Chemical speciation of metals in estuarine sediments and their accumulation in estuarine benthic organisms

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DOCTOR OF PHILOSOPHY

In

Marine Sciences

By

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Dedicated to My Family and

Teachers

Statement

As required under the University ordinance **OA-19.8 (v-viii)**, I state that the present thesis entitled "*Chemical speciation of metals in estuarine sediments and their accumulation in estuarine benthic organisms''* is the original research work carried out by me. I further declare that this work has not been previously submitted and is not concurrently submitted for any other degree at Goa University or at any other university or institutions.

The literature related to the problem investigated has been cited. Due acknowledgments have been made wherever facilities and suggestions have been availed off.

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8th May 2019

Certificate

This is to certify that the thesis entitled "*Chemical speciation of metals in estuarine sediments and their accumulation in estuarine benthic organisms''* submitted by Darwin Devidas Ramteke to the Goa University for the degree of Doctor of philosophy, is based on his original studies carried out by him under my guidance. The thesis or any part of this work has not been previously submitted for any other degree or diploma in any university or institution.

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Preface

A first comprehensive study was performed to establish the linkage between metal speciation in tropical estuarine sediments and their bioaccumulation in benthic organisms. An effort was also made to identify the environmental factors that control metal speciation in tropical estuarine systems. In this thesis, the concentrations of bioaccumulated Cu and Ni in mangrove roots (pneumatophores) from Mandovi estuary of Goa was found to be high. Chemical speciation study of Cu and Ni in estuarine sediments (from mangrove areas) suggests that Fe/Mn hydroxide and organic phases were the primary hosting phase for non-residual Cu and Ni. Cu was found strongly associated with the sedimentary organic phase and controlled Cu bioavailability in the sediment systems. The combination of two chemical speciation methods suggests that non-residual Cu and Ni were labile with fast dissociation rate constants. Labile complexes were found to be bioavailable in the sediment system. Based on the speciation study of Cu and Ni, a plausible disjunctive pathway of metal uptake from sediments by the mangrove roots was suggested.

The thesis suggests that Cd speciation in finer sediments compare to the bulk sediments provides better information on bioavailable Cd in estuarine sediments. Finer sediments were found to have higher concentrations of sedimentary organic matter and total Cd concentration. The level of bioaccumulated Cd in oysters tissue was elevated and was above the maximum permissible limit for human consumption. Cd speciation study of finer particles suggests that exchangeable, carbonate, and bicarbonate forms of Cd in the finer sediment, were labile in nature. Labile Cd-sediment complexes increased

total bioaccumulated Cd concentration in oysters. Chemical speciation of Cd in finer sediment was found to play important roles in controlling Cd bioaccumulation in an oyster at the tropical estuarine system.

The thesis also presents the influence of environmental factors on metal speciation in tropical estuarine systems. Variation of pH in the overlying water column significantly influenced Cu speciation at the bottom estuarine sediments. An increase in pH was found to increase Cu association with the organic phase and further increased the water-soluble Cu from the sediments. The study suggests that an increase in pH may cause leaching of Cu organic complexes from sediment which may enter the water column and could increase Cu mobility and its bioavailability in the sediment system.

Variation in pH and salinity of overlying water column showed no effect on Cd speciation, however, an increase in salinity has increased the total bioaccumulated concentration of Cd in oyster (*Magallana* sp.) species. The increased Cd bioaccumulation was due to an increase in filtering activity (a favorable condition during high salinity). Thus, the study indicated that the environmental parameter of the overlying water plays a significant role in influencing metal speciation (bioavailability) and its accumulation in the estuarine organisms.

The scientific outcome of this thesis work provides a relationship between chemical speciation and their bioavailability in the estuarine sediment system. This thesis also suggests that metal speciation study in sediments (rather than total metal concentration) can be used as an important tool to delineate metal bioaccumulation in tropical estuarine systems.

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Introduction

1.1 Overview

Estuary, the most dynamic environment of the coastal zone, is identified as unique ecotones that connect land and ocean, here seawater is diluted by freshwater brought from the land. An estuary is a buffer region between riverine (freshwater) and oceanic environment (saltwater) and is influenced by tidal oscillations. They are highly dynamic and subject to changes over very short periods to geological periods. An estuary is defined in many ways (Dalrymple et al., 1992; Dionne, 1963; Kjerfve and Medeiros, 1989; Perillo, 1995; Pritchard, 1967), but the most accepted and relevant definition of an estuary was proposed by Potter et al., 2010:

An estuary is a partially enclosed coastal body of water that is either permanently or periodically open to the sea and which receives at least periodic discharge from a river(s), and thus, while its salinity is typically less than that of natural seawater and varies temporally and along its length, it can become hypersaline in regions when evaporative water loss is high and freshwater and tidal inputs are negligible.

Since estuaries are transition zone, they are ecologically the most productive ecosystem on the earth. Estuaries are nursery grounds for many marine and riverine organisms and provide life-cycle accomplishment of many invertebrates and fish species. Various habitats could be found in and around estuaries such as salt marshes, sandy beaches, mud and sand flats, rocky shores, mangrove forests, river deltas, tidal ponds, and seagrass beds.

Estuarine areas have been greatly influenced by the human population since the beginning of civilization. Augmented growth of human settlement and development in the coastal areas, accompanied by increased industrialization and urbanization has created combined anthropogenic impacts on the ecological health of many estuaries. Around 4 billion people are

now estimated to inhabit within 60 km of the world's coastline area, which has placed tremendous pressure on the sensitive estuarine ecosystem (Goldberg, 1995; Kennish, 2002, 2000, 1996; McIntyre, 1995). Various human activities such as an increase in demand for energy, mineral resources, houses for human settlement, increased output of sewage and other waste products (Flemer and Champ, 2006) have greatly impaired estuarine water quality, and lead to habitat loss. The Joint Group of Experts on the Scientific Aspects of Marine Pollution (GESAMP), an international committee authorized by the United Nations (UN), has periodically assessed the condition of the worldwide marine environment and observed that pollutants enter estuarine environments through mainly six pathways: (1) land run-off (2) direct release of pollutants through pipeline (3) inflow through rivers (4) atmospheric deposition (5) maritime transportation and (6) straight waste dumping at sea (Goldberg, 1995; Kennish, 1996; McIntyre, 1992).

Once the pollutants enter an estuarine environment, they are exposed to a variety of physical, chemical, geological, and biological processes that could degrade or could ultimately remove the pollutant from the system. But persistent chemicals, like metals, do not break down and may cause serious environmental complications in an estuary. Metals are natural elements and are generally present in very low concentrations. Some of the metals are essential for the biological functions of an organism and few of them are purely beneficial, but it may also turn toxic if taken or enters in the biological system of a living organism in high concentration. While some of the metals have no biological function and are toxic even in low concentration. In the estuarine environment, the metals of greatest concern are Copper, Nickel, Zinc, Cadmium, Mercury, Chromium. Toxic metals are often cumulative toxins and continue to increase in concentration and may be found abundantly in tissues of a biota. The accumulation

of toxic metals may result in food chain magnification of concentrations in animals at higher trophic levels.

Toxic metals in estuaries derive from both natural and anthropogenic sources. Anthropogenic inputs to estuarine waters originate from mining and smelting operations, refining and electroplating, dye and paint manufacture, and fossil-fuel burning activities. River discharges, urban run-off, and atmospheric deposition transport bulk of the total toxic metal that ultimately brought and accumulates in the estuarine region (Bothner et al., 1998; Cearreta et al., 2000; de Groot, 1995; Rozan and Benoit, 1999; Turner, 2000). The metal pollutant which is transported and accumulated in an estuarine system is majorly deposited to the bottom sediments. In an estuary, the sediments are majorly brought and or deposited through rivers, waves, and tidal action. Rivers bring the majority of fine-grained sediments from the continents into the estuaries. While waves and especially tides (tidal current) provide sturdy energy that transport coarser marine sediments to and fro from sea to the estuary. The erosion, transportation, and burial of fine-grained sediments in an estuary are majorly governed by three chief processes: estuarine mixing, aggregation (flocculation), and primary particle properties (Edzwald and O'Melia, 1975).

Mangroves, one of the highly ecologically important ecosystems supported by an estuary are the major site for sediment deposition and retention of metals. Mangroves are the group of tree and shrub species that inhabits the intertidal coastal zone. It is distributed along the sheltered estuarine area, of the tropical and subtropical regions (Dwivedi and Padmakumar, 1983). The unique transitional coastal ecosystem provides many natural services, such as shoreline protector (from devastation storm, hurricanes, erosion), acting as the nursery (providing nourishment and refugee for shrimp, molluscan, crustaceans and fish), asylum to threatened

and endangered floral and faunal species and a potential source of renewable material (source of wood, tannins, dyes, traditional medicine, apiculture). Mangrove species compare to its territorial counterpart has evolved successfully in the saline conditions of the estuary with the help of their specialized aerial and dense root systems. These roots afford strong anchoring in the sediment and infiltrate oxygen deep to the submerged roots. Physically, the complex structure of the mangrove root system behaves as an active particle-trapper. It reduces the energy of river water flow and water brought during tidal flood resulting in the settlement of suspended material carried by the water column to the bottom sediment. This activity leads to the high deposition of fine-grained (< 63 μm) sediments in the mangrove system. It also increases the carbon sequestration in the mangrove sediments, brought from the rivers and adjacent ecosystem along with the carbon contributed by mangroves (leaf and litter of roots and leaf). It is estimated that the coastal region is responsible for burying 50 times more carbon than those in tropical rainforests (Bouillon, 2011). Mangroves account for 14% of carbon sequestered in the world's ocean (Alongi, 2012).

The deposited anaerobic and reduced fine sediments with high sulfide and organic matter in the mangrove habitat favors the entrapment of various pollutants, especially the toxic metals (Huerta-Diaz and Morse, 1992; Qiu et al., 2011) brought by various human activities. Fine-grained mangrove sediments (< 63 μm) due to its large surface area-to-grain size and organic content favors the (Adriano, 1986; Horowitz and Elrick, 1987; Moore et al., 1989) high accumulation of toxic metals to its sediments. The metals which are bound to the fine sediments are probably more bioavailable compare to bulk sediments due to high cation exchange capacity (Bradl, 2004; Fernandez-Bayo et al., 2008).

1.2 Toxic metal behavior in Estuary

Metals are basically cations (electro positively charged particles) forming complexes with negatively charged surfaces (anion) of clay particles, organic matter, and Fe and Mn oxides,etc. in the sediments (Evans, 1989). The mobility and bioavailability of metal from estuarine sediments will decrease with an increase in the complex formation and therefore retaining a high concentration of metal (Du Laing et al., 2009). The destiny and transport of metals once entered in the estuarine environment are dependent on several factors such as redox conditions, ionic strength, pH, the temperature of the water column, particle size and total surface available for adsorption (John and Leventhal, 1995; Wang and Fisher, 1999). For example, an increase in ionic strength influences metal transformation processes such as adsorption or desorption, flocculation, coagulation, precipitation, and biotic assimilation or excretion.

Removal of metal from water by adsorption on sediments (sediment as a sink) or desorption of metals from sediment to the water column (sediment as source) usually occurs during estuarine mixing. Toxic metals that are adsorbed on to suspended particles are scavenged from the water column and brought down ultimately to bottom sediments (Daskalakis and O'Connor, 1995; Karickhoff, 1984; Lee et al., 1998).

Metals that are bound to estuarine bed sediments could release back to the water column by various processes such as re-suspension of bed sediments, desorption of metals from sediment particles, and diffusion of pore water metals to the overlying water (Liu, 1996). Thus, estuarine sediments act as both sink and source of toxic metals, acting as filters to the pollutants between land and sea and proved to be an excellent indicator of environmental pollution. The mobility of toxic metals associated with sediments entirely depends on their chemical and physical speciation. In an environment, 'Bioavailable metal' comprises of metal species that probably

are bio accessible and have the potential for distribution, metabolism, elimination, and bioaccumulation by a specific organism (McGeer et al., 2004). While 'Bioaccumulated metal' defined as the metal fraction which is internalized (from the outside environment to inside biological system through membrane or tissue) by organisms.

Estuarine sediments which often accumulate high concentration of toxic metals brings a high risk on the health condition of associated biotic communities and the organisms in higher trophic levels through bioaccumulation in their tissues (Marsden and Rainbow, 2004). As a result, biotic communities may endure considerable changes, including the ecological loss of rare or sensitive species, decrease in abundance of species, alterations in the age structure, and altered trophic interactions (Rainbow, 2002). The toxicity of metal from sediments will greatly impact benthic organisms (flora and fauna which lives in, on, near the bottom sediment) as they are generally sedentary and long-lived organism which could not escape when exposed to the sediments with a high concentration of toxic metals. Hence the organism with chronic exposure develops several biological disorders such as dysfunction of feeding, digestive and respiratory activities, effect on calcium ion transport channel, functional loss of enzyme activities, abnormal physiological and neurological activities, impact on reproductive activity, effect on ribosomal activities, protein homeostasis, increase in microbial infections, degeneration of tissues and damage of genetic material [DNA (Deoxyribonucleic acid) and RNA (Ribonucleic acid)]. Toxic substances that are bioaccumulated in the specific trophic level organism will be transported and biomagnified in the entire estuarine food chain also ultimately pose a potential health threat to humans who consume the contaminated fish and invertebrates (Kennish, 2002).

1.3 Toxic metal speciation

Over the years, determination and quantification of total metals are used to assess the impact of metals and its toxicity from estuarine sediments. But, recently determination of total toxic metal concentration in sediments is considered as only the simplified way of expressing metals toxicity (Benson et al., 2013). The environmental behavior, mobility, bioavailability, and toxicity of any metal is a function of their chemical speciation (presence of its different chemical form). Speciation of an individual metal reflects its reactivity, solubility, and the nature of uptake.

The International Union of Pure and Applied Chemistry (IUPAC) has defined Chemical speciation as follows:

1. *Chemical species*. Chemical elements: a specific form of an element defined as to isotopic composition, electronic or oxidation state, and/or complex or molecular structure.

2. *Speciation analysis*. Analytical chemistry: analytical activities of identifying and/or measuring the quantities of one or more individual chemical species in a sample.

3. *Speciation of an element*. Speciation: Distribution of an element among defined chemical species in a system.

However, it is normally not possible to determine the concentrations of all the different chemical species of an element in a given sample. In such a case, the term fractionation is used

4. *Fractionation*. Process of classification of an analyte or a group of analytes from a certain sample according to physical (e.g., size, solubility) or chemical (e.g., bonding, reactivity) properties.

Chemical speciation analysis helps us to understand the association of various metals with different binding phases of sediments. Not all the total metal associated with estuarine sediment participate in geochemical processes or bioavailability, only a part of the total metal is bioavailable and could bioaccumulate in an organism (Li et al., 2000; Peijnenburg et al., 2007; Singh et al., 2005). Chemical speciation analysis helps us to understand the association of various metals with different binding phases of sediments.

In the sediment system, metal present as water soluble, exchangeable complexes, metals associated with carbonates, metals bound to oxyhydroxide of Fe and Mn, and metals associated with organic matter and sulfides are considered as non-residual complexes and are the good indicator of metal bioavailability (Chakraborty, 2012; Peijnenburg et al., 2007). Major binding phases of metal in sediment are organic and Fe and Mn oxyhydroxides (Chakraborty, 2012; Chakraborty et al., 2011) which could significantly influence the bioavailability of metal in the estuarine system. Metals that are naturally present within the sediment structure and minerals are considered as residual metal complexes and are inert and doesn't participate in bioavailability to biota (Chakraborty et al., 2014a, 2014c; Chakraborty et al., 2015a).

There are numerous methods which are employed for the determination and estimation of different forms of metals in sediments. The detail description of different methodology and protocols are vividly described by Kersten and Förstner, 1989. Some of these methods (Agemian and Chau, 1976; Loring, 1976; Malo, 1977) used a single extractant, commonly used to provide a swift and effective evaluation of residual and non-residual metals in sediments.

The recent approaches include Diffusive Gradients in Thin films (Chakraborty et al., 2009; Town et al., 2009; Zhang et al., 2014), flow techniques (Shiowatana et al., 2001; Silva et al., 2007), batch techniques (Beauchemin et al., 2002; Bermond and Varrault, 2004), kinetic extraction methods (Chakraborty et al., 2012a, 2011, 2009; Chakraborty and Chakrabarti, 2006), which are being widely used for metal speciation study in estuarine and marine sediment. Some of the additional techniques used in metal speciation study are Cation-exchange dynamic mode (CED, Driscoll, 1984), Donnan membrane technique (DMT, Kalis et al., 2006), Differential pulse anodic stripping voltammetry (DPASV, Coale and Bruland, 1988), ion-exchange column technique (IET, Fortin et al., 2010; Fortin and Campbell, 1998), ion-selective electrode method (ISE, Zirino et al., 1998), and Permeable liquid membrane (PLM) technique (Bayen et al., 2007; Parthasarathy et al., 1997).

However, the most widely used techniques for metal speciation study in sediment is based on sequential extraction protocols (an operationally defined, equilibrium-based method). The method involves several selective chemical reagents, to extract operationally defined phases from the sediment in a set sequence (Harrison et al., 1981; Izquierdo et al., 1997; Kersten and Förstner, 1986; Rauret et al., 1999; Salomons and Förstner, 1980; Tessier et al., 1979). Primarily, the different sequential extraction procedures proposed by Tessier et al., 1979; Salomons and Förstner, 1980;Kersten and Förstner, 1986 had been recognized but were not commonly accepted and used for the study. This created a lack of standardization, in comparing the results obtained. Accordingly, the group of scientific experts under the support of Commission of the European Communities, Communautaire de Reference (BCR, now superseded by the Standards, Measurement and Testing Programme) (BCR), started a program in 1987 to harmonize the extraction methodology. The key outputs from the "Workshop on

Sequential Extraction in Soil and Sediments" held at Sitges, Spain in 1992 (Mossop and Davidson, 2003; Usero et al., 1998) was:

1. proposal of a simple, three-step sequential extraction protocol for extraction of metals in different geochemical phases of the sediment

2. Lake sediment reference material (BCR CRM 601) certified for metals extractable by the protocol

Ultimately, the sequential extraction method helps us in understanding the detailed information about mode of existence, biological and physio-chemical availability, mobility and transportation of toxic metals (Campbell and Tessier, 1989; Howard and Vandenbrink, 1999; Li et al., 2001; Ure et al., 1993).

Another method, Competing Ligand Exchange method (Kinetic extraction method) has been now increasingly used to understand the lability and mobility of metal-sediment complexes. It is a simple and sensitive time-dependent metal extraction study from sediments and has been described to be effectively useful in determining bioavailable and mobile metal complexes in an anthropogenic or naturogenic polluted soils/sediments (Beauchemin et al., 2002; Bermond and Varrault, 2004; Fangueiro et al., 2002). This method helps us to estimate dynamic metal complexes from marine sediments (Chakraborty et al., 2012; Chakraborty and Chakrabarti, 2006).

Study of chemical speciation of toxic metals in the estuarine sediments is highly significant, the speciation of metal in the sediments is influenced by the regular changes in the various estuarine environmental conditions such as salinity, pH and redox potential (Calmano et al., 1993; Kersten and Förstner, 1986; Lam et al., 1997).

1.3.1 Influence of pH

pH is the most vital environmental factor in governing speciation, solubility, transport, and bioavailability of metal. It affects adsorption-desorption reactions and increases the percentage of the free metal ion with a decrease in pH. Lower pH mainly affects the adsorption of metal to organic matter and metal hydroxides, which are the major binding site of metal in sediments. Once the pH decreases, the negatively charged surface of the organic matter becomes less available (due to protonation) for metal adsorption. In the case of metal hydroxide, low pH enhances the solubility of metal hydroxide leading to increase in the concentration of free/dissolve metals, consequently making more metal available for interaction and assimilation in the biological activity of estuarine organisms (Millero et al., 2009; Salomons, 1995). However, the effect of pH on speciation of metals also depends upon the nature of the metal. For example, in the case of Cu speciation, the higher pH compare to the lower pH of the overlying water column has shown an effect on Cu speciation. A study by Jayachandran et al., 2018 showed that an increase in pH of the overlying water column increased the concentration of water-soluble fraction of Cu from mangrove sediments that may, therefore, increase Cu mobility in estuarine sediment system.

1.3.2 Influence of Salinity

Particularly in an estuarine environment, salinity plays an influencing factor on the speciation of metals. Several studies (Liang and Wong, 2003; Riba et al., 2003, 2005a) have reported that lower salinity generally tends to increase the bioavailability of metals compared to the higher salinity value. The study also revealed that the effect of salinity on speciation of metal differs from one metal to another and majorly rely upon desorption rate from sediments to water and coagulation, flocculation and precipitation nature of metal. For example, Cd metal is identified

to be more mobile with an increase in salinity. Increase in Chloride (Cl⁻) content increases the complexation of chloride with Cd ions and forms stable compounds as $CdCl^+$ and $CdCl_0^2$ (Zhao et al., 2013). This stable complex has comparatively higher stability and solubility than the affinity of Cd with the different binding phase of the sediment.

1.3.3 Influence of Redox potential

The change of redox potential in sediments is also one of the significant factors in controlling the speciation (mobility) of toxic metal (Kersten, 1988; Salomons et al., 1987). When estuarine sediments are exposed to the aerobic/oxidic environment, the decomposition rate of metal organic complexes increases, and therefore increases the mobility of metal associated majorly with organic matter. Concurrently the metal mobility decreases in an oxic environment with sorption and co-precipitation of metals by Fe/Mn hydroxides. In reducing environment, the reverse activity takes place as first Mn, and then Fe gets reduced and release adsorbed and co-precipitated metals (Zwolsman et al., 1993). The precipitation of metal sulfides increases within a stronger reducing environment and so decrease the mobility and toxicity of metal from sediments (Popenda, 2014).

Thus, the knowledge about the speciation of metals from sediments and the environmental factors influencing the metal speciation is very important to understand the bioavailability of metal from sediments to the inhabited biological species in a given/estuarine environment.

1.4 Interaction of toxic metal with biological species

Exposure of toxic metal and its interaction from the outside environment with the biological system of an aquatic species is a very complex pathway. Different model has been proposed and developed to understand the pathway of metal uptake and its accumulation in different

phyla of aquatic organisms such as the free ion activity model (FIAM), biotic ligand model (BLM), and the biokinetic model (BK) (Brown and Markich, 2000; Di Toro et al., 2001; Thomann, 1981).

Interaction of toxic metals with the aquatic species and its internalization in the biological system generally includes three steps. First, the diffusion of metal takes place from the water to the surface of the cell membrane. Second, after diffusion, the complexation of metal with the active ligands present on the cell membrane takes place. Third, after metal biotic ligand complexion, metal will be transported inside the biological system and utilized for various biological functions and if not required would be bioaccumulated for detoxification (Campbell, 1994; Chakraborty, 2007).

The free ion activity model (FIAM) was initially developed and was based on justifying the experimental observation that the activity of free metal ion and not the concentration of dissolved metal complexes in bulk solution is the governing factor in defining the bioavailability, accumulation, and toxicity of metals to aquatic organisms (Morel and Hering, 1993). The detailed key assumptions under the FIAM model and the critical review of the model were provided in detail by Campbell, 1994; Brown and Markich, 2000 and explained in more detail in Chakraborty, 2007. The schematic presentation of the metal interaction with the biota based on FIAM model is provided (Figure 1.1).
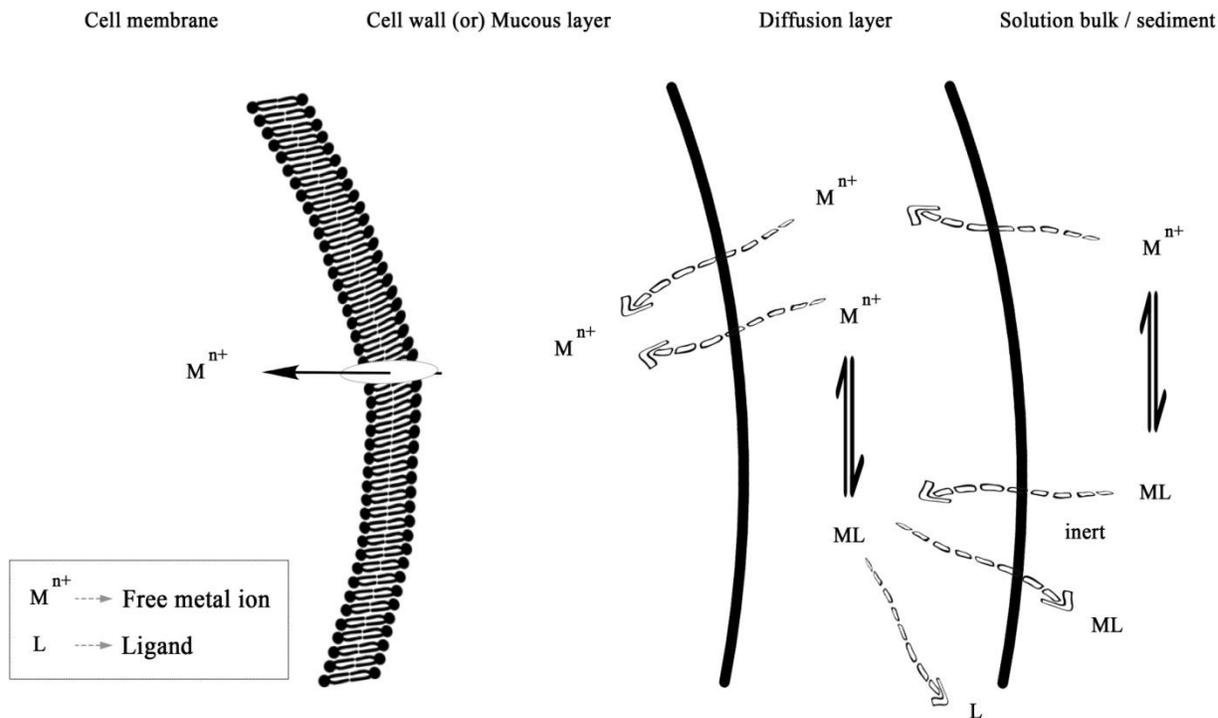
Figure 1.1: The schematic presentation of the metal interaction with the biota, adopted and modified based on the FIAM model (Campbell, 1994)**.**

The main highlight of the proposed FIAM model was about the speciation of metal and their interaction with the aquatic organisms. But the model lacked in providing adequate knowledge about the role of biotic ligands present on the cell membrane and its interaction with a different constituent of metal from the environment. The information was necessary and highly important to clearly understand the metal bioavailability. The interaction of metals in an environment is highly complex; various components such as organic matter, metal hydroxides, and carbonates are present which in turn provides a large range of complexing sites for metal. Moreover, other major metals will compete with toxic metal and influence the number of binding sites available within the cell membrane.

Later for better understanding extension based on the FIAM model came such as Fish Gill Surface Interaction Model (GSIM), Biotic Ligand Model (BLM) in which the concept of biotic ligand was included to predict metal bioavailability. The approach recognized the competition between the environmental ligands, biotic ligands in an organism, and the competing cations (Bell et al., 2002; Campbell et al., 2002; Di Toro et al., 2001). The BLM is fundamentally a chemical-equilibrium based model. It assumes and suggests that,

- Metal species in the bulk solution is in equilibrium with those at the cell surface of an organism. The rate of formation of M-X-cell (M-metal, X-cell- cellular ligand) is much faster than the rate of metal bio uptake and expression of the biological response.

- The primary site of metal interaction with the biota is plasma membrane and only via. a ligand exchange reaction M-X-cell.

- The concentration of M-X-cell surface complex is very important in determining the internalization flux of metal into the biota and to understand its biological response (metal uptake, toxicity).

- The nature of the biological surface (plasma membrane) or the ion transporters will always remain constant and will not undergo any transition during the exposure of any given metal.

The interaction of toxic metals from sediment, and pore water with biological species based on sediment ligand model (sBLM) (Di Toro et al., 2005) was presented in Figure 1.2.
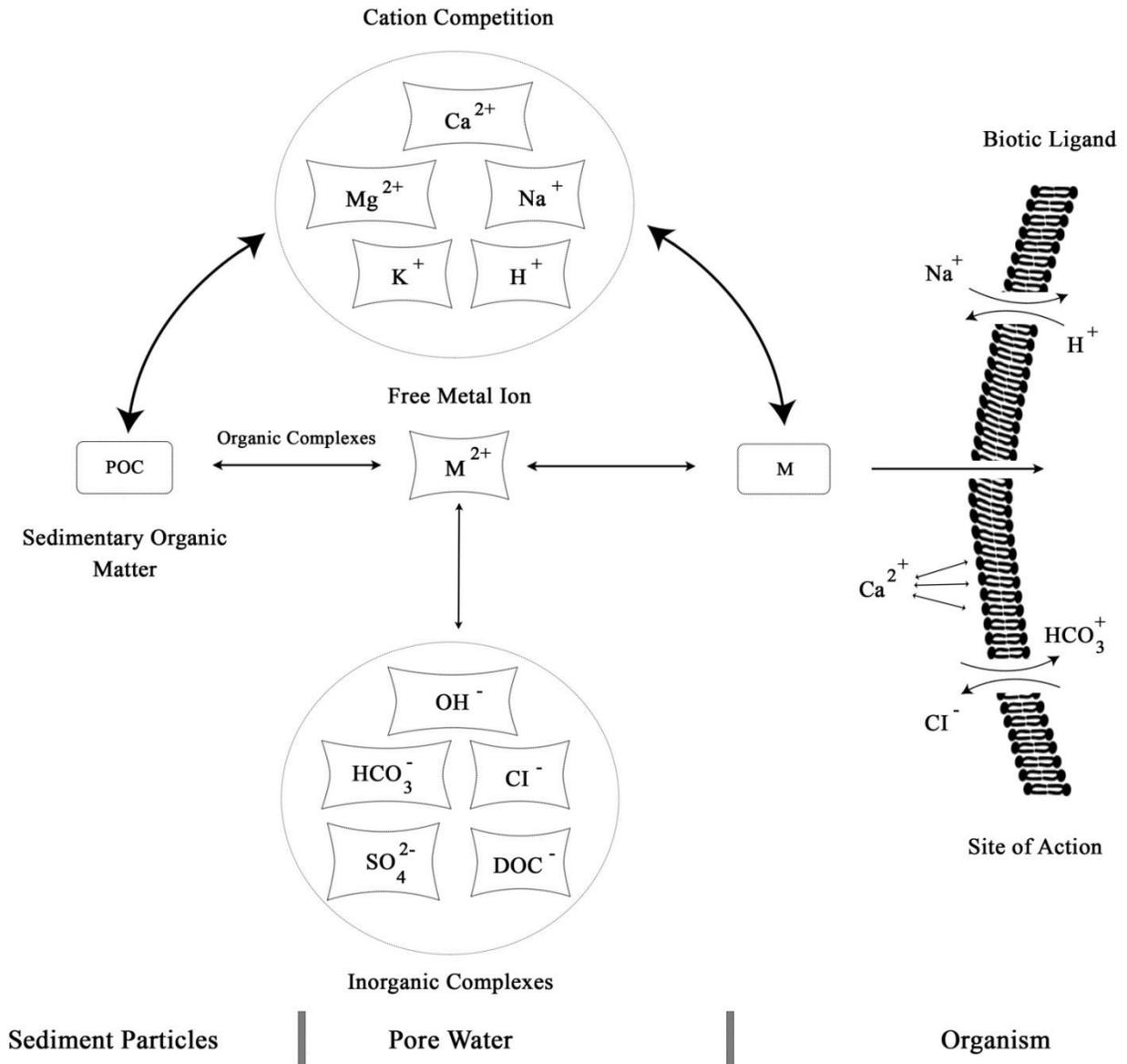
Figure 1.2: Schematic diagram based on sediment biotic ligand model (sBLM) adopted from Di Toro et al., 2005. POC = particulate organic carbon, DOC = dissolved organic carbon, HCO_3^- = bicarbonate, OH^- = hydroxide, Cl^- = chloride, SO_4^{2-} = sulfate, H^+ = hydrogen ion, M^{2+} = free metal, M = metal.

Metals are highly essential for the normal biological function of the aquatic biota. However, the same metal becomes toxic when exposed to a high concentration. Some metals are non-essential and turn toxic at very low concentration. The toxicity of any metal from the sediment depends on its speciation (chemical and physical form).

1.5 Current state of knowledge

Over the past few decades, numerous studies have been involved in determining the concentration of total metals in various estuarine and mangrove sediments around the world. The study was carried out basically to understand the toxicity of metals in the sediment environment. Till now, various authors have determined the total content of various metals [Aresnic (As), Cadmium (Cd), Selenium (Se), Zinc (Zn), Nickel (Ni), Copper (Cu), Chromium (Cr), Lead (Pb), Mercury (Hg) and Cobalt (Co)] from the estuarine sediment. The total concentration of essential metals Se, Zn, Ni, Cu, Cr, and Co reported from the different estuarine region of the earth ranged from 0.8 to 174 $mg.kg^{-1}$, 0.28-2372 $mg.kg^{-1}$, 0.3-208.4 $mg.kg^{-1}$, 0.01 to 4050 $mg.kg^{-1}$, 0.55-6240 $mg.kg^{-1}$ and 0.6 to 58 $mg.kg^{-1}$ respectively. In case of non-essential metals which are highly toxic even at low concentration, the range of value reported was 8-130 $mg.kg^{-1}$ in case of As, 0.01-87 $mg.kg^{-1}$ for Cd, 2-1810 $ng.g^{-1}$ for Hg and 0.08-1950 $mg.kg^{-1}$ for Pb. The value reported above were from the different estuarine/mangrove environment of various countries such as Kenya, Indonesia, Persian Gulf, Gulf of Oman, India, China, Brazil, Vietnam, Tanzania, Senegal, Australia, England, New Caledonia, and Nigeria. (Alam et al., 2010; Amin et al., 2009; Benson et al., 2017; Bodin et al., 2013; Canuto et al., 2013; Davari et al., 2010; Ding et al., 2009; Diop et al., 2015; Fernandes et al., 2011; Kamau, 2002; Kruitwagen et al., 2008; Kwokal et al., 2008; Lewis et al., 2011; Li et al., 2018; Marchand et al., 2011; Olivares-Rieumont et al., 2012; Prajith et al., 2016; Preda and Cox,

2002; Sharifinia et al., 2018; Tue et al., 2012; Turner and Taylor, 2018; Vane et al., 2009; Wang et al., 2017).

In India, the research about the contamination of metal from the estuarine sediments has started way back in 1982. The variation in the metal contamination levels in estuarine surface sediments with respect to time showed that the estuaries of India on which major urban population resides are more contaminated with the metal pollutant. The estuarine sediment situated along the east coast of India is comparatively less contaminated with the metal pollutant of the west coast. The detail information about the total metal concentration in surface sediments of major estuaries is provided in Table 1.1 and 1.2 (Chakraborty et al., 2014b). The elevated total metal concentration reported need not be essentially hazardous as they could probably not be released and may not be available for biological intake from the contaminated sediments. The bioavailability of any metal depends on the chemical speciation, which helps us in a clear understanding of metal accumulation and its toxicity to inhabiting organisms.

Table 1.1: Toxic metal concentrations (mg.kg^{-1}) in the sediments from the major estuaries of the East Coast of India.

Estuary	References	Cr	Ni	Cu	Zn	Pb	Cd	Hg	Mn	Fe	Co	As	No. of Samples	Year of sampling	Methods
Ganges	Subramanian et al., 1988	67	32	26	71	29			553	31036	36		19	January 1980	EDXRF*
	Subramanian, 1993			26	71	29			550	31000			19	Winter Season	AAS *
	Ramesh et al., 1999	61.46	37.5		64.38	10.49	0.45				10.76	0.08	11		TIMS*
	Banerjee et al., 2012	40.11	33.97	21.64	53.42	23.45	2.01		502.42	28600	18.23		17	June 2008	AAS*
	Sarkar et al., 2017	39	24	27	54			0.02	461	23340	11	34.4	7		ICP-AES* and DMA*
	Samanta et al., 2017	44	40	34	83	31			782	41200	16.2		1 (core)	December 2013	ICPMS*
Godavari	Ramesh et al., 1999	50.88	23.55		27.28	10.15	0.18				6.04	0.05	6		TIMS*
	Ray et al., 2006	1.73	20.7	40.2		46.7	9		429	4254	30.7		4	September 2001	AAS*
	Krupadam et al., 2007		25.1	25.8	197.1	14.4					21.9		10	2004-2005	AAS*
	Chakraborty et al., 2012	71.2	63.8	103.4	3876.7	424	24.8				25.5			2009	ICPMS*
	Prasad et al., 2019		82.1	78.7	832	44.5	25.1			1194.5			7	2016	AAS*
Krishna	Subramanian et al., 1985	68	30	49	31	9			1040	42280	29		19	October to December 1980	EDXRF*
	Ramesh et al., 1989	82	32	35	26				906	25100	32		14	August 1984	XRF*
	Ramesh et al., 1999	148.46	94.67		171.12	4.81	0.99				37.8	0.14	9		TIMS*
Cauvery	Seralathan,1987 & Sreralathan and Seetaramaswamy, 1987	249.5	120.7	94.5	118.7	61			3100		14		6 & 15	1976-1977	colorimetrically and AAS*
	Ramanathan et al.,	68.3	79.4	21.7					607.2	35552	46.1		18	July 1986	AAS*

Estuary	References	Cr	Ni	Cu	Zn	Pb	Cd	Hg	Mn	Fe	Co	As	No. of Samples	Year of sampling	Methods
Cauvery	1988														
	Subramanian et al., 1989	229	379	33	75	38	1.85	0.118	1310	33500			18	July 1986	AAS* and Hydride system
	Ramanathan et al., 1993	42.9		19.6	25.4	30.3	0.22		567.2	16285			16	June -1987 & April-1989	AAS*
	Ramesh et al., 1999	73.39	34.16		49.36	15.12	0.73				9.49	0.35	22		TIMS*
	Dhanakumar et al., 2013	49.5	13.5	29.5	30	8.5			160	5228.5			10	May-2008 & November 2009	AAS*
	Dhanakumar et al., 2015	112.6	4	24.3	53.7	2.92			253	5513.3			3	2012	AAS*

*AAS-Atomic absorption spectrometry, TIMS- Thermal ionization mass spectrometer, XRF (EDXRF)-Energy dispersive X-ray fluorescence, ICPMS- Inductively coupled plasma mass spectrometry, ICP-AES- Inductively coupled plasma atomic emission spectroscopy, DMA-Direct mercury analyzer

Table 1.2: Toxic metal concentrations (mg.kg^{-1}) in the sediments from the major Estuaries of the West Coast of India.

Estuary	References	Cr	Ni	Cu	Zn	Pb	Cd	Hg	Mn	Fe	Co	As	No. of Samples	Year of Sampling	Methods
Cochin	Venugopal et al., 1982		48.1	28	205				152.7		22.3			1976-1977	AAS *
	Nair, 1992	29.99	21.15	12.2	70.4	14.86	1.66		66.03	8325	9.23		120	1988& 1989	AAS*
	Jayasreeand Nair, 1995	1	3	0.4	14	2.7	0.51		41	803	2.1		10	January - April 1994	AAS *
	Balachandran et al., 2005			53.15	1266	71.28	14.94			61800				November 2000	AAS*
	Balachandran et al., 2006	82.3	53.7	30.8	562.3	38.7	5.91		229.1	44700	18.3		17	November 2000	AAS*
	Martin et al., 2012	131.9	57.0	43.4	422.7	39.8	9.1		1147.8	29100.0	26.1		56	2005	AAS *
	George et al., 2016		48.1	38.5	378	30.6	4.7		562.6	66949.7	23.9		27	2010-2011	AAS*
	Salas et al., 2017	134	31.1	26.7	386.1	21.9	5.1		273.8	31784	13.8		15	2009-2010 & 2012	AAS*
	Shyleshchandran et al., 2018			45.5	629.5	17.9	6.4	0.76					12	2012-2014	VMA* and CVAFS*
Ulhas	Sahuand Bhosale, 1991	42.1	101.2	109.7	143.6	57.0	3.4	0.7	731.0	66571.3	42.6		11	1984-86	AAS* and Hg analyzer
	Rokade, 2009	282.6	93.2	132.6	862.3				1232.2	95000	47.4		10	1996	AAS*
	Rokade, 2009	312.3	96.6	124.3	400.9				1301.8	84000	63.3		13	1998	AAS*
	Rokade, 2009	496.3	98.0	130.9	217.5				1151.5	78000	64.1		11	2000	AAS*
	Fernandes and Nayak, 2014	307		264	133	95			1902	85600	109		1 (core)		AAS*
	Fernandes and Nayak, 2016	239		173.3	179.7	81.6			2076	90200	78.9		1 (core)		AAS*

Estuary	References	Cr	Ni	Cu	Zn	Pb	Cd	Hg	Mn	Fe	Co	As	No. of Samples	Year of Sampling	Methods
Narmada	Subramanian et al., 1985	55	23	46	50	5			514	31400	36		3	October-December 1980	EDXRF*
	Sharma and Subramanian, 2010	199.3	200.3	188.3	196.2	13.9	1.1		1214	89577	25.9	1.6	9	2004	WDXRF* and EDXRF*
Tapti	Subramanian et al., 1985	108	60	126	118	5			1300	10900	64		3	October-December 1980	EDXRF*
	Sharma and Subramanian, 2010	212.6	205.5	326.2	216.7	25	0.5		1498	91128	27	1.7	5	2004	WDXRF* and EDXRF*
	Shah et al., 2013	55.2	81	148.3	143.6	56.7	0.83			67900	18.34		12	2011	AAS*

*AAS-Atomic absorption spectrometry, EDXRF- Energy dispersive x-ray fluorescence, WDXRF- Wavelength dispersive X-ray fluorescence, VMA- Voltametric trace metal analyzer, CVAFS- Cold vapor atomic fluorescence spectrometer.

1.5.1 Metal Speciation study

The study of chemical speciation of metal along with the total metal concentration from the estuarine sediments is now well recognized by many authors. Several studies have majorly involved the use of a sequential extraction method, to understand the distribution of various toxic metals with the different geochemical phases of the sediment [Anagboso et al., 2013 (England); Chakraborty, 2012 (India); Chakraborty et al., 2012(India); Chakraborty et al., 2016a (India); Chakraborty and Babu, 2015 (India);Cuong and Obbard, 2006 (Singapore); Davutluoglu et al., 2011 (Turkey); de Andrade Passos et al., 2010 (Brazil); Fernandes and Nayak, 2014 (India); Fernandes and Nayak, 2015 (India); Gu et al., 2014 (China); Hiếu et al., 2013 (Vietnam); Jayachandran et al., 2018 (India); Liu et al., 2018 (China); Martínez-Fernández et al., 2011 (Spain); Massolo et al., 2012 (India); Ngiam and Lim, 2001 (Malaysia); Noronha-D'Mello and Nayak, 2016 (India); Okoro et al., 2017 (Nigeria); Pejman et al., 2017 (Persian Gulf); Prajith et al., 2016 (India); Prartono et al., 2016 (Indonesia); Renjith et al., 2016 (India); Rosado et al., 2016 (Spain); Sundaray et al., 2011 (India); Van Thinh et al., 2018 (Vietnam); Venkatramanan et al., 2015 (India); Wang et al., 2010 (China); Yuan et al., 2004 (China)]. Thus, the authors/geochemist widely used the sequential extraction method in successfully understanding the speciation and mobility of metals in sediments. But the metals which are considered as mobile/bioavailable from the experimental study need not be always necessary to be taken into the biological system of the inhabited floral and faunal species and get bioaccumulated. To understand how much metal was bioavailable and accumulated into the biota the estimation of total metal from the estuarine organism is necessary.

1.5.2 Toxic metal concentration in aquatic organisms

When the geochemists were estimating the total metal concentration and performing metal speciation study to understand metal toxicity, biologists were exploring the different organisms as a candidate species to understand the metal bioaccumulation and its toxicity. A wide range of biotic species was employed by the biologist to estimate the total metal content in the tissues. The authors estimated the total metal concentration from the sediments and tried to correlate the metal content analyzed from the associated biota. Hornung et al., 1989 analyzed toxic metal in sediments and associated benthic fauna of Hafiya Bay, Israel. Nicolaidou and Nott, 1998 studied the accumulation of different metals from sediments to seagrass (*Cymodocea nodosa* leaves, roots) and gastropods (*Cerithium vulgatum* and *Monodonta mutabilis*) from Larymna bay in Greece. Birch et al., 2014; Birch and Apostolatos, 2013 used the oyster (*Saccostrea glomerata*) and black mussel (*Mytilus galloprovincialis*) tissues to determine the relationship between metals in estuarine sediments and metal bioaccumulation from Sydney estuary, Australia. He et al., 2014 used mangrove plants (leaves and stems) to understand the threat of toxic metal from sediment cores (China). Nath et al., 2014 adopted pneumatophore (aerial root) tissues of *Avicennia marina* to assess the sediment quality in terms of metals. The author found a significant positive correlation between metals in sediments and pneumatophores suggesting pneumatophores a bio-indicator of estuarine metal contamination. Ismail and Ramli, 1997 analyzed toxic metal in gastropod (*Nerita lineata* and *Cherithidea obtusa*) and sediments to understand metal pollution due to pig farming activities. Sun et al., 2015 studied the toxic metal levels in intertidal surface sediments and marsh plants (*Suaeda salsa*, *Phragmites australis*, *Tamarix chinensis*, *Sparganium minimum*, *Potentilla supina*, *Calamagrostis pseudophragmites*, and *Imperata cylindrical*) situated at the intertidal

zone of northern Yellow River estuary, China. Bordon et al., 2016 investigated the relationship between the metal accumulated in crab (*Callinectes danae*) tissue and metal concentration in local surface sediment of Santos estuarine system, Brazil. Benson et al., 2017 determined the metal content in the tissue of crab *Leptodius exarate* and surface sediments from the intertidal region of Qua Iboe estuary. Marques et al., 2018 used Manila clam *Ruditapes philippinarum* to signalize trace element contamination from Tagus estuary, Portugal. From India, Bhattacharya et al., 1994 analyzed a total of 23 organisms (seaweeds, zooplankters, mollusks, and fishes) to understand the toxic metal bioaccumulation form the water, sediments of Hooghly estuary. Kesavan et al., 2010 estimated metal from the sediment and gastropod shells and tissues (*Telescopium telescopium*) from Vellar estuary. Kesavan et al., 2013 analyzed and compared the metal concentration in sediments, tissues, and shells of the mollusks *Meretrix meretrix*, *Crassostrea madrasensis,* and *Cerithidea cingulata.* Jayaprakash et al., 2015 investigated the accumulation of metals in water, sediment and fish species (*Sillago sihama*, *Liza parsia*, *Etroplus suratensis*, *Oreochromis mossambicus*, *Arius parkii* and *Gerres oyena*) from Ennore creek, Chennai. Chowdhury et al., 2015 used mangrove plant organs (*Avicennia officinalis*, *A. marina*, *A. alba*, *Bruguiera gymnorrhiza*, *Ceriops tagal*, *Rhizophora apiculate*, *Aegiceras corniculatum*, *Excoecaria agallocha*, *Lumitzera racemosa*, *Heritiera fomes*, and *Sonneratia caseolaris*) to establish a correlation between metals in sediment and mangrove plant tissues. Dange and Manoj 2015 studied the heavy metal accumulation in sediment, polychaetes worms, mudskipper, and mud crab from Purna river estuary, Gujarat. Al-Usmani et al., 2015 assessed the metal pollution in water, sediment, and bivalves (*Paphia malabarica*, *Perna viridis*, and *Saccostrea cucullata*) from Mandovi and Chapora estuaries of Goa. Ramasamy et al., 2017 used water, pore water, sediment and 17 species of fish (*Arius arius*, *Parambasis dayi*, *Liza*

macrolepis, *Gazza minuta*, *Cynoglossus semifasciatus*, *Synaptura orientalis*, *Platycephalus indicus*, *Etroplus suratensis*, *Megalops cyprinoides*, *Gerres setifer*, *Oreochromis mossambica*, *Glossogobius giuris*, *Lutjanus johnii*, *Scatophagus argus*, *Caranx affinis*, *Stolephorus commersonnii* and *Thryssa mystax*) to analyze metal accumulation from Vembanad Lake, Kerala. Sankar et al., 2018 assessed the heavy metal contamination in water, sediments, and marine shell fish and fin fish (*Penaeus indicus*, *Sardinella longiceps*, *Rastrelliger kanagurta*, *Scomberomorous guttatus*, *Parastromateus niger* and *Perna viridis*) from Parangipettai and Nagapattinam, Tamil Nadu. All the above studies have focused mainly to establish the relationship between the total metal from sediment and metal bioaccumulation in different estuarine and coastal organisms.

