Metal bioavailability in sediments, bioaccumulation in edible bivalves and phyto-remediation potential of mangrove plants in lower region of the Sal Estuary, Goa

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DECLARATION BY STUDENT

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

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COMPLETION CERTIFICATE

This is to certify that the dissertation report "Metal bioavailability in sediments, bioaccumulation in edible bivalves and phyto-remediation potential of mangrove plants in lower region of the Sal Estuary, Goa" is a bonafide work carried out by Mr. Rahul Ramchandra Gaonkar 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 estuaries are invaluable ecosystems along the coast where freshwater from rivers mixes with the sea water **(Silva et al. 2011).** The most widely quoted definition of an estuary was given by **Pritchard (1967):** "An estuary is a semi-enclosed coastal body of water which has a free connection with the open sea and within which sea water is measurably diluted with fresh water derived from land drainage." Certainly, one of the characteristic attributes of most coastal areas is the action of the tide. Pritchard's definition makes no specific mention of tide, although the mixing of seawater and fresh water implies this. There are, however, many non-tidal or minimally tidal seas, such as the Mediterranean Sea and the Black Sea, where fresh and salt water mix. So Pritchard's definition of estuary excludes some coastal areas where estuarine ecology is studied today. In an attempt to address the limitations of Pritchard's definition, **Fairbridge (1980)** gave a more comprehensive definition of an estuary: An estuary is an inlet of the sea reaching into a river valley as far as the upper limit of tidal rise, usually being divisible into three sectors: (i) a marine or lower estuary, in free connection with the open sea; (ii) a middle estuary subject to strong saline and fresh water mixing; And (iii) an upper or fluvial estuary, characterized by fresh water but subject to daily tidal action. The limits between these sectors are variable and subject to constant changes in the river discharge.

The estuaries may be classified according to their geomorphology as coastal plain, fjord, bar-built and tectonic. (a) Coastal plain estuaries, also called drowned river valleys, are those that were formed as a result of the pleistocene increase in sea level, starting ~15,000 years ago. Originally rivers, these estuaries formed during flooding over several millennia by rising sea levels. Their shape resembles that of present-day rivers, although much wider. They are typically wide (on the order of several kilometers) and shallow (on the order of 10 m), with large width/depth aspect ratios. (b) Fjords are associated with high latitudes where glacial activity is intense. They are characterized by an elongated, deep channel with a sill. The sill is related to a moraine of either a currently active glacier or an extinct glacier. In the sense of the glacier activity, it could be said that there are riverine and glacial fjords. The riverine fjords are related to extinct glaciers and their main source of buoyancy comes from river inputs. They are usually found equatorward of glacial fjords. The glacial fjords are found in high latitudes, poleward of riverine fjords. They are related to active glaciers and their main source of buoyancy is derived from melting of the glacier and of snow and ice in mountains nearby. The fjords are deep (several hundreds of meters) and narrow (several hundreds of meters) and have low width/depth aspect ratios with steep side walls. (c) The bar-built estuaries, originally embayments, became semi-enclosed because of littoral drift causing the formation of a sand bar or spit between the coast and the ocean/sea. Some of the~~se~~ bars are joined to one of the headlands of a former embayment and display one small inlet (on the order of a few hundred meters) where the estuary communicates with the ocean/sea. Some other sand bars may be detached from the coast and represent islands that result in two or more inlets that allow communication between the estuary and the ocean/sea. In some additional cases, sand bars were formed by rising sea level. (d) The tectonic estuaries were formed by earthquakes or by fractures of the earth's crust, and creases that generated faults in regions adjacent to the ocean. Faults cause part of the crust to sink, forming a hollow basin. An estuary is formed when the basin is filled by the ocean.

According to water column stratification or salinity vertical structure, the estuaries can be classified as salt wedge, strongly stratified, weakly stratified or vertically mixed. This classification considers the competition between buoyancy forcing from river discharge and mixing from tidal forcing. Large river discharge and weak tidal forcing results in salt wedge estuaries. Such systems are strongly stratified during flood tides, when the ocean/sea water intrudes in a wedge shape. They may lose the~~ir~~ salt wedge nature during dry periods. Typical tidally averaged salinity profiles exhibit a sharp pycnocline (or halocline), with mean flow~~s~~ dominated by outflow throughout most of the water column and weak inflow in a near-bottom layer. The mean flow pattern results from relatively weak mixing between the inflowing ocean/sea water and the river water.

The weakly stratified or partially mixed estuaries result from moderate to strong tidal forcing and weak to moderate river discharge. The mean salinity profile either has a weak pycnocline or continuous stratification from surface to bottom, except near the bottom mixed layer. The mean exchange flow is most vigorous because of the mixing between river and ocean/sea waters. Strong tidal forcing and weak river discharge result in vertically mixed estuaries. The mean salinity profiles in mixed estuaries are practically uniform and mean flow is unidirectional with depth. In wide and shallow estuaries, inflow may develop on one side across the estuary and outflow on the other side, especially during the dry season.

The estuaries can be classified according to their mean tidal range as (a) Microtidal (mean tidal Range <2 m), (b) Mesotidal (mean tidal range between 2 and 4 m), (c) Macrotidal (mean tidal range between 4 and 6 m), and (d) Hypertidal (mean tidal range > 6 m) (**Dyer 1997**). The difference in the tidal range confers distinct characteristics of the estuarine dynamics.

Mudflats, mangroves and sandflats are sub-environments found in the intertidal regions of the estuaries.

(i) The mudflats cover large non-vegetated areas that are exposed during low tide and submerged during high tide **(Nayak and Noronha-D'Mello 2018).** The mudflats generally support very little vegetation other than green algae. They are coastal wetlands formed in sheltered shores where greater amounts of sediments~~,~~ and detritus are deposited by rivers or tides. They are frequently developed within estuaries, and are usually situated adjacent to mangroves. The intertidal mudflats play a critical role in the estuarine exchange of marine and continental supplies of nutrients and sediments. The sediments consist mainly of fine particles, mostly silt and clay fractions. The intertidal mudflats support communities characterized by polychaetes, bivalves and oligochaetes, birds and fish. They provide an important nursery and feeding ground for many fish species.

(ii) The mangroves, on the other hand, are salt-tolerant shrubs and plants associated with a unique horizontal root network **(SotoJime'nez and Pa'ez-Osuna 2001; Kumaran et al. 2004)**. They primarily consist of fine sediment deposits and are influenced by tides, waves and fluvial processes **(Lesueur et al. 2003; Dalrymple et al. 1992)**. Along with finer sediments, favour the deposition of higher organic matter content. The fine cohesive sediments favour the accumulation of numerous pollutants **(Cundy and Croudace 1995; Szefer et al. 1995; Delacerd 1983)**. Similar to mudflats, the mangroves are important nursery grounds and breeding sites for fish, crustaceans, birds, reptiles, mammals, and many other semi-terrestrial and estuarine organisms. Several juvenile species spend their early part of the life cycle at mangrove habitat. The mangrove region receives ample of nutrient supply being in proximity to terrestrial environment and thus, attracts marine fauna. Moreover, the intrinsic root system of mangrove plants enables the species to protect themselves from predators. They help to ameliorate coastal erosion and the impacts of extreme events, viz., tsunamis and cyclones. They are a renewable source of wood for fuel and housing, and of food and traditional medicines. Also, they are biogeochemically important sites of sediment, carbon, nutrient and contaminant accumulation **(Alongi 2015)**.

(iii) The sandflat is an expanse of unconsolidated material found primarily in the lower intertidal zone. It is an unstable environment defined by the constant resuspension of sand caused by tidal flood and ebb currents. The tidal force~~s~~ dominates other hydrodynamic processes, resulting in well-sorted sand deposits. The amount of mud found in the sediment of a sandflat is determined by the bed slope, the amount of fine sediments derived from land, and the strength of the current. The sandflat-mudflat threshold is determined by the aforementioned factors. Finer muddy sediments accumulate during neap tides, while coarser sandy sediments accumulate during spring tides. The hydrodynamic conditions at sandflat preclude development of submerged aquatic vegetation; however, there are important resident communities of burrowing invertebrates (worms, bivalves and crustaceans) that are fundamentally important for local trophic networks and the recycling of nutrients. The ~~s~~andflats can be found adjacent to other types of habitats, viz., saltmarshes located in the upper intertidal zone **(Perillo et al. 2009).**

The estuary being largely covered by terrestrial region receives numerous pollutants from natural and anthropogenic means. The metal is one of the important pollutants introduced in estuaries via natural processes viz., weathering of catchment area rocks and aeolian transport (**Hwang and Yang 2003**). Also, its rate of discharge in a dynamic ecosystem viz., an estuary is enhanced in recent years due to input from various human-induced activities (mineral ore mining, industrial effluents, agricultural discharge, municipal and domestic wastes, etc.) (**Jordao et al. 2002**). Also, metals are introduced in estuaries through atmosphere fallout **(Yuan et al. 2012)**. The metals released into estuaries are initially suspended in the water column (**Gonzalez et al. 2011**) and later get adsorbed onto suspended particulate matter (**Nasnodkar and Nayak 2015**). It eventually sinks down the water column and become a part of cohesive bottom sediments **(Ra et al. 2013)**. The size of the sediment particles is crucial in the distribution of metals in sediments. The finer sediments viz., silt and clay owing to higher surface area/volume ratio facilitate high concentration of metals in sediments (**Horowitz and Elrick 1987**). The coarser sediments lack in free binding surfaces and therefore, causes dilution of metals in sediments (**Gadkar et al. 2019**). Additionally, the finer sediments favour adsorption of organic matter in sediments, which in turn enhances adsorption of metals through organo-metallic complexes (**Ramanathan et al. 1999**).

The estuary being a dynamic ecosystem, there are frequent changes in physico-chemical parameters of water viz., salinity, pH, Eh, ionic composition, microbial activity, etc., (**Elliotta and McLusky 2002**). These properties have ability to disturb adsorbed metal on sediment surface, and cause mobilization/desorption of metals to water column or pore water. The metals are remobilized by digenesis in sediment or resuspended to the overlying water **(Ra et al. 2011).** The estuarine hydrodynamics govern the distribution and overall fate of metals in sediments. According to **Tesser et al. (1979)**, a given metal in sediment is present in residual and bioavailable (exchangeable, carbonate, Fe-Mn oxide and organic/sulphide) forms. The metals of natural origin are strongly embedded in the lattice structure of minerals in sediments and are referred as the residual form of metals (**Purushothaman et al. 2007**). They are highly stable and environmentally unreactive. On the contrary, the metals adsorbed on sediment surface are loosely bound and are mostly derived from anthropogenic sources. They are considered as bioavailable, and are unstable and susceptible to mobilization by changes in physico-chemical properties of water. For instance, the salinity gradient is one of the main estuarine characteristics responsible for non-conservative metal behaviour. It results in several major changes in water chemistry and ionic strength **(Deep and Nasnodkar 2023)**, accounting for metal mobilization. At higher salinity, the proportion of major cations viz., Na^+ and Cl^- increases that compete for adsorption sites on sediment surface (**Acosta et al. 2011**). Thus, replaces metal cations from sediments and releases them to water column. Also, complexation of metal cations with major ions prevents their adsorption onto sediments. The mixing of fresh and saline water within estuaries also induces change in the hydrogen ion concentration. At low pH, the high concentration of H^+ ions compete with metal cations for binding surfaces on sediments (**Gadkar et al. 2019**). It facilitates the desorption of metals from sediments and enhances their bioavailability. Similarly, variations in oxygen level are often encountered in the estuarine environment. The oxic-anoxic conditions regulate stability of Fe-Mn in their oxide/hydroxide form. The Fe-Mn oxides/hydroxides are precipitated under oxygen rich environment and form coatings on sediment surface. They act as scavengers of other metals from the estuarine water and increase the adsorption of metals in sediments (**Prabhu Dessai et al. 2023**). However, under anoxic conditions these oxides/hydroxides are unstable and result in dissolution. It increases their dissolved concentration, so as the level of scavenged trace metals in water. The oxygen level also governs metals associated with sulphides in sediments (**Boulegue 1982**). Also, the microbial activity degrades organic matter in sediments and, releases metals to water column.

The estuarine sub-environments viz., mudflat and mangrove are suitable beds for bivalves and other sediment associated fauna. The bivalves are filter feeders and consume metals associated with sediments in their bioavailable form and also, through ingestion of water. They are known to intake metals through aqueous/dietary phases which include the suspended particulate matter and sediment from the water-sediment interface. They feed through their gills **(Jitar et al. 2013)** and, therefore, can take the metals from the dissolved phase, particulate phase, and sediment. The characteristics of bivalves viz. sedentary, ability to retrieve metals with ease from water, sediment and other substratum, and the susceptibility of metals in tissues to various biotic and abiotic factors makes them an ideal organism in the biomonitoring studies **(Rodrigues et al. 2021)**. The main purpose of monitoring the concentrations of metals in biota is to determine the toxicological risk faced by marine organisms and even by humans through the ingestion of contaminated edible species that are rich in nutritional content. The use of particular organisms as biomonitors of metal bioavailability in coastal water allows comparison to be made over space and time, as biomonitors provide integrated measures of the ecotoxicologically significant fraction of ambient metal in water **(Phillips and Rainbow 1993; Rainbow 1995).** The uptake of metals by bivalves, and subsequent bioavailability, are highly dependent on geochemical and biological factors. Among the biological factors, there are major differences in bioaccumulation between and within a bivalve species. The accumulation of metals in bivalves can be a function of age, size, sex, genotype, phenotype, feeding activity, and reproductive state. The geochemical factors that influence bioaccumulation are organic matter content in sediments, water hardness, temperature, pH, dissolved oxygen, sediment grain size, and hydrologic features of the system **(Elder and Collins 1991)**.

Owing to growing concern of metal pollution in estuaries and other coastal water bodies numerous scientific studies are carried out to remediate metal pollution using natural techniques. One of such kinds is the application of mangrove plants in phytoremediation technique widely preferred to curb metal pollution in estuaries. The phytoremediation is an in-situ emerging solar-driven technology that utilizes the inherent abilities of living plants and rhizosphere microorganisms for removal or transformation of the contaminated soils/sediments. This could be recognized as an eco-friendly, cost-effective method of immobilizing, stabilizing, degrading, transferring, removing, or detoxifying both inorganic (e.g., metals/metalloids) and organic pollutants (e.g., organochlorine pesticides, hydrocarbons, etc.) as well as nutrients and organic matter **(Sarkar 2018)**. The metal tolerance in plants may be defined as the ability of plants to survive at conditions that are toxic to other plants **(Cai and Wang 2003)**.

