Assessment of metal toxicity through bioavailability and bioaccumulation in middle region of the Sal Estuary, and to decipher phyto-remediation ability of mangrove plants

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DECLARATION BY STUDENT

I hereby declare that the data presented in this dissertation report entitled, “Assessment of metal toxicity through bioavailability and bioaccumulation in middle region of the Sal Estuary, and to decipher phyto-remediation ability of mangrove plants” is based on the results of investigations carried out by me in the 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 "Assessment of metal toxicity through bioavailability and bioaccumulation in middle region of the Sal Estuary, and to decipher phyto-remediation ability of mangrove plants" is a bonafide work carried out by Mr. Gaurav Shamu Gawas 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 1

INTRODUCTION

1.1 Introduction

The coastal environment is the interface between land and sea or ocean, where the marine environment influences the terrestrial environment and the terrestrial environment influences the marine environment **(Wolanski 2007).** The Earth has a coastline of 620,000 km, out of which India shares about 7500 km. The various geomorphological features occur in the coastal environment, viz., lagoons, beaches, rocky shores, islands, headlands, estuaries, etc. An estuary is the transition zone between the river and the sea or ocean, wherein there is an inflow of freshwater from the river and saltwater from the sea or ocean. This interaction, along with tidal influence, geology, climatic conditions, and the flora and fauna of the region, creates a unique, complex, and dynamic ecosystem with sub-environments such as mudflats, sandflats, deltas, mangrove and saltmarsh ecosystems. The estuary provides habitat for a wide range of diverse groups of organisms. They are called as "nursery grounds" because many marine fauna reproduce and spend their early part of the life cycle in estuaries.

The word "estuary" comes from the Latin word "aestuarium," which means tidal inlet of the sea, which in itself is derived from the term 'aestus', meaning tide. Various attempts have been made to define an estuary, and among those, the most widely accepted definition in the scientific literature was given by **Prichard (1967),** who states that "an estuary is a semi-enclosed coastal body of water, which has a free connection with the open sea, and within which seawater is measurably diluted with fresh water derived from land drainage." Pritchard's definition of estuary excludes some coastal water bodies where estuarine ecology is studied today. In an attempt to address the limitations of Prichard's definition, **Fairbridge (1980)** defined an estuary as "an inlet of the sea, reaching into the river valley as far as the upper limit of tidal rise, usually being divisible into three sectors: a) a marine or lower estuary, in free connection with the open sea; b) a middle estuary, subject to strong salt and freshwater mixing; and c) an upper or fluvial estuary, characterized by freshwater but subject to daily tidal activity." The limits between these sectors are variable and subject to constant changes with temporal variations in tidal amplitude, river flow, seasons, winds, waves, and geomorphology.

The estuaries are classified based on their geology, circulation pattern, and tidal range. Based on geology (existing geology or their geological origin), the estuaries are classified as: (a) coastal plain estuaries - these are also called drowned or submerged river valley estuaries as they were formed due to rising sea level flooding the existing river valley. These types of estuaries are funnel or V-shaped, usually shallower (rarely exceeding 30 m depth), and may exhibit meandering characteristics in the middle portion of the estuary. There are special types of drowned river valley estuaries with special characteristics of the presence of rocky islands in the mouth region, known as Rias; for example, Hooghly Estuary; (b) Bar-built or restricted mouth estuaries - where there is the formation or occurrence of sandbars or barrier islands at the mouth of an estuary. Sand bars are formed when there is a very high rate of sedimentation at the mouth of an estuary, separating the sea or ocean from the estuary. The bar may grow into barrier beaches or islands, and the estuary may become blocked, forming a blind estuary. The bar-built estuaries typically have a very high flow of river water during the monsoon period. A large volume of water flowing down the river can also completely wash away small bars and re-open the mouth of the estuary; (c) Delta estuaries – in such a type of estuary, there is the formation of a delta at the mouth of the estuary. Deltas are large, flat, fan-shaped deposits of sediment formed due to rapid load of sedimentation by the river at the mouth of an estuary; (d) Fjords - are the estuaries that were formed due to glacial erosion and retreat. The glaciers leave deep channels carved into the earth with a shallow, narrow sill near the sea/ocean. When the glaciers retreat, seawater floods the deeply incised valleys, creating estuaries; (e) Tectonic estuaries – are formed due to tectonic activities like earthquakes. The disturbance in the Earth’s crust due to tectonic activities can create depressions in the coastal terrestrial landmass, which, if they are formed below sea level, can create tectonic estuaries wherein there is a mixture of seawater from the sea or ocean and freshwater from the land run-off. These estuaries are found along major fault lines, for example, in the San Francisco Bay area of California.

According to the circulation pattern, estuaries are classified into: (a) highly stratified, salt wedge estuary – typically, these estuaries have a small tidal range and a very large flow of river water that is not mixed with saltwater, forming the highly stratified layers of freshwater above denser saltwater; (b) Moderately stratified, partially mixed estuary – they have a high tidal range and low inflow of freshwater, experiences much more mixing of saltwater and freshwater, showing a decreasing salinity profile from bottom to surface waters, and higher salinity at the mouth region with a decreasing trend towards upstream; and (c) a vertically or fully mixed estuary – in such estuaries, the salinity remains the same at all depths due to very strong tidal currents, eliminating the vertical layering of freshwater floating above the denser seawater. This occurs when the flow of the river is low and tidal currents are moderate to strong. The flow of river changes with seasons, and tides changes periodically; therefore, the estuary can change from one type to another with time or season.

Dyer (1997) classified estuaries based on the tidal range as follows: (a) microtidal estuary with a mean tidal range < 2 m; (b) mesotidal estuary with a mean tidal range between 2 m and 4 m; (c) macrotidal estuary with a mean tidal range between 4 m and 6 m; and (d) hyper-tidal estuary with a mean tidal range > 6 m.

The sources of sediment in the estuary are in situ, marine, terrestrial, and anthropogenic. Among these, the terrestrial source is the main contributor of sediments in the estuary, which originates due to the weathering of rocks in the catchment area. The rivers are dominant transporting medium of sediment to estuaries **(Gaonkar and Matta 2020)**. The erosion and transportation of weathered material through streams, rivers, or wind bring the particulate, dissolved, and colloidal material into the estuary. The inorganic and organic species introduced through various sources in an estuary undergo various biogeochemical processes before becoming part of the cohesive bed sediments in the estuarine sub-environment. For example, during the mixing of fresh and saline waters in an estuary, the finer sediment particles settle out of suspension by the process of flocculation **(Dessai and Nayak 2009)**.

The mudflat and mangrove sub-environments are formed due to the process of deposition and accumulation of sediments over a period of time in an estuary. **Klein (1985)** defined tidal flats as "low relief environments containing unconsolidated and unvegetated sediments that accumulate within the intertidal range, including the supratidal zone." In simple words, mudflats are depositional sub-environments in the intertidal regions formed in low-energy areas that promote the higher deposition of sediments and are unvegetated or have little vegetation other than green algae. The growth rate of an unvegetated mudflat depends on a number of factors, including wave activity, shape and size of fringing wetlands, local geology, sediment availability, minerology, and biological activity, such as the growth of the surface diatom layer (bio-film), which diminishes the erosion rate **(Wolanski 2007)**. Over a period of time, the mud flats are vegetated with salt-tolerant species of mangroves and mangrove associates, eventually evolving into mangrove ecosystems. Mangroves are vascular halophytic (salt-tolerant) plants adapted to growing in high-saline, water-logged, and anaerobic soil conditions. Their growth is restricted in tropical and sub-tropical coastal environments. The mangrove plants are highly adaptive having a specialized root system. They have special roots called pneumatophores that help them deal with anaerobic waterlogged soil by absorbing oxygen from the air during low-tide exposure to the atmosphere (**Al-Khayat and Alatalo 2021**). Mostly mangrove and mudflat sub-environments are found in the lower and middle intertidal regions of the estuary, where vigorous mixing of fresh and saline water takes place and a huge amount of sediment is kept in suspension. The specialized root system of mangroves promotes the higher deposition of sediments in this ecosystem. The sediment grains that are accumulated in the mudflat and mangrove sediments are in size from less than 0.002 mm to more than 4 mm, with a predominance of finer sediments in most estuaries and high organic matter, supporting a higher accumulation of metals in the sediments. Both the sub-environment provides a habitat and supports communities characterized by polychaetes, oligochaetes, bivalve mollusks (e.g., clams, oysters), gastropods (e.g., telescope snails), crustaceans (e.g., crabs, shrimps), and many other fish and birds. The mangrove ecosystem is a nursery ground for many fish **(El-Regal and Ibrahim 2014).**

Estuaries provide many goods and services to the large human population. They are sites for port, industrial, urban, and recreational development. Many local fishermen's livelihoods are dependent on estuarine ecosystem. Estuaries are known as sinks for many environmentally concerned pollutants. Having immense ecological and economic importance, the quality of this fragile ecosystem around the world is deteriorating due to the negligence of humans, as these are utilized as dumping and disposal sites for human-generated waste, believing its natural dilution by waves and tides.

Among the various pollutants introduced in estuaries, metals are noxious pollutants with characteristics such as persistent behaviour, non-biodegradability, bioavailability, bioaccumulative nature, and toxicity potential. Many studies have been carried out around the world assessing the pollution of the metals, their effect on the health of the estuarine ecosystem, and the risk to human health. The metals are introduced to an estuary through the weathering of rocks in the catchment area. Additionally, anthropogenic activities viz., mining, fishing activities, ship scrapping and repair, industrial activities, domestic and municipal sewage discharge, agricultural, aquaculture, tourism, and recreational activities contribute to significant level of metals in estuaries **(Gavhane et al. 2021; Nasnodkar and Nayak 2017)**. The metals introduced in soluble or colloidal forms into estuarine water tend to either precipitate or be adsorbed to suspended inorganic or organic particulate matter **(Nasnodkar and Nayak 2019)**. They can even form organometallic complexes with the organic matter, which can later get adsorbed onto the particulate matter. The precipitated, adsorbed, or complex metals may be deposited in bioavailable form on the surface of sediment grains. In general, 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 **(Kumar et al. 2014)**. 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 **(Pereira et al. 2023)**. The metals that are released into estuaries because of human activities are adsorbed on sediment surface and 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 several 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. The changes in the physico-chemical parameters (pH, ionic composition, redox potential) and biological activity (organic matter decomposition) in the estuary can result in remobilization of the deposited metals from sediments to the interstitial pore water or into the water column, making it easily available for uptake by sediment-associated organisms. The 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 bivalves viz., oysters and clams are benthic organisms most often found in estuarine mudflat and mangrove regions. They are filter feeders, feeding through their gills, and obtain metals not only from food and water but also from ingestion of inorganic particulate materials **(Yusof et al. 2004)**. The bivalves are used as a bio-indicator for monitoring the metal pollution status, and also to assess the metal toxicity in the environment, because the concentration of metal in the bivalve tissues is found to be corelated with concentration of metal in the ambient environment. The accumulation of metal in the bivalve is depended on extrinsic and intrinsic factors. The extrinsic factors include all the physico-chemical parameters, and the concentration of the bioavailable or labile form of the metal, occurring outside the body of an organism, which will determine availability of the metal for the uptake of biota. The internal factors include, feeding activity, age, size, sex, genotype, phenotype, reproductive stage, concentration of metallothionein in the bivalve tissue and affinity of the metal towards metallothionein etc, which will determine the ability of an organism to accumulate metal in their body tissue. The concentration of metal, essential or non-essential, above threshold concentration can have a harmful effect on the biota, such as alterations of growth, disease development, reproductive impairment, abnormal development in embyro, etc, **(Yuzereroglu et al. 2010; Rodrigues et al. 2021; Bhutia et al. 2023)**.

Observing the increasing trend of metal pollution in the estuarine environment and the fact that the presence of environmentally active metals in mudflat and mangrove sediments above the threshold concentration can have detrimental effects on this fragile ecosystem, there is a need to acquire control measures. Across the world, scientists and researchers are working conscientiously to curb the metal pollution in estuaries using various physical, chemical, and biological methods. The phyto-remediation is one of the biological techniques that is widely gaining popularity in the remediation of metal pollution in estuaries by using naturally occurring mangrove plants in the estuarine environment.

The phyto-remediation is an in-situ, emerging solar-driven technology that utilizes the inherent abilities of living plants and rhizosphere microorganisms for the removal or transformation of contaminated soils and sediments. This is recognized as an eco-friendly, cost-effective method of immobilizing, stabilizing, degrading, transferring, removing, or detoxifying both inorganic (e.g., heavy metals/metalloids) and organic pollutants (e.g., organochlorine pesticides, hydrocarbons, etc.) as well as nutrients and organic matter **(Sarkar 2019).** Mangrove plants can transfer metals from sediments and concentrate them in their tissues, and are convenient means for phyto-remediation **(Nguyen et al. 2020). Chai et al. (2017)** asserted that the growth of mangrove species (*Kandelia obovata, Avicennia marina, and Sonneratia caseolaris*) may alter the sediment environment and influence the ecological risk posed by heavy metals due to changed concentrations. **Ivorra et al. (2021)** concluded that mangrove areas tend to have lower organochlorinated pesticide (OCP's) than non-mangrove areas, indicating mangrove ecosystems might be a potential tool to deal with persistent contamination. The mangrove plants have adapted different cohesive mechanisms to grow in stressful environments, such as in metal contaminated sediments, without any adverse impact on plant growth. But a highly stressful environment can have a harmful effect on them **(Nguyen et al. 2020).** The mechanism and efficiency of phyto-remediation depend on the type of species, the type of pollutant, and its bioavailability and toxicity (**Mahar et al. 2016**). The mangrove plants uptake the metals through their root system, which either utilizes, accumulates, or excretes them at the root level, or they are transported to the aerial organs of the plant. Similarly, in the aerial organs, it will be utilized, excreted, or accumulated in the plant tissue. For a phyto-remediation, the mangroves should be able to accumulate a higher concentration of metals in their tissues without having the adverse effect on the plant. The plant should accumulate a higher concentration of metals in the aerial parts. Some of the mangrove species are found to be hyperaccumulators of metals. Therefore, such species should be identified who will have higher translocation ability and accumulation of metals from the contaminated sediments. This will promote the afforestation of such species in the estuarine environment to tackle metal pollution naturally.

1.2 Study area

The Sal River is one of the important rivers flowing in Goa, India. The river originates from Udear springs in Verna, Salcete taluka in South Goa district, and flows southwards parallel to the coast **(Panandiker et al. 2020).** The mainstream of the Sal River passes through the areas of Verna, Cansaulim, Majorda, Nuvem, Duncolim, Seraulim, Mulgao, Margao (Khareband), Benaulim, Varca, Telaulim, Orlim, Sirlim, Navelim, Carmona, Cavelossim, Assolna, Velim, and Mobor. The Navelim and Cuncolim are two significant tributaries of the Sal River **(Pradhan 2016)**. The river experiences tidal influence as it discharges water into the Arabian Sea, forming an estuarine ecosystem stretch of 14 km long from Khareband to Mobor, near Betul Fort **(Saha et al. 2021; Action Plan Report on River Sal RRC 2019).** The catchment area (301 km^2) of the Sal River receives maximum rainfall during the southwest monsoon (June to September), with annual rainfall of approximately 300 cm. The mean river flow during the wet season is 6.96 $m^3 s^{-1}$ which reduces during the dry period, transforming the estuary from partially mixed to well mixed **(Fernandes et al. 2019)**. The Sal Estuary is a drowned river valley type of estuary having microtidal (< 2 m) characteristics with semi-diurnal tides **(Fernandes et al. 2018; Saha et al. 2021)**. There is a presence of sand bar with a narrow mouth opening connecting the Arabian Sea on west. The sand bars can sometimes extend completely isolating the estuary from the sea during the wet season, which becomes dissipated during the dry season **(Fernandes et al. 2019)**. The intertidal mangrove and mudflat sediments within the Sal Estuary are prominent beds of oysters, clams, and other bivalves. The edible bivalves are widely harvested in the Sal Estuary for commercial and local consumption, supporting the livelihood of fishermen community. The mouth of the estuary has a fishing jetty and therefore, metallic waste from such activities might enhance metal concentration in the Sal Estuary. Additionally, the urbanization along the banks with establishment of restaurants has increased the organic debris into the estuary which might facilitate metal deposition in sediments. The narrow mouth of the Sal Estuary hinders the transport of domestic and other wastes into the sea, and as a result, metals and other wastes settle along the banks **(Fernandes et al. 2018)**.

1.3 Objectives

- To determine metal bioavailability in mangrove sediments in the middle region of the Sal Estuary.
- To study the bioaccumulation of metals in edible bivalves.
- To understand the phyto-remediation potential of mangrove plants.

1.4 Literature review

Fernandes et al. (2019)	The spatial and seasonal distribution of metals (V, Cr, Co, Cu, Ni, Zn, Pb, Mn, Fe, Al and Ti) in the suspended particulate matter (SPM) and bottom sediments of the Sal Estuary, Goa was investigated. The study suggested anthropogenic input of metals in the Sal Estuary. The Mn showed significant to very high enrichment in particulate matter during wet and dry seasons, and the Zn showed significant enrichment in sediments during dry season. The sediments were polluted with Mn according to the geo-accumulation index (Igeo).
Nasnodkar and Nayak (2017)	They studied metals (Fe, Mn, Ni, Co and Cr) in mudflat core sediments of Mandovi and Sharavati estuaries, and reported higher concentration of trace metals in the Mandovi Estuary than the Sharavati Estuary. It was attributed to adsorption of metals on finer sediment, organic matter and clay minerals. The metal speciation indicated anthropogenic sources of Mn, Ni, Co, Cr, and potential toxicity to the sediment-associated biota.
Nasnodkar and Nayak (2015)	The grain size, organic carbon, and total metals in mudflat core sediments from Mandovi, Sharavati and Gurupur estuaries were studied to understand the processes and factors determining the distribution of metals. The changes in the hydrodynamics of Mandovi and Sharavati estuaries in recent years promoted higher deposition of finer sediments and metals. In the Gurupur Estuary, higher deposition of coarser sediments diluted metal concentration in

	recent years. The sediment grain size, organic matter, Fe-Mn oxides regulated the concentration of metals in estuaries.
Gaonkar and Matta (2020)	The spatial and seasonal assessment of metals (Fe, Mn, Zn, Cr, Cu and Pb) in the surface sediments of Zuari Estuary was carried out to understand the factors affecting the distribution of metals and their sources, and to study the impact of mining on the Zuari Estuary. There was no significant seasonal variation of metals except for Zn and Pb, but, the substantial spatial variations were observed in the surface sediments of the estuary. The Fe-Mn oxides/hydroxides, organic carbon and sediment texture played an important role in abundance of metals in the sediments. The metal pollution indices indicated a moderate contamination of Pb and Cr in the estuary, which was attributed to release of these metals from industrial waste and handling of coal in the estuary. The ban of mining had reduced the concentrations of Fe and Mn in the surface sediments.
Nasnodkar and Nayak (2019)	The speciation Fe, Ni, Co, Cu and Cr in the mudflat core sediments of the Vaghotan Estuary was studied to understand the bioavailability, sources and toxicity of metals. The natural weathering of basalt and laterite was the main source of the metals in the estuary, with > 80 % of metal proportion in the residual fraction in the sediment. The Screenig Quick Reference Table (SQUIRT) revealed possible toxicity of Co and to certain extent of Cu to the sediment associated biota.