1.6 Knowledge gap

Despite various studies about the total metal concentration from estuarine sediments, metal content in the tissues and shells of estuarine biota, and the metal speciation study from the sediments. The linkage between the bioavailable, labile metal complexes in sediments, and its uptake and accumulation in the estuarine organism is poorly studied. Recently, few authors Castiglioni et al., 2018; Dias and Nayak, 2016; Fan et al., 2014; Noronha-D'mello and Nayak, 2016; Van Hop et al., 2017 has approached an assessment of trace metal bioavailability in estuarine sediments and bioaccumulation in estuarine organisms. However, the study done is scarce and there is a significant need to understand the relationship between metal speciation, and their bioavailability in an estuarine system. To understand the relationship between metal speciation and their bioavailability, the selection of specific organisms for the determination of metal accumulation is required. All levels of biotic communities are important in estuarine functioning. It has been reported that benthic biota forms a very important component of

estuarine ecosystems as they are amongst the most sensitive biotic components. They are considered as the most beneficial component for assessing ecosystem integrity (Chapman, 2007; Resh and Rosenberg, 1993).

1.7 Objectives

Keeping the above contents in mind, **the main objective of the thesis was to test the hypotheses that**

1. Metal speciation in estuarine sediments determines metal bioavailability and their bioaccumulation in benthic organisms.

2. Influences of environmental factors from the overlying water column determine the speciation and bioavailability of metals from estuarine sediments.

1.8 Thesis framework

Comprehensive abstract of the thesis is elucidated as follows

Chapter 1 covers the general introduction about metals with special reference to the estuarine ecosystem. Toxic metal behavior and the importance of metal speciation over total metal concentration are described. This chapter also describes the environmental factors influencing metal speciation and bioavailability in estuarine sediments and how the metal from the sediment interact with the aquatic flora and fauna. This chapter provides the current state of knowledge on the anthropogenic increase of metal input in estuaries, the use of different organisms to understand metal bioaccumulation from sediment, applications of chemical

extraction techniques to determine metal bioavailability. The knowledge gaps based on the current state of knowledge are put forward. The objectives of this research work are set in this context demonstrating that metal speciation in estuarine sediment determines bioavailability and bioaccumulation in the associated biota.

The review of the anthropogenic increase of metal in estuarine sediments of India was published in Marine Pollution Bulletin Journal (Chakraborty et al., 2014).

Chapter 2 presents detailed information about the study area, the procedure of sampling, storage of sediment and tissue of biological organisms, laboratory procedure, analytic techniques used in understanding the metal speciation, and the statistical analysis used for the results obtained.

Chapter 3 presents the speciation of Cu and Ni from the mangrove sediments and its bioaccumulation in the mangrove roots from Divar Island, Mandovi estuary. This chapter presents that Fe/Mn oxyhydroxide and organic phases in the sediments are the major hosting phases for Ni and Cu and control its bioavailability in the mangrove system. Further, this study has proposed a plausible pathway of Cu and Ni uptake by mangrove roots from the mangrove sediments. This work was published in Marine Pollution Bulletin Journal (Chakraborty et al., 2014 and 2015)

Chapter 4 describes Cd speciation from the estuarine sediments and its bioaccumulation in the edible oyster (*Magallana* sp.). Total Cd concentration in water, suspended particulate matter (SPM) and sediment was determined. This chapter presents the geochemical fractionation study of bulk and finer (< 63 μm) estuarine sediments to estimate Cd bioavailability (chemically). To understand the Cd bioaccumulation, estimation of Cd concentration in the soft

tissues of oysters was carried out. This chapter also describes the relationship between Cd in water, SPM, and bulk sediments with bioaccumulated Cd in oyster (*Magallana* sp.). This chapter concludes that bioaccumulation of Cd in the oyster increases with the increasing association of Cd in water-soluble, carbonate/bicarbonate, and exchangeable forms in the finer particles of the sediments.

Chapter 5 deals with the Kinetic speciation of Cd in the estuarine sediments and its bioaccumulation in the edible oyster (*Magallana* sp.). This chapter presents the Kinetic speciation study of Cd in finer fraction of (< 63 µm) estuarine sediments and its relationship with Cd accumulation in soft tissues of the edible oyster. The chapter suggests that sedimentary Cd, especially Cd in the finer fraction (< 63 µm) of the sediment could be responsible for Cd availability from the environment (as reported in Chapter 4) and may probably enter into the oyster system through its filter-feeding behavior and bioaccumulated in their soft tissues.

Chapter 6 presents the role of environmental factors (such as pH, salinity, Temperature) in governing the chemical speciation of Cu and Cd and its bioaccumulation. This chapter describes the effect of overlying water column pH on Cu distribution in different geochemical phases of the sediment. It also describes the influence of salinity on Cd accumulation in oyster tissue.

Chapter 7 summarizes the results and provides the overall conclusion of the thesis.

Chapter 2

Materials and Methods

2.1 Study area and sampling

For the present study, four different study locations were chosen to understand the speciation of metals from the estuarine sediments and its bioaccumulation in benthic organisms. The selected sites were Divar Island at Mandovi estuary, Agacaim, and Sancoale at Zuari estuary and a station (Virnoda) in Chapora estuary in Goa, West coast of India.
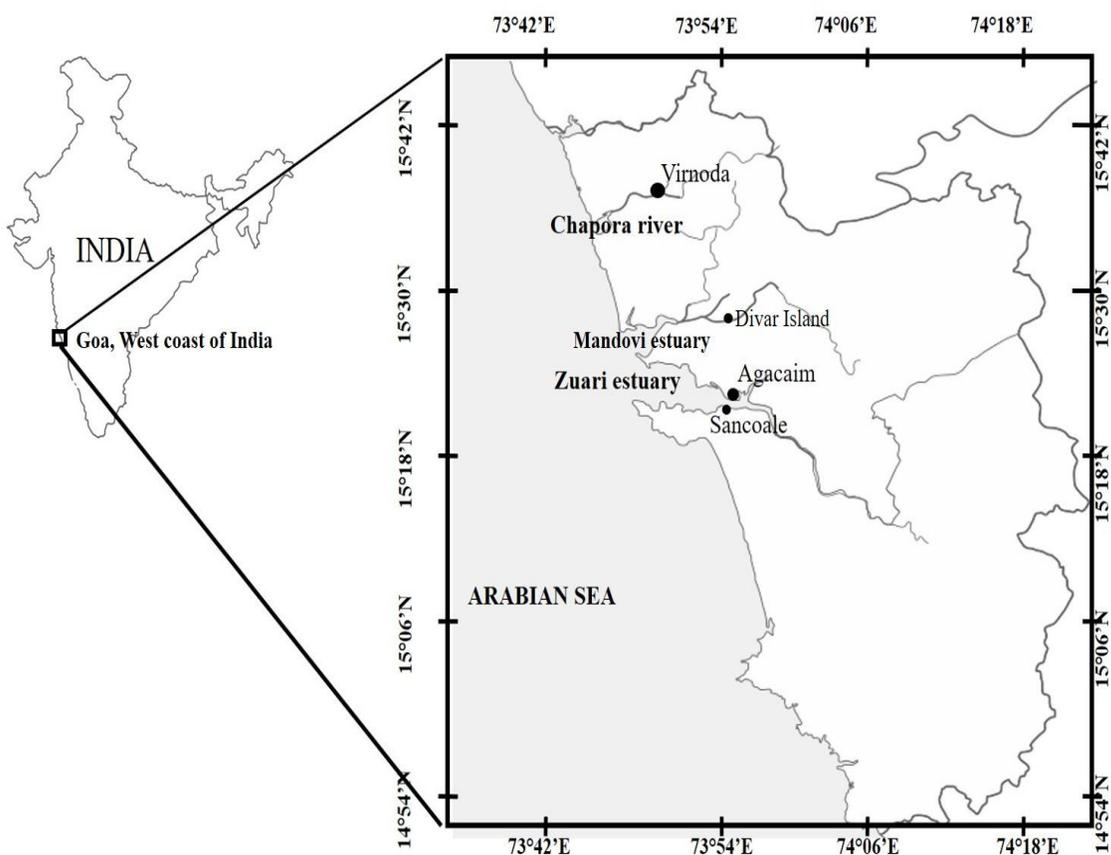
Figure 2.1: Map of the sampling location, West coast of India.

Mandovi estuary

The Mandovi River which originates from the Parwa Ghat in Karnataka of the Sahyadri hills forms an important estuarine system in Goa state. The estuary is approximately 50 km long with the width of 3.2 km (at the estuary mouth), and in few place confines to 0.25 km finally drains into the eastern Arabian Sea. The average depth of the Mandovi estuary is 5 m. It is a coastal plain, monsoonal estuary (Shetye et al., 2007). The estuary receives abundant freshwater river discharge ($\sim$ 258 m^3s^{-1}) during the SW monsoon (June-September) and minor river discharge ($\sim$ 6 m^3s^{-1}) in the non-monsoon period (Vijith et al., 2009). Throughout the non-monsoon period, the estuary is regulated by the semi-diurnal tides and wind-induced currents, resulting in an extension of saline conditions to around 45 km landward from the mouth (Sundar and Shetye, 2005). The Mandovi estuarine channel is influenced by human activities, it was used to transport a huge amount of iron and ferromanganese ores throughout the year from the mining site (located upstream) to the Marmugao harbor, located in the mouth of Zuari estuary (Arabian Sea). The ores which are stored at various points in the estuarine area and are being transported get eroded and flushed into the estuaries and ultimately get accumulated in the estuarine sediments (Alagarsamy, 2006; Dessai et al., 2009a; Shynu et al., 2012). Presently the mining activity has been banned in Goa. The ban was initiated from 2012, (Kessarkar et al., 2015) and was due to various environmental threats possessed due to mining as well as mined ore transportation activities.

The selected location Divar Island in the Mandovi estuary (Figure 2.1) is a deltaic Island and is majorly surrounded by tidal sandflats, clam beds, and dense mangrove forests. The dominant mangrove species found in the Island are *Rhizophora mucronata, R. apiculata, Avicennia marina, A. officinalis, A. alba, Sonneratia alba, S. caseolaris* and *Acanthus ilicifolius* (Borges and Shanbhag, 2008).

Total of five different sampling stations selected along the mangrove forest in Divar Island. At each station surface sediment samples and mangrove roots were collected during the low tide period in December 2013. Sediment samples were collected by hand (with gloves) using PVC core (inner diameter 7.5 cm). Mangrove aerial roots (pneumatophores) of equal size from *Avicennia officinalis* species were picked from all the five sampling locations. Triplicate samples were collected at each station. The details of the sampling site are given in Table 2.1 and Figure 2.2.
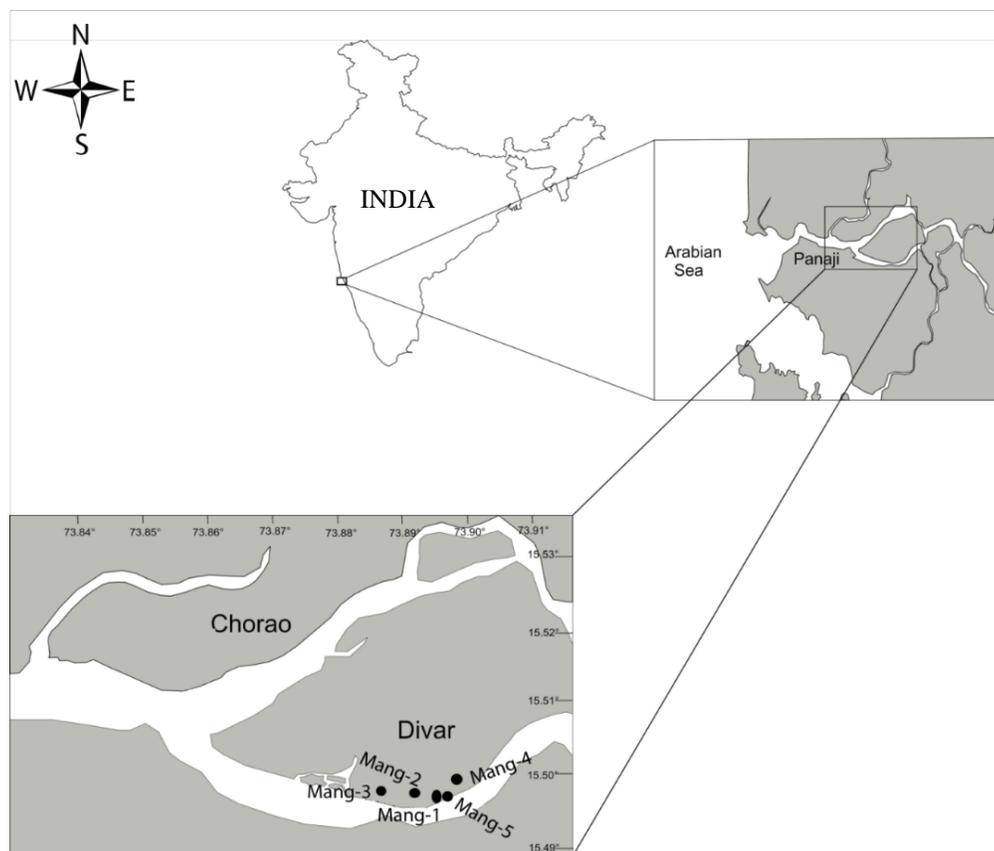
Figure 2.2: Sampling locations from the mangrove ecosystem at Divar Island, Mandovi estuary, West coast of India.

Figure 2.3: Sampling site location in Divar Island, Mandovi estuary.
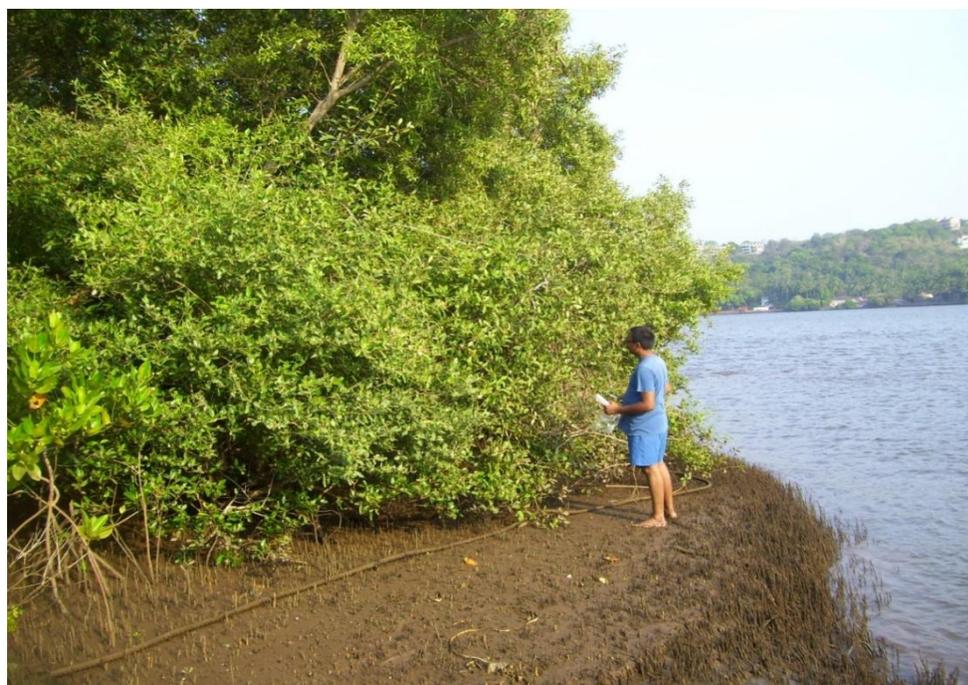
Figure 2.4: Sampling site location in Divar Island, Mandovi estuary.

Zuari estuary

The Zuari River is about 70 km in length originates in Hemad-Barshem in the Western Ghats. Similar to Mandovi it also receives heavy freshwater runoff during the southwest monsoon (June-September). It is a tide-dominated estuary and geomorphologically termed as drown river valley estuary (Murty et al., 1976). The influence of saltwater is seen around 65 km landward side in May, reduced to about 20 km in June (Qasim and Sen Gupta, 1981). The average depth of the estuary is ~5m. The neighborhood of Zuari estuary is capped by rocks belonging to the Dharwar Super Group of the Archaean-Proterozoic age characterized by metamorphosed basic and acid volcanic rocks with sediments at the base, and covered by greywacke suite of rocks, which is followed by pyroclasts and tuffs with the associated chemogenic precipitates of lime, manganese, and iron and again superimposed by greywacke suite of rocks (Dessai et al., 2009b; Gokul et al., 1985). The two rivers Kushavati and Sanguem are the major source of freshwater to the Zuari estuary. The major harbor of Goa, Mormugao is located near to the Zuari estuarine mouth. The two estuaries Mandovi and Zuari are connected through the 17 km long Cumbarjua canal, which is around 14 km from the Zuari estuarine mouth. Similar to Mandovi, Zuari catchment area is recognized for open cast mining of ferromanganese ores. The mined ores are transported through barges via Zuari estuary to the Mormugao harbor for loading to larger ships. Presently the mining activity has been stopped in Goa. The ban was initiated from the year 2012 (Kessarkar et al., 2015) due to various environmental threats. Apart from the mining activities, barge constructing and mending industries, several jetties, and construction activities, waste discharges from the industrial and domestic sectors along the estuarine area (Nayak, 2002) lead to the release of huge amounts of organic and inorganic contaminants to the estuary.

Along the Zuari estuarine area, about 900 ha of mangrove forest is found. The mangroves found in the Zuari estuary are the fringing type and the species composition varies depending on topography and environmental conditions. Some of the common mangrove species found in the area are *Avicennia officinalis, A. alba, Acanthus ilicifolius, Aegiceras corniculatum, Exocecaria agallocha, Sonneratia alba, S. caseolaris, Rhizophora mucronata, R. apiculata*, and *Bruguiera gymnorrhiza*. (www.forest.goa.gov.in/pdf/mangroves/ch2.pdf).

Two different sampling areas were selected from the Zuari estuary. The Agacaim sampling site is located below the Zuari Bridge and covered naturally with dense mangrove vegetation. *Avicennia offcinalis, Rhizophora mucronata, R. apiculata, Sonneratia alba*, *and Acanthus ilicifolius* are some of the mangrove species found in the Agacaim area (www.forest.goa.gov.in/pdf/mangroves/ch2.pdf). Two stations were selected from the Agacaim area, Station 1 (St-1) was located close to the area of the artisanal fisherman boat area, the place is located little inside from main estuarine channel and sheltered with mangrove trees. Whereas, Station 2 (St-2) is located in an area with no human activities around its immediate surrounding compared to St-1 (Figure 2.5). Comparatively, St-2 has less mangrove cover than St-1 and receives freshwater runoff from adjacent land. In both the stations, there is the presence of dense oyster bed (*Crassostrea* sp. Sacco, 1897, recently the oyster name has been changed to *Magallana* sp. Salvi and Mariottini, 2016) (http://www.marinespecies.org/aphia.php?p=taxdetails&id=836032), which were harvested by local fishers for their consumption and sometimes for marketing.
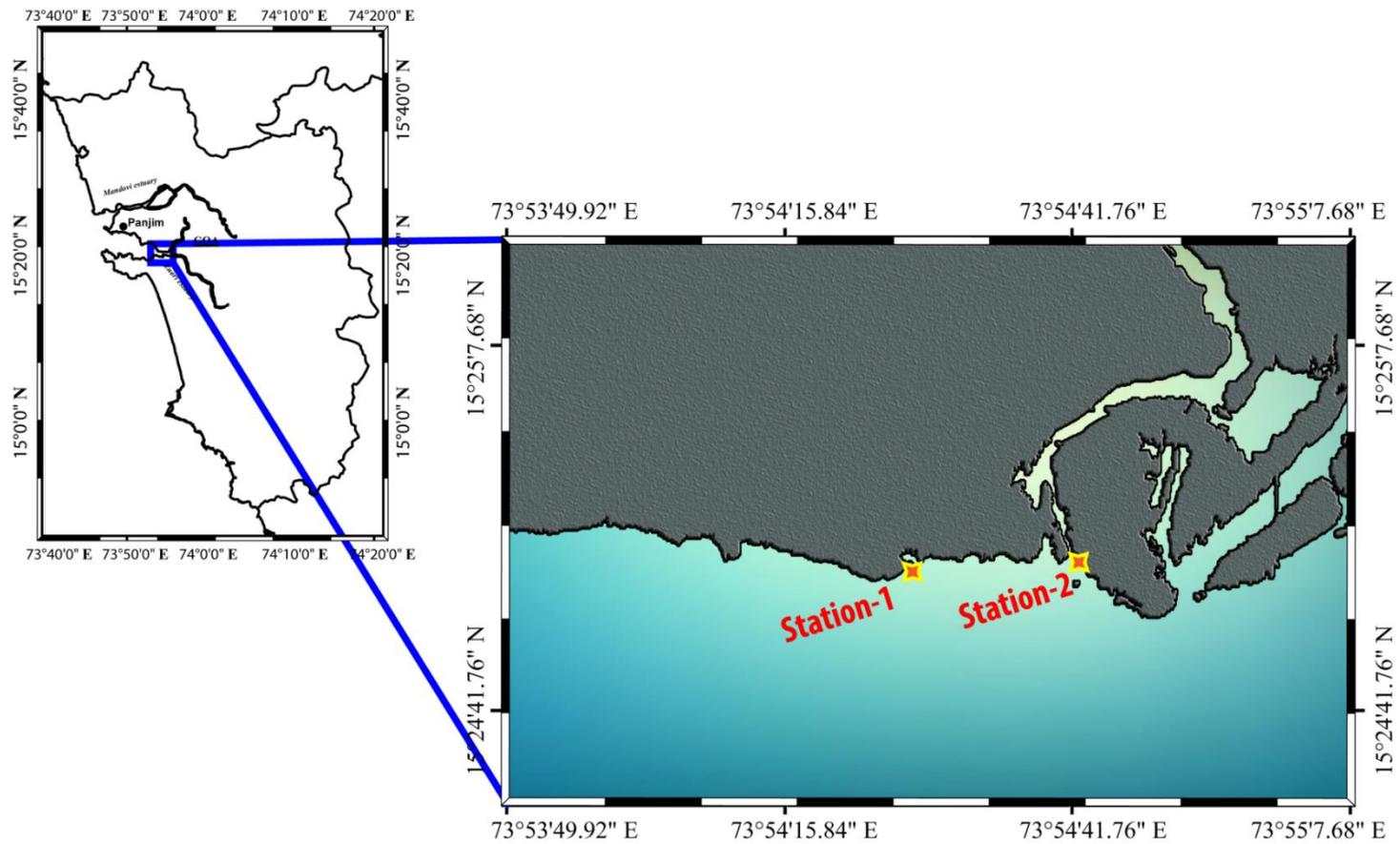
Figure 2.5: Sampling locations from the estuarine area of Zuari (at Agacaim), West coast of India.

The Sancoale sampling site is located opposite to the Agacaim site in the Zuari estuary. The sampling stations were located within a 200 m radius of Sancoale (a small civic place in the South Goa district) area and also found covered with dense mangrove vegetation. Six sampling station was selected from the Sancolae study area. The complete details of both the sampling location (with stations) in Zuari estuary is given in Table 2.1.

In Agacaim, the sampling was performed every month during the low tide period from the year 2014-2016. Water samples, surface sediments, SPM (suspended particulate matter), and oysters (benthic organisms) were collected for metal analysis. Surface sediments were collected with the help of PVC core (inner diameter 7.5 cm) by hand wearing gloves. 30-60 no's of live edible oyster *Magallana* sp. (of equal size) attached to the small rocks/stones were hand-picked from the fixed sampling stations (St-1 and St-2). Water samples adjacent to the exact sediment collection site were collected in 2.2 L Teflon bottle and 5 L in HDPE bottles for metal analysis in water and SPM respectively. SPM was obtained after filtering the collected water through 0.45 µm MCE Millipore filter paper. Multiple sampling was performed at each station.

In Sancoale the sampling was carried in post-monsoon period in 2015. Undisturbed surface sediment samples were collected in triplicates with the help of PVC core (inner diameter 7.5 cm) at each sampling location.

Figure 2.6: Image of the sampling location (Station-1) at Agacaim, Zuari Estuary.

Figure 2.7: Image of the water sample collection at Station-1, Agacaim, Zuari Estuary.

Figure 2.8: Image of the sampling location (Station-2) at Agacaim, Zuari Estuary.

Figure 2.9: Image of the oyster bed at Station-2, Agacaim, Zuari Estuary.

Chapora estuary

The Chapora estuary is 30 km long and situated in the northern part of the Goa state. The estuary is comparatively narrower to the main estuary of Goa (Mandovi and Zuari). The maximum width of the estuary is around 900 m and has a narrow opening to the sea (width~600 m). It experiences mixed semi-diurnal tide and receives maximum freshwater input in monsoon and minimum during the rest of the period (Varma and Cherian, 1975). Some of the common mangrove species found in the Chapora estuary is *Avicennia officinalis*, *Acanthus illicifolius, Sonneratia alba, Rhizophora mucronata,* and *R. apiculata*. The estuary is predominantly rich with *Bruguiera gymnorrhiza* and *Aegiceras corniculatum* species at Virnoda and Caturlim (www.forest.goa.gov.in/pdf/mangroves/ch2.pdf). The Virnoda was selected as the sampling site from the Chapora estuary. The undisturbed surface sediments (in triplicates) were collected from Virnoda with the help of PVC corer during the peak monsoon period (July 2015).

Table 2.1 Latitude and Longitude of the sampling stations from the Mandovi and Zuari estuary, Goa.

Station ID	Latitude	Longitude
Mandovi estuary (Divar Island)		
Mang-1	15°30'31.45''N	73°54'43.75''E
Mang-2	15°30'29.60''N	73°54'40.91''E
Mang-3	15°30'28.59''N	73°54'38.81''E
Mang-4	15°30'33.60''N	73°54'46.46''E
Mang-5	15°30'32.45''N	73°54'45.34''E
Zuari estuary (Agacaim)		
St-1	15°24'52.77''N	73°54'25.90''E
St-2	15°24'53.71''N	73°54'41.94''E
Zuari estuary (Sancoale)		
SC-1	15°24'23.16''N	73°53'18.55''E
SC-2	15°24'21.78''N	73°53'19.14''E
SC-3	15°24'21.04''N	73°53'16.22''E
SC-4	15°24'20.88''N	73°53'12.84''E
SC-5	15°29'19.28''N	73°53'13.21''E
SC-6	15°29'19.91''N	73°53'15.74''E

2.2 Determination of physical parameters in the overlying water column

pH, Salinity, DO (Dissolved oxygen) and water temperature of the overlying water column on the sediments during low tide period was measured. The pH of the overlying water samples was determined by using pH meter (Metrohm, 827 pH Lab, Switzerland). The salinity and water temperature were determined with the help of a waterproof portable meter (Eutech Instruments Cyberscan series-600). In case when the waterproof portable meter was not available, salinity was

measured through ERMA Salinity Refractometer (Model RHS-10 ATC). DO of the water sample was analyzed by Winkler's Iodometric method. The procedure for the estimation of DO is given in Appendix 1.1.

2.3 Analysis of Sediment texture

The texture of the sediment sample, after sieving through the 63µm sieve was determined by Laser Particle Size Analyzer (LPSA, Malvern Mastersizer 2000, Worcestershire, UK). The detailed description of the method has been given in literature by Ramaswamy and Rao, 2006. The brief procedure is provided in Appendix 1.2. The data obtained were presented as weight percentage (wt%) for sand and the silt and clay in volume (%) in this study.

2.4 Elemental analysis in sediments

The total carbon (TC) and total nitrogen (TN) of dried homogenized, ground-milled sediment samples were analyzed with the help of Flash 2000 CHN elemental analyzer (Thermo Fisher Scientific Incorporation, USA) with the precision within ± 5%. The soil NC content was used as the certified reference material (CRM: Soil NC, UK). Total inorganic carbon (TIC) was analyzed by coulometry (UIC Coulometrics). Anhydrous calcium carbonate (12% C) was used as certified reference material. The relative standard deviation of the analysis was within ± 2%. The total sedimentary organic carbon (C_{org}) was derived by abstracting TIC from TC. $CaCO_3$ content was calculated pretending that the majority of inorganic carbon (or carbonate) is associated with Ca.

2.5 Isotopic Analysis

To understand the source of sedimentary C_{org}, stable C and N isotopic compositions from sediments were measured by Eurovector elemental analyzer (EURO3000) connected to Isotope-ratio mass spectrometer (Thermo DELTA V PLUS) in a continuous-flow mode. Subsamples of sediment were

acidified using 10% HCl (suprapur grade) and 7-10 mg of decalcified dried samples were filled in tin cups and combusted at 1050 °C. ε-Amino-n-Caproic acid, ($C_6H_{15}NO_2$; ACA) laboratory standard was used for calibration of elemental data as well as for $\delta^{13}C_{org}$ and $\delta^{15}N$. Reported ACA values of $\delta^{13}C_{org}$ and $\delta^{15}N$ are −25.3‰ and 4.6 ‰ respectively.

Carbon and nitrogen isotopic ratios of sedimentary organic matter are expressed as δ values ($\delta^{13}C_{org}$ and $\delta^{15}N$) with reference to V-PD Band atmospheric N_2, respectively.

$$\delta^{13}C_{org} \text{ and } \delta^{15}N = [(R_{sample}-R_{standard})/R_{standard}) \times 1000$$

where $R = {}^{13}C/{}^{12}C$ and ${}^{15}N/{}^{14}N$.

The accuracy of analytical replicates (n=3) was < 2% for C_{org} and TN, for $\delta^{13}C_{org}$ and $\delta^{15}N$ it was < 0.17%

2.6 Separation of finer particles from bulk sediments

Finer particles (silt and clay) fractions were separated from the bulk sediment by the Pipette-sieve method as described in Folk, 1980. The total bulk sampled sediment was taken in a measuring cylinder and made up to 1000 mL, the silt and clay fractions were then pipetted out at the measured time and depth corresponding to particle diameters of 62 to 2 and <2 μm (Ramaswamy and Rao, 2006). The collected finer fraction of the sediment was freeze-dried and finely grounded (homogenized) for metal analysis.

2.7 Metal analysis in the sediments and Suspended Particulate Material (SPM)

The total concentration of toxic metals in sediments was evaluated by disintegrating it with a mixture of strong acids HF, $HClO_4$, and HNO_3. Sediment samples after bringing to the laboratory were freeze-dried and homogenized (ground-milled) into a fine powder with the help of mortar

pestle. 0.05g of homogenized sediment samples were then transferred in 15 mL of HF, HClO$_4$, and HNO$_3$ acid mixture (in 7:3:1 ratio) in acid-cleaned Teflon beaker. The beaker containing samples were heated on a hot plate around 180° to 200°C for complete digestion. The dried residues in the beaker were re-dissolved with 2% HNO$_3$ for the determination of total metal.

Suspended particulate matter (SPM) collected on the 0.45 µm MCE Millipore filter paper was dried and removed carefully for metal determination. 0.05 g homogenized SPM were taken in hot plate and digested with the same procedure as for sediments. The dried residues of SPM re-dissolved in 2% HNO$_3$ for total metal analysis.

Total metal in sediment and SPM samples were analyzed in ETAAS and FAAS (Electrothermal and Flame Atomic absorption spectrometry, Model: PinAAcle 900T, PerkinElmer) at CSIR-National Institute of Oceanography (CSIR-NIO). MAG-1, a fine-grained gray-brown clayey mud with low carbonate content, from the Wilkinson Basin of the Gulf of Maine (obtained from United States Geological Survey, Virginia, USA) and PACS-3, marine sediment obtained from National Research Council Canada (in absence of MAG-1) were used as certified sediment standard material. The recovery of the metals from certified material in all digestion procedure was above 95%. The reproducibility of the analysis was < 3% for all the metals.

2.8 Analysis of metal in the water sample

Collected surface water samples in the Teflon beaker (400 ml) were filtered immediately (within 72 hrs) in a cleaned laboratory through 0.45mm filters (MCE membrane, MF-Millipore) and acidified with ultra-pure HNO$_3$ to pH 2.5 as a preservative. The slightly modified method described in Tessier et al., 1980 and Satyanarayana et al., 1985 was used to extract dissolved metals in the filtrates. In the first stage of the method, dissolved metals were chelated with 10 mL of 1% (w/v)

Ammonium pyrrolidinedithiocarbamate (APDC) and extracted by 15 mL of methyl-isobutyl-ketone (MIBK). In the second stage of extraction the volume APDC and MIBK was reduced to 5 mL and 10 mL respectively. APDC is a non-specific chelating agent and forms stable complexes with transition metals in the water samples. The addition of MIBK allows APDC to dissolve in the organic phase. After the successful phase separation in the experiment, the metal complexes in MIBK was back extracted by 10 mL of 2% HNO_3 acid and analyzed in ETAAS and FAAS (Electrothermal and Flame Atomic absorption spectrometry, Model: PinAAcle 900T, PerkinElmer) at CSIR-National Institute of Oceanography (CSIR-NIO).

2.9 Metal analysis in benthic biota

Mangrove pneumatophores (aerial roots) were collected very close to the respective surface sediments. In the laboratory, the roots were immediately cleaned with ultra-pure water to completely remove fine sediment on its surface. Cleaned roots were freeze-dried until constant weight obtained.

Benthic fauna, live oysters (*Magallana* sp.) were collected from the sampling site and washed gently with seawater to remove the fine sediment deposition on its uneven shell surface. After cleaning, the live oysters were depurated for 24 hours in the plastic buckets/trays filled with filtered seawater brought from the sampling site. Once depurated, soft tissues were removed with the help of ultrapure pre-washed stainless-steel forceps and rinsed again with ultrapure water for the entire removal of fine sediments. Washed tissues were freeze-dried until the constant weight is obtained.

All the biological samples after drying were homogenized. 0.1 g of homogenized floral samples were taken in the acid-washed Teflon beaker and digested with 10 ml of an acid mixture of HF, HNO_3, $HClO_4$ (in 7:3:1) ratio on a hot plate at 80°C to 100°C. Faunal (soft tissue) samples were

also digested in the same method but with the different acid mixture of HNO_3 and H_2O_2 (1:1 ratio). The dried residues after complete evaporation were re-dissolved in 2% HNO_3 for metal analysis in ETAAS and FAAS (Electrothermal and Flame Atomic absorption spectrometry, Model: PinAAcle 900T, PerkinElmer) at CSIR-National Institute of Oceanography (CSIR-NIO). DORM 4, fish protein certified reference material (obtained from National Research Council Canada) were used as certified reference materials to check the metal recoveries.

2.10 Sequential extraction Method

The modified BCR sequential extraction protocol (originally proposed by Ure et al., 1993) was used to understand the distribution of metals in different geochemical phases of the sediment. BCR analytical protocol was first modified by Quevauviller et al., 1997, followed by Sahuquillo et al., 1999 and lastly by Rauret et al., 1999. The protocol after consequent modification was further slightly improved by Chakraborty et al., 2015b for our present study in which water-soluble metal complexes (a good indicator of bioavailability) in the sediments were separated. This step aids in the removal of the excess salt content present in the estuarine sediments. The modified BCR analytical procedure involving several chemicals of different strength help us in partitioning and determination of water-soluble metal complexes (Fr.1); ion-exchangeable and carbonate/bicarbonate metal forms (Fr.2); metals associated with Fe/Mn oxyhydroxides, the reducible fraction and believed as the sink of toxic metals in the sediment (Fr.3); metals bound to organic phases, oxidizable fraction and considered to remain for longer periods in the sediment (Fr. 4) and residual metal fraction, associated with silica matrix (Fr. 5) in the sediment. The extraction processes were performed in Teflon containers. The Suprapur grade (of Merck) or better (ultrapure) reagents were used in this analytical extraction method. The detailed procedure of the experiment is presented graphically as Figure 2.10.

The isolated solution in each step and the final residual fraction (in 2% ultrapure HNO_3 solution) were analyzed in the Electrothermal atomic absorption spectrometer (ETAAS) for metal concentration. BCR-701 certified sediment reference material (CRM) (produced by IRMM-Institute Reference material and Measurements, Geel, Belgium, and marketed by Sigma Aldrich) was used for the validation of metal extracted. In BCR-701 certificate no certified values for the water-soluble fraction are mentioned, therefore Fr.1 and Fr.2 (analyzed by this modified method) were added and compared with the certified reported values. Affirmation of the method was also done by correlating the total metal concentration in the sediment (determined by ETAAS) with the total sum concentration of each metal fractions obtained through the modified sequential extraction protocols. The comparability (near to 100% recovery) of both the values proves the BCR sequential extraction method is reliable and legitimate. Triplicate samples were used to ensure repeatability of results. The reproducibility of each measurement (n=3) was <1% for all the elements.
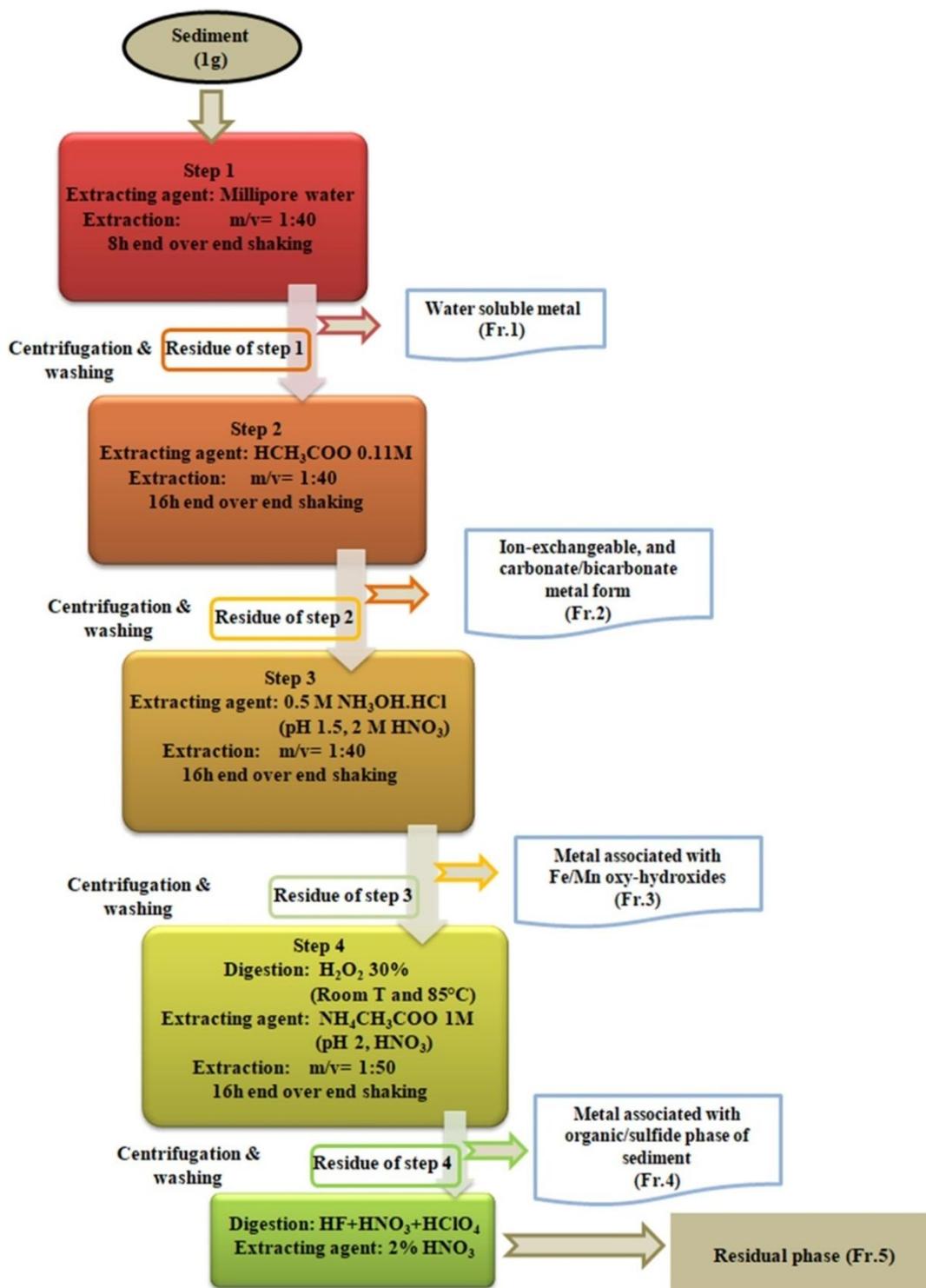
Figure 2.10: Schematic presentation of the modified BCR protocol used in this study.

2.11 Kinetic extraction study

The understanding of the stability of sedimentary metal complexes is very important to identify the bioavailability of metals from sediments. The kinetic model proposed by Olson and Shuman, 1985 is used in the present study to determine kinetically distinguishable forms of metals in the sediments. Ethylenediaminetetraacetate (EDTA) was chosen as the extracting agent for the toxic metal determination (from the sediments) as a function of time.

Experimental Procedure

2.0 g of sediment samples was added to 200 mL of 0.05 M EDTA (MerckPvt. Ltd.) solution (at pH 6.0) in a 400 mL capacity Teflon beaker (400 cm^3). The mixture was constantly stirred with a Teflon-coated magnetic stirring bar until the end of the experiment. The ratio of the mass of sediment to the volume of EDTA solution (mass/volume) was set at 0.01, as this ratio provided sufficiently high metal concentrations in the extract to be accurately quantified while requiring a minimum amount of sediment. A homogeneous suspension was maintained to avoid any change in the mass/volume ratio during sampling. Larger mass/volume ratio is undesirable, as they can cause problems with filtration. At set time intervals (0 min, 5 min, 10 min, 30 min, 45min, 60 min, 2 hr, 6 hr, 12 hr, 24 hr, 48hr, 72 hr), 2 mL of aliquots of the suspension were filtered through a 0.2 ml syringe filter (Millex, Millipore). The initial time for the kinetic measurement (i.e. t = 0 s) was taken as the time just before the sediment was added to the EDTA solution. The filtrate samples (1.0 mL) were then (in 2% ultrapure HNO_3) analyzed by GFAAS to monitor the change in concentrations of metals in the extracted solution as a function of time. The kinetic extraction experiments were performed in triplicate for all the samples to ensure the repeatability of the results. The pictorial presentation of the experimental set up is provided in Appendix 1.3.

EDTA the selected extracting reagent is a well-characterized, non-specific, strong chelating agent which allows excellent control of experimental variables. The EDTA-metal chelate complexes in the experiment are formed quickly and with great stability (Welcher, 1958). The major advantage of EDTA over other complexants is that a single 1:1 complex species is formed relatively than a stepwise formation of complexes, helping in simplifying calculations and analysis of results (Welcher, 1958). EDTA is a hexaprotic acid and naturally has large pH buffering capacity. It forms negatively charged metal complexes at most pH values, which helps to maintain extracted metals in the solution by reducing the chance of readsorption of extracted metals back to the sediment. The kinetic extraction experiments were performed in triplicate for all the samples to ensure repeatability of results.

Theory

The kinetic speciation experiment in this study is based on the kinetic model proposed by Olson and Shuman, 1985. The model is based on the assumption that each sediment sample has n different components, in which each component, M-sediment$_i$, exists in equilibrium with its dissociation products: M^{2+} or extractable M complexes, and naturally occurring heterogeneous complexant, sediment$_i$. The subscript, i, denotes different binding sites of the naturally-occurring heterogeneous complexant.

The extraction of metals from sediment using EDTA is presented by the following reactions (charges are omitted for simplicity):

$$M - sediment_i + EDTA \xrightarrow{k_i} M - EDTA + sediment_i \tag{1}$$

M-sediment$_i$ represent the metal ion (M), bound to a sediment binding site, sediment$_i$ and M-EDTA, represent the metal ion (M) bound by EDTA. If EDTA is added in large excess, the M is

extracted from the original sediment binding site with a rate constant k_i. The variation in concentration of M sediment$_i$ with respect to time is provided by the following pseudo first-order rate law.

$$-\frac{dc_{M-sediment_i}}{dt} = k_i c_{M-sediment_i} \tag{2}$$

The integrated rate law derived from equation 2, expressed in terms of the concentration of M−EDTA. The concentration of metal extracted, in solution (M−EDTA), rises exponentially over time to a limiting value, as shown in Eq. (3). Inopportunely, it is not feasible to study the individual binding sites separately or have a priori knowledge about the available number of distinct binding sites, as there could be a nearly continuous distribution of binding sites in the sediment. Hence, Eq. (3) is written as a summation of exponentials.

$$C_{M-EDTA}(t) = \sum_{i=1}^{n} C_{M-sediment_i}(1 - e^{-k_i \cdot t}) \tag{3}$$

This system described by Eq. (3) can be approximated by a two or three-component first-order reaction model.

$$C_{M-EDTA}(t) = c_1(1 - e^{-k_1 t}) + c_2(1 - e^{-k_2 t}) \tag{4}$$

Where,

$C_{M-EDTA}(t)$- Concentration of metal extracted by EDTA at time t.

c_1 & c_2- Concentrations of EDTA-extractable metal primarily bound to labile (quickly extracted), and stable (with respect to the time scale of measurement) sediment binding sites, respectively.

$k_1 \& k_2 -$ Dissociation rate constants of c_1 and c_2 respectively.

The kinetic model presume that 1) the reactions in the experiment are first-order or pseudo first-order; 2) reaction between M and EDTA is much faster than reaction involving dissociation of M–sediment complexes, so that the dissociation reaction is the rate-determining step, and the measured kinetics represent the kinetics of the dissociation of the metal complex, M–sediment$_i$3) M–sediment does not directly (i.e. without pre dissociation) interact with the EDTA, and 4) the ratio between the concentrations of complexed metal and free metal is much larger than unity (i.e. $c_{M–Sediment} /c_M \gg 1$).

Optimization of pH

The dependency of metal extraction by EDTA on pH is due to competitive extraction by H$^+$ ions (Bermond et al., 1998; Fangueiro et al., 2002), which is represented as below.

$$Sediment - M^{2+} + 2H^+ \leftrightarrow Sediment - 2H^+ + M^{2+}$$
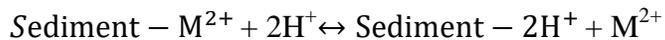
Hence, it is very important to determine at what pH value the competition between the extracted metal and H$^+$ ions could be minimized. The pH value should be optimized in such a way that it minimizes two unwanted effects due to low and high pH conditions. At very low pH condition, EDTA complexation efficiency could be reduced due to protonation of the EDTA molecule, at the same time at very high pH, an increasing tendency for the metal ions to hydrolyze to form slightly soluble metal hydroxides takes place.

