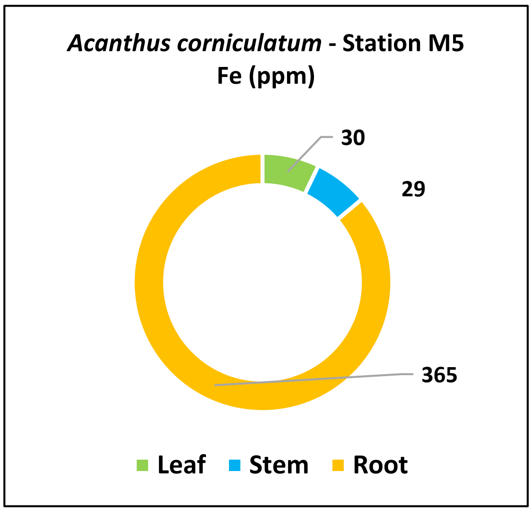
Stations	**Mangrove Species**	**Mangrove parts**	**Metals concentration (ppm)**						
			Fe	**Mn**	**Zn**	**Cu**	**Co**	**Ni**	**Hg (ppb)**
M3	*Sonneratia alba*	Root	100	14	5	2	ND	2	4
		Stem	627	142	10	3	ND	3	7
		Leaves	52	31	6	3	ND	1	64
M4	*Rhizophora mucronata*	Root	2755	378	27	28	0.755	4	88
		Stem	ND	15	9	2	ND	2	12
		Leaves	894	96	11	5	ND	3	45
	Acanthus ilicifolius	Stem	21	42	16	7	ND	2	11
		Leaves	293	21	6	9	ND	1	82
M5	*Avicennia corniculatum*	Root	365	30	10	19	ND	2	29
		Stem	29	76	10	2	ND	2	33
		Leaves	30	53	0	3	ND	2	67
M6	*Avicennia officinalis*	Root	2211	528	32	7	1.12	5	66
		Stem	1054	183	15	3	ND	2	22
		Leaves	74	525	8	1	ND	1	65
	Rhizophora mucronata	Stem	631	378	11	3	ND	2	58
		Leaves	1048	498	8	3	ND	3	48
	Acanthus ilicifolius	Stem	900	183	21	3	ND	2	19
		Leaves	503	15	41	1	ND	2	73
M7	*Avicennia marina*	Root	263	41	24	13	ND	2	103
		Stem	4174	384	29	6	0.585	4	28
		Leaves	1504	141	35	16	ND	2	90
M8	*Rhizophora mucronata*	Root	1827	765	51	6	1.34	7	64
		Stem	897	549	17	1	ND	2	13
		Leaves	603	327	10	1	ND	2	54

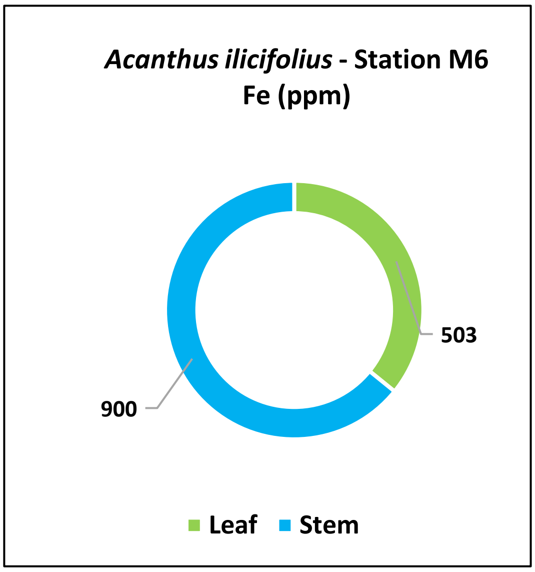
In *Sonneratia alba* (Station M3), metal concentrations—specifically Fe, Mn, Zn, Cu, and Ni were highest in the stem compared to the root and leaves except for Hg which was highest in leaves. Conversely, in *Rhizophora mucronata* (Station M4), all metal concentrations—Fe, Mn, Zn, Cu, Co, Ni and Hg were highest in the root compared to the stem and leaves. For *Acanthus ilicifolius* (Station M4), Fe, Cu and Hg were highest in the leaves, while Mn, Zn, and Ni were highest in the stem. In *Avicennia corniculatum* (Station M5), Fe and Cu concentrations were highest in the root, while Mn and Zn were highest in the stem. Ni concentrations were equal and highest in both leaves and root, and Hg was highest in leaves. In *Avicennia officinalis* (Station M6), metal concentrations—Fe, Mn, Zn, Cu, Co, Ni and Hg were highest in the root, followed by the stem, and then the leaves. The level of hg was also similar in leaves. In *Rhizophora mucronata* (Station M6), metal concentrations – Fe, Mn, Ni and Hg were highest in leaves, while Zn and Cu were highest in stem. Lastly, in *Acanthus ilicifolius* (Station M6), metal concentrations – Fe, Mn, Cu and Ni were highest in stem and exceeded those in the leaves, whereas Zn and Hg showed their highest concentration in the leaves. In *Avicennia marina* (Station M7), metal concentrations – Fe, Mn, Co and Ni were highest in stem, whereas Zn and Cu showed its highest concentration in the leaves and Hg in root. In *Rhizophora mucronata* (Station M8), metal concentrations—Fe, Mn, Zn, Cu, Co, Ni and Hg were highest in the root, followed by stem (except Hg) and leaves. Metal accumulation varied across different parts of mangroves among different species. Additionally, within the same species, metal concentrations differed across different stations (e.g., *Rhizophora mucronata* at Stations M4, M6 and M8). The variations in metal uptake by mangrove species may result from differences in sources, bioavailability, physico-chemical factors influencing metal bioavailability, species type, age, and other factors **(Chowdhury and Maiti 2016)**. Mangroves showed a greater preference for Fe and Mn. The Fe is essential for chlorophyll production, aiding in protein synthesis and root growth. Similarly, Mn plays a significant role in various biological processes, including photosynthesis **(Millaleo et al. 2010).** On the other hand, Hg was not preferred by mangroves (showed low accumulation than other metals) owing to its toxic characteristics in the environment. It hinders seed germination and seedling growth in various species. Additionally, Hg inhibits photosynthesis and speeds up the loss of photosynthetic pigments **(Sengar et al. 2010).**