The mangrove plants adopt different intricate cohesive mechanisms to grow in metal-stressed environment with no adverse impact on plant growth. Some plants exclude the metals/metalloids from metabolically active sites by restricted uptake or root–shoot transfer of metals **(Küpper et al. 1999)**. They can tolerate high metal concentrations in their tissues through binding of metals with organic compounds, metal compartmentalization at cellular and subcellular levels, and metabolic alternations **(Wei et al. 2005)**. The hyperaccumulator species of mangroves have the efficiency in absorption of metal(loid)s by adopting certain specific mechanisms such as (1) high-affinity transport systems across the plasma membranes, (2) roots growing efficiently in the metal-stressed regions, and (3) developing dense root systems with intricate fine roots helping a large surface area to absorb metals in metal-contaminated soils/sediments. They absorb metals adopting the following successive steps: (1) adsorption and transportation of metals across the membrane of roots cells, (2) loading the metals into the xylem and translocation to the aerial organs (shoots), and (3) metal detoxification within plant tissues, preferably in the epidermis, trichomes, and cuticles (**Yang et al. 2005**). The mechanisms and efficiency of phytoremediation depend on the type of contaminant, bioavailability, and sediment properties **(Laghlimi et al. 2015)**. There are several ways by which mangrove plants clean up or remediate contaminated sites. The uptake of contaminants in plants occurs primarily through the root system, in which the principal mechanisms for preventing toxicity are found. The root system provides an enormous surface area that absorbs and accumulates water and nutrients essential for growth along with other non-essential contaminants **(Raskin and Ensley 2000)**. The different techniques of phytoremediation involving mangroves include phytoextraction (the use of plants to remove contaminants from soils), phytovolatilization (the use of plants to make volatile chemical species of metals in sediments), rhizofiltration (the use of plant roots to remove contaminants from flowing water) and phytostabilization (the use of plants to transform metals in sediments to less toxic forms, but not remove the metal from the sediments).

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. 2021).** 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 40 km long from Khareband to Mobor, near Betul Fort **(Saha et al. 2021).** 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 study the bioavailability of metals in intertidal sediments of the lower Sal Estuary.

- To understand the bioaccumulation of metals in edible bivalves.

- To determine the phyto-remediation potential of mangrove plants.

Nasnodkar and Nayak (2015)	Examined sediment cores from the lower region~~s~~ of Mandovi, Sharavathi, and Gurupur estuaries for sedimentological and geochemical characteristics. The concentration of finer sediments increased in Mandovi and Sharavathi estuaries in the recent years, while coarser sediments increased in the Gurupur Estuary. The high deposition of finer sediments facilitated enrichment of metals in the Mandovi and Sharavathi estuaries in recent years, while coarser sediments caused dilution of metals in the Gurupur Estuary.
Cruz et al. (2020)	Studied the concentration of metals (Fe, Mn, Zn and Pb) in sediment cores collected from middle and upper regions of the Zuari Estuary, India. Metal distribution varied due to many sources and hydrodynamic differences in the middle and upper portions. The contamination factor revealed moderate level of contamination in the middle (Fe, Mn and Zn) and upper (Fe, Zn and Pb) estuary. The Pollution Load Index (PLI) value >1 indicated metal pollution at both the stations. The metals viz., Mn, Zn and Pb were significantly present in bioavailable forms. Furthermore, the concentration of Mn > 5% in the labile fraction in sediment cores indicated a high bioavailability to sediment-associated biota with changes in ionic composition and pH.
Fernandes and Nayak (2014)	Investigated the distribution of metals (Fe, Mn, Cu, Co, Cr, Pb and Zn) in intertidal sediments of the Ulhas Estuary and Thane Creek, Mumbai. The findings demonstrated a substantial significance of grain size and organic matter as metal transporters. Based on metal concentrations and several sediment quality assessment tests, the Ulhas

	Estuary had higher levels of metal contamination than the Thane Creek.
Nayak (2020)	The increased mud and organic matter deposition in recent years has promoted the growth of mangroves in the Zuari Estuary. The hydrodynamic conditions and anthropogenic activities were crucial in the formation of intertidal mudflats in the Zuari Estuary. The fluctuating tidal and freshwater discharge, interactions, and hydrodynamic circumstances resulted in a calmer environment that facilitated the deposition of finer sediments and organic matter, which trapped and maintained higher metal concentrations in the middle region of the Zuary Estuary.
Magesh et al. (2013)	The trace metal contamination was investigated in estuarine sediments along the Tuticorin Coast-Gulf of Mannar, on India’s southeast coast. The enrichment factor, metal pollution index, and geo-accumulation index indicated contamination of Cd, As, Zn, Hg and Pb in sediments. The factor analysis revealed riverine input as the source of Fe and Mn in sediments, whereas untreated industrial effluents were the sources of As and Hg.
Gadkar et al. (2019)	The study examined metal enrichment and bioavailability in mudflat and mangrove sediment cores obtained from the confluence point of the Cumbarjua Canal and the Zuari River, contaminated with open-cast mining waste. Fe, Mn, Cu and Co indicated moderate contamination in mangroves, while Fe, Mn, and Co were found contaminated in mudflat sediments. Metal speciation revealed the bioavailability of Fe in mangroves and Mn in both mangrove and mudflat sediments. The sediment quality values suggested toxicity to sediment-associated biota.

Jiang et al. (2020)	The distribution and ecological risk of Cd, Co, Cr, Cu, Mn, Ni, Pb and Zn in mangrove sediments of the Maowei Sea were investigated. The results showed that Mn concentrations increased dramatically with depth in the mudflat. The various environmental risk indices revealed that the investigation region was heavily contaminated, with Cd being the main contributor to possible ecological harm. The analysis suggested that Cd, Co, Cr, Cu, Ni, Pb and Zn were derived from anthropogenic sources, whereas Mn had an input from natural processes.
Fernandes et al. (2014)	The depositional environment of mudflats and mangroves in a tropical estuary was investigated (Shastri). Four sediment cores from the estuary's lower and upper middle areas were analyzed for sediment composition (sand, silt, and clay), total organic carbon, and total metal concentration. The hydrodynamic characteristics of upper and middle estuary sediments varied significantly. Total organic carbon and total metal concentrations were found to be extremely high, which was attributed to tributary input.
Kesavan et al. (2013)	The concentrations of metals (Mg, Cu, Co, Zn and Co) were measured in sediments, shells, and tissues *of Meretrix meretrix, Crassostrea madrasensis,* and *Cerithidea cingulata* at two sites in the Uppanar Estuary on India's southeast coast. Metal concentrations in sediments, shells, and tissues varied across both locations. Mg was found to be higher in shell and tissue, although Zn and Cu levels were not alarmingly high. Sediment at both locations had the highest Fe level, followed by shells and tissues. Mg was the highest, whereas Cd and Co were the lowest in *Cerithidea cingulata* shells. The human-induced activities were the primary sources of metals.

Adjei-Boateng (2010)	The concentration of metals (Fe, Mn, Zn and Hg) in the clam *Galatea paradoxa* and in the Volta Estuary sediments in Ghana were compared. The concentration~~s~~ of metals in organs of clam and sediments varied significantly. However, the concentration of Fe in tissues of the various clam size classes from the two sampling points was comparable.
Kumari et al. (2006)	Investigated the bioaccumulation of trace metals (Fe, Cu, Zn, Pb and Cd) in a short-neck clam, *Paphia malabarica*, from the Mandovi Estuary, Goa. The cadmium was most concentrated in mantle and adductor muscle, lead in the foot muscle, copper in the digestive glands and gonads, and zinc and iron in the gills. The variable correlation coefficient between distinct coupling metals revealed a shift in accumulation ability based on the organism's size and organ location.
Joseph et al. (2016)	The study examined the bioaccumulation of metals (Cu, Cd, Zn, Pb, Ni and Fe) in soft tissues (foot, digestive caecum, total body and operculum) and shell of three different species of molluscs namely (*Telescopium telescopium*, *Cerithidea obtusa* and *Cerithidea cingulate*) using the Atomic Absorption Spectrophotometer (AAS). The molluscs had a significant amount of metals, with the exception of Cd, Pb and Ni.
Kesavan et al. (2010)	Estimated the concentration of metals (Fe, Cu, Zn and Mg) in sediment and biota (*Telescopium telescopium)* from the Vellar Estuary. The concentration of metals in sediments exhibited the following decreasing order Fe > Mg > Zn > Cu. The concentration of metals showed decreasing order of Mg > Fe > Zn > Cu in the tissues and shells of the biota.

McFarlane et al. (2003)	*Avicennia marina*, a grey mangrove prevalent in Australia's estuarine wetlands, was investigated for metal accumulation and dispersion. Metal concentrations (Zn, Cu and Pb) were assessed in mangrove sediments, roots, and leaf tissues. *Avicennia marina* roots and leaves were found to be biological indicators of Cu, Pb, and Zn exposure in the environment.
Chowdhury et al. (2015)	Trace metal (As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb and Zn) accumulation was investigated in mangrove plants from the Indian Sundarbans wetland. The uptake, accumulation and partitioning of trace metals in plant tissues (leaves, bark, and roots/pneumatophore) were also studied and it was found that *Excoecaria agallocha* had the greatest bio-concentration factor (15.5) for Cd, indicating that it is an extremely efficient plant for metal retention.

CHAPTER 2

MATERIALS and METHODS

2.1 Introduction

A sample of a specific quantity represents the biogeochemical processes prevailing in theenvironment. The samples are collected from a desired site(s), stored and then analyzed 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 justthe environment but the issues pertaining in the environment. Thus, the materials, methods and the techniques used must be of internationally accepted standards. To meetthe objectives of the study, the following methodology was adopted:

2.1 Sampling

The field sampling was carried out in the Sal Estuary, Goa in May 2023. Four short sediment cores of 10-20 cm length were retrieved from the mangrove (S5, S6 and S7) and sandflat (S8) 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, stem and pneumatophores) were collected from the vicinity of sedimentcore 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-5	15° 9' 54.7092" N	73° 56' 55.8168" E
S-6	15° 9' 34.2576" N	73° 57' 6.9408" E
S-7	15° 9' 11.3508" N	73° 57' 10.0224" E
S-8	15° 8' 46.2084" N	73° 57' 2.4912" 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 andmangrove samples were collected in labelled cloth bags. The sediment, biota andmangrove samples were transported to the laboratory in an ice box. Upon reaching the laboratory, the sediment and mangrove samples were stored at 4℃ in a refrigerator whereas, the biota samples were stored at -20℃ 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 species	Biota species
S – 5	• *Avicennia alba*	• *Saccostrea cucullate* • *Polymesoda erosa* • *Placenta placenta* • *Telescopium Telescopium*
S – 6	• *Avicennia marina*	• *Saccostrea cucullate* • *Paphia (not identified)* • *Crassostrea madrasensis*
S – 7	Nil	• *Telescopium Telescopium* • *Paphia (not identified)*
S – 8	Nil	• *Donax scortum* • *Donax (not identified)*

2.2 Sedimentological and geo-chemical analysis

The sediment core sub-samples were oven dried at 60°C 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. This method 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 transferredinto the 1000 mL glass beaker. Then the glass beaker was filled with distilled water andwas mixed well. Later, the sediments were allowed to settle at the bottom of the beaker 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 were 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 dis-integrate 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 2.5: 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) ×100X = (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 matterin retention and bioavailability of metal in sediments. A modified Walkey-Black method**(Gaudette et al. 1974)** was followed to estimate the concentration of TOC 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 MillQ 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 dropbrilliant 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 solutionT = 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. 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 waskept 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 wasfollowed 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. 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 sediment 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 **Tessieret 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 thebioavailable form of metal which is environmentally reactive, whereas the residual formof 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 compositionof 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 wasthen 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 acetateat 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 andthe 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. Thesediment residue (II) in centrifuge tube was washed with MilliQ water and was used fornext step.

2.3.4.3 Fe-Mn oxides fraction (F3)

In the marine environment, the dissolved Fe and Mn under oxic conditions precipitate asoxides 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 environmentas 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 throughWhatman filter paper into the 25 mL volumetric flask and the final volume was made to25 mL using MilliQ water. The leachate was stored in a pre-cleaned polyethylene bottleat refrigerated temperature until further analysis. The sediment residue (III) in centrifugetube 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_2at pH 2 was added to the mixture and was kept back in a water bath for additional 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 storedin a pre-cleaned polyethylene bottle at refrigerated temperature until further analysis. Thesediment residue (IV) in centrifuge tube was washed with MilliQ water and was used fornext 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 leadsto complete digestion of the residual fraction.

The residue (IV) was treated with 10 mL of acid mixture (HF, HNO_3, $HClO_4$) in the ratioof 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 sediment fractions was determined using the (AAS).

2.4 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 andkept separately for drying in an oven at 60℃ 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 mLof H_2O_2 at 90℃ 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-cleanedpolyethylene bottle and stored in a refrigerator until metal (Fe, Mn, Zn, Cu, Co and Ni) analysis using the (AAS).

2.5 Biota digestion

The analysis of metals in the soft tissues of bivalves (oysters and clams) was carried outas 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℃ for 72 hours. The dried biota sample was powdered using an 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℃ 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 (AAS).

2.6 Metal Pollution Indices

The following pollution indices were used to understand the contamination and pollution of metals in the 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 **(Barbieri 2016)** as follow:

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

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

2.6.2 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:

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

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 isthe 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 possibilityof 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.

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

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

The mBSAF of < 1 suggest that the biota is de-concentrator of metal, whereas the valueranging from 1 to 2, and > 2 suggest biota as micro-concentrator and macro-concentratorof 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 ofmetals 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 tissuesof biota with the standard permissible limits (Table 2.6).

Table 2.6: Standard permissible limits of metals.

Metals	Standard Permissible Limit	References
Fe (%)	0.0034 − 0.0107	Charbonneau & Nash (1993)
Mn (ppm)	0.01	WHO (1989)
Zn (ppm)	40	WHO (1989)
Cu (ppm)	30	FAO/WHO (2004)
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 thesoftwares viz., Microsoft Excel 16, Q-GIS , Grapher 14 and STATISTICA.

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-5, S-6, S-7 and S-8.