Dessai and Nayak (2009)	The metals viz., Fe, Mn, Cu, Zn, Cr and Co in surface sediments of the Zuari Estuary, Goa, were studied seasonally from head to mouth. The distribution of metals was regulated by finer sediments and organic carbon. Mn was highly, and Co was significantly associated with the bioavailable forms in the sediments. The metals were mainly associated with the Fe-Mn oxides phase, except for Cu which was present more in the organic and sulfide bound phase. The SQUIRT indicated potential risk of Mn and Co to the aquatic life.
Siraswar et al. (2021)	The core sediments from main and sub-channels of the Mandovi Estuary were analysed for sediment components, bulk metals (Fe, Mn, Zn, Co and Pb) and their geochemical phases. The finer sediments (~94%) were dominant at both channels. The concentration of organic carbon was high in surface sediments and declined with depth due to microbial decomposition. The bulk metal concentration was above global standards, except for Zn. The toxicity of Mn, Co and Pb to biota was revealed through SQUIRT and Risk Assessment Code (RAC).
Nasnodkar et al. (2021)	The assessment of metals in sediment cores from the Zuari Estuary revealed higher concentration of total metals (Fe, Mn, Cr, Zn, Cu, Cd and Pb), and bioavailable form of metals (Mn, Cd, and Pb) during spring tide than neap tide, which suggested low mobility of metals under flooded conditions. It was supported by higher concentration of metals

	accumulated in *Polymesoda erosa* during neap tide than subsequent spring tide.
Abhilash et al. (2013)	The metal concentrations (Zn, Cu, Fe, Pb & Ni) in seawater, sediment and in the tissues of *Saccostrea cucullata,* from Port Blair, Andaman Islands was determined. The metals were present in higher concentration in sediments than in overlying water. The Zn displayed high bioconcentration potential as compared to other metals which is due to its importance in shell mineralization, reproduction, etc. The concentrations of metals in *Saccostrea cucullata* were within background limits, indicating that the ecosystem is not affected by anthropogenic inputs.
Yuzereroglu et al. (2009)	The metals (Fe, Zn, Cu, Ni, Pb, Cd, Co) in *Patella caerula* displayed higher concentration at polluted sites in the Mediterranean area, Turkey. The metals were highest during winter and spring months, attributed to environmental and biological factors.
Rodrigues et al. (2021)	The level of metals (Fe, Mn, Zn & Cu) in soft tissue of edible biota *Saccostrea cucullata* from the Zuari Estuary exceeded the standard permissible limit. Thus, indicated the risk of metal toxicity to biota and their consumers. The spatial and temporal variations in the concentration of metals in the tissues of *Saccostrea cucullata* was attributed to bioavailability of metal, affinity to metallothionein in the bivalve tissue, sex types, age, habitat, etc.
Yusof et al. (2004)	The bivalves *Anadara granosa* and *Perna viridis* collected from 22 sampling sites along the Malaysian

	coast were analysed to determine the bio-indicator potential in assessing trace metals (As, Cd, Cr, Cu, Pb, Se and Zn) pollution. The study indicated *Anadara granosa* as an effective bio-indicator of Pb, Cd and Se pollution, while *Perna viridis* for Cr pollution.
Nath et al. (2014)	The distribution of As, Cd, Co, Cr, Cu, Ni, Pb and Zn in sediments and pneumatophores of *Avicennia marina* in the Syndney Estuary was studied to understand the metal contamination and potential use of the mangrove pneumatophores as a bio-indicator. The concentration of metals exceeded the background level indicating an anthropogenic input. The Cu, Pb and Zn were very severely enriched and highly present in bioavailable form in sediments. A strong relationship between metals (Cd, Cr, Pb and Zn) in pneumatophores and sediments was observed. The accumulation of metals in pneumatophores was regulated by total sediment chemistry and physiological requirements. The study suggested application of pneumatophores of *A. marina* as a bio-indicator of metal pollution.
Pi et al. (2011)	A laboratory experiment was carried out wherein *Bruguiera gymnorrhiza, Excoecaria agallocha* and *Acanthus ilicifolius* species of mangrove plants were grown in ex-situ conditions. The species were subjected to different concentrations of synthetic wastewater containing metal pollutants. The study revealed Fe-plaque formation on the mangrove roots which increased with an increase in the concentrations of metal pollutants in the sediments. It

	facilitated immobilisation of metals as Fe-plaque traps the metals, acting as a physical ‘barrier’ for the protection of the plant under metal stress.
Hossain et al. (2022)	The metal concentration (Fe, Mn, Cu, Zn and Sr) in sediment and mangrove species (*Excoecaria agallocha, Avicennia officinalis* and *Sonneratia apetala*) of the Sunderban region was studied. A low to moderate degree of metal contamination in sediments was observed. The metals accumulated in the roots were translocated to leaves, with higher Translocation Factor (TF) for Sr, Mn and Zn. The mangrove species were found as phyto-extractors, especially *A. officinalis,* while *E. agallocha* was phyto-stabilizer of Mn.

CHAPTER 2

MATERIALS

and

METHODS

2.1 Introduction

A sample of a specific quantity represents the biogeochemical processes prevailing in the environment. The samples are collected from a desired site(s), stored and then analysed for several parameters, upon reaching the laboratory. The analysis must be carried out using the standard analytical procedure to obtain accurate results which represent not just the environment but the issues pertaining in the environment. Thus, the materials, methods and the techniques used must be of internationally accepted standards. To meet the objectives of the study, the following methodology was adopted:

2.2 Sampling

The field sampling was carried out in the Sal Estuary, Goa in May 2023. Four short sediment cores of 20 cm length were retrieved from the mangrove (S-1, S-2, S-3 and S-4) intertidal sub-environments of the Sal Estuary using a mortar canoe (Fig 2.1). The sediment cores were collected with the help of a hand-operated PVC corer of 5 cm diameter. Additionally, sediment associated biota (oysters and clams) and mangrove samples (leaves, stems and pneumatophores) were collected from the vicinity of sediment core sampling station. The location co-ordinates of the sampling stations (Table 2.1) were marked using the Global Positioning System (GPS).

Table 2.1: The sampling locations in the Sal Estuary.

Sampling stations	**Latitude**	**Longitude**
S-1	15.181302°N	73.961949°E
S-2	15.177137°N	73.961137°E
S-3	15.173373°N	73.953870°E
S-4	15.172144°N	73.947323°E

The sediment cores were sub-sampled at 2 cm interval using a plastic knife and the sub-samples were then transferred into pre-labelled zip-lock polyethylene bags. The biota and mangrove samples were collected in pre-labelled cloth bags. The sediment, biota and mangrove samples were transported to the laboratory in an ice box. Upon reaching the laboratory, the sediment and mangrove samples were stored at 4°C in a refrigerator whereas, the biota samples were stored at -20°C in the deep freezer until further analysis. Later, the biota and mangrove samples were identified which are listed in Table 2.2.

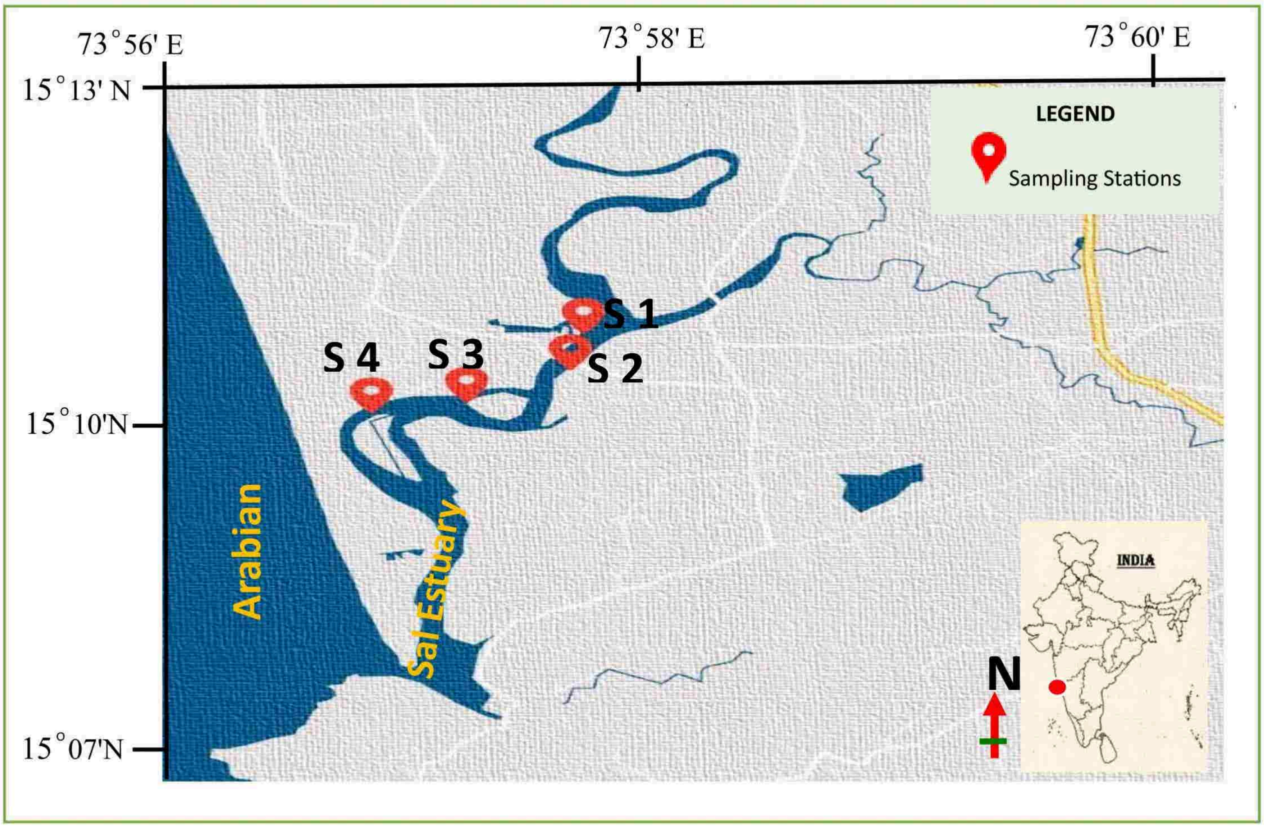


Fig 2.1: The sampling locations of sediment cores, biota and mangrove samples in the Sal Estuary.

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

Sampling stations	Mangrove	Biota
S – 1	• *Sonneratia alba* • *Avicennia alba*	• *Saccostrea cucullata* • *Villorita cyprinoides* • *Meretrix casta*
S – 2	• *Sonneratia alba* • *Avicennia marina*	• *Saccostrea cucullata* • *Meretrix meretrix* • *Meretrix casta* • *Circe scripta*
S – 3	• *Avicennia marina*	• *Saccostrea cucullata* • *Crassostrea madrasensis* • *Villorita cyprinoides* • *Meretrix meretrix*
S – 4	• *Avicennia marina*	• *Saccostrea cucullata* • *Crassostrea madrasensis*

2.3 Sedimentological and geo-chemical analysis

The sediment core sub-samples were oven dried at 60℃ for 96 hours. A portion of the dried 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 Grain size analysis

To understand the composition of sand:silt:clay in sediment the pipette technique proposed by **Folk (1974)** was adopted. It involves the separation of silt and clay based on the settling velocity principle of the particles following the Stoke's law. The settling velocity of a sediment particle depend on its size and density, and the viscosity of the liquid. In this method, 10 g of the dried bulk sediment sub-sample was weighed and transferred into the 1000 mL glass beaker. Then the glass beaker was filled with distilled water and was mixed well. After that, the sediments were allowed to settle at the bottom for almost 24 hours. Once all the sediment particles were settled at the bottom of the beaker, water was decanted using a decanting pipe without disturbing the settled sediments. This step was repeated 4 to 5 times until there was no traces of salinity observed which was verified with $AgNO_3$ solution. Later, the sediment sample was treated with 10 mL of 10 % sodium hexametaphosphate and was kept overnight to disintegrate the grain particles. On the next day, the sediment sample was treated with 5 mL of 30 % H_2O_2 and was kept overnight to oxidize the organic matter. The treated sediment sample was wash-sieved through 63 µm mesh size sieve using distilled water to separate sand component from silt and clay. The sand retained on sieve was transferred into the 100 mL pre-weighed beaker and was kept for oven drying. The filtrate containing silt and clay was collected in the 1000 mL measuring glass cylinder. The volume of filtrate in glass cylinder was made to 1000 mL with distilled water and the solution was homogenised using stirrer for 2 minutes. The homogenised solution in the measuring cylinder was allowed to settle. After measuring the room temperature using thermometer the extraction time (from stirring to pipetting) was calculated at 8 phi size 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 kept in an oven for drying. 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)

2.3.2 TOC analysis

The organic matter has the ability to adsorb metals in the estuarine environment. They readily form organo-metallic complexes in mangrove sediments. The microbial degradation of organic matter can desorb or release metals in the interstitial pore water and water column. The analysis of TOC enables to understand the role of organic matter in retention and bioavailability of metal in sediments. A modified Walkey-Black method **(Gaudette et al. 1974)** was followed to estimate the TOC concentration in sediments. It involves exothermic heating and oxidation of organic carbon in the sediment with $K_2Cr_2O_7$ and concentrated H_2SO_4 respectively. The excess of $K_2Cr_2O_7$ which does not participate in the reaction is titrated against 0.5 N ferrous ammonium sulphate solution to a sharp one drop brilliant green end point.

All the glass-wares were cleaned with chromic acid prior to use. The 0.5 g of powdered sediment sub-sample was transferred into 500 mL conical flask. To this 10 mL of 1 N $K_2Cr_2O_7$ solution was added, followed by 20 mL of H_2SO_4 and $AgSO_4$ mixture. The conical flask was gently swirled and was kept for 30 minutes reaction time. After 30 minutes, 200 mL of MilliQ water, 10 mL of 85 % of ortho-phosphoric acid and 0.2 g of NaF was added, and was mixed well. After adding few drops of diphenylamine indicator, the solution was titrated against 0.5 N ferrous ammonium sulphate solution till one drop brilliant green end point was observed. The same procedure was followed for standardization of blank without sediment sample.

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 = (Normality of $K_2Cr_2O_7$ × milliequivalent weight of carbon× 100)/Sample weight

Milliequivalent weight of carbon= 12/4000

F is equal to 0.6, when the sample weight is exactly equal to 0.5 g

2.3.3 Total metal

The total metal in the bulk sediment is the concentration of the metal present in residual fraction as well as in the bioavailable fraction of the sediment. To determine the total metal concentration in the sediment, the acid digestion method proposed by **Jarvis and Jarvis (1985)** which was later modified by **Sholkovitz (1990)** was adopted.

The apparatus were cleaned with chromic acid prior to use. 0.2 g of the powdered sediment sample was taken in a Teflon beaker, to which 10 mL of acid mixture of HF, HNO_3, $HClO_4$ in the ratio of 7:3:1 was added, respectively. Later, the Teflon beaker was kept on a hot plate at 150°C and the mixture was dried completely. Additional, 5 mL of the same acid mixture was added to the Teflon beaker and further dried for 1 hour. It was followed by complete digestion of sample with 2 mL of concentrated HCl. Further, the digested sample was extracted with 10 mL of 1:1 HNO_3 for few minutes. The solution was then filtered through Whatman filter paper in 50 mL volumetric flask. The volume of filtrate (extracted sample) was made to 50 mL using MilliQ water and was transferred into the pre-cleaned polyethylene bottle.

The concentration of metals viz., Fe, Mn, Zn, Cu, Co and Ni in the digested sample was determined using the flame Atomic Absorption Spectrophotometer (AAS) Thermo Fischer, iCE 3000 Series.

2.3.4 Metal speciation

The speciation of metals enables to segregate bioavailable forms of metal and residual form of metal in the sediment sample. To determine the different forms of metal (exchangeable, carbonate, Fe-Mn oxide, organic matter/sulphide, and residual fractions) in the sediment sample, the 5-steps sequential extraction procedure proposed by **Tessier et al (1979)** which was later modified by **Dessai and Nayak (2009)** was adopted. The first four fractions in this method are considered as bioavailable forms of metal. It is the bioavailable form of metal which is environmentally reactive, whereas the residual form of metal is environmentally unreactive as the metal is firmly held in the lattice structure of the mineral. The speciation study provides valuable information on source, mobilisation and bioavailability of metal in the sediment and its possible toxicity to the sediment associated biota **(Nasnodkar and Nayak 2019; Nasnodkar and Nayak 2017).**

2.3.4.1 Exchangeable fraction (F1)

The metal present in this fraction is most loosely bound to the sediment particles and can get desorb and release into the adjacent environment due to changes in ionic composition of water. The exchangeable fraction was extracted as follows:

1 g of the powdered sediment sub-sample was treated with 8 mL of 1N $MgCl_2$ into 50 mL centrifuge tube for 1 hour with continuous agitation in the orbital shaker at room temperature. Later, the treated sample was centrifuged at 8000 rpm for 10 minutes. The supernatant was filtered through Whatman filter paper into the 25 mL volumetric flask and the final volume was made to 25 mL using MilliQ water. The extracted sample was then stored in pre-cleaned polyethylene bottle at refrigerated temperature until further analysis. The sediment residue (I) in centrifuge tube was washed with MilliQ water and was used for next step.

2.3.4.2 Carbonate fraction (F2)

The second step is the extraction of metals associated with the carbonate fraction. The metal in this 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 whose pH was adjusted to 5 with a glacial acetic acid. The mixture was continuously agitated in an orbital shaker for 5 hours. Later, the sample was centrifuged at 8000 rpm for 10 minutes. The supernatant was filtered into the 25 mL volumetric flask using Whatman filter paper and the final volume was made to 25 mL using MilliQ water. The extracted sample was stored in a pre-cleaned polyethylene bottle at refrigerated temperature until further analysis. The sediment residue (II) in centrifuge tube was washed with MilliQ water and was used for next step.

2.3.4.3 Fe-Mn oxides fraction (F3)

In the marine environment, the dissolved Fe and Mn under oxic conditions precipitate as oxides and hydroxides and forms coatings on the surface of sediment particles. The Fe and Mn oxides are referred as effective scavengers of metals in the marine environment as they hold high concentration of metals viz., Cu, Zn, Cr, Ni, Co, etc. The metals associated with the Fe-Mn oxides fraction was extracted as follows:

The residue (II) was treated with 20 mL of 0.04 M hydroxylamine hydrochloride in 25 % acetic acid and was kept in a water bath at 95± 3°C for 6 hours with frequent gentle shaking in between the extraction time. Later, the treated sample was allowed to cool down and centrifuged at 8000 rpm for 10 minutes. The supernatant was filtered through Whatman filter paper into the 25 mL volumetric flask and the final volume was made to 25 mL using MilliQ water. The leachate was stored in a pre-cleaned polyethylene bottle at refrigerated temperature until further analysis. The sediment residue (III) in centrifuge tube was washed with MilliQ water and was used for next step.

2.3.4.4 Organic matter/sulphide fraction (F4)

Organic matter/sulphide fraction includes the metals bound to organic matter and sulphides. The metal in this fraction was extracted as follows:

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 and was kept in a water bath at 85°C for 2 hours. Later, additional 3 mL of 30 % H_2O_2 at pH 2 was added to the mixture and was kept back in a water bath for 3 hours. Further, the mixture was treated with 5 mL of 3.2 M ammonium acetate in 20 % HNO_3 and was subjected to continuous agitation for 30 minutes in an orbital shaker. After agitation, the sample mixture was centrifuged at 8000 rpm for 10 minutes. The supernatant was filtered through Whatman filter paper into 25 mL volumetric flask and the final volume was made to 25 mL using MilliQ water. The extracted sample was stored in a pre-cleaned polyethylene bottle at refrigerated temperature until further analysis. The sediment residue (IV) in centrifuge tube was washed with MilliQ water and was used for next step.

2.3.4.5 Residual fraction (F5)

The residue IV contains detrital silicate minerals, resistance sulfides and small quantity of refractory organic materials. The treatment with HF, HNO_3, $HClO_4$ acid mixture leads to complete digestion of the residual fraction.

The residue (IV) was treated with 10 mL of acid mixture (HF, HNO_3, $HClO_4$) in the ratio of 7:3:1, respectively in a Teflon beaker and was acid digested following the same protocol as that of total metal extraction. 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 MilliQ water and was transferred into a pre-cleaned polyethylene bottle and was stored at refrigerated temperature until further analysis.

The concentration of Fe, Mn, Zn, Cu, Co and Ni in all fractions was determined using the flame Atomic Absorption Spectrophotometer (AAS) Thermo Fischer, iCE 3000 Series

2.4 Biota digestion

The analysis of metals in the soft tissues of bivalves (oysters and clams) was carried out as follows:

The frozen biota samples were de-frozen. The bivalves were break-open and soft tissue was separated from the hard shell using the plastic knife. The soft tissue was kept for oven drying at 60°C for 72 hours. The dried biota sample was powdered using the agate mortar and pestle.

1 g of the powdered biota sample was acid digested using 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).** After complete digestion, few mL of MilliQ water was used for extraction of the digested sample into solution form. The solution was then filtered through Whatman filter paper into the 25 mL volumetric flask and the final volume was made to 25 mL with MilliQ water. It was then transferred into the pre-cleaned polyethylene bottle and was stored in a refrigerator until further analysis. The concentration of Fe, Mn, Zn, Cu, Co and Ni in the biota sample was analysed using the flame Atomic Absorption Spectrophotometer (AAS) Thermo Fischer, iCE 3000 Series.

2.5 Mangrove analysis

To analyse the concentration of metals in the pneumatophores, stems and leaves of the mangrove plants the following technique was adopted:

The mangrove samples were washed and cleaned using MilliQ water to remove the dirt adhered on it. After cleaning, pneumatophores, stems and leaves were cut into pieces and kept separately for drying in an oven at 60°C in the glass petri-plates for 20 hours. Later, the dried samples were finely powdered using an agate mortar and pestle **(Nath et al. 2014)**.