To optimize pH, the experiment involving the extraction of Cu and Ni in different pH from estuarine sediments was carried out. The obtained result showed that within a pH range of around 5–9, no significant variation in the concentration of metal extracted were observed (Figure 2.11), indicating that the metals were chiefly extracted by EDTA and not by H^+. Further, after 24 h of extraction, no significant changes in pH were observed for any of the pH values studied. From this optimization study pH 6 was chosen an optimized pH as the value is acidic enough to prevent precipitation of metal hydroxides, as well as alkaline enough to decrease the competitive extraction by H^+. pH 6 also optimizes EDTA buffering capacity as pKa, 3 of EDTA is 6.16, which certifies minimal differences in pH throughout the kinetic extraction study.
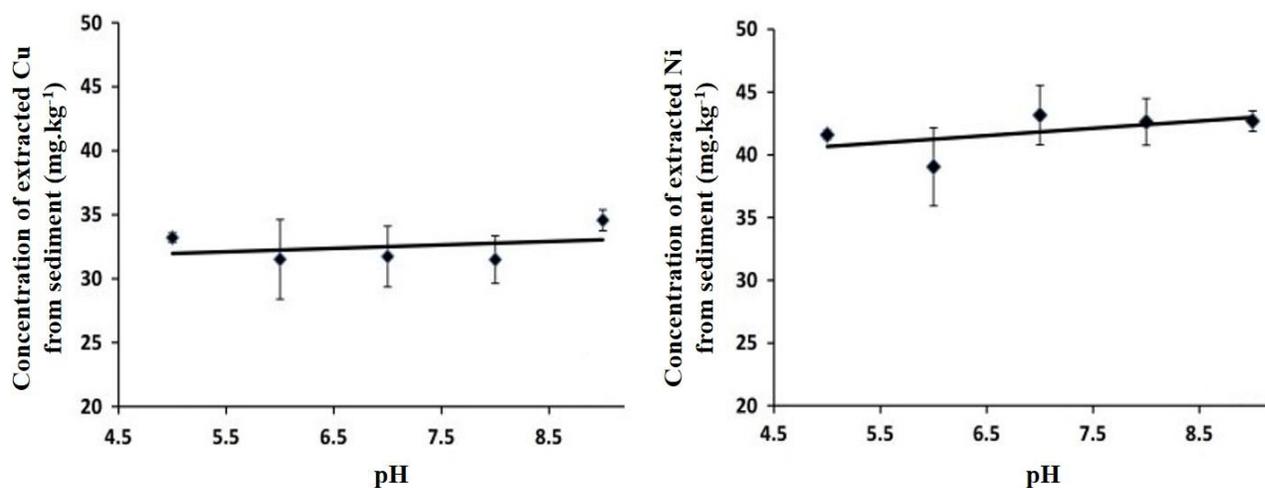
Figure 2.11: Changes in Cu and Ni concentrations respectively, as a function of pH.

Optimization of concentration of EDTA

EDTA is known as non-specific complexing agent and extracts a wide range of cations present in the sediments. Henceforth, the ratio of R, between the EDTA concentration and the total concentration of all extracted cations is a very important parameter

$$R = \frac{EDTA}{\sum [M_i^{n+}]}$$

where M_i^{n+} is considered as any major cation. Whenever $R \ll 1$, there is a lack of EDTA, and increasing concentrations of EDTA will extract greater concentrations of cations. However, when $R \gg 1$, EDTA is considered in excess and has attained maximum extraction efficiency. Hence, any further increases in EDTA concentration will not increase the concentrations of extracted cation (Bermond et al., 1998; Fangueiro et al., 2002). An excess requirement of EDTA is an obligatory condition for the kinetic model experiment to be valid, as it helps to minimize the suppression of metal solubilization due to competition by other major cations for complexation by EDTA (Ghestem and Bermond, 1998).

The optimization experiments for EDTA concentration indicates that the concentrations of Cu and Ni extracted by EDTA increased with the increasing concentrations. The concentrations of both the metals were found maximum in presence of 0.05 mol dm^{-3} of EDTA, indicating that 0.05 mol dm^{-3} of EDTA represents an excess of EDTA. Similar concentration has also been recommended for the harmonization of extraction techniques under the auspices of the Community Bureau of Reference (BCR) (Ure et al., 1993).

The lability criteria in kinetic study

The conception of lability illustrates the ability of complexes to maintain equilibrium with the free metal ion, M, within the perspective of an ongoing interfacial process in which a particular species, usually M, is consumed. Consider sediment of n components, in which each component, designated M-sediment$_i$, presents in equilibrium state with its dissociation products:

$$M - sediment_i \; \underset{k_{-1}}{\overset{k_1}{\underset{\longleftarrow}{\longrightarrow}}} \; M + \; sediment_i \; (slow) \tag{1}$$
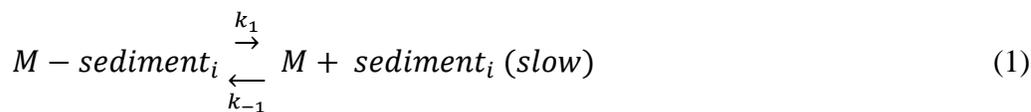
Where, k_1 and k_{-1} are the rate constants for complex association and dissociation respectively and the thermodynamic stability constant, K, equals k_1/k_{-1}.

Under conditions of sufficient availability of excess ligand (L) over metal (M), the association reaction is quasi-monomolecular with rate constant $k'_1 = k_1 c_L$. On time scales, t, much larger than the characteristic lifetimes of M $(\frac{1}{k'_1})$ and M-sediment$_i$$(\frac{1}{k_{-1}})$, a given metal ion undergoes frequent interchange between M and M-sediment$_i$. The complex system is then sufficiently dynamic to maintain bulk equilibrium and obeys the double condition (Gaabass et al., 2009).

$$k'_1 t, k_{-1} t \gg 1 \tag{2}$$

In the usual situation of practical interest, $K'(= Kc_{sediment})k'1/k_{-1} > 1$, so equation 2 reduces to $k_{-1}t \gg 1$. Thus, metal complexes in sediment will be called labile if their $k_1 t \gg 1$.

2.12 Statistical Analysis

All the data obtained from the study is an average of three replicates. Errors are shown along with the average values. All the data are presented with 95% confidence interval. Regression analysis, and Pearson's correlation coefficient were used to statistically evaluate the relationship between total metal in sediments, metal associated with different geochemical phases of the sediment (from sequential extraction study), labile metal sediment complexes (from kinetic speciation study), and accumulated metal concentrations in biological tissues of benthic organism.

Chapter 3

Speciation of Cu and Ni from the estuarine sediments

and its bioaccumulation in the mangrove roots

3.1 Introduction

In this study, an effort was made to understand the distribution and speciation of Copper and Nickel from mangrove sediments and their bioavailability to the mangrove species. BCR sequential extraction procedure and Kinetic extraction method were employed to understand how much metal (Cu and Ni) is bioavailable from sediments. Mangrove roots, pneumatophores are used in this study to determine bioaccumulated metals, which in turn could be a good indicator of bioavailable metal from sediments. Pneumatophores are selected as they are a perennial tissue emerging from and above the sediments and have an elongated period of trace element accumulation compared to other organs (Ismail, 2002; Silva et al., 1990). It also naturally comprises a high concentration of tannins in their tissue which are comparatively higher and favors enhanced binding of trace metals (Ismail, 2002). This study aimed to prove the hypothesis that non-residual (derived from sequential extraction method) and labile metal complexes (derived from kinetic extraction study) are responsible for bioavailable metal flux and upsurge the bioaccumulation rate in the mangrove roots. An effort was made to establish a linkage between metal (copper and nickel) speciation, and their bioavailability in a mangrove ecosystem.

3.2 Results and Discussions

The physical parameters of the sediment, sediment texture, total organic carbon (C_{org}), total nitrogen (TN), and total metal concentrationin the mangrove sediments are shown in Table 3.1. Stations, Mang-1 and Mang-2 were dominated with finer sediments (< 63 μm), while Mang-3, Mang-4, and Mang-5 sediments were dominated with the sand fraction. The average total Cu concentrations in the sampled sediments varied around 41.3-58.7 mg.kg^{-1}.

The total concentration of Pb in the sediments varies from 14.7 to 23.1 mg.kg^{-1}. Total Ni concentration in the sediments ranged from 52.6 to 76.5 mg.kg^{-1}. While the total concentration of Fe and Mn was found to vary 8.4% to 11.5% and 0.03 to 0.5 % respectively. The high concentration of Fe in sampled sediments could probably due to the high transportation activity of Fe ore through Mandovi estuary from the mines situated upstream region of Mandovi river. Pearson's correlation analysis has shown a significant positive correlation between the total Cu, and Ni in the mangrove sediments and the fine (clay, silt) particles content in the bulk sediments (Table 3.2). The comparatively higher metal concentration in the finer sediments is probably due to phenomena like sorption, co-precipitation, complexation which are found more predominant with finer particles due to more surface area (Chakraborty et al., 2014a, 2014d). A strong positive correlation was found between the finer particles (silt and clay) and C$_{org}$ content in the mangrove sediments indicating that finer sediment particles have high sorption capacity and affinity of organic carbon in an aquatic ecosystem (Hedges and Keil, 1995). A strong positive correlation was found between C$_{org}$ and TN in the sampled sediments which shows that majority of TN was associated with sedimentary organic carbon and could be regarded as total organic nitrogen (TON) (Gireeshkumar et al., 2013; Paropkari et al., 1987).

It is well acknowledged that the determination of total metal concentrations in sediments is inadequate to understand its speciation and bioavailability in a system. To further understand the metal speciation, geochemical fractionation and kinetic extraction study were carried for Cu and Ni from the mangrove sediments.

Table 3.1: Sediment texture, total organic carbon (C_{org}), total nitrogen (TN), total concentrations of metals and their variation in the mangrove sediments collected at Divar Island, Mandovi estuary.

Station	Sand (%)	Silt (%)	Clay (%)	C_{org} (%)	TN (%)	Cu_T (mg.kg^{-1})	Ni_T (mg.kg^{-1})	Pb_T (mg.kg^{-1})	Mn (%)	Fe (%)
Mang 1	19.6	48.7	31.8	8.8	0.42	57.6 ± 4.0	76.5 ± 4.9	21.4 ± 0.1	0.40 ± 0.01	9. 9 ± 0.1
Mang 2	17.7	50.2	32.1	7.5	0.39	58.7 ± 0.4	74.8 ± 0.6	23.1 ± 1.1	0.50 ± 0.01	11.5 ± 0.1
Mang 3	71.7	17.6	10.8	1.9	0.13	43.6 ± 1.8	59.2 ± 2.1	14.7 ± 0.2	0.26 ± 0.01	9.3 ± 0.1
Mang 4	67.3	20.9	11.8	2.1	0.13	41.3 ± 0.3	52.6 ± 2.3	15.6 ± 0.2	0.28 ± 0.01	10.6 ± 0.1
Mang 5	44.0	35.0	21.0	5.0	0.26	45.6 ± 0.2	57.0 ± 1.4	18.9 ± 0.3	0.03 ± 0.0	8.4 ± 0.1
MAG-1*						29.2 ± 0.8	53.8 ± 0.3			

Table 3.2: Pearson correlation between sediment texture, total organic carbon (C_{org}), total nitrogen (TN), and total Cu and Ni concentration in sediments.

	Sand (%)	Silt (%)	Clay (%)	C_{org} (%)	TN (%)	$[Cu]_T$ (mg.kg^{-1})	$[Ni]_T$ (mg.kg^{-1})
Sand (%)	1.00						
Silt (%)	-1.00	1.00					
Clay (%)	-1.00	1.00	1.00				
C_{org} (%)	**-0.98**	**0.98**	**0.99**	1.00			
TN (%)	**-0.99**	**0.99**	**1.00**	1.00	1.00		
$[Cu]_T$ (mg.kg^{-1})	**-0.95**	**0.95**	**0.96**	**0.94**	**0.96**	1.00	
$[Ni]_T$ (mg.kg^{-1})	**-0.89**	**0.88**	**0.91**	**0.90**	**0.92**	**0.98**	1

3.2.1 Sequential extraction of Cu and Ni in the mangrove sediments

Fractionation of Cu in the different geochemical binding phases of the sediment is shown in Figure 3.1. The study showed that various concentration of Cu has different affinities with binding phases of the sediments. The water-soluble Cu complexes (Fr. 1) from the studied sediments were found in very low concentration from all the sampling stations. The concentration of Cu exists as carbonate, bicarbonate and exchangeable forms (Fr. 2) in the sediments were in the range of 0.4 to 3.0 %. Around 1.2 to 5.9 % of total Cu was found associated with Fe/Mn oxyhydroxide phases (Fr. 3). The data acquired through the sequential extraction method found that the majority of non-residual Cu was associated with organic phases (Fr. 4) of the sediments which were relatively high and varied about 9.8% to 19.5 % of the total Cu in the studied sediments (Table 3.3). The Cu concentration in residual (Fr. 5) fraction of the studied sediments were higher and found in the range of 78.4% to 82.7% of the total Cu content in the studied sediments. The absolute Cu concentrations in the residual phase of the sediment varied from 35.5 to 46.0 mg.kg^{-1}, (Table 3.4) which were similar to the value of Cu described in the continental crust and shale (28.0–50.0 mg.kg^{-1}) (Nyakairu and Koeberl 2001; Rudnick and Gao 2003). The fractionation study showed that the stability and lability of Cu-sediment complexes in our mangrove sediments depend on the total concentration of Cu in the sediments. The same type of observation has also been reported in various literature (Flemming and Trevors, 1989; Lu and Allen, 2002; Robertson and Leckie, 1999).
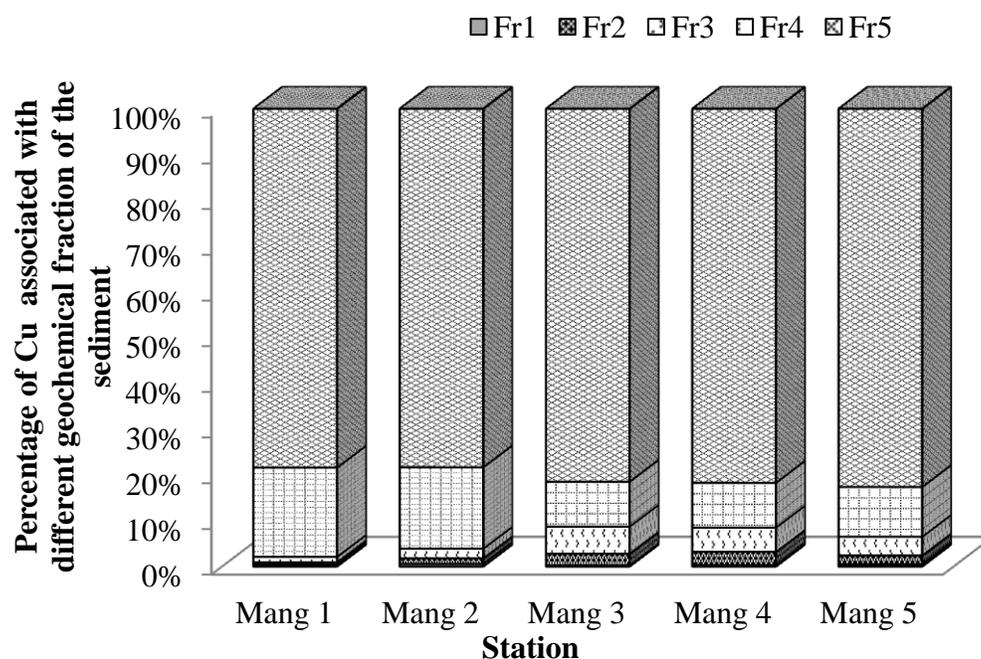
Figure 3.1: Geochemical fractionation of Cu from mangrove sediment.

Table 3.3: Distributions of Cu (relative % of the total metals content) in different phases of the mangrove sediments. Metals associated with different phases are presented as: water soluble (Fr.1), exchangeable + carbonates metal fraction (Fr. 2); fraction of metal associated with Fe and Mn oxides (Fr. 3): fraction of metal bound to organic matter (Fr. 4) and residual fraction (Fr. 5). $[Cu]_T$ and $[Cu]_{man}$ is total metal concentration in sediment and mangrove roots respectively $(mg.kg^{-1})$.

Station	Fr.1 (%)	Fr.2 (%)	Fr.3 (%)	Fr.4 (%)	Fr.5 (%)	$[Cu]_T$ $(mg.kg^{-1})$	$[Cu]_{man}$ $(mg.kg^{-1})$
Mang 1	0.5±0.0	0.4±0.0	1.2±0.0	19.5±0.9	78.4±3.9	57.6±2.8	7.5±0.3
Mang 2	0.6±0.0	1.2±0.0	2.1±0.1	17.8±0.8	78.4±3.9	58.7±2.9	12.2±0.6
Mang 3	0.2±0.0	2.6±0.1	5.9±0.2	9.8±0.4	81.4±4.0	43.6±2.1	10.9±0.5
Mang 4	0.3±0.0	2.9±0.1	5.3±0.2	9.8±0.4	81.8±4.0	41.3±2.0	13.7±0.6
Mang 5	0.5±0.0	1.9±0.0	4.1±0.2	10.9±0.5	82.7±4.1	45.6±2.2	11.1±0.5

Table 3.4: Cu concentration (mg.kg^{-1}) in different phases of the mangrove sediments. Metals associated with different phases are presented as: water soluble (Fr.1), exchangeable + carbonates metal fraction (Fr. 2); fraction of metal associated with Fe and Mn oxides (Fr. 3): fraction of metal bound to organic matter (Fr. 4) and residual fraction (Fr. 5). $[Cu]_T$ and $[Cu]_{man}$ is total metal concentration in sediment and mangrove roots respectively (mg.kg^{-1}).

Station	Fr.1 (mg.kg^{-1})	Fr.2 (mg.kg^{-1})	Fr.3 (mg.kg^{-1})	Fr.4 (mg.kg^{-1})	Fr.5 (mg.kg^{-1})	$[Cu]_T$ (mg.kg^{-1})	$[Cu]_{man}$ (mg.kg^{-1})
Mang 1	0.3 ± 0.0	0.2 ± 0.0	0.7 ± 0.0	11.2 ± 0.9	45.2 ± 3.9	57.6 ± 2.8	7.5 ± 0.3
Mang 2	0.4 ± 0.0	0.7 ± 0.0	2.1 ± 0.1	10.4 ± 0.8	46.0 ± 3.9	58.7 ± 2.9	12.2 ± 0.6
Mang 3	0.1 ± 0.0	1.1 ± 0.1	2.6 ± 0.2	4.3 ± 0.4	35.5 ± 4.0	43.6 ± 2.1	10.9 ± 0.5
Mang 4	0.1 ± 0.0	1.2 ± 0.1	2.2 ± 0.2	4.0 ± 0.4	33.8 ± 4.0	41.3 ± 2.0	13.7 ± 0.6
Mang 5	0.2 ± 0.0	0.9 ± 0.0	1.9 ± 0.2	5.0 ± 0.5	37.7 ± 4.1	45.6 ± 2.2	11.1 ± 0.5

The geochemical fractionation of Ni from the mangrove sediments showed that Ni also had different affinities for different binding-phases in the sediments (Figure 3.2). The concentrations of extracted water-soluble Ni complexes (Fr. 1) were found negligible. The concentration of carbonate, bicarbonate, and exchangeable Ni complexes (Fr. 2) in the studied sediments were also found very low and were in the range of 2.1-2.9% of total Ni. This low concentration of Ni complexes (Fr. 1 and Fr. 2) indicates that the mobility of Ni in the mangrove sediments is very less. Around 3.2–7.2% of total Ni was found associated with Fe–Mn oxyhydroxide phases (Fr. 3) in the sediments. It was found that 3.4–10.3% of the total Ni concentration was complexed with organic phases (Fr. 4) of the sediments (Table 3.5). The majority of the total Ni (varying 79.8% to 91.3%) were found to present as a residual fraction (Fr. 5). This sequential extraction study showed that the absolute Ni concentrations in the residual phase of the sediment varied from 48 mg.kg^{-1}to 61.6 mg.kg^{-1}, (Table 3.6) which were similar to the value of Ni described in the upper continental crust (UCC) (47.0 mg.kg^{-1}) and post archean Australian Shale (PAAS) (55.0 mg.kg^{-1}) (Nyakairu and Koeberl 2001; Rudnick and Gao 2003).
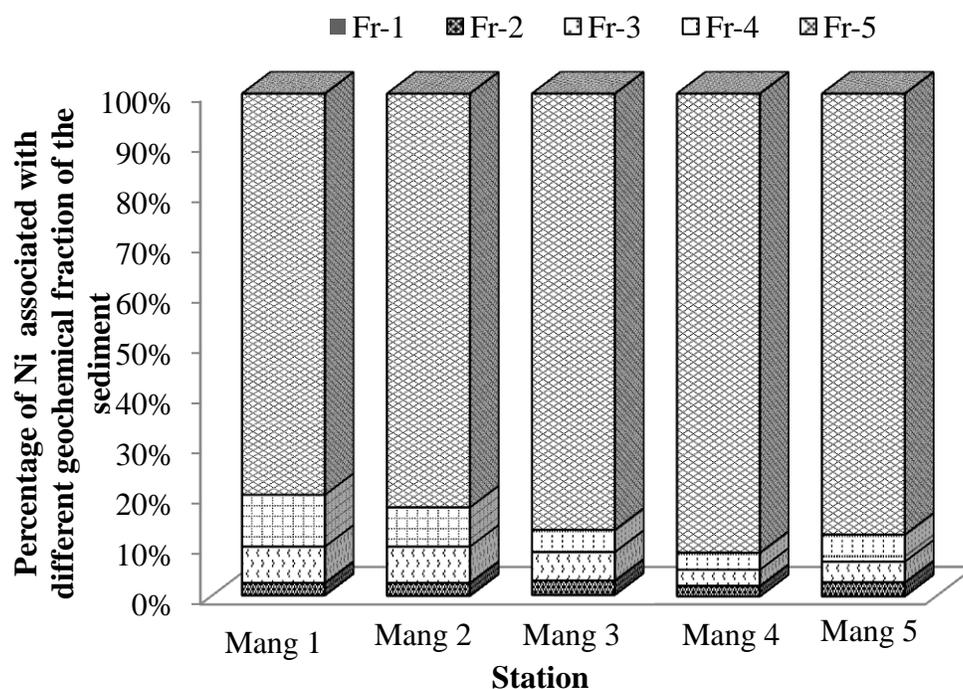
Figure 3.2: Geochemical fractionation of Ni from mangrove sediment.

Table 3.5: Distributions of Ni (relative % of the total metals content) in different phases of the mangrove sediments. Metals associated with different phases are presented as: water soluble (Fr.1), exchangeable + carbonates metal fraction (Fr. 2); fraction of metal associated with Fe and Mn oxides (Fr. 3): fraction of metal bound to organic matter (Fr. 4) and residual fraction (Fr. 5). Ni_T and $[Ni]_{man}$ is total metal concentration in sediments and mangrove roots respectively $(mg.kg^{-1})$.

Station	Fr.1 (%)	Fr.2 (%)	Fr.3 (%)	Fr.4 (%)	Fr.5 (%)	$[Ni]_T$ $(mg.kg^{-1})$	$[Ni]_{man}$ $(mg.kg^{-1})$
Mang 1	0.2±0.0	2.5±0.1	7.2±0.3	10.3±0.5	79.8±3.9	76.5±3.8	8.3±0.4
Mang 2	0.1±0.0	2.6±0.1	7.2±0.3	7.8±0.3	82.3±4.1	74.8±3.7	6.8±0.3
Mang 3	0.3±0.0	2.9±0.1	5.7±0.2	4.5±0.2	87.7±4.3	59.2±2.9	5.8±0.2
Mang 4	0	2.1±0.1	3.2±0.1	3.4±0.1	91.3±4.5	52.6±2.6	6.2±0.3
Mang 5	0	2.8±0.1	4.1±0.2	5.4±0.2	87.6±4.3	57.0±2.8	3.5±0.1

Table 3.6: Concentration of Ni (mg.kg^{-1}) found in different phases of the mangrove sediments. Metals associated with different phases are presented as: water soluble (Fr.1), exchangeable + carbonates metal fraction (Fr. 2); fraction of metal associated with Fe and Mn oxides (Fr. 3): fraction of metal bound to organic matter (Fr. 4) and residual fraction (Fr. 5). Ni_T and $[Ni]_{man}$ is total metal concentration in sediments and mangrove roots respectively (mg.kg^{-1}).

Station	Fr.1 (mg.kg^{-1})	Fr.2 (mg.kg^{-1})	Fr.3 (mg.kg^{-1})	Fr.4 (mg.kg^{-1})	Fr.5 (mg.kg^{-1})	$[Ni]_T$ (mg.kg^{-1})	$[Ni]_{man}$ (mg.kg^{-1})
Mang 1	0.2±0.0	1.9±0.1	5.5±0.3	7.9±0.5	61.0±3.9	76.5±3.8	8.3±0.4
Mang 2	0.1±0.0	1.9±0.1	5.4±0.3	5.8±0.3	61.6±4.1	74.8±3.7	6.8±0.3
Mang 3	0.2±0.0	1.7±0.1	3.4±0.2	2.7±0.2	51.9±4.3	59.2±2.9	5.8±0.2
Mang 4	0	1.1±0.1	1.7±0.1	1.8±0.1	48.0±4.5	52.6±2.6	6.2±0.3
Mang 5	0	1.6±0.1	2.3±0.2	3.1±0.2	49.9±4.3	57.0±2.8	3.5±0.1

Figure 3.3 presents the changes in the distribution of Cu and Ni between Fe/Mn oxyhydroxide and organic phases in the sediments with varying total metal concentrations. Cu associated with the organic binding phases gradually increased with the increasing Cu concentration (loading) in the sediments. However, it is compelling to observe that the Cu associated with Fe/Mn oxyhydroxide phases decreases with the increasing Cu loading in the sediments suggesting that Cu prefers to associate with the organic phase of the sediments as compare with the Fe/Mn oxyhydroxide phase. The present observation indicates that non-residual Cu has a higher affinity for the organic binding phases in all the studied sediments. The similar observation has been observed by various authors (Cao et al., 2004; Chakraborty et al., 2016a, 2009; Chakraborty and Chakrabarti, 2006; Meima et al., 1999) describing that Cu has a strong affinity for the binding sites of organic phases in sediments and with the dissolved organic carbon in natural systems.

Association of Ni in both the phases (Fe/Mn oxyhydroxide phase and organic phase) gradually increased with the increasing total Ni loading in the sediments and probably indicates that both the sediment binding phases have sufficient sites to bind increasing Ni concentrations in the sediments (Figure 3.3). Thus, the sequential extraction study showed that these two geochemical phases of the sediments were found to be the major controlling factors in the case of Ni speciation and their bioavailability, whereas organic phases controlled Cu bioavailability in the studied mangrove sediments.
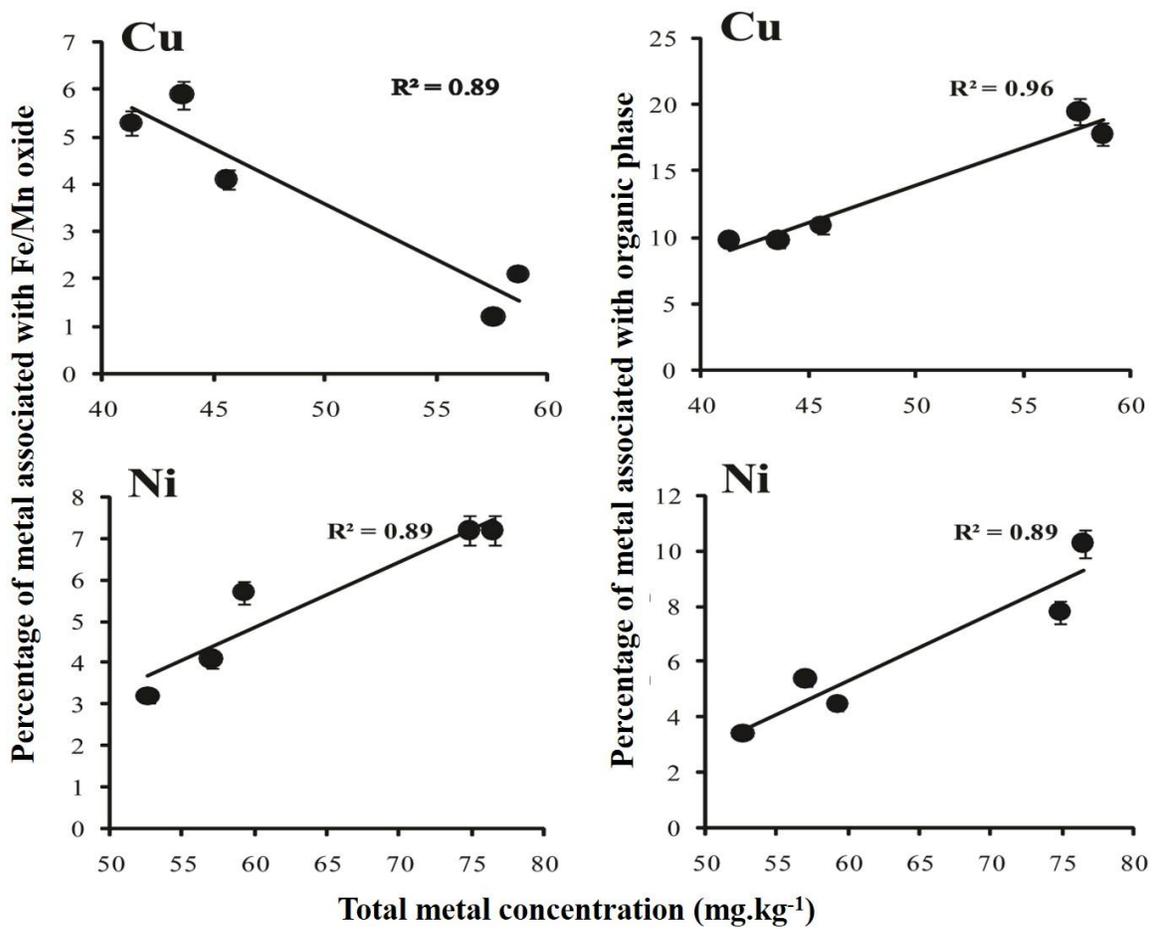
Figure 3.3: Variation of Cu and Ni association with Fe/Mn oxyhydroxide and organic phases in the sediments as a function of total metal concentration.

The larger association of Cu and Ni within the residual fraction in the studied sediments was further reanalyzed by leaching the non-residual metals complexes from the sediments by heating the sampled sediments at 80°C in 50% hydrochloric acid (HCl) for 4 hrs. This procedure could unable to leach out Cu, and Ni associated with more resistant silicates and aluminosilicates (the residual fraction). A comparable concentration of Cu and Ni in residual fraction (by following BCR protocol) and residual part (after leaching with 50% HCl) of the sediments were found. This study confirms that major fractions of both the metals were relatively immobile species and were in high concentrations in the studied mangrove sediments. To validate the modified sequential extraction method results, BCR-701 standard reference material was analyzed. The recoveries in each fraction were in agreement with the certified values for both the metals.

3.2.2 Kinetic speciation of Cu and Ni in the mangrove sediments

Figure 3.4 a & b represents the variation in concentrations of extracted Cu and Ni in 0.05 M EDTA solution as a function of time. Each curve shows an exponential increase in the Cu and Ni concentrations within the extracting EDTA solution with respect to time. The kinetic extraction data obtained from the curves were then fitted mainly into two-component model as it was the simplest model and provides an adequate statistical and visual fit to the data. Though the employment of more components in the model, the data are often statistically fit better, the additional components were often of negligible concentration or had a high degree of uncertainty. The two-component system in this experiment could not be presumed that there are only two distinct sediment binding sites present.

Figure 3.4 a shows two distinguishable features, a quickly rising section that denotes the rapid dissociation rate (k_{d1}) of weak Cu-sediment complexes (c_1), and another part lying

almost parallel to the x-axis which represents relatively strong Cu sediment complexes (c_2) with slower dissociation rate constant (k_{d2}). The initial part of all curves obtained from the experimental study are found nearly indistinguishable from one another, signifying that they all represent dissociation of one or more Cu complexes with very similar fast dissociation rate constants. All these Cu complexes with a fast dissociation rate could probably have low thermodynamic stability and were dynamic in nature (within the time scale of the measurement). The detailed information about the dissociation rate constants and dynamic fractions of Cu sediment complexes obtained in this study are presented in Table 3.7. Fractions of weak Cu-sediment complexes (c_1) having low thermodynamic stability were found in the range of 33.4% to 54.9%. The k_{d1} values of fast dissociating Cu-sediment complexes were found to vary from 2.0×10^{-3} to 5.3×10^{-3} respectively. While slow dissociating Cu complexes (c_2) were found to be varied from 47% to 66%. However, dynamic fast dissociating Cu-sediment complexes (c_1) obtained from the study could probably be considered as a good representative of bioavailable Cu from the mangrove sediments

Each curve in Figure 3.4 b represents similar two distinguishable features: a quickly rising section [rapid dissociation rate (k_{d1}) of weak Ni-sediment complexes (c_1)], and another part lying parallel to x-axis [relatively stronger Ni complexes (c_2) with slower dissociation rate constant (k_{d2})]. The first part of all the curves obtained from the experimental study are found almost indistinguishable, suggesting that they represent dissociation of one or more Ni complexes (c_1) with very similar fast dissociation rate constants (k_{d1}); probably indicating that all Ni complexes are having low thermodynamic stability and are dynamic (within the time scale of the measurement) in nature.

The percentage of thermodynamically weak Ni-sediment complexes (c_1) were found in the range of 33.8% to 55.8% of the total dynamic extractable Ni in the studied mangrove sediments. The variation in the respective dissociation rate constants (k_{d1}) of Ni-sediments complexes were found in the range of 1.3×10^{-3} to 8.1×10^{-4}. These weak Ni-sediment complexes (c_1) with low thermodynamic stability could probably considered as a good representative of bioavailable Ni in the mangrove sediment (Table 3.7). The concentrations of relatively strong Ni-sediment complexes (c_2) with higher thermodynamic stability were found to vary from 44.2% to 66.6% (of the total extractable Ni) with slow dissociation rate constants (k_{d2}) varying from 2.3×10^{-5} to 3.2×10^{-5} in the studied sediments.
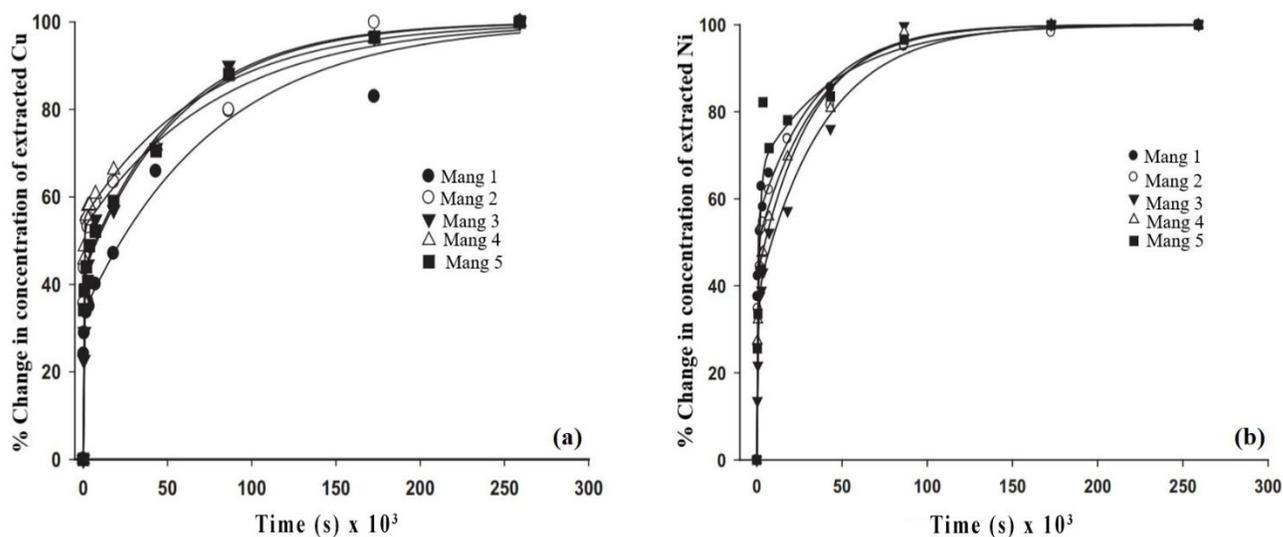
Figure 3.4: Release of extractable dynamic Cu (a) and Ni (b) complexes from the sampled sediments with respect to change in time in presence of 0.05 M EDTA at pH 6.

Table 3.7: Kinetically distinguishable components of Cu and Ni complexes in mangrove sediments and their respective dissociation rate constants.

Metal	Station	Total dynamic fraction (mg.kg^{-1})	c_1(%)	k_{d1} (s^{-1})	c_2(%)	k_{d2} (s^{-1})	Concentration of metals in mangrove roots (mg.kg^{-1})
	Mang 1	29.1±1.3	33.4±2.2	(3.8±0.2) x10^{-4}	66.6±4.7	(1.3±0.2) x10^{-5}	7.5±0.2
	Mang 2	26.2±2.7	52.7±1.6	(3.4±0.3) x10^{-3}	47.4±3.4	(1.2±0.2) x10^{-5}	12.2±0.4
Cu	Mang 3	13.2±0.9	43.1±2	(2.0±0.2) x10^{-3}	56.9±3	(1.8±0.2) x10^{-5}	10.9±0.1
	Mang 4	14.3±1.1	54.9±1.2	(5.3±0.4) x10^{-3}	45.1±2.6	(1.4±0.2) x10^{-5}	13.7±0.2
	Mang 5	17.8±1.3	52.7±1.6	(3.4±0.4) x10^{-3}	47.4±3.4	(1.2±0.3) x10^{-5}	11.1±0.3
	Mang 1	33.8 ± 0.8	55.8±3.0	(3.0±0.7) x10^{-3}	44.2±6.9	(2.5±0.7) x10^{-5}	8.3±0.1
Ni	Mang 2	33.4±2.2	49.5±4.6	(2.1±0.6) x10^{-3}	50.5±5.8	(2.7±0.6) x10^{-5}	6.8±0.4
	Mang 3	15.6±1.8	37.6±2.7	(1.4±0.3) x10^{-3}	62.4±3.2	(2.6±0.4) x10^{-5}	5.8±0.2
	Mang 4	16.8±1.9	41.9±1.9	(1.3±0.5) x10^{-3}	58.2±2.3	(3.2±0.4) x10^{-5}	6.2±0.1
	Mang 5	25.2±3.1	33.8±1.1	(8.1±1.2) x10^{-4}	66.6±11.9	(2.3±0.2) x10^{-5}	3.5±0.2

3.2.3 Comparison of non-residual Cu and Ni (sequential extraction study) with dynamic metal fraction (kinetic extraction method)

In this segment, an attempt was made to understand the dynamic behavior and bioavailability of Cu and Ni in the mangrove sediments with help of combining the data obtained by the sequential extraction method and the kinetic speciation study. The use of these two different methods, with different measurement timescales and detection windows, will provide better information about the speciation of metals in natural systems (Chakraborty, 2010; Chakraborty et al., 2011, 2009).

The total concentration of non-residual (Fr. 1 + Fr. 2 + Fr. 3 + Fr. 4) Cu and Ni found from sequential extraction method and dynamic fractions of Cu (c_1) and Ni (c_1) obtained from kinetic extraction study were compared (Table 3.8).The data obtained from two above mentioned methods were found comparable suggesting that the Cu and Ni complexes in Fr. 1, Fr. 2, Fr. 3 and Fr. 4 (obtained from sequential extraction study) in the studied sediments were probably are dynamic in nature having lower thermodynamic stability compare to their EDTA complexes. Therefore, inferring that (by comparing the non-residual and dynamic concentrations of these metals) that a fraction of metal found associated with Fe/Mn oxyhydroxide and organic phases of the sediments were dynamic in nature (within the time scale of measurement in the kinetic extraction study).

The statistical correlation analysis between the non-residual metal fractions, obtained from the sequential extraction study and dynamic fractions, obtained from the kinetic extraction study for both Cu and Ni were performed (the data is presented in Table 3.9 a and

b). The analysis revealed that a strong correlation was found between the dynamic fractions (kinetic extraction study) and non-residual fractions (sequential extraction study) for both the metals. Thus, the statistical analysis probably confirms that the data obtained for Cu and Ni (non-residual and dynamic fraction) from the studied sediments using both the method could be a strong representative of bioavailable metals in the sediments. Accumulation of Cu and Ni in mangrove roots was further determined to establish the linkage between speciation of Cu and Ni and their bioavailability in the mangrove sediments.

Table 3.8: Comparison between the sequentially extracted non-residual fractions of Cu and Ni with the kinetically extracted Cu and Ni dynamic metal fractions from the sediments.

Station	Metal	Sequentially extracted non-residual fractions (Fr. 1+Fr.2+Fr. 3+Fr. 4) (mg.kg^{-1})	Kinetically extracted dynamic metal fractions (c_1) (mg.kg^{-1})
Mang 1		12.4 ± 0.5	9.7 ± 0.2
Mang 2		13.6 ± 0.7	13.8 ± 0.4
Mang 3	Cu	8.1 ± 0.1	5.7 ± 0.2
Mang 4		7.5 ± 0.3	7.8 ± 0.3
Mang 5		8.0 ± 0.6	9.4 ± 0.1
Mang 1		15.5 ± 0.2	18.9 ± 0.9
Mang 2		13.3 ± 0.3	16.5 ± 0.6
Mang 3	Ni	7.9 ± 0.4	5.9 ± 0.3
Mang 4		4.6 ± 0.2	6.9 ± 0.4
Mang 5		7.0 ± 0.1	8.5 ± 0.2

Table 3.9 a: Pearson Correlation coefficient derived from the analysis between dynamic Cu complexes, labile Cu complex (c_1), non-residual Cu fractions (Fr.1+Fr.2+Fr.3+Fr.4).

	Total dynamic complex, Cu (mg.kg^{-1})	c_1 (mg.kg^{-1})	Fr.1 (mg.kg^{-1})	Fr.2 (mg.kg^{-1})	Fr.3 (mg.kg^{-1})	Fr.4 (mg.kg^{-1})	Non-residual Cu (mg.kg^{-1})
Total dynamic complex, Cu (mg.kg^{-1})	1.00						
c_1 (mg.kg^{-1})	0.75	1.00					
Fr.1 (mg.kg^{-1})	0.91	**0.93**	1.00				
Fr.2 (mg.kg^{-1})	-0.94	-0.50	-0.74	1.00			
Fr.3 (mg.kg^{-1})	**-0.79**	-0.30	-0.48	0.91	1.00		
Fr.4 (mg.kg^{-1})	**0.99**	**0.74**	0.91	-0.91	-0.71	1.00	
Non-residual Cu (mg.kg^{-1})	**0.93**	**0.81**	0.95	-0.79	-0.52	0.97	1.00

Table 3.9 b: Pearson Correlation coefficient derived from the analysis between dynamic Ni complexes, labile Ni complex (c_1), non-residual Ni fractions (Fr.1+Fr.2+Fr.3+Fr.4)

	Total dynamic complex, Ni (mg.kg^{-1})	c_1 (mg.kg^{-1})	Fr.1 (mg.kg^{-1})	Fr.2 (mg.kg^{-1})	Fr.3 (mg.kg^{-1})	Fr.4 (mg.kg^{-1})	Non-residual Ni (mg.kg^{-1})
Total dynamic complex, Ni (mg.kg^{-1})	1.00						
c_1 (mg.kg^{-1})	0.95	1.00					
Fr.1 (mg.kg^{-1})	0.21	0.40	1.00				
Fr.2 (mg.kg^{-1})	0.73	0.70	0.68	1.00			
Fr.3 (mg.kg^{-1})	**0.81**	**0.89**	0.70	0.88	1.00		
Fr.4 (mg.kg^{-1})	**0.91**	**0.97**	0.56	0.79	0.92	1.00	
Non-residual Ni (mg.kg^{-1})	**0.88**	**0.95**	0.65	0.87	0.98	0.98	1.00

3.2.4 Bioaccumulation of Cu and Ni in mangrove pneumatophores

To analyze and understand the actual bioavailability of metals in a system, the determination of total metal concentrations in living organisms habited in that particular natural system is very important (Chapman et al., 2003; Schloter et al., 2003; Zhou et al., 2008). Mangrove roots especially pneumatophores are known to be efficient indicators of metal pollution from sediment (Ismail, 2002; Silva et al., 1990). The assimilation of Cu and Ni in the mangrove roots from the sediments was entirely anticipated to depend on their speciation in the sediments. The total concentrations of Cu and Ni accumulated in the sampled mangrove roots system were found in the range of 7.5 to 13.7 mg.kg^{-1} and 3.5 to 8.3 mg.kg^{-1} respectively (Table 3.3 and 3.5). The change in concentration of both the bioaccumulated metals in mangrove roots as a function of their concentrations in different geochemical fractions is shown in Figure 3.5. It shows that the accumulation of Cu in mangrove roots decreased with the increasing association of Cu with the organic phases of the sediment. While increased in the association of Cu with Fe/Mn oxyhydroxide phase has increased the Cu accumulation in mangrove roots. This suggests that Cu associated with the organic phase of the sediment were not bioavailable whereas Cu associated with Fe/Mn oxyhydroxide phase was bioavailable.

In case of Ni, accumulation rate in the mangrove root gradually increased with the increase in the association of Ni with Fe/Mn-oxyhydroxide and organic phases of the sediment, indicating that a part of Ni, associated with Fe/Mn oxyhydroxide and organic phases, were probably bioavailable from the sediments.
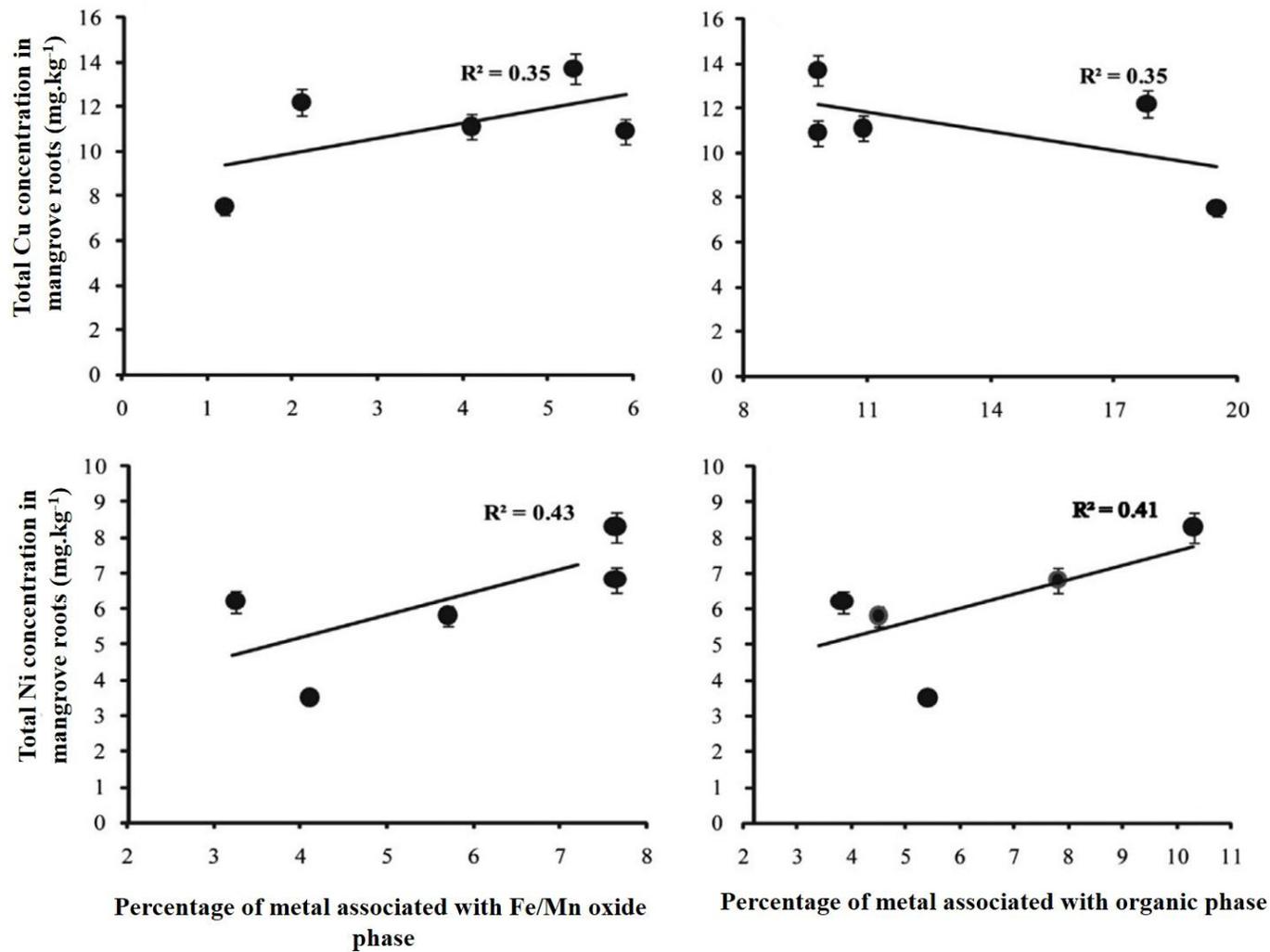
Figure 3.5: Changes in the total concentration of Cu and Ni accumulations in the mangrove roots as a function of Cu and Ni associated with Fe/Mn oxyhydroxide and organic phases of the sediment.