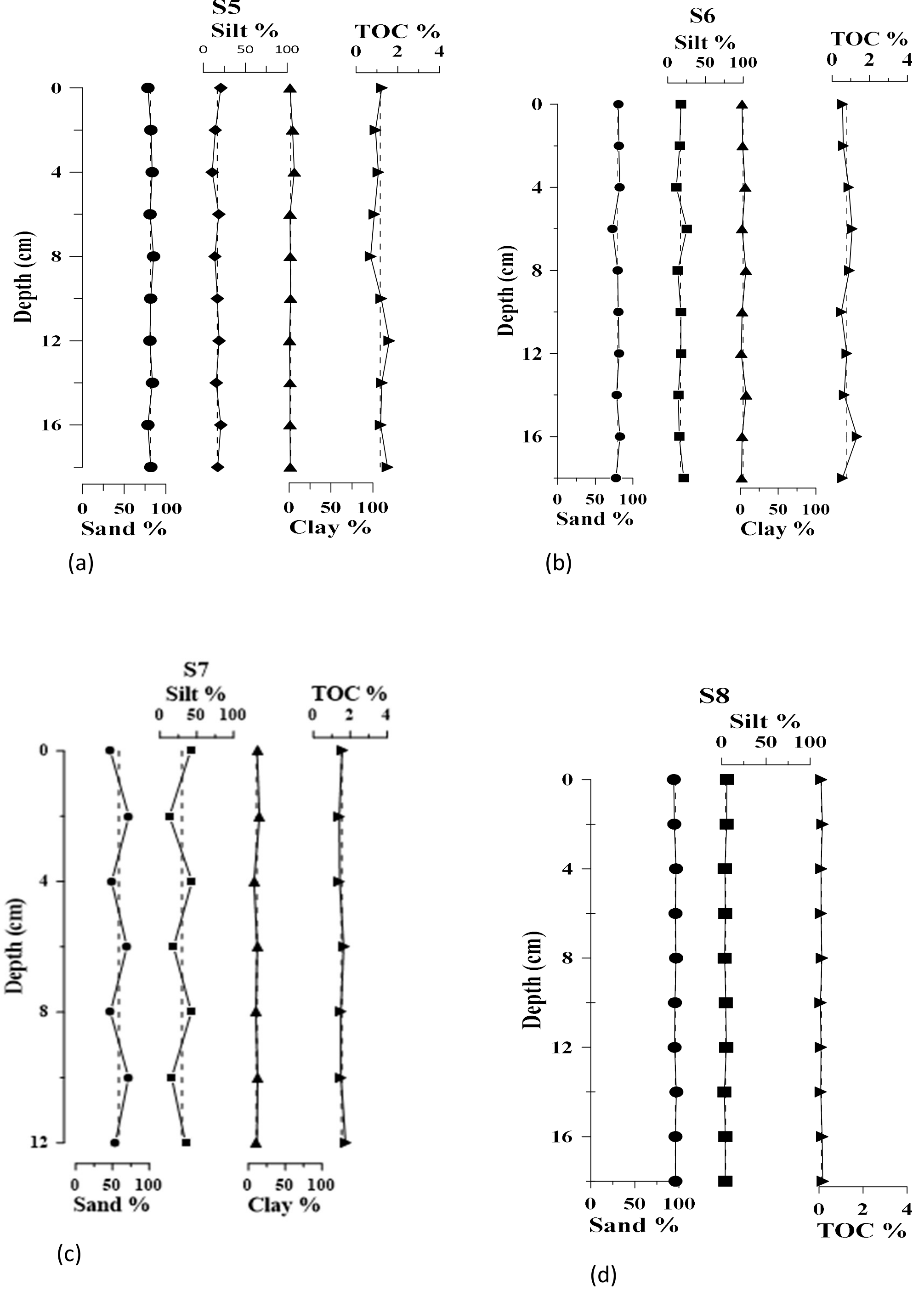
Sediment cores		Sand %	Silt %	Clay %	TOC %
S-5	Range	78.34–83.86	10.52-20.86	0.8-6	0.70-1.59
	Average	81.63	16.61	1.76	1.15
S-6	Range	72.75-82.66	11.41-25.29	0.92-7.48	0.50-1.32
	Average	79.71	16.91	3.38	0.78
S-7	Range	47.03-72.31	12.73-43.53	7.6-14.96	1.40-1.74
	Average	58.65	29.85	11.50	1.53
S-8	Range	94.22-97.10	2.89-5.78	Nil	0.07-0.18
	Average	95.80	4.20	Nil	0.11

The range and average concentration of sediment components and TOC in cores S-5, S-6, S-7 and S-8 are presented in Table 3.1. The sand was highest (95.80 %) in the core S-8 collected from the close proximity to the mouth region and was relatively low (58.65 %) in the core S-7. Further, the sand in cores S-5 and S-6 was 81.63 % and 79.71 %, respectively. The silt showed an increase from S-5 to S-7 followed by a decrease in the core S-8, while an overall increase in clay was observed from S-5 to S-7. The core S-8 was only composed of sand and silt particles. TOC was highest (1.53 %) in the core S-7 and was lowest (0.11 %) in the core S-8.

The sampling stations were dominated (50 %) with sand particles which was attributed to higher hydrodynamics due to strong waves and tides in the lower estuary. The most significant mechanism influencing the movement, deposition, and resuspension of sediments and pollutants in estuaries is hydrodynamics **(Chen et al. 2017; Yang et al. 2017)**. It might have facilitated the retention of coarser sediments in the lower estuary, while finer sediments were transported to the middle estuary **(Yang et al. 2017)**. The high bottom shear stress causes resuspension of fine-grained particles, while coarser sediments are kept in place for longer duration **(Fernandes and Nayak 2015)**. The dominance of coarser particles diluted the concentration of silt and clay. The TOC was relatively higher in the core S-7 than other cores. It was mainly due to considerable proportion of finer sediments (40 %) at station S-7. However, the concentration of TOC in all the cores was below 2 % and it was attributed to dilution of organic matter due to enrichment of coarser particles (sand) in the lower estuary. In general, the distribution of TOC in sediments is related by the grain size composion of sediments, especially by the finer particles **(Chatterjee et al. 2007).** The finer sediments favour the adsorption of organic matter in sediments due to large surface area/volume ratio **(Ramanathan et al. 1999)**.

3.1.2 Vertical distribution of sediment component and TOC

In cores S-5 and S-6 (Fig 3.1a and b), the vertical distribution of sand, silt and clay was nearly constant from bottom to surface of the core, while the TOC exhibited a slight fluctuating trend. In core S-7 (Fig 3.1c), sand displayed fluctuations from bottom to surface of the core with a decrease near the surface. The distribution pattern of sand was well compensated by silt. On the other hand, clay and TOC were constant along the core length. The concentration of sand, silt and TOC was constant throughout the depth of the core S-8 (Fig 3.1d). The nearly constant concentration of sand, silt and clay at stations S-5, S-6 and S-8 indicated uniformity in the energy of waves and tides over a period of time. On the contrary, fluctuating trends of sand and silt suggested changes in grain size. It might be due to human interference as the sampling site S-7 was close to the human establishments along the estuarine bank. The similarity in the distribution of silt or clay with TOC revealed adsorption of organic matter onto finer sediments. The finer particles have a large surface area relative to their volume, providing more opportunities for organic matter to interact and adhere **(Li et al. 2015).**



(a)

(b)

(c)

(d)

Fig 3.1: The vertical distribution of sediment components and TOC with average line in cores of S-5 (a), S-6 (b), S-7 (c) and S-8 (d)

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-5	Range	1.03-8.10	159-2635	72-287	22-64	18-32	97-259
	Average	2.36	462	102	31	25	129
S-6	Range	1.84-4.82	448-589	73-252	26-43	20-30	98-132
	Average	3.36	494	139	32	24	113
S-7	Range	2-4.6	216-309	65-81	31-40	14-19	136-147
	Average	2.84	254	73	36	17	144
S-8	Range	0.09-0.37	75-99	19-70	9-20	3-10	162-202
	Average	0.23	85	42	17	7	177
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-5, S-6, S-7 and S-8 are presented in Table 3.2. The average concentration of metals viz., Fe, Mn and Zn was highest in the core S-6, while the Cu was highest in the core S-7. The Co and Ni were highest in cores S-5 and S-8 respectively. The lower concentration of most of the metals (Fe, Mn, Zn, Cu and Co) was in the core S-8 and was attributed to abundance (95.80 %) of coarser sediments and low TOC (0.11 %) content. The presence of coarser sediments tends to dilute metal concentration, while finer sediments owing to large surface area/volume ratio facilitate the adsorption of metals (**Nasnodkar and Nayak 2019).** Therefore, the grain size variations might have influenced the distribution of metals in the Sal Estuary.

The concentration of Fe (S-6), Zn (S-5, S-6 and S-7), Cu (S-5, S-6, S-7 and S-8), Co (S-5, S-6 and S-7) and Ni (S-5, S-6, S-7 and S-8) exceeded the upper crustal average value indicating enrichment of these metals in sediments of the Sal Estuary. It suggested anthropogenic input of metals in the estuary. The Sal Estuary is impacted by various human activities, including the discharge of paint particles from trawlers at fishing jetties, the scraping of ship hulls, and the release of domestic, agricultural and aquaculture waste **(Fernandes et al. 2018).**

3.2.2 Vertical distribution of total metals

In the core S-5 (Fig 3.2a), the concentration of metals viz., Fe, Mn, Zn, Cu and Ni was nearly constant along the core length. However, these metals showed a sharp positive peak at 6 cm depth. The vertical profile of Co showed fluctuating trend throughout the core length with a slight decrease near the surface. In core S-6 (Fig 3.2b), the metals viz., Fe and Zn displayed decrease in their concentration from bottom to 14 cm depth. Further, Fe showed a gradual increase up to 6 cm depth, while Zn increased till 4 cm depth. Both showed a decrease near the surface. The Mn, Cu and Ni were nearly constant along the core length. The Co displayed slight fluctuations from bottom to surface of the core S-6. In case of core S-7 (Fig 3.2c), the metals viz., Mn, Zn, Cu, Co and Ni were almost constant from bottom to surface. Fe remained constant from bottom to 10 cm depth, followed by a slight increase at 8 cm depth. Further, it showed a gradual decrease towards the surface. Even in core S-8 (Fig 3.2d), the distribution profiles of metals were nearly constant from bottom to surface.

The vertical profiles of most of the metals in sediment cores representing the lower region of the Sal Estuary were nearly constant. It indicated similar sources of metals in the Sal Estuary. Moreover, the profiles of Zn, Cu and Ni at station S-5, Cu and Ni at station S-6, Zn, Cu, Co and Ni at stations S-7 and S-8 were identical to either Fe and/or Mn. The Fe and Mn are redox sensitive elements and are effective scavengers of trace metals in the estuarine environment **(Asa et al. 2013).** They precipitate as oxides or hydroxides under oxic conditions and facilitate adsorption of trace metals **(Zhu et al. 2013).**

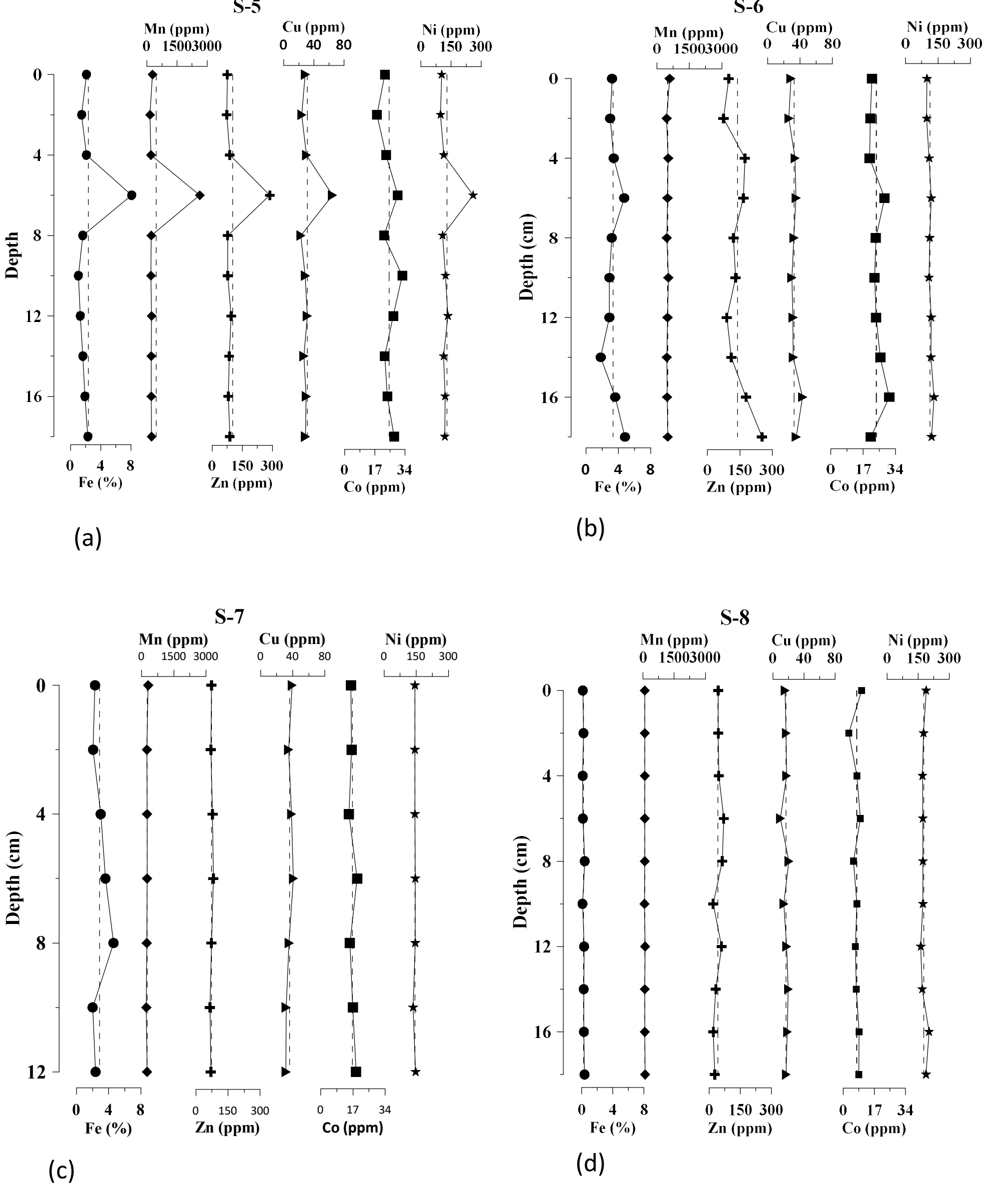


Fig 3.2: The vertical distribution of total metals with average line in cores S-5 (a), S-6 (b), S-7 (c) and S-8 (d)

3.2.3 Metal speciation

The range and average of trace metals in different geochemical fractions in sediment cores S-5, S-6, S-7 and S-8 is presented in Table 3.3. In core S-5, the metals viz., Fe, Mn, and Ni were highest (>40 %) in the residual fraction, whereas Zn (33.73 %) and Co (34. 65 %) were highest in the Fe-Mn oxide fraction. The concentration of Cu was highest (44.85 %) in the organic matter/sulphide bound fraction. The Zn and Co were significantly (>25 %) bound to the residual fraction. Even Cu was considerably associated with the residual fraction. The metals viz., Fe, Mn and Ni were more than 10 % in Fe-Mn oxide and organic matter/sulphide fractions. Similarly, the concentration of Zn and Co was more than 10 % in the organic matter/sulphide fractions. Moreover, the concentration of Mn, Zn, Cu, Co and Ni was > 10 % in the labile (exchangeable and carbonate) fraction.

In core S-6, the concentration of metals viz., Fe, Zn, Cu, Co and Ni was highest (>35 %) in the residual fraction, while Mn was more than 85 % in the Fe-Mn oxide fraction. Additionally, Zn and Co were significantly (>25 %) bound to the Fe-Mn oxide fraction. Also, Cu was more than 25 % in the organic/sulphide fraction. The concentration of Mn, Zn, Cu and Ni was > 10 % in the labile fraction.

In the core S-7, Fe, Mn and Ni showed highest (>35 %) concentration in the residual fraction, while Zn (39.48 %) and Co (29.11 %) were highest in the Fe-Mn oxide fraction. The Cu was highest (43.86 %) in the exchangeable fraction. The metals viz., Mn, Zn and Cu were also significantly (>25 %) bound to the residual fraction. Also, Fe and Ni in the Fe-Mn oxide fraction, and Zn and Cu in the organic matter/sulphide fraction were present in considerable (>10%) amount. The concentration of Mn, Zn, Cu, Co and Ni was > 10 % in the labile fraction.

In the core S-8, the concentration of metals viz., Fe, Mn, Zn and Ni was highest (>40 %) in the residual fraction. Also, Co was highest (21.88 %) in the residual fraction. The Cu reported highest (55.62 %) concentration in the exchangeable fraction. Additionally, Co and Ni were greater than 20 % in the carbonate fraction. The metals viz., Mn, Zn, Co and Ni were considerably bound (>10 %) to the Fe-Mn oxide fraction.