1 g of the powdered mangrove sample was acid digested using 10 mL of HNO_3 and 5 mL of H_2O_2 at 90°C on a hot plate for 2 hours **(MacFarlane and Burchett 2002).** After complete digestion of the sample, few mL of MilliQ water was added and the solution was filtered through Whatman filter paper into 25 mL volumetric flask and the final volume was made to 25 mL with MilliQ water. It was then transferred into the pre-cleaned polyethylene bottle and stored in a refrigerator until metal (Fe, Mn, Zn, Cu, Co and Ni) analysis using the flame Atomic Absorption Spectrophotometer (AAS) Thermo Fischer, iCE 3000 Series.

2.6 Metal Pollution Indices

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

2.6.1 Contamination Factor (CF)

To assess the level of contamination of metals in the sediments, the CF was calculated **(Gaonkar et al. 2019)** 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.

2.6.2 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

2.7 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.

2.7.1 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~~s~~ 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.

2.7.2 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)

2.7.3 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)**.

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

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.

2.8 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:

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

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.

2.9 Human health risk assessment

The edible bivalves collected from the Sal 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 (ppm)	34 − 107	Charbonneau & Nash (1993)
Mn (ppm)	0.01	WHO (1989)
Zn (ppm)	40	WHO (1989)
Cu (ppm)	30	FAO/WHO (2004)
Ni (ppm)	70 – 80	USFDA (1993)
Co (ppm)	0.1− 5	FAO/WHO (2004)

2.10 Statistical analysis

The processing of raw data and plotting of various parameters were carried out using the softwares viz., Microsoft Excel 16 and Grapher 14.

CHAPTER 3

RESULTS and DISCUSSION

3.1 Sedimentological analysis

3.1.1 Grain Size and TOC

Table 3.1: Range and average of sediment components and TOC in sediment cores S-1, S-2, S-3 and S-4.

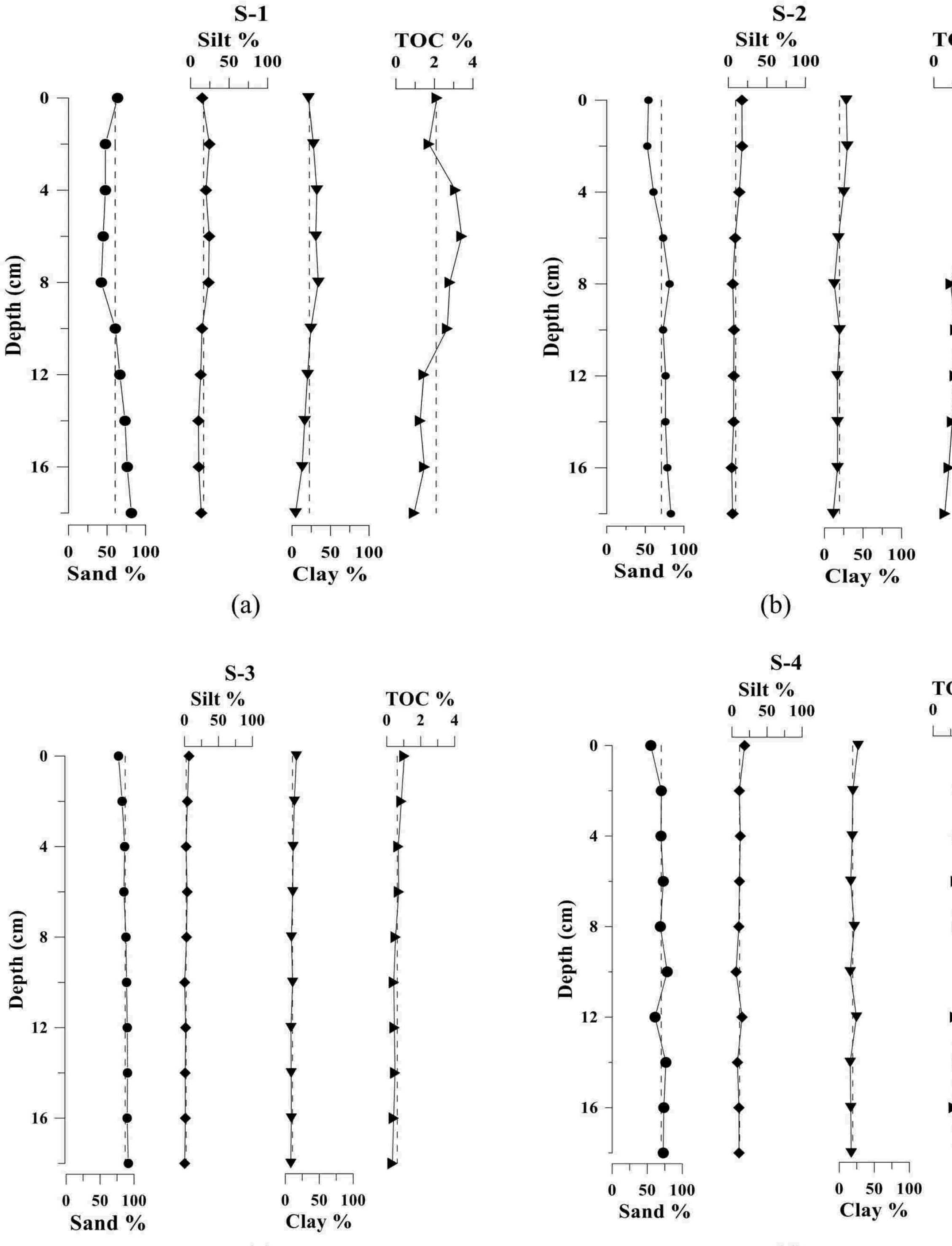
Sediment Cores		Sand %	Silt %	Clay %	TOC %
S-1	Range	42.58 - 81.60	10.20 - 24.40	4.64 - 34.16	0.95 - 3.41
	Average	60.52	16.93	22.55	2.09
S-2	Range	52.62 - 83.14	4.46 - 17.82	11.4 - 29.56	0.57 - 1.99
	Average	70.92	9.55	19.53	1.26
S-3	Range	77.08 - 91.61	0.25 - 6.56	8.12 - 16.36	0.33 - 1.03
	Average	86.97	2.44	10.60	0.62
S-4	Range	55.10 -78.43	5.89 - 17.98	15.6 - 26.92	1.16 - 1.99
	Average	69.8987	10.8893	19.212	1.50

The range and average concentration of sediment components and TOC in cores S-1, S-2, S-3 and S-4 are given in Table 3.1. The percentage of sand was highest in all four cores with an average concentration greater than 60 %. The sand was highest (86.97 %) in the core S-3, while reported lowest concentration in core S-1 (60.52 %). The silt and clay were highest in the core S-1 and were lowest in the core S-3. However, the concentration of silt or clay were below 25 % at a given station. The percentage of TOC was highest in the core S-1 (2.09 %) and was lowest in the core S-3 (0.62 %). The core S-1 was retrieved from upstream end of the middle estuarine channel, whereas core S-4 represented downstream end of middle estuarine region. In the present study, the dominance of sand (> 60 %) was contradictory to general distribution pattern of grain size in the middle estuary wherein there is often dominance of finer sediments. It may be attributed to high hydrodynamics conditions near the sampling sites. The Sal Estuary is a short estuary with a length of ~ 14 kms. All Sampling stations were at a distance of 5 to 7 kms from the mouth region. Therefore, the sampling stations were subjected to significant amount of wave and tidal energy capable of transporting coarser (sand) material The estuarine hydrodynamics is an important factor regulating the sediment grain size within the estuary **(Nasnodkar and Nayak 2015)**. The dominance of sand in the middle estuary caused dilution of finer sediments. The higher proportion of TOC at station S-1 relative to other stations was due to considerable proportion of silt and clay particles that might have facilitated the adsorption of TOC. The finer sediments have high surface area by volume ratio than coarser sediments which provides ample of free-binding surface for the adsorption of organic matter **(Burone et al. 2003)**. The previous study conducted by **Fernandes et al. (2019)** in the Sal Estuary had reported TOC in the range of 0.18 – 4.02 %.

3.1.2 Vertical distribution of sediment components and TOC

In core S-1 (Fig 3.1a), the percentage of sand decreased from bottom to 8 cm depth and, then remained nearly constant up to 2 cm depth, followed by an increase near the surface. The concentration of silt was more or less constant throughout the core length. The clay showed a slight increase from bottom to 8 cm depth, and then remained nearly constant up to 2 cm depth, followed by decrease near the surface. The TOC profile fluctuated around the average value with a slight increase reported at 10 – 4 cm depth. The temporal change in grain size, mainly the dominance of sand from bottom to 12 cm depth caused dilution of TOC, while the depletion of sand and a slight increase in silt from 8 to 2 cm depth facilitated adsorption of TOC. The availability of free binding surfaces for TOC particles is higher in finer sediments owing to high surface area by volume ratio **(Nasnodkar and Nayak 2015)**.

In core S-2 (Fig 3.1b), the sand showed overall decrease from bottom to surface of the core. The distribution profile of sand was compensated by clay, while silt maintained nearly constant concentration throughout the core. The TOC profile was more or less similar to clay. Therefore, the clay size particles seem to have regulated the distribution of organic matter in sediments.



(a) (b) (c) (d)

Fig 3.1: The vertical distribution of sediment components and TOC with average line in cores S1 (a), S2 (b), S3 (c) and S4 (d).

In core S-3 (Fig 3.1c), the distribution of sand, silt and clay was uniform along the core length. Also, TOC displayed constant concentration from bottom to surface of the sediment core, similar to finer sediments. In case of core S-4 (Fig 3.1d), the grain size and TOC maintained almost constant concentration from bottom to surface.

3.2 Geochemical analysis

3.2.1 Total metals

Table 3.2: Range and average concentration of total metals in sediment cores.

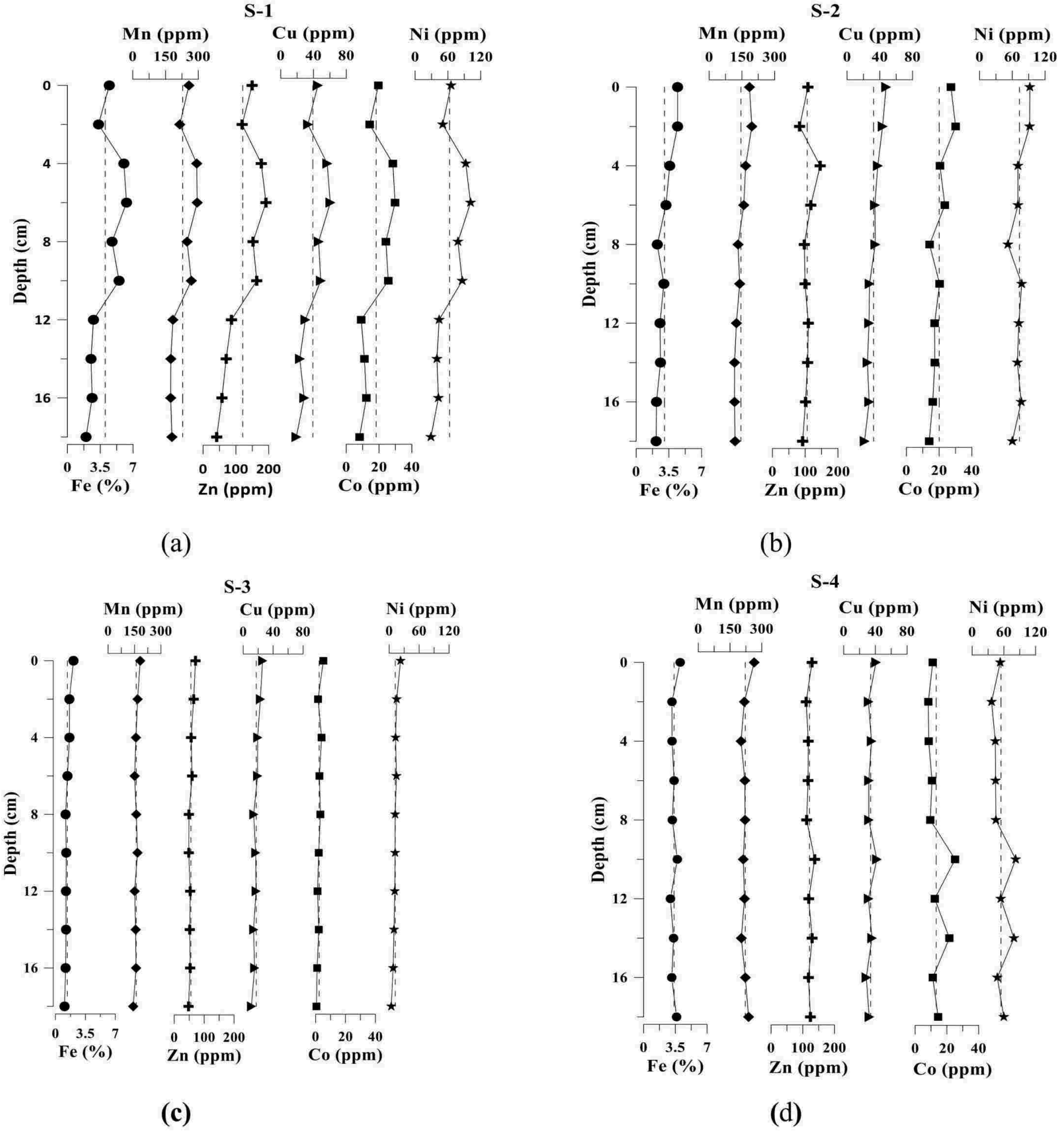
Sediment cores		**Fe (%)**	**Mn (ppm)**	**Zn (ppm)**	**Cu (ppm)**	**Co (ppm)**	**Ni (ppm)**
S-1	Range	1.98 -6.3	174 – 295	41 – 192	19 - 60	8 - 30	29 - 101
	Average	4.03	229	121	39	18	63
S-2	Range	2.13 - 4.43	116 – 195	83 - 146	21 - 48	14 - 30	52 - 92
	Average	3.03	14	106	32	20	73
S-3	Range	1.06 - 2.10	142 – 182	48 - 71	11 - 26	0.6 - 5	4 - 23
	Average	1.39	159	56	17	2	12
S-4	Range	2.96 - 4.03	202 -264	111 - 137	28 - 42	8 - 25	37 – 82
	Average	3.36	222	121	34	13	5
Upper crustal average **(Wedepohl 1995)**		3.08	527	52	14.3	11.6	18.6

The range and average concentration of total metals in cores S-1, S-2, S-3 and S-4 are presented in Table 3.2. The average concentration of metals viz., Fe, Mn, Zn and Cu was highest in the core S-1, while the metals viz., Co and Ni showed highest concentration in core S-2. The concentration of Zn at station S-4 was similar to station S-1. The higher concentration of most of the metals (Fe, Mn, Zn and Cu) in the core S-1 was attributed to relatively higher concentration of finer sediments and TOC at this station than others. The variability in the concentration of metals at different stations might be the result of differential sources of metals and factors influencing metal adsorption onto sediments viz., grain size, TOC, Fe-Mn oxides, clay minerals, etc., **(Gadkar et al. 2019)**. The concentration of Fe (S-1 and S-4), Zn (S-1, S-2, S-3 and S-4), Cu (S-1, S-2, S-3 and S-4), Co (S-1, S-2 and S-4) and Ni (S-1, S-2 and S-4) exceeded the upper crustal average value. Thus, indicated an enrichment of these metals in sediments of the Sal Estuary. The anthropogenic sources of metals in the Sal Estuary include input from fishing jetty through scrapping of paint particles from trawlers and due to contribution from domestic waste, agricultural and aquaculture waste, electronic waste disposal etc.

3.2.2 Vertical distribution of total metal

In core S-1 (Fig 3.2a), the concentration of all metals was below average value from bottom to 12 cm depth, and was more than the average value from 10 - 4 cm depth. Further, it decreased at 2 cm depth, followed by an increase near the surface. The distribution of all metals was similar to TOC and was opposite to sand particles. Therefore, the TOC might have regulated the distribution of metals along with clay particles. The metals tend to get adsorbed on organic matter, often forming organo-metallic complexes and also on clay size particles due to ample of free binding surfaces **(Gaonkar and Matta 2021)**. Additionally, the distribution profiles of metals viz., Zn, Cu, Co and Ni were similar to Fe and Mn. The chemistry of Fe and Mn is regulated by redox potential in the estuarine environment. Under oxic conditions, Fe and Mn precipitate as oxides or hydroxides onto sediment particles and in turn, scavenge other trace metals (Zn, Cu, Co and Ni) in the estuarine environment **(Fernandes and Nayak 2016)**. Further, upon depletion of oxygen level or under anoxic conditions both Fe-Mn oxides/hydroxides and scavenged trace metals are released back to water column in their dissolved form **(Dessai et al. 2023)**. Therefore, the role of Fe-Mn oxides/hydroxides in the distribution of trace metals cannot be ignored as disclosed through similar distribution pattern of metals.

In core S-2 (Fig 3.2b), the metals viz., Fe, Mn, Cu, Co and Ni displayed an increase in their concentration from 4 cm depth to surface of the core. An increase in the level of metals in recent sediments might be due to their fresh additions from anthropogenic sources. Even, the presence of relatively high TOC near the surface of the core might have facilitated high adsorption of metals in sediments **(Dessai et al. 2023)**. The distribution of metals in core S-2 (Fe, Mn, Cu, Co and Ni) (Fig 3.2b) from bottom to 8 cm depth, S-3 (Fe, Mn, Zn, Cu, Co and Ni) (Fig 3.2c) and S-4 (Fe, Mn, Zn and Cu) (Fig 3.2d) along the core length displayed nearly constant concentration. Also, Co and Ni exhibited constant concentration from 8 cm depth to surface. The lack of noticeable variability in the concentration of metals in cores S-2, S-3 and S-4 revealed no change in the sources of metals over a period of time.



(a)

(b)

(c)

(d)

Fig 3.2: The vertical distribution of total metals with average line in cores S-1 (a), S-2 (b), S-3 (c) and S-4 (d).

3.2.3 Metal speciation

The range and average of trace metals in different geochemical fractions in sediment cores S-1, S-2, S-3 and S-4 is presented in Table 3.3. In core S-1, the metals viz., Fe, Mn, and Ni were highest in the residual fraction, while Zn and Co were highest in the Fe-Mn oxide fraction. The Cu reported highest concentration in the organic matter/sulphide bound fraction. Also, Mn was more than 25 % in the Fe-Mn oxide fraction in sediments. The metals viz., Mn, Zn, Cu, Co and Ni were more than 10 % in the labile (exchangeable and carbonate) fraction. The Fe and Ni were more than 10 % in Fe-Mn oxide fraction, while Fe, Zn, Co and Ni were associated in more than 10 % with the organic matter/sulphide fraction.

In core S-2, metals viz., Fe, Mn, Cu and Ni were highest in the residual fraction, whereas Zn and Co were highest in the Fe-Mn oxide fraction. Also, Cu and Ni were more than 25 % in organic matter/sulphide and Fe-Mn oxide fractions respectively. The metals viz., Fe and Mn were more than 10 % in the Fe-Mn oxide fraction, while Fe, Zn, Co and Ni were above 10 % in the organic matter/sulphide fraction. Additionally, Mn, Zn, Cu, Co and Ni were more than 10 % in the labile fraction.

In core S-3, Fe, Mn, Zn and Ni were highest in the residual fraction. The metals viz., Cu and Co were highest in exchangeable and Fe-Mn oxide fractions respectively. The Zn was more than 25 % in the Fe-Mn oxide fraction. The metals viz., Fe and Mn were more than 10 % in the Fe-Mn oxide fraction, while Zn, Cu and Co were above 10 % in the organic sulphide fraction. The concentration of Zn, Cu and Ni was more than 10 % in the labile fraction.

In case of core S-4, Fe, Mn, Cu and Ni displayed highest concentration in the residual fraction, while Zn and Co were highest in the Fe-Mn oxide fraction. The Mn and Cu were more than 25 % in Fe-Mn oxide and organic matter sulphide fraction respectively. Also, Fe and Ni were more than 10 % in the Fe- Mn oxide fraction, and also Zn, Co and Ni in the organic matter/sulphide fraction. The metals viz., Mn, Zn, Cu and Ni were greater than 10 % in the labile fraction.