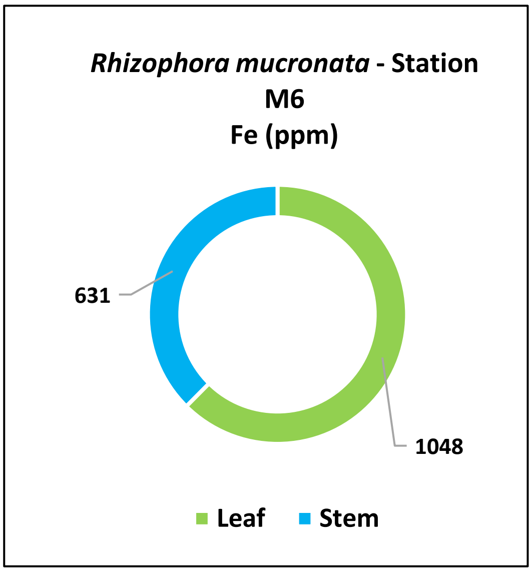


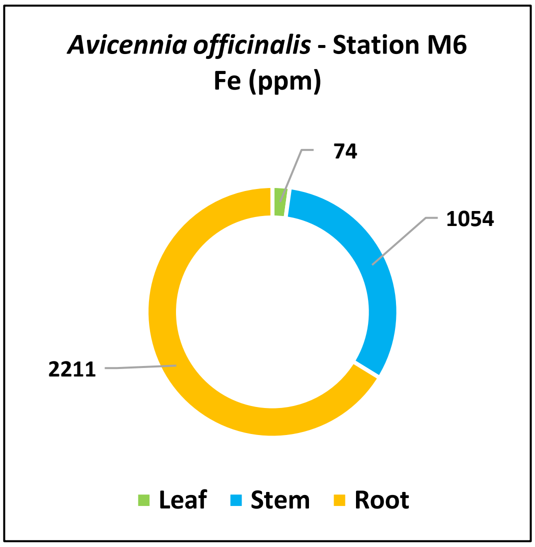


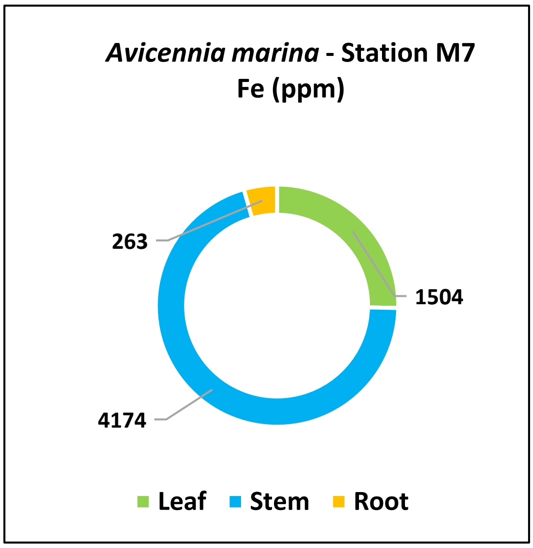

Avicennia ilicifolius - Station M4
Fe (ppm)
21
293
Leaf
Stem

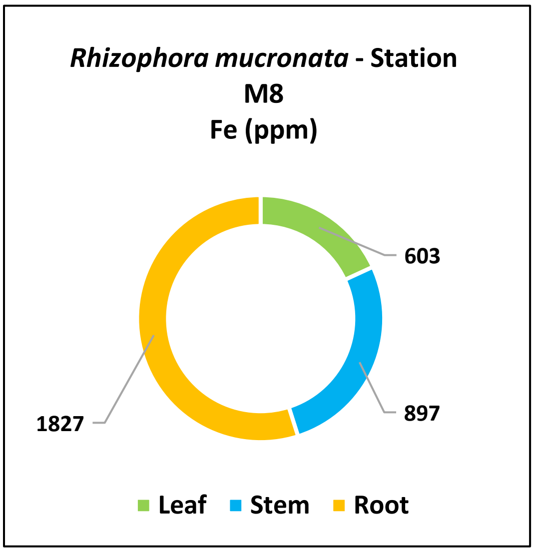

Acanthus corniculatum - Station M5
Fe (ppm)
30
29
365
Leaf
Stem
Root

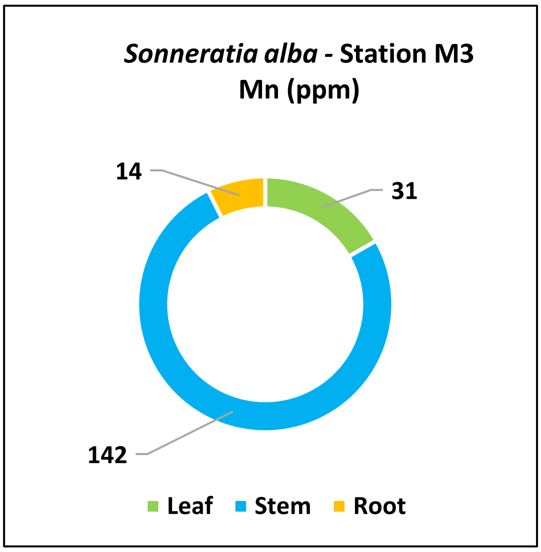

Acanthus ilicifolius - Station M6
Fe (ppm)
503
900
Leaf
Stem

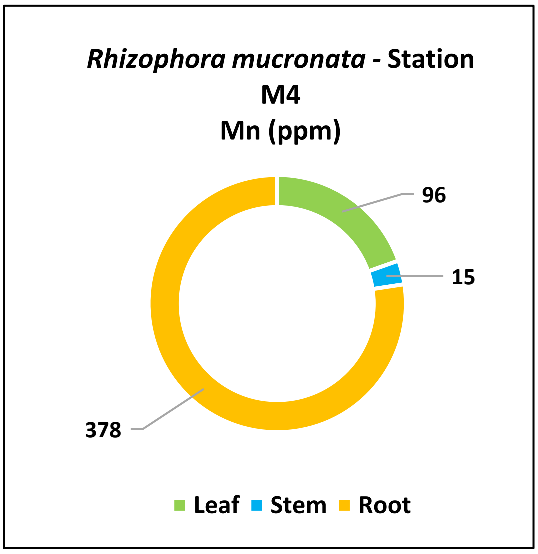

Rhizophora mucronata - Station M6
Fe (ppm)
631
1048
Leaf
Stem

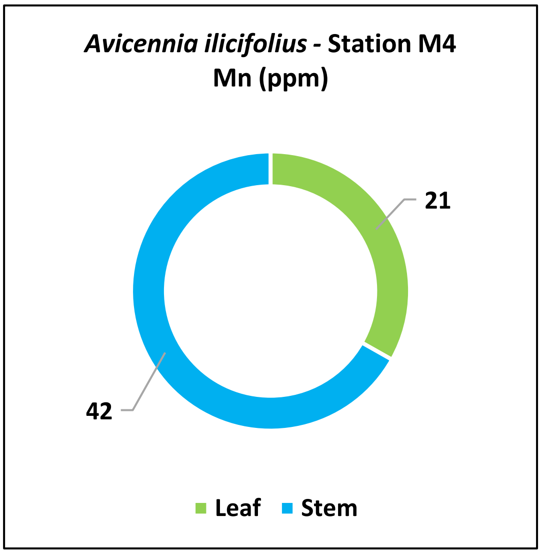

Avicennia officinalis - Station M6
Fe (ppm)
74
1054
2211
Leaf
Stem
Root

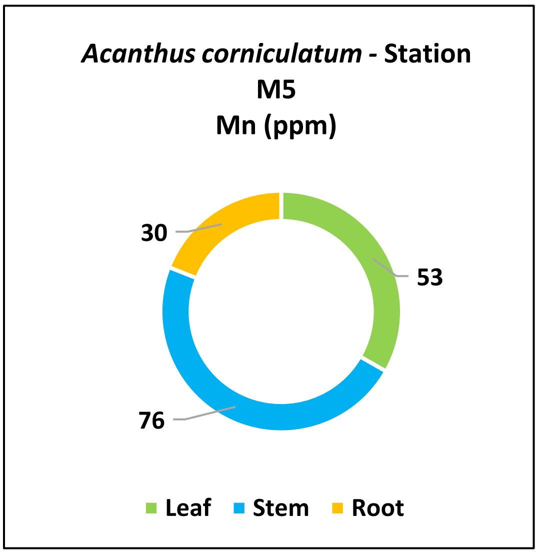

Avicennia marina - Station M7
Fe (ppm)
263
1504
4174
Leaf
Stem
Root

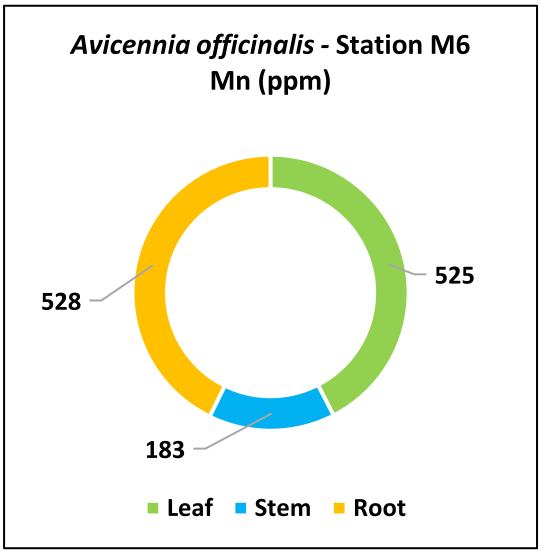