Also, Co was more than 10 % in the organic matter/sulphide fraction. Moreover, the concentration of Mn, Zn, Cu, Co and Ni was > 10 % in the labile fraction.

Table 3.3: Range and average of geochemical forms of metals in sediment cores S-5, S-6, S-7 and S-8.

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 – 5	Fe	0.07-0.14	0.10	0.09-0.59	0.23	3.08-14.44	10.42	8.23-23.24	14.38	67.67-79. 63	74.87
	Mn	3.51-8.88	5.88	2. 67-28.09	8.04	11.93-19.56	15.88	5.04-22.08	11.99	38.87-76.01	58.21
	Zn	5.88-11.19	8.09	10. 68-15.59	13.41	29.21-37.25	33.73	10.33-15.51	13.07	28.53-35.15	31.70
	Cu	4. 61-34.31	20. 66	4. 65-7.56	5. 64	4.26-9.91	6.19	27.75-59.55	44.85	17. 65-30.32	22. 66
	Co	0.00-10.82	6.30	9.42-22.30	16.34	28.16-41.11	34. 65	13.44-21.54	17.01	21.89-29.36	25.70
	Ni	10.81-15.35	12. 66	14. 61-17.77	16.24	12.86-20.22	17.03	9.72-16.27	11.73	39.05-48.10	42.35
S – 6	Fe	0.05-0.10	0.06	0.004-0.14	0.08	7.30-14.20	10.20	2. 61-1239	5.72	77.43-88.85	83.95
	Mn	1.09-6. 65	3.16	9.76-40.33	23.92	48. 64-66.47	87.37	3.96-13.86	8.99	3.39-10.18	6.56
	Zn	1.90-8.07	5.46	7.02-16.37	11.07	29.96-50. 64	35.76	6.11-10. 65	8.57	24.93-49.82	39.15

	Cu	4.80-36.86	11.84	4.41-10.90	7.13	5.41-11.66	8. 65	18.32-38.51	25.75	32.72-53.11	46. 63
	Co	0.00-5.71	1.66	2.74-14.11	7. 60	28.53-44.71	35.27	0.00-14.28	5.26	31.06-67.36	50.21
	Ni	6.36-13.96	10.79	11.71-19.17	15.10	15.01-20.79	17.75	9.88-11.59	10. 63	39.62-50.89	45.73
S – 7	Fe	0.06-0.10	0.09	0.07-0.13	0.10	11.29-15.76	14.18	2.73-10.99	5.58	76.62-84.20	80.05
	Mn	1.27-3.91	3.09	13.81-17.98	15.44	31.32-36.54	34.46	6.94-16.57	9.82	34.50-38.89	37.15
	Zn	6.38-8.42	7.34	8.77-12.07	10.14	35.23-43.54	39.48	9.73-11.50	10.87	29.56-38.55	32.17
	Cu	30. 67-55.38	43.86	3.30-19. 68	6.25	3.18-6.00	4. 66	14.74-28.49	19.43	19.98-31. 66	25.78
	Co	12.55-15.33	13. 64	15.03-17.92	15.96	27.39-30.77	29.11	16.22-19.19	17. 66	22.48-27.52	23. 64
	Ni	9.00-11.30	9.82	10.76-12.73	11.90	13.57-17.41	15.30	8.52-9.41	9.14	51.37-57. 63	53.84
S – 8	Fe	0.36-0.77	0.52	0.31-0.58	0.43	4.31-16.81	9. 69	0.50-0.86	0.70	81.40-94.34	88. 67
	Mn	3.71-8. 64	6.56	0.10-9.97	6.59	6.59-23.37	16.41	4. 60-11.53	6.56	53.41-84.11	63.88
	Zn	2.46-17.53	12.50	1.71-17.94	10.15	2.35-17.4	12.38	1.39-9.90	6.25	47.91-92.09	58.73
	Cu	13. 64-81.03	55. 62	4.32-41.9	16.34	3.05-13.97	5.70	2.79-11.39	5.22	8.80-43.56	17.11

	Co	17.01-23.88	20.52	15.86-21.03	18.49	19.30-22.73	20.56	15.96-20.50	18.55	19.77-26.41	21.88
	Ni	10.19-22.71	20.09	10.55-22.73	19.66	5.42-17.02	10.47	3.89-8.83	7.41	35.56-69.96	42.38

The metals viz., Fe, Mn and Ni in cores S-5 and S-7, Fe, Zn, Cu, Co and Ni in the core S-6, and Fe, Mn Zn, Co and Ni in the core S-8 were highest in the residual fraction. The metals bound to the residual fraction are held in the lattice structure of the minerals in sediments and are of natural origin **(Rauret et al. 2001)**. Therefore, it indicated natural weathering of catchment area rocks as the major source of metals in the Sal Estuary. Additionally, the metals were also present in significant (>25 %) or considerable (>10 %) amount in Fe-Mn oxide and organic matter/sulphide fractions. It revealed anthropogenic sources of metals in the estuary (**Zeng et al. 2003).** The metals derived through anthropogenic means are loosely adsorbed on the surface of the sediments particle. A slight fluctuation in the level of dissolved oxygen and degradation of organic matter by microorganisms can cause desorption of metals from sediments (bound to Fe-Mn oxide and organic matter/sulphide fractions) to water column **(Nasnodkar and Nayak 2019)**. Moreover, considerable proportion of Mn, Zn, Cu, Co and Ni in the labile fraction suggested their possible bioavailability with alterations in ionic composition and pH of the water. The metals bound to exchangeable and carbonate fractions are highly susceptible to mobilization with changes in ionic composition and hydrogen ion concentration of water **(Deep and Nasnodkar 2023)**. Thus, the speciation of metals in the sediments of the Sal Estuary revealed bioavailability and their possible mobilization from sediments to water column.

3.2.4 Metal adsorption-desorption signatures from vertical profile

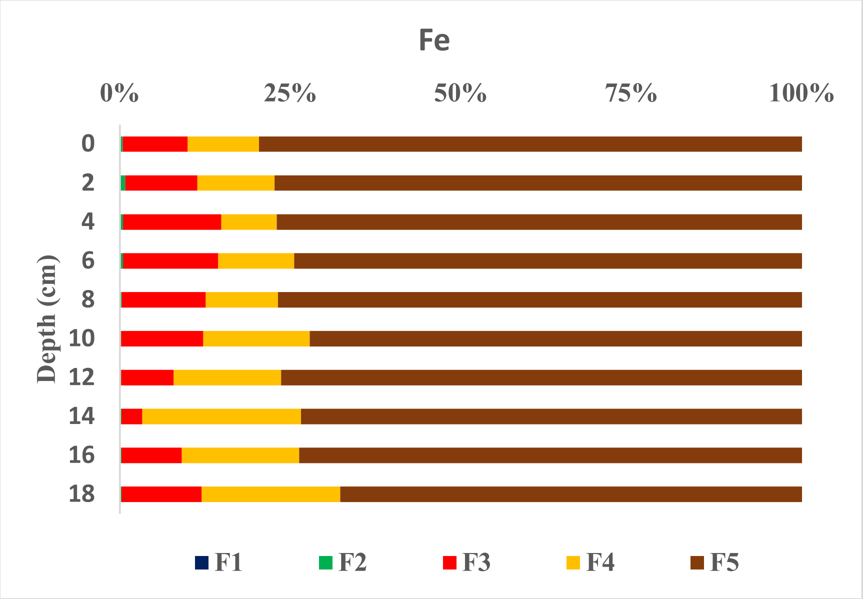


Fig 3.3.1a: The vertical profile of speciation of Fe in core S-5.

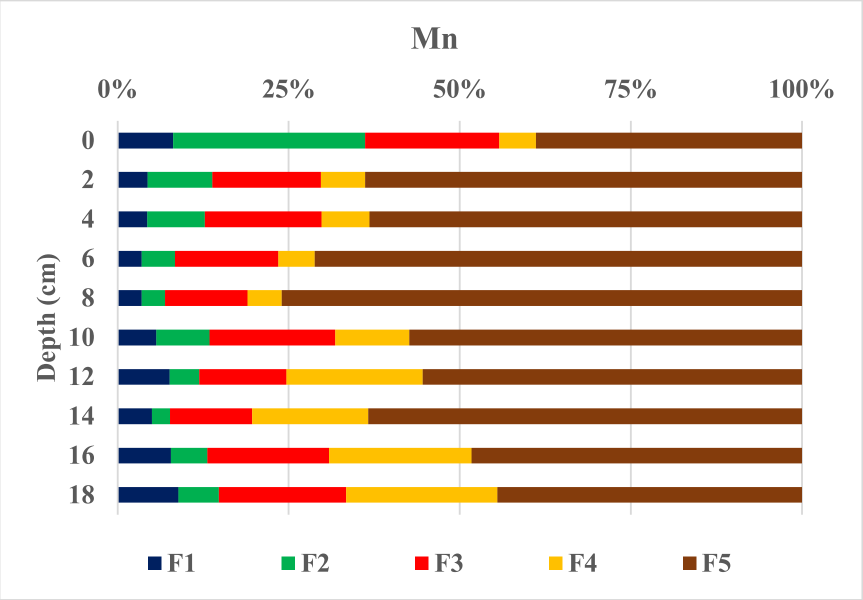


Fig 3.3.1b: The vertical profile of speciation of Mn in core S-5.

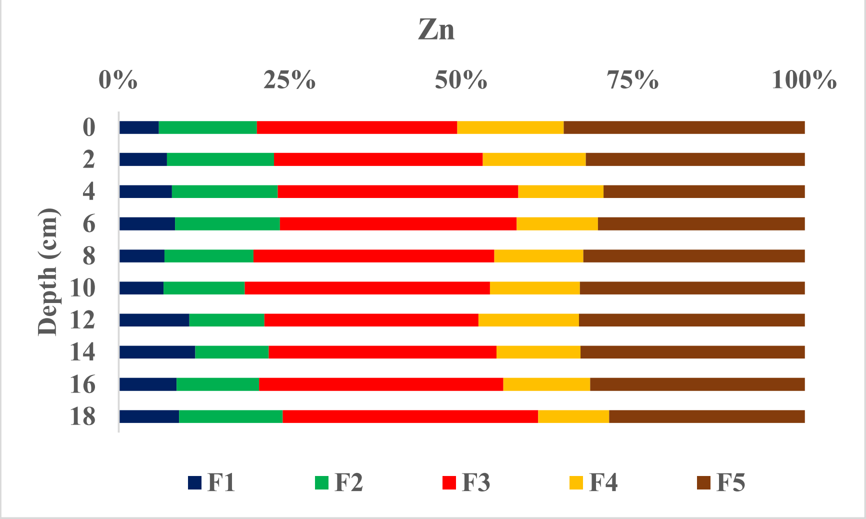


Fig 3.3.1c: The vertical profile of speciation of Zn in core S-5.

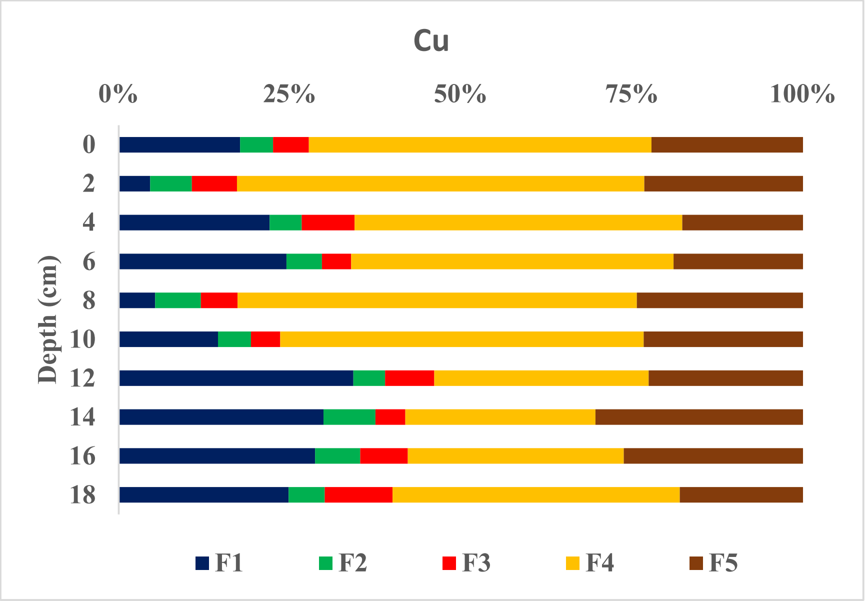


Fig 3.3.1d: The vertical profile of speciation of Cu in core S-5.

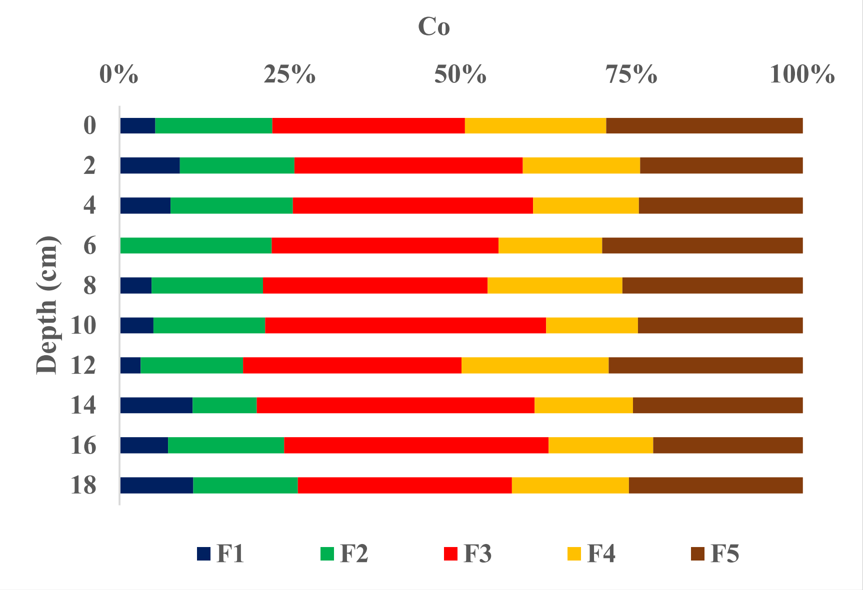


Fig 3.3.1e: The vertical profile of speciation of Co in core S-5.

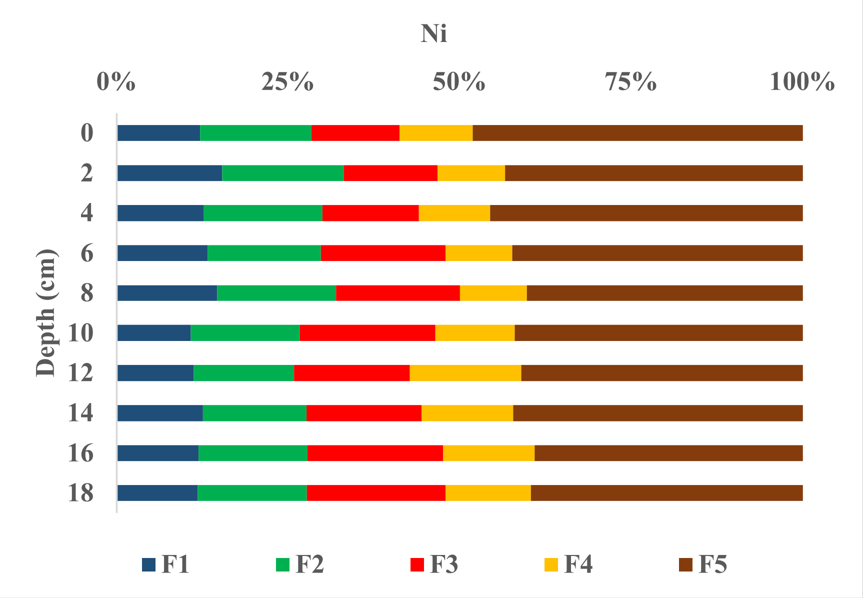


Fig 3.3.1f: The vertical profile of speciation of Ni in core S-5.