The geochemical forms of metals in the sediments of the Sal Estuary showed highest concentration in the residual fraction in cores S-1 (Fe, Mn and Ni), S-2 (Fe, Mn, Cu and Ni), S-3 (Fe, Mn, Zn and Ni) and S-4 (Fe, Mn, Cu and Ni). The residual fraction is considered to be environmentally unreactive fraction as the metals are strongly held in the lattice structure of the minerals **(Tessier et al. 1979)**. The metals derived from the natural weathering of rocks constitute the residual fraction in sediments **(Nasnodkar and Nayak 2019)**. Therefore, the significant concentration of metals in the residual fraction indicated their natural sources in the Sal Estuary. The metals were also associated with the bioavailable fractions viz., exchangeable, carbonate, Fe-Mn oxide and Organic sulphide fractions in the sediments of the Sal Estuary. In general, the metals bound to the bioavailable fraction are mainly adsorbed on the surface of the sediment particles, and are of anthropogenic nature. A slight change in ionic composition and pH can trigger the mobilization of metals from exchangeable and carbonate fraction respectively **(Giraldo 2018)**. Further, the redox potential and microbial activity regulates the adsorption and desorption of metals from Fe-Mn oxide and organic matter/sulphide fraction respectively **(Giraldo 2018)**. Additionally, one or more metals were available in more than 10 % in the labile fraction which is the most susceptible fraction in terms of mobilization of metals. Thus, it indicated strong bioavailability of metals to the sediment associated biota.

Among the bioavailable fractions, metals were mainly associated with the Fe-Mn oxide fraction followed by organic matter/ sulphide fraction. The association of metals in the Fe- Mn oxide fraction was attributed to their scavenging property in the Sal Estuary **(Nasnodkar and Nayak 2018)**. Zn was significantly (25 %) associated with the Fe-Mn oxide fraction in all sediment cores retrieved from the Sal Estuary, suggesting its adsorption onto precipitated Fe-Mn oxides/hydroxides in sediments. Moreover, the organic matter played an important role in the adsorption of metals through the formation of organo-metallic complexes **(Nasnodkar and Nayak 2015; Gaonkar and Matta 2021)**. The prevalence of anoxic conditions can cause dissolution of oxides and hydroxides, and other scavenge trace metals. Also, the degradation of organic matter by microbial activity can release metals bound to organic matter/sulphide fraction **((Fernandes and Nayak 2016)**. Thus, the speciation of metals in the sediments of the Sal Estuary revealed bioavailability of metals to sediment dwelling biota.

Table 3.3: Range and average of various forms of metals in sediment cores S-1, S-2, S-3 and S-4.

Station	Metals	Exchangeable (F1)		Carbonate (F2)		Iron Manganese (F3)		Organic matter/sulphide (F4)		Residual (F5)	
		Range (%)	**Avg. (%)**	**Range (%)**	**Avg. (%)**	**Range (%)**	**Avg. (%)**	**Range (%)**	**Avg. (%)**	**Range (%)**	**Avg. (%)**
S – 1	Fe	0.01 - 0.08	0.03	0.13 - 0.57	0.28	10.62 - 15.35	13.74	10.94 - 16.86	13.19	68.81 - 76.79	72.76
	Mn	5.67 - 13.76	10.04	4.41 - 20.04	13.58	15.12 - 36.59	26.33	3.96 - 11.73	7.24	25.84 - 65.09	42.81
	Zn	1.97 - 15.93	6.31	8.28 - 17.56	12.78	30.11 - 50.23	37.90	9.75 - 14.30	12.21	21.68 - 39.13	30.81
	Cu	1.27 - 36.92	13.38	2.97 - 6.63	4.66	2.52 - 6.38	4.86	22.91 - 49.49	39.48	28.12 - 43.39	37.62
	Co	1.06 - 7.33	3.53	9.89 - 17.49	12.72	27.92 - 39.77	35.03	13.99 - 24.63	18.41	27.64 - 32.34	30.32
	Ni	4.63 - 15.39	8.74	6.58 - 12.18	9.93	13.22 - 15.81	14.20	6.06 - 14.80	10.47	51.71 - 60.13	56.66
S – 2	Fe	0.02 - 0.05	0.03	0.03 - 0.07	0.05	9.71 - 15.02	12.21	8.97 - 15.93	13.32	70.59 - 81.27	74.39
	Mn	8.95 - 15.83	12.82	3.15 - 5.30	4.45	18.67 - 26.78	24.25	4.13 - 8.70	6.44	45.33 - 61.64	52.04
	Zn	7.70 - 20.83	13.17	6.85 - 11.69	9.54	33.95 - 50.81	40.06	9.36 - 14.33	11.50	21.64 - 30.50	25.72
	Cu	1.56 - 15.97	3.85	3.77 - 45.48	21.98	2.32 - 5.33	3.86	18.40 - 37.89	29.20	27.11 - 54.57	41.11
	Co	5.97 - 9.01	7.49	5.22 - 10.73	7.58	40.96 - 53.92	46.44	14.63 - 20.39	17.48	14.80 - 28.17	21.01
	Ni	7.35 - 19.03	12.01	5.38 - 10.70	7.26	19.09 - 40.45	27.35	8.31 - 13.44	11.42	27.33 - 52.47	41.97

S – 3	Fe	0.03 - 0.09	0.06	0.06 - 0.11	0.07	9.23 - 14.40	11.76	3.45 - 6.73	5.00	80.11 - 86.71	83.11
	Mn	3.14 - 5.92	4.27	0.44 - 9.73	2.24	4.49 - 25.96	10.66	1.20 - 5.54	2.54	52.85 - 90.18	80.28
	Zn	4.78 - 29.87	18.47	6.13 - 10.07	8.32	20.75 - 51.64	29.04	7.89 - 18.96	11.85	24.75 - 36.76	32.32
	Cu	3.40 - 55.22	37.70	4.16 - 7.01	5.49	3.28 - 5.67	4.68	12.68 - 39.38	20.76	22.82 - 45.70	31.36
	Co	0.00 - 7.92	2.12	0.00 - 3.37	0.34	35.98 - 48.79	43.91	0.00 - 21.41	10.82	33.55 - 50.53	42.81
	Ni	9.54 - 22.42	15.71	8.13 - 11.70	10.23	6.25 - 12.21	9.88	4.58 - 13.76	8.24	48.95 - 58.47	55.95
S – 4	Fe	0.01 - 0.04	0.02	0.04 - 0.12	0.08	10.83 - 17.79	13.93	5.53 - 10.88	8.29	74.59 - 80.02	77.67
	Mn	2.44 - 11.42	5.61	3.34 - 15.06	6.59	14.82 - 36.44	26.16	3.28 - 7.27	5.22	36.91 - 74.77	56.43
	Zn	4.09 - 11.75	7.00	8.06 - 11.49	10.32	32.73 - 37.69	36.04	13.75 - 17.59	15.73	28.71 - 35.43	30.91
	Cu	3.42 - 48.39	24.49	2.05 - 28.81	7.08	3.04 - 6.74	4.29	20.03 - 30.95	26.43	25.67 - 60.07	37.71
	Co	0.00 - 0.97	0.12	2.22 - 8.57	5.77	44.25 - 50.63	47.61	13.69 - 22.53	18.22	22.01 - 33.08	28.28
	Ni	6.04 - 9.02	7.48	6.93 - 10.90	8.63	15.49 - 29.79	21.58	9.40 - 16.74	12.42	38.45 - 59.08	49.90

3.2.4 Metal adsorption-desorption signatures from vertical profile

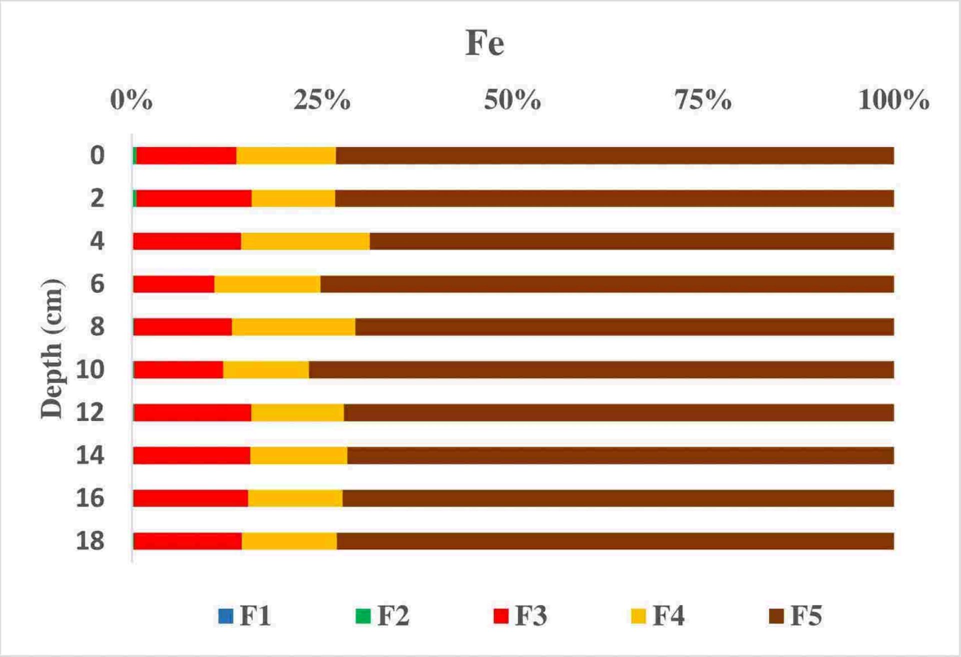


Fig 3.3.1a: The vertical profile of speciation of Fe in core S-1.

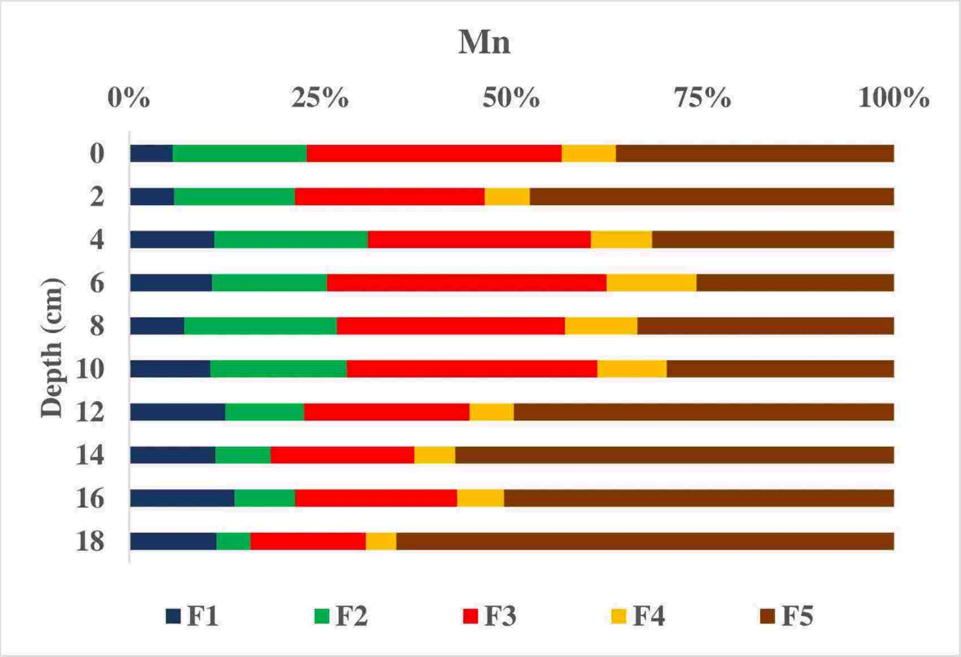


Fig 3.3.1b: The vertical profile of speciation of Mn in core S-1.

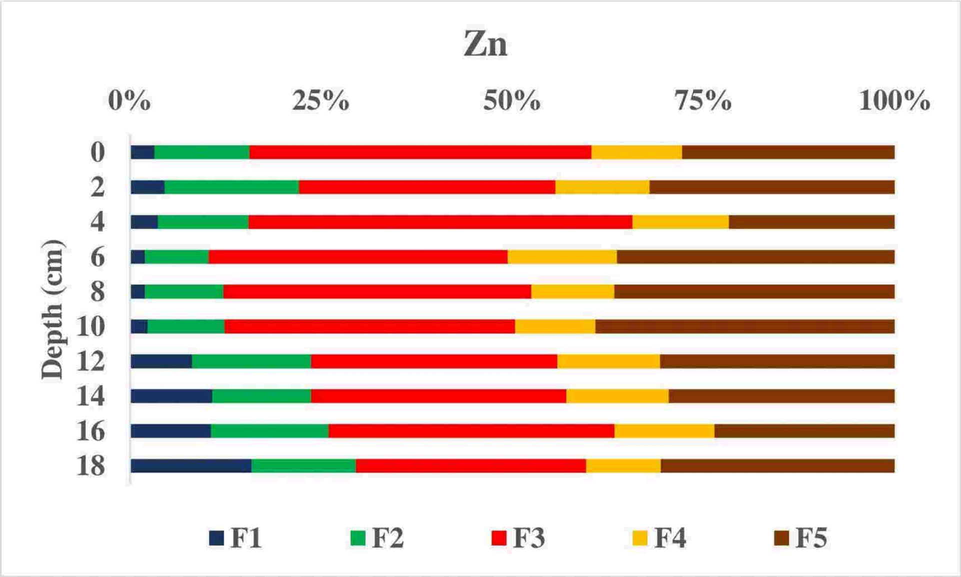


Fig 3.3.1c: The vertical profile of speciation of Zn in core S-1.

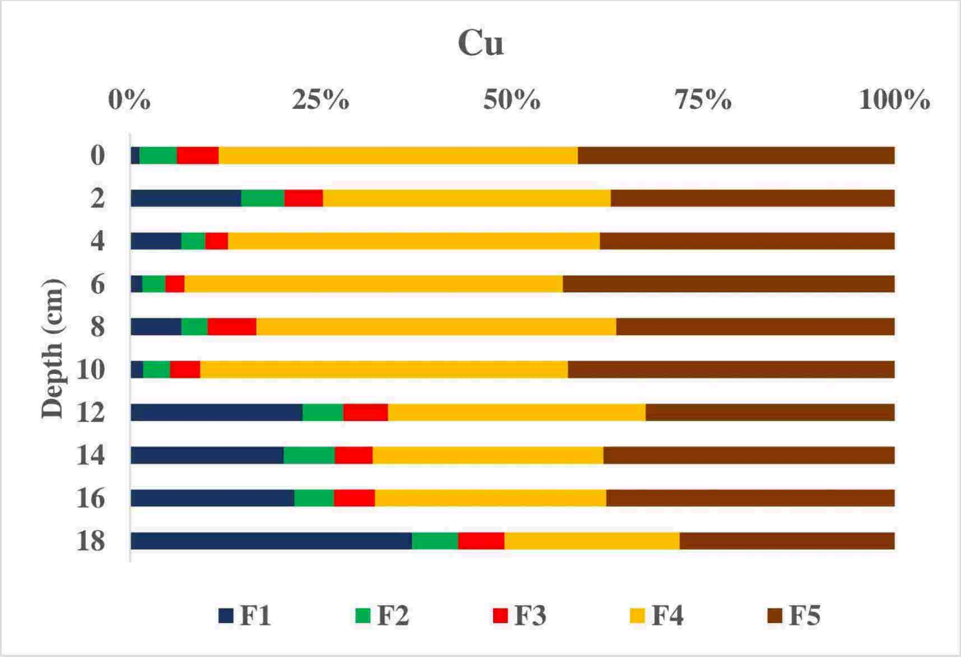


Fig 3.3.1d: The vertical profile of speciation of Cu in core S-1.

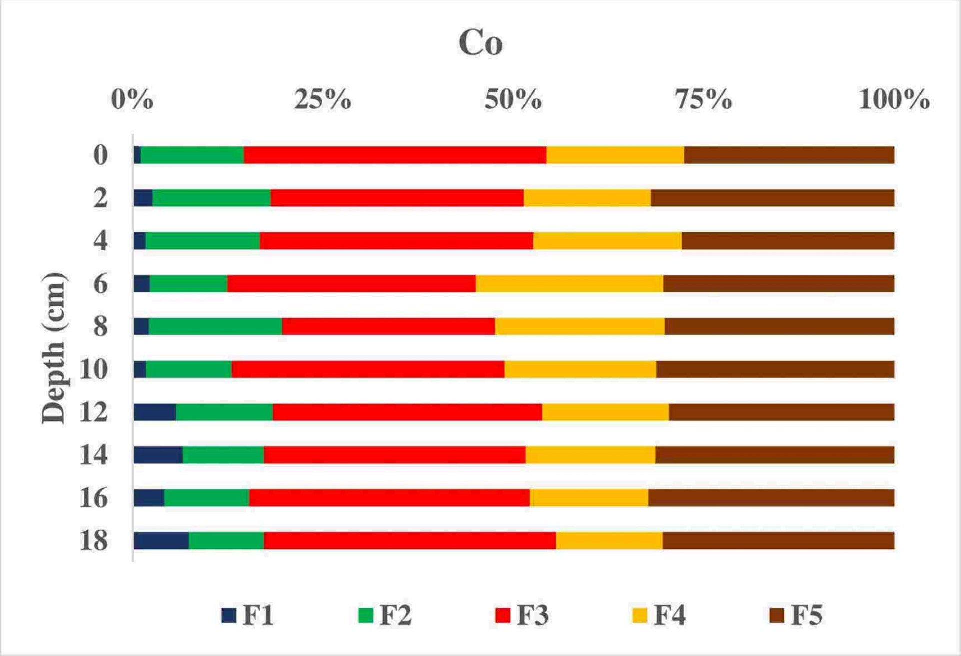


Fig 3.3.1e: The vertical profile of speciation of Co in core S-1.

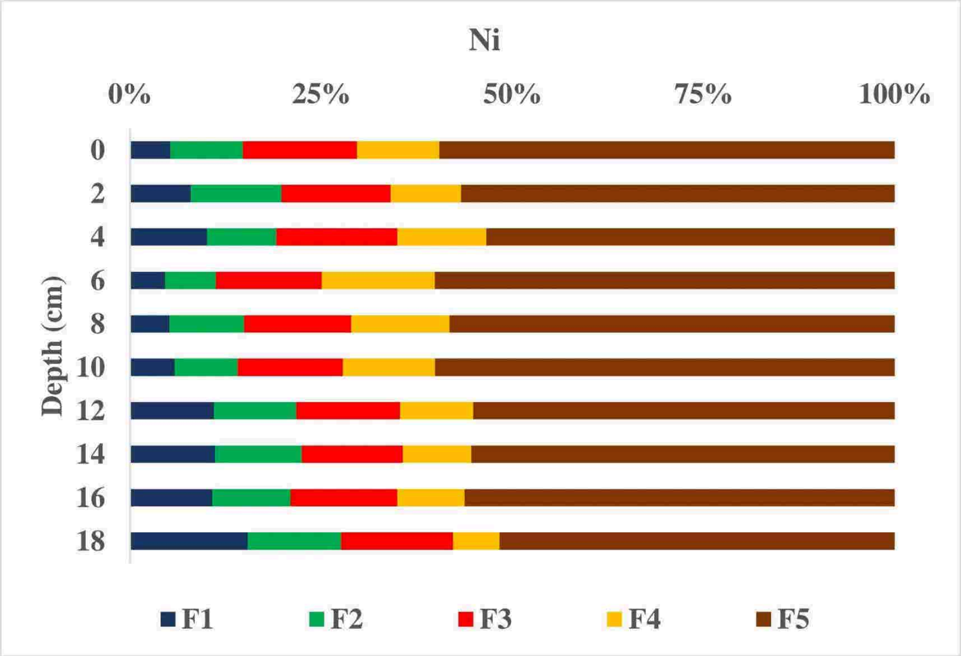


Fig 3.3.1f: The vertical profile of speciation of Ni in core S-1.

In core S-1 (Fig. 3.3.1), the concentration of Fe did not show much variations from bottom to surface with small positive spikes observed at 10 and 6 cm depth. It displayed sub-surface depletion from 10 to 6 cm depth followed by an overall increase towards the surface suggesting precipitation of Fe in its ferric form in surface sediments under oxic conditions. Contradictory to the Fe-Mn oxide fraction, the concentration of Fe in organic metal/sulphide fraction showed decline in its concentration from 4 cm to surface. Thus, indicated desorption of metals from organic matter due to microbial activity. The Mn showed an overall increase in concentration in the Fe-Mn oxide fraction from bottom to surface. It indicated presence of Mn in form of oxides or hydroxides near the surface and its strong bioavailability in sediments. Even, Mn showed an increase in its concentration near the surface in the carbonate bound fraction. It was attributed to substitution of Ca^{2+} by Mn^{2+} in carbonate sediments due to similarity in ionic radii **(Kanetkar et al. 2022)**. In case of the exchangeable fraction, Mn exhibited a decline in its concentration near the surface. Thus, suggested mobilisation of Mn from the exchangeable fraction due to ion-exchange reactions (**Nasnodkar and Nayak 2019**). Further, the concentration of Zn bound to Fe-Mn oxide and carbonate fractions showed an overall increase in the top 4 cm. It suggested bioavailability of Zn to biota with change in Eh and pH, respectively. A sharp decline was observed in the exchangeable fraction in the top 10 cm which indicated mobilisation of Zn to pore water or water column. Cu showed an overall increase near the surface associated with the organic matter/sulphide fraction. The Cu is often, associated with the organic matter fraction due to formation of organo-metallic complexes in the estuarine environment **(Nasnodkar et al. 2021).** The concentration of Cu in the exchangeable fraction was more than 20 % from bottom to 12 cm depth, it showed drastic decrease at 10 cm depth followed by an overall increase up to 2 cm depth and later decreased at the surface sediments. The vertical profile of Cu displayed fluctuating trend in the exchangeable fraction which might be due to adsorption and desorption of Cu as a function of changes in ionic composition of water. Co showed an overall increase and decrease from 8 cm to surface in Fe-Mn oxide and organic matter/sulphide fractions. The surface sediments in a core are enriched in oxygen level due to close contact with the overlying water column. The presence of oxic conditions in the surface sediments might have facilitated the adsorption of Co onto the Fe-Mn oxide or hydroxides, and caused mobilization of Co due to microbial degradation of organic matter. The high concentration of Co in the carbonate fraction near the surface was indication of its adsorption on carbonate minerals, while decline in its concentration (top 10 cm) revealed its mobilization from sediments to water column. Ni showed a gradual decrease in its concentration in the top 4 cm bound to the exchangeable fraction that suggested its mobilization from sediment to water. On the other hand, Ni in the carbonate fraction revealed an increase towards the surface in sediments. The mobilisation of Ni from the exchangeable fraction might have facilitated its adsorption in the carbonate sediments.