Rhizophora mucronata - Station M8
Fe (ppm)
603
897
1827
Leaf
Stem
Root

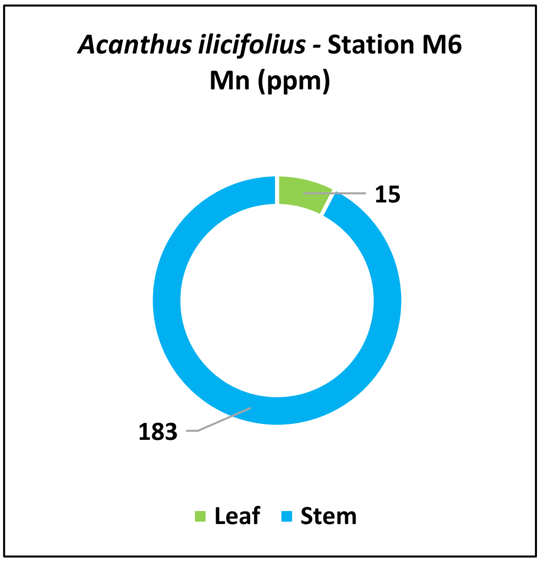

Sonneratia alba - Station M3
Mn (ppm)
14
31
142
Leaf
Stem
Root

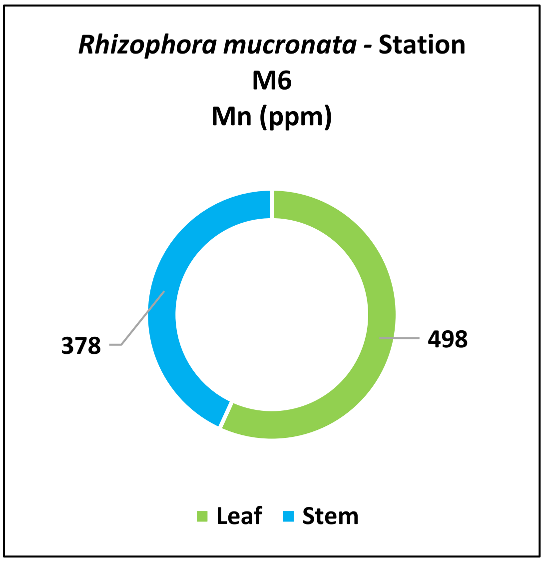

Rhizophora mucronata - Station M4
Mn (ppm)
96
15
378
Leaf
Stem
Root

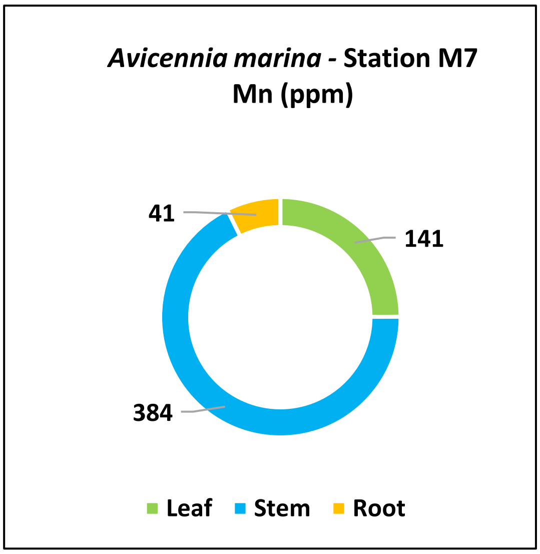

Avicennia ilicifolius - Station M4
Mn (ppm)
21
42
Leaf
Stem

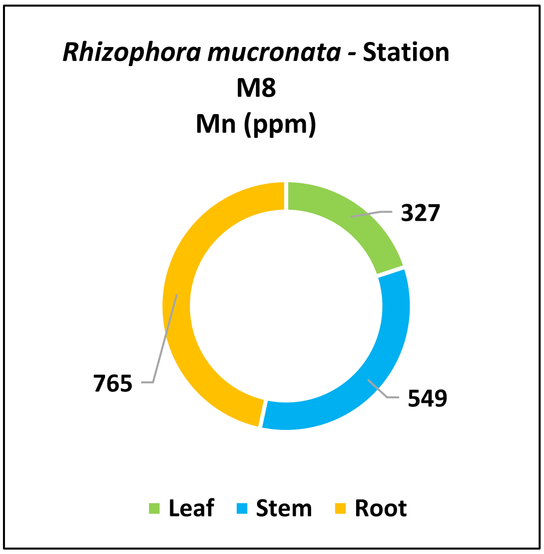

Acanthus corniculatum - Station M5
Mn (ppm)
30
53
76
Leaf
Stem
Root

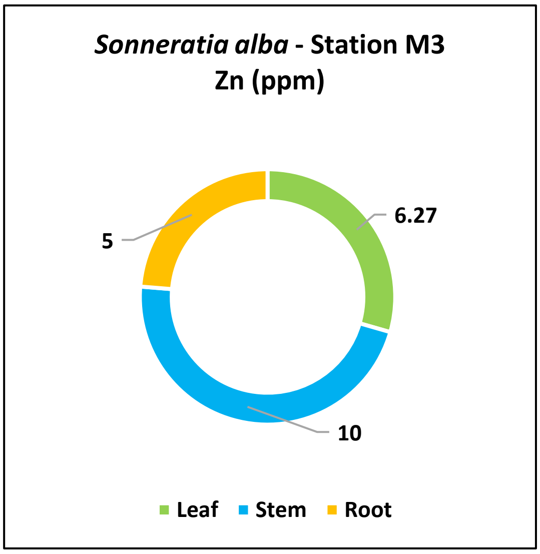

Avicennia officinalis - Station M6
Mn (ppm)
525
528
183
Leaf
Stem
Root

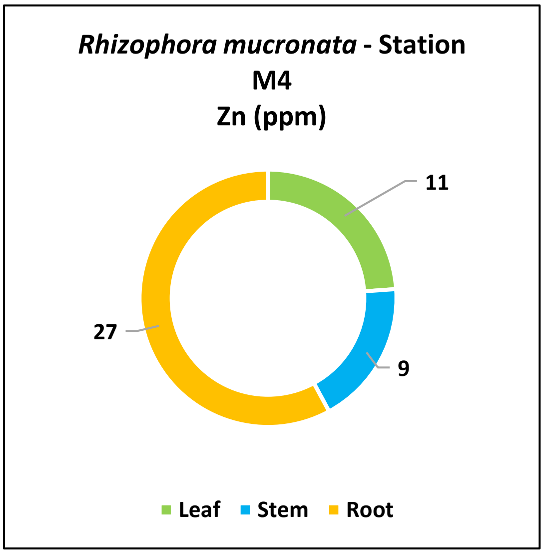

Acanthus ilicifolius - Station M6
Mn (ppm)
15
183
Leaf
Stem

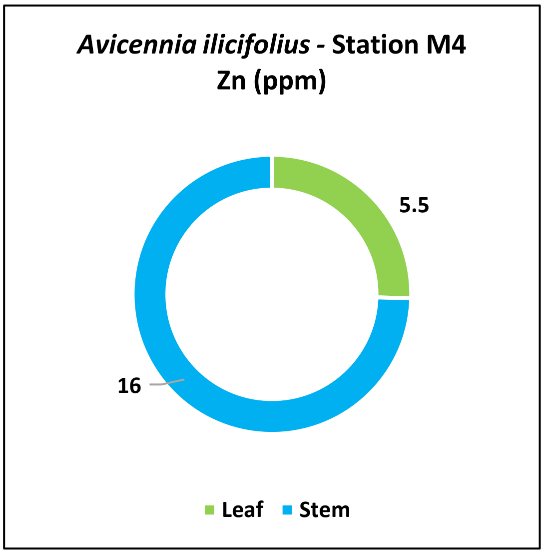

Rhizophora mucronata - Station M6
Mn (ppm)
378