In core S-5 (Fig 3.3.1), the concentration of Fe in the residual fraction showed an overall increase from bottom to surface, while in the organic matter/sulphide fraction showed an increase from bottom to 14 cm depth, followed by an overall decrease towards the surface. It showed a decrease from bottom to 14 cm depth in the Fe-Mn oxide fraction, followed by an increase till 4 cm depth. Later, it decreased near the surface. The Mn showed a decreasing trend from bottom to surface in the organic matter/sulphide fraction. Mn was relatively more in bottom and surface sediments, while displayed slight fluctuations in the sub-surface sediments. Its concentration showed an increase towards the surface in the labile fraction. The Zn associated with carbonate and organic matter/sulphide fractions showed an overall increase from bottom to the surface. A slight decrease in Zn bound to exchangeable and Fe-Mn oxide fractions was observed from 6 cm depth to the surface. The Cu displayed an increase near the surface bound to the organic matter/sulphide fraction. In the exchangeable fraction, an overall increase in Cu was seen from bottom to 12 cm depth. Further, it showed an overall decrease with fluctuations towards the surface. The Co associated with carbonate and organic matter/sulphide fractions showed an overall increase near the surface. A slight decrease in Co in the Fe-Mn oxide fraction was observed near the surface, while displayed a fluctuating trend in the exchangeable fraction. Ni bound to Fe-Mn oxide and organic matter/sulphide fractions showed slight decrease towards the surface, whereas a slight increase of Ni near surface was evident in the labile fraction. An increase in Mn, Zn, Cu, Co and Ni in one or more bioavailable fractions near the surface indicated their possible mobilization from sediments with changes in physico-chemical properties of water **(Pereira et al. 2023)**. The fluctuating trend of Fe in the Fe-Mn oxide fraction might be due to variations in the oxygen level with time **(Luther et al. 2008)**. Similarly, fluctuations observed in the exchangeable fraction for Cu suggested its mobilization from sediments with changes in ionic composition of water **(Niu et al. 2015)**.

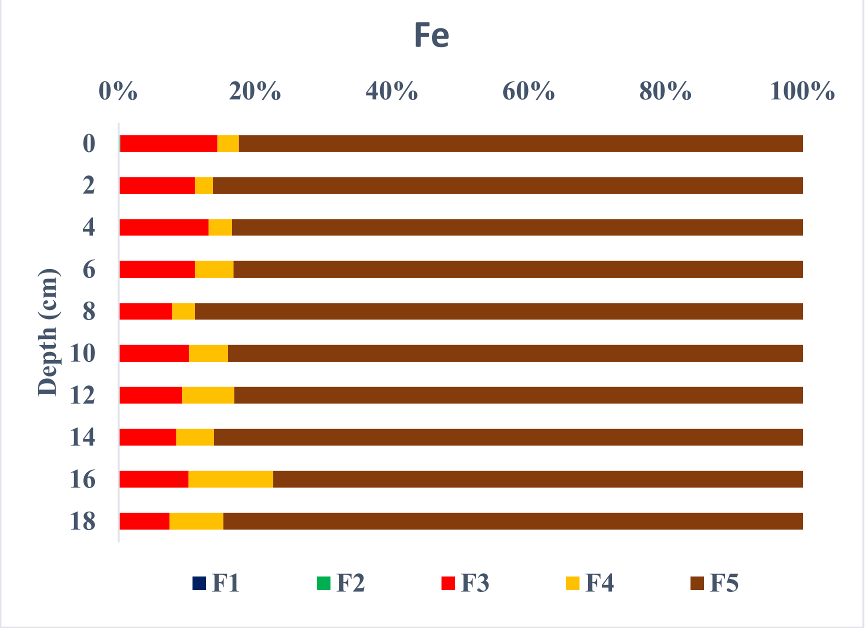


Fig 3.3.2a: The vertical profile of speciation of Fe in core S-6.

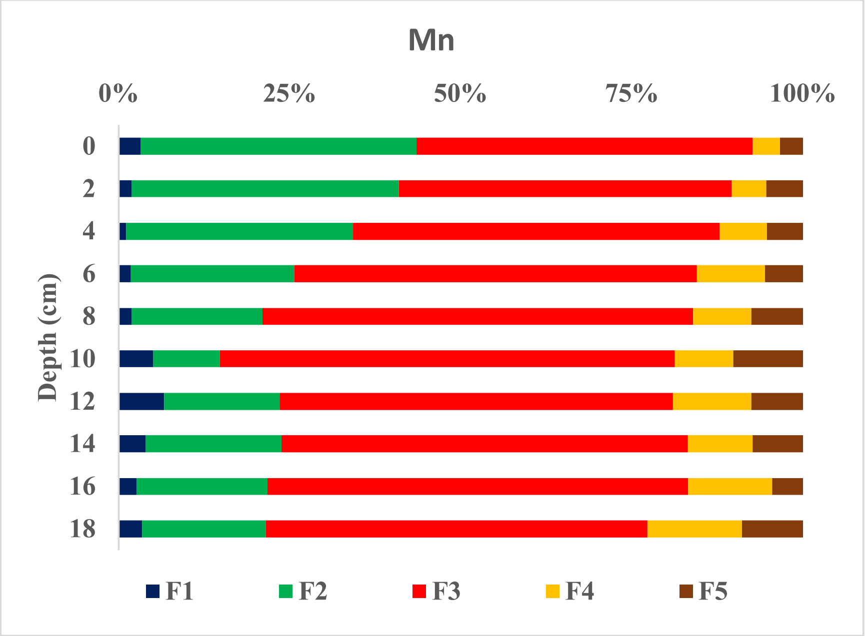


Fig 3.3.2b: The vertical profile of speciation of Mn in core S-6.

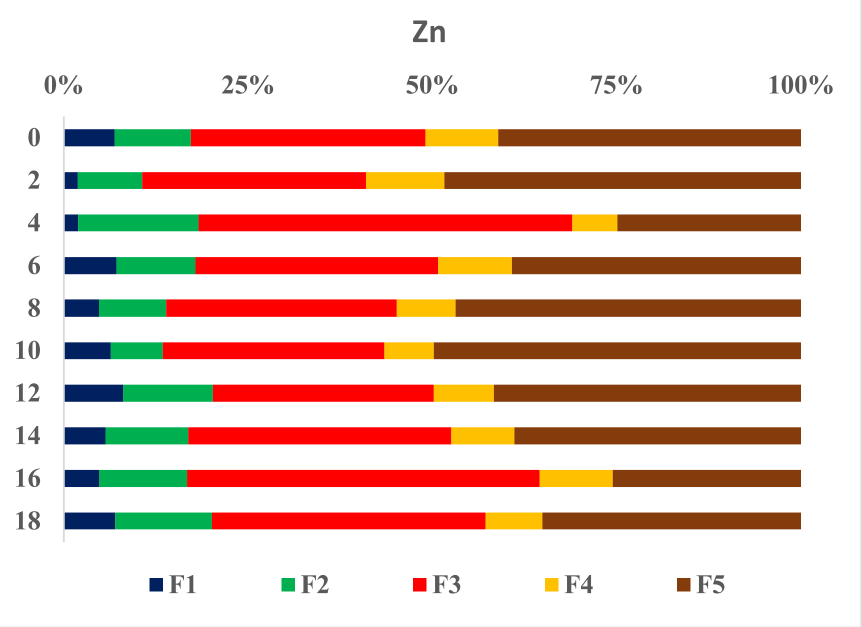


Fig 3.3.2c: The vertical profile of speciation of Zn in core S-6.

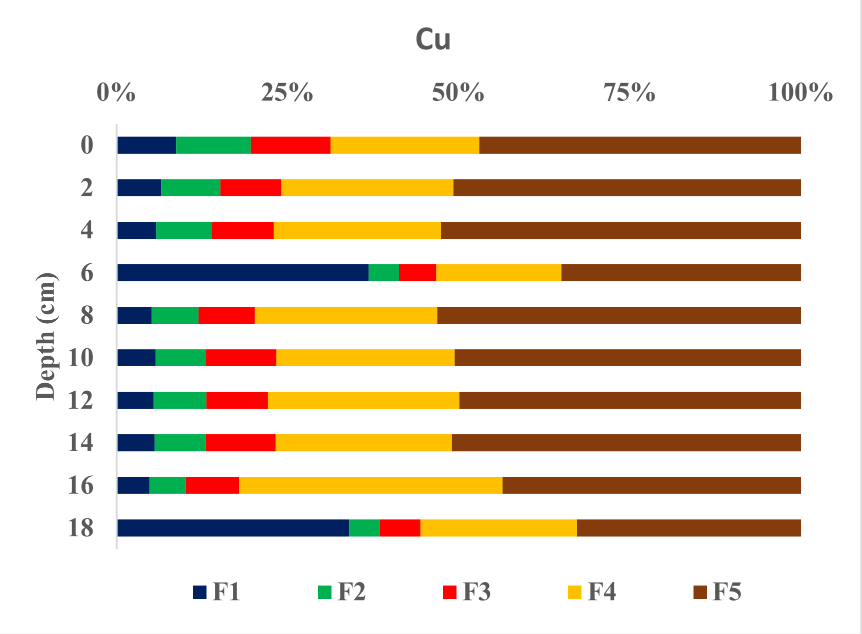


Fig 3.3.2d: The vertical profile of speciation of Cu in core S-6.

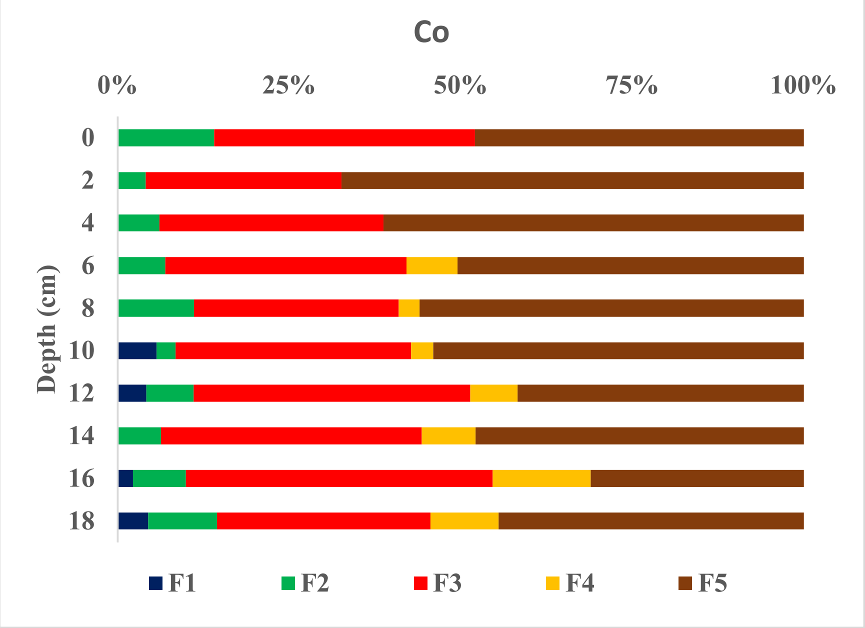


Fig 3.3.2e: The vertical profile of speciation of Co in core S-6.

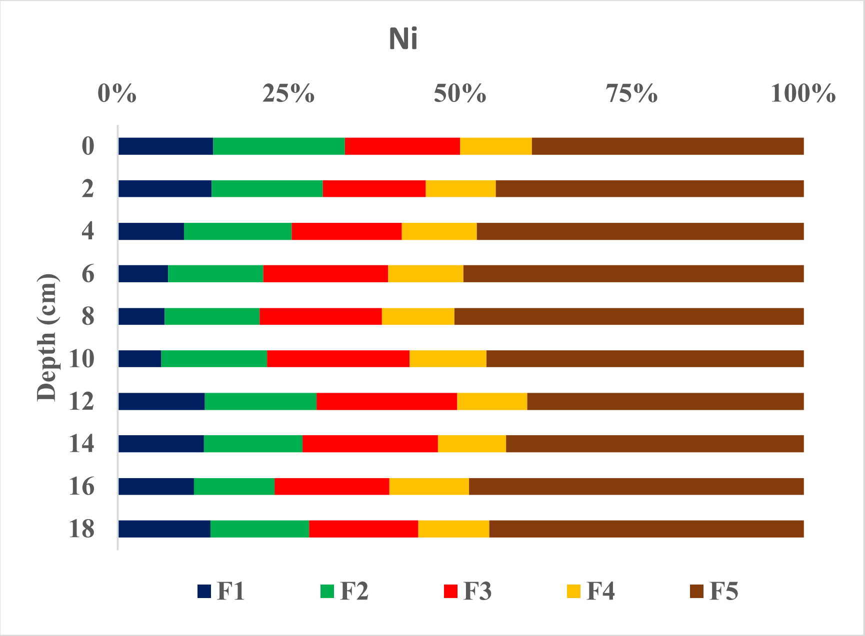


Fig 3.3.2f: The vertical profile of speciation of Ni in core S-6.

In core S-6 (Fig 3.3.2), the concentration of Fe in the residual fraction showed fluctuating trend from bottom to surface, while it decreased in the organic matter/sulphide fraction. Fe in the Fe-Mn oxide fraction showed an overall increase from bottom to surface. The Mn showed an overall decrease from bottom to surface in the organic matter/sulphide fraction, while it slightly decreased towards the surface in the Fe-Mn oxide fraction. Mn associated with carbonate fraction decreased from bottom to 10 cm depth, followed by a prominent increase towards the surface. The Zn associated with the Fe-Mn oxide fraction showed slight fluctuations along the core length, while it slightly increased near the surface in the organic matter/sulphide fraction. The Zn in the labile fraction showed fluctuating trend with an increase in the surface sediments. The Cu associated with the organic matter/sulphide fraction showed slight decrease at the surface, while a slight increase in its concentration was observed in the surface sediments in exchangeable, carbonate and Fe-Mn oxide fractions. The prominent positive peaks of Cu were seen in the exchangeable fraction at 18 and 6 cm depths. The Co in exchangeable and Fe-Mn oxide fractions showed an overall decrease from bottom to 2 cm depth, followed by an increase near the surface. Also, Co in organic matter/sulphide fraction decrease from bottom to the surface. Ni bound to bioavailable fractions showed an increase in top 2 cm of the core. The Fe in Fe-Mn oxide fraction, Mn in Fe-Mn oxide and carbonate fractions, Zn, Cu, Co and Ni in one or more bioavailable fractions exhibited an increase in the surface sediments and thus, indicated their bioavailability in the estuarine biota.