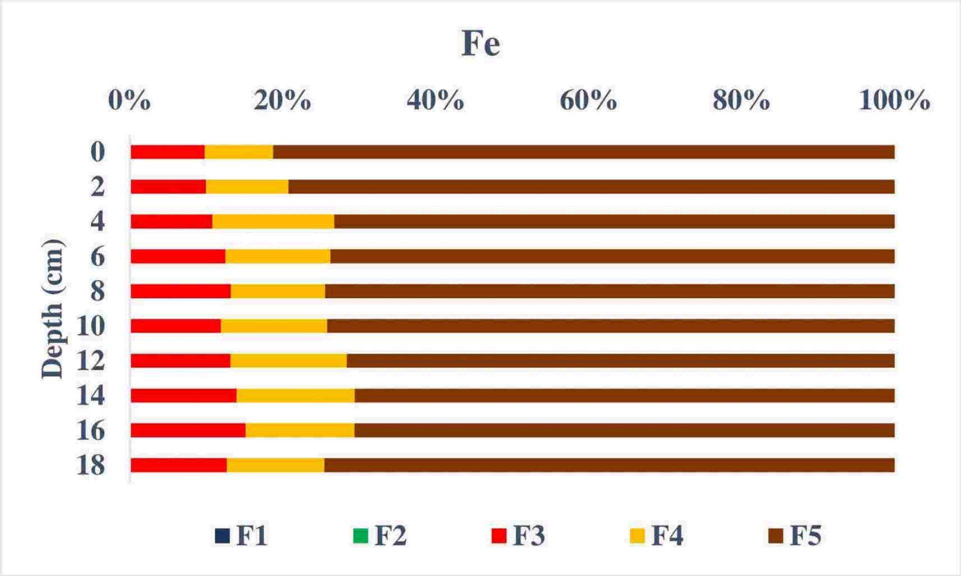


Fig 3.3.2a: The vertical profile of speciation of Fe in core S-2.

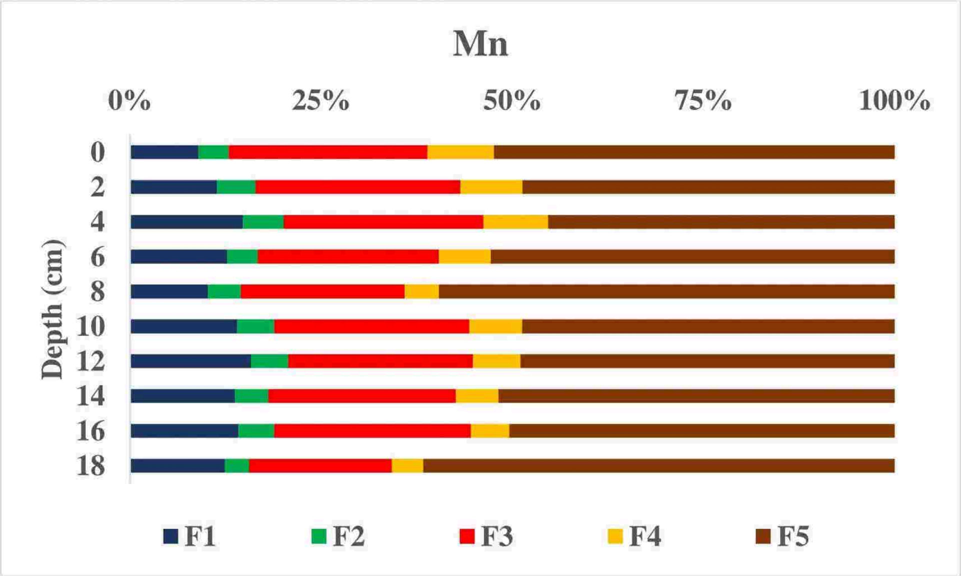


Fig 3.3.2b: The vertical profile of speciation of Mn in core S-2.

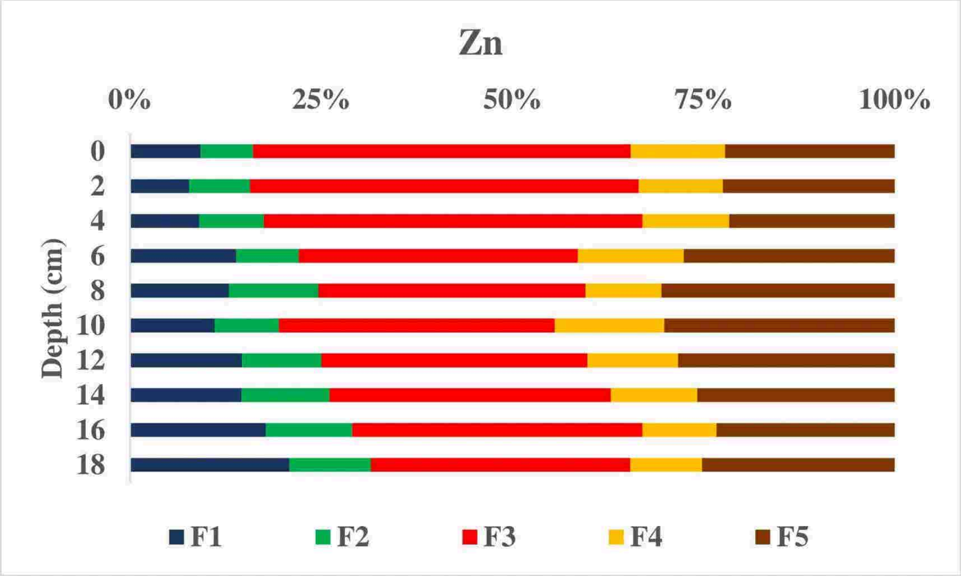


Fig 3.3.2c: The vertical profile of speciation of Zn in core S-2.

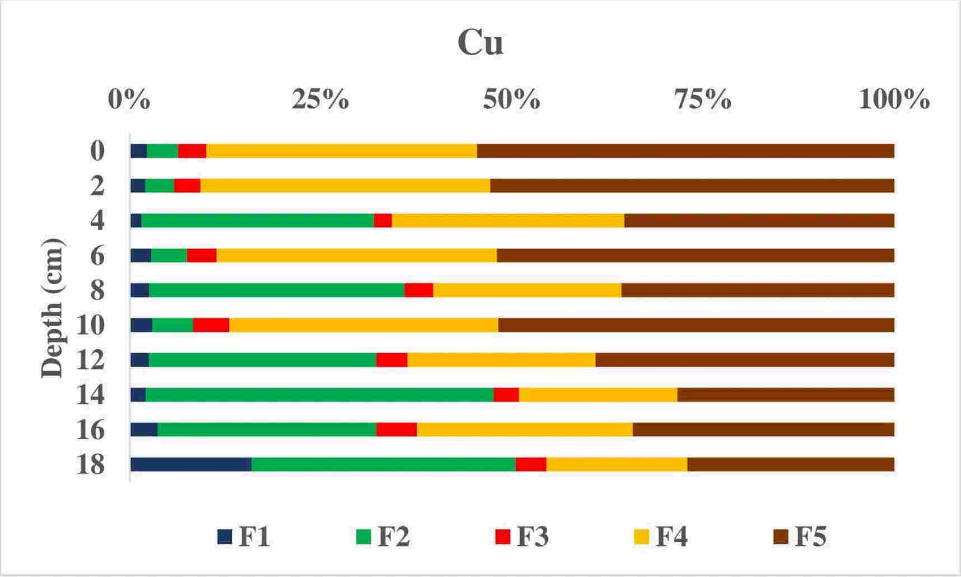


Fig 3.3.2d: The vertical profile of speciation of Cu in core S-2.

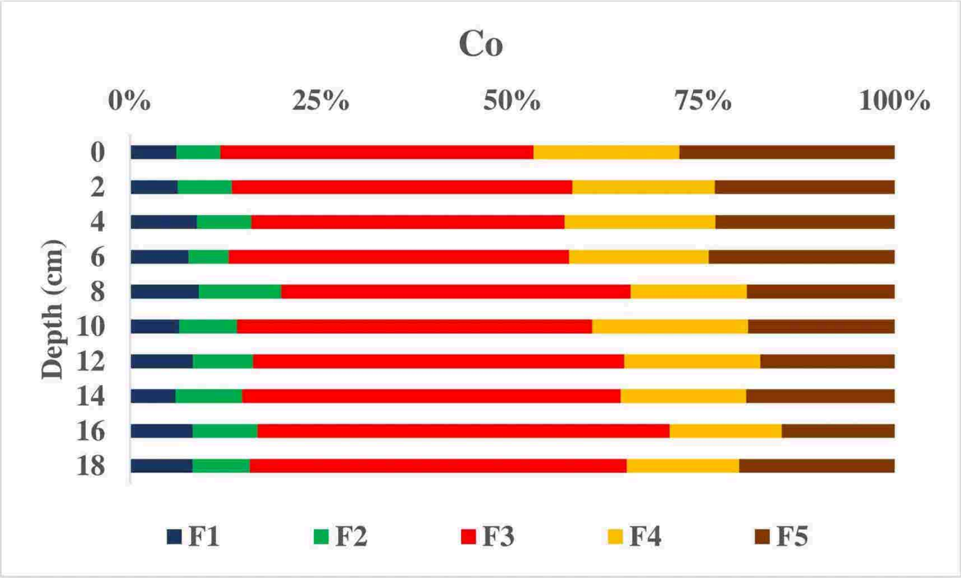


Fig 3.3.2e: The vertical profile of speciation of Co in core S-2.

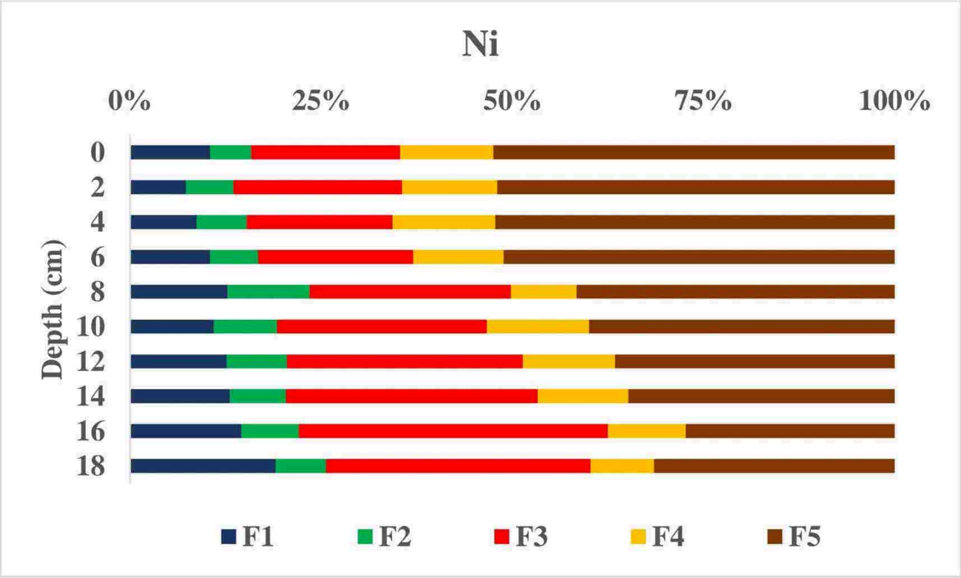


Fig 3.3.2f: The vertical profile of speciation of Ni in core S-2.

In core S-2 (Fig 3.3.2), there was an overall increase in the residual fraction towards the surface, while there was a decrease in Fe-Mn oxide and organic matter/sulphide bound fractions. The surface depletion of Fe in these bioavailable fractions indicated its mobilization from sediment to water. Mn showed an overall increase in surface sediments bound to Fe-Mn oxide and organic matter sulphide fractions indicating its adsorption onto oxide and hydroxides of Fe and/or Mn, and organic matter. The fluctuating trend of Mn in the exchangeable fraction was due to its adsorption and desorption as a function of change in ionic composition of water. Zn showed an increase in its adsorption onto Fe-Mn oxide and organic matter/sulphide fractions indicating strong bioavailability in the surface sediments. On the other hand, its concentration decreased in the labile fraction near the surface which reflected its mobilisation to the water column. Cu showed a gradual increase in the organic matter/sulphide fraction from bottom to surface. It highlighted the presence of organo-metallic complexes in the sediments. The ionic composition and acidic pH of the water might have caused the desorption of metals from the labile fraction in the surface sediments. Co displayed an increase in the organic matter/sulphide fraction in the surface sediments and suggested its adsorption on organic matter. Rest of the bioavailable fractions exhibited fluctuating trend of Co along the core length. The Ni bound to the Fe-Mn oxide fraction showed its desorption with decrease in its concentration in the surface sediments, while there was an overall increase in concentration of Ni bound to the organic matter fraction. It displayed a gradual decrease in its concentration from bottom to 2 cm depth, followed by an increase near the surface.

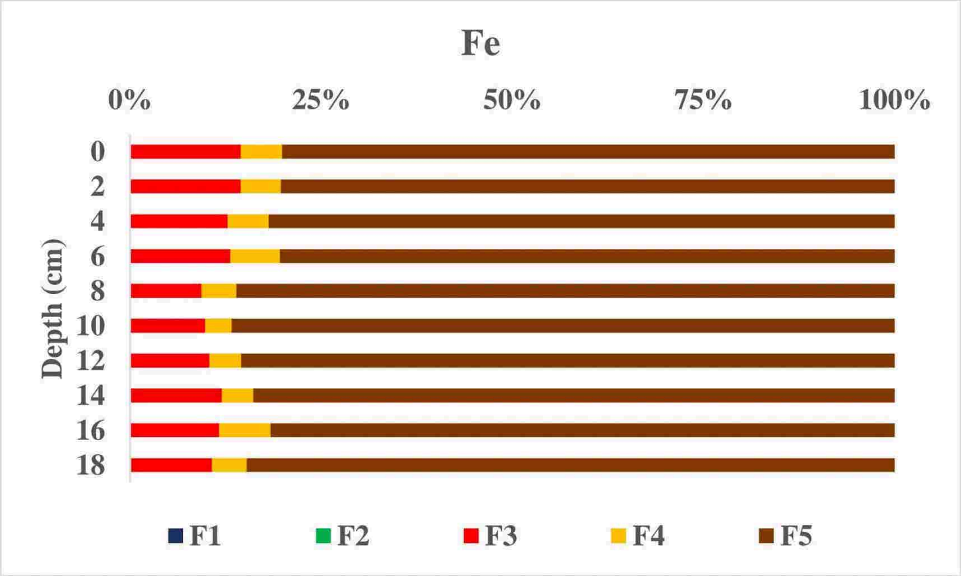


Fig 3.4.3a: The vertical profile of speciation of Fe in core S-3.

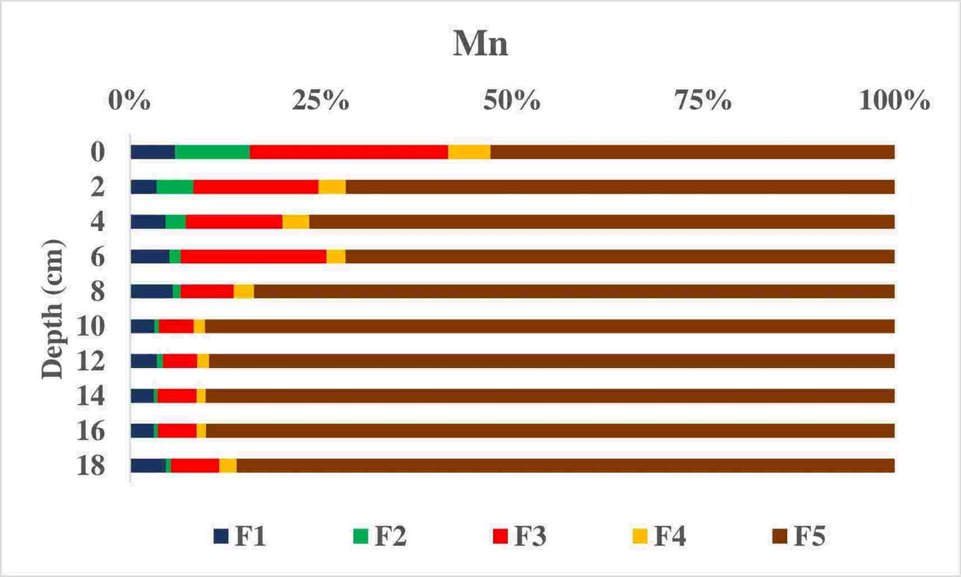


Fig 3.4.3b: The vertical profile of speciation of Mn in core S-3.

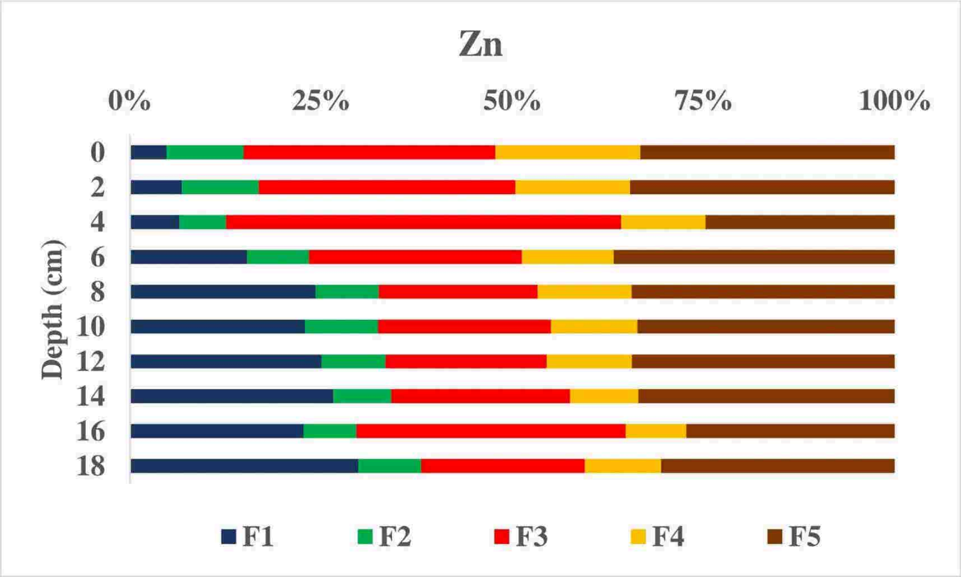


Fig 3.4.3c: The vertical profile of speciation of Zn in core S-3.

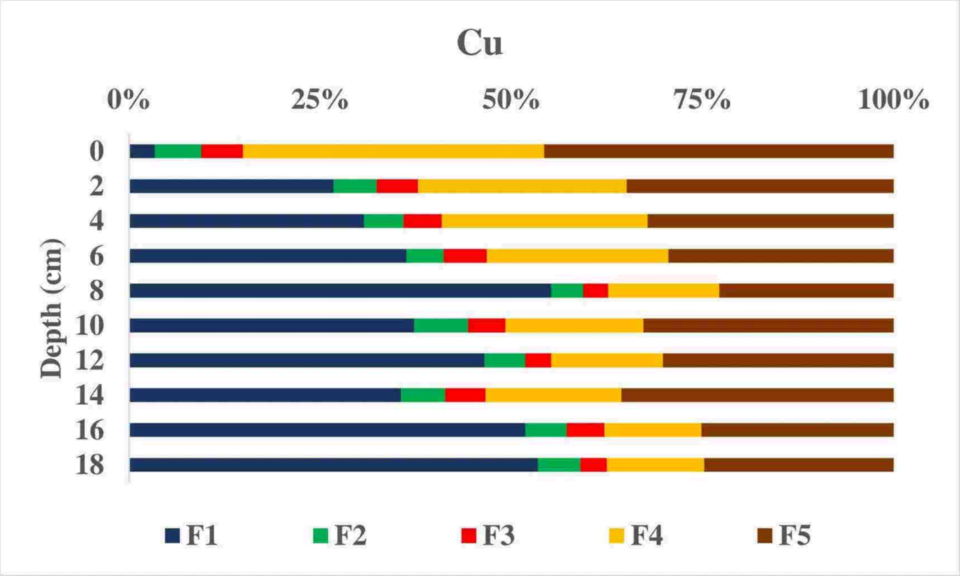


Fig 3.4.3d: The vertical profile of speciation of Cu in core S-3.