498
Leaf
Stem

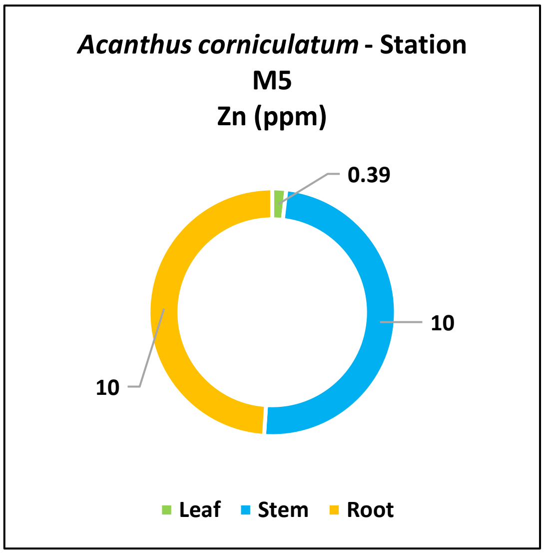

Avicennia marina - Station M7
Mn (ppm)
41
141
384
Leaf
Stem
Root

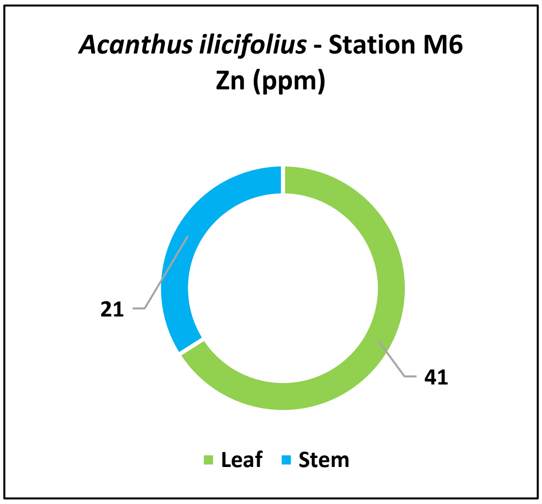

Rhizophora mucronata - Station M8
Mn (ppm)
327
765
549
Leaf
Stem
Root

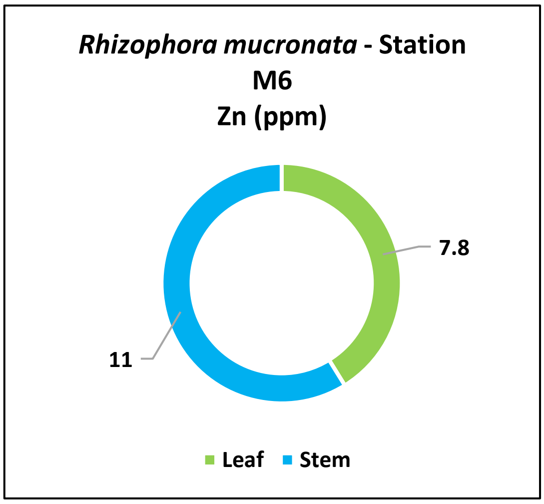

Sonneratia alba - Station M3
Zn (ppm)
6.27
5
10
Leaf
Stem
Root

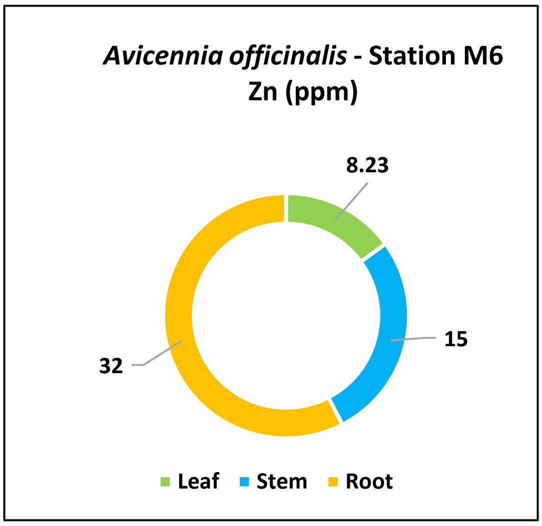

Rhizophora mucronata - Station M4
Zn (ppm)
11
27
9
Leaf
Stem
Root

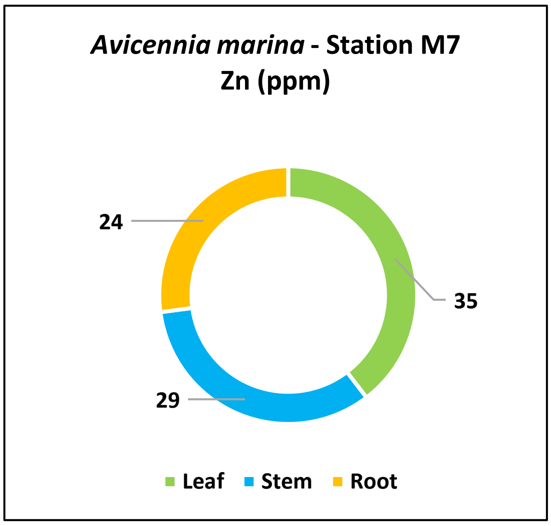

Avicennia ilicifolius - Station M4
Zn (ppm)

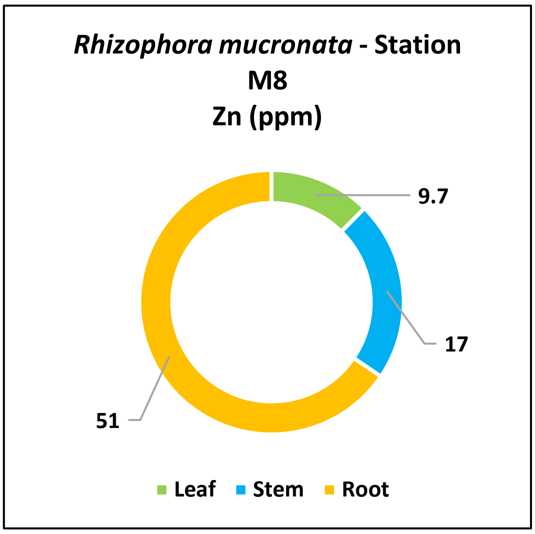

Acanthus corniculatum - Station M5
Zn (ppm)

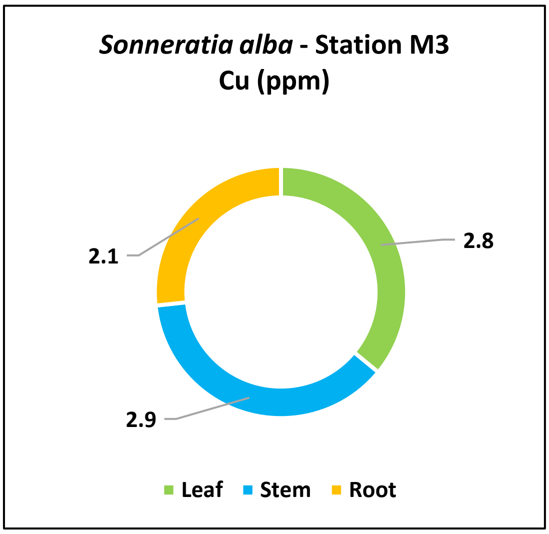

Acanthus ilicifolius - Station M6
Zn (ppm)

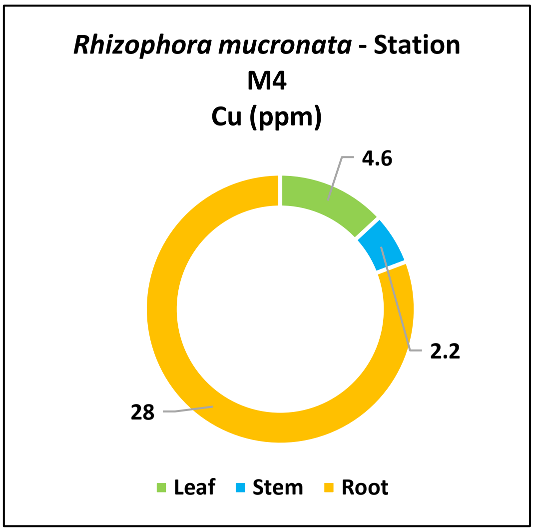

Rhizophora mucronata - Station M6
Zn (ppm)