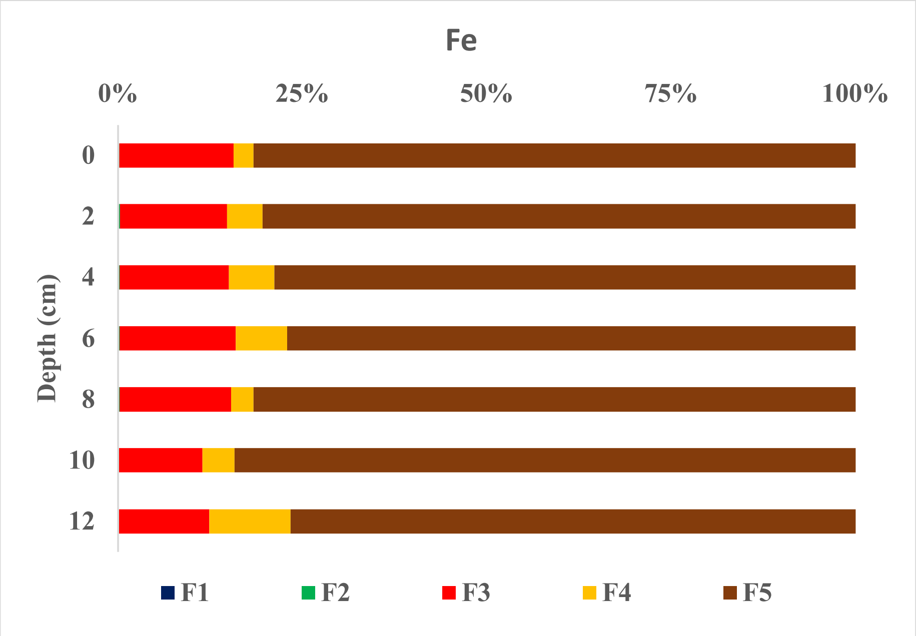


Fig 3.3.3a: The vertical profile of speciation of Fe in core S-7.

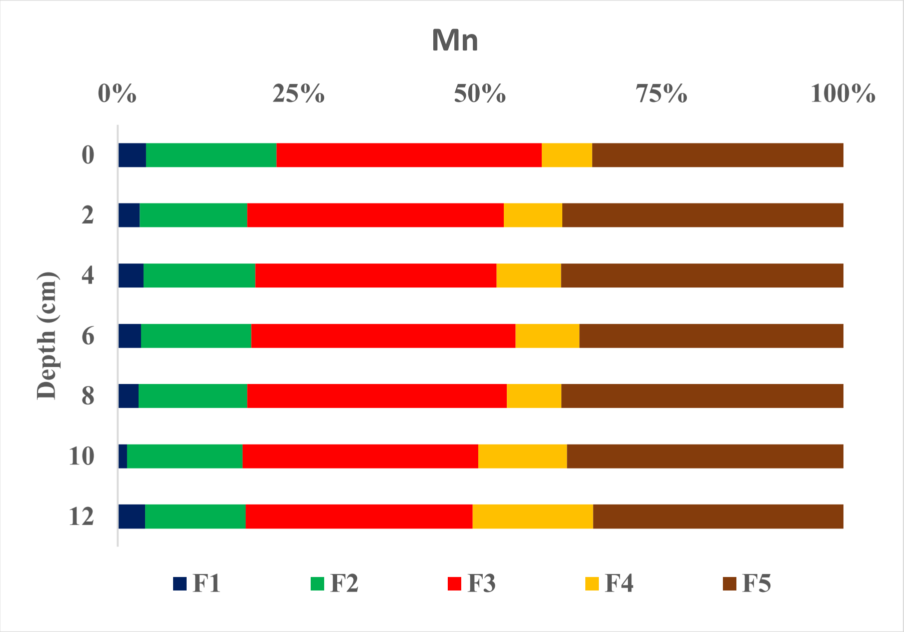


Fig 3.3.3b: The vertical profile of speciation of Mn in core S-7.

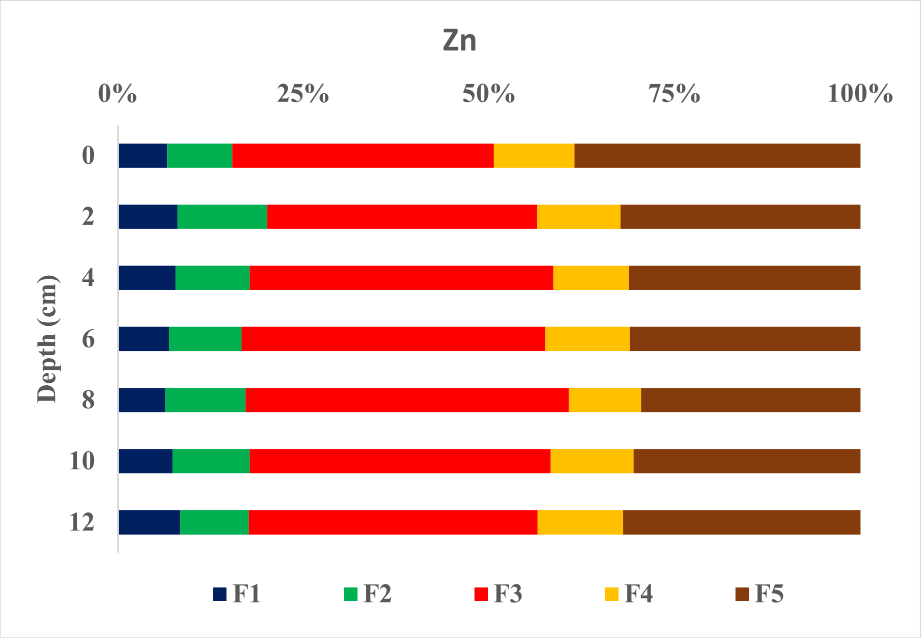


Fig 3.3.3c: The vertical profile of speciation of Zn in core S-7.

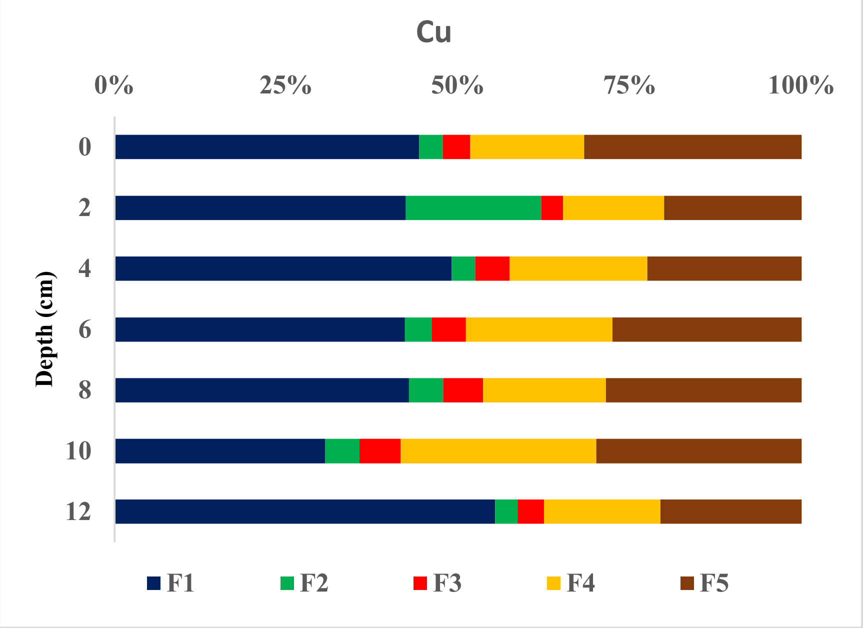


Fig 3.3.3d: The vertical profile of speciation of Cu in core S-7.

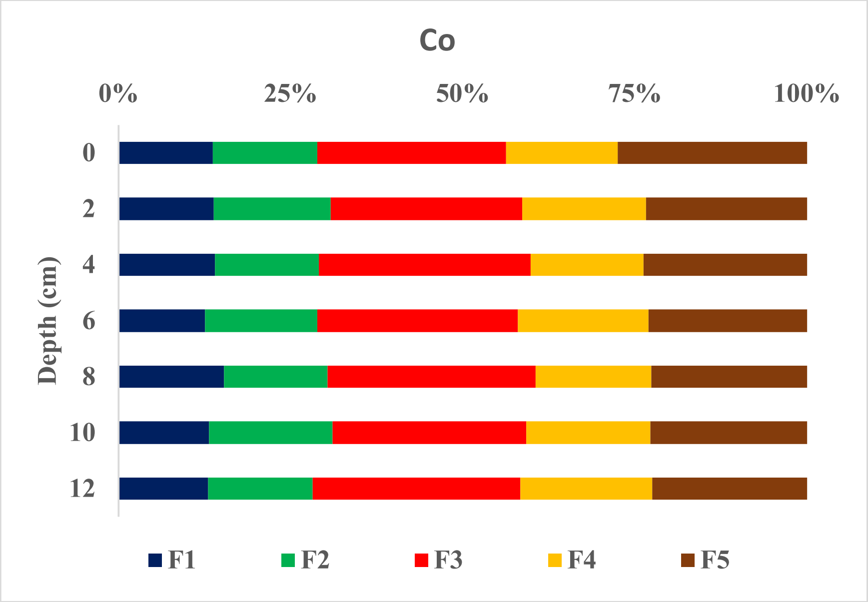


Fig 3.3.3e: The vertical profile of speciation of Co in core S-7.

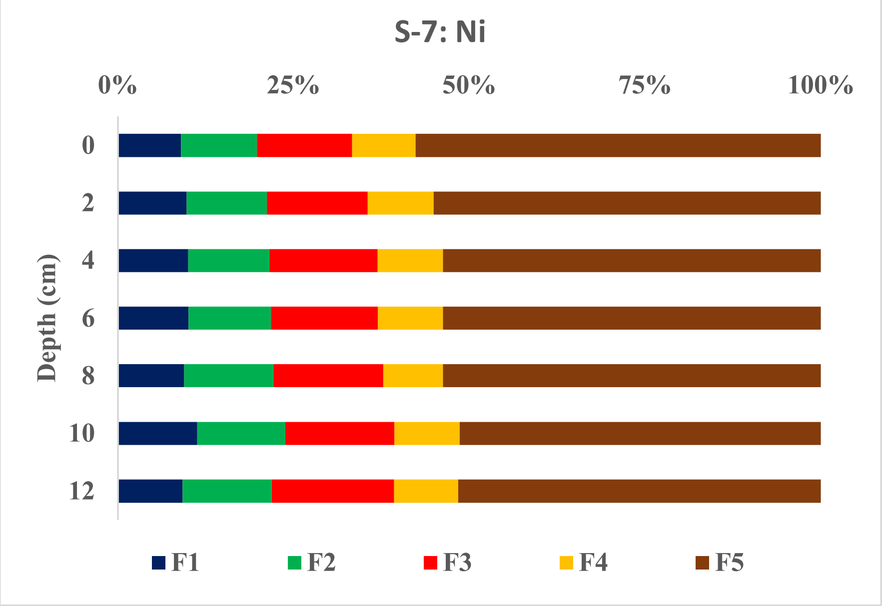


Fig 3.3.3f: The vertical profile of speciation of Ni in core S-7.

In core S-7 (Fig 3.3.3), the Fe associated with the Fe-Mn oxide fraction was higher in the top 6 cm of the core than bottom sediments. On the other hand, Fe in the organic matter/sulphide fraction showed an overall decrease from bottom to the surface. The Mn associated with carbonate and Fe-Mn oxide fractions showed an increase in the surface sediments, while its concentration decreased in the organic matter/sulphide fraction near the surface. The concentration of Zn in the bioavailable fractions showed a overall decrease in the surface sediments, while its concentration increase in the residual fraction. The Cu associated with the exchangeable fraction showed an overall increase from 10 cm depth to surface. The Cu bound to the organic matter/sulphide fraction displayed an overall decrease near the surface as compared to bottom sediments. Not much variations in the bioavailable concentration of Co was observed from bottom to surface sediments, however a slight decrease in the surface sediments was seen. The Ni showed an overall decrease in the bioavailable fraction from bottom to surface. The Fe bound to the Fe-Mn oxide fraction, Mn in carbonate and Fe-Mn oxide fractions, Zn in Fe-Mn oxide and organic matter/sulphide fractions and Cu in the exchangeable fraction displayed an increase in their bioavailable concentration near the surface and thus, indicated bioavailability and possible mobilization from sediment to water column with changes in physico-chemical properties of the water.

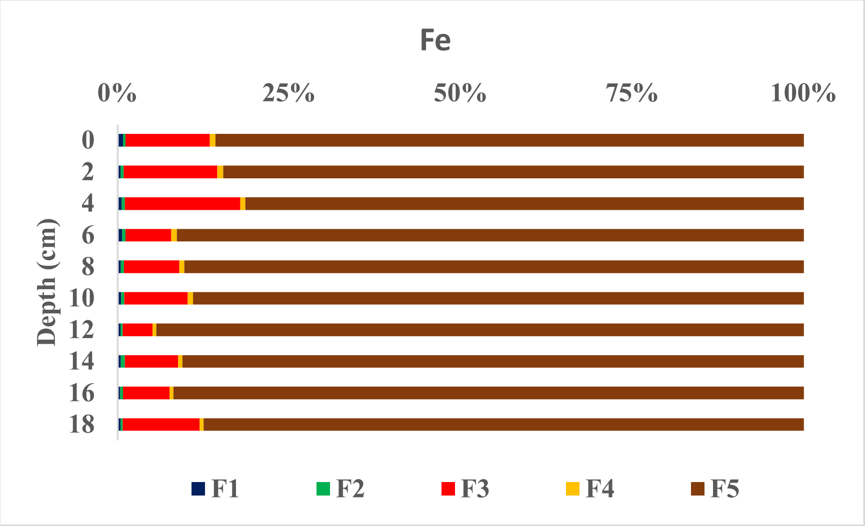


Fig 3.3.4a: The vertical profile of speciation of Fe in core S-8.

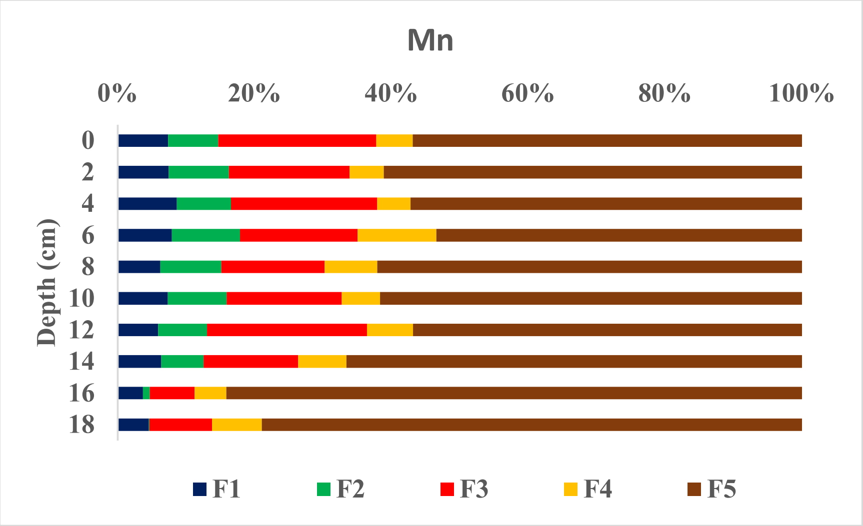


Fig 3.3.4b: The vertical profile of speciation of Mn in core S-8.

