STUDIES ON TRANSFER FACTORS OF TRACE METALS IN MARINE ECOSYSTEM

By

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DECLARATION

I, hereby declare that the investigation presented in the thesis has been carried out by me. The work is original and has not been submitted earlier as a whole or in part for a degree/diploma at this or any other Institution / University.

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List of Publications arising from the thesis

Journals

1. **Sukanta Maity**, S. K. Sahu and G. G. Pandit, (2014). "Distribution of uranium and thorium in fractionated sediment samples obtained from different locations across Thane Creek area (Mumbai, India)". Journal of Radioanalytical and Nuclear Chemistry, 302, 1363-1370.

2. **Sukanta Maity**, S. K. Sahu and G. G. Pandit, (2015). "Standardization of solvent extraction procedure for determination of uranium in seawater". Journal of Radioanalytical and Nuclear Chemistry, 303, 33-37.

3. **Sukanta Maity**, S. K. Sahu and G. G. Pandit, (2016). "Determination of heavy metals and their distribution in different size fractionated sediment samples using different analytical techniques". Soil and Sediment Contamination: An International Journal, 25 (3), 332-345.

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5. **Sukanta Maity**, S. K. Sahu and G. G. Pandit, (2017). “Determination of traces of Pb, Cu and Cd in seawater around Thane Creek by Anodic Stripping Voltammetry method”. Bulletin of Environmental Contamination and Toxicology, DOI 10.1007/s00128-016-2025-y

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2. **Sukanta Maity**, C. B. Dusane, S. K. Sahu and G. G. Pandit. Standardization of solvent extraction procedure for determination of uranium in seawater. In the proceedings of Third International Conference, "Application of RadiotraCers and Energetic Beams in Sciences (ARCEBS-14), January 12-18, 2014. p 223-224.

3. **Sukanta Maity**, S. K. Sahu and G. G. Pandit. Distribution of uranium & thorium in fractionated sediment samples obtained from different locations across Thane Creek area, Mumbai, India. In the proceedings of DAE-BRNS Fifth Symposium on Nuclear Analytical Chemistry (NAC-V), January 20-24, 2014. P 455-456.

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5. **Sukanta Maity**, R.C. Bhangare, M. Tiwari, P.Y. Ajmal, S. K. Sahu and G. G. Pandit. Effect of pH in the extraction efficiency of solvent extraction procedure for determination of trace metals in seawater.19th National Symposium on Environment (NSE-19), December 11-13, 2014. p 57-58.

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Dedicated to my family

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LIST OF ABBREVIATIONS

MHB- Mumbai Harbor Bay

USEPA- United States Environmental Protection Agency

FAO-Food and Agriculture organization of the United Nations

WHO-World Health Organization

AAS- Atomic Absorption Spectrometry

HCL-Hollow Cathode Lamp

EDL-Electrode less Discharge Lamp

PMT-PhotoMultiplier Tube

ICP-AES- Inductively Coupled Plasma Atomic Emission Spectrometry

FAES-Flame Atomic Emission Spectrometry

DPASV- Differential Pulse Anodic Stripping Voltammetry

APDC-Ammonium Pyrolidine Dithicarbamate

MIBK-Methyl Isobutyl Ketone

DDDC-Di-ethylammonium DiethylDithioCarbamate

OC-Organic carbon

TC- Total Carbon

EF-Enrichment Factor

$\mathbf{I_{geo}}$-Geoaccumulation index

CF- Contamination Factor

PLI-Pollution Load Index

SQGs-Sediment Quality Guidelines

NOAA-National Oceanic and Atmospheric Administration

ERL-Effect range- low

ERM-Effect-range-medium

SEP-Sequential Extraction Procedures

BCRSEP-Bureau of Reference sequential extraction procedure

RAC-Risk Assessment Code

MAC-Maximum Allowable Concentration

RfD-Reference Dose

NOAEL- No Observable Adverse Effect Level

IRIS-Risk Information system

ATSDR-Agency for Toxic Substances and Disease Registry

HQ-Hazard Quotient

HI-Hazard Index

Synopsis

Marine environment around the world receives large input of trace metals due to rapid industrialization and human activities. Sediments not only reflect the current quality of surface waters, but also provide vital information on the transportation and fate of contaminants. The trace metals can be transported through water, sediment and biota pathways to human beings and may possess potential health risk. In aquatic environment depending on the environmental conditions sediments can act either as a sink or a source for trace metals. Geochemical composition of bottom sediments is very good indicator of surface water quality, particularly as regard to trace metal content. Changing environmental conditions, such as the presence of organic chelators may render the remobilization of metals from sediments.