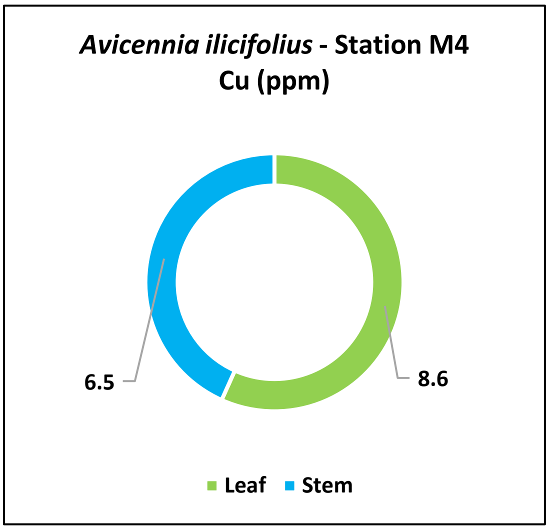

Avicennia officinalis - Station M6
Zn (ppm)

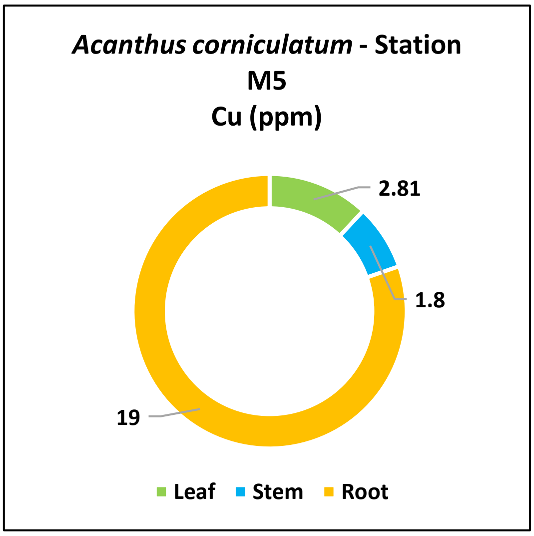

Avicennia marina - Station M7
Zn (ppm)