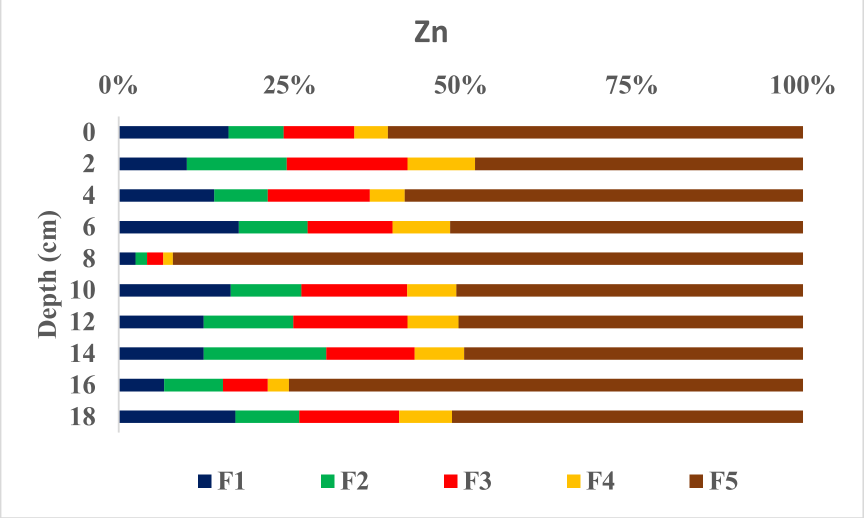


Fig 3.3.4c: The vertical profile of speciation of Zn in core S-8.

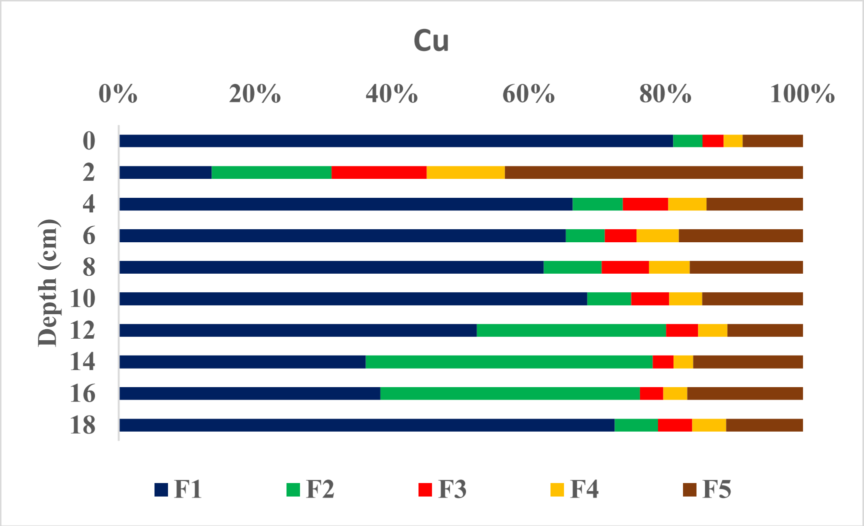


Fig 3.3.4d: The vertical profile of speciation of Cu in core S-8.

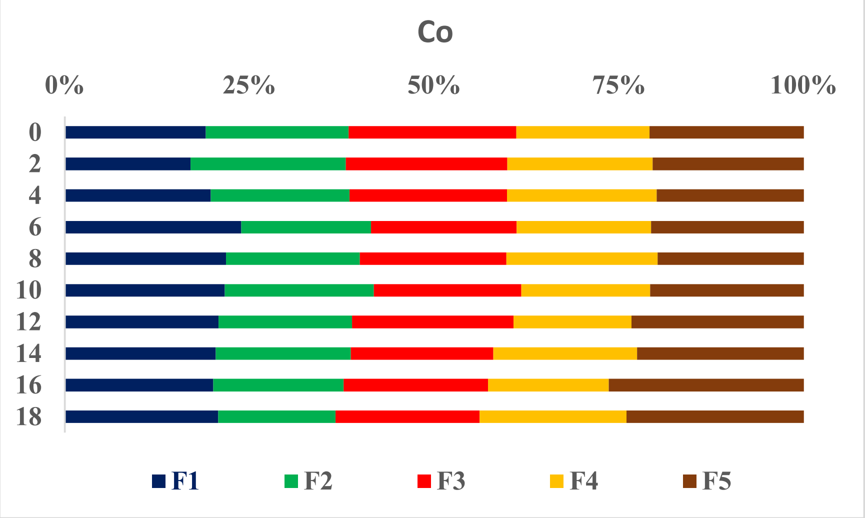


Fig 3.3.4e: The vertical profile of speciation of Co in core S-8.

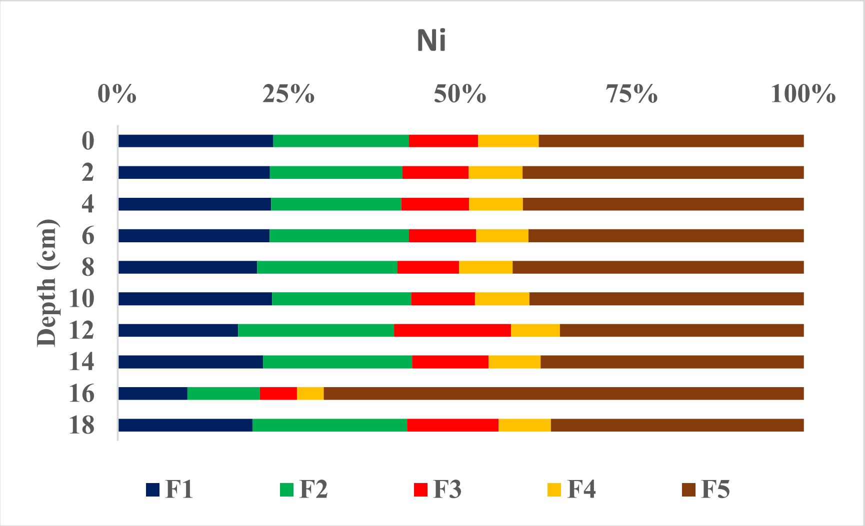


Fig 3.3.4f: The vertical profile of speciation of Ni in core S-8.

In core S-8 (Fig 3.3.4), the Fe associated with the Fe-Mn oxide fraction displayed an overall increase from 16 cm to 4 cm depth, followed by a gradual decrease near the surface. The Mn bound to exchangeable, carbonate and Fe-Mn oxide fractions showed an overall increase from bottom to surface sediments. In the organic matter/sulphide fraction Cu reported slightly higher concentration in the sub-surface (6 cm depth) sediments and later, decreased near the surface. The Zn associated with exchangeable, carbonate and Fe-Mn oxide fractions exhibited fluctuating trend along the core length.

A slight increase in the exchangeable fraction was observed in the surface sediments, while its concentration in the remaining bioavailable fraction decrease near the surface. The Cu bound to the exchangeable fraction showed an overall increase from 16 cm depth to the surface. Its concentration in the carbonate fraction was high in the bottom (16-12 cm depth) sediments. The Co bound to carbonate, Fe-Mn oxide and organic matter/sulphide fractions showed an increase near the surface sediments. Its concentration in the exchangeable fraction slightly increased from bottom to 6 cm depth, followed by a gradual decrease up to 2 cm depth and further increased near the surface. Ni in the bioavailable fraction showed an overall increase in the bioavailable fraction in the surface sediments. The increase in the concentration of Mn, Zn, Cu, Co and Ni in one or more bioavailable fractions indicated bioavailability and possible desorption of metals from sediment to water column with alteration in physical and chemical properties of water.

3.3 Bioaccumulation of metals

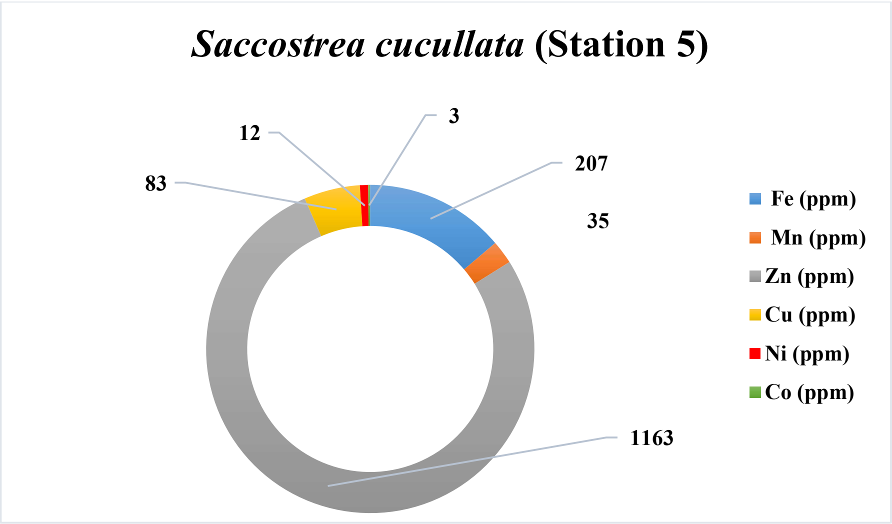


Fig 3.4.1a: The concentration of metals in soft tissue of bivalve *Saccostrea cucullata* at station S-5.

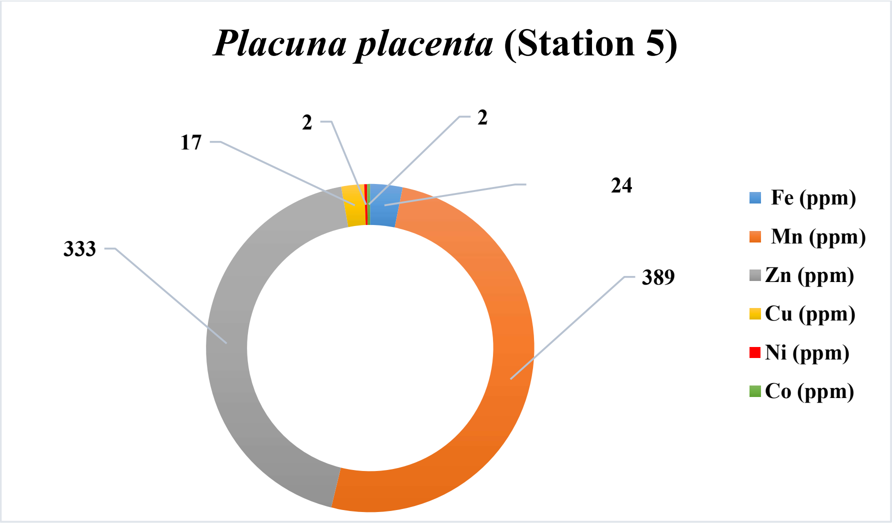


Fig 3.4.1b: The concentration of metals in soft tissue of bivalve *Placuna placenta* at station S-5.

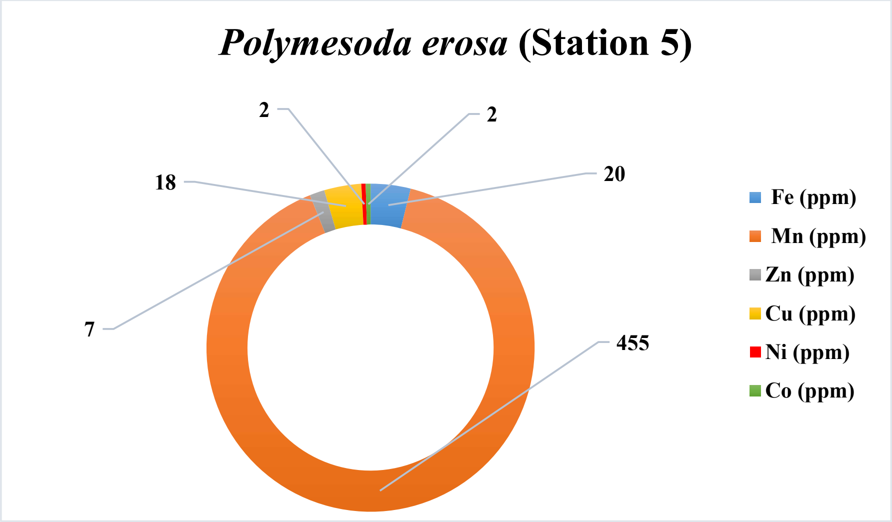


Fig 3.4.1c: The concentration of metals in soft tissue of bivalve *Polymesoda erosa* at station S-5.

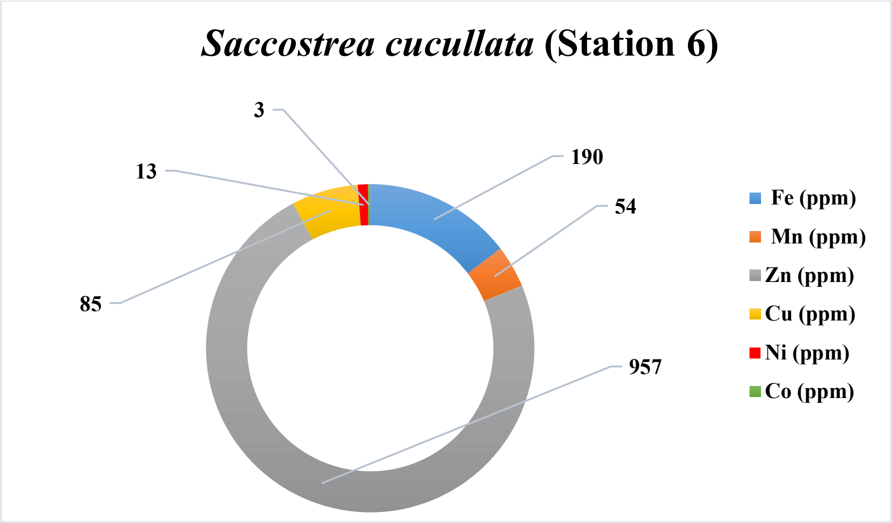


Fig 3.4.2a: The concentration of metals in soft tissue of bivalve *Saccostrea cucullata* at station S-6.

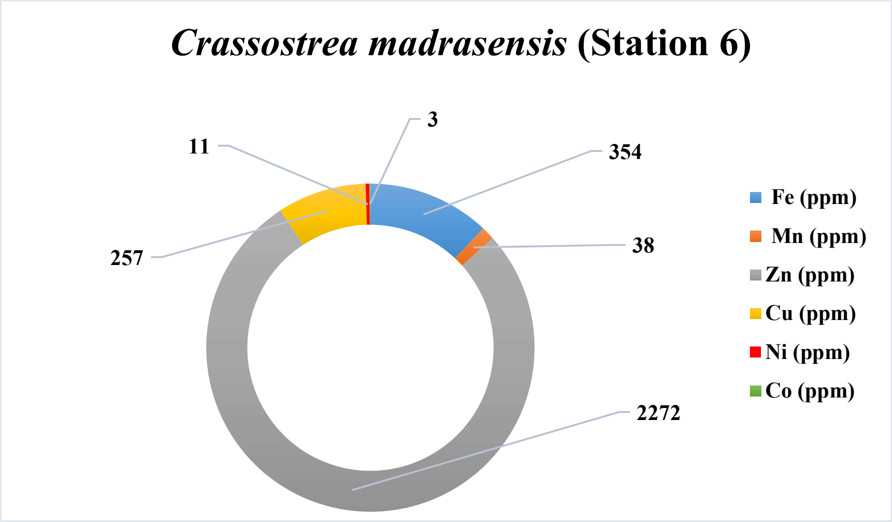


Fig 3.4.2b: The concentration of metals in soft tissue of bivalve *Crassostrea madrasensis* at station S-6.