Table 3.10 represents the correlation analysis between the accumulated concentrations of metals (Cu and Ni) in mangrove roots and the non-residual metals (Cu and Ni) concentration in the sediment. A significant positive correlation was found between the Cu fraction (Fr. 2 and Fr.3) with the accumulated Cu in the mangrove roots. A negative correlation was found between the total Cu, Cu associated with the organic phase, and bioaccumulated Cu in mangrove roots. Thus, the analysis showed that the accumulation of Cu in mangrove roots did not gradually increase with the increasing total Cu loading and Cu associatied with the organic phases in the sediments, indicating that organic binding phase in the mangrove sediments could probably be acted as a buffer to control Cu bioavailability in the system.

The correlation analysis showed the concentrations of Ni associated with Fe/Mn oxyhydroxide (Fr. 3) and organic phases (Fr. 4) gradually increased with the total increase in the Ni concentration of the sediments. Thus, suggesting that these two geochemical phases of the sediments were the key controlling factors for Ni speciation and its bioavailability in the mangrove sediments.

Table 3.10: Pearson correlation between the accumulated metals (Cu and Ni) in the mangrove roots collected from sampled stations and metals (Cu and Ni) associated with different geochemical fractions in the studied sediments. $[Cu]_{sediment}$ and $[Ni]_{sediment}$ represents total Cu and Ni concentration from sediments respectively, $[Cu]_{Mangrove}$ and $[Ni]_{Mangrove}$ represents total Cu and Ni concentration from mangrove roots respectively.

	$[Cu]_T$ sediment (mg.kg^{-1})	Fr.1 (%)	Fr.2 (%)	Fr.3 (%)	Fr.4 (%)	Fr.5 (%)	$[Cu]_T$ Mangrove (mg.kg^{-1})
$[Cu]_T$ sediment (mg.kg^{-1})	1.00						
Fr.1 (%)	0.80	1.00					
Fr.2 (%)	-0.93	-0.77	1.00				
Fr.3 (%)	**-0.95**	-0.85	0.97	1.00			
Fr.4 (%)	**0.98**	0.74	-0.95	-0.97	1.00		
Fr.5 (%)	-0.93	-0.55	0.80	0.83	-0.94	1.00	
$[Cu]_T$ Mangrove (mg.kg^{-1})	-0.52	-0.24	**0.76**	**0.59**	**-0.60**	0.46	1.00

	$[Ni]_T$ sediment (mg.kg^{-1})	Fr.1 (%)	Fr.2 (%)	Fr.3 (%)	Fr.4 (%)	Fr.5 (%)	$[Ni]_T$ Mangrove (mg.kg^{-1})
$[Ni]_T$ sediment (mg.kg^{-1})	1.00						
Fr.1 (%)	0.37	1.00					
Fr.2 (%)	0.12	0.50	1.00				
Fr.3 (%)	**0.95**	0.62	0.34	1.00			
Fr.4 (%)	**0.95**	0.29	0.10	0.84	1.00		
Fr.5 (%)	-0.98	-0.39	-0.21	-0.93	-0.98	1.00	
$[Ni]_T$ Mangrove (mg.kg^{-1})	**0.70**	0.45	-0.40	**0.66**	**0.64**	-0.63	1.00

Figure 3.6 represents the analysis in the change in concentration of Cu and Ni accumulation in the mangrove roots against the dynamic Cu and Ni complexes (c_1) and their dissociation rate constant (k_{d1}) in the sediments. The results showed that the accumulation of Cu in mangrove roots gradually increased with the increasing concentrations of labile Cu-sediment complexes (c_1) and their dissociationrate constants (k_{d1}). The accumulation of Ni in mangrove roots also gradually increased with the increasing concentrations of labile Ni-sediment complexes (c_1) and their dissociation rate constants (k_{d1}) in the studied sediments. Thus, suggesting that labile metal-sediment complexes (determined by kinetic speciation study) could also be a good indicator of bioavailability in a mangrove system.

Based on the result of two speciation methods (sequential extraction method and kinetic extraction method) employed in this study to understand the bioavailability of Cu and Ni from sediments and its relationship with accumulated metals in mangrove roots, a plausible pathway has been suggested.
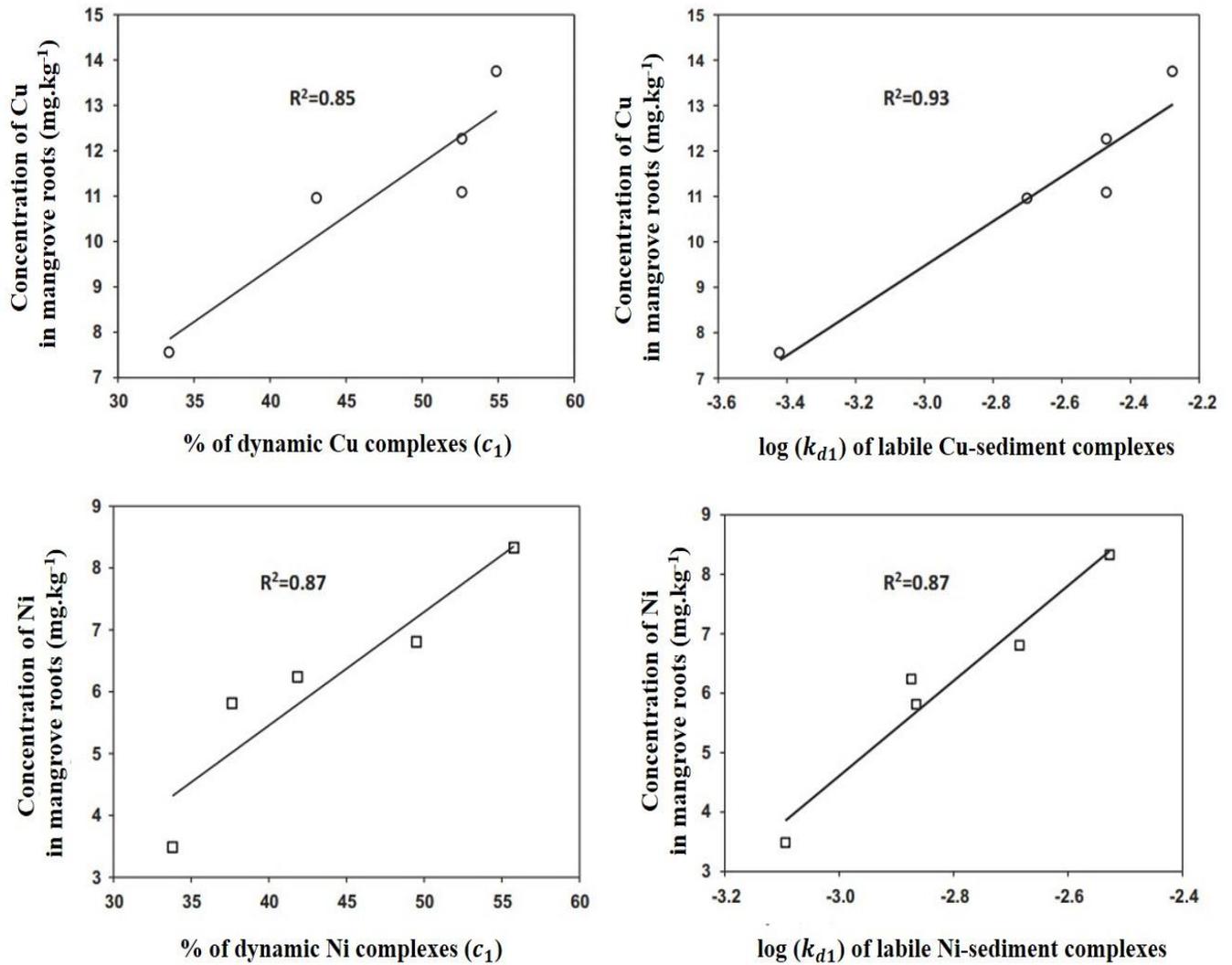
Figure 3.6: Variation in the total concentration of metals (Cu and Ni) accumulation in the mangrove roots against the concentration of dynamic metal complexes (Cu and Ni) (c_1) and their respective dissociation rate constant ($\log k_{d1}$) in the mangrove sediments.

Generally, in a natural system, the dissociation of M-sediment complexes is a fundamental process. The uptake route of metal by mangrove roots (pneumatophores) from metal-sediment complexes could basically happen in two different pathways (1) disjunctive and (2) adjunctive pathways (Kraemer et al., 2006). In disjunctive pathways, the dissociation of metal-sediment complexes initially takes place, after the dissociation the metal ion then probably enters into the sediment pore water and further taken up inside the root system. Whereas, in the adjunctive pathway, the direct association of biotic binding ligands (from mangrove roots) may occur to form a ternary complex (root-metal-sediment) with the metal followed by loss of the sediment (as a ligand) (as shown below).

$$M - sediment + Pneumatophores \leftrightarrow Pneumatophores - M - Sediment$$

$$\rightarrow M - Pneumatophores + Sediments$$
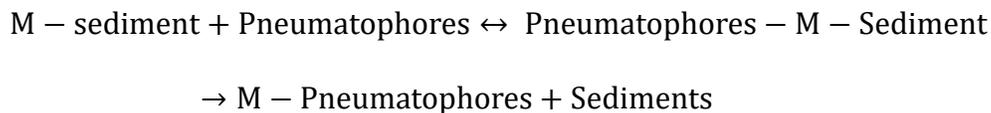
The total concentration of Cu and Ni were determined in the sampled mangrove sediment pore waters to provide information for the anticipated metal uptake mechanism by mangrove roots. The concentration of these metals in the pore water was found to depend on the lability of both metals. Concentrations of Cu and Ni in pore water increased with the increasing lability of the metal-sediment complexes and their dissociation rate constants. At the same time, distinct increases in metal accumulation were also found in the mangrove roots with the increasing lability and dissociation rate of metal-sediment complexes. Therefore, a plausible pathway of metal uptake and its accumulation in mangrove roots from sediment is proposed and shown in Figure 3.7. Overall, based on the result obtained in this study; it is proposed that the disjunctive pathway could be one of the most plausible mechanisms for metal uptake by mangrove roots from the sediments.
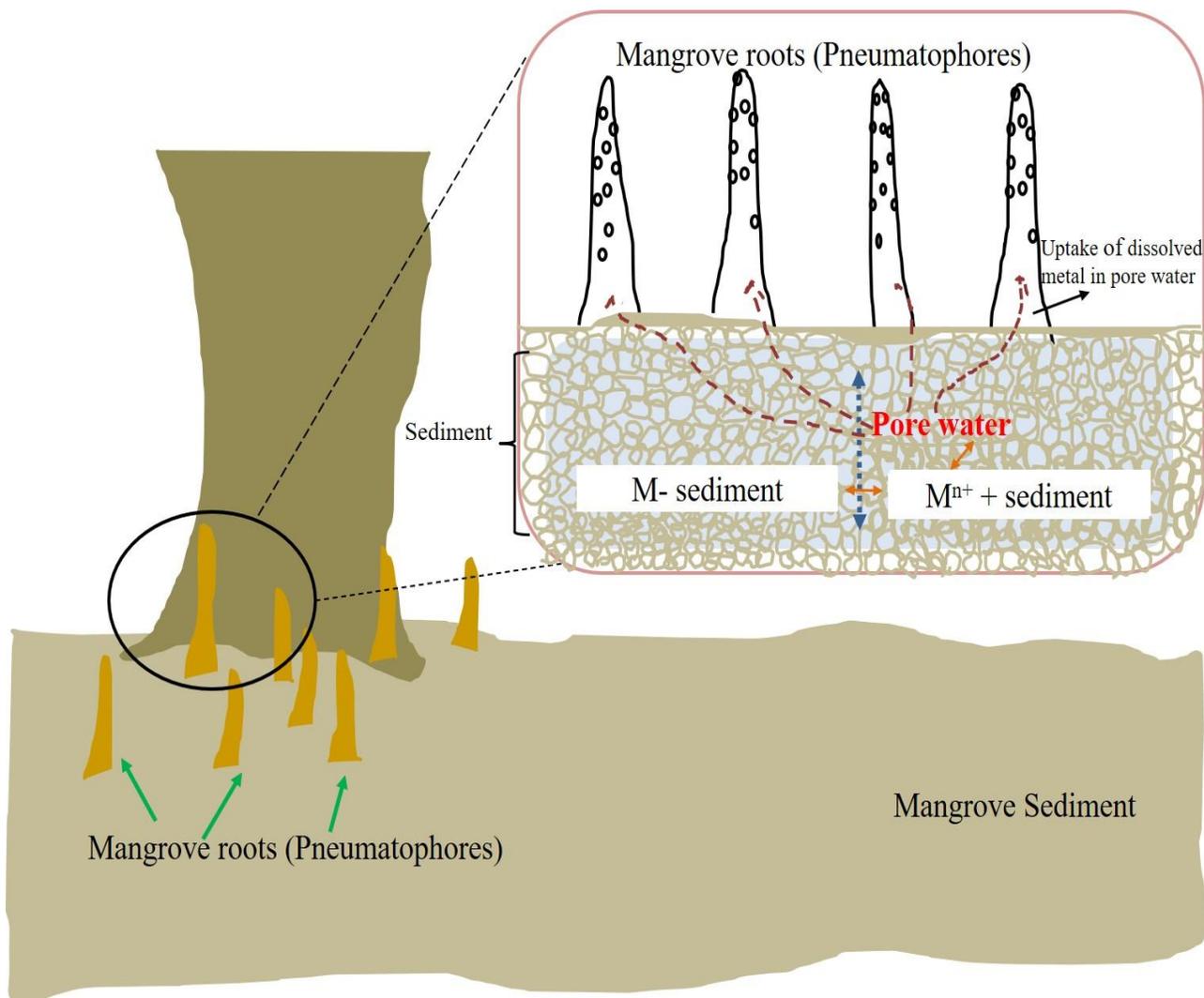
Figure 3.7: A plausible, disjunctive pathway for uptake of metal by mangrove roots (pneumatophores) from mangrove sediments.

3.3 Conclusion

This study shows the linkage between the speciation of Cu and Ni (bioavailability) from the mangrove sediments and its accumulation in the mangrove roots (pneumatophores). The study related the speciation of Cu and Ni (performed through sequential extraction and kinetic extraction methods) in the mangrove sediments to their bioavailability in the system. Geochemical fractionation study has shown that Fe/Mn oxide and organic phases in the sediments are major binding phases for non-residual Cu and Ni. However, Cu was found to have strongly interacted with different binding ligands present in the organic phases of the sediments. Thus, acting as a buffer to control the bioavailability of Cu from the mangrove system. In the case of Ni, a part of Ni associated with Fe/Mn oxide and organic phases in the sediments were bioavailable and were the major controlling factors for Ni speciation and its bioavailability. The total concentration of Cu and Ni accumulated in the mangrove roots increases with increasing concentrations of labile complexes of Cu and Ni in mangrove sediments. Dissociation rate constants (obtained through kinetic study) of metal-sediment complexes in mangrove sediments influenced metal accumulation in mangrove roots, indicating that labile metal-complexes and their dissociation rate constants could be a good indicator of metal bioavailability in a mangrove system. Thus, chemical speciation of Cu and Ni from the mangrove sediments played an important role in bioaccumulation of Cu and Ni in mangrove roots from a tropical estuarine system.

Chapter 4

Speciation of Cd from the estuarine sediments

and its bioaccumulation in the edible oyster

4.1 Introduction

In the previous chapter, I have employed mangrove roots, (pneumatophores) as a good indicator of bioavailable metal from sediments. In this study, I have used mollusk (bivalves) to understand the bioavailability of toxic metals from sediments. Bivalve mollusks (especially oysters, mussels) are efficient indicators of sediment contamination by toxic metals (Spooner et al., 2003). Due to their filter-feeding nature, they concentrate high concentration of toxic metals in their soft tissues. The consumption of such toxic metal-contaminated sea food has been described as a vital path of human exposure to toxic metals in India (Sankar et al., 2006; Sivaperumal et al., 2007; Vinodhini and Narayanan, 2008). Oysters were selected as a suitable candidate to determine toxic metal accumulation in this study, as it is known to naturally bioaccumulate contaminants (Geffard et al., 2007; Jeon et al., 2010). In an environment, bioaccumulation of toxic metals in oysters takes place via three major routes, (i) suspended sediments, (ii) water column, and/or (iii) diet (Rand, 1995). Lee et al., 2015 have reported that the diet of oysters comprises 79% of fine sediment and 21% algae. Studies by Ward and Shumway, 2004 have also shown that bivalve ingests sediments along with food. Presently, only a few studies have been carried out to understand the sedimentary metal speciation and their relation to bioaccumulation in a tropical estuarine system. The main aim of this study was to recognize the importance of toxic metal distribution and speciation in the water column, suspended particulate matter (SPM), and bulk, as well as finer particles of tropical estuarine sediments, in interpreting toxic metal bioaccumulation in edible oysters (*Magallana* sp.).

In this study, an attempt was made to understand the relationship between geochemical fractionation of metals in a tropical monsoon fed estuarine system (Zuari estuary) and its relation with bioavailability. BCR sequential extraction procedure was employed to understand how much metal is bioavailable from sediments.

4.2 Results and Discussions

Sampling from August-December 2014

Initially, we have carried out the geochemical fractionation study from the estuarine sediments in a single station (Station-2) from Agacaim, from August to December 2014. Variation of the environmental, physical parameters of the sediment, and total metal concentration in the estuarine sediments are shown in Table 4.1. The pH of the overlying water column varied from 7.4 to 8.2. Whereas, the salinity of the overlying water column was in the range of 10-30 PSU. Lower pH and salinity in the monsoon period were due to the influence of freshwater influx in the estuary. The DO (dissolved oxygen) and water temperature of the overlying water column have shown not much variation. Sediment texture was dominated with finer sediments (< 63 µm) in the month of August, November, and December, while in September and October the sediments were dominated with the sand fraction. The average total concentration of Cu, Ni, Pb, and Cd concentrations in the sampled sediments were lower (except Pb) in the monsoon period (August-October) compare to post-monsoon (November-January). The total concentration of Cu, Ni, Pb and Cd in the sediments varied from 37.9 to 88.3 mg.kg^{-1}, 59.6 to 149.6 mg.kg^{-1}, 13.7 to 69.6 mg.kg^{-1}, and 0.17 to 0.49 mg.kg^{-1} respectively.

Table 4.1: Sediment texture, environmental parameters of overlying water column and total metal concentration in estuarine sediments.

Sampling station	Sand (%)	Silt (%)	Clay (%)	pH	Salinity (PSU)	Water Temperature (°C)	Dissolved Oxygen (ml.l^{-1})	Cu_T (mg.kg^{-1}) Sediment	Ni_T (mg.kg^{-1}) Sediment	Pb_T (mg.kg^{-1}) Sediment	Cd_T (mg.kg^{-1}) Sediment
Aug St-2	25.3	18.7	56.7	7.4	11	31.7	4.6	69.4 ± 1.7	93.7 ± 2	69.6 ± 1.5	0.17 ± 0.01
Sep St-2	41.3	17.3	41.4	7.6	10	31.8	4.8	38.2 ± 0.02	63.5 ± 1.5	43.5 ± 0.6	0.26 ± 0.01
Oct St-2	62	7	30.9	7.9	28	32	6.2	37.9 ± 0.3	59.6 ± 1.2	39.8 ± 0.5	0.17 ± 0.01
Nov St-2	29.7	14.7	55.6	8.1	28	32	5.8	67.4 ± 0.5	104.5 ± 2.3	13.7 ± 0.1	0.49± 0.06
Dec St-2	10.3	15.6	74	8.2	30	32.2	7.3	88.3 ± 0.7	149.6 ± 0.9	19.2 ± 0.02	0.32± 0.11
ERL (mg.kg^{-1})								34	20.9	46.7	1.2
ERM (mg.kg^{-1})								270	51.6	218	9.6

Table 4.2: Total Organic Carbon (C_{org}), total nitrogen (TN), molar C/N ratio (C:N$_{molar}$), and δ^{15}N, and $\delta^{13}C_{org}$ in the mangrove sediments.

Station	C_{org} (%)	TN (%)	C:N$_{molar}$	δ^{15}N (‰)	$\delta^{13}C_{org}$ (‰)
Aug St-2	2.4	0.20	13.9	5.2	-24.6
Sep St-2	0.8	0.08	11.3	4.2	-22.4
Oct St-2	2.1	0.13	19.2	3.0	-26.8
Nov St-2	1.9	0.17	13.1	4.3	-24.2
Dec St-2	3.1	0.30	12.7	5.5	-24.0

The analysis of ERL (Effect range low) and ERM (Effect range median) was performed based on total metal concentration from the sediments, to understand the estuarine sediment quality. The metal concentration found below ERL value shows not to cause any adverse effects, while a value greater than ERM indicates it to be very toxic. A station is rated as "good" if the concentrations of the metal are below the ERL limit, "intermediate" applies if any metal concentration is between ERL and ERM limit and a "poor" rating for the metal that exceeds ERM limit (Garcia et al., 2011; Long et al., 1995; MacDonald et al., 2000). The present study site was rated poor as the Ni concentration found in all the months was greater than the ERM value.

4.2.1 Nature of Sedimentary Organic Carbon

Total organic carbon (C_{org}) and TN (total nitrogen) in sediment samples varied from 0.8-3.1 % and 0.08-0.3% respectively. The lowest and highest value (for C_{org} and TN) was found in the month of September and December respectively. The regression analysis for variation of sedimentary TN against C_{org} showed a linear relation. Thus, the $C:N_{molar}$ ratio reported in this study is strictly C_{org}/total-N, they can be taken to approximate C_{org}/N_{org} ratio.

The total organic carbon to nitrogen ratio is widely used to determine the origin of organic matter in sediments (Hélie and Hillaire-Marcel, 2006; Perdue and Koprivnjak, 2007). Terrestrial plants have a higher $C:N_{molar}$ (> 20), while plankton and bacteria have lower values (6-7) and (2-4) respectively. Microbial degradation also alters the $C:N_{molar}$ ratio of the sediments. $C:N_{molar}$ ratio of terrestrial plants decreases with mineralization and planktonic origin may increase upon diagenesis. The $C:N_{molar}$ ratio was found in the range of 11.3 and 19.2 (Table 4.2). This clearly shows that sedimentary organic matter from sampled sediments was influenced by both marine and terrestrial derived OM.

Stable organic Carbon and Nitrogen isotopes are the most important tool for source organic matter identification (Carreira et al., 2002). $\delta^{13}C_{org}$ ranged -22.4‰ to -26.8 ‰, while $\delta^{15}N$ varied 3.0 to 5.5 ‰. Among the sampled months, October recorded the most depleted $\delta^{13}C_{org}$ ($-26.8‰$) and $\delta^{15}N$ (3.0‰) values corresponding with the highest C:N$_{molar}$ value (19.2) (Table 4.2) which indicates that the influence of terrestrial derived organic matter in the total organic carbon of sampled sediments was maximum in the month of October. This study indicates that organic matter in the C$_{org}$ from sampled sediments is derived from both terrigenous and marine sources input.
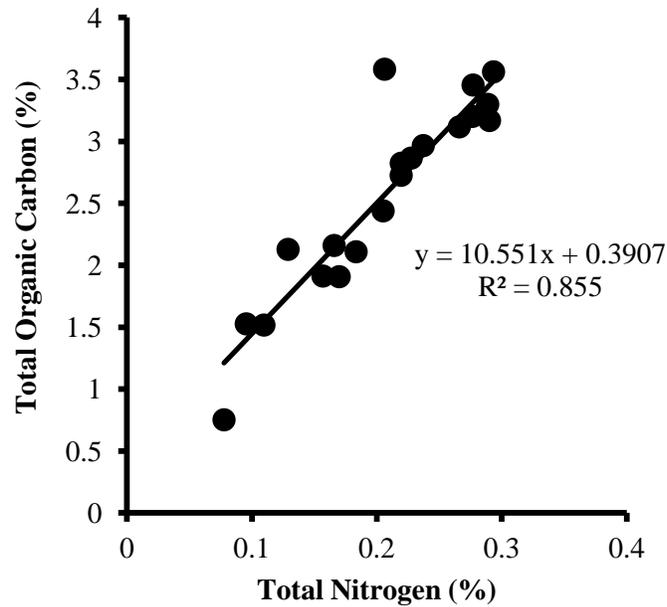
Figure 4.1: Variation of total nitrogen and total organic carbon in the sediments.

4.2.2 Bioaccumulation of metals in oyster

Analyses of total metal concentrations in benthic organisms were carried out. Benthic organisms are selected as they are the biota which habitats the bottom sediments and will be an excellent candidate to assess the actual bioavailability of metals from a sediment system (Long et al., 1995; Neff, 2002; Paul et al., 1992). They are also known to be efficient accumulation indicators of sediment contamination by toxic metals (Pinheiro et al., 2012). In this study oyster *Magallana* sp. was selected to determine bioaccumulated total toxic metal concentration. The total concentration of Cu in the oyster tissue was found in the range of $209.6 – 310.5$ mg.kg^{-1}. Total Ni concentration varied 0.7 to 2.2 mg.kg^{-1}, while total Cd concentration varied 3.2 to 12.2 mg.kg^{-1} (Table 4.3). The total concentration of Pb in oysters was found very low (< 0.05 mg.kg^{-1}). The concentration of Cu and Cd was found to be very higher in oyster tissue as compare to their sediment concentration. The higher Cd concentration found in the sampled oysters was uncommon as it is a non-essential metal and is not required for any biological function in the oyster system.

Overall, the sampled oysters showed high bioaccumulation of metals (especially Cu and Cd) in their soft tissue. Oysters in nature are selective suspension filter-feeders, feeding exclusively on phytoplankton, suspended materials, sediments, and aggregates containing detritus, fecal matter, and microorganisms by filtering huge volumes of seawater via their gills. Due to this high filtering activity, metals from the environment (water, sediment, suspended materials) can easily enter into its biological system and bioaccumulate. However, the physiology of oyster plays an important role in very high metal bioaccumulation compare to environmental metal concentration.

Wang et al., 2018 suggested that in oyster several specific genes are involved in the metal bioaccumulation process. The ATP-binding cassette (ABC) transporter family genes (membrane-bound proteins) are identified in transporting Cu and Cd in oyster cells, once inside the cells these

metals are complexed by different ligands. In the oyster system, metals are recognized to present largely in soluble pools, metallothioneins (MTs), and the insoluble pools, metal-rich granules (MRGs). Metallothioneins (MTs) are low molecular weight, cysteine-rich, metal-binding proteins involved in the process of metal detoxification (Wang et al., 2018). In oyster, Cu was found in strongly inducing the production of MTs. Metals such as Cr, Ni, and Pb was detoxified by binding with MRGs. (Wang et al., 2011). Apart from this, considerable amounts of metals are found associated with low molecular weight compounds such as taurine, lysine, and homarine, and mainly in large volumes in hemolymph (Wang et al., 2018).

Shi et al., (2019) in their study showed that hyper-accumulation of Cu and Zn in oysters takes place to increases its host resistance to pathogens. The high concentration of Cu and Zn in oyster tissue, expose the invading pathogen to a toxic environment and concurrently upsurge host resistance by producing hydroxyl radical and increasing metalloenzymes activity. The *Vibrio* culture experiments showed that high concentrations of Cu inhibited the growth of pathogen *V. harveyi*. In oyster, cellular localization, the chemical bindings of different metals in different biological compounds, and innate immunity all contribute to the high metal bioaccumulation.

Oyster is considered a culinary delicacy and is being consumed by the human population throughout the world. The maximum acceptable level of Cd in oysters for consumption is 2 mg.kg^{-1} in India (FSSAI, 2011), while in other countries such as the Unites States (US) it is 4 mg.kg^{-1}, 2 mg.kg^{-1} in Australia, and New Zealand and 1 mg.kg^{-1} in Malaysia (Broten, 1998; Kruzynski, 2001) (Table 4.4). The present result suggests that all the sampled oysters are contaminated by Cd and are not fit for human consumption.

Table 4.3: Total metal concentration in soft tissues of oyster samples.

Station	Cu Oyster (mg.kg⁻¹)	Ni Oyster (mg.kg⁻¹)	Cd Oyster (mg.kg⁻¹)
Aug St-2	232.2 ± 0.3	1.2 ± 0.1	7.3 ± 0.4
Sept St-2	209.6 ± 0.6	0.8 ± 0.6	3.2 ± 0.03
Oct St-2	310.5 ± 0.7	2.2 ± 1.3	8.9 ± 0.05
Nov St-2	249.2 ± 0.9	0.7 ± 0.2	11.8 ± 0.3
Dec St-2	271.3 ± 0.4	1.2 ± 0.2	12.2 ± 0.2

Table 4.4: Permissible limit for Cd in oystersrecommended by various worldwide organizations.

Authorities	Cd (mg.kg⁻¹) maximum permissible limit
Malaysian Food Act (Act 281) and Food Regulation (1985)	1
European Commission (2001)	1
FDA (2007)	4
Australia New Zealand Food Standards Code (2016)	2
Codex Alimentarius Commission (2006)	2
FSSAI (2011)	2
Present study	3.5-12.2

*FDA- Food and Drug Administration, FSSAI- Food Safety and Standard Authority of India.

From the present obtained result, the understanding of such high Cd bioaccumulation in oysters from the environment (sediments) is necessary. It is well acknowledged that the determination of total metal concentrations in sediments is inadequate to understand its speciation and bioavailability in a system. To further understand the metal speciation, geochemical fractionation study was carried for Cd from the estuarine sediments.

4.2.3 Geochemical fractionation of Cd in estuarine sediments

Fractionation of Cd in the different geochemical binding phases of the sediment is shown in Figure 4.2. The water-soluble Cd complexes (Fr. 1) from the studied sediments were found comparatively low in concentration from all the sampling stations. It was found to vary from 0.8 to 8.2% of the total Cd in sediments. The concentration of Cd exists as carbonate, bicarbonate and exchangeable forms (Fr. 2) in the sediments were in the range of 6.6 to 38.6%. The concentration of labile Cd complexes (Fr. 1 & Fr. 2) was found higher, suggesting the possibility of higher Cd mobility in the studied sediments. Around 13.9 to 26.8% of total Cd was found associated with Fe/Mn oxyhydroxide phases (Fr. 3). The data acquired through the sequential extraction method found that the majority of non-residual Cd was found associated with Fe/Mn oxyhydroxides phases of the sediment. In organic phases (Fr. 4) of the sediments, about 4.5% to 17.9% of the total Cd was found associated in the studied sediments (Table 4.5). The Cd concentration in residual (Fr. 5) fraction were highest and found in the range of 35.6% to 60.2% of the total Cu content in the studied sediment. The residual fraction of Cd (average absolute concentration, 0.13 mg.kg^{-1}, Table 4.6) was expected to be non-bioavailable.
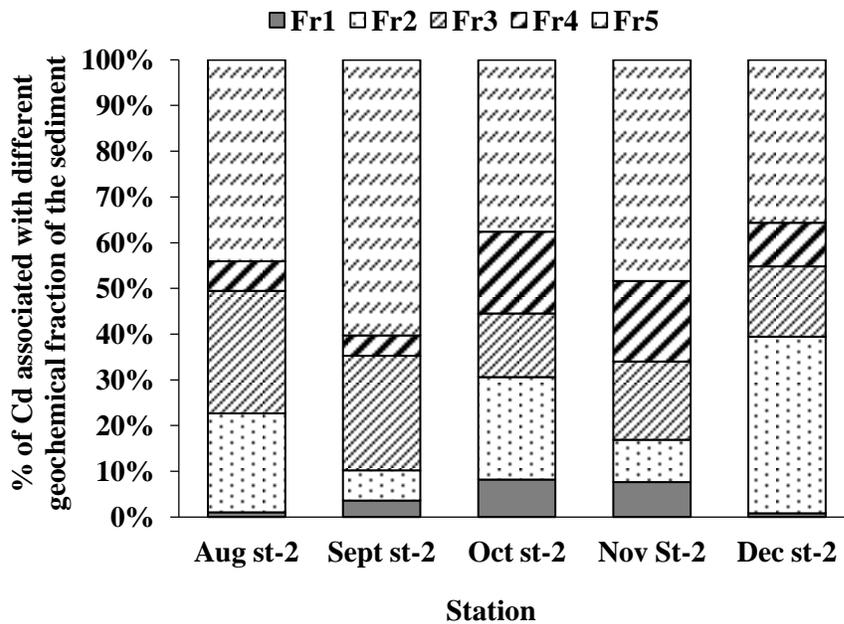
Figure 4.2: Geochemical fractionation of Cd from sampled sediments.

Table 4.5: Distribution of Cd (relative % of the total metals content) in different phases of the mangrove sediments. Metals associated with different phases are presented as: water soluble (Fr.1), exchangeable + carbonates metal fraction (Fr. 2); fraction of metal associated with Fe and Mn oxides (Fr. 3): fraction of metal bound to organic matter (Fr. 4) and residual fraction (Fr. 5). $[Cd]_T$ and $[Cd]_{oyster}$ is total metal concentration in sediment and oyster tissue respectively $(mg.kg^{-1})$.

Station	Fr.1 (%)	Fr.2 (%)	Fr.3 (%)	Fr.4 (%)	Fr.5 (%)	$[Cd]_T$ $(mg.kg^{-1})$	$[Cd]_{oyster}$ $(mg.kg^{-1})$
Aug St-1	1.0 ± 0.1	21.7 ± 0.03	26.8 ± 0.02	6.5 ± 0.02	44.1 ± 0.1	0.17 ± 0.01	7.3 ± 0.4
Sept St-1	3.6 ± 0.1	6.6 ± 0.1	25.1 ± 0.03	4.5 ± 0.02	60.2 ± 0.1	0.26 ± 0.01	3.2 ± 0.03
Oct St-1	8.2 ± 0.1	22.4 ± 0.1	13.9 ± 0.02	17.9 ± 0.03	37.6 ± 0.1	0.17 ± 0.01	8.9 ± 0.05
Nov St-1	7.7± 0.1	9.3 ± 0.1	17.1 ± 0.06	17.6 ± 0.1	48.4 ± 0.1	0.49± 0.06	11.8 ± 0.3
Dec St-1	0.8 ± 0.1	38.6 ± 0.08	15.4 ± 0.1	9.6 ± 0.1	35.6 ± 0.1	0.32± 0.11	12.2 ± 0.2

Table 4.6: Cd concentration (mg.kg^{-1}) in different phases of the mangrove sediments. Metals associated with different phases are presented as: water soluble (Fr.1), exchangeable + carbonates metal fraction (Fr. 2); fraction of metal associated with Fe and Mn oxides (Fr. 3): fraction of metal bound to organic matter (Fr. 4) and residual fraction (Fr. 5). Cd]$_T$ and [Cd]$_{oyster}$ is total metal concentration in sediment and oyster tissue respectively (mg.kg^{-1})**.**

Station	Fr.1 (mg.kg^{-1})	Fr.2 (mg.kg^{-1})	Fr.3 (mg.kg^{-1})	Fr.4 (mg.kg^{-1})	Fr.5 (mg.kg^{-1})	[Cd]$_T$ (mg.kg^{-1})	[Cd]$_{oyster}$ (mg.kg^{-1})
Aug St-1	0.002 ± 0.1	0.04 ± 0.03	0.05 ± 0.02	0.01 ± 0.02	0.07 ± 0.1	0.17 ± 0.01	7.3 ± 0.4
Sept St-1	0.01 ± 0.1	0.02 ± 0.1	0.07 ± 0.03	0.01 ± 0.02	0.16 ± 0.1	0.26 ± 0.01	3.2 ± 0.03
Oct St-1	0.01 ± 0.1	0.04 ± 0.1	0.02 ± 0.02	0.03 ± 0.03	0.06 ± 0.1	0.17 ± 0.01	8.9 ± 0.05
Nov St-1	0.04 ± 0.1	0.05 ± 0.1	0.08 ± 0.06	0.09 ± 0.1	0.24 ± 0.1	0.49± 0.06	11.8 ± 0.3
Dec St-1	0.003 ± 0.1	0.12 ± 0.08	0.05 ± 0.1	0.03 ± 0.1	0.11 ± 0.1	0.32± 0.11	12.2 ± 0.2

The changes in the distribution of Cd in different geochemical phases of the sediments with varying total Cd concentration are shown in Figure 4.3. The concentration of water-soluble Cd (Fr. 1) and Cd in exchangeable, carbonate and bicarbonate forms (Fr. 2) gradually increased (Fr.1+Fr.2) (~ 10.0 to 39.2% of total Cd) with the increasing Cd concentration (loading) in the sediments. A similar observation of Cd distribution in coastal sediments was reported by Chakraborty et al., (2012) from the east coast of India. The present result indicates that at low Cd loading, Cd probably advanced to undergo complexation reaction with strong binding sites (in sediments). However, when the sediment Cd concentration starts increasing, this toxic metal starts to form water soluble, exchangeable and carbonate and bicarbonate forms of Cd (after saturating relatively stronger binding sites) in the sediment. This analysis suggests that Cd mobility and bioavailability from sediments might increase with the increasing Cd loading inthe study area. However, Cd associated with Fe/Mn oxyhydroxide phases and organic phases also progressively increased with the increase in total Cd in the sediments. The present observation indicates that Fe/Mn oxyhydroxide phases was one of the major binding phases for non-residual Cd in the studied sediments. It was surprising to observe that Cd associated with non-residual and residual fraction of the sampled sediments increased with increasing total Cd loading, suggesting that Cd in different sediment phases were possible of similar nature with low thermodynamic stability.
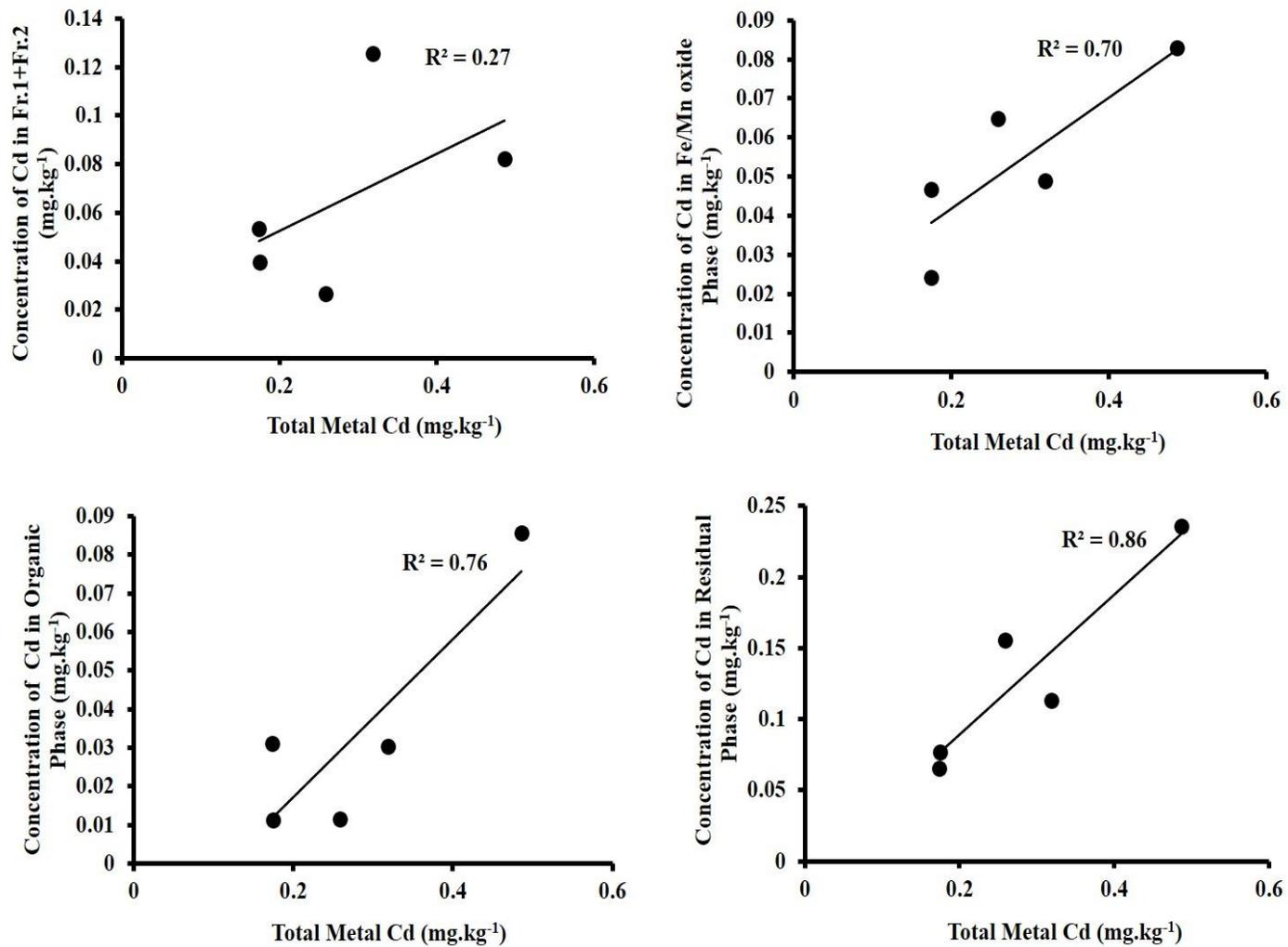
Figure 4.3: Variation of Cd in different geochemical phases of the sediments as a function of total Cd loading.

4.2.4 Relationship between Cd in estuarine sediments and oyster tissue

The changes in the concentration of bioaccumulation Cd in the oyster samples with respect to the concentration of Cd associated with different geochemical binding phases of the sediments are shown in Figure 4.4. The concentration of Cd in oyster soft tissues (*Magallana* sp.) was found to be gradually increased with the increasing total Cd concentrations. Increasing concentrations of water-soluble Cd (Fr. 1), Cd as exchangeable, carbonate, and bicarbonate forms (Fr. 2) in the sediments (Fr. 1+Fr. 2) has significantly increased the Cd accumulation in the oyster system. The analysis suggests that water soluble, exchangeable, carbonate, and bicarbonate forms of Cd could be a good indicator of Cd bioavailability in the sediment system.

However, a poor correlation was found between the Cd associated with Fe/Mn oxyhydroxide binding phase (Fr. 3, a major binding phase for non-residual Cd in the studied sediments) and the total Cd accumulated in the oyster. This suggests that an increase in Cd association with Fe/Mn oxyhydroxide phases of the sediment may probably decrease the Cd bioavailability and reduce the accumulation of Cd in the oyster tissue. The Cd accumulation in the oyster was found to increase (exponentially) with the increasing association of Cd with the organic phases (Fr. 4) of the sediments, suggesting that a part of Cd associated with the organic phase was possibly bioavailable.

The geochemical fractionation study of Cd showed that water soluble, exchangeable, and carbonate and bicarbonate forms of Cd and Cd associated with organic phases of the sediments might be a good indicator of bioavailability in the system.
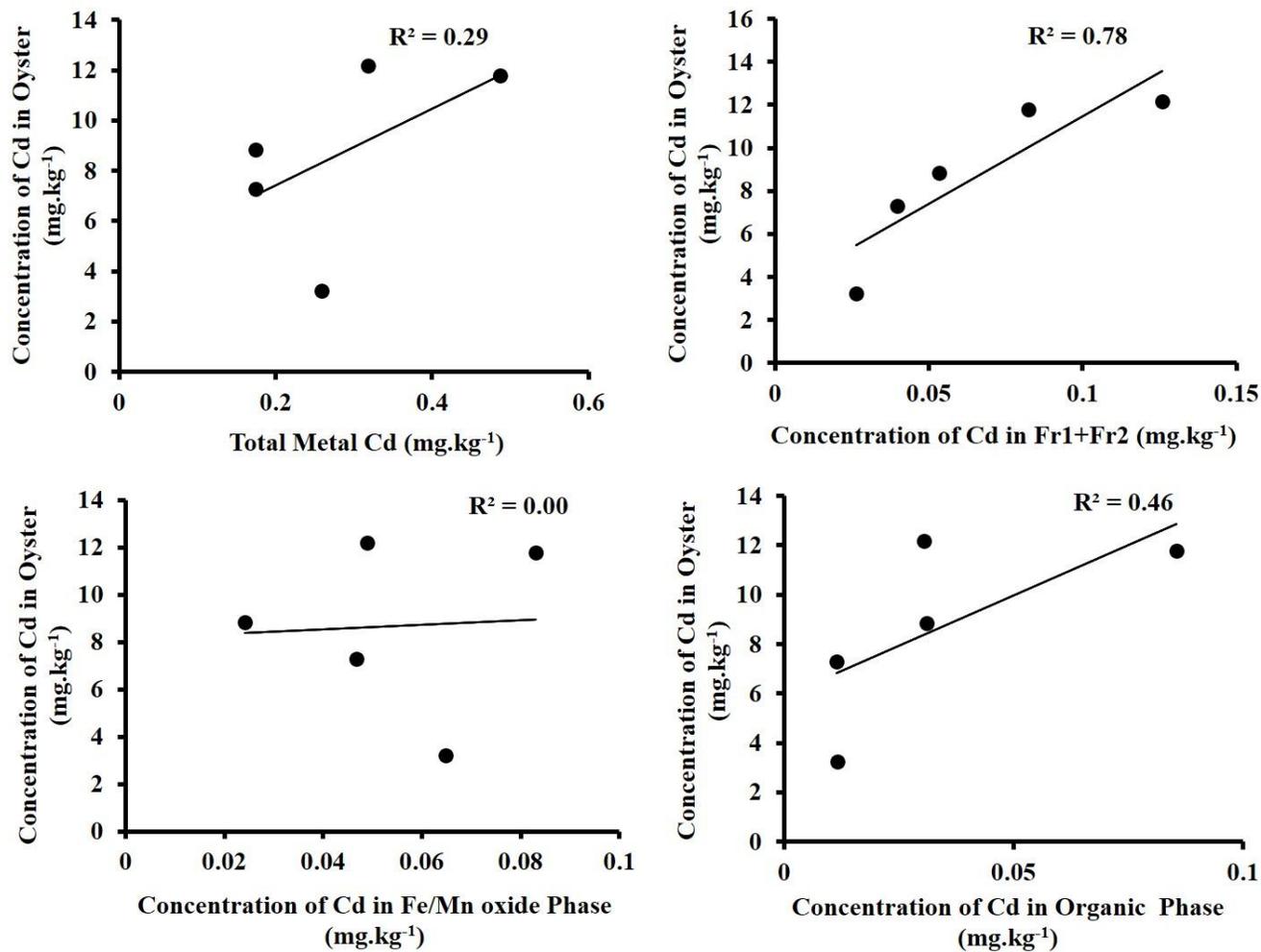
Figure 4.4: Bioaccumulation of Cd in oyster as a function of total Cd concentration and concentration of Cd in different geochemical phases of the sediments as a function of total Cd loading.

In the previous section (sampling from August-December 2014) we have carried out Cd speciation study to understand Cd speciation and its accumulation in the oyster system. Oysters are filter-feeders in nature and they filter huge amount of water for consuming food particles while filtering water finer sediments along with food particles (plankton and bacteria) enters into the oyster system. Hence, to have a better understanding of such high Cd bioaccumulation in the oyster, along with speciation of Cd from sediments, determination of Cd in water and SPM samples were also carried. The study was performed for one year with an additional sampling station (Station-1) from the same Agacaim area.