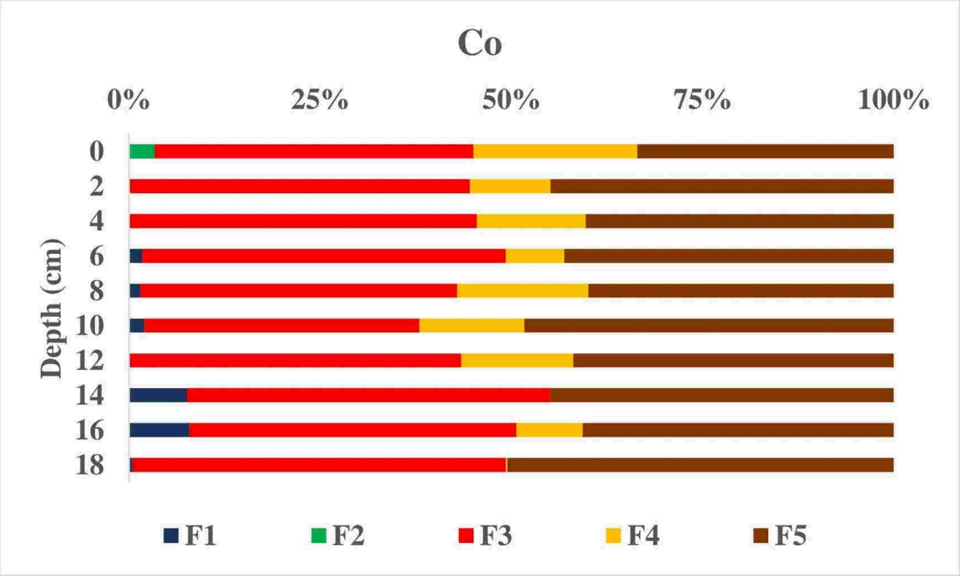


Fig 3.4.3e: The vertical profile of speciation of Co in core S-3.

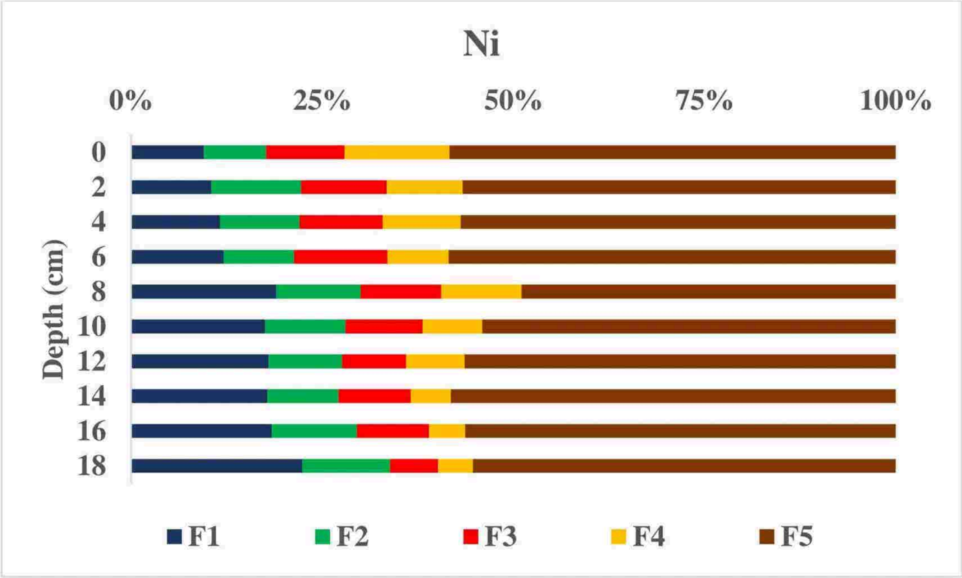


Fig 3.4.3f: The vertical profile of speciation of Ni in core S-3

In core S-3 (Fig 3.3.3), the Fe associated with the Fe-Mn oxide fraction showed an overall increase in the top 6 cm and thus, suggested its precipitation under oxic conditions. Similar results were also observed in case of Mn bound to the Fe-Mn oxide fraction. An increase in Mn bound to the carbonate fraction was evident in the surface sediments similar to organic matter/sulphide fraction. The Zn bound to the exchangeable fraction decreased in the top 4 cm indicating its mobilisation from sediment to water column. The mobilised Zn might have adsorbed onto other bioavailable fractions in the surface sediments viz., carbonate and Fe-Mn oxide fractions. A gradual increase in the concentration of Zn bound to the organic matter/sulphide fraction was attributed to microbial degradation of organic matter. Similar trend of Cu bound to exchangeable and organic matter/sulphide fraction was reported. Co was mainly associated with the Fe-Mn oxide fraction with slight fluctuations in the surface sediments. Its decrease in the organic matter/sulphide fraction with depth was a result of microbial action. The Ni displayed declined in its concentration in the exchangeable fraction, while an overall increase was evident in Fe-Mn oxide and organic matter/sulphide fractions. The physico-chemical properties of estuary might have regulated the adsorption-desorption of Ni in sediments. The differential environmental conditions and diagenetic processes over a period of time brings changes in physical and chemical properties in the sediments. Therefore, the fluctuations in metals in their bioavailable forms along the core depth can be attributed to physico-chemical propeties, besides other factors like sediment texture, organic matter and biota **(Siraswar et al. 2021)**.

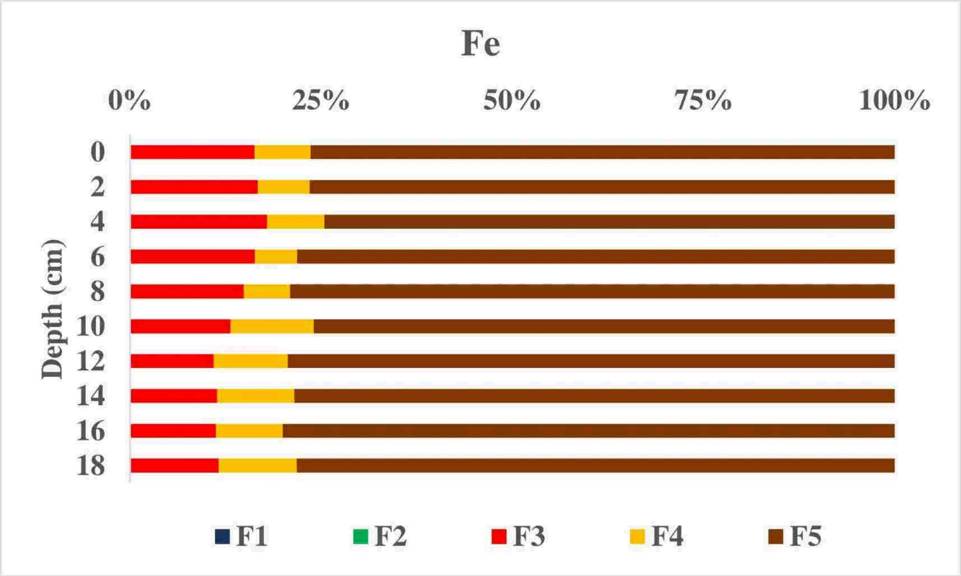


Fig 3.4.4a: The vertical profile of speciation of Fe in core S-4.

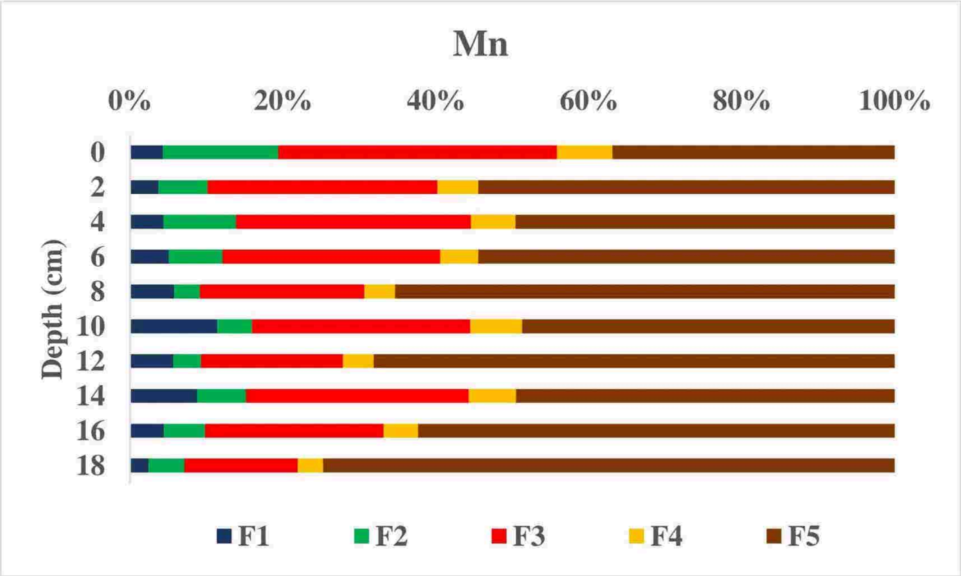


Fig 3.4.4b: The vertical profile of speciation of Mn in core S-4.

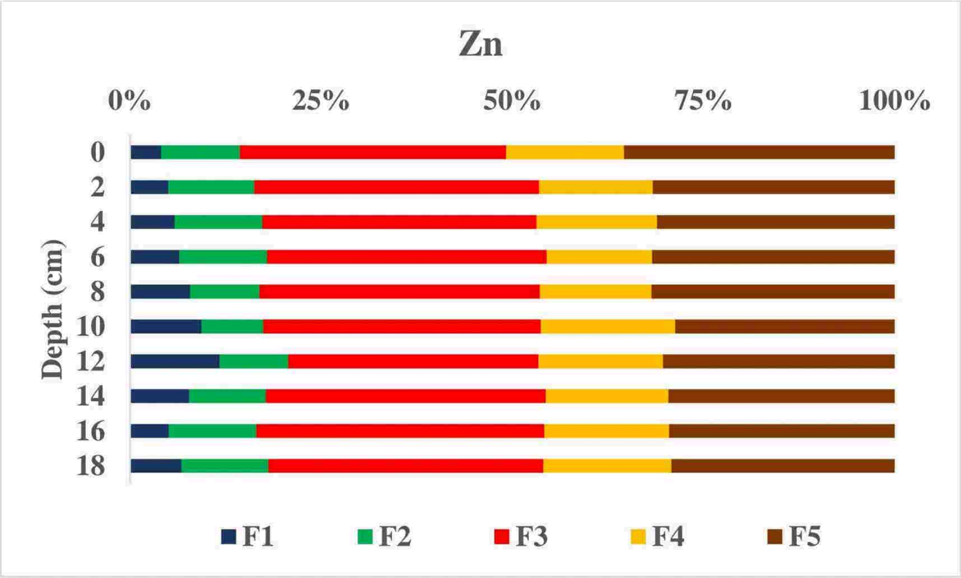


Fig 3.4.4c: The vertical profile of speciation of Zn in core S-4.

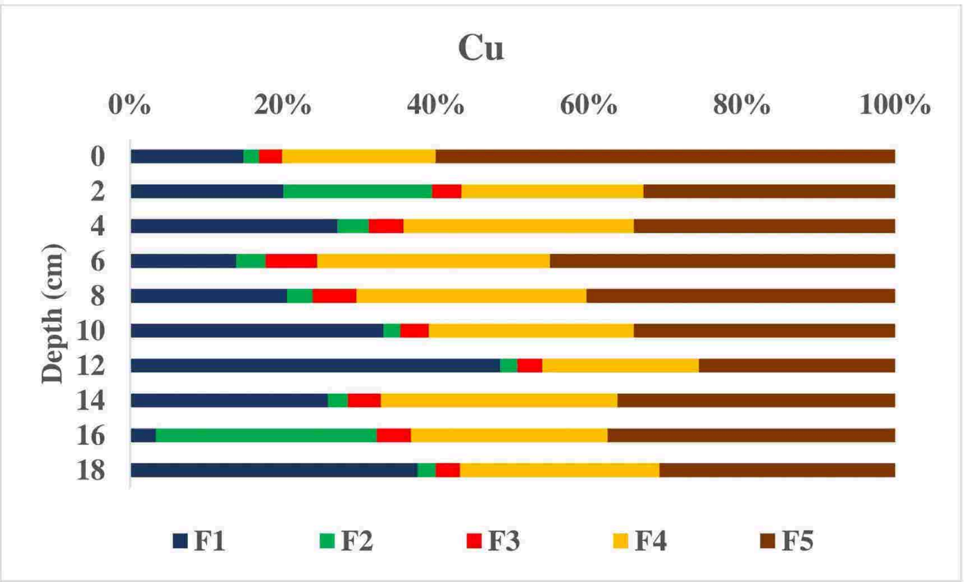


Fig 3.4.4d: The vertical profile of speciation of Cu in core S-4.

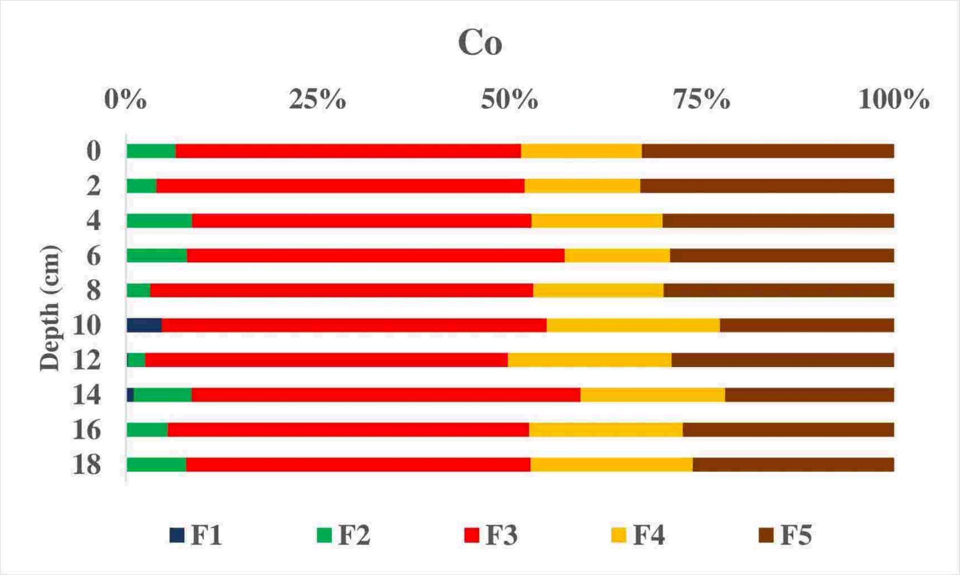


Fig 3.4.4e: The vertical profile of speciation of Co in core S-4.

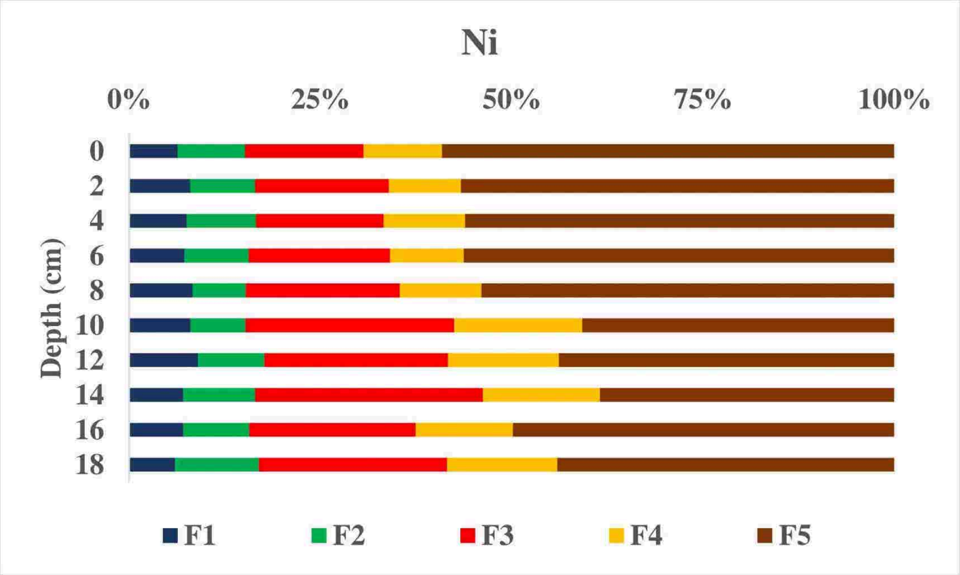


Fig 3.4.4f: The vertical profile of speciation of Ni in core S-4.

In core S-4 (3.3.4), an increase in the concentration of Fe bound to the Fe-Mn oxide fraction was observed from bottom to surface. It was attributed to precipitation of Fe as oxide and hydroxide in surface sediments under oxic conditions. On the contrary, a decrease in the concentration of Fe from bottom to surface of the core was observed. Therefore, a slight enrichment of Fe in the organic matter/sulphide fraction in the bottom half of the core, and a subsequent decline in the Fe-Mn oxide fraction reflected association of Fe with sulphide in the form of pyrite under anoxic condition **(Nasnodkar and Nayak 2019)**. Mn showed a prominent increase from bottom to surface in its level bound to the Fe-Mn oxide fraction which indicated its adsorption on surface sediments and bioavailability to the sediment associated biota. Similarly, an overall increase in Mn in the carbonate fraction near surface might be due to replacement of Ca^{2+} in the $CaCO_3$ and prevalence of favourable (alkaline) conditions. The replacement of Ca^{2+} by Mn^{2+} in $CaCO_3$ was attributed to the similar ionic radii between these metals **(Kantekar et al. 2022)**. The Zn bound to exchangeable and carbonate fractions showed an overall decrease and increase in concentration from 12 cm depth to surface. It might be regulated by ionic composition and pH conditions in the water column. No drastic variations in Zn bound to the bioavailable fractions was observed along the core length. The Cu associated with exchangeable, carbonate and organic matter/sulphide fractions displayed fluctuating trend along the core length. Thus, suggested fluctuations in the physico-chemical parameters with time in the estuary. Its significant proportion in the exchangeable fraction indicated strong bioavailability, while formation of organo-metallic complexes with reference to organic matter/sulphide bound fraction **(Dessai et al. 2023)**. Co indicated fluctuating trend in the carbonate fraction along the core length while, an overall decrease near the surface in the organic matter/sulphide fraction was observed. A slight fluctuating trend of Ni was observed in the labile fraction, whereas an overall decrease in the Fe-Mn oxide and organic matter/sulphide fractions was noted. It indicated mobilisation of metals from sediment to water column.

In general, the metals bound to exchangeable, carbonate, Fe-Mn oxide and organic/sulphide fractions can be desorbed from the sediments with changes in ionic composition, pH, Eh and microbial degradation of organic matter respectively. Thus, releasing metals in the interstitial pore waters or into ambient water column making it easily available for the uptake of sediment associated biota, which can be harmful to them and as well can have effect on the overall health of an estuarine ecosystem **(Tessier et al. 1979**; **Rodrigues et al. 2021)**.

3.3 Bioaccumulation of metals

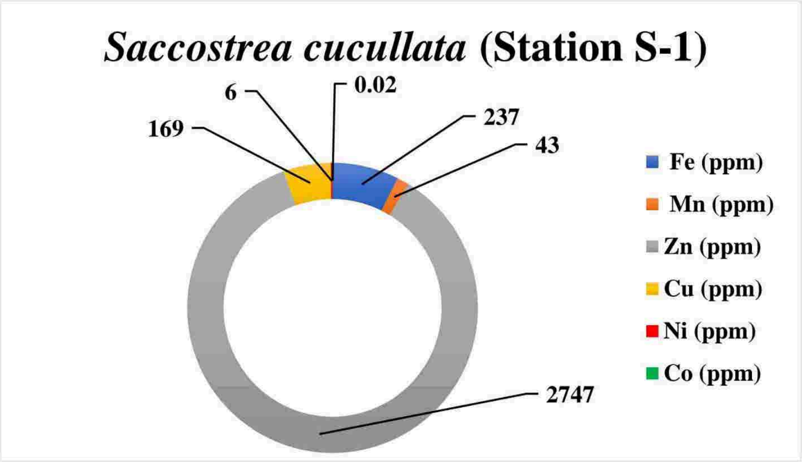


Fig 3.4.1a: The concentration of metals in soft tissue of bivalve *Saccostrea cucullata* at station S-1.