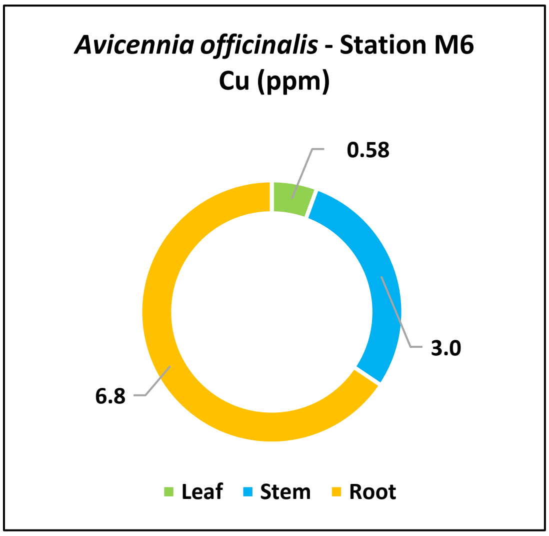

Rhizophora mucronata - Station M8
Zn (ppm)
9.7
17
51
Leaf
Stem
Root

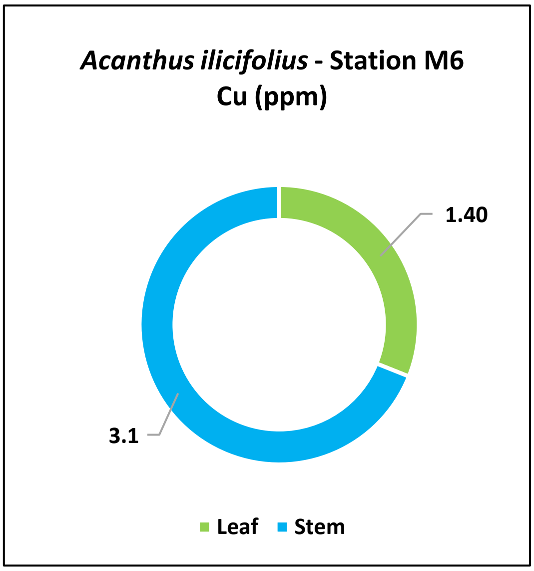

Sonneratia alba - Station M3
Cu (ppm)
2.1
2.8
2.9
Leaf
Stem
Root

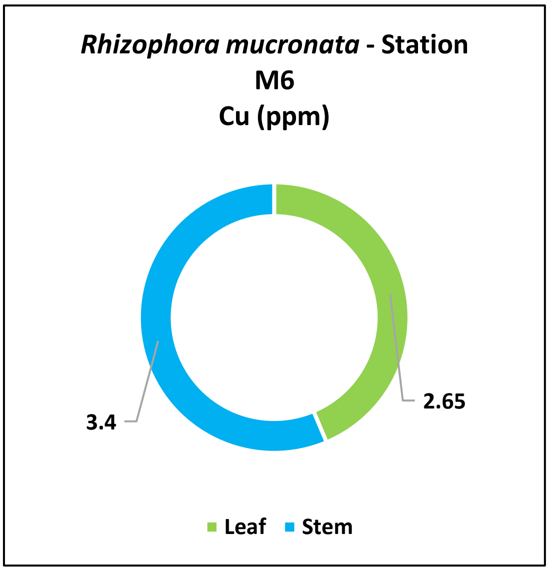

Rhizophora mucronata - Station M4
Cu (ppm)
4.6
2.2
28
Leaf
Stem
Root

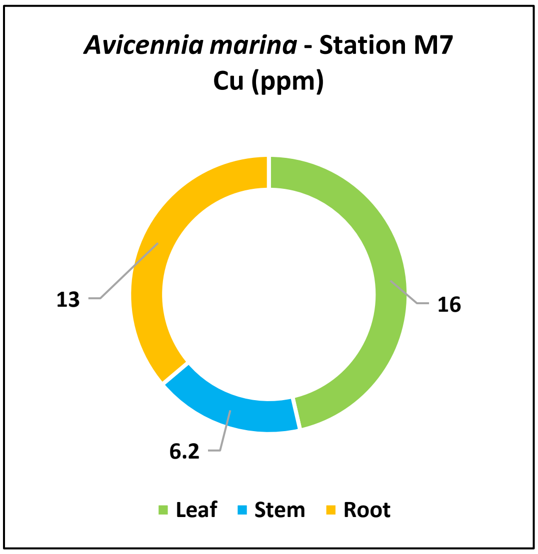

Avicennia ilicifolius - Station M4
Cu (ppm)
6.5
8.6
Leaf
Stem

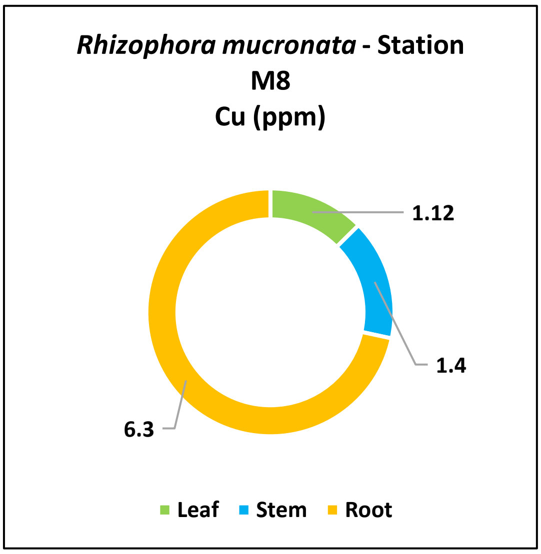

Acanthus corniculatum - Station M5
Cu (ppm)
2.81
1.8
19
Leaf
Stem
Root

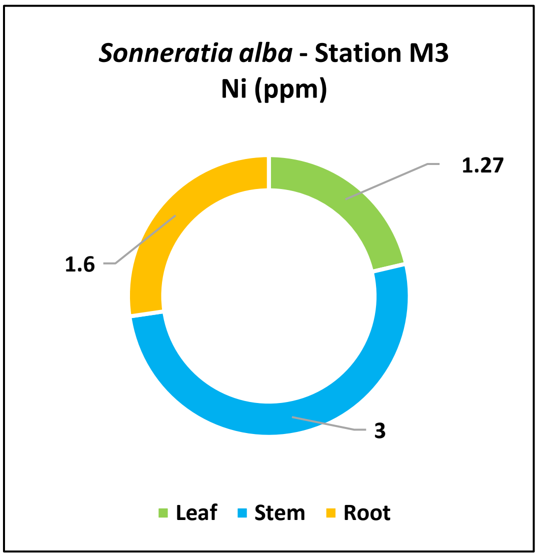

Avicennia officinalis - Station M6
Cu (ppm)
0.58
3.0
6.8
Leaf
Stem
Root

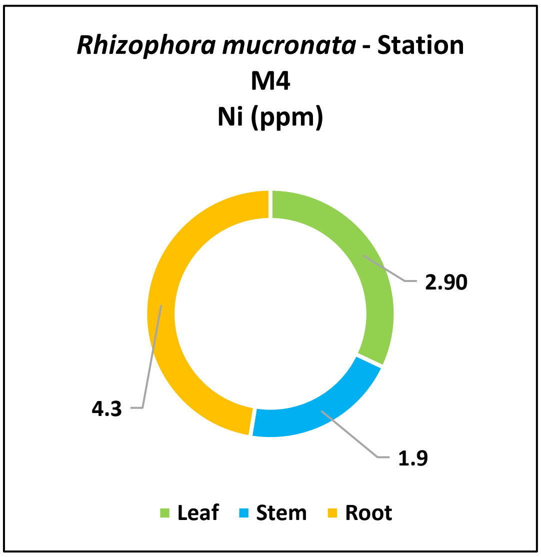

Acanthus ilicifolius - Station M6
Cu (ppm)
1.40
3.1
Leaf
Stem

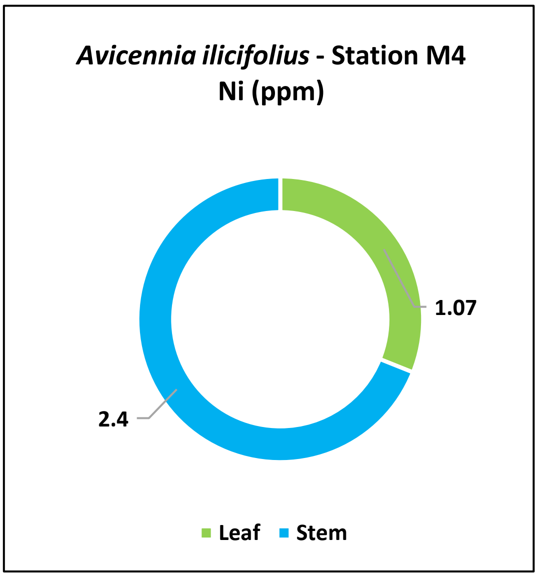

Rhizophora mucronata - Station M6
Cu (ppm)
2.65
3.4
Leaf
Stem

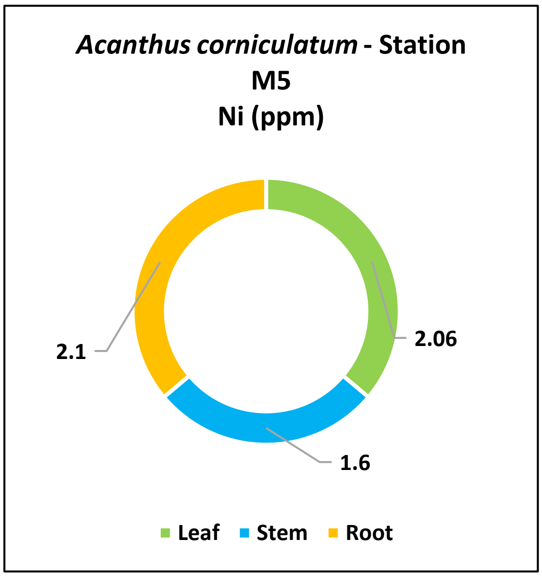

Avicennia marina - Station M7
Cu (ppm)
13
16
6.2
Leaf
Stem
Root

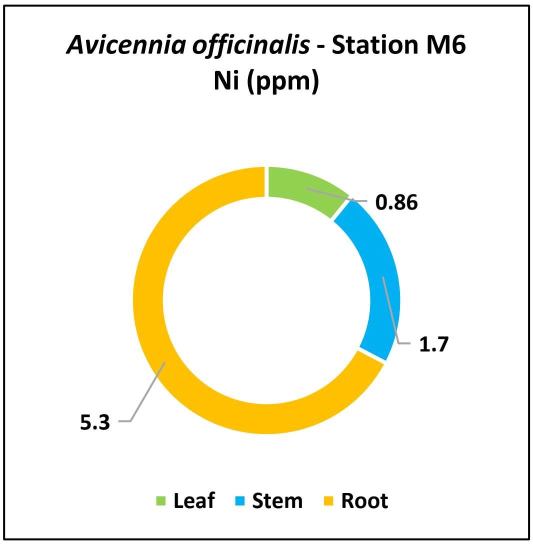

Rhizophora mucronata - Station M8
Cu (ppm)
1.12
1.4
6.3
Leaf
Stem
Root

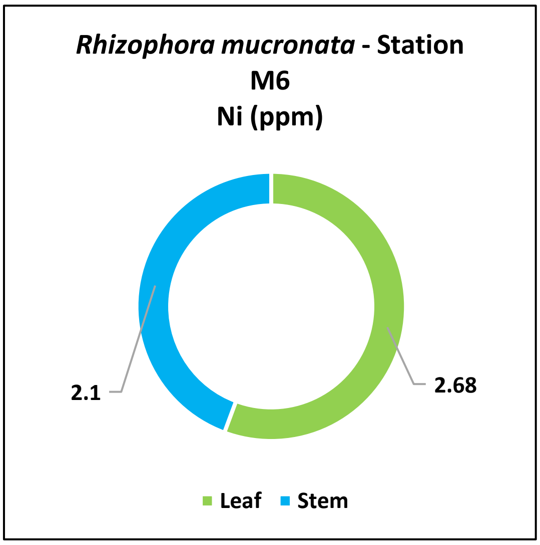

Sonneratia alba - Station M3
Ni (ppm)
1.27
1.6
3
Leaf
Stem
Root

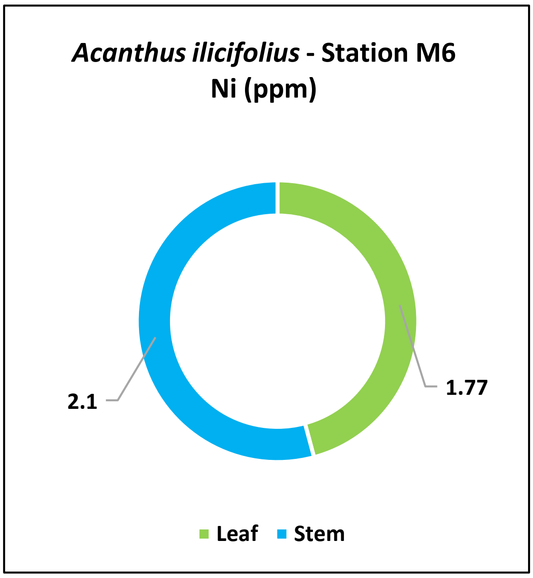

Rhizophora mucronata - Station M4
Ni (ppm)
2.90
4.3
1.9
Leaf
Stem
Root

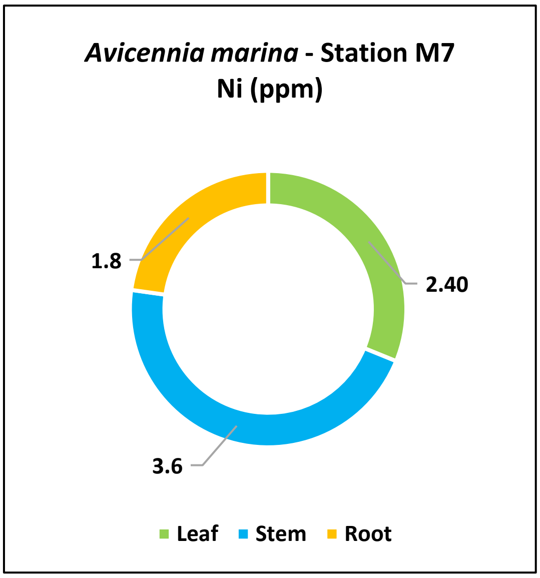

Avicennia ilicifolius - Station M4
Ni (ppm)
1.07
2.4
Leaf
Stem

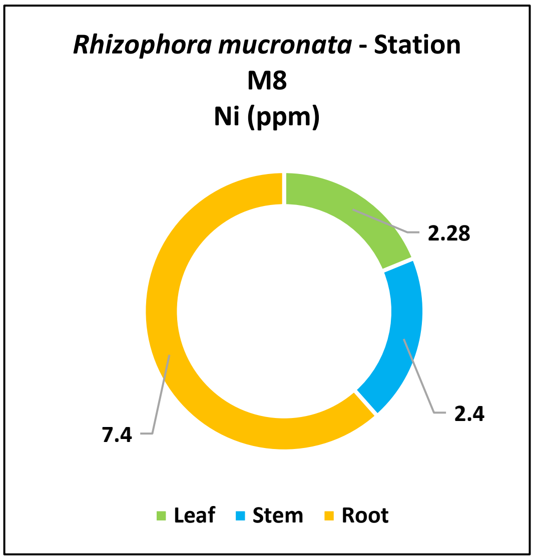

Acanthus corniculatum - Station M5
Ni (ppm)
2.1
2.06
1.6
Leaf
Stem
Root

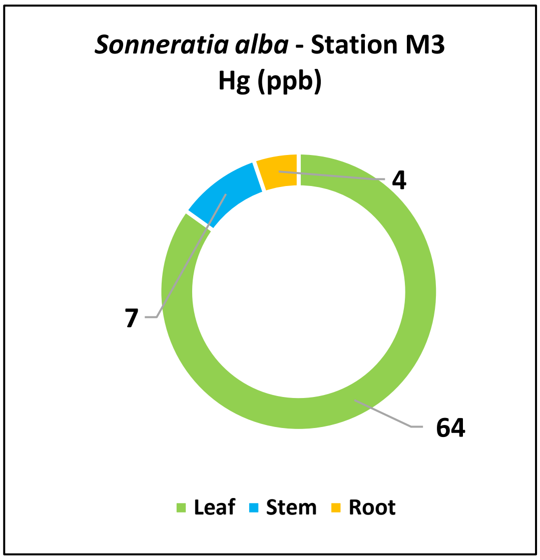

Avicennia officinalis - Station M6
Ni (ppm)
0.86
1.7
5.3
Leaf
Stem
Root

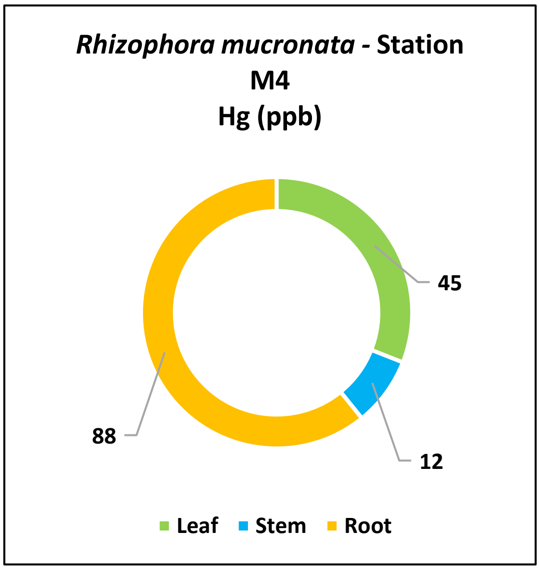

Rhizophora mucronata - Station M6
Ni (ppm)
2.1
2.68
Leaf
Stem

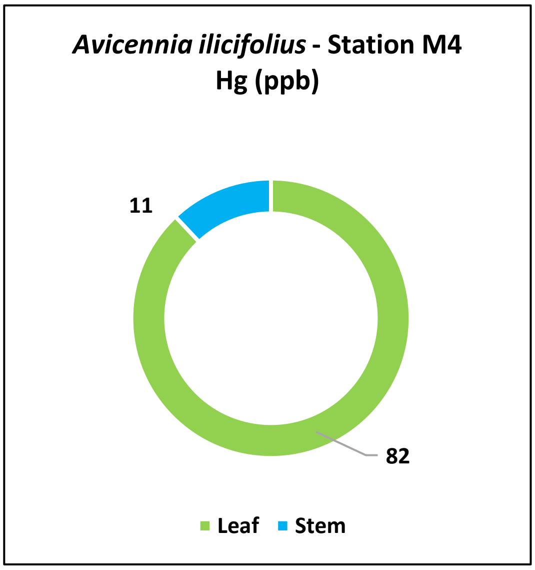

Acanthus ilicifolius - Station M6
Ni (ppm)
2.1
1.77
Leaf
Stem

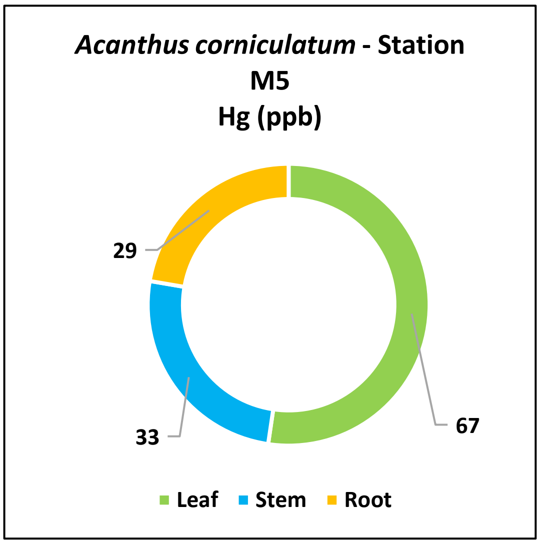

Avicennia marina - Station M7
Ni (ppm)
1.8
2.40
3.6
Leaf
Stem
Root

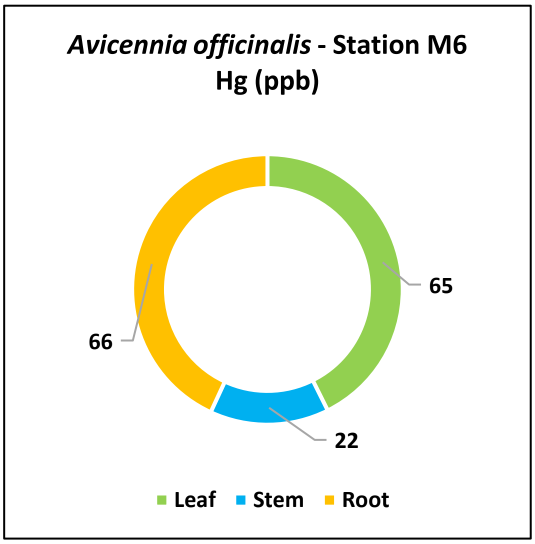

Rhizophora mucronata - Station M8
Ni (ppm)
2.28
2.4
7.4
Leaf
Stem
Root


Sonneratia alba - Station M3
Hg (ppb)
4
7
64
Leaf
Stem
Root


Rhizophora mucronata - Station M4
Hg (ppb)
45
12
88
Leaf
Stem
Root


Avicennia ilicifolius - Station M4
Hg (ppb)
11
82
Leaf
Stem


Acanthus corniculatum - Station M5
Hg (ppb)
29
33