4.2.5 Physical and Chemical composition

The monthly variation in the pH, salinity, temperature of the overlying water column, and grain size distribution of the studied sediment in both the stations (St-1 and St-2) are shown in Table 4.7. The pH of the overlying water column was in the range of 7.4-7.9. Salinity varied from 10-34.3 PSU. Lower salinity values found during the monsoon period was due to the influx of freshwater into the estuary. There was no considerable variation in the temperature of the overlying water column in both the stations. The results show that except in October-December in St-1, both the sampling stations (St-1 and St-2) sediments were dominated with finer particles (silt + clay).

Table 4.7: Sediment texture, total organic carbon (C_{org}), total nitrogen (TN), molar C/N ratio ($C:N_{molar}$), environmental parameter of overlying water column (pH, salinity, water temperature) and their variation in the sediments from Zuari estuarine region.

Stations	Sand (%)	Silt (%)	Clay (%)	C_{org} (%)	TN (%)	$C:N_{molar}$	pH	Salinity (PSU)	Water Temperature (°C)
Feb-15 st-1	7.6	47.6	44.9	2.1	0.2	12.5	7.7	31	30.2
Mar-15 st-1	25.7	38.4	36	1.9	0.18	12.4	7.6	30.8	31
Apr-15 st-1	18.4	39.9	41.7	3	0.26	13.7	7.5	27.5	31.4
May-15 st-1	9.6	47.9	42.6	2.9	0.25	13.6	7.6	28.8	30.8
Jun-15 st-1	34.5	31.2	34.3	2.1	0.17	14.4	7.7	13.4	29.8
Jul-15 st-1	22.7	41.5	35.8	2.5	0.21	13.7	7.7	10.8	28.9
Aug-15 st-1	25.4	37.3	37.3	3.2	0.27	13.7	7.9	18.7	30.9
Oct-15 st-1	49.7	20.8	29.5	1.9	0.18	12.5	7.6	28.4	30.4
Nov-15 st-1	47.6	19.3	33.1	2.1	0.2	12	7.5	32.3	30.3
Dec-15 st-1	60.6	18.1	21.3	1.8	0.17	11.8	7.7	32.4	28.9
Jan-16 st-1	38.3	25.7	36	2.4	0.23	12.5	7.7	33.5	31.9
Feb-15 st-2	12	43.6	44.3	3.1	0.29	12.3	7.6	31.4	30.8
Mar-15 st-2	11.8	16.2	72	3.1	0.29	12.2	7.6	30.8	31.2
Apr-15 st-2	21	41.6	37.4	2.9	0.27	12.3	7.5	29.1	31.6
May-15 st-2	13.9	49.8	36.3	3.3	0.31	12.2	7.4	33.6	32
Jun-15 st-2	13.3	44.3	42.3	3.2	0.31	12	7.7	10.6	30.1
Jul-15 St-2	22.4	30.1	47.4	3.3	0.31	12.3	7.7	10.1	30.2
Aug-15 st-2	11.7	27.8	60.5	3.4	0.34	11.8	7.8	22.1	32.1
Oct-15 st-2	40.9	14.1	45	2.4	0.24	11.9	7.8	29	31.8
Nov-15 st-2	13.5	17.7	68.8	3.5	0.33	12.4	7.7	32.4	31.5
Dec-15 st-2	18.9	0	81.1	3.3	0.32	12.3	7.6	33.6	28
Jan 16 st-2	27.4	25.2	47.4	2.9	0.27	12.6	7.8	34.3	32.3

4.2.6 Intra-annual variation of total organic matter, total nitrogen and C:N$_{molar}$ ratio

The monthly variation of total organic carbon (C$_{org}$), total nitrogen (TN), and C:N$_{molar}$ in the studied sediments are presented in Table 4.7. The concentration of C$_{org}$varied from 1.8-3.2% in the St-1 and 2.4-3.5% in the St-2. The sedimentary TN contents varied from 0.17%-0.27% and 0.27-0.34% in the St-1 and the St-2 respectively. The highest concentrations of sedimentary C$_{org}$ and TN were found in the month of August in both the stations. A positive correlation was observed between the C$_{org}$ and TN with the finer fraction of the sediment (Table 4.8) and indicates that C$_{org}$ and TN had a strong affinity towards the clay fractions of the sediments. A linear relation with regression of R^2= 0.94 was found between the concentrations of TN and C$_{org}$ in the studied sediments and suggesting that a major part of TN was associated with C$_{org}$ in the sediments. These observations suggest that TN can be considered as organic N.

The C:N$_{molar}$ ration of the sediments were used to determine the sources of the sedimentary organic matter. It has been reported that organic matter from the planktonic source has low C:N$_{molar}$ ratio (ranging 4-10) while, higher C:N$_{molar}$ ratio (20 and above) are mainly derived from mangrove/terrestrial plants (Jennerjahn and Ittekkot, 1997; Prahl et al., 1994; Yamamuro, 2000). The C:N$_{molar}$ ratio ranged between 11.8 and 14.4 in the studied sediments, which are intermediate values and indicates an autochthonous and terrestrial input of organic matter. The low C:N$_{molar}$ ratio values from mangrove sediments could be due to bacterial activity and degradation of plant detritus. This clearly shows that sedimentary organic matters in the studied sediments were of mixed origin. The stable isotopic ratio of C$_{org}$, TN data obtained during 2014 sampling (August-December 2014) has also shown that sedimentary organic matter from the studied sediments is of mixed origin.

Table 4.8: Pearson correlation between the sediment texture, total organic carbon (C_{org}), TN and $C:N_{molar}$ ratio of the sediment

	Sand (%)	Silt (%)	Clay (%)	C_{org} (%)	TN (%)	$C:N_{molar}$
Sand (%)	1.00					
Silt (%)	-0.47	1.00				
Clay (%)	-0.57	-0.45	1.00			
C_{org} (%)	**-0.71**	0.07	**0.65**	1.00		
TN (%)	**-0.66**	-0.04	**0.70**	**0.97**	1.00	
$C:N_{molar}$	-0.11	0.35	-0.21	-0.06	-0.29	1.00

4.2.7 Total metal concentration in bulk sediment samples

The total concentration of Cu, Ni, Cr, Pb, and Cd in the sampled sediments was presented in Table 4.9. Concentration of total Cu from sampled sediments were in the range of 33.0 – 70.0 mg.kg^{-1} in St-1, while in St-2 the concentration ranged from 51.8 to 85.2 mg.kg^{-1}. Total Ni concentration in sediments of St-1 and St-2 showed to vary from 38.5-82.5 mg.kg^{-1} and 76.1-121.6 mg.kg^{-1} respectively. In case of Cr, the total concentration in St-1was found to vary 118.7 mg.kg^{-1}to 165.7 mg.kg^{-1} and in St-2 it varied 129.6 mg.kg^{-1} to 172.1 mg.kg^{-1}. Total concentration of Pb in the sampled sediments was found in the range of 10.9-25.9 mg.kg^{-1} (in St-1) and 11.7-33.6 mg.kg^{-1} (in St-2). Concentration of total Cd in the sediments were in the range of 0.09 – 0.15 mg.kg^{-1} in St-1, while in St-2 it ranged 0.11 to 0.19 mg.kg^{-1}. Certified reference material (MAG-1) was applied in this study to validate method recovery. A good recovery (more than 99%) for both the metals was found from the MAG-1.

The ERL (Effect range low) and ERM (Effect range median) analysis were performed again based on total metal concentration from the sediments. The metal concentration found below ERL value shows not to cause any adverse effects, while values greater than ERM indicates it to be

very toxic. A station is rated as "good" if the concentrations of the metal are below the ERL limit, "intermediate" applies if any metal concentrations are between ERL and ERM limit and a "poor" rating for the metal that exceeds ERM limit (Garcia et al., 2011; Long et al., 1995; MacDonald et al., 2000). In the present study (Feb 2015-Jan 2016) both the station was rated poor, in terms of Ni, where the concentration was found greater than ERM value. In the case of an individual metal rating, the sampling station was rated intermediate for Cu and Cr metal and rated good in terms of Cd and Pb total loading. The high concentration of Ni in sampled sediments could possibly due to the influence of human activities from adjacent areas.

One-way ANOVA analysis was used to understand if any significant seasonal influence (variation) on the total metal concentration in studied sediments is present. The analysis result (Table 4.10) has shown that the total loading of Cu and Pb in the studied sediments significantly varied ($P < 0.05$) with the seasonal changes. Total Cu concentration has increased in studied sediments from monsoon to post-monsoon period, while in pre-monsoon the concentration has decreased. Whereas, the total concentration of Pb decreased in post-monsoon compare to monsoon and pre-monsoon period. The low concentration of Pb in post-monsoon could probably due to the dilution effect by the high influx of freshwater during the monsoon period.

Table 4.9: Total metal concentration of Cu_T, Ni_T, Cr_T, Pb_T and Cd_T from sediment samples of Zuari estuary, Goa.

Stations	Cu_T (mg.kg^{-1}) in sediment	Ni_T (mg.kg^{-1}) in sediment	Cr_T (mg.kg^{-1}) in sediment	Pb_T (mg.kg^{-1}) in sediment	Cd_T (mg.kg^{-1}) in sediment
Feb-15 st-1	34.9 ± 0.4	46.0 ± 0.2	118.7 ± 1.5	15.6 ± 0.3	0.12 ± 0.03
Mar-15 st-1	33.0 ± 0.1	38.5 ± 0.4	119.0 ± 0.1	14.8 ± 0.7	0.12 ± 0.03
Apr-15 st-1	51.6 ± 0.4	71.7 ± 0.3	153.6 ± 1.2	25.9 ± 0.2	0.13 ± 0
May-15 st-1	48.7 ± 0.2	68.0 ± 0.0	145.7 ± 1.4	22.7 ± 0.1	0.15 ± 0.01
Jun-15 st-1	41.4 ± 1.4	63.3 ± 0.1	138.8 ± 0.4	11.6 ± 0.0	0.13 ± 0.02
Jul-15 st-1	57.7 ± 0.1	78.0 ± 0.9	146.8 ± 0.3	16.8 ± 0.2	0.12 ± 0
Aug-15 st-1	70.0 ± 0.7	82.5 ± 0.4	165.7 ± 1.7	16.9 ± 0.7	0.12 ± 0.01
Oct-15 st-1	46.4 ± 0.5	58.6 ± 0.6	129.7 ± 1.1	13.4 ± 0.5	0.10 ± 0.01
Nov-15 st-1	52.8 ± 0.9	65.1 ± 0.4	140.5 ± 0.4	13.4 ± 0.2	0.10 ± 0.01
Dec-15 st-1	52.5 ± 0.1	57.2 ± 0.4	151.9 ± 0.5	10.9 ± 0.0	0.09 ± 0.02
Jan-16 st-1	61.9 ± 0.9	70.6 ± 0.4	127.7 ± 0.1	12.0 ± 0.1	0.11 ± 0
Feb-15 st-2	53.8 ± 0.5	79.6 ± 0.3	159.3 ± 1.2	22.4 ± 0.0	0.16 ± 0.03
Mar-15 st-2	56.1 ± 0.2	88.5 ± 0.2	166.6 ± 0.4	23.5 ± 0.2	0.16 ± 0.01
Apr-15 st-2	51.8 ± 0.4	121.6 ± 0.0	172.1 ± 1.7	33.6 ± 0.7	0.19 ± 0.02
May-15 st-2	56.5 ± 0.2	79.5 ± 0.0	162.7 ± 0.7	25.4 ± 0.7	0.14 ± 0.01
Jun-15 st-2	77.2 ± 2.5	98.4 ± 0.2	165.5 ± 0.5	17.7 ± 0.9	0.15 ± 0.02
Jul-15 St-2	77.9 ± 0.6	101.4 ± 1.2	166.0 ± 1.0	20.7 ± 0.4	0.14 ±0.02
Aug-15 st-2	77.3 ± 1.4	100.0 ± 0.4	156.8 ± 1.0	16.9 ± 0.9	0.15 ± 0.01
Oct-15 st-2	61.4 ± 0.6	76.1 ± 0.2	129.6 ± 1.3	11.7 ± 0.5	0.11 ± 0
Nov-15 st-2	85.2 ± 0.6	91.0 ± 0.4	149.3 ± 0.2	15.7 ± 0.0	0.13 ± 0.01
Dec-15 st-2	79.3 ± 0.2	87.5 ± 1.0	159.6 ± 0.7	15.4 ± 0.2	0.15 ± 0.02
Jan 16 st-2	74.3 ± 3.5	92.8 ± 1.4	162.1 ± 0.6	15.2 ± 0.2	0.17 ± 0.01
ERL	34	20.9	81	46.7	1.2
ERM	270	51.6	370	218	9.6

*ERL- Effects Range Low, ERM- Effects Range-Median

Table 4.10: Analysis of variance (ANOVA) performed to analyze the variation in total metal concentration from bulk sediments with change in the seasons (pre-monsoon, monsoon and post-monsoon).

Source of Variation	Metal	SS	df	MS	F	P-value	F crit
Between Groups	Cu	1509.7	2	754.9	4.7	**0.02**	3.5
Within Groups		3042.9	19	160.2			
Total		4552.6	21				
Between Groups	Ni	711.0	2	355.5	0.9	0.41	3.5
Within Groups		7225.1	19	380.3			
Total		7936.1	21				
Between Groups	Cr	562.7	2	281.3	1.07	0.36	3.5
Within Groups		4997.5	19	263.0			
Total		5560.1	21				
Between Groups	Pb	372.2	2	186.1	11.2	**0.0006**	3.5
Within Groups		316.0	19	16.6			
Total		688.1	21				
Between Groups	Cd	0.003	2	0.0014	2.6	0.11	3.5
Within Groups		0.01	19	0.0005			
Total		0.013	21				

*SS- Sum of squares, df- Degree of freedom, MS- Mean square, F- F Variable

4.2.8 Bioaccumulated metals in oyster tissue

In oyster tissue, the total metal concentration of Cu, Ni, Cr, Pb, and Cd were determined and presented in Table 4.11. Most of the time, juvenile oysters were collected from both the stations and were due to non-accessibility of adult oysters from the sampling site. The morphometry of sampled oysters is provided in Table 4.12. The high concentration of total Cu from sampled oysters was found, in St-1 the value ranged 216.5–674.4 mg.kg^{-1}, while in St-2 the concentration ranged from 382.6 to 722.4 mg.kg^{-1}. The very high bioaccumulation of Cu in the oyster system in our study could possibly be due presence of specific transporter family genes (responsible for transportation of Cu in the oyster cell) in oyster, increase in metallothionein production for detoxification, and to increase its self-immunity power against microbial pathogens (Shi et al., 2019; Wang et al., 2018). Total Ni concentration in oysters collected from St-1 and St-2 showed to vary from 1.6-6.4 mg.kg^{-1} and 0.8-2.6 mg.kg^{-1} respectively. In case of Cr, the bioaccumulated concentration in St-1 was found to vary 0.5 to 5.0 mg.kg^{-1} and in St-2 it varied 0.7 mg.kg^{-1} to 2.0 mg.kg^{-1}. Total concentration of Pb in the oyster tissues were found very low and was in the range of 0.0-0.52 mg.kg^{-1} (in St-1) and 0.04-0.42 mg.kg^{-1} (in St-2). The concentration of total Cd in the oyster samples was found very much higher compare to sediment concentration and was similar to what was observed during 2014 sampling, the concentration was in the range of 2.4–7.9 mg.kg^{-1} in St-1, while in St-2 it ranged 5.4 to 8.9 mg.kg^{-1}.

One-way ANOVA analysis was carried out to understand if any significant seasonal influence (variation) on the total metal accumulation in oysters is present. The analysis result (Table 4.13) showed no influence of seasonal variation ($P > 0.05$) on the total metal accumulation of Cu, Ni, Cr, Pb and Cd within the oyster system.

Table 4.11: Total metal concentration of (Cu$_T$, Ni$_T$, Cr$_T$, Pb$_T$ and Cd$_T$) from soft tissues of oyster (*Magallana* sp.) collected from Zuari estuary, Goa.

Stations	Cu$_T$ (mg.kg^{-1}) in oyster	Ni$_T$ (mg.kg^{-1}) in oyster	Cr$_T$ (mg.kg^{-1}) in oyster	Pb$_T$ (mg.kg^{-1}) in oyster	Cd$_T$ (mg.kg^{-1}) in oyster
Feb-15 st-1	674.4 ± 0.1	2.9 ± 0.6	2.0 ± 0.3	0.20 ± 0.01	7.1 ± 0.4
Mar-15 st-1	423.3 ± 0.4	1.6 ± 0.3	1.4 ± 0.7	0.23 ± 0.01	5.4 ± 0.1
Apr-15 st-1	495.2 ± 0.2	NA	0.5 ± 0.2	0.08 ± 0.01	5.1 ± 0.2
May-15 st-1	442.9 ± 1.4	3.2 ± 0.1	1.6 ± 0.0	0.19 ± 0.01	5.2 ± 0.2
Jun-15 st-1	459.5 ± 0.1	2.4 ± 1.5	2.6 ± 1.0	0.28 ± 0.01	7.0 ± 0.1
Jul-15 st-1	556.2 ± 0.8	1.7 ± 0.6	2.0 ± 0.1	0.00 ± 0.00	2.4 ± 0.0
Aug-15 st-1	610.9 ± 1.5	3.3 ± 0.5	2.8 ± 0.8	0.52 ± 0.01	7.9 ± 0.3
Oct-15 st-1	628.9 ± 0.9	2.5 ± 0.4	5.0 ± 0.5	0.18 ± 0.02	6.8 ± 0.0
Nov-15 st-1	216.5 ± 0.4	2.4 ± 0.5	1.4 ± 0.5	0.09 ± 0.02	6.1 ± 0.2
Dec-15 st-1	423.2 ± 0.2	6.4 ± 0.7	2.4 ± 0.5	0.66 ± 0.02	7.1 ± 0.0
Jan-16 st-1	305.4 ± 0.5	1.7 ± 0.4	2.4 ± 0.5	0.35 ± 0.02	7.6 ± 0.2
Feb-15 st-2	513.9 ± 0.5	2.2 ± 0.2	1.0 ± 0.0	0.30 ± 0.01	6.9 ± 0.1
Mar-15 st-2	505.5 ± 0.3	1.3 ± 0.6	1.1 ± 0.5	0.27 ± 0.01	7.7 ± 0.0
Apr-15 st-2	452.0 ± 0.2	2.1 ± 0.2	1.6 ± 0.1	0.22 ± 0.01	8.9 ± 0.3
May-15 st-2	382.6 ± 0.5	2.3 ± 0.5	1.1 ± 0.8	0.10 ± 0.01	6.0 ± 0.0
Jun-15 st-2	503.8 ± 0.4	1.0 ± 0.1	1.1 ± 0.0	0.35 ± 0.01	6.4 ± 0.1
Jul-15 St-2	653.1 ± 0.1	1.4 ± 0.1	1.5 ± 0.9	0.19 ± 0.01	5.4 ± 0.1
Aug-15 st-2	622.1 ± 0.2	2.4 ± 1.2	2.0 ± 1.0	0.42 ± 0.02	7.5 ± 0.0
Oct-15 st-2	722.4 ± 2.1	2.6 ± 1.4	1.9 ± 0.1	0.04 ± 0.01	8.6 ± 0.1
Nov-15 st-2	511.5 ± 0.2	1.9 ± 1.2	0.7 ± 0.1	0.23 ± 0.02	8.9 ± 0.2
Dec-15 st-2	581.2 ± 0.3	1.8 ± 0.4	1.1 ± 0.0	0.24 ± 0.01	7.2 ± 0.2
Jan 16 st-2	477.3 ± 0.2	0.8 ± 0.2	0.7 ± 0.1	0.30 ± 0.01	5.8 ± 0.3

Table 4.12: Number of oysters (*Magallana* sp.) collected in each station and oyster morphometry from the sampling location in Zuari estuarine region.

Stations	Numbers of oyster collected	Oyster Morphometry		
		Average Length (cm)	Average Width (cm)	Average tissue wet weight (g)
Feb-15 st-1	28	3.3 ± 0.3	2.2 ± 0.3	1.9 ± 0.1
Mar-15 st-1	32	3.6 ± 0.4	2.4 ± 0.3	1.6 ± 0.5
Apr-15 st-1	35	3.6 ± 0.6	2.5 ± 0.4	1.8 ± 0.7
May-15 st-1	36	3.6 ± 0.5	2.4 ± 0.4	1.9 ± 0.2
Jun-15 st-1	62	3.0 ± 0.7	2.5 ± 0.5	1.6 ± 0.6
Jul-15 st-1	45	3.0 ± 0.4	2.1 ± 0.4	1.3 ± 0.6
Aug-15 st-1	29	3.7 ± 0.6	2.9 ± 0.3	1.6 ± 0.4
Oct-15 st-1	33	4.3 ± 0.5	2.9 ± 0.3	1.8 ± 0.2
Nov-15 st-1	19	6.3 ± 2.8	4.4 ± 2	6.1 ± 5
Dec-15 st-1	38	4.3 ± 0.4	2.5 ± 0.3	1.7 ± 0.4
Jan-16 st-1	56	4.2 ± 0.3	2.8 ± 0.2	2.2 ± 0.5
Feb-15 st-2	51	3.4 ± 0.5	2.2 ± 0.3	1.5 ± 0.3
Mar-15 st-2	43	3.8 ± 0.6	2.6 ± 0.5	1.8 ± 0.6
Apr-15 st-2	56	3.5 ± 0.6	2.3 ± 0.5	1.9 ± 0.6
May-15 st-2	29	3.7 ± 0.7	2.6 ± 0.5	1.6 ± 0.7
Jun-15 st-2	36	3.4 ± 0.7	2.8 ± 0.4	1.8 ± 0.6
Jul-15 St-2	41	3.5 ± 0.8	2.6 ± 0.5	1.8 ± 0.8
Aug-15 st-2	43	3.8 ± 0.7	2.9 ± 0.4	1.9 ± 0.7
Oct-15 st-2	17	10.8 ± 1.2	7.2 ± 1.3	15.6 ± 3.4
Nov-15 st-2	43	4.2 ± 0.4	3.1 ± 0.6	2.4 ± 0.6
Dec-15 st-2	39	4.5 ± 0.5	2.6 ± 0.2	1.8 ± 0.2
Jan 16 st-2	33	4.2 ± 0.6	2.7 ± 0.4	1.7 ± 1

Table 4.13: Analysis of variance (ANOVA) performed to analyze the influence of seasons on total accumulation of various metals in oyster tissue from Zuari estuary, Goa.

Source of Variation	Metal	SS	df	MS	F	P-value	F crit
Between Groups	Cu	29977.7	2.0	14988.9	1.0	0.4	3.5
Within Groups		277641.8	19.0	14612.7			
Total		307619.5	21				
Between Groups	Ni	0.8	2.0	0.4	0.3	0.8	3.6
Within Groups		25.7	18.0	1.4			
Total		26.6	20				
Between Groups	Cr	2.4	2.0	1.2	1.3	0.3	3.5
Within Groups		17.4	19.0	0.9			
Total		19.8	21				
Between Groups	Pb	0.03	2.0	0.02	0.7	0.5	3.5
Within Groups		0.46	19.0	0.02			
Total		0.50	21				
Between Groups	Cd	4.9	2.0	2.4	1.1	0.3	3.5
Within Groups		41.4	19.0	2.2			
Total		46.3	21				

*SS- Sum of squares, df- Degree of freedom, MS- Mean square, F- F Variable

Oyster is considered a culinary delicacy and is being consumed by humans throughout the world. Oyster is a bivalve mollusk and is known to accumulate high concentration of pollutants. The consumption of such contaminated oysters will possess a great danger to human health. Keeping this in mind, various organizations in the world has provided a strict guideline in which maximum acceptable level of various metals (Cr, Pb, and Cd) in oysters for human consumption are provided (Table 4.14).

According to Indian governmental organization (FSSAI), in terms of Cd metal, oyster from both the stations in any month of the year is not suitable for human consumption.

Table 4.14: Permissible limit for Cr, Pb and Cd in oystersrecommended by various worldwide organizations.

Authorities	Cr (mg.kg^{-1}) maximum permissible limit	Pb (mg.kg^{-1}) maximum permissible limit	Cd (mg.kg^{-1}) maximum permissible limit
Malaysian Food Act (Act 281) and Food Regulation (1985)		1	1
European Commission (2001)			1
FDA (2007)	1		4
Australia New Zealand Food Standards Code (2016)		2	2
Codex Alimentarius Commission (2006)			2
FSSAI (2011)	12	1.5	2
Present study	0.5-5.0	0.0-0.52	2.4-8.9

*FDA- Food and Drug Administration, FSSAI- Food Safety and Standard Authority of India.

From the result observed, we have further determined the concentration of Cd in SPM and overlying water column. Speciation (geochemical fractionation) study of Cd in bulk as well as in finer sediments was also carried out.

4.2.9 Concentration of Cd in the suspended particulate matter (SPM) and the overlying water column

The concentration of total Cd in the SPM varied from 0.05 to 0.61 mg.kg^{-1}(Table 4.15). The highest concentration of Cd in the SPM samples was reported in the month of December in St-1 and in April from St-2 (0.61 and 0.3 mg.kg^{-1} respectively). Lowest concentration was found in August (0.05 mg.kg^{-1}) in St-1 and in November (0.1 mg.kg^{-1}) in St-2. The concentration of Cd in the overlying water column varied from 0.02 to 0.07 µg.L^{-1}.

4.2.10 Geochemical fractionation of Cd in surface sediments

The total concentration of Cd in the bulk sediments ranged from 0.09-0.19 mg.kg^{-1} (Table 4.15). Percentage of total Cd associated with different binding phases of the bulk sediment is presented in Figure 4.5. It showed that Cd had bound to different geochemical phases of the sediment with different affinity. The concentration of Cd as exchangeable, carbonate and bicarbonate form (Fr. 1) varied from 0.04 to 0.07 mg.kg^{-1} (23.7-42.1%) of total Cd in St- 1 and in St- 2 it varied from 0.04-0.08 mg.kg^{-1} (26.8-44.2%) of total Cd. High concentrations of Cd complexes in Fr. 1 possibly indicates high bioavailability of Cd from sampled sediments. The association total Cd with Fe/Mn oxyhydroxide phases (Fr. 2) was found to be the highest (0.03-0.08 mg.kg^{-1}) ~25.2-48% in St-1 and (0.04-0.09 mg.kg^{-1}) ~28.4-50.4% in St-2 in the sampled sediments. The concentration of Cd bound to sedimentary organic phase (Fr. 3) was much lower. In St-1 only ~0.5–8.4% of Cd was present in Fr. 3, while in St-2 it ranged ~3.3-8.4%. The concentration of residual fraction (inert fraction) of Cd (Fr. 4) was found in the range of ~15 to 32.2% in St-1 and in St-2 it ranged (~6 to 27.3%).

Table 4.15: Total Cd concnetration in bulk and finer sediment, total Cd in suspended particulate matter (SPM) and their variation in the mangrove sediments from Zuari estuarine region.

Stations	$[Cd]_T$ bulk sediment (mg.kg^{-1})	$[Cd]_T$ finer sediment (mg.kg^{-1})	$[Cd]_T$ SPM (mg.kg^{-1})
Feb-15 st-1	0.12 ± 0.03	0.10 ± 0.02	
Mar-15 st-1	0.12 ± 0.03	0.15 ± 0.01	
Apr-15 st-1	0.13 ± 0	0.11 ± 0.03	0.52 ± 0
May-15 st-1	0.15 ± 0.01	0.09 ± 0	0.22 ± 0.02
Jun-15 st-1	0.13 ± 0.02	0.12 ± 0	0.25 ± 0.02
Jul-15 st-1	0.12 ± 0	0.08 ± 0.01	0.19 ± 0.01
Aug-15 st-1	0.12 ± 0.01	0.12 ± 0.02	0.05 ± 0.01
Oct-15 st-1	0.10 ± 0.01	0.12 ± 0.01	0.14 ± 0
Nov-15 st-1	0.10 ± 0.01	0.10 ± 0.02	0.14 ± 0.01
Dec-15 st-1	0.09 ± 0.02	0.11 ± 0.03	0.61 ± 0.04
Jan-16 st-1	0.11 ± 0	0.09 ± 0.01	0.24 ± 0.01
Feb-15 st-2	0.16 ± 0.03	0.10 ± 0.03	
Mar-15 st-2	0.16 ± 0.01	0.10 ± 0.01	
Apr-15 st-2	0.19 ± 0.02	0.10 ± 0.02	0.28 ± 0.01
May-15 st-2	0.14 ± 0.01	0.10 ± 0.01	0.18 ± 0.06
Jun-15 st-2	0.15 ± 0.02	0.11 ± 0	0.16 ± 0
Jul-15 St-2	0.14 ±0.02	0.11 ± 0.01	0.20 ± 0
Aug-15 st-2	0.15 ± 0.01	0.11 ± 0.02	0.25 ± 0.02
Oct-15 st-2	0.11 ± 0	0.10 ± 0.04	0.26 ± 0.01
Nov-15 st-2	0.13 ± 0.01	0.12 ± 0	0.12 ± 0
Dec-15 st-2	0.15 ± 0.02	0.11 ± 0.01	0.19 ± 0
Jan 16 st-2	0.17 ± 0.01	0.07 ± 0.01	0.16 ± 0.04
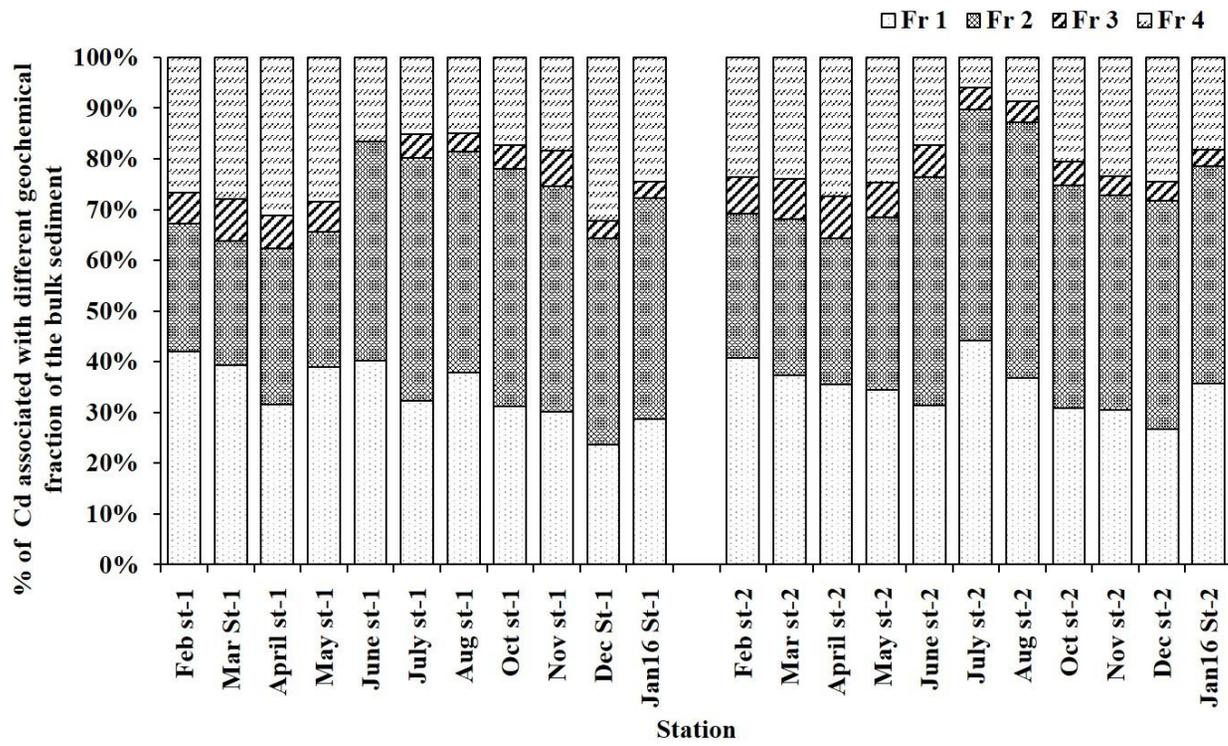
Figure 4.5: Geochemical fractionation of Cd in the bulk sediments.

4.2.11 Geochemical fractionation of Cd in finer sediments

The geochemical fractionation study of Cd was carried out in the finer particles of the sediment by considering the fact that finer particles of sediments have higher concentrations of metals and a major portion of metals are embedded in silt and clay fractions. Finer fractions of sediments are assumed to resuspend from the surface sediments to the overlying water column and become available to sessile organisms (like oysters) while filter-feeding. The total concentration of Cd in the finer sediments was found in the range of 0.07–0.15 mg.kg^{-1} (Table 4.15). The total concentration of Cd in the studied sediments was much lower than the ERL value (1.2 mg.kg^{-1}) recommended by the sediment quality guideline (MacDonald et al., 2000). The concentrations of Cd in the finer sediments were found very similar to the Cd concentration in the bulk sediment. This observation suggests that Cd was also equally distributed in the coarser particle of the sediment. It has been reported in the literature that Cd can be associated with the coarser particle (in the form of carbonate) in the estuarine and marine sediments (Salomons and Förstner, 2012; Tessier et al., 1980).

The geochemical fractionation of the total Cd in different binding phases of the finer sediment is presented in Figure 4.6 and Table 4.16. The data indicates that Cd had a different affinity towards different geochemical binding phases of the sediment. The concentrations of sedimentary Cd complexes in Fr. 1 has been reported to provide an indication of Cd's mobility and bioavailability within the sediment systems (Chakraborty et al., 2016c). The concentration of Cd complexes in Fr 1 (i.e., exchangeable, carbonate and bicarbonate form of Cd) varied from 0.04 to 0.08 mg.kg^{-1} (39.8-50.4 % of total Cd) and 0.04-0.07 mg.kg^{-1} (39.4-54.0 % of total Cd) in St-1 and St-2 respectively (Table 4.17).

The concentrations of Cd associated with Fe/Mn oxyhydroxide binding phases (Fr. 2) in the sediment was high and ranged from 0.02-0.07 mg.kg^{-1} (~24.1-39.6% of the total Cd) in St-1 and 0.02-0.04 mg.kg^{-1} (~19.2-27.7% of the total Cd) in St-2. The concentration of Cd bound to sedimentary organic phase (Fr. 3) was low. In St-1, only ~5.5–8.4% of the total Cd was present in Fr 3, while in St-2 it ranged ~4.2-9.2% (Table 4.16) of the total sedimentary Cd. The concentration of residual fraction (inert fraction) of Cd was found in the range of ~8.7 to 25.1% of the total sedimentary Cd in the St-1 and ~16.4 to 28.1% of the total Cd in the St-2. The residual Cd is assumed to be inert and not bioavailable in the estuarine system. It is also proposed that the residual fraction carries the signature of source rocks.
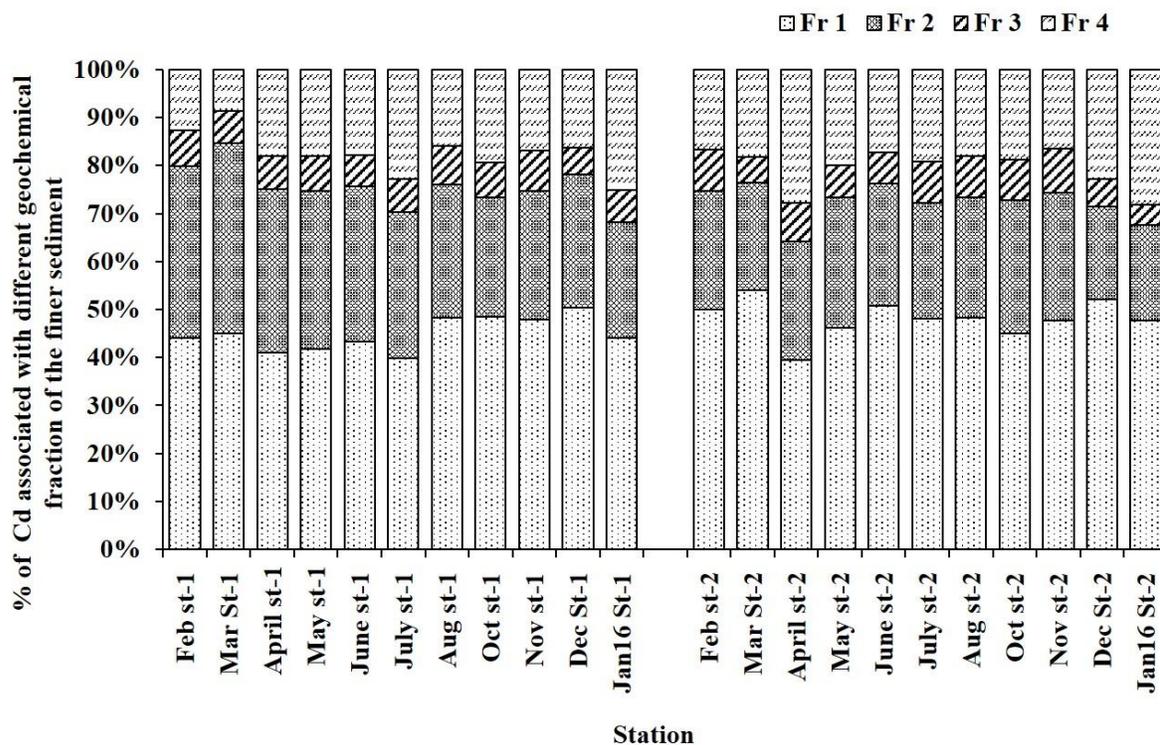
Figure 4.6: Geochemical fractionation of Cd in the finer sediments.

Table 4.16: Concentration of Cd (mg.kg^{-1}) associated with different geochemical fractionation in finer fraction of the sediment. Cd total represents the total Cd concentration in finer sediments.

Stations	Fr.1 (mg.kg^{-1})	Fr.2 (mg.kg^{-1})	Fr.3 (mg.kg^{-1})	Fr.4 (mg.kg^{-1})	Cd Total (mg.kg^{-1})
Feb st-1	0.05 ± 0.04	0.04 ± 0.1	0.01 ± 0.02	0.01 ± 0	0.10 ± 0.02
March St-1	0.08 ± 0.02	0.07 ± 0.07	0.01 ± 0.02	0.02 ± 0.01	0.15 ± 0.01
April st-1	0.05 ± 0.03	0.05 ± 0.1	0.01 ± 0	0.02 ± 0	0.11 ± 0.03
May st-1	0.05 ± 0.05	0.04 ± 0.05	0.01 ± 0	0.02 ± 0	0.09 ± 0
June st-1	0.07 ± 0.02	0.05 ± 0.04	0.01 ± 0.03	0.03 ± 0	0.12 ± 0
July st-1	0.04 ± 0.01	0.03 ± 0	0.01 ± 0.01	0.02 ± 0	0.08 ± 0.01
Aug st-1	0.07 ± 0.05	0.04 ± 0.05	0.01 ± 0.02	0.02 ± 0	0.12 ± 0.02
Oct st-1	0.07 ± 0.01	0.04 ± 0.1	0.01 ± 0.01	0.03 ± 0	0.12 ± 0.01
Nov st-1	0.06 ± 0.02	0.03 ± 0.03	0.01 ± 0.02	0.02 ± 0.02	0.10 ± 0.02
Dec St-1	0.06 ± 0.02	0.03 ± 0.05	0.01 ± 0	0.02 ± 0	0.11 ± 0.03
Jan16 St-1	0.05 ± 0	0.02 ± 0.03	0.01 ± 0.03	0.03 ± 0.01	0.09 ± 0.01
Feb st-2	0.06 ± 0.06	0.03 ± 0.03	0.01 ± 0.02	0.02 ± 0.02	0.10 ± 0.03
March St-2	0.06 ± 0.03	0.03 ± 0.05	0.01 ± 0.02	0.02 ± 0.01	0.10 ± 0.01
April st-2	0.04 ± 0.02	0.03 ± 0.03	0.01 ± 0	0.03 ± 0	0.10 ± 0.02
May st-2	0.05 ± 0.04	0.03 ± 0.05	0.01 ± 0.01	0.02 ± 0.01	0.10 ± 0.01
June st-2	0.07 ± 0.01	0.04 ± 0.03	0.01 ± 0.01	0.02 ± 0	0.11 ± 0
July st-2	0.07 ± 0	0.03 ± 0.02	0.01 ± 0.01	0.03 ± 0.01	0.11 ± 0.01
Aug st-2	0.06 ± 0.03	0.03 ± 0.05	0.01 ± 0.01	0.02 ± 0.01	0.11 ± 0.02
Oct st-2	0.05 ± 0.02	0.03 ± 0.00	0.01 ± 0.02	0.02 ± 0.01	0.10 ± 0.04
Nov st-2	0.07 ± 0.01	0.04 ± 0.03	0.01 ± 0	0.02 ± 0.01	0.12 ± 0.01
Dec St-2	0.07 ± 0.09	0.02 ± 0.01	0.01 ± 0.03	0.03 ± 0.01	0.11 ± 0.01
Jan16 St-2	0.04 ± 0.02	0.02 ± 0.01	0.004 ± 0.03	0.03 ± 0.01	0.07 ± 0.01

Table 4.17: Percentage of Cd associated with different geochemical fractionation in finer fraction of the sediment.

Stations	Fr.1 (%)	Fr.2 (%)	Fr.3 (%)	Fr.4 (%)
Feb-15 st-1	44.1	35.8	7.5	12.7
Mar-15 st-1	45.1	39.6	6.6	8.7
Apr-15 st-1	40.9	34.2	6.9	17.9
May-15 st-1	41.7	33.1	7.3	18.0
Jun-15 st-1	43.3	32.4	6.5	17.8
Jul-15 st-1	39.8	30.5	6.9	22.8
Aug-15 st-1	48.3	27.7	8.0	16.0
Oct-15 st-1	48.5	24.9	7.2	19.5
Nov-15 st-1	47.9	26.8	8.4	16.9
Dec-15 st-1	50.4	27.7	5.5	16.3
Jan-16 st-1	44.1	24.1	6.7	25.1
Feb-15 st-2	49.9	24.8	8.5	16.8
Mar-15 st-2	54.0	22.4	5.3	18.3
Apr-15 st-2	39.4	24.8	8.1	27.7
May-15 st-2	46.1	27.2	6.6	20.0
Jun-15 st-2	50.8	25.4	6.5	17.3
Jul-15 St-2	48.1	24.1	8.6	19.1
Aug-15 st-2	48.3	25.1	8.6	18.1
Oct-15 st-2	45.1	27.7	8.5	18.8
Nov-15 st-2	47.6	26.7	9.2	16.4
Dec-15 st-2	52.2	19.2	5.8	22.9
Jan 16 st-2	47.7	19.9	4.2	28.1

Figure 4.7 shows the variation of Cd concentrations in different binding phases of the finer sediment with increasing total Cd concentrations in the sediments. The concentrations of Cd in Fr.1 (exchangeable, carbonate, and bicarbonate forms) linearly increased with increasing Cd loading in the finer sediment (R^2=0.74, p<0.05, n = 22). A very similar observation has been reported in the literature (Chakraborty et al., 2016c, 2012). This observation probably suggests that strong Cd-binding sites were probably unavailable in the finer particles of the sediments. It is also suggested that increasing Cd loading might increase the mobility and bioavailability of Cd in the sediment system. However, a statistically significant increase in Cd association with Fe/Mn-oxyhydroxide

phase (R^2=0.68, p<0.05, n=22) and sedimentary organic matter phase (R^2=0.58, p<0.05, n=22) also were observed with increasing Cd loading in the finer fraction of the sediment. But, the concentration of Cd present in the organic binding phase (Fr.3) was much lower than the concentration of Cd in Fr.1 and Fr.2 phases of the sediments. The geochemical fractionation study of Cd in the finer sediments suggests that there were no specific binding sites for Cd in the estuarine sediment. Therefore, the concentration of Cd in Fr.1, Fr.2, and Fr.3 gradually increased with increasing total Cd loading in the sediment. This observation also suggests that these Cd complexes were not thermodynamically stable.
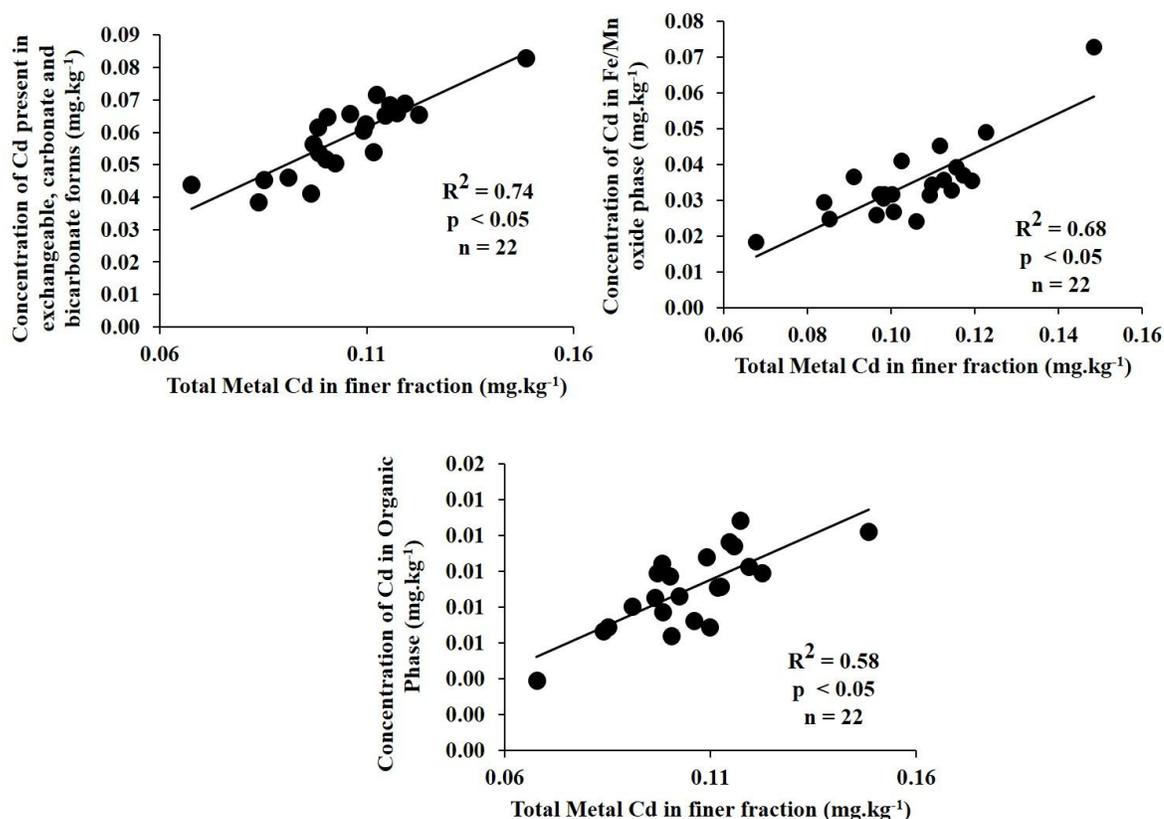
Figure 4.7: Variation of Cd present in different geochemical phases of finer sediments as a function of total Cd concentration.

4.2.12. Relationship between Cd in water, SPM and finer sediments with bioaccumulated Cd in oyster (*Magallana* sp.)

To understand the high bioaccumulation of Cd in the oyster system the relationship between Cd in water, SPM, and finer sediments with bioaccumulated Cd in oyster (*Magallana* sp.) was carried out. Normally, Cadmium is a divalent ion (Cd^{2+}) which resembles the divalent Ca (Ca^{2+}) ion due to its similar radius and charge. This similarity enables Cd to enter through the calcium channels present on the cells of oyster gills (Adams et al., 2011).

The changes in concentration of bioaccumulated Cd in the oyster tissues with varying concentration of Cd in the SPM is shown in Figure 4.8. Surprisingly, no significant correlation found between the concentration of bioaccumulated Cd in the oyster and the increasing concentration of Cd in the SPM from the study area. A similar observation has been reported by Lekhi et al., 2008. They have reported that Cd in SPM is not a significant source of bioaccumulated Cd in oysters. Wang and Wong, 2003 have reported that Cd assimilation efficiency in bivalves depends on food quality than quantity. This suggests that Cd accumulation in the oyster from the SPM was low, even though the Cd concentration was relatively high in the SPM. There was no significant relationship found between the concentration of dissolved and bioaccumulated Cd in the study area.