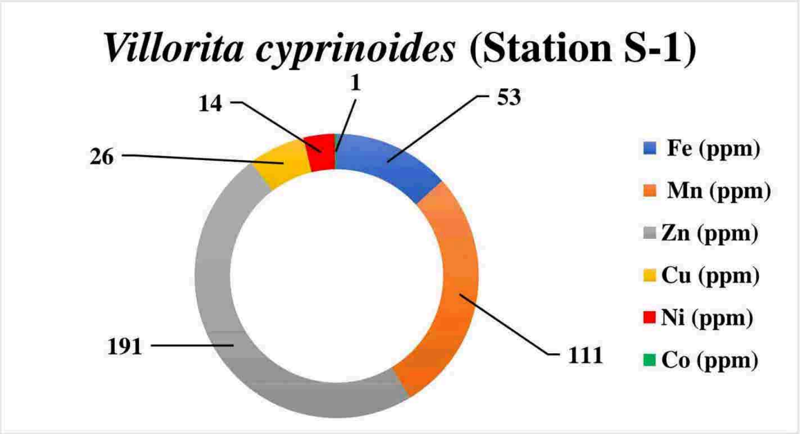


Fig 3.4.1b: The concentration of metals in soft tissue of bivalve *Villorita cyprinoides* at station S-1.

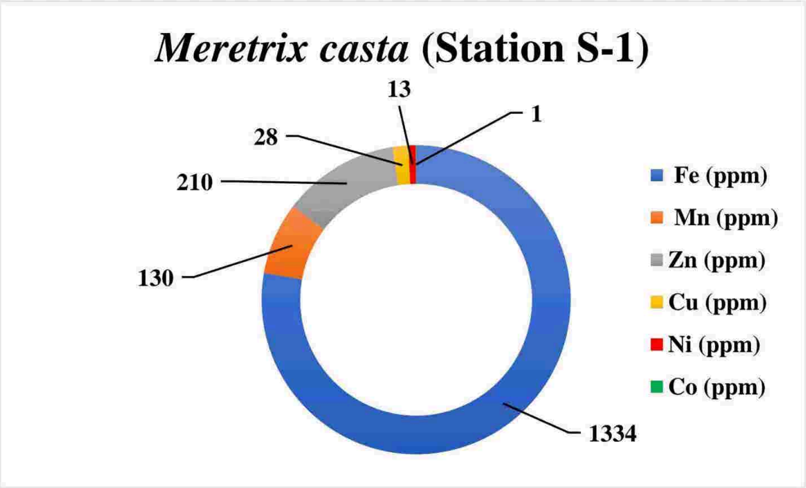


Fig 3.4.1c: The concentration of metals in soft tissue of bivalve *Meretrix casta* at station S-1.

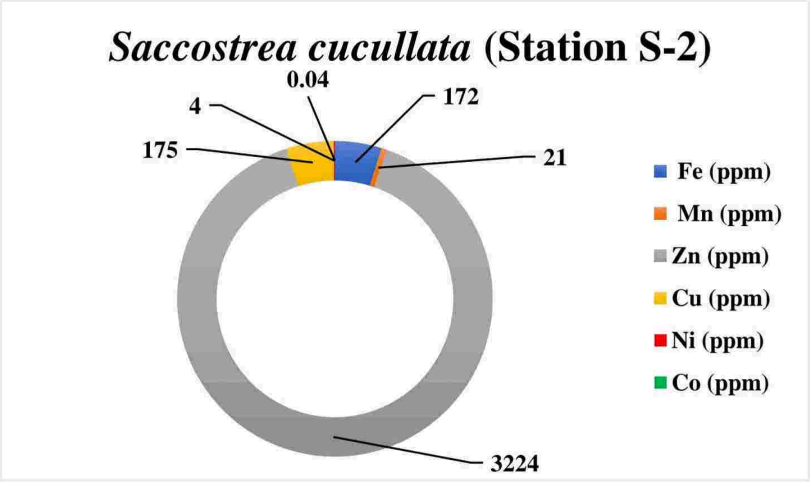


Fig 3.4.2a: The concentration of metals in soft tissue of bivalve *Saccostrea cucullata* at station S-2.

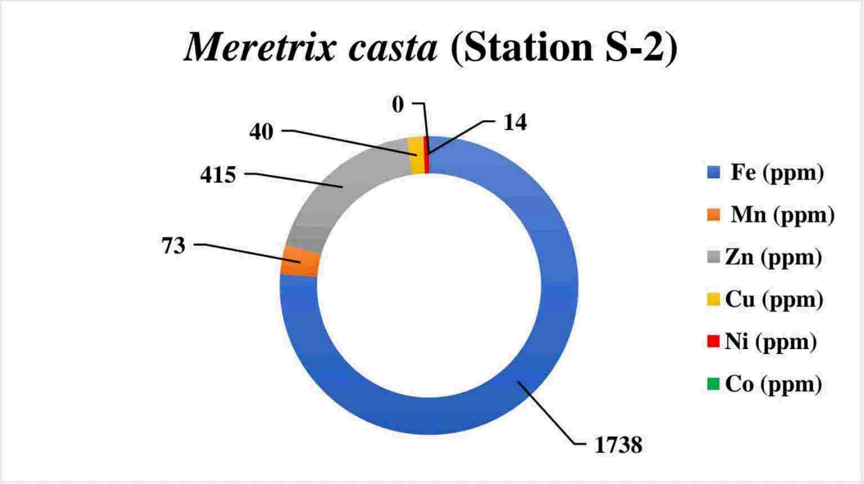


Fig 3.4.2b: The concentration of metals in soft tissue of bivalve *Meretrix casta* at station S-2.

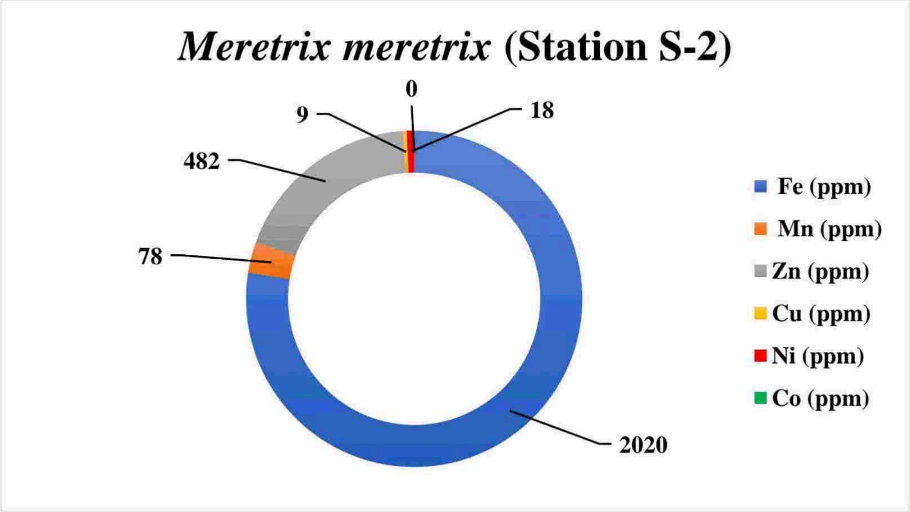


Fig 3.4.2c: The concentration of metals in soft tissue of bivalve *Meretrix meretrix* at station S-2.

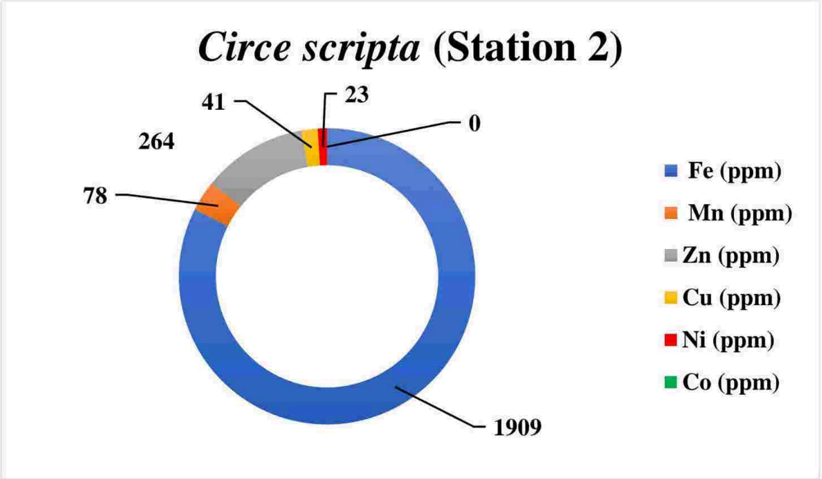


Fig 3.4.2d: The concentration of metals in soft tissue of bivalve *Circe scripta* at station S-2.

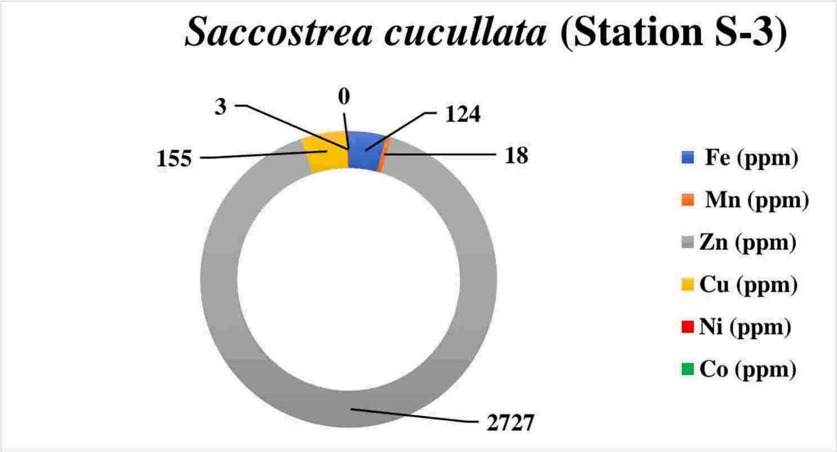


Fig 3.4.3a: The concentration of metals in soft tissue of bivalve *Saccostrea cucullata* at station S-3.

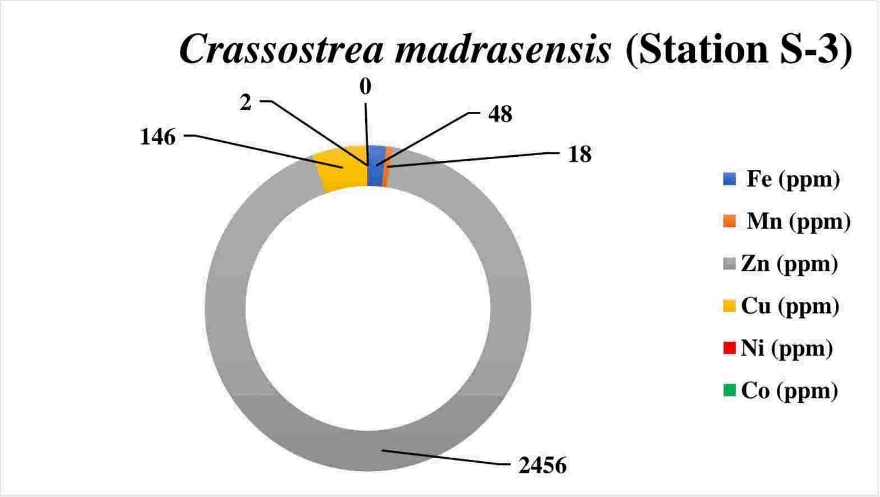


Fig 3.4.3b: The concentration of metals in soft tissue of bivalve *Crassostrea madrasensis* at station S-3.

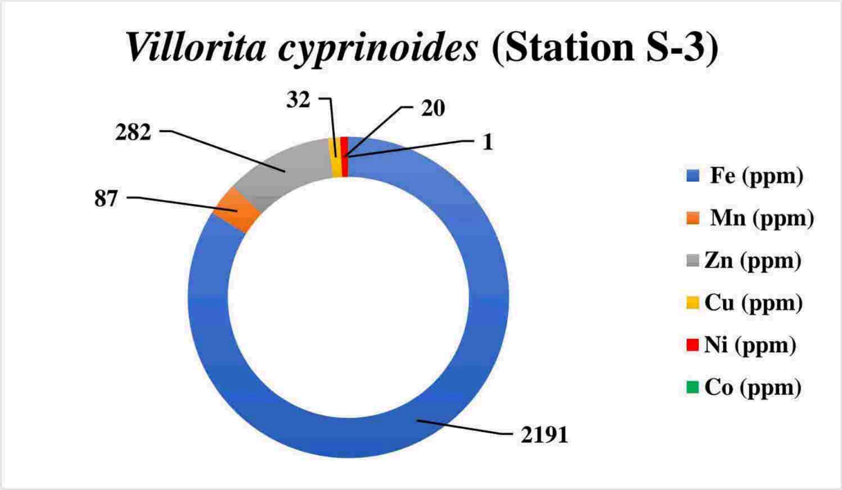


Fig 3.4.3c: The concentration of metals in soft tissue of bivalve *Villorita cyprinoides* at station S-3.

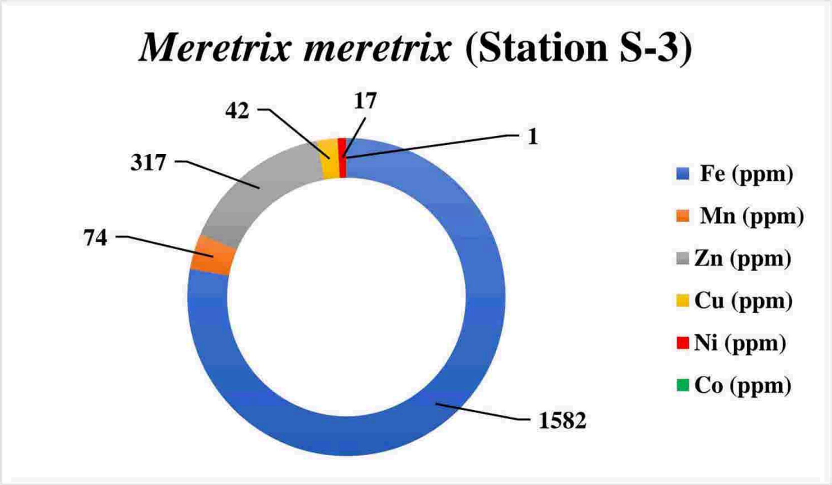


Fig 3.4.3d: The concentration of metals in soft tissue of bivalve *Meretrix meretrix* at station

S-3.

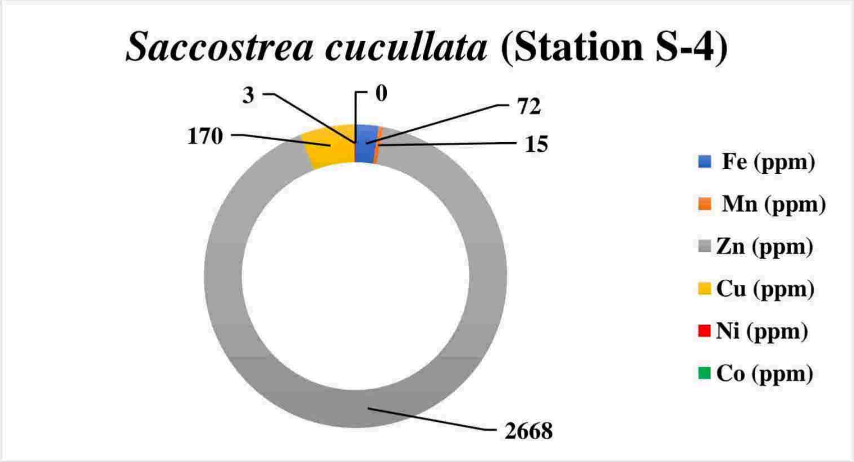


Fig 3.4.4a: The concentration of metals in soft tissue of bivalve *Saccostrea cucullata* at station S-4.

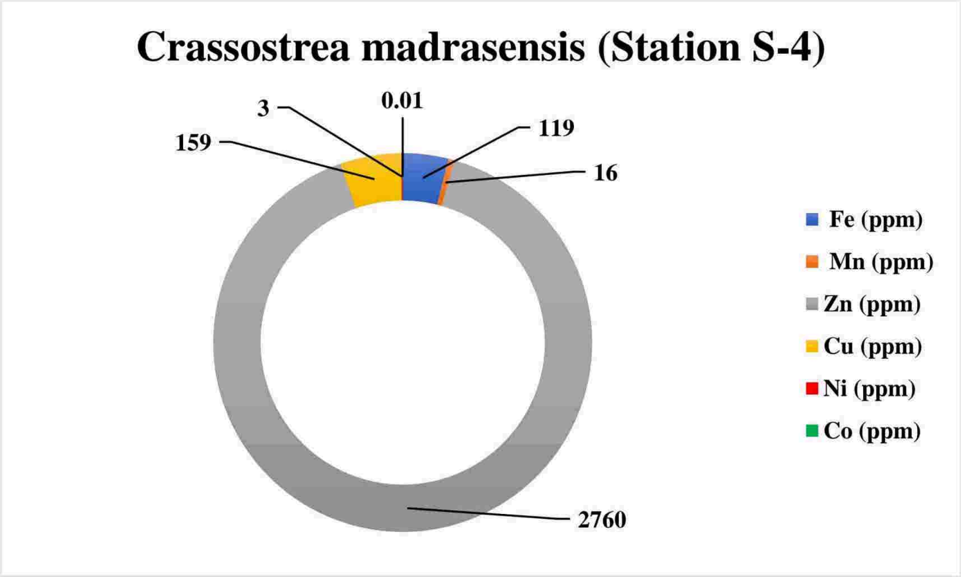


Fig 3.4.4b: The concentration of metals in soft tissue of bivalve *Crassostrea madrasensis* at station S-4

The concentration of Zn was highest in soft tissues of *Saccostrea cucullata* and *Villorita cyprinoides* at station S-1, whereas, Fe was highest in *Meretrix casta* (Fig 3.4.1). All species had lowest concentration of Co. The accumulation of metals was in the decreasing order of Zn > Fe > Cu > Mn > Ni > Co in *Saccostrea cucullata,* while *Villorita cyprinoides* displayed a decreasing order of Zn > Mn > Fe > Cu > Ni > Co. In *Meretrix casta,* the accumulation of metals was in the decreasing order of Fe > Zn > Mn > Cu > Ni > Co.

The concentration of Zn was highest in soft tissues of *Saccostrea cucullata*, while Fe was highest in *Meretrix casta, Meretrix meretrix* and *Circe scripta* (Fig 3.4.2). All species showed lowest concentration of Co. The accumulation of metals was in the decreasing order of Zn > Cu > Fe > Mn > Ni > Co in *Saccostrea cucullata*. The *Meretrix casta* and *Circe scripta* showed decreasing order of accumulation of Fe > Zn > Mn > Cu > Ni > Co. In case of *Meretrix meretrix*, the metals were in the decreasing order of Fe > Zn > Mn > Ni > Cu > Co.

The concentration of Zn was highest in soft tissues of *Saccostrea cucullata* and *Crassostrea madrasensis*, while Fe was highest in *Villorita cyprinoides* and *Meretrix meretrix* (Fig 3.4.3). Similar to stations S-1 and S-2, all species showed lowest concentration of Co at station S-3. The accumulation of metals was in the decreasing order of Zn > Cu > Fe > Mn > Ni > Co in *Saccostrea cucullata* and *Crassostrea madrasensis*. The *Villorita cyprinoides* and *Meretrix meretrix* showed decreasing order of accumulation of Fe > Zn > Mn > Cu > Ni > Co.

The soft tissues of *Saccostrea cucullata* and *Crassostrea madrasensis* showed highest and lowest concentration of Zn and Co respectively (Fig 3.4.4). Both species displayed similar decreasing order of metals as Zn > Cu > Fe > Mn > Ni > Co.