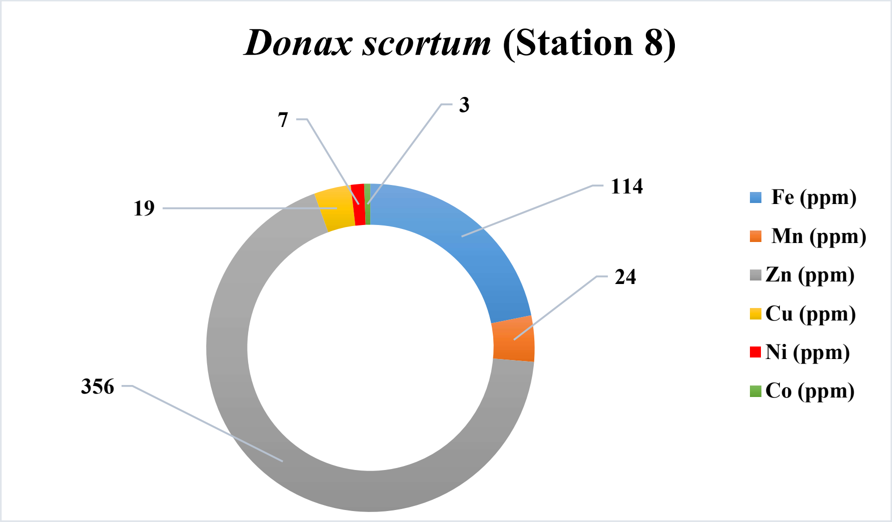


Fig 3.4.3a: The concentration of metals in soft tissue of bivalve *Donax scortum* at station S-8.

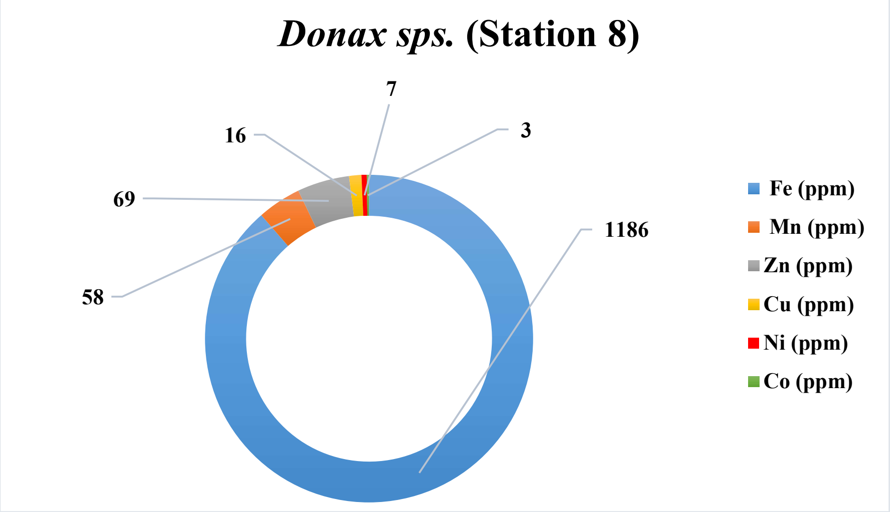


Fig 3.4.3b: The concentration of metals in soft tissue of bivalve *Donax sps.*at station S-8.

The concentration of Zn was highest in soft tissues of *Saccostrea cucullata* at station S-5, whereas, Mn was highest in *Placuna placenta* and *Polymesoda erosa* (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 *Placuna placenta* showed a decreasing order of Mn > Zn> Fe > Cu > Ni > Co. In *Polymesoda erosa,* the accumulation of metals was in the decreasing order of Mn > Fe > Cu > Zn>Ni > Co.

The concentration of Zn was highest in soft tissues of *Saccostrea cucullata* and *Crassostrea madrasensis*, whereas, the concentration of Co was lowest in both the species (Fig 3.4.2). The accumulation of metals was in the decreasing order of Zn > Fe > Cu > Mn > Ni > Co in *Saccostrea cucullata* and *Crassostrea madrasensis.*

The concentration of Zn was highest in soft tissues of *Donax scortum*, while Fe was highest in *Donax* sps. (Fig 3.4.3). Similar to stations S-5 and S-6, all species showed lowest concentration of Co at station S-8. The accumulation of metals was in the decreasing order of Zn > Fe > Mn > Cu> Ni > Co in *Donax scortum,* while *Donax sps.* showed a decreasing order of Fe > Zn > Mn > Cu > Ni > Co.

3.4 Metal Pollution Indices

3.4.1 Geo-accumulation Index (Igeo)

Table 3.4: Geo-accumulation Index (Igeo) for metals in sediments.

Sediment cores	Igeo					
	Fe	Mn	Zn	Cu	Co	Ni
S-5	-0.97	-0.77	0.40	0.55	0.53	2.21
S-6	-0.46	-0.68	0.84	0.61	0.46	2.02
S-7	-0.70	-1.63	-0.10	0.75	-0.05	2.37
S-8	-4.33	-3.20	-0.87	-0.35	-1.23	2.67

The Igeo values for Fe (S-5, S-6, S-7 and S-8), Mn (S-5, S-6, S-7 and S-8), Zn (S-7 and S-8), Cu (S-8) and Co (S-7 and S-8) were < 0, indicating no pollution from these metals (Table 3.4). The Igeo values for Zn (S-5 and S-6), Cu (S-5, S-6 and S-7) and Co (S-5 and S-6) 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 2 to 3 at stations S-5, S-6, S-7 and S-8. It revealed moderate to strong level of pollution of Ni in sediments of the Sal Estuary.

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-5	0.77	0.88	1.97	2.20	2.16	6.93
S-6	1.09	0.94	2.68	2.30	2.06	6.10
S-7	0.93	0.48	1.40	2.52	1.44	7.73
S-8	0.07	0.16	0.82	1.18	0.64	9.55

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 Co exhibited low to moderate level of contamination in the intertidal sediments, while Cu showed moderate level of contamination in the sediments. The Ni showed very high level of contamination in the Sal Estuary.

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 (> 50 %) in the sediments of the 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-5	**S-6**	**S-7**	**S-8**
Fe (%)	-	-	-	-	22 (Neanthes)	0.35	0.32	0.37	0.03
Mn (ppm)	-	-	-	-	260 (Neanthes)	107	51	25	83
Zn (ppm)	124	150	271	410	410 (Infaunal community impacts)	48	37	88	10
Cu (ppm)	18.7	34	108	270	390 (Microtox& Oyster larvae)	21	18	14	24
Co (ppm)	-	-	-	-	10 (Neanthes)	33	41	34	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-5, S-6, S-7 and S-8. It revealed no toxicity of these metals to edible bivalves. On the contrary, the concentration of bioavailable Co at stations S-5, S-6 and S-7 exceeded the AET value, and levelled AET value at station S-8. 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: exchangeable and carbonate)**			
	S-5	**S-6**	**S-7**	**S-8**
Fe	0.33	0.14	0.19	0.95
Mn	13.92	27.08	18.53	13.15
Zn	21.5	16.52	17.49	22.65
Cu	26.3	18.97	50.11	71.96
Co	22.64	9.26	29.6	39.01
Ni	28.9	25.89	21.72	39.75

The RAC revealed no potential adverse effects of Fe on the estuarine biota with values < 1% (Table 3.7). The metals viz., Mn and Zn showed medium potential adverse effect at all stations. The Ni displayed medium potential adverse effects on estuarine biota at stations S-5, S-6 and S-7. It revealed high risk of Ni at station S-8. The risk of Cu to estuarine biota was medium at stations S-5 and S-6, while it showed very high potential adverse effect at stations S-7 and S-8. The RAC indicated low level of risk for Co at station S-6, medium level at stations S-5 and S-7 and high level of risk at station S-8. The analysis of RAC indicated medium-high-very high risk of Mn, Zn, Cu, Co and Ni to the sediment-dwelling biota. The metals in the labile fraction are loosely bound on the sediment surface, and can easily mobilize to water with changes in ionic composition and pH of water **(Nasnodkar et al. 2021)**.

3.5.3 Implications of metal toxicity to humans

Table 3.8: The concentration of metal in soft tissues of edible bivalves.

Stations	Species	Fe (ppm)	Mn (ppm)	Zn (ppm)	Cu (ppm)	Co (ppm)	Ni (ppm)
S-5	*Saccostrea cucullata*	207	35	1163	83	12	3
	Placuna placenta	24	389	333	17	2	2
	Polymesoda erosa	20	456	7	18	2	2
S-6	*Saccostrea cucullata*	190	54	957	85	13	3
	Crassostrea madrasensis	354	38	2273	258	11	3
S-8	*Donax scortum*	114	24	356	19	7	3
	Donax sps.	1186	58	69	16	7	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-5, the concentration of Fe, Mn, Zn, Cu and Co exceeded the standard permissible limit in *Saccostrea cucullata*. Also, the concentration of metals viz., Mn and Zn was more than the consumption safety limit in *Placuna placenta and* Mn in *Polymesoda erosa.* The *Saccostrea cucullata* and *Crassostrea madrasensis* accumulated metals (Fe, Mn, Zn, Cu and Co) at higher level than the permissible limit at station S-6. The concentration of Fe, Mn, Zn and Co exceeded the permissible limit in *Donax scortum* and *Donax sps.* station S-8.

The Fe, Mn, Zn, Cu and Co 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, Cu and Co to humans. Iron toxicity can cause organ damage, particularly to the liver, heart, and pancreas, and it may increase the risk of diabetes, arthritis, and certain cancers. Symptoms of cobalt toxicity may include cardiomyopathy thyroid dysfunction, neurological symptoms and skin disorders.

3.5.4 modified Biota Sediment Accumulation Factor (mBSAF)

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

Sediment core	**Species**	**mBSAF**					
		Fe	**Mn**	**Zn**	**Cu**	**Co**	**Ni**
S-5	*Saccostrea cucullata*	0.06	0.73	35.65	2.80	0.37	0.46
	Placuna placenta	0.01	8.09	10.21	0.57	0.25	0.08
	Polymesoda erosa	0.01	9.48	0.21	0.61	0.25	0.08
S-6	*Saccostrea cucullata*	0.06	1.45	23.10	6.22	0.62	0.57
	Crassostrea madrasensis	0.11	1.02	54.86	18.88	0.62	0.48
S-8	*Donax scortum*	0.37	2.48	37.02	0.79	0.27	0.41
	Donax sps.	3.80	6.00	7.17	0.67	0.27	0.41

According to mBSAF, all edible bivalve species retrieved from the Sal Estuary were de-concentrator of Fe, Co and Ni, except for *Donax sps*. (station S-8) which was macro-concentrator of Fe (Table 3.9). At station S-5, *Saccostrea cucullata* was de-concentrator of Mn, whereas *Placuna placenta* and *Polymesoda erosa* were macro-concentrator of Mn. Also, at station S-5 *Saccostrea cucullata* and *Placuna placenta* were macro-concentrator of Zn, whereas *Polymesoda erosa* was de-concentrator of Zn. *Saccostrea cucullata* was macro-concentrator of Cu at station S-5, while *Placenta placenta* and *Polymesoda erosa were* de-concentrator. *Saccostrea cucullata* and *Crassostrea madrasensis* were micro-concentrator of Mn and macro-concentrator of Zn and Cu at stations S-6. *Donax scortum* and *Donax sps.* were macro-concentrator of Mn and Zn at station S-8, while both species were de-concentrator of Cu. Considering the results of mBSAF the edible bivalves had high affinity towards Zn followed by Mn and Cu.

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.

Station	**Species**	**Mangrove plant parts**	**Metal (ppm)**					
			Fe	**Mn**	**Zn**	**Cu**	**Co**	**Ni**
S–5	*Avicennia alba*	Pneumatophores	1904	104	28	36	5	9
		Stem	189	10	14	24	3	4
		Leaves	150	45	33	27	2	6
S-6	*Avicennia marina*	Pneumatophores	2641	77	104	35	4	8
		Stem	112	31	19	29	3	5
		Leaves	150	37	24	28	3	5

The concentration of metals in different parts of mangrove plants is presented in Table 3.10. Among the metals, Fe 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. (2015)** 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 viz., respiration, chlorophyll biosynthesis, and photosynthesis, and serves as a cofactor for enzymes involved in electron or oxygen transfer **(Kobayashi et al. 2019)**.

In the present study, the higher concentration of Fe observed in pneumatophores 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)**. Also, the development of Fe-plaque might have facilitated the adsorption of other metals on the surface of pneumatophores that were higher in concentration in pneumatophores than in stem or leaves. The Co displayed lowest accumulation in different parts of the mangrove plants. The variations in the concentration of metals between different species collected from different sampling stations of the Sal Estuary was attributed to 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 and 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.

Stations	**Species**	**Ratio**	**TF**					
			Fe	**Mn**	**Zn**	**Cu**	**Co**	**Ni**
S–5	*Avicennia alba*	Leaves/root	0.079	0.43	1.17	0.74	0.40	0.64
S-6	*Avicennia marina*	Leaves/root	0.06	0.48	0.23	0.81	0.75	0.64

The TF values for metals are presented in the Table 3.11. According to TF, *Avicennia alba* was accumulator of Zn at station S-5, while extruder of metals such Fe, Mn, Cu, Co and Ni. Similarly, *Avicennia marina* was extruder of all the metals at station S-6. Thus, the present study revealed the application of mangrove species viz., *Avicennia alba* in the phyto-remediation of Zn 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 sediments, bioaccumulation in edible bivalves, and to understand the phyto-remediation potential of mangrove plants in the lower region of the Sal Estuary. Four sediment cores namely, S-5, S-6, S-7 and S-8 of 10-20 cm length were retrieved from the inter-tidal region. Also, edible bivalves and mangrove plant samples were collected in the proximity to sampling sites. The percentage of sand was > 50 % in the lower 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-6), Zn (S-5, S-6 and S-7), Cu (S-5, S-6, S-7 and S-8), Co (S- 5, S-6 and S-7) and Ni (S-5, S-6, S-7 and S-8) exceeded the upper crustal average value. It indicated their enrichment in sediments due to anthropogenic input from discharge of paint particles from trawlers at fishing jetties, the scraping of ship hulls, and the release of domestic, agricultural and aquaculture waste. 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-5, S-6, S-7 and S-8), Zn (S-5, S-6, S-7 and S-8), Cu (S-5, S-6, S-7 and S-8), Co (S-5, S-7 and S-8), Ni (S-5, S-6, S-7 and S-8) 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 very high risk of Cu to the sediment dwelling biota at stations S-7 and S-8. The mBSAF indicated high affinity of edible bivalves towards Zn and Mn. The bivalves were found as macro-concentrator of Zn, except for *Polymesoda erosa* at station S-5. 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 *Avicennia alba* in the phyto-remediation of Zn in the Sal Estuary.

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