The correlation between bioaccumulated Cd in the oyster tissues and the variation in the concentration of Cd associated with different geochemical phases of the bulk sediments are presented (Table 4.18 and Figure 4.9). There was no significant correlation found between the bioaccumulated Cd in the oyster and increasing Cd concentrations in the non-residual fraction of the bulk sediments.
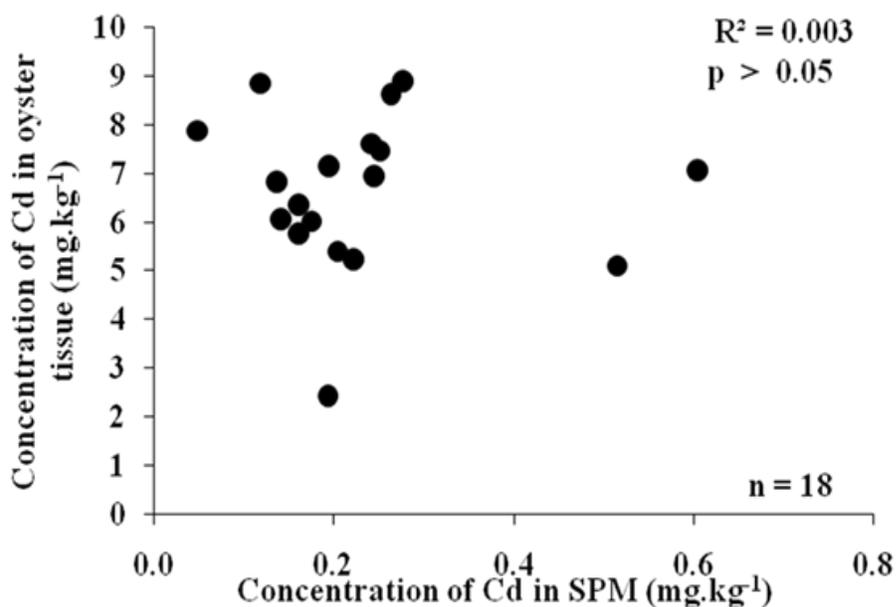
Figure 4.8: Bioaccumulated Cd in the soft tissues of oysters versus Cd concentration in the suspended particulate matter (SPM).

Table 4.18: Pearson correlation between the accumulated Cd in oyster tissue and Cd in different geochemical fractions of the bulk sediments.

	Fr.1 (%)	Fr.2 (%)	Fr.3 (%)	Fr.4 (%)	$[Cd]_T$ sediment $(mg.kg^{-1})$	$[Cd]_T$ in oyster $(mg.kg^{-1})$
Fr.1 (%)	1					
Fr.2 (%)	-0.46	1.00				
Fr.3 (%)	0.47	-0.73	1.00			
Fr.4 (%)	-0.34	-0.65	0.26	1.00		
$[Cd]_T$ sediment $(mg.kg^{-1})$	**0.50**	-0.34	**0.45**	-0.11	1.00	
$[Cd]_T$ in oyster $(mg.kg^{-1})$	-0.03	-0.07	0.05	0.11	0.15	1.00
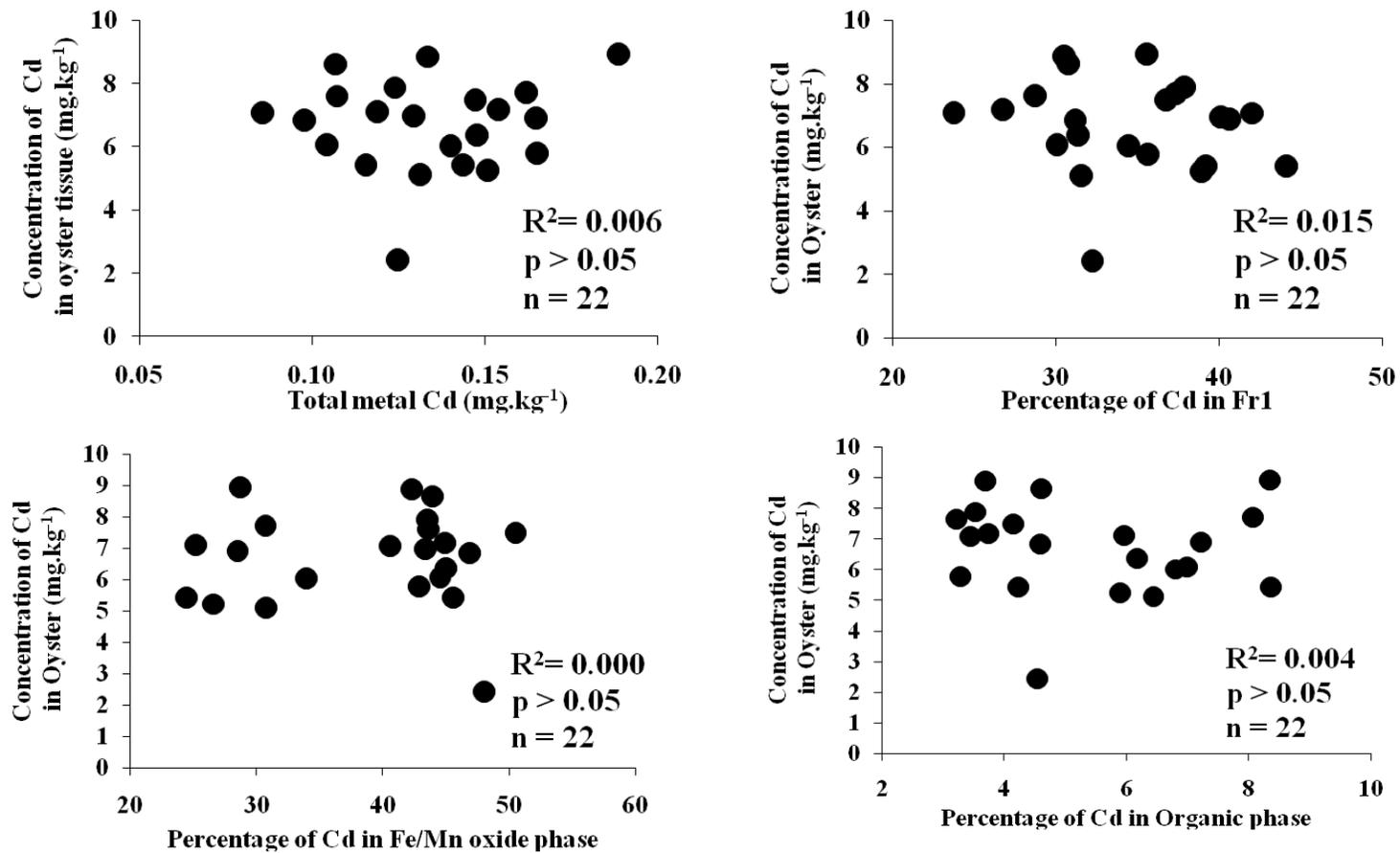
Figure 4.9: Relationship between the bioaccumulated Cd in the soft tissues of oyster and Cd in different geochemical phases of the bulk sediments.

The changes in the concentration of Cd in the soft tissues of the oyster with changing Cd concentration in the finer particles of the sediments are shown in Figure 4.10. The concentration of bioaccumulated Cd in the oyster was found to increase with the increasing association of Cd in Fr 1 (R^2 = 0.26, p<0.05, n=21) of the finer particles of the sediments. This clearly indicates that Cd accumulation in the oyster tissues increases with increasing labile Cd (Fr. 1) concentration. The correlation indicates that exchangeable and carbonate and bicarbonate forms of Cd (Fr.1) (in the finer fraction) can be a good indicator of bioavailable Cd in estuarine systems. However, increasing association of Cd with Fe/Mn oxyhydroxide phases showed a decreasing trend in the Cd bioaccumulation. Although the negative relationship was not statistically significant (Figure 4.10), the trend shows that bioavailability of Cd may get decreased by its association with Fe/Mn oxyhydroxide phases in the sediments. There was no correlation found between the percentage of Cd in the organic and residual phase and Cd concentration in the oysters (Table 4.19). Speciation study showed that the Cd present in exchangeable and carbonate and bicarbonate forms from the finer sediments compare to the bulk sediment is mainly responsible for bioavailable Cd from the sediment system.
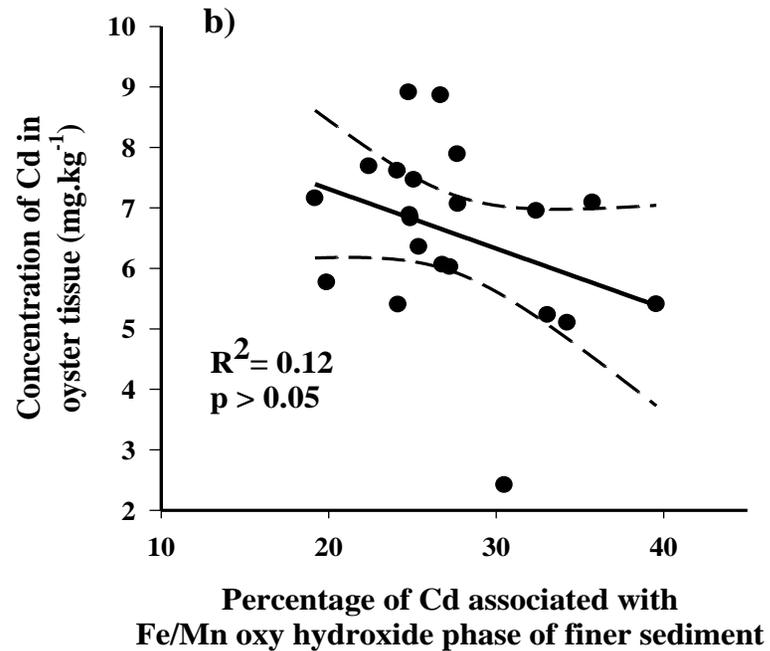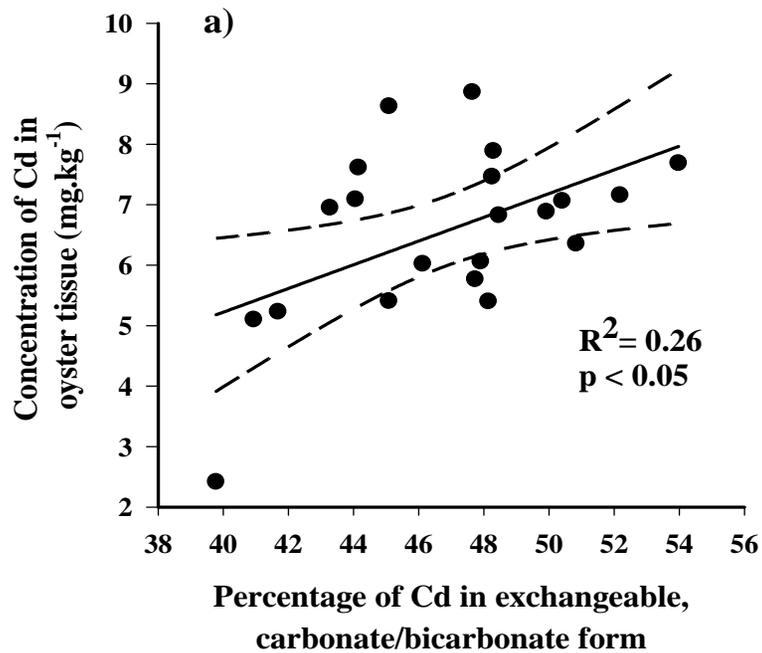
Figure 4.10: Relationship between the concentration of bioaccumulated Cd in the soft tissues of oysters and the concentration of Cd in different geochemical phases of finer sediments (a) exchangeable, carbonate/bicarbonate forms of Cd in sediment and (b) Cd associated with Fe/Mn oxy hydroxide phases of the finer sediments.

Table 4.19: Pearson correlation between the accumulated Cd in oyster tissue and Cd in different geochemical fractions in the finer fraction of sediments.

	Fr.1 (%)	Fr.2 (%)	Fr.3 (%)	Fr.4 (%)	$[Cd]_T$ sediment $(mg.kg^{-1})$	$[Cd]_T$ in oyster $(mg.kg^{-1})$
Fr.1 (%)	1					
Fr.2 (%)	-0.68	1				
Fr.3 (%)	-0.16	0.18	1			
Fr.4 (%)	-0.002	-0.71	-0.41	1		
$[Cd]_T$ sediment $(mg.kg^{-1})$	0.12	0.46	0.27	-0.77	1	
$[Cd]_T$ in oyster $(mg.kg^{-1})$	**0.51**	-0.31	0.24	-0.14	0.19	1

Thus, this study suggests that high Cd accumulation in the oyster was probably due to carbonate/bicarbonate, exchangeable, and water-soluble Cd complexes in the finer particle of the sediment. It has been reported that Cd can also enter in an oyster body directly via water (dissolved Cd), food (Phytoplankton, Bacteria) and suspended finer sediment (Borchardt, 1983; Dixon et al., 2006; Ettajani et al., 2001; Lekhi et al., 2008). It has been reported that Cd associated with phytoplankton can be taken up quickly after solubilization in the gut, during the digestion process (Amiard et al., 2007). However, Cd associated with phytoplankton has been reported to bind with depuration proteins such as phytochelatins and metallothioneins (Lee and Morel, 1995; Payne and Price, 1999). A study by Lekhi et al., 2008 has suggested that Cd associated with phytoplankton can be quickly depurated. This experimental data also suggest that Cd associated with the SPM and dissolved total Cd was probably not the major sources for Cd bioaccumulation in the oysters. Several studies have shown that metal associated with suspended sediments is one of the major sources for metals accumulation in molluscs (Luoma, 1989; Wang and Fisher, 1999; Wu et al., 2012). Shulkin et al., 2003 have tried to establish a linkage between fractionated metals (leached by acid) from sediment and their bioaccumulation in oyster (*Crassostrea gigas*). Birch and Hogg, 2011 have also reported that leachable metals by 1M HCl from fine fraction of surficial sediments can be a good indicator of metals bioavailability in natural systems. In this study, the correlation between bioaccumulated Cd in the oyster tissues and the variation in the concentration of Cd associated with different geochemical phases of the finer sediments (Figure 4.10) suggests that exchangeable, carbonate and bicarbonate forms of Cd in the finer sediment were bioavailable to the oyster. Based on the result, a schematic diagram has been proposed (Figure 4.11) about the possible uptake route of Cd into the oyster system from the environment.
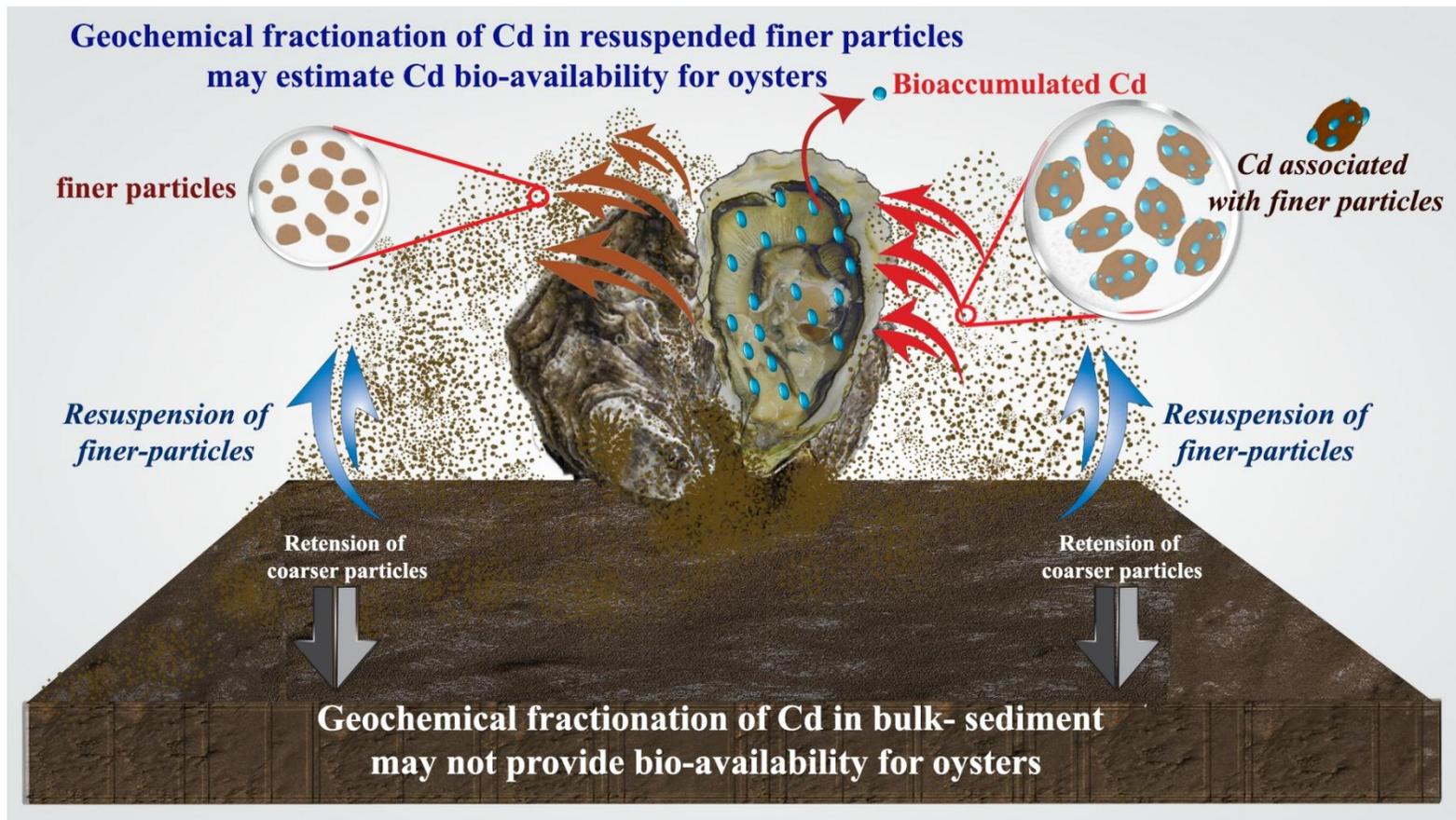
Figure 4.11: Based on overall result a schematic diagram about the possible uptake route of Cd into the oyster system from the environment has been proposed.

4.3 Conclusion

Thus, this study has attempted to establish a linkage between the Cd speciation from the studied sediments (especially finer sediments) and Cd bioaccumulation in edible oysters from a tropical estuarine system. The concentration of Cd was found higher in the edible oysters throughout the year from the Zuari estuary and was above the maximum limit for human consumption. However, the concentration of Cd in the water (0.02-0.07 μg.L^{-1}), SPM (0.05-0.61 mg.kg^{-1}) and sediments were not high (0.09-0.19 mg.kg^{-1}). This study indicates that exchangeable, carbonate and bicarbonate forms of Cd were bioavailable in the sediment system. The geochemical fractionation study of Cd in finer sediments provides better information about Cd bioavailability in estuarine sediments than the bulk sediments. Ingestion of finer particle of the surface sediment can increase bioaccumulation of Cd in edible oysters from a mangrove estuarine system. Chemical speciation of Cd in finer sediment plays a key role in controlling Cd bioaccumulation in oysters from the tropical estuarine system.

Chapter 5

Kinetic speciation of Cd in the estuarine sediments

and its bioaccumulation in the edible oyster

5.1 Introduction

Geochemical fractionation (or functional speciation) of sedimentary Cd (bulk and finer sediments) has been performed and discussed in the previous chapter. Chapter 4 provides the information on major hosting phases of Cd in the estuarine sediments and its bioavailability to the benthic organism (oyster). However, understanding of geochemical fractionation of Cd in sediment is not adequate to comprehend the stability or lability of Cd complexes in a given estuarine sediment system. Henceforth, in this chapter kinetic fractionation study of Cd was carried out to understand the nature of the Cd-sediment complexes (labile or inert) and their corresponding dissociation rate constants (k_d).

Cadmium (Cd) is a non-essential trace metal, it arrives in an estuarine systemmainly through runoff from land, river discharge, and aerial deposition (Hall et al., 1998; Jayaraju et al., 2011). After entering into an estuary, the dissolved Cd is swiftly scavenged by negatively charged suspended particulate matter (SPM) such as clays, carbonates, Fe and Mn oxides, and hydroxides and finally deposited to bed sediments (Förstner and Salomons, 1980; Kranck, 1975). However, the toxicity (mobility and bioavailability) of Cd in estuarine sediments does not entirely depend on total Cd concentration but the physicochemical forms of Cd in the accumulated sediments (Tack and Verloo, 1995).

Cd is known to generally form weaker complexes (labile) with sedimentary organic matter (SOM) in natural systems (Almås et al., 2000; Chakraborty et al., 2012; Eggleton and Thomas, 2004). Chakraborty et al., (2012) has reported the formation of kinetically labile complexes of Cd in sediments. It was found that the increase in the total concentration of Cd in estuarine and coastal sediments may increase the lability and bioavailability of Cd (Chakraborty et al., 2012).

Therefore, sediment samples collected from the Agacaim area (St-1 and St-2), Zuari estuary were selected for the kinetic fractionation study of Cd. The sampling location map is already provided in the Chapter-2. The results obtained throughthe kinetic fractionation study of Cd-sediment complexes are further combined with the result of the geochemical fractionation study (presented in the previous chapter) to identify the labile Cd-complexes from estuarine sediments and its bioavailability to oyster (*Magallana* sp.).

5.2 Results and Discussions

In our previous chapter, we have carried out geochemical fractionation of Cd in two different periods, August-December 2014 and February 2015-January 2016. We concluded that speciation of Cd (especially finer sediments) are mainly responsible for bioavailable Cd and its accumulation in oyster tissue. Similarly, in this chapter, we have carried out kinetic speciation of bulk sediments (August-December 2014) as well as finer sediments (February 2015-January 2016) to understand the nature of Cd-sediment complexes (labile or inert).

5.2.1 Kinetic speciation of sediment samples (during August-December 2014)

The physical and environmental parameters (sediment texture, total organic carbon (C_{org}), total nitrogen (TN), pH, salinity, and water temperature (overlying water column) are described in detail in the previous chapter (Chapter 4).

Kinetic speciation of Cd from studied sediments was carried out. Changes in concentrations of extracted Cd from the sediments by EDTA (complexing agent) with respect to time are shown in Figure 5.1. The experimental data obtained were fitted to two component models. Each curve shows two distinct features: a quickly rising section which represents the rapid dissociation rate (k_1) of weak Cd-sediment complexes (c_1) and the last part of each curve which lies almost parallel to x-

axis represents slow dissociation (k_2) of strong Cd-sediment complexes (c_2). The first part of all curves in Figure 5.1 is nearly indistinguishable from one another, indicating that they represent dissociation of one or more Cd complexes with a similar fast dissociation rate constant; they represent Cd complexes with low thermodynamic stability and are dynamic (within the time scale of the measurement). The dissociation rate constant and labile fractions of Cd-sediment complexes are presented in Table 5.1.
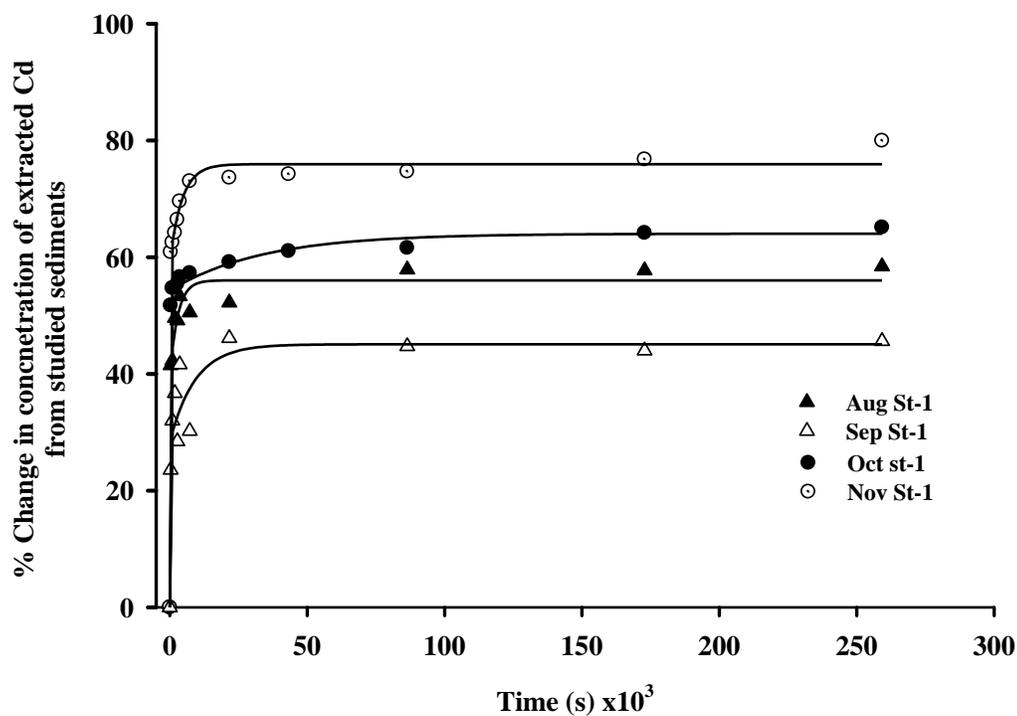
Figure 5.1: Release of extractable dynamic Cd complexes from the studied sediments as afunction of time in presence of 0.05 M EDTA solution at pH 6.

Table 5.1: Kinetically distinguishable components of Cd complexes in studied sediments and their respective dissociation rate constants.

Stations	C_1 (%)	k_{d1} (s^{-1})	C_2 (%)	k_{d2} (s^{-1})	Total dynamic fraction (mg.kg^{-1})	Total Cd (mg.kg^{-1})	Cd in oyster (mg.kg^{-1})
Aug St-1	49.8	5.7 x 10^{-3}	50.2	9.31 x 10^{-7}	0.10 ± 0.02	0.17 ± 0.01	7.3 ± 0.4
Sep St-1	36.4	3.2 x 10^{-3}	63.6	7.6 x 10^{-7}	0.12 ± 0.01	0.26 ± 0.01	3.2 ± 0.3
Oct St-1	56.5	8.2 x 10^{-3}	43.5	1.0 x 10^{-6}	0.11 ± 0.04	0.17 ± 0.01	8.9 ± 0.5
Nov St-1	68.3	7.3 x 10^{-3}	31.7	2.0 x 10^{-6}	0.39 ± 0.03	0.49 ± 0.06	11.8 ± 0.3

The percentage of labile Cd-sediment complexes (c_1) varied from 36.4 to 68.3% of the total Cd in the studied sediments. The dissociation rate (k_1) of labile Cd-sediment complexes varied from ~ 8.2 $\times$ 10^{-3} to 3.2 $\times$ 10^{-3} s^{-1}. The lowest labile fraction of Cd complexes were found in the month of September (~36.4% of the totalCd with k_1 value of ~3.2 $\times$ 10^{-3} s^{-1}), whereas the highest fraction of labile Cd sediment complexes was shown in the month of November (~68.3% of the total Cd with k_1 value of ~7.3 $\times$ 10^{-3} s^{-1}) (Table 5.1). The high concentration of labile Cd sediment complexes may alter the delicate balance between the speciation, bioavailability, and toxicity of this metal. Zero ligand field stabilization energy (LFSE) of Cd^{2+} was probably responsible for forming thermodynamically weak Cd-sediment complexes. The weak stability of Cd-humate complexes has also been attributed to poorly effective nuclear charge, the polarizability of Cd, and the ligand in

the system (Chakraborty, 2010). The concentration of labile Cd complexes (c_1) with its fast dissociation rate constant (based on lability criterion) obtained through this study was probably considered as a good indicator of bioavailable Cd in estuarine sediments.

Regression analysis was performed to understand the relationship between bioavailable Cd from sediments and bioaccumulated Cd in the soft tissue of oysters. The variation of total Cd concentration accumulated in the soft tissues of the oysters with varying concentrations of labile Cd complexes (c_1) and their dissociation rate constant (k_{d1}) in the sediments are shown in Figures 5.2 a & b respectively. The correlation showed that the accumulation of Cd in oysters gradually increased with the increasing concentrations of labile Cd-sediment complexes (c_1) and their dissociation rate constants (k_{d1}). Thus, the kinetic speciation study of Cd has shown that the accumulation of Cd in oysters depends upon the available concentrations of labile Cd-complexes from the studied sediments. The concentration of Cd present in oyster tissue depends on the dissociation rate constantof Cd-sediment complexes. Thermodynamically stable (inert) Cd sediment complexes did not contribute in the Cd bioaccumulation process in sampled oysters from the study area.
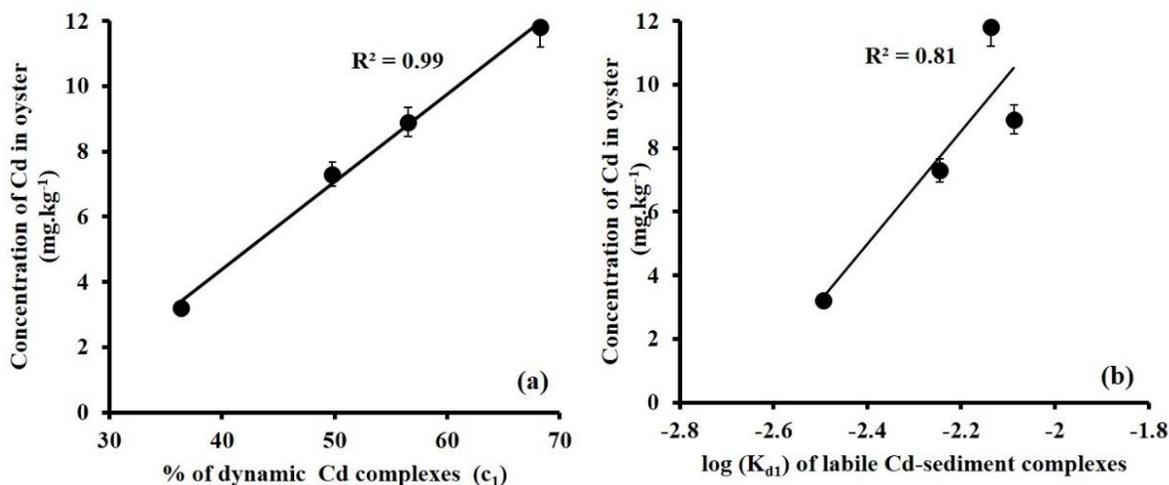
Figure 5.2: Variation in concentration of Cd accumulated in the soft tissues of oysters with varying concentrations of **(a)** labile Cd complexes (c_1) and **(b)** their dissociation rate constant (k_{d1}) in the sediments.

5.2.2 Kinetic speciation of sediment samples (during February 2015- January 2016)

Kinetic speciation of Cd from the finer fraction of the sediment sampled from the Zuari estuarine area was performed. Changes in concentrations of extracted Cd from the sediments by EDTA (complexing agent) with respect to time are shown in Figure 5.3 a & b. The experimental data obtained were fitted to two component models. Each curve in Figure 5.3 a & b showed two distinct features: a quickly rising section which represents the rapid dissociation rate (k_1) of weak Cd-sediment complexes (c_1) and the last part of each curve which lies almost parallel to x-axis represents slow dissociation (k_2) of strong Cd-sediment complexes (c_2). The first part of all curves in Figure 5.3 a & b is nearly indistinguishable from one another, indicating that they represent dissociation of one or more Cd complexes with a similar fast dissociation rate constant, they

represent Cd complexes with low thermodynamic stability and are dynamic (within the time scale of the measurement) in nature. The dissociation rate constant and labile fractions of Cd-sediment complexes are presented in Table 5.2.

The labile Cd-sediment complexes (c_1) with low thermodynamic stability varied from 37.8 to 75.8 % of the total Cd in the St-1 sediments. In St-2, the labile Cd-sediment complexes (c_1) varied from 34.8 to 63.1%. The dissociation rate (k_1) of labile Cd-sediment complexes in St-1 and St-2 sediments varied from ~ 2.0×10^{-2} to 3.1×10^{-3} s^{-1} and ~ 2.4×10^{-2} to 1.3×10^{-3} s^{-1}. The lowest labile fraction of Cd complexes in St-1 was found in the month of July (~37.8% of the total Cd with k_1 value of ~9.3×10^{-3} s^{-1}), whereas the highest fraction of labile Cd sediment complexes was shown in the month of November (~75.8% of the total Cd with k_1 value of ~$7.5 \times 10{-3}$ s^{-1}) (Table 5.2). In St-2, the lowest labile fraction of Cd complexes was found in October month (~34.8% of the total Cd with k_1 value of ~2.4×10^{-3} s^{-1}), whereas the highest fraction of labile Cd sediment complexes was found in November month (~63.1% of the total Cd with k_1 value of ~1.3×10^{-3} s^{-1}) The concentration of labile Cd-sediment complexes (c_1) in finer fraction of the sediment were found to depend on the total concentration of Cd from finer sediments (Figure 5.4) and were considered as a good indicator of bioavailable Cd in estuarine sediments with fast dissociation rate constant (based on lability criterion).

The high concentration of labile Cd-sediment complexes found in this study could be due to HSAB (Hard Soft Acid Base theory) and LFSE (Ligand field stabilization energy) of Cd^{2+}. According to HSAB theory, Cd^{2+} ion is a soft acid and always has a preference to softer base (S- and N- containing groups in organic binding phases) of the sediment to form stable Cd-sediment complexes with higher conditional stability constant values. In sedimentary organic substances/humic substances, 60–90% of the acid groups which are estimated are carboxylic and

the rest are phenolic (Chakraborty, 2007), and the remaining 1–10% of the total binding sites are stronger binding sites that form strong metal-sediment complexes (Chakraborty and Chakrabarti, 2008). The number of soft base ligands found to be very little (as minor sites) and it is not possible that all the Cd could bind. Hence, the remaining Cd presents tend to binds with available major sites, such as –COOH and –OH groups, giving them low conditional stability constant (Chakraborty and Chakrabarti, 2008). Zero ligand stabilization energy (LFSE) of Cd^{2+} was also probably responsible for the formation of thermodynamically weak Cd-sediment complexes. The weak stability of Cd-sediment complexes is due to poorly effective nuclear charge, the polarizability of Cd, and the ligand in the system. Competition of other trace metals in the natural system with Cd^{2+} for binding with different sites (cation exchange and nonionic sorption sites) of sediments might also lead to the upsurge in the lability of Cd-sediment complexes (Chakraborty, 2010; Chakraborty et al., 2015c). The last part of the curve which is almost parallel to the x-axis denotes the slow dissociation of inert Cd-sediment complexes (c_2) and thus considered as non-bioavailable.
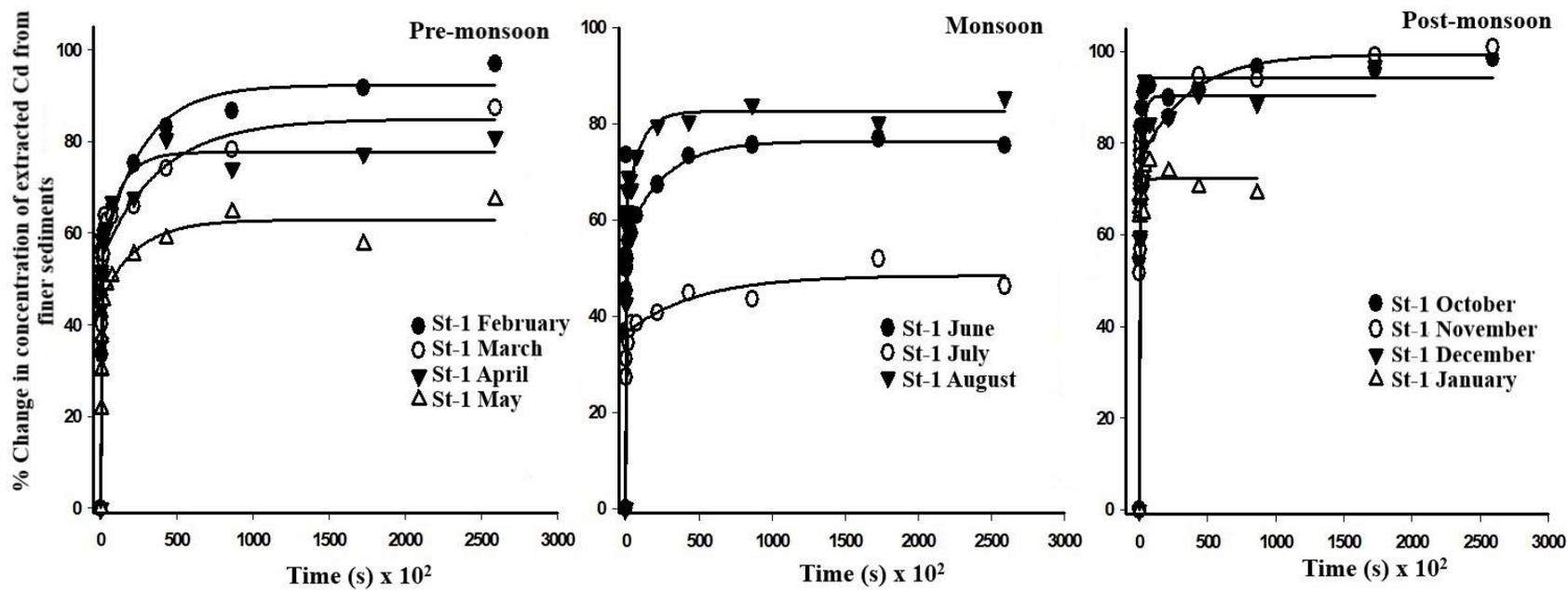
Figure 5.3 a: Release of extractable dynamic Cd complexes from the studied sediments (Station -1) as a function of time in the presence of 0.05 M EDTA solution at pH 6.
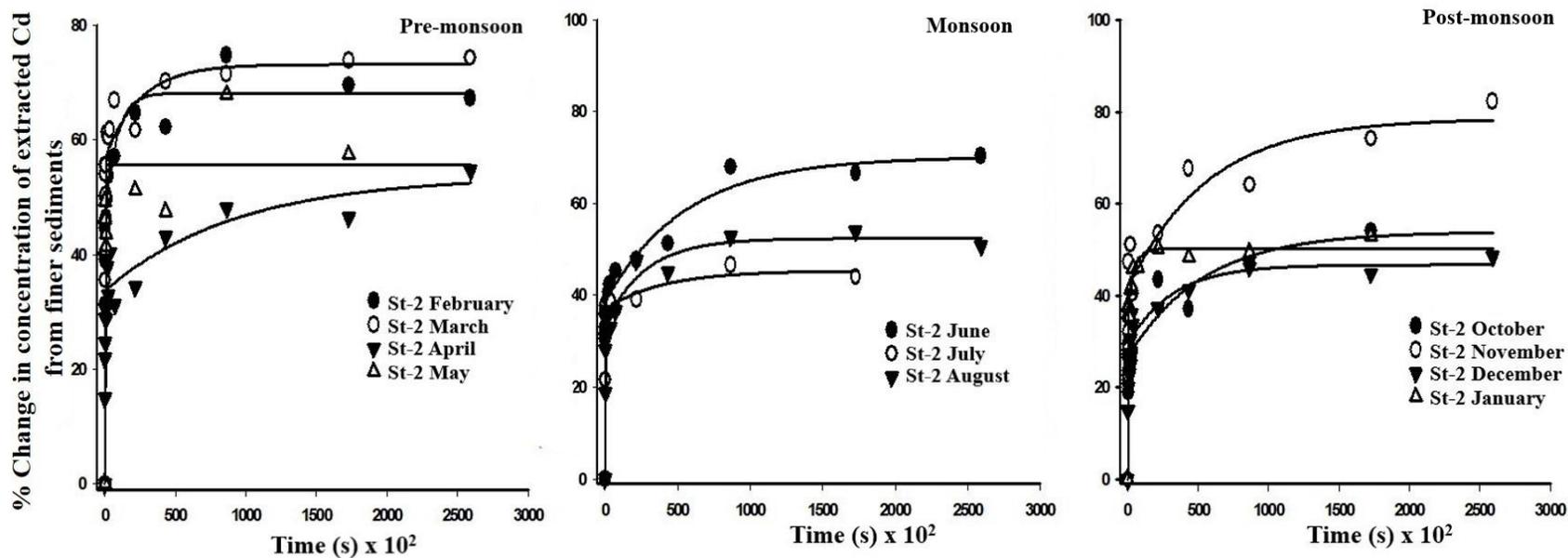
Figure 5.3 b: Release of extractable dynamic Cd complexes from the studied sediments (Station-2) as a function of time in the presence of 0.05 M EDTA solution at pH 6.

Table 5.2: Kinetically distinguishable components of Cd complexes in finer sediments and their associated dissociation rate constants.

Stations	C_1 (%)	K_{d1} (s^{-1})	C_2 (%)	K_{d2} (s^{-1})	Total Cd in finer sediment (mg.kg^{-1})	Cd oyster (mg.kg^{-1})
Feb-15 st-1	56.6	5.4 x 10^{-3}	43.4	1.9 x 10^{-5}	0.10 ± 0.02	7.1 ± 0.4
Mar-15 st-1	58.9	6.5 x 10^{-3}	41.1	1.3 x 10^{-5}	0.15 ± 0.01	5.4 ± 0.1
Apr-15 st-1	61.8	4.9 x 10^{-3}	38.2	3.7 x 10^{-6}	0.11 ± 0.03	5.1 ± 0.2
May-15 st-1	49.6	3.1 x 10^{-3}	50.4	2.2 x 10^{-6}	0.09 ± 0	5.2 ± 0.2
Jun-15 st-1	57.2	1.0 x 10^{-2}	42.8	3.8 x 10^{-6}	0.12 ± 0	7.0 ± 0.1
Jul-15 st-1	37.8	9.3 x 10^{-3}	62.2	9.3 x10^{-7}	0.08 ± 0.01	2.4 ± 0.0
Aug-15 st-1	68.6	7.3 x 10^{-3}	31.5	4.3 x 10^{-6}	0.12 ± 0.02	7.9 ± 0.3
Oct-15 st-1	74.7	1.0 x 10^{-2}	25.4	3.2 x 10^{-4}	0.12 ± 0.01	6.8 ± 0.0
Nov-15 st-1	75.8	7.5 x 10^{-3}	24.2	2.6 x 10^{-5}	0.10 ± 0.02	6.1 ± 0.2
Dec-15 st-1	73.5	4.1 x 10^{-3}	26.5	1.0 x 10^{-5}	0.11 ± 0.03	7.1 ± 0.0
Jan-16 st-1	68.8	2.0 x 10^{-2}	31.3	3.6 x 10^{-6}	0.09 ± 0.01	7.6 ± 0.2
Feb-15 st-2	54.0	5.0 x 10^{-3}	46.0	1.9 x 10^{-6}	0.10 ± 0.03	6.9 ± 0.1
Mar-15 st-2	59.6	7.7 x 10^{-3}	40.4	2.5 x 10^{-6}	0.10 ± 0.01	7.7 ± 0.0
Apr-15 st-2	35.0	3.7 x 10^{-3}	65.0	1.4 x 10^{-6}	0.10 ± 0.02	8.9 ± 0.3
May-15 st-2	52.4	1.0 x 10^{-2}	47.6	5.7 x 10^{-6}	0.10 ± 0.01	6.0 ± 0.0
Jun-15 st-2	40.5	1.2 x 10^{-2}	59.5	3.6 x 10^{-6}	0.11 ± 0	6.4 ± 0.1
Jul-15 St-2	36.8	6.8 x 10^{-3}	63.2	1.8 x 10^{-6}	0.11 ± 0.01	5.4 ± 0.1
Aug-15 st-2	37.4	5.4 x 10^{-3}	62.6	1.5 x10^{-6}	0.11 ± 0.02	7.5 ± 0.0
Oct-15 st-2	34.8	2.4 x 10^{-3}	65.2	2.3 x 10^{-6}	0.10 ± 0.04	8.6 ± 0.1
Nov-15 st-2	63.1	1.3 x 10^{-3}	36.9	2.2 x 10^{-5}	0.12 ± 0	8.9 ± 0.2
Dec-15 st-2	42.4	1.8 x 10^{-3}	57.6	4.0 x 10^{-7}	0.11 ± 0.01	7.2 ± 0.2
Jan 16 st-2	40.0	2.4 x 10^{-2}	60.1	2.7 x10^{-6}	0.07 ± 0.01	5.8 ± 0.3
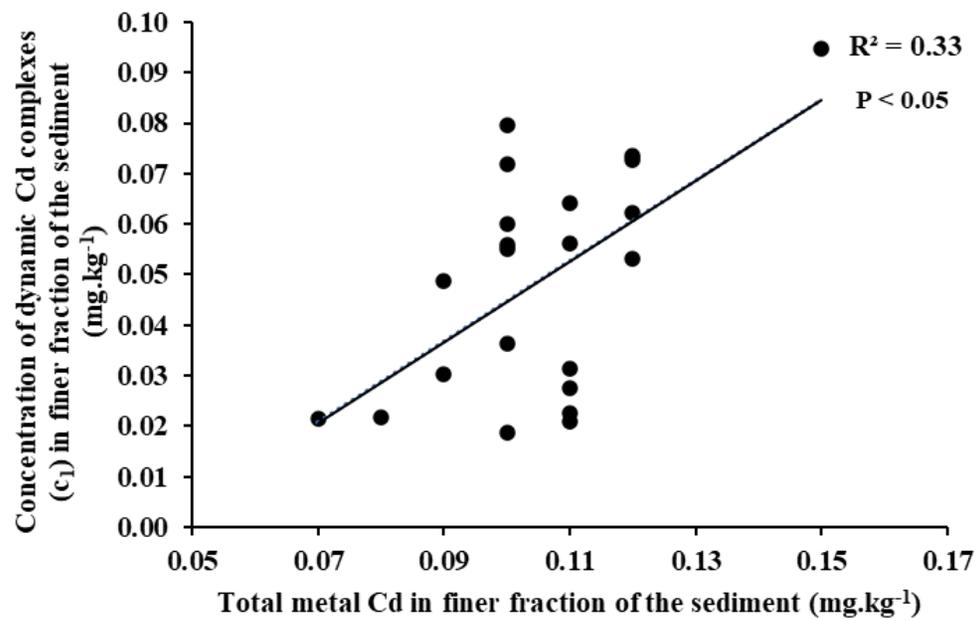
Figure 5.4: Variation in concentration of labile Cd-sediment complexes (finer sediment) as a function of total Cd loading in finer sediments.

5.2.3 Comparison of non-residual fraction of Cd obtained from sequential extraction method with labile Cd-sediment complexes obtained from the kinetic extraction method

It is well recognized that the use of different analytical speciation protocols with different time scales has advantages over a single speciation technique in analyzing environmental samples. It provides us different detection windows, which always offers us better information on metal speciation in natural systems (Chakraborty et al., 2011; Chakraborty et al., 2012). In this section, an effort was made to understand the dynamic behavior and bioavailability of Cd in the mangrove sediments by combining the data obtained by the sequential extraction method (previous study) and the kinetic speciation study (this study). In the previous chapter, in the geochemical fractionation study of Cd (in the same mangrove sediments), results showed that exchangeable, carbonate, and bicarbonate forms of Cd in the finer fraction of the sediment could be a good indicator of bioavailable Cd in the sediment system.