67
Leaf
Stem
Root


Avicennia officinalis - Station M6
Hg (ppb)
65
66
22
Leaf
Stem
Root

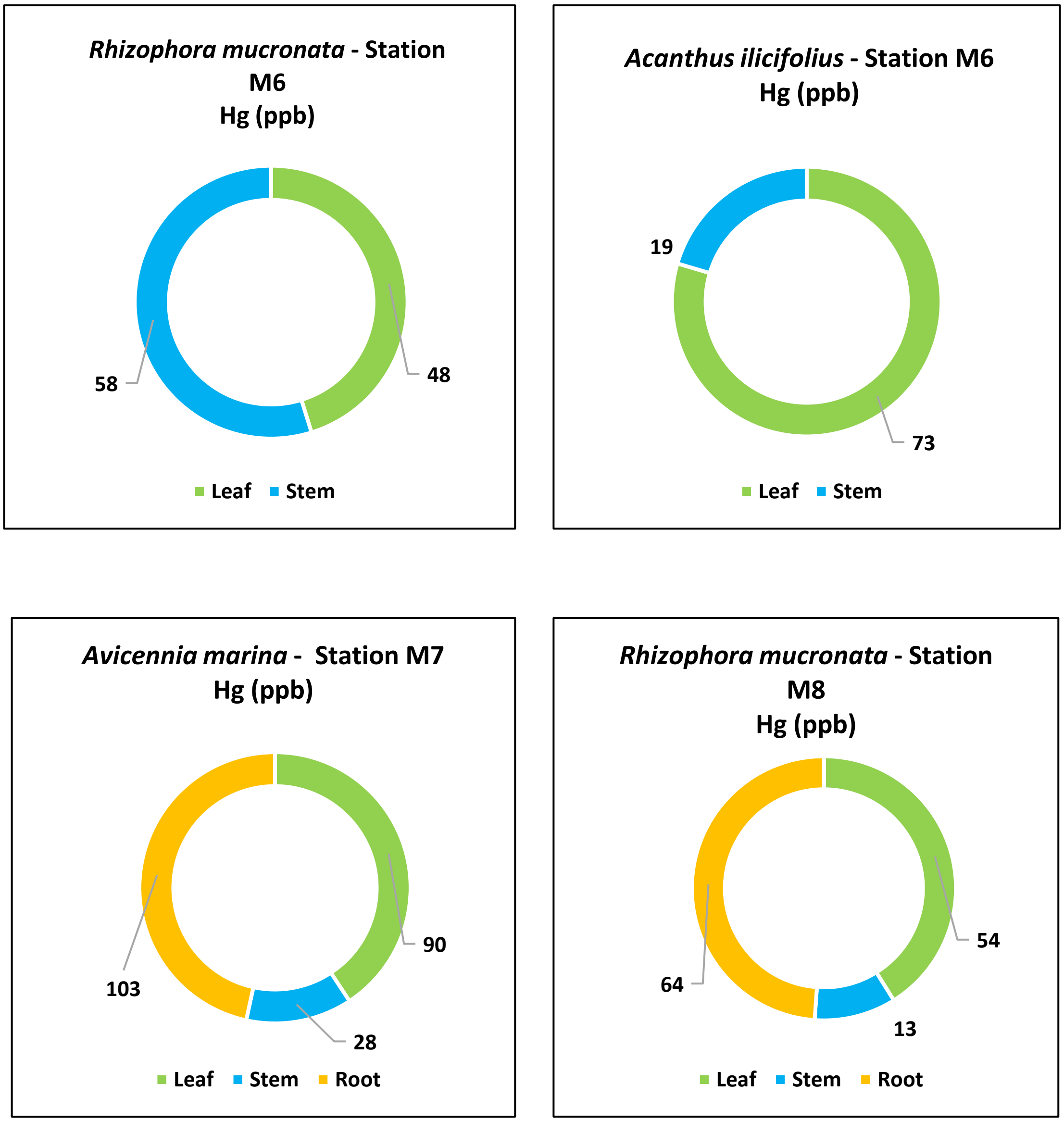


Fig. 3.7 The concentration of metals in different parts of mangrove plants.

3.3.1 Translocation Factor (TF)

The TF values for metals are presented in the Table 3.13.

Table 3.13 The Translocation Factor (TF) for metals in mangrove plants.

Stations	**Mangrove species**	**TF**						
		Fe	**Mn**	**Zn**	**Cu**	**Co**	**Ni**	**Hg**
M3	*Sonneratia alba*	0.52	2.26	1.24	1.35	ND	0.78	16.18
M4	*Rhizophora mucronata*	0.32	0.25	0.41	0.16	ND	0.68	87.60
	Acanthus ilicifolius	13.65	0.50	0.34	1.31	ND	0.45	7.32
M5	*Avicennia corniculatum*	0.08	1.75	0.04	0.15	ND	1.00	2.35
M6	*Avicennia officinalis*	0.03	0.99	0.26	0.09	ND	0.16	0.99
	Rhizophora mucronata	1.66	1.32	0.70	0.78	ND	1.26	0.83
	Acanthus ilicifolius	0.56	0.08	1.94	0.45	ND	0.85	3.89
M7	*Avicennia marina*	5.72	3.41	1.46	1.28	ND	1.37	0.87
M8	*Rhizophora mucronata*	0.33	0.43	0.19	0.18	ND	0.31	0.84

The TF analysis identified *Sonneratia alba* (Station M3) as an accumulator of Mn, Zn, Cu and Hg, while an extruder of Fe, Co and Ni. *Rhizophora mucronata* (Station M4) was found to be an extruder of Fe, Mn, Zn, Cu, Co and Ni, but an accumulator of Hg. *Acanthus ilicifolius* (Station M4) was an accumulator Fe, Cu and Hg, while was an extruder of Mn, Zn, Co and Ni. *Avicennia corniculatum* (Station M5) was an accumulator Mn, Ni and Hg, but extruded Fe, Zn, Cu and Co. *Avicennia officinalis* (Station M6) was identified as an extruder of all metals. *Rhizophora mucronata* (Station M6) was an accumulator of Fe, Mn and Ni, but extrudes Zn, Cu, Co, and Hg. Similarly, *Acanthus ilicifolius* from station M6 was an accumulator of Zn. *Avicennia marina* at station M7 was accumulator of Fe, Mn, Zn, Cu and Ni, but extrudes Co and Hg. *Rhizophora mucronata* at station M8 was found to be an extruder of all metals. Overall, *Sonneratia alba* showed potential for Mn, Zn, Cu and Hg remediation, while *Avicennia marina* was effective for Fe, Mn, Zn, Cu and Ni remediation at station M7, and *Avicennia corniculatum* was promising for Mn, Ni and Hg remediation at station M5. However, the variations in phyto-remediation potential between *Avicennia* species might be attributed to variations in the conditions at two sampling sites, age, size, metal bioavailability, etc.