In the Sal Estuary, the studied bivalve species exhibited lowest concentration of Co in their soft tissues. On the other hand, Fe and Zn were preferred by bivalves to a greater extent. The study carried out by many researchers namely, **Rodrigues et al. (2021)**, **Abhilash et al. (2013)**, **Yuzereroglu et al. (2010)** had reported Zn and Fe as highly preferred metals by the biota in the marine environment. The metals viz., Fe and Zn are considered as essential metals to marine biota that have importance in terms of growth and biological functions **(Jakimsha et al. 2011; Abhilash et al. 2013)**. The *Saccostrea cucullata* retrieved from stations S-1, S-2, S-3 and S-4 had highest accumulation of Zn, while *Meretrix casta* at stations S-1 and S-2 showed highest preference for Fe. Even, *Meretrix meretrix* displayed high affinity towards Fe at stations S-2 and S-3. The *Crassostrea madrasensis* showed high affinity towards Zn at stations S-3 and S-4. However, *Villorita cyprinoides* had high affinity towards Zn at station S-1 and disclosed high preference for Fe at station S-3. In the current study, oysters (*Saccostrea cucullata* and *Crassostrea madrasensis)* recorded highest concentration of Zn which suggested their very high ability to accumulate Zn and, might be due to several zinc metalloenzymes which are involved in their shell mineralization **(Abhilash et al. 2013)**. Overall, the different species of bivalves revealed their affinity towards specific metal in the Sal Estuary. The accumulation of metals in the soft tissues of bivalve is governed by various intrinsic (genotype, phenotype, species, age, size, sex, etc.) and extrinsic (bioavailability of metals regulated by salinity, pH, Eh, ionic composition, sulphides, clay minerals, microbial activity, etc.) factors **(Rodrigues et al. 2021; Rajeshkumar and Xiaoyu 2018)**. Such factors might have regulated the discrepancies in the concentration of metals in bivalves **(Khaled 2004)**.

3.4 Metal Pollution Indices

3.4.1 Geo-accumulation Index (Igeo)

Table 3.4: Geo-accumulation Index (Igeo)

Sediment Cores	Igeo					
	Fe	**Mn**	**Zn**	**Cu**	**Co**	**Ni**
S-1	-0.20	-1.79	0.63	0.87	0.07	1.18
S-2	-0.61	-2.44	0.44	0.60	0.20	1.39
S-3	-1.73	-2.31	-0.49	-0.29	-2.86	-1.20
S-4	-0.46	-1.83	0.64	0.66	-0.39	0.97

The Igeo values for Fe (S-1, S-2, S-3 and S-4), Mn (S-1, S-2, S-3 and S-4), Zn (S-3), Cu (S-3), Co (S-3 and S-4) and Ni (S-3) were < 0, indicating no pollution from these metals at sampling stations (Table 3.4). The Igeo values for Zn (S-1, S-2 and S-4), Cu (S-1, S-2 and S-4), Co (S-1 and S-2) and Ni (S-4) were between 0 to 1. Thus, these metals fell under the category of unpolluted to moderately polluted class. Further, Igeo value for Ni ranged from 1 to 2 at stations S-1 and S-2. It revealed moderate level of pollution of Ni in sediments of the Sal Estuary at stations S-1 and S-2.

3.4.2 Contamination Factor (CF)

Table 3.5: Contamination Factor (CF) of metals in sediments.

Station	CF					
	Fe	**Mn**	**Zn**	**Cu**	**Co**	**Ni**
S-1	1.31	0.43	2.33	2.74	1.57	3.39
S-2	0.98	0.28	2.04	2.27	1.73	3.93
S-3	0.45	0.30	1.07	1.22	0.21	0.65
S-4	1.09	0.42	2.33	2.38	1.14	2.93

The concentration of Fe in sediments of the Sal Estuary indicated low to moderate level of contamination, while Mn showed low contamination (Table 3.5). The metals viz., Zn and Cu exhibited moderate contamination in the intertidal sediments. The level of Co in sediments ranged from low to moderate degree of contamination. The Ni showed low and moderate degree of contamination at stations S-3 and S-4 respectively, while was deposited at considerable level of contamination at stations S-1 and S-2.

The determination of Igeo and CF in the sediments of the Sal Estuary revealed pollution and contamination of Ni compared to other metals. The Ni was significantly associated with the bioavailable fractions (> 40 %) in the sediments of Sal Estuary, which indicated its enrichment due to anthropogenic sources. The major sources of Ni in the Sal Estuary might be from scrapping and repairing shipyards, electronic waste disposal, fertilizers containing Ni used in aquaculture and agriculture.

3.5 Metal toxicity assessment

3.5.1 Screening Quick Reference Table (SQUIRT)

Table 3.6: Screening Quick Reference Table (SQUIRT) for metals in sediments and bioavailable metal concentration.

Metals	**Threshold Effect Level (TEL)**	**Effect Range Low (ERL)**	**Probable Effect Low (PEL)**	**Effect Range Median (ERM)**	**Apparent Effect Threshold (AET)**	**Bioavailable concentration (F1+F2+F3+F4)**			
						S-1	**S-2**	**S-3**	**S-4**
Fe (%)	-	-	-	-	22 (Neanthes)	0.92	0.69	0.22	0.62
Mn (ppm)	-	-	-	-	260 (Neanthes)	107	51	25	83
Zn (ppm)	124	150	271	410	410 (Infaunal community impacts)	80	73	31	58
Cu (ppm)	18.7	34	108	270	390 (Microtox & Oyster larvae)	21	18	14	24
Co (ppm)	-	-	-	-	10 (Neanthes)	14	18	2	10
Ni (ppm)	15.9	20.9	42.8	51.6	110 (Echinoderm Larvae)	21	32	8	26

The SQUIRT was used to understand the toxicity of bioavailable form of metals to edible bivalves (Table 3.6). The bioavailable concentration of metals viz., Fe, Mn, Zn, Cu and Ni was lower than the AET at stations S-1, S-2, S-3 and S-4. Also, Co was lower than the AET at Station S-3. It revealed no toxicity of these metals to edible bivalves. On the contrary, the concentration of bioavailable Co at stations S-1 and S-2 exceeded the AET value, and levelled AET value at station S-4. Thus, suggested toxicity of Co to estuarine bivalves. The metal speciation study had revealed bioavailability of Co, and the CF had suggested contamination and anthropogenic sources of Co in the Sal Estuary. Though, Co is required in small concentration to the marine biota, the high bioavailability and subsequent accumulation of Co above the threshold limit can influence the growth rate and the Ca-uptake in marine organisms **(Nasnodkar and Nayak 2019)**.

3.5.2 Risk Assessment Code (RAC)

Table 3.7: Risk Assessment Code (RAC) for metals in sediments.

Metals	**RAC % (labile fraction: F1+ F2)**			
	S-1	**S-2**	**S-3**	**S-4**
Fe	0.30	0.08	0.12	0.10
Mn	23.62	17.27	6.51	12.19
Zn	19.09	22.72	26.79	17.32
Cu	18.04	25.83	43.20	31.56
Co	16.24	15.07	2.46	5.90
Ni	18.67	19.27	25.94	16.11

The RAC revealed no potential adverse effects of Fe on the estuarine biota with values < 1% (Table 3.7). The Mn indicated medium potential adverse effect at stations S-1, S-2 and S-4, while showed low potential adverse effects at S-3. The metals viz., Zn and Ni displayed medium potential adverse effects on estuarine biota at all stations. The risk of Cu to estuarine biota was medium level at stations S-1 and S-2, while it showed high potential adverse effect at stations S-3 and S-4. The RAC indicated low and medium level of risk from Co at stations S-3 and S-4, and S-2 and S-3, respectively. The analysis of RAC indicated high risk of Cu to the sediment-dwelling biota at station S-3 and S-4. The metal is loosely bound to the labile fraction, and can easily dissociate from the estuarine sediments with changes in ionic composition and pH of water **(Nasnodkar et al. 2021)**. Thus, Mn (S-1, S-2 and S-4), Zn (S-1, S-2, S-3 and S-4), Cu (S-1, S-2, S-3 and S-4), Co (S-1 and S-2) and Ni (S-1, S-2, S-3 and S-4) indicated the possible risk of toxicity to edible bivalves in the Sal Estuary.

3.5.3 Implications of metal toxicity to humans

Table 3.8: The concentration of metal in soft tissues of edible bivalves. ND = not detected.

Stations	Biota name	Fe (ppm)	Mn (ppm)	Zn (ppm)	Cu (ppm)	Co (ppm)	Ni (ppm)
S-1	*Saccostrea cucullata*	237	43	2747	169	0.02	6
	Villorita cyprinoides	53	111	191	26	1	14
	Meretrix casta	1334	130	210	28	1	13
S-2	*Saccostrea cucullata*	172	21	3224	175	0.04	4
	Meretrix casta	1738	73	415	40	ND	14
	Meretrix meretrix	2020	78	482	9	ND	18
	Circe scripta	1909	78	264	41	ND	23
S-3	*Saccostrea cucullata*	124	18	2727	155	ND	3
	Crassostrea madrasensis	48	18	2456	146	ND	2
	Villorita cyprinoides	2191	87	282	32	1	20
	Meretrix meretrix	1582	74	317	42	1	17
S-4	*Saccostrea cucullata*	72	15	2668	170	ND	3
	Crassostrea madrasensis	119	16	2760	159	0.01	3
Standard Permissible limits		34 – 107 **Charbonneau & Nash (1993)**	0.01 **WHO (1989)**	40 **WHO (1989)**	30 **FAO/ WHO (2004)**	0.1 - 5 **FAO/ WHO (2004)**	70 – 80 **USFDA (1993)**

The concentration of metals in soft tissues of edible bivalves was compared with the standard permissible limits in order to understand implications of metal toxicity to the consumers (humans) of bivalves (Table 3.8). At station S-1, the concentration of Fe, Mn, Zn and Cu exceeded the standard permissible limit in *Saccostrea cucullata*. Also, the concentration of metals viz., Fe, Mn and Zn was more than the consumption safety limit in *Villorita cyprinoides and Meretrix casta.* The *Saccostrea cucullata, Meretrix casta, Meretrix meretrix* and *Circe scripta* accumulated metals (Fe, Mn and Zn) at higher level than the permissible limit at station S-2. Also, the level of Cu was above the permissible limit in *Saccostrea cucullata*, *Meretrix casta* and *Circe scripta*. The concentration of Fe, Mn, Zn and Cu exceeded the permissible limit in *Saccostrea cucullata*, *Villorita cyprinoides* and *Meretrix meretrix* at station S-3, while in *Crassostrea madrasensis* Mn, Zn, Cu exceeded the consumption safety limit. At station S-4, the concentration of Mn, Zn and Cu was above the permissible limit in *Saccostrea cucullata*, while metals viz., Fe, Mn, Zn and Cu exceeded the consumption safety limit in *Crassostrea madrasensis*.

The Fe, Mn, Zn and Cu are essential metals required for well-functioning of human body. However, the concentration of these metals above the standard permissible limit in the tissues of edible bivalves can cause potential risk to humans. The high accumulation of Mn in human body can cause damage to the nervous system. An overdose of Zn can cause fatigue/weakness, nausea, anaemia and also may affect immune system, while Cu can cause brain, kidney and liver failures or damage, demolition of blood cells, etc., **(Gavhane et al. 2021)**. Therefore, the consumption of edible bivalves might impart toxicity of Fe, Mn, Zn and Cu to humans.

3.5.3 modified Biota Sediment Accumulation Factor (mBSAF)

Table 3.9: modified Biota Sediment Accumulation Factor (mBSAF).

Sediment core	Biota name	mBSAF					
		Fe	Mn	Zn	Cu	Co	Ni
S-1	*Saccostrea cucullata*	0.03	0.40	34.26	8.15	0.00	0.29
	Villorita cyprinoides	0.01	1.03	2.38	1.23	0.06	0.68
	Meretrix casta	0.14	1.21	2.62	1.34	0.05	0.64
S-2	*Saccostrea cucullata*	0.03	0.42	44.11	9.55	0.00	0.14
	Meretrix casta	0.25	1.44	5.68	2.19	0.00	0.43
	Meretrix meretrix	0.29	1.53	6.59	0.49	0.00	0.58
	Circe scripta	0.28	1.53	3.61	2.21	0.00	0.73
S-3	*Saccostrea cucullata*	0.06	0.72	88.00	10.93	0.00	0.32
	Crassostrea madrasensis	0.02	0.70	79.27	10.30	0.00	0.26
	Villorita cyprinoides	0.99	3.46	9.10	2.24	0.44	2.51
	Meretrix meretrix	0.71	2.92	10.22	2.97	0.23	2.15
S-4	*Saccostrea cucullata*	0.01	0.18	45.62	7.01	0.00	0.12
	Crassostrea madrasensis	0.02	0.20	47.19	6.55	0.00	0.13

According to mBSAF, all edible bivalve species retrieved from the Sal Estuary were de-concentrator of Fe and Co, while they were macro-concentrator of Zn (Table 3.9). At station S-1, *Saccostrea cucullata* was de-concentrator of Mn, whereas *Villorita cyprinoides and Meretrix casta* were micro-concentrator of Mn. Also, *Meretrix casta, Meretrix meretrix* and *Circe scripta* were micro-concentrator of Mn at station S-2. *Saccostrea cucullata* and *Crassostrea madrasensis* were de-concentrator of Mn at stations S-3 and S-4. *Villorita cyprinoides and Meretrix meretrix* were macro-concentrator of Mn at station S-3. The *Saccostrea cucullata* at station S-1, *Saccostrea cucullata*, *Meretrix casta* and *Circe scripta* at station S-2, *Saccostrea cucullata*, *Crassostrea madrasensis*, *Villorita cyprinoides* and *Meretrix meretrix* at station S-3, and *Saccostrea cucullata* and *Crassostrea madrasensis* at S-4 were macro-concentrator of

Cu. The *Villorita cyprinoides* and *Meretrix casta* were micro-concentrator of Cu at station S-1, while *Meretrix meretrix* was de-concentrator of Cu at S-2. The *Villorita cyprinoides* and *Meretrix meretrix* were macro-concentrator of Ni at station S-3, while rest of the species retrieved from different sampling stations of the Sal Estuary were de-concentrator of Ni. Considering the results of mBSAF the edible bivalves had high affinity towards Zn followed by Cu and to a certain extent for Mn and Ni.

3.6 Phyto-remediation

3.6.1 Accumulation of metals in mangroves

Table 3.10: The concentration of metals in different parts viz., pneumatophores, stem and leaves of mangroves. ND = not detected.

Station	**Species**	**Mangrove plant parts**	**Metal**					
			Fe (ppm)	**Mn (ppm)**	**Zn (ppm)**	**Cu (ppm)**	**Co (ppm)**	**Ni (ppm)**
S–1	*Sonneratia alba*	Pneumatophores	1043	5	10	22	ND	0.3
		Stem	139	24	19	22	ND	1
		Leaves	75	11	27	26	ND	2
	Avicennia alba	Pneumatophores	4453	26	12	19	ND	2
		Stem	107	8	15	18	ND	0.3
		Leaves	136	43	26	16	ND	1
S-2	*Sonneratia alba*	Pneumatophores	1351	12	12	20	ND	1
		Stem	131	6	9	18	ND	0
		Leaves	138	9	13	14	ND	0.1
	Avicennia marina	Pneumatophores	1351	12	10	12	ND	1
		Stem	74	12	8	7	ND	0.1
		Leaves	99	75	24	10	ND	0.4
S-3	*Avicennia marina*	Pneumatophores	965	31	18	31	ND	1
		Stem	69	11	20	16	ND	0.2
		Leaves	108	16	20	10	ND	0.1
S-4	*Avicennia marina*	Pneumatophores	933	13	19	20	0.017	11
		Stem	144	9	12	18	ND	ND
		Leaves	209	14	15	30	0.001	1

The concentration of metals in the different parts of mangrove plants are presented in Table 3.10. Among the metals, it was Fe which was highly accumulated in the mangrove pneumatophores, stem and leaves compared to other metals. The similar results were reported by **Hossain et al. (2022)** and **Chowdhury et al. (2016)**, and it was attributed to the physiological requirement of the plant and bioavailability of metal in the sediments. The plant utilizes Fe for various cellular processes such as respiration, chlorophyll biosynthesis, and photosynthesis, and serves as a cofactor for enzymes involved in electron or oxygen transfer **(Kobayashi et al. 2018)**. In the present study, the higher concentration of Fe observed in roots than aerial organs might be because of Fe-plaque formation as an adaptive response to metal stress conditions and restricted mobility of the metal at the root level **(Pi et al. 2011)**. The Co displayed lowest accumulation in different parts of the mangrove plants. In most of the mangrove species Mn showed highest concentration in leaves than roots which can be attributed to high translocation ability of the metal and physiological requirements in aerial organs **(Thanh-Nho et al. 2019)**. The variations in the concentration of metals between different species parts, and also in similar species parts collected from different sampling stations of the Sal Estuary was evident. The accumulation of metals in mangrove plants depends upon factors viz., sources of metals and their bioavailability at a given sampling site, excluder mechanism, affinity of mangrove plant to specific metal (species-specific metabolic requirement) which is in turn regulated by size, age, diversity in the given area, etc., metal tolerance capability **(Dessai et al. 2023; Nath et al. 2014)**

3.6.2 Translocation Factor (TF)

Table 3.11: The Translocation Factor (TF) for metals in mangrove plants. ND = not detected.

Stations	**Species**	**Ratio**	**TF**					
			Fe	**Mn**	**Zn**	**Cu**	**Co**	**Ni**
S – 1	*Sonneratia alba*	Leaves/root	0.07	2.02	2.66	1.14	ND	8.24
	Avicennia alba	Leaves/root	0.03	1.66	2.11	0.86	ND	0.57
S-2	*Sonneratia alba*	Leaves/root	0.10	0.73	1.09	0.71	ND	0.06
	Avicennia marina	Leaves/root	0.07	6.42	2.38	0.87	ND	0.34
S-3	*Avicennia marina*	Leaves/root	0.11	0.53	1.11	0.32	ND	0.07
S-4	*Avicennia marina*	Leaves/root	0.22	1.10	0.79	1.50	0.06	0.06

The TF values for metals are presented in the Table 3.11. According to TF, *Sonneratia alba* was accumulator of Mn, Zn, Cu and Ni at station S-1 and also accumulator of Zn at station S-2. *Avicennia alba* was accumulator of Mn and Zn at station S-1. The *Avicennia marina* revealed ability to accumulate Mn and Zn at station S-2, and to accumulate Zn and Cu at stations S-3 and S-4 respectively. Thus, the present study construed the application of mangrove species viz., *Sonneratia alba*, *Avicennia alba* and *Avicennia marina* in the phyto-remediation of metals namely, Mn, Zn, Cu and Ni in the Sal Estuary.

CHAPTER 4

SUMMARY and CONCLUSIONS

The present study was conducted in the Sal Estuary, Goa with objectives to determine metal bioavailability in mangrove sediments, bioaccumulation in edible bivalves, and to understand the phyto-remediation potential of mangrove plants in the middle region of the Sal Estuary. Four mangrove sediment cores namely, S-1, S-2, S-3 and S-4 of 20 cm length were retrieved from the Sal Estuary. Also, edible bivalves and mangrove plant samples were collected in the proximity to sampling sites. The percentage of sand was > 60 % in the middle region of the Sal Estuary which was attributed to estuarine hydrodynamics. The grain size regulated the distribution of TOC in sediments. The average concentration of total metals viz., Fe (S-1), Zn (all stations), Cu (all stations), Co (S-1, S-2 and S-4) and Ni (S-1, S-2 and S-4) exceeded the upper crustal average value. It indicated their enrichment in sediments due to anthropogenic input from scrapping and repairing shipyard, electronic waste disposal, domestic sewage waste discharge, agricultural and aquacultural activities. The bioavailable metals were significantly associated with Fe-Mn oxide and organic matter/sulphide fractions. Thus, suggested their bioavailability with changes in Eh and microbial degradation of organic matter. The metals viz., Mn (S-1, S-2 and S-3), Zn (all stations), Cu (all stations), Co (S-1 and S-2), Ni (all stations) were present in > 10 % in the labile fraction in sediments that revealed high potential risk to the sediment associated biota with changes in ionic composition and pH. The edible bivalves from the Sal Estuary showed high affinity towards Fe and Zn, and least towards Co. It was attributed to essential role of Fe and Zn in growth and metabolic activity. The Igeo and CF revealed moderate level of pollution and considerable contamination of Ni in sediments. Thus, construed its anthropogenic sources in the estuary. Similarly, Fe, Zn, Cu and Co showed moderate level of contamination at one or more stations. The SQUIRT suggested toxicity of Co to estuarine biota. The analysis of RAC indicated high risk of Cu to the sediment dwelling biota at station S-3 and S-4. The mBSAF indicated high affinity of edible bivalves towards Zn and Cu. All bivalves were found as macro-concentrator of Zn. Further, the accumulation of metals in mangrove plants revealed high concentration of Fe in pneumatophores, stem and leaves which was attributed to its essentiality in biological functions. The TF construed the application of *Sonneratia alba*, *Avicennia alba* and *Avicennia marina* in the phyto-remediation of Mn, Zn, Cu and Ni in the Sal Estuary.

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