Non-residual fraction (exchangeable, carbonate, and bicarbonate forms of Cd) obtained from the sequential extraction study and the dynamic fractions of Cd (c_1) obtained from this kinetic extraction study were compared (Figure 5.5). The statistical analysis has shown a significant positive correlation between non-residual Cd (Cd in exchangeable, carbonate and bicarbonate forms) and labile Cd-sediment complexes. The concentration of non-residual Cd (Fr. 1, exchangeable, carbonate and bicarbonate forms) showed a gradual increase with the increase of labile Cd-sediment complexes, which clearly indicates that non-residual Cd (Cd in exchangeable, carbonate, and bicarbonate forms) were comparable and probably labile and bioavailable in the estuarine system.
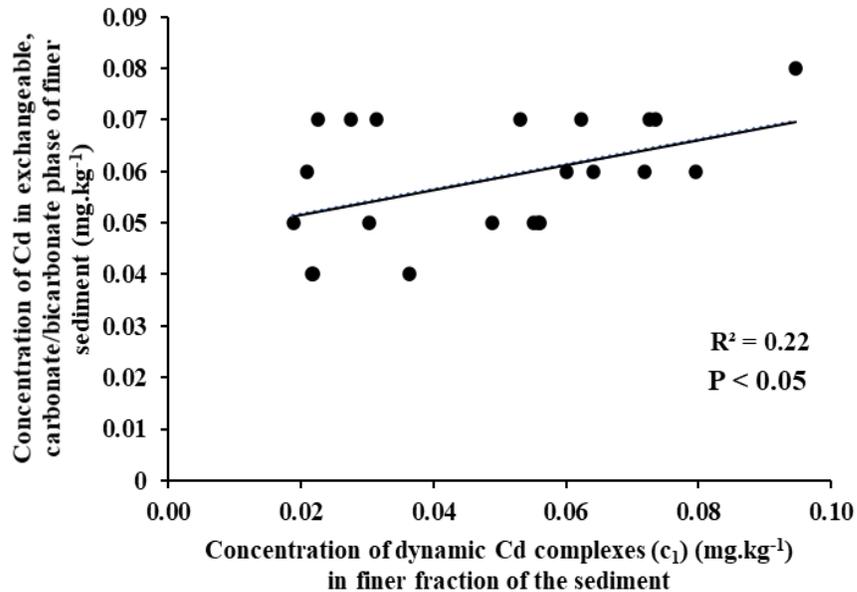
Figure 5.5: Variation of labile Cd complexes (c_1) concentration with concentration of Cd in exchangeable, carbonate/bicarbonate phase of mangrove sediments.

5.2.4 Kinetic speciation of Cd in finer sediments and its accumulation in oyster

Oysters are filter-feeders by nature, they filter a huge amount of surrounding water for their food. It has been reported that during the filtering process, fine sediment particles from the bottom sediments also get ingested along with plankton and the metals associated with these particles are considered as a major route of metal accumulation (Luoma, 1989; Reinfelder et al., 1997; Wang and Fisher, 1999; Wu et al., 2012). The relationship between Cd bioaccumulation in soft tissues of oyster with the varying concentration of labile Cd (c_1) complexes in finer particles of the sediments was studied (Figure 5.6). It shows that Cd concentration in the oysters gradually increased with the increasing concentration of labile Cd-sediment complexes (c_1) in the sediments. Bioaccumulation of Cd in oyster increases with increasing concentrations of labile Cd-complexes in the finer sediments. Thus, the kinetic speciation study of Cd from finer sediments has shown that the accumulation of Cd in oysters from the sampled sediment system depends upon the concentrations

of labile Cd-complexes in the finer particles of the sediments. Further, bioaccumulation of Cd in oysters will increase with increasing total Cd concentrations in the finer particles of the sediment.
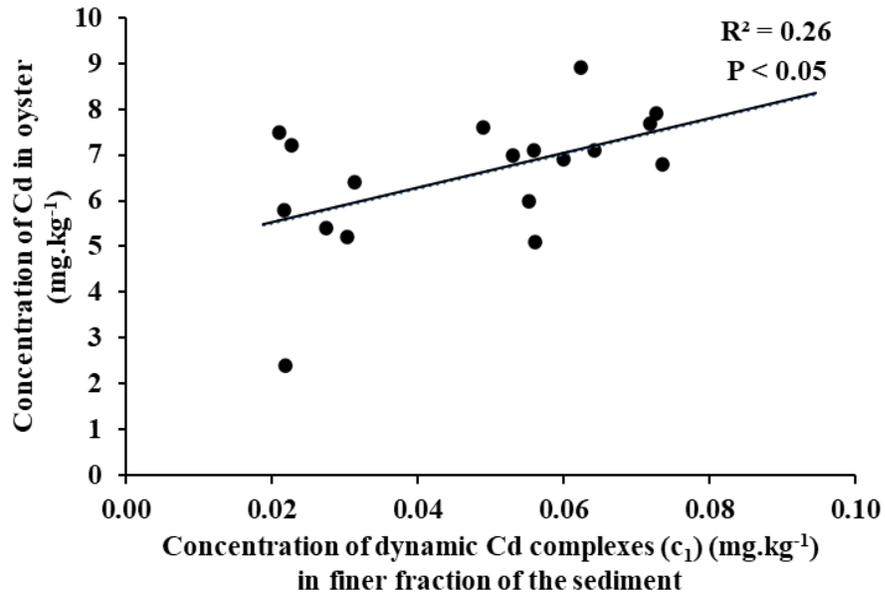
Figure 5.6: Variation of bioaccumulated Cd concentration in oyster with change in concentration of labile Cd complexes (c_1) of finer sediment.

5.3 Conclusion

Sedimentary Cd complexes in the sampled estuarine sediments were contained of two kinetically distinguishable forms (labile and inert Cd complexes). The study suggests that bioaccumulation of Cd in oyster increases with increasing concentrations of labile Cd-sediment complexes in studied sediments. Low thermodynamic stability and high bioavailability of Cd in the estuarine sediment were responsible for high bioaccumulation of Cd in edible oysters (2.4 to 8.9 mg.kg^{-1}) even though the total concentration of Cd in the finer sediment was low (0.07–0.15 mg.kg^{-1}).

A combination of sequential and kinetic extraction methods, with their associated measurement timescales and detection windows, suggests that increasing uptake of finer particles suspended from the surface sediments may increase Cd accumulation in the oyster system. Cd-speciation (chemical speciation) of the finer surface sediments may play a significant role in increasing Cd bioaccumulation in edible oysters from the study region.

Chapter 6

Influence of environmental factors on speciation and

bioavailability of sedimentary metals in estuarine systems

6.1 Introduction

It is well known that the estuarine ecosystem is a unique ecotone, (i.e.) a transition zone between land and ocean where consistent interaction between the freshwater (brought from the river and land discharge) and seawater brought from tidal action takes place. It is subjected to seasonal and day-to-day variations of various physicochemical parameters (e.g, salinity, pH, DO, etc.) (Ringwood and Keppler, 2002). Salinity is an important dynamic gradient in an estuary that varies daily or periodically and is majorly controlled by the river discharge, local rainfall, and tidal cycles (Chakraborty et al., 2010). Dissolved oxygen and pH may also change on a regular basis owing to photosynthetic and metabolic processes. pH fluctuation in the estuarine environment could be attributed to the poor buffering capacity of river bed clay mineral, freshwater, and episodic mixing of alkaline saltwater by tides.

The speciation of the toxic metal associated with estuarine sediment is influenced by the pH, salinity, DO, and concentration of suspended solids of the overlying water column (Simpson et al., 2004). Contaminants which are partitioned between the aqueous and solid phase are majorly controlled by two key variables-pH and salinity. These two variables are important in governing the bioavailability and the toxicity of metals bound to the estuarine sediments (Riba et al., 2003).

pH is one of the most vital factors that govern speciation, solubility, transport, and bioavailability of metal. pH affects adsorption-desorption reactions of metals on sediment/soil surfaces and controls free metal ion concentration in the estuarine sediment system. Generally, in the sediment system with the decrease in pH value, the competition between H^+ ions and dissolved metals (free metal ions) for ligands (e.g., natural organic ligands, such as humic substances, Fe/Mn oxyhydroxide, etc.) becomes more and more important. At lower pH value H^+ (or H_3O^+) will occupy more adsorption sites compare to metals, this, in turn, reduces the adsorption abilities of

metals and thus, increase the mobility of toxic metals (Li et al., 2013). Lower pH also affects the adsorption of metal to natural ligands, which are the major binding site of metal in sediments. Many studies have reported that lower pH of the overlying water column has significantly increased metal mobilization (Atkinson et al., 2007; Calmano et al., 1993; Håkansson et al., 1989; Loska and Wiechula, 2000; Rauret et al., 1991; Steinberg and Högel, 1990) from sediments. Lower pH is known to enhance the solubility of metal hydroxide leading to an increase in the concentration of free/dissolve metals, consequently making more metal available for interaction and assimilation in the biological activity of estuarine organisms (Millero et al., 2009; Salomons, 1995).

The salinity of the overlying water column, another important environmental parameter, affect the speciation of metal at the bottom sediments. Many studies (Liang and Wong, 2003; Riba et al., 2010, 2005b, 2004, 2003) have reported that lower salinity usually increases the bioavailability of metals in an estuarine system. But the effect of salinity on the bioavailability of an individual metal differs from metal to metal and mainly depend on their desorption rate from sediments to water and coagulation, flocculation and precipitation nature of metals. For example, field and laboratory experiments (Hatje et al., 2003; Paalman et al., 1994) study have shown that an increase in salinity increases the mobilization of metals. In the case of Cd metal, it is identified to be more mobile with an increase in the salinity, since Cd chlorocomplexation takes place which decreases the activity of free Cd^{2+}ions and increases desorption process (Hatje et al., 2003; Paalman et al., 1994). Cd speciation then move towards $CdCl^+$, $CdCl_2$, $CdCl^{3-}$ and $CdCl_4^{2-}$forms (Helmke, 1999). The mobility of Cd will further take place due to competition with Ca^{2+} ions for binding sites on suspended particles (Paalman et al., 1994).

Change in the pH and salinity values is expected to have a significant impact on the accumulation of metals to estuarine biota. A study by Riba et al., 2003 has shown that lower pH and salinity values have increased mortality and increased the concentration of metallothionines (MTs) associated with toxic metals. Metallothioneins (MTs) are known as low-molecular-weight, cysteine-rich metal-binding proteins, and ensue in most of the faunal species (Langston et al., 1998). Its primary role is in the homeostasis of essential metals such as Cu and Zn in a biological system of an organism. However, the increase in Cd (non-essential metal) could also induce MTs synthesis and dislocate essential metal from pre-existing MTs (Stillman et al., 1999).

Acidified pH may also directly damage tissues of invertebrates and will influence toxic metals accumulation. Toxic metal enters into a biological system first through the apical epithelial membrane, lower pH causes damages to epithelial tissues which in turn will increase the penetration of metals into cells. Study on Bivalve, *Mytilus edulis* has revealed that lower pH of seawater has significantly reduced the lysosomal health which would damage cellular pathways and increase the fragility of the membrane and consequently increased metal accumulation due to weakened defense system (Shi et al., 2016).

Thus, various studies have emphasized that pH and salinityare the two important variables in estuarine systems that regulate speciation and bioavailability of toxic metals and metal toxicity. Lower pH and salinity affect the chemical speciation of the metals, therefore, producing more bioavailable species and it also influences the sensitivity of the organisms to the metals, through the increase of distribution of some non-essential metals through the protein membranes and to the organism (Riba et al., 2003).

In this chapter, the influence of environmental parameters (pH and salinity) on speciation and bioavailability of metals (Cu and Cd) from the tropical estuarine sediments was carried out.

The study was performed with the sediments sampled from the Zuari and the Chapora estuaries. Both the estuary are tropical estuaries and annually experiences two major phases one is wet and another dry. The wet phase is around 4-5 months (June-September) and characterized by a high input of freshwater brought due to rain via the river. The dry phase is 7-8 months long (October-May) where a very low influx or negligible influx of freshwater takes place (Shetye et al., 2007). The main aim of the study was to understand how environmental factors influence the speciation and bioavailability of metals in tropical estuarine sediment systems.

6.2 Results and Discussions

6.2.1 Impact of varying pH of overlying water column on copper speciation from estuarine sediments

Sediment characteristics comprising of texture, total organic carbon (C_{org}), total nitrogen (TN), pH, salinity (overlying water), and the total concentration of Cu metal from mangrove sediments are shown in Table 6.1. In the Sancolae area (Zuari estuary), the sediment texture in St-1, St-2, and St-5 were dominated by coarser particles (sandy) and finer sediments dominated (< 63 µm) at St-3, St-4, and St-6. In the case of Agacaim, finer particles (< 63 µm) dominated in both the sampling stations except during the month of September and October. In Virnoda (Chapora estuary) station, the sediment texture was completely dominated by finer particles.

The measured pH of the overlying water column ranged from 6.9-8.3 in the sampling stations. The lowest pH value (6.9) was found at the Virnoda station where the salinity was zero and dominated by freshwater. The highest pH of the overlying water column was 8.3 at Agacaim during the post-monsoon period (December). The total concentration of Cu in the studied sediments varied from 31.1-103.7 mg.kg^{-1}. The high concentration of sedimentary Cu (103.7 mg.kg^{-1}) was observed

during the post-monsoon at Sancoale area in Zuari estuary. The lowest concentration of Cu (31.1 mg.kg^{-1}) was found during the monsoon period in Agacaim station. Overall, total Cu concentration in the estuarine sediments was low in the monsoon than the post-monsoon period. A decrease in river discharge (freshwater dilution) during the post-monsoon period could be the possible reason for the high total Cu concentration in mangrove sediments.

Table 6.1: Sediment texture, environmental parameter and total metal concentration of Cu in estuarine sediments.

Station	Sand (%)	Silt (%)	Clay (%)	C_{org} (%)	TN (%)	pH	parameter of overlying water column	
							Salinity (PSU)	$[Cu]_T$ sediment (mg.kg^{-1})
Sancolae St-1	69.9	5.7	24.4	2.2	0.2	7.7	20	91.7 ± 0.3
Sancolae St-2	80.1	4.3	15.6	1.5	0.1	7.6	30	103.7 ± 0.3
Sancolae St-3	18.3	6.1	75.5	3.2	0.3	7.6	25	95.6 ± 0.2
Sancolae St-4	37.0	12.2	50.8	2.8	0.2	7.6	25	97.4 ± 0.1
Sancolae St-5	53.0	7.8	39.2	3.6	0.2	7.5	25	80.6 ± 0.1
Sancolae St-6	7.0	18.2	74.8	3.6	0.3	7.7	20	88.6 ± 0.2
Agacaim Aug St-1	29.6	19.2	51.2	3.0	0.2	7.3	8	52.1 ± 1.5
Agacaim Sep St-1	65.8	6.5	27.6	1.5	0.1	7.8	10	31.1 ± 1.6
Agacaim Oct St-1	45.0	13.2	41.8	1.9	0.2	8	27	51.1 ± 0.5
Agacaim Nov St-1	38.1	14.2	47.7	2.1	0.2	7.9	28	60.8 ± 1.1
Agacaim Dec St-1	24.4	16.1	59.6	3.1	0.3	8.3	29	74.3 ± 0.9
Agacaim Aug St-2	25.3	18.0	56.7	2.4	0.2	7.4	11	69.4 ± 1.7
Agacaim Sep St-2	41.3	17.3	41.4	0.8	0.1	7.6	10	38.2 ± 0.1
Agacaim Oct St-2	62.0	7.0	30.9	2.1	0.1	7.9	28	37.9 ± 0.3
Agacaim Nov St-2	29.7	14.7	55.6	1.9	0.2	8.1	28	67.4 ± 0.5
Agacaim Dec St-2	10.3	15.6	74.0	3.2	0.3	8.2	30	88.3 ± 0.6
Virnoda St-1	0.3	70.8	28.9	18.7	1.0	6.9	0	34.2 ± 0.1

To understand the impact of pH on Cu loading in the estuarine sediments, the correlation between the changes in sedimentary Cu concentration with changing pH of overlying water was tested (Figure 6.1). Figure 6.1 shows that there were two distinct relationships existed between pH and sedimentary Cu concentration in the sampling stations. **Group a** stations with the pH value range from 6.9 to 7.7 and **group b** the pH value ranged 7.6-8.3. However, it was not possible to understand the change in the binding capacity of Cu in the sediments. Further studies were carried out to understand the impact of pH on Cu distribution and speciation in sediments.
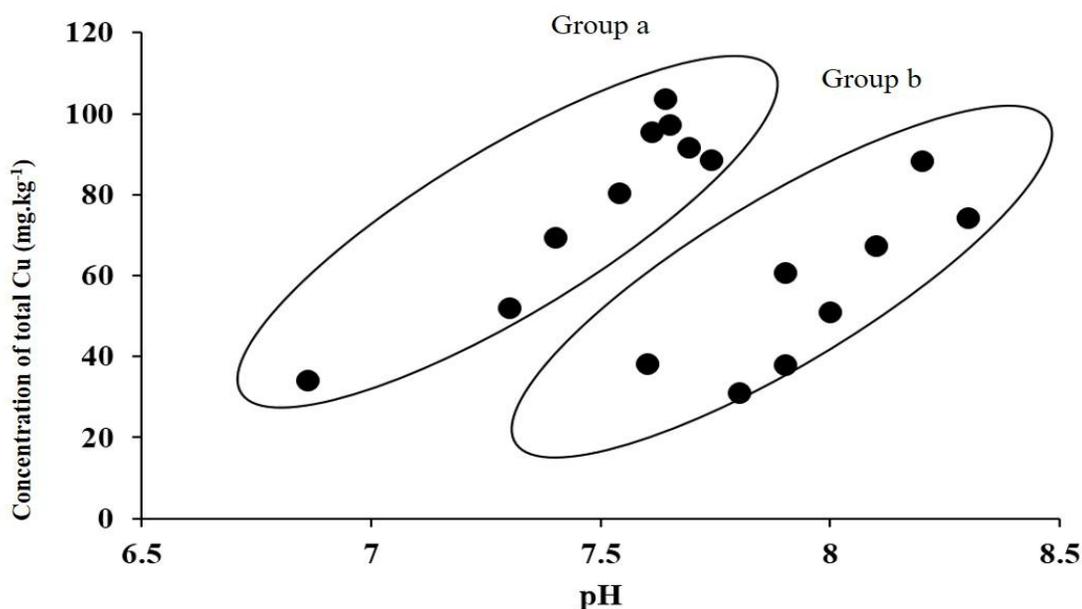
Group a- Virnoda St-1, Sancolae St-1, Sancolae St-2, Sancolae St-3, Sancolae St-4, Sancolae St-5, Sancolae St-6, Agacaim (Aug St-1), Agacaim (Aug St-2)

Group b- Agacaim (Sep St-1), Agacaim (Sep St-2), Agacaim (Oct St-1), Agacaim (Oct St-2), Agacaim (Nov St-1), Agacaim (Nov St-2), Agacaim (Dec St-1), Agacaim (Dec St-2)

Figure 6.1: Variation of total Cu concentration in sediments as a function of pH value of the overlying water column.

Nature of Sedimentary Organic Carbon

Total organic carbon (C_{org}) and TN varied from 1.5- 18.7 % and 0.1-1% respectively in the **group a** sediment. Whereas, in **group b**, C_{org}varied in the range of 0.8-3.2% and TN ranged 0.1-0.3%. The concentrations of C_{org} and TN were higher in the sediments from group a stations than the group b (Table 6.2). Strong positive correlations of C_{org} and TN with the finer fraction of the sediments (silt+clay) from both the groups were observed (Table 6.3 and 6.4), confirming again that finer sediment has more surface area to adsorb C_{org} and TN.

The total organic carbon to nitrogen molar ratio is widely used to determine the origin of organic matter in sediments (Hélie and Hillaire-Marcel, 2006; Perdue and Koprivnjak, 2007). Terrestrial plants have a higher $C:N_{molar}$ ratio (> 20), while plankton and bacteria have lower values (6-7) and (2-4) respectively. Microbial degradation also alters the $C:N_{molar}$ ratio of the sediments. $C:N_{molar}$ ratio of terrestrial plants decreases with mineralization and planktonic origin may increase upon diagenesis. The $C:N_{molar}$ ratio from the **group a** sediments were in the range of 13.5 and 23. However, the $C:N_{molar}$ ratio in the sediment for **group b** varied from 11 to 19 (Table 6.2). This clearly shows that sedimentary organic matter in the sampling stations was influenced by both marine and terrestrially derived OM, except station in Virnoda and in Sancoale which has value > 20 showing the dominance of OM derived from mangrove trees (terrestrial).

Stable organic Carbon and Nitrogen isotopes are the most important tool for the identification of the source of sedimentary organic matter (Carreira et al., 2002). $\delta^{13}C_{org}$ ranged in -24.4 to -28.5 ‰ **group a**, however, it ranged -22.4‰ to -26.8 ‰ in **group b**. $\delta^{15}N$ varied in 1.9 to 5.7‰ **group a** and varied from2.8 to 5.5 ‰ in **group b**. This indicates that variation in Cu distribution due to pH variation (as in group a and b) was probably due to differences in the nature of sedimentary organic matter.

Table 6.2: Total Organic Carbon (C_{org}), total nitrogen (TN), C/N ratio, and $\delta^{15}N$ and $\delta^{13}C_{org}$ from the estuarine sediments.

Station	C_{org} (%)	TN (%)	$C:N_{molar}$	$\delta^{15}N$	$\delta^{13}C_{org}$
Group a					
Virnoda St-1	18.7	1.0	22.7	1.9	-28.5
Sancolae St-1	2.2	0.2	15.2	5.3	-25.1
Sancolae St-2	1.5	0.1	16.1	4.4	-24.9
Sancolae St-3	3.2	0.3	13.5	5.7	-24.4
Sancolae St-4	2.8	0.2	15.0	5.4	-24.8
Sancolae St-5	3.6	0.2	20.3	5.4	-26.2
Sancolae St-6	3.6	0.3	14.2	5.6	-25.7
Agacaim Aug St-1	3.0	0.2	14.6	5.2	-25.1
Agacaim Aug St-2	2.4	0.2	13.9	5.2	-24.6
Group b					
Agacaim Sep St-1	1.5	0.1	18.6	2.8	-26.2
Agacaim Sep St-2	0.8	0.1	11.3	4.2	-22.4
Agacaim Oct St-1	1.9	0.2	14.2	4.7	-24.7
Agacaim Oct St-2	2.1	0.1	19.2	3.0	-26.8
Agacaim Nov St-1	2.1	0.2	13.4	5.1	-24.5
Agacaim Nov St-2	1.9	0.2	13.1	4.3	-24.2
Agacaim Dec St-1	3.1	0.3	13.7	5.4	-24.7
Agacaim Dec St-2	3.2	0.3	12.7	5.5	-24.0

Table 6.3: Pearson correlation coefficient between the sediment texture, C_{org}, TN and C:N_{molar} ratio from stations in group a.

	Sand (%)	Silt (%)	Clay (%)	C_{org} (%)	TN (%)	C:N_{molar}
Sand (%)	1.00					
Silt (%)	-0.64	1.00				
Clay (%)	-0.66	-0.15	1.00			
C_{org} (%)	-0.56	**0.96**	-0.22	1.00		
TN (%)	**-0.63**	**0.95**	-0.11	**0.99**	1.00	
C:N_{molar}	-0.09	**0.67**	-0.54	0.78	0.70	1.00

Table 6.4: Pearson correlation coefficient between the sediment texture, C_{org}, TN and C:N_{molar} ratio from stations in group b.

	Sand (%)	Silt (%)	Clay (%)	C_{org} (%)	TN (%)	C:N_{molar}
Sand (%)	1.00					
Silt (%)	-0.82	1.00				
Clay (%)	-0.99	0.73	1.00			
C_{org} (%)	**-0.63**	0.17	**0.72**	1.00		
TN (%)	**-0.87**	0.58	**0.90**	**0.86**	1.00	
C:N_{molar}	0.13	-0.19	-0.10	0.16	0.08	1.00

Distribution of Cu in different geochemical fraction of sediments

Geochemical fractionation of Cu associated with different binding phases of the studied mangrove sediments shown in Figure 6.2. The concentration of water-soluble Cu complexes (Fr.1) was found in very low concentration (< 0.9%) from all the sediment samples (except in Agacaim, September st-2 and December St-1). Cu associated with carbonate, bicarbonate, and exchangeable phase (Fr.2) in sediments of group a were found all below 1.7% whereas in group b it varied ~1.2-4.2% of total Cu from the sediments. The concentration of Cu associated with Fe-Mn oxyhydroxide phases (Fr.3) was found to be varied ~0.2-9.5% in the stations from the group a and in group b, it ranged ~3.4- 17.4% of the total Cu in the sediments. The total concentration of Cu from the sampled sediments associated with organic phases (Fr.4) were found in the range of ~9.5 to 18.6 % in stations of group a, and ~9.4-20.7% in group b. Overall the concentration of Cu-organic complexes in the group a sediments was comparatively higher than group b. This could probably due to the relatively high concentration of total sedimentary organic matter (C_{org}) found in the stations of group a than group b. The majority of total Cu in all studied estuarine sediments were present in the residual fraction (Fr.5), in group a Cu as residual from varied ~75.6- 88.3 % and in group b it varied (~65-85.4%). The absolute concentration of residual Cu concentration varied ~20.2 to 88.9 mg.kg^{-1}.

The relationship between the change in total Cu and the variation of Cu associated with different binding phases of the sediment is shown in Figure 6.3. It was found, that the association of Cu with water-soluble and exchangeable, carbonate, and bicarbonate phases (Fr.1+Fr.2) (thermodynamically weak complexes) in the sediments from both the groups was not significantly related with an increase in total Cu in the respective sediments. Variation of total Cu concentration in both the groups also did not show any significant effect/relationship with Cu associated in Fe/Mn

oxyhydroxide phase. However, Cu association with the organic phase progressively increased with the increasing Cu loading in the sediments. It is interesting to note that both groups showed a similar trend. The highest association of Cu was within the residual fraction in both the groups. The concentration of Cu in residual fraction increased with the increase in total concertation of Cu in both the groups which probably suggests that Cu analyzed from the sampled sediments is basically from the source rocks (geogenic source).

The fractionation study clearly shows that whenever the total Cu loading in the sediment has increased the association of Cu with the organic phase has also been increased. The higher association of non-residual Cu in the organic phase was due to the formation of thermodynamically stable Cu-organic complexes (Chakraborty et al., 2016a). Various authors have described the higher affinity of Cu with the binding ligands in the organic phases of sediments and dissolved organic carbon in the natural system (Cao et al., 2004; Chakraborty et al., 2009; Chakraborty and Chakrabarti, 2006; Smith et al., 2002). A similar observation was also been observed in the previous study (Chapter 3).
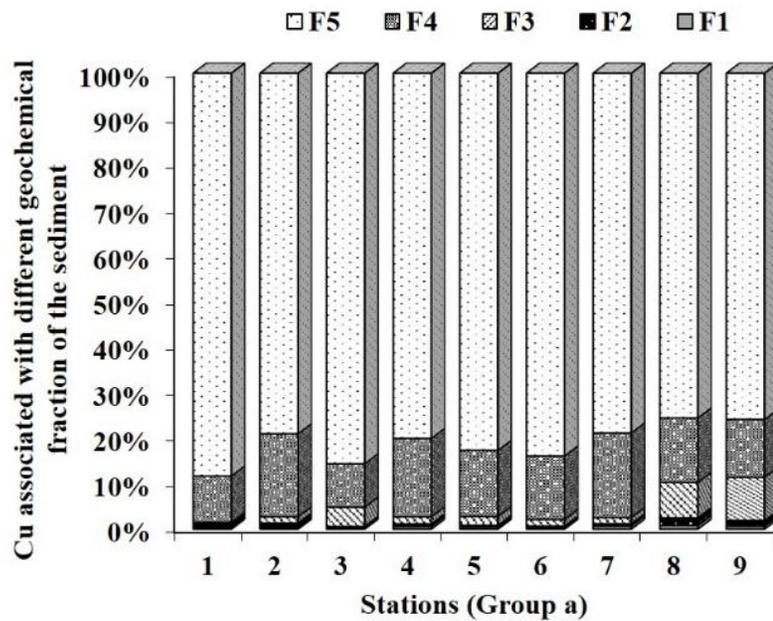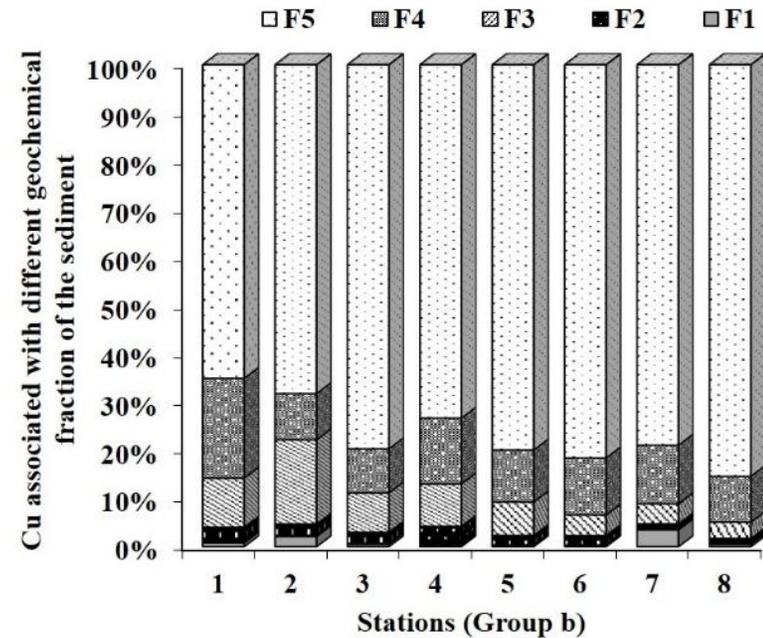
Figure 6.2: Geochemical fractionation of Cu in the mangrove sediments (i) group a and (ii) group b.
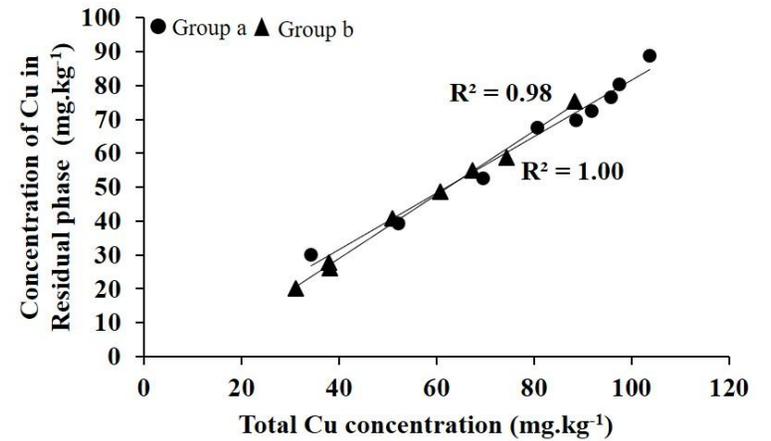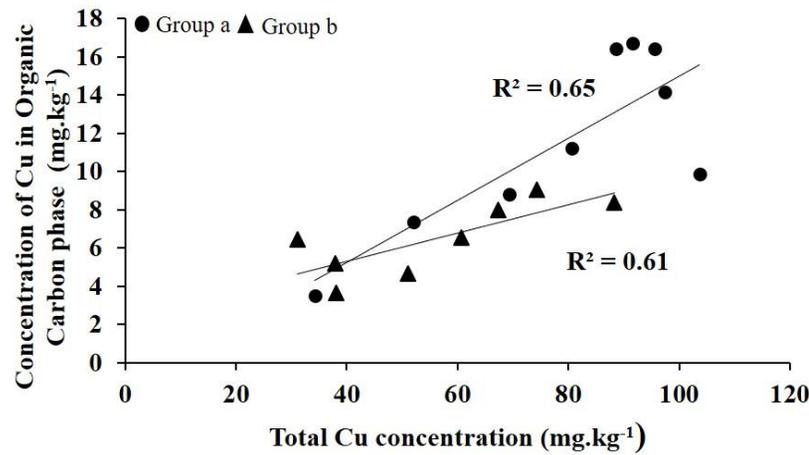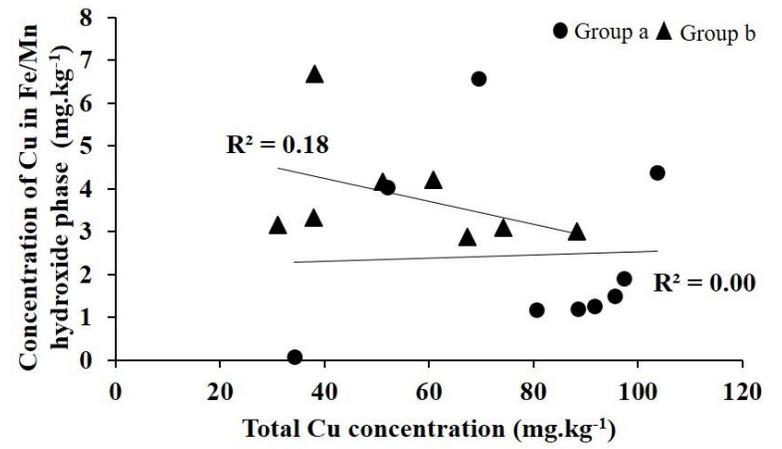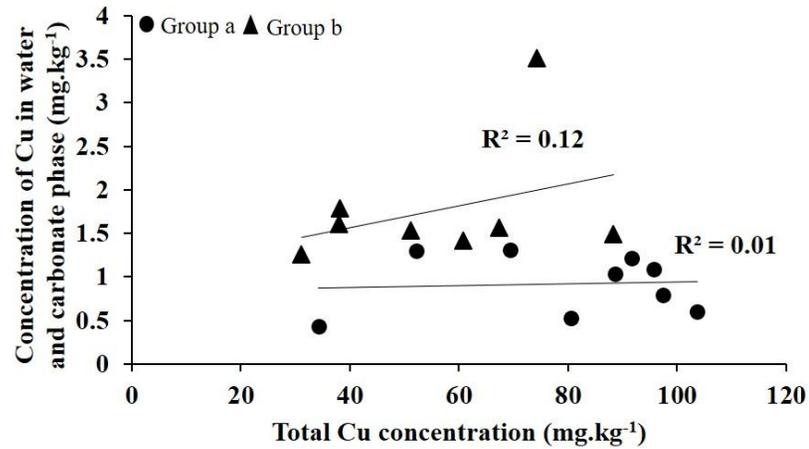
Figure 6.3: Variation of Cu distribution in different geochemical phases of the sediment as a function of total Cu loading.

Sedimentary organic matters are polyfunctional, polyelectrolyte, and polydisperse in nature. An experimental study by Jayachandran et al., (2018) has shown that pH variation of the overlying water column has affected the mobility of Cu from mangrove sediments. Therefore, statistical analysis was performed to understand the impact of measured pH (from natural samples, overlying water column) on Cu speciation from the sediments.

Figure 6.4 shows the effect of the pH of the overlying water column on the association of Cu in different binding phases of the sediment. Cu present in the water-soluble phase (Fr.1) showed a positive increasing trend with an increase in pH in both the groups. Cu associated with carbonate, exchangeable phase (Fr.2), and Fe/Mn ox hydroxide phase (Fr.3) of the total Cu did not show any significant effect due to variation of pH in overlying water in both the group sediments. In the organic phase of the sediment, a significant increase in Cu concentration was observed with the increase in pH, which probably suggests that the pH of the overlying water column has a decisive effect on Cu speciation and its bioavailability in the mangrove sediments.

The pH of the overlying water column showed a significant influence on the association of Cu with the organic phase and water-soluble phase in the studied sediments. Usually, low pH is known to increase the solubility of metals (Chuan et al., 1996; Ram and Verloo, 1985; Thornton, 1996). However, this study showed that increasing pH increased water-soluble Cu (mobility) concentration in sediments. The same trend was observed in a laboratory-based controlled study (Jayachandran et al., 2018). Jayachandran et al., 2018 have found that variation in pH redistributes Cu within different non-residual binding phases of sediment. Cu concentration associated with organic phases increased with an increase in pH, simultaneously an increase in the water-soluble fraction of Cu was also observed due to disaggregation and

leaching a part of sedimentary Cu-SOM at higher pH. Thus, our present study proves and confirms the experimental study carried out with the sediments from Zuari estuarine area by Jayachandran et al., 2018. This study suggests that an increase in pH value (measured on sampling site) increased the concentration of Cu associated with the organic phase and leached a part of Cu-SOM complexes as water-soluble Cu.

Table 6.5: Pearson correlation coefficient between Cu associated with different geochemical phases of the sediment, total Cu concentration in sediments (Cu_T) and pH of the overlying water column from stations in group a.

	Fr.1 (mg.kg^{-1})	Fr.2 (mg.kg^{-1})	Fr.3 (mg.kg^{-1})	Fr.4 (mg.kg^{-1})	Fr.5 (mg.kg^{-1})	$[Cu]_T$ (mg.kg^{-1})	pH
Fr.1 (mg.kg^{-1})	1.00						
Fr.2 (mg.kg^{-1})	0.18	1.00					
Fr.3 (mg.kg^{-1})	0.19	0.48	1.00				
Fr.4 (mg.kg^{-1})	0.63	0.07	-0.25	1.00			
Fr.5 (mg.kg^{-1})	0.33	-0.27	0.00	0.74	1.00		
$[Cu]_T$ (mg.kg^{-1})	0.43	-0.16	0.04	0.81	0.99	1.00	
pH	**0.58**	0.03	0.07	**0.89**	**0.89**	**0.94**	1.00

Table 6.6: Pearson correlation coefficient between Cu associated with different geochemical phases of the sediment, total Cu concentration in sediments (Cu_T) and pH of the overlying water column from stations in group b.

	Fr.1 (mg.kg^{-1})	Fr.2 (mg.kg^{-1})	Fr.3 (mg.kg^{-1})	Fr.4 (mg.kg^{-1})	Fr.5 (mg.kg^{-1})	$[Cu]_T$ (mg.kg^{-1})	pH
Fr.1 (mg.kg^{-1})	1.00						
Fr.2 (mg.kg^{-1})	-0.63	1.00					
Fr.3 (mg.kg^{-1})	-0.02	-0.23	1.00				
Fr.4 (mg.kg^{-1})	0.45	-0.29	-0.76	1.00			
Fr.5 (mg.kg^{-1})	0.31	-0.19	-0.44	0.77	1.00		
$[Cu]_T$ (mg.kg^{-1})	0.37	-0.24	-0.43	0.78	1.00	1.00	
pH	**0.47**	-0.07	**-0.74**	**0.85**	**0.83**	**0.84**	1.00
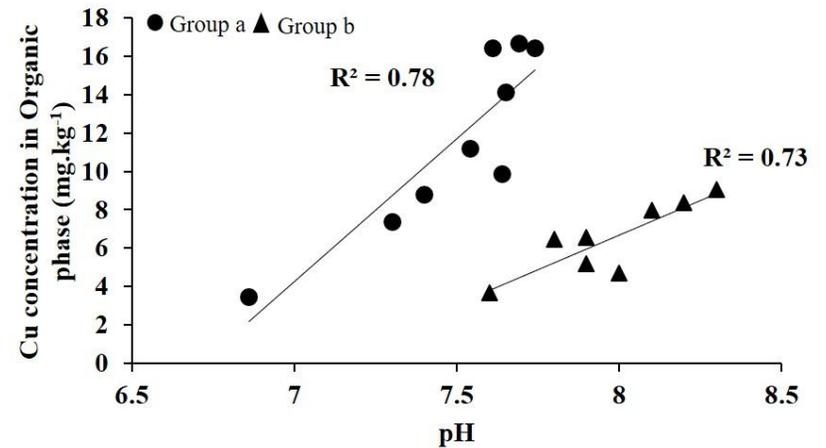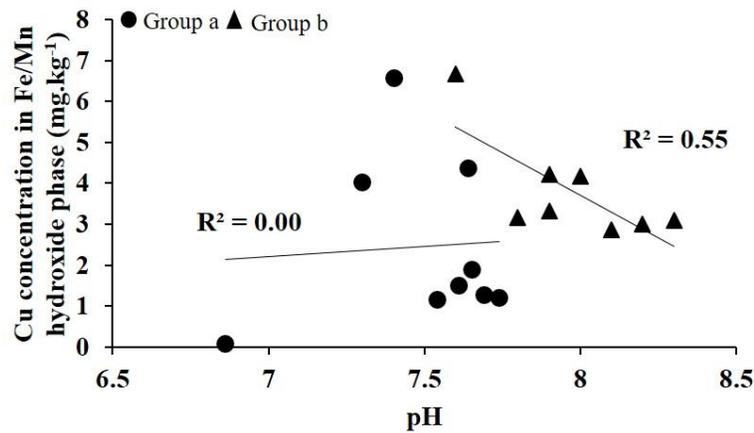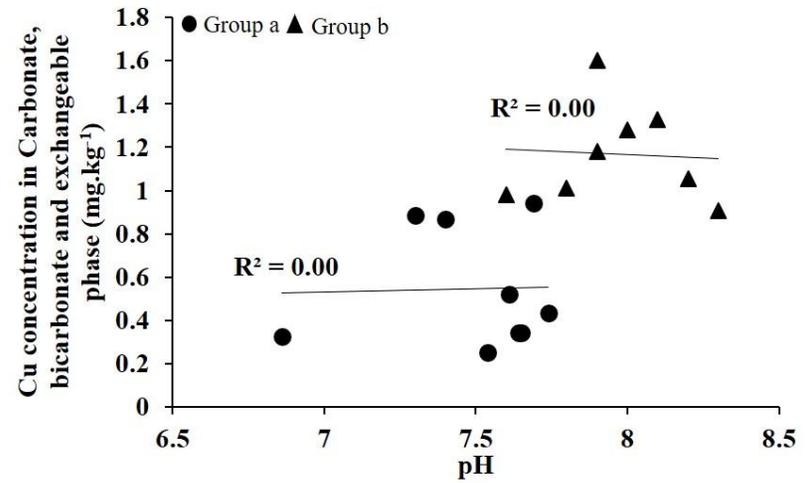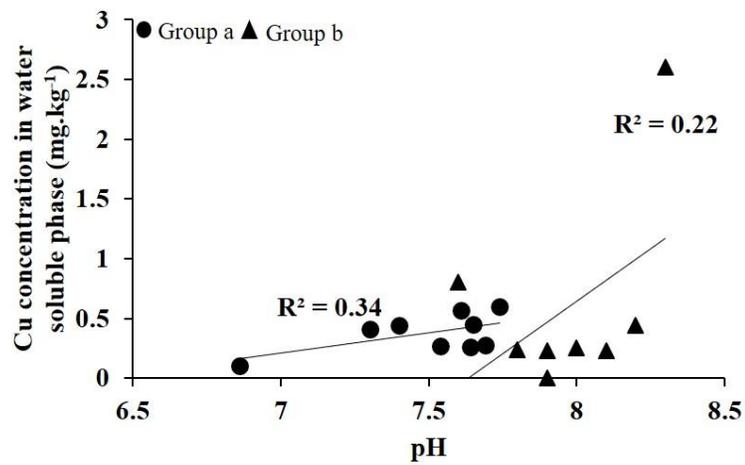
Figure 6.4: Variation of Cu distribution in non-residual phases of the sediment as a function of pH (overlying water column).

6.2.2 Impact of pH of overlying water column on cadmium speciation from estuarine sediments

Variation in the total concentration of Cd in the bulk and finer sediments and suspended particulate matter (SPM) along the pH, salinity, and water temperature are shown in Table 6.7. pH value of the overlying water column varied 7.7 to 7.9 in St-1, while in st-2 the pH ranged between 7.4 to 7.8.

ANOVA (analysis of variance) analysis was performed to understand whether there was any statistically significant variation in pH values with the change in the season (pre-monsoon, monsoon, and post-monsoon). The data are represented in Table 6.8; the analysis showed that there was no significant variation of pH found with the change of the season in St-1 (P-value > 0.05). In St-2, a significant variation of pH was found with the change of the season (P-value < 0.05), in pre-monsoon, the pH value was found low compare to post-monsoon and monsoon period.

Table 6.7: pH, salinity, water temperature of overlying water column and total Cd concentration in estuarine sediments and suspended particulate matter (SPM).

Agacaim Stations	pH	Salinity (PSU)	Water Temperature (°C)	$[Cd]_T$ bulk sediment (mg.kg^{-1})	$[Cd]_T$ finer sediment (mg.kg^{-1})	$[Cd]_T$ SPM (mg.kg^{-1})	$[Cd]_T$ oyster (mg.kg^{-1})
Jan-15 st-1	7.8	32.5	30.8	0.18 ± 0.05			6.1 ± 0.2
Feb-15 st-1	7.7	31	30.2	0.12 ± 0.03	0.10 ± 0.02		7.1 ± 0.4
Mar-15 st-1	7.6	30.8	31	0.12 ± 0.03	0.15 ± 0.01		5.4 ± 0.1
Apr-15 st-1	7.5	27.5	31.4	0.13 ± 0	0.11 ± 0.03	0.52 ± 0	5.1 ± 0.2
May-15 st-1	7.6	28.8	30.8	0.15 ± 0.01	0.09 ± 0	0.22 ± 0.02	5.2 ± 0.2
Jun-15 st-1	7.7	13.4	29.8	0.13 ± 0.02	0.12 ± 0	0.25 ± 0.02	7.0 ± 0.1
Jul-15 st-1	7.7	10.8	28.9	0.12 ± 0	0.08 ± 0.01	0.19 ± 0.01	2.4 ± 0.0
Aug-15 st-1	7.9	18.7	30.9	0.12 ± 0.01	0.12 ± 0.02	0.05 ± 0.01	7.9 ± 0.3
Oct-15 st-1	7.6	28.4	30.4	0.10 ± 0.01	0.12 ± 0.01	0.14 ± 0	6.8 ± 0.0
Nov-15 st-1	7.5	32.3	30.3	0.10 ± 0.01	0.10 ± 0.02	0.14 ± 0.01	6.1 ± 0.2
Dec-15 st-1	7.7	32.4	28.9	0.09 ± 0.02	0.11 ± 0.03	0.61 ± 0.04	7.1 ± 0.0
Jan-16 st-1	7.7	33.5	31.9	0.11 ± 0	0.09 ± 0.01	0.24 ± 0.01	7.6 ± 0.2
Jan-15 st-2	7.8	32.2	30.2	0.17 ± 0			9.5 ± 0.2
Feb-15 st-2	7.6	31.4	30.8	0.16 ± 0.03	0.10 ± 0.03		6.9 ± 0.1
Mar-15 st-2	7.6	30.8	31.2	0.16 ± 0.01	0.10 ± 0.01		7.7 ± 0.0
Apr-15 st-2	7.5	29.1	31.6	0.19 ± 0.02	0.10 ± 0.02	0.28 ± 0.01	8.9 ± 0.3
May-15 st-2	7.4	33.6	32	0.14 ± 0.01	0.10 ± 0.01	0.18 ± 0.06	6.0 ± 0.0
Jun-15 st-2	7.7	10.6	30.1	0.15 ± 0.02	0.11 ± 0	0.16 ± 0	6.4 ± 0.1
Jul-15 St-2	7.7	10.1	30.2	0.14 ±0.02	0.11 ± 0.01	0.20 ± 0	5.4 ± 0.1
Aug-15 st-2	7.8	22.1	32.1	0.15 ± 0.01	0.11 ± 0.02	0.25 ± 0.02	7.5 ± 0.0
Oct-15 st-2	7.8	29	31.8	0.11 ± 0	0.10 ± 0.04	0.26 ± 0.01	8.6 ± 0.1
Nov-15 st-2	7.7	32.4	31.5	0.13 ± 0.01	0.12 ± 0	0.12 ± 0	8.9 ± 0.2
Dec-15 st-2	7.6	33.6	28	0.15 ± 0.02	0.11 ± 0.01	0.19 ± 0	7.2 ± 0.2
Jan 16 st-2	7.8	34.3	32.3	0.17 ± 0.01	0.07 ± 0.01	0.16 ± 0.04	5.8 ± 0.3

Table 6.8: Analysis of variance (ANOVA) performed to analyze the difference in pH of the overlying water column with respect to three seasons (pre-monsoon, monsoon, and post-monsoon).