The intrinsic factors related to a mangrove species play vital role in assimilation of metals **(Chowdhury and Maiti 2016).** *Rhizophora mucronata* can be used for the remediation of pollution arising from metals Fe, Mn and Ni at station M6 and Hg at station M4.

CHAPTER – IV

SUMMARY AND CONCLUSIONS

The present study was conducted in the Mandovi Estuary, Goa with objectives to understand the metal bioavailability and bioaccumulation in edible biota. Also, to understand the potential of microplastics as adsorption site for metals and to study the phyto-remediation potential of mangrove plants. The total metals (Fe, Mn, Zn, Cu, Co, Ni and Hg) were enriched in sediments that was attributed to human-induced activities viz., mining, industrial, sewage, and agricultural inputs. The dominance of Fe, Mn, Zn, Cu, Co and Ni in the residual fraction indicated their natural origin from weathering of rocks. However, the significant proportion of Mn, Cu, Co and Ni in the bioavailable fraction suggested their anthropogenic origin and potential bioavailability. The labile form of Mn and Co revealed toxicity risks to estuarine biota. The concentration of metals (Fe, Mn, Zn, Cu and Hg) in soft tissues of estuarine biota (*M. meretrix*, *P. erosa*, *C. madrasensis*, *S. cucullata* and *S. serrata*) exceeded the permissible limit and indicated potential toxicity to both biota and humans. Therefore, the consumption of bivalves and crustaceans from the estuary might cause health risks. Further, the fiber type of microplastics were widespread in the Mandovi Estuary than film and fragment which was attributed to input from fishing activities and discharge of laundry waste. The PLI suggested microplastic pollution in the Mandovi Estuary. The chemistry of microplastics disclosed their potential to adsorb metals on the surface and construed them as vectors of metals in the estuarine environment. The mangrove plants viz., *Sonneratia alba* was effective in remediation of Mn, Zn, Cu and Hg at station M3, while *Avicennia marina* was effective in removal of Fe, Mn, Zn, Cu and Ni at station M7. Also, *Avicennia corniculatum* was promising in remediation of Mn, Ni and Hg at station M5, and *Rhizophora mucronata* at station M4 (Fe, Mn and Ni).

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