Source of Variation	Station	SS	df	MS	F	P-value	F crit
Between Groups		0.04	2	0.02	2.5	0.14	4.26
Within Groups	Agacaim, St-1	0.08	9	0.008			
Total		0.12	11				
Between Groups		0.14	2	0.069	11.7	**0.0032**	4.26
Within Groups	Agacaim, St-2	0.05	9	0.06			
Total		0.19	11				

*SS- Sum of squares, df- Degree of freedom, MS- Mean square, F- F Variable

Geochemical fractionation of Cd from the mangrove sediments of Agacaim was carried out and explained in detail in the previous Chapter 4. Fractionation study of Cd was performed in both the bulk and finer sampled sediments (< 63 µm). In this segment, the effect of pH variation of the overlying water column at different season on Cd speciation in the bulk and finer sediments were examined.

Pearson correlation and the regression analysis were performed between the pH measured and Cd associated in different geochemical phases of the sediment (Figure 6.5; 6.6 and Table 6.9; 6.10). The statistical analysis data clearly showed there was no significant impact of overlying water column pH on total Cd concentration in bulk and finer sediments. The analysis between the pH and Cd in different binding phases of the sediments also showed no significant correlation except on Cd associated with Fe/Mn oxyhydroxide phase in bulk sediment. This study suggests that measured pH had no influence on the Cd speciation and its bioavailability from the bulk and finer mangrove sediments.
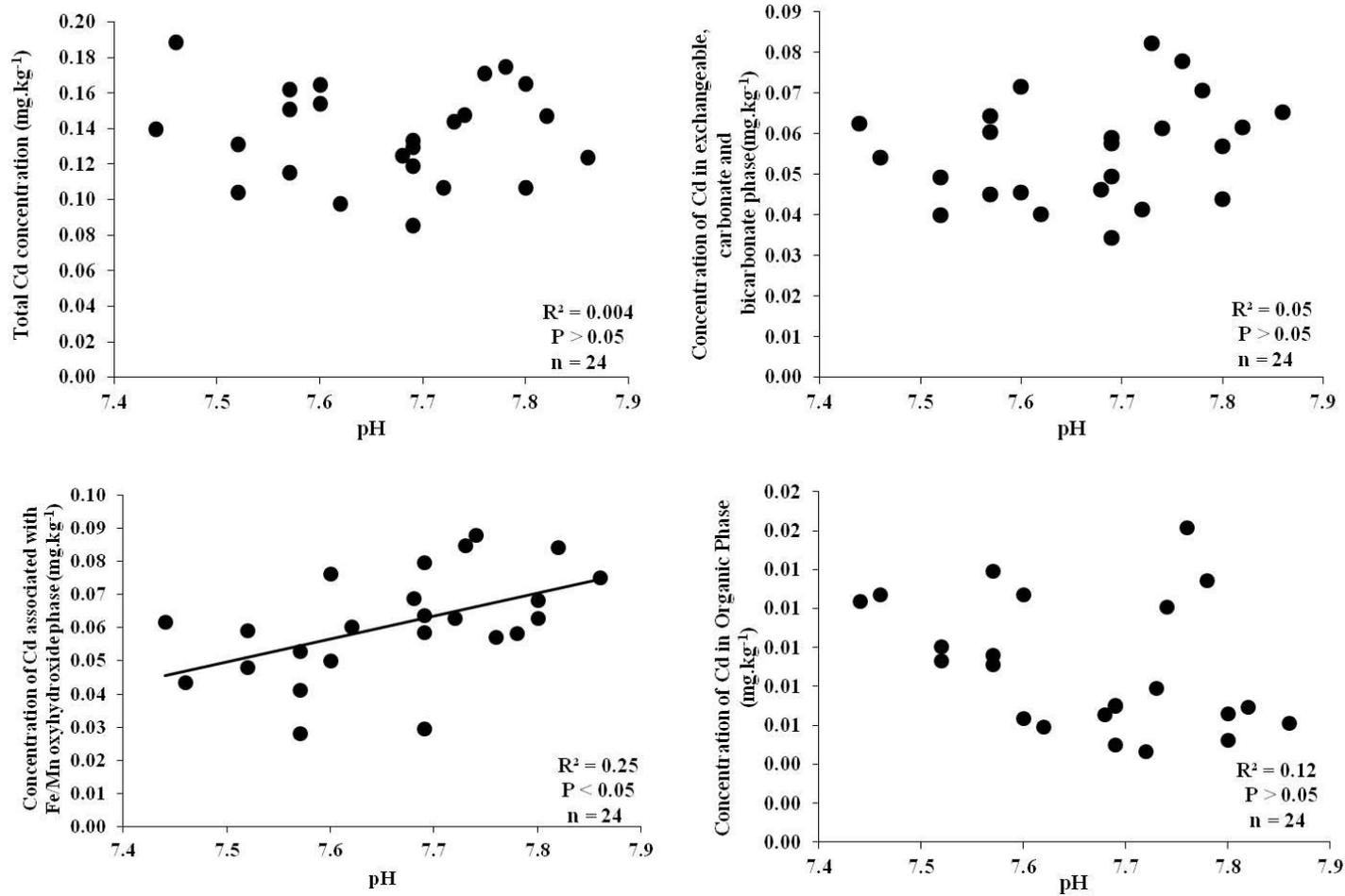
Figure 6.5: Variation of Cd associated with different geochemical phases of the bulk sediment as a function of pH value of overlying water column.
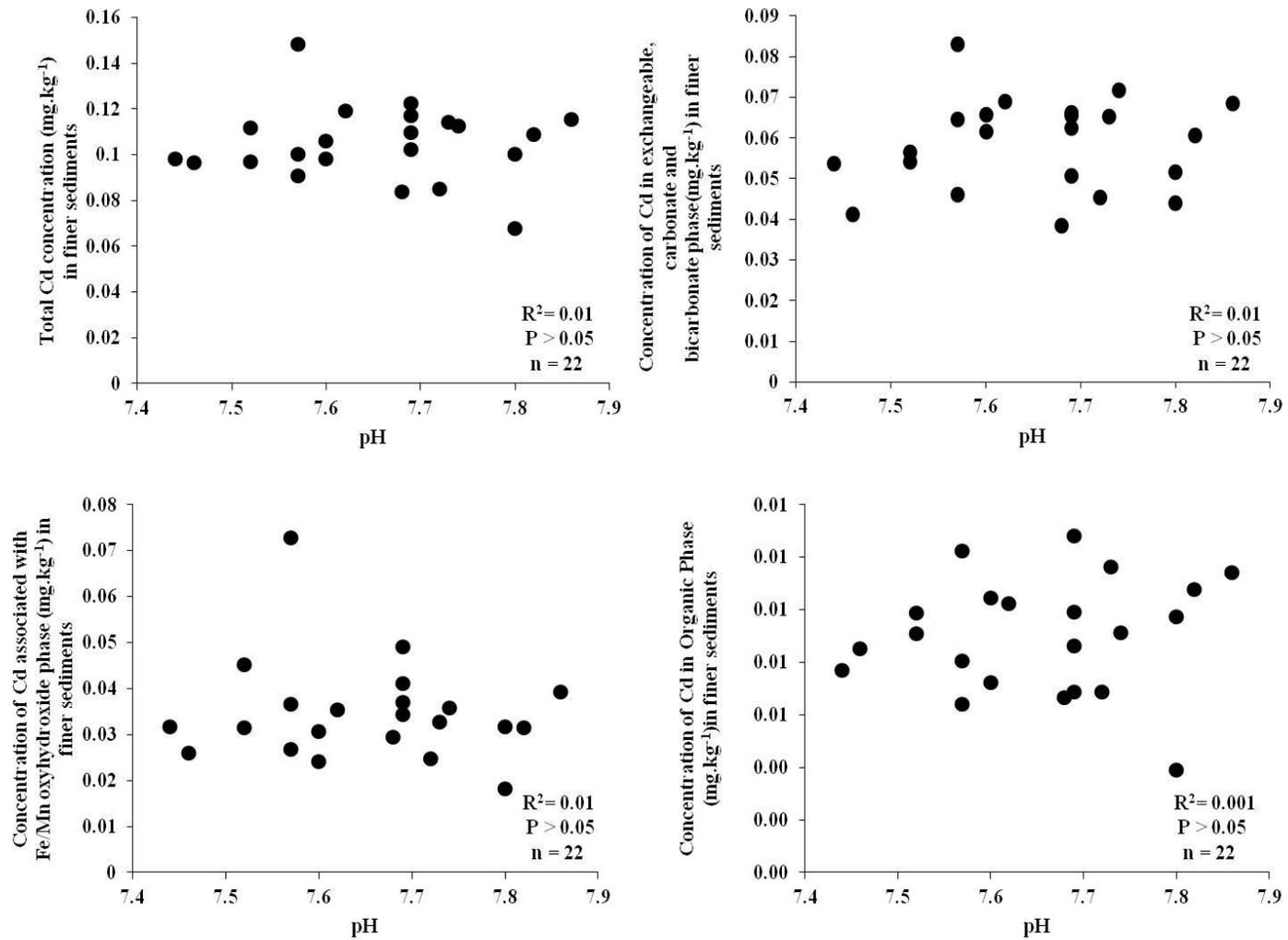
Figure 6.6: Variation of Cd associated with different geochemical phases of the finer sediment (< 63 μm) as a function of pH value of overlying water column.

Table 6.9: Pearson correlation coefficient between Cd associated with different geochemical phases of the sediment, total Cd concentration in bulk sediments $[Cd]_T$ and pH of the overlying water column from Agacaim.

	Fr.1 (mg.kg^{-1})	Fr.2 (mg.kg^{-1})	Fr.3 (mg.kg^{-1})	Fr.4 (mg.kg^{-1})	$[Cd]_T$ bulk (mg.kg^{-1})	pH
Fr.1 (mg.kg^{-1})	1.00					
Fr.2 (mg.kg^{-1})	0.24	1.00				
Fr.3 (mg.kg^{-1})	0.59	-0.26	1.00			
Fr.4 (mg.kg^{-1})	-0.14	-0.39	0.31	1.00		
$[Cd]_T$ bulk (mg.kg^{-1})	0.70	0.03	0.65	0.16	1.00	
pH	0.23	**0.50**	-0.35	-0.52	-0.07	1.00

Table 6.10: Pearson correlation coefficient between Cd associated with different geochemical phases of the sediment, total Cd concentration in finer sediments $[Cd]_T$ and pH of the overlying water column from Agacaim.

	Fr.1 (mg.kg^{-1})	Fr.2 (mg.kg^{-1})	Fr.3 (mg.kg^{-1})	Fr.4 (mg.kg^{-1})	$[Cd]_T$ finer (mg.kg^{-1})	pH
Fr.1 (mg.kg^{-1})	1					
Fr.2 (mg.kg^{-1})	0.58	1.00				
Fr.3 (mg.kg^{-1})	0.61	0.59	1.00			
Fr.4 (mg.kg^{-1})	-0.10	-0.48	-0.18	1.00		
$[Cd]_T$ finer (mg.kg^{-1})	0.86	0.82	0.76	-0.17	1.00	
pH	0.10	-0.11	0.09	0.00	-0.04	1

6.2.3 Impact of salinity of overlying water column on copper speciation in estuarine sediments

The measured salinity of the overlying water column ranged from 0-30 PSU (Table 6.1). The lower value of salinity in sampling stations was found probably due to the increased inflow of freshwater during the monsoon period in the estuary. The lowest salinity (0 PSU) was found in the Virnoda station of Chapora estuary during the peak monsoon period.

To understand the impact of salinity (overlying water column) on total Cu loading in estuarine sediments, the correlation between total Cu concentration of the sample sediments and the salinity of overlying water column from the study area was performed (Figure 6.7). The analysis clearly shows that there was no significant influence of salinity on total Cu loading in studied sediments. The analysis between the measured salinity and Cu associated in the different geochemical phases of the sediment (Figure 6.8) also exhibited a similar trend and has shown no significant correlation proving that the measured salinity of overlying water column does not influence Cu speciation and its bioavailability from sampled mangrove sediments.
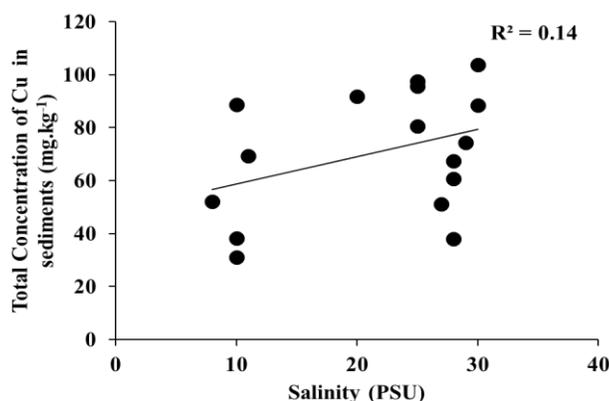
Figure 6.7: Variation of total Cu concentration in sediments as a function of salinity of the overlying water column.
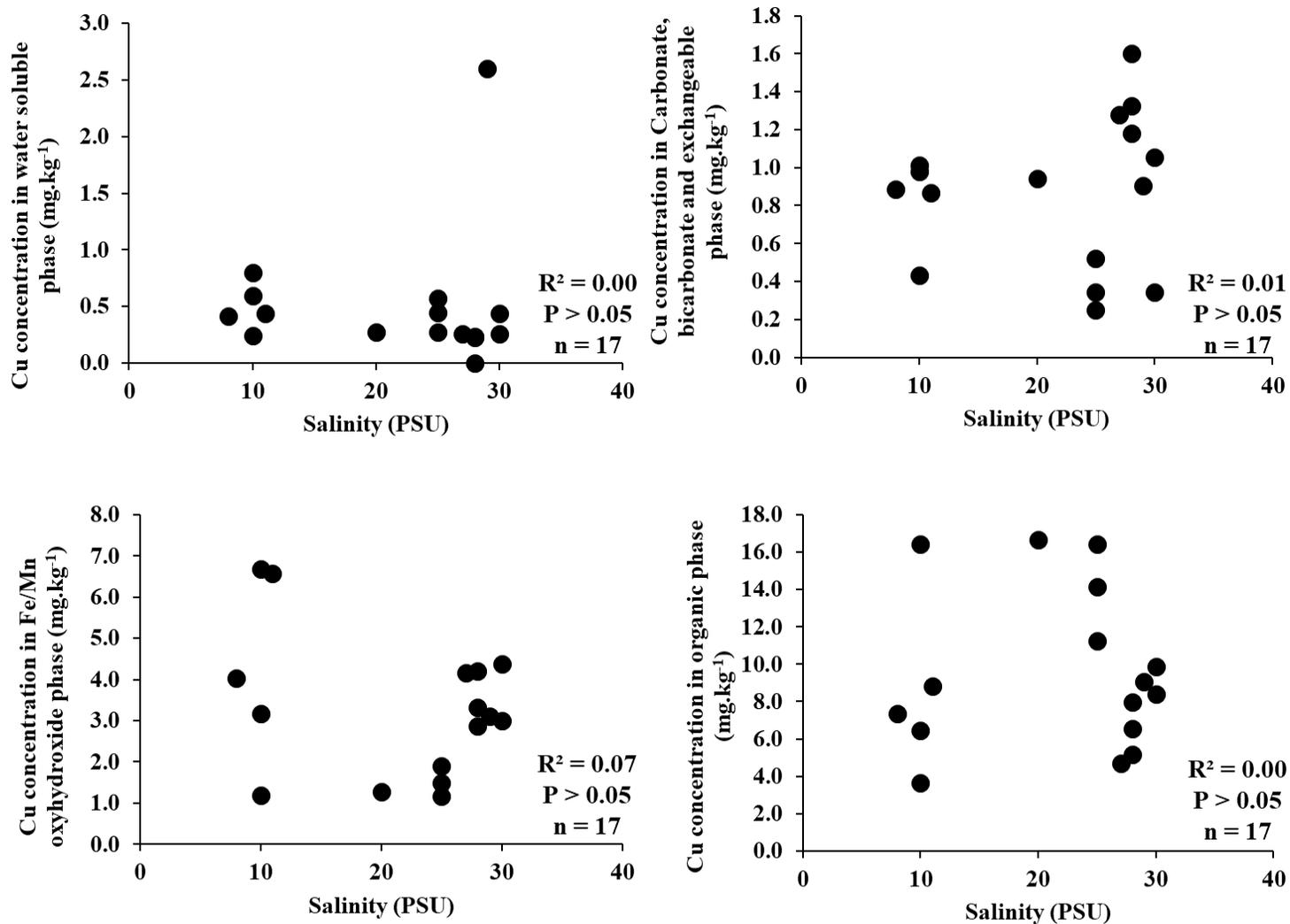
Figure 6.8: Variation of Cu associated with different geochemical phases of the sedimentas a function of salinity of overlying water column.

6.2.4 Impact of overlying water column salinity on cadmium speciation from estuarine sediments

Salinity value measured from the overlying water column varied around 10.8- 33.5 PSU in St-1 and 10.1 to 34.3 PSU in St-2 (Table 6.7). ANOVA analysis showed a significant variation in the salinity value in both the stations (St-1 and 2) with respect to change in season (P-value < 0.05) (Table 6.11). Lower salinity values were found during the monsoon period in both the sampling station and were due to the influx of freshwater into the estuary.

Table 6.11: Analysis of variance (ANOVA) performed to analyze the difference in salinity of the overlying water column with respect to three seasons (pre-monsoon, monsoon, and post-monsoon).

Source of Variation	Station	SS	df	MS	F	P-value	F crit
Between Groups	Agacaim, St-1	623.8	2	311.9	49.17	**0.00001**	4.26
Within Groups		57.1	9	6.3			
Total		680.8	11				
Between Groups	Agacaim, St-2	697.7	2	348.9	26.44	**0.000171**	4.26
Within Groups		118.7	9	13.2			
Total		816.5	11				

*SS- Sum of squares, df- Degree of freedom, MS- Mean square, F- F Variable

Pearson correlation analysis and the regression analysis were performed between the salinity measured and Cd associated with different geochemical phases of the sediment (Table 6.12; 6.13 and Figure 6.9; 6.10). The analysis data indicated that there was no significant impact of overlying water column salinity on total Cd concentration in the bulk and finer sediments. The

analysis between the salinity and Cd concentrations in different binding phases of the sediments showed no significant correlation, except on Cd associated with Fe/Mn oxyhydroxide phase in bulk sediment. This study showed that the salinity of the overlying water column had no influence on Cd speciation and its bioavailability in the mangrove sediments.

Table 6.12: Pearson correlation coefficient between Cd associated with different geochemical phases of the sediment, total Cd concentration in bulk sediments $[Cd]_T$ and salinity of overlying water column from Agacaim.

|	Fr.1 $(mg.kg^{-1})$	Fr.2 $(mg.kg^{-1})$	Fr.3 $(mg.kg^{-1})$	Fr.4 $(mg.kg^{-1})$	$[Cd]_T$ bulk $(mg.kg^{-1})$	Salinity (PSU)
Fr.1 $(mg.kg^{-1})$	1.00					
Fr.2 $(mg.kg^{-1})$	0.24	1.00				
Fr.3 $(mg.kg^{-1})$	0.59	-0.26	1.00			
Fr.4 $(mg.kg^{-1})$	-0.14	-0.39	0.31	1.00		
$[Cd]_T$ bulk $(mg.kg^{-1})$	0.70	0.03	0.65	0.16	1.00	
Salinity (PSU)	-0.27	**-0.48**	0.09	0.57	0.04	1.00

Table 6.13: Pearson correlation coefficient between Cd associated with different geochemical phases of the sediment, total Cd concentration in finer sediments $[Cd]_T$ and salinity of overlying water column from Agacaim.

	Fr.1 $(mg.kg^{-1})$	Fr.2 $(mg.kg^{-1})$	Fr.3 $(mg.kg^{-1})$	Fr.4 $(mg.kg^{-1})$	$[Cd]_T$ finer $(mg.kg^{-1})$	Salinity (PSU)
Fr.1 $(mg.kg^{-1})$	1					
Fr.2 $(mg.kg^{-1})$	0.58	1.00				
Fr.3 $(mg.kg^{-1})$	0.61	0.59	1.00			
Fr.4 $(mg.kg^{-1})$	-0.10	-0.48	-0.18	1.00		
$[Cd]_T$ finer $(mg.kg^{-1})$	0.86	0.82	0.76	-0.17	1.00	
Salinity (PSU)	-0.14	-0.15	-0.26	-0.19	-0.21	1.00
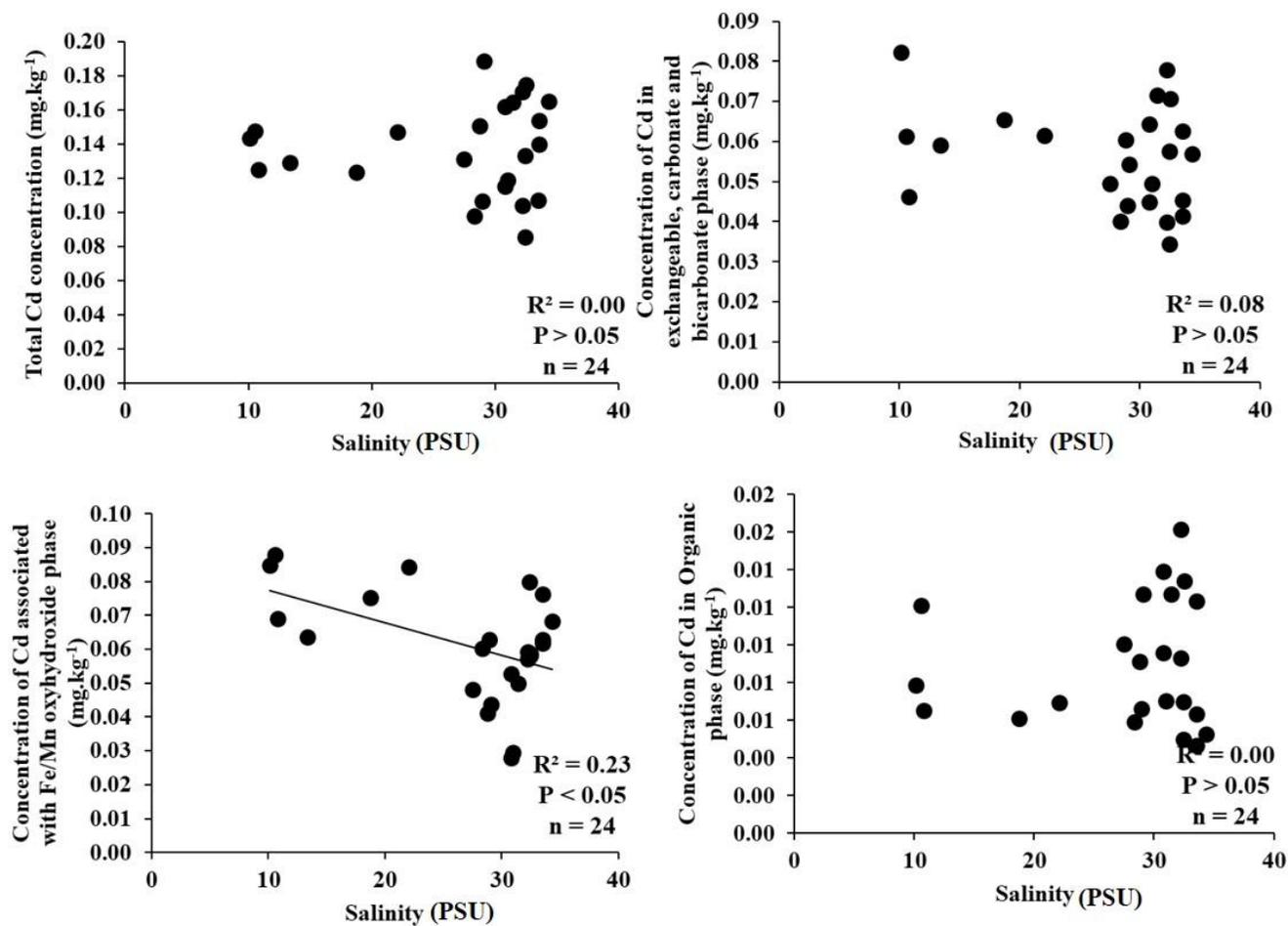
Figure 6.9: Variation of Cd associated with different geochemical phases of the bulk sediment as a function of salinity of overlying water column.
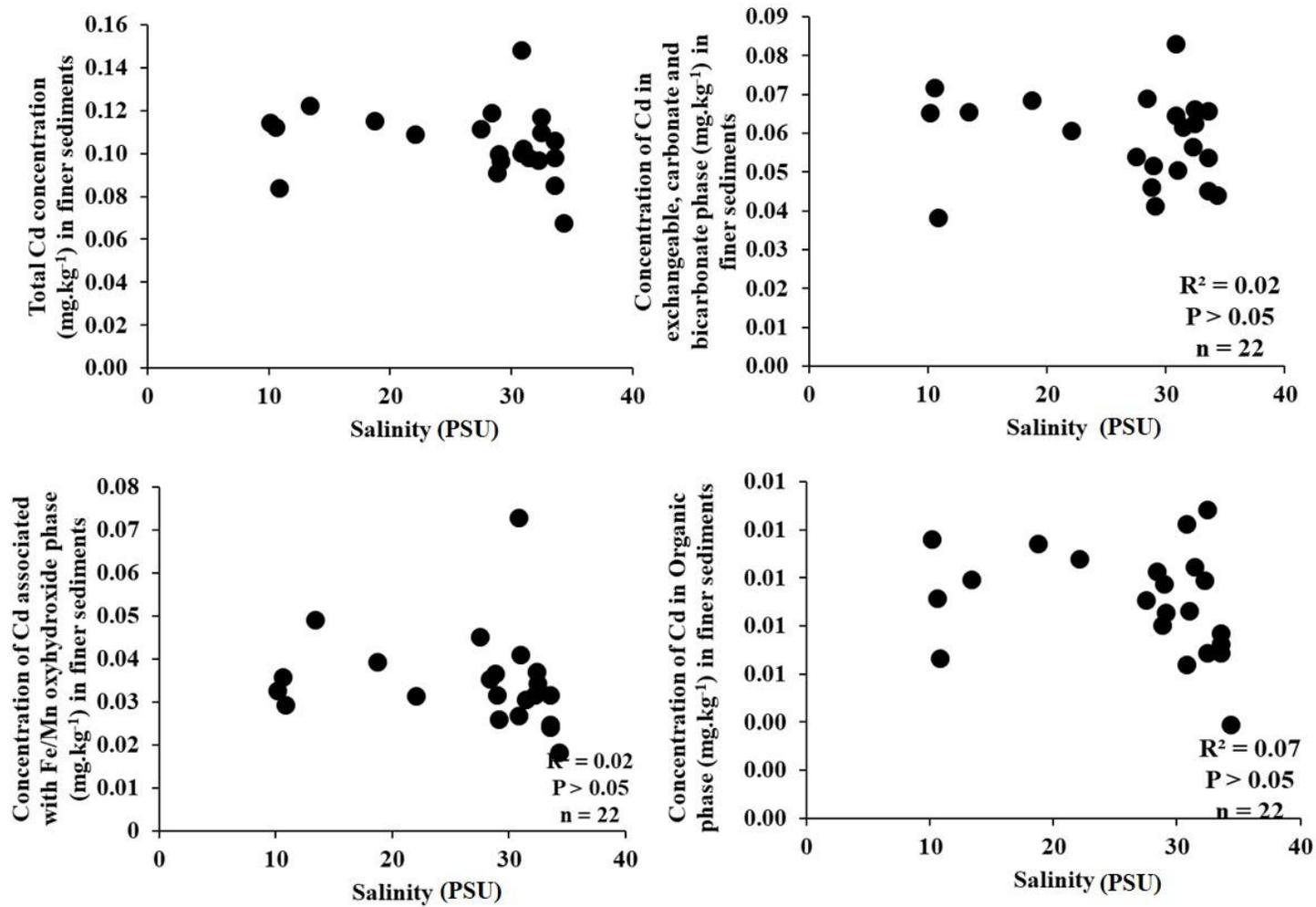
Figure 6.10: Variation of Cd associated with different geochemical phases of the finer sediment (< 63 μm) as a function of salinity of overlying water column.

6.2.5 Impact of overlying water column temperature on Cu and Cd speciation from estuarine sediments

Determination of water temperature of the overlying water column in all the sampling stations, in different seasons, was carried out during the low tide period. The measured value of water temperature showed no statistical relationship with metal speciation (Cu and Cd) parameters.

6.2.6 Impact of environmental parameters (pH, salinity) on metal accumulation in the oyster system (*Magallana* sp.)

The correlation between the change in the pH, salinity of the overlying water column, and the Cd accumulation in oysters are carried out and shown in Table 6.14. The increase in the pH value of the overlying water column did not show any statistically significant increase or decrease in the concentration of Cd accumulation in oyster tissue (Figure 6.11). However, an increase in salinity showed a significant ($p < 0.05$) increase in Cd concentration in the oyster system (Figure 6.12). The increase in Cd accumulation with high salinity values could probably due to the filter-feeding nature of oysters. Mane, 1978 has studied and explained how the different salinity values (overlying water column) can affect the oyster survival and behavior nature from Ratnagiri, west coast of India. The study showed that oyster filtration capacity, metabolic rate, and assimilation efficiency increased with more saline conditions (favorable condition), whereas in low salinity condition oysters may experience stress and reduces the filtration activity and cease the feeding.

Cadmium is a divalent ion (Cd^{2+}) which resembles the divalent Ca (Ca^{2+}) ion due to its similar radius and charge. This similarity probably enabled Cd to enter through the calcium

channels present on the cells of oyster gills (Adams et al., 2011). Thus, an increase in bioavailable Cd from the finer fraction of the sediments along with the increase in filtration rate at higher salinity might have increased the Cd bioaccumulation in oyster tissue.

No significant effect of variation ofthe water temperature of the overlying water column was observed on the Cd accumulation in the oyster.

Table 6.14: Pearson correlation coefficient between environmental parameters of the overlying water column and total Cd concentration in oyster tissue.

	pH	Salinity (PSU)	Water Temperature (°C)	$[Cd]_T$ in oyster $(mg.kg^{-1})$
pH	1.00			
Salinity (PSU)	-0.31	1.00		
Water Temperature (°C)	0.02	0.29	1.00	
$[Cd]_T$ in oyster $(mg.kg^{-1})$	0.20	**0.37**	0.25	1.00
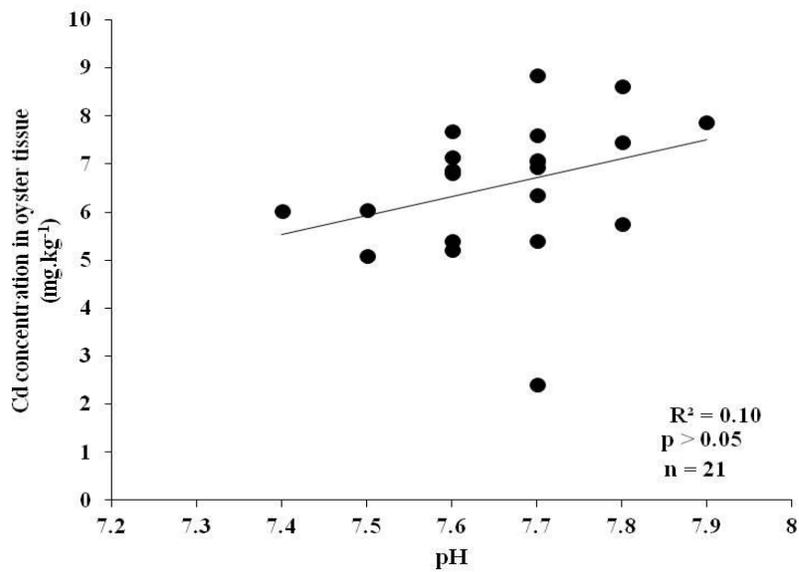
Figure 6.11: Cd bioaccumulation in oyster tissue as a function of pH of the overlying water column.
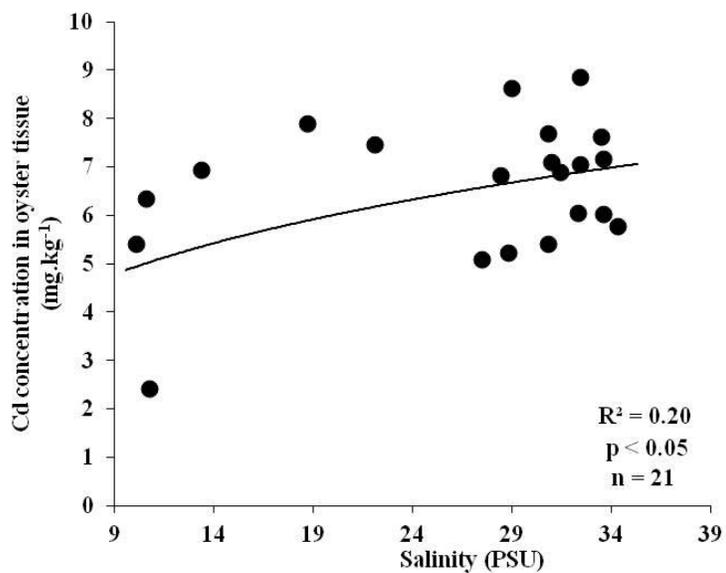
Figure 6.12: Cd bioaccumulation in oyster tissue as a function of salinity of the overlying water column.

6.3 Conclusion

The present study provided an understanding of the influence of environmental parameters (pH, salinity, and temperature) on the speciation of Cu and Cd from the estuarine sediments. The pH of the overlying water column has significantly affected the Cu speciation. Geochemical fractionation of Cu showed that organic carbon was the major binding site for non-residual Cu in the studied sediments. The concentration of Cu associated with the organic phase of the sediment increased with the increase in the pH. Further, an increase in the water-soluble Cu from the sampled sediments was also observed with an increase of pH and was probably due to the higher solubility of humic acid at higher pH. Thus, an increase in pH value of the overlying water column may leach out Cu complexes which may enter the water column and could increase Cu mobility from the sediment system. The pH of the overlying water column has not shown any influence on Cd speciation.

In the case of the salinity of the overlying water column, it did not show any impact on the distribution of Cu as well as on Cd in the different geochemical binding phases of the sediments. However, an increase in salinity has increased the bioaccumulation of Cd in oyster (*Magallana* sp). Thus, the study showed that the environmental parameter of the overlying water column plays an important role in influencing metal speciation (bioavailability) and its accumulation in the estuarine organisms. However different environmental parameters (pH in case of Cu and salinity in case of Cd accumulation in oysters) had an influence on metal speciation and its bioavailability from the sediments of the tropical estuarine system.

Chapter 7

Summary and Conclusions

An estuarine ecosystem is a unique ecotone of tropical and subtropical coasts. This ecologically important productive ecosystem in recent decades has been under tremendous pressure (especially due to larger cities) by the human population. They receive various pollutants brought from land and seas, and deposition through the atmosphere. Among various pollutants, metals are of serious concern as they are non-degradable and could be bioavailable, bioaccumulate, and biomagnified to different trophic level organisms. The majority of metals in an estuarine system are deposited on bottom sediments and thus, act as sink and also a source. The bioavailability of metals in sediment depends on their chemical speciation. Environmental parameters of the overlying water column can also control metal speciation in sediments. Unfortunately, there are very few reports available that tried to link chemical speciation of metals and their bioaccumulation in tropical marine systems.

This thesis presents the first comprehensive research on metal speciation study in delineation metal bioaccumulation in tropical estuarine sediment. This thesis also identifies the environmental parameters that influence metal speciation and its bioavailability in tropical estuarine sediments.

Chemical speciation parameters of Cu and Ni in mangrove sediments were used to delineate their bioaccumulation in benthic floral tissues (mangrove pneumatophores). Fe/Mn oxide and sedimentary organic phases were the major hosting phases for non-residual Cu and Ni in the mangrove sediments from Goa. Cu showed a strong association with the organic fraction of the sediment. Faster k-w and higher ionic potential in combination with the extra stability of Cu-ligand (from organic phase) complexes due to the Jahn-Teller effect increased Cu association with organic phase compare to Ni. The total concentration of Cu and Ni determined in the mangrove pneumatophores was found higher. The study revealed that Cu associated with Fe/Mn hydroxides are bioavailable, while sedimentary organic matter acted as a buffer to control Cu bioavailability in

mangrove sediments. In the case of Ni, a part of Ni associated with Fe/Mn oxyhydroxide and organic phases were probably bioavailable from the sediments and may responsible for Ni accumulation in mangrove roots.

Kinetic speciation study showed that the nature of metal-sediment complexes (labile or inert) and their respective dissociation rate constant determine metal bioaccumulation in mangrove roots. Increase in labile concentration of Cu and Ni with their respective fast dissociation rate constant increased bioaccumulation of Cu and Ni in mangrove roots. This study suggests that geochemical fractionation study and kinetic speciation study can provide useful information to estimate metal bioavailability (Cu and Ni) in mangrove sediments. The study proposes that disjunctive is the most plausible pathway through which bioavailable metals from mangrove sediments enter and probably accumulates in the mangrove root system.

In this thesis, an effort was also made to establish a linkage between Cd speciation in mangrove sediments and its bioaccumulation in edible oysters from a tropical estuarine system.

The fractionation study of both bulk and finer sediments showed that exchangeable, carbonate, and bicarbonate forms of Cd, especially from finer sediments were bioavailable in the sediment system. Since oyster is benthic filter-feeders, an increase in ingestion of finer particle of the surface sediment along with food particles could increase bioaccumulation of Cd in edible oysters in mangrove estuarine systems.

Kinetic speciation study of Cd from estuarine sediments (finer sediments) found that increasing concentration of labile Cd-sediment complexes significantly increases bioaccumulation of Cd in the oyster. This study suggests that low thermodynamic stability of Cd complexes in estuarine sediment can increase Cd bioaccumulation in edible oysters.

Finer surface sediments have been found to play a significant role in increasing Cd bioaccumulation in the edible oyster in mangrove sediments.

This thesis successfully used chemical speciation data of metals in mangrove sediment system to delineate metal bioaccumulation in different sessile organisms.

This thesis also tried to identify the key factors that control metal speciation in tropical estuarine sediment systems. It was found that Cu speciation in estuarine sediments significantly influenced by the pH of overlying water. The organic carbon phase of the sediment was the major binding phase for non-residual Cu. An increase in the pH has increased Cu association with the sedimentary organic phase, further it also increased water-soluble Cu complexes in the studied sediments. Thus, an increase in pH of the overlying water column may probably cause leaching of Cu organic complexes from sediment which may increase the mobility of Cu at the overlying bottom water.

However, the pH and salinity of the overlying water column have not shown any impact on Cd speciation. But, an increase in salinity increased Cd bioaccumulation in oyster (*Magallana* sp.) species. The increase of Cd bioaccumulation could be due to the increase in filtering activity (feeding behavior) of oysters during high salinity conditions. The environmental parameters of the overlying water column play a key role in influencing metal speciation (bioavailability) and its accumulation in estuarine organisms.

This thesis showed that edible oysters from the mangrove system of Goa contained high Cd concentration. Thus, based on the present study, it is strongly recommended to carry out further detailed study on the pathways of Cd bioaccumulation (by isotopic study) in the oyster.

The scientific outcome of this thesis work provides a relationship between chemical speciation and bioavailability of metals in the estuarine sediment system. Metal speciation study of sediments (rather than total metal concentration) is recommended as an important tool to delineate metal bioaccumulation in tropical estuarine systems.

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- Chakraborty, P., **Ramteke, D**., Chakraborty, S., Nath, B. N., 2014. Changes in metal contamination levels in estuarine sediments around India–an assessment. Marine Pollution Bulletin78(1-2), 15-25.

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- **Darwin Ramteke**., Parthasarathi Chakraborty., Kartheek Chennuri., Arindam Sarkar. Geochemical fractionation study in combination with equilibrium based chemical speciation modelling of Cd in finer sediments provide a better description of Cd bioavailability in tropical estuarine systems. (*manuscript communicated*).

- Darwin Ramteke., Parthasarathi Chakraborty., Kartheek Chennuri. Influence of overlying water column pH on distribution of Cu in different geochemical phases of tropical estuarine sediments: a field case study. (***manuscript communicated***)

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Appendix

In this segment, the procedure of determination of dissolved oxygen in seawater, the method for analysis of sediment texture and the experimental set up of kinetic fractionation study are described in detail.

Appendix1.1: Determination of Dissolved oxygen by Winkler's method

Principle:

Determination of Dissolved oxygen (DO) in the water is the measure of the amount of dissolved (or free) oxygen present at the time. Do is determined with the help of titrimetric method developed by Winkler.

1. In the water, dissolved molecular oxygen is not capable to react with KI, hence manganese hydroxide is used as oxygen carrier. $Mn(OH)_2$ is produced by the action of KOH on $MnSO_4$.

2. $Mn(OH)_2$ which is produced reacts with dissolved oxygen molecules and form a brown precipitate of basic manganic oxide, $MnO(OH)_2$.

3. $MnO(OH)_2$ then reacts with concentrated sulphuric acid and releases nascent oxygen.

4. The released nascent oxygen results in oxidation of KI to I_2.

5. The liberated iodine is then titrated against standard sodium thiosulphate solution by using starch as an indicator.

6. Thiosulphate in the solution, reduces iodine to iodide ions and itself gets oxidized to tetrathionate ion. (the reactions are mentioned below)

Reactions:

$$2NaOH + MnSO_4 \rightarrow Mn(OH)_2 + K_2SO_4 \qquad \text{........ (1)}$$

$$2Mn(OH)_2 + O_2 \rightarrow 2MnO(OH)_2 \qquad \text{........ (2)}$$

$$MnO(OH)_2 + H_2SO_4 \rightarrow MnSO_4 + 2H_2O + [O] \qquad \text{........ (3)}$$

$$2KI + H_2SO_4 + [O] \rightarrow K_2SO_4 + H_2O + I_2 \qquad \text{........ (4)}$$

$$I_2 + I^- \rightarrow I_3^- \qquad \text{........ (5)}$$

$$2S_2O_3^{2-} + I_3^- \rightarrow 3I^- + S_4O_6^{2-} \qquad \text{........ (6)}$$
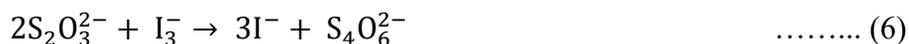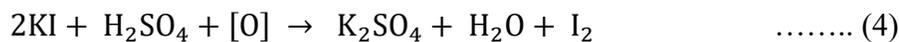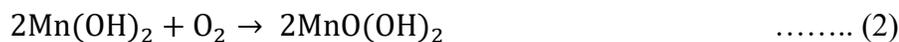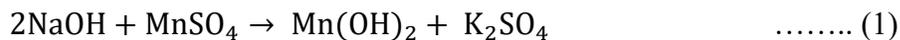
Calculations:

1000 mL of 1N $Na_2S_2O_3$ = 8g oxygen

V mL of 0.025 N EDTA = V x 0.025 x 8/ 1000 g oxygen per 1000 mL water sample

= V x 0.025 x 8 mg oxygen per 100 mL water sample

= V x 0.025 x 8 x 10 mg oxygen per 1000 mL water sample

Precautions:

1. During sampling water for DO analysis do not allow air to trap.

2. Dip the tip of the pipette just at the bottom of the DO bottle and then gently release the chemical reagents.

3. While shaking/swirling the bottle, make sure the chemicals do not leak from the DO bottle.

4. During DO reaction, observe any change in colour.

Appendix 1.2: Procedure for sediment texture analysis

The method used for sediment texture analysis is a combination of wet sieving of the sediment fraction >63 μm and Laser Diffraction method for the <63 μmfraction, free with carbonate and organic carbon.

Initially, the salt content of thesampled sediments were removed with the help of deionized water (by repeated washing). After washing, the sediment samples were dried at 45°C. 2 to 5g of dried samples were then disaggregated in a beaker with deionized water and treated overnight in 30% H_2O_2 to remove the sediment organicmatter content. The treated samples were then sieved (wet sieving) through 63 μm sieve to separate the sand from the silt+clay fraction. The materials which are retained after sieving, on the sieve represents the sand fraction. The sand fraction was then dried and weighed to determine total sand content in a sample.

The size distribution of the mud fraction (silt and clay) was measured with the help of Malvern laserparticle size analyzer (Mastersizer 2000) which is based on Laser Diffraction Method. The basic principle of this method is that particles of a given size diffract light through a given angle, the angle increases with decreasing particle size and vice-versa. In this technique, a narrow beam of monochromatic light is made to pass through a sample cell containing an upward moving suspensionof particles. The diffracted light after passing through particles is focused on to

detectors, which detect the angular distributionof scattered light intensity from which distribution of the particle size is calculated.

To measure the percentage of silt (<63 to >4 μm) and clay (<4 μm) content in the sample, a small aliquot of the mud fraction sample was taken in a beaker and diluted with 1 L of particle free water. The size distribution was then measured while the suspension was continuously pumped around. This process confirms random orientation of most particles relative to the laser beam, and the corresponding spherical cross-sectional diameter is measured. Obscuration of the light beam by the mud solution was maintained between 8 and 20%. Pump and stirrer speeds were kept constant throughout the analysis. Calibration of the instrument was carried out using various Duke's latex and glass sphere standards in the range of 0.2 to 62μm. Before the measurement of mud fraction, the samples were ultrasonicated for 1 to 2 min. The silt and clay data obtained are presented in vol (%).
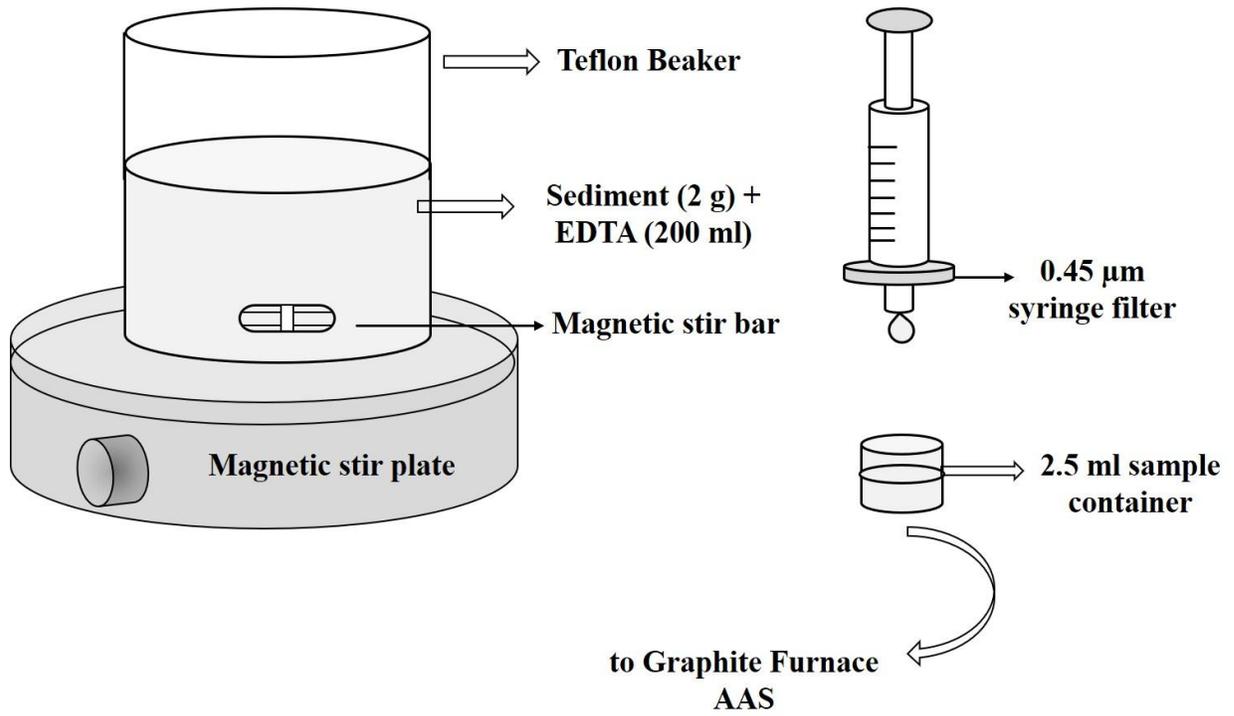
Appendix 1.3: Experimental setup for performing kinetic fractionation study