As of now various chemical industries are located on coastal region. Therefore it is very important to study the impact of environmental releases of trace metals on the various matrices of the coastal environment, since bays and estuaries are known to be biologically productive and strongly influenced by human activities. India has a long coastline of 8,129 km of which 6,000 km is rich in estuaries, creeks, brackish water and lagoons. Due to their environmental persistence and biogeochemical recycling and ecological risks, trace metals are of particular concern for the countries with such huge coastlines. The bay at Trombay is called as Mumbai Harbor Bay (MHB). The average area of seawater surface of the bay is about 215 km^2 at high tide level and about 160 km^2 at low tide level.

The thesis describes the discharges of trace metals from different industries situated nearby Thane Creek area, Mumbai, India and their concentration distribution in different compartments (seawater, sediment and biota) of the marine ecosystem. Methodology was optimized for determination of trace metals in different matrices of marine environment. In the thesis it is also discussed about the dependency of trace metal concentrations on physico-chemical parameters in sediment samples. Total trace metals concentration does not reflect exact toxicity in sediment samples, so binding behavior of trace metals in sediment samples were studied using sequential extraction procedure. Transfer factor/ concentration ratio of trace metals from seawater to biota was also studied. Intake of trace metals due to consumption of biota by human beings was also studied. The work embodied in this thesis has been divided into seven chapters.

- Introduction.
- Materials and methods.
- Distribution of trace metals in different compartment of marine ecosystem.
- Dependency of trace metal concentrations in sediment on physico-chemical parameters.
- Binding behavior of trace metals in marine ecosystem.
- Transfer factor/ concentration ratio and intake of trace metals through consumption of biota.
- Summary and Conclusions.

CHAPTER 1: INTRODUCTION

This chapter encompasses an overview of the study area which includes the principal discharges to the Thane Creek, water flow and tidal action in the creek and trace metals distribution in the marine ecosystem. The principal industrial discharge channels into the creek from the city are Airoli, Ghansoli, Koperkhairane, Vashi and Juinagar situated in the east side of the creek. In extreme north, the creek also receives outlet from Ulhas River. Extensive development provides a large amount of flux into the Thane Creek. The Mumbai harbour in the west and New Nava Sheva port in the east, which handle more than 30 million tonnes of goods per year, additionally contribute to the pollutants in the creek by way of leakage, spill and corrosion. The bay is subjected to semidiurnal tides and there exists asymmetry in both their periods and range. The water movements are mainly governed by the tidal actions.

This chapter discussed about the various studies on trace metals in marine ecosystem carried out in recent past of India and other countries. The introduction chapter also briefly discusses the main objectives of the present study such as, distribution of trace metals in different compartment of marine ecosystem across the sampling area, trace metals concentration dependency with respect to different physico-chemical parameters, binding behavior of trace metals in the sediment samples, intake of trace metals to human beings through consumption of biota and estimation of transfer factor/ concentration ratios for trace metals from seawater to biota.

CHAPTER 2: MATERIALS AND METHODS

This chapter mostly discussed various instruments and methodologies used in the present study. Seawater and sediment samples were collected from fourteen different locations across Thane Creek area, Mumbai, India. Different types of biota samples (lizard fish, shrimps, prawns and crab) were also collected.

Sediment samples were freeze-dried and then sieved below 2 mm using an electromagnetic sieve shaker (EMS 8). The fraction of < 2 mm (grab sediment) is sieved in the size fractions 250 μm and 53 μm to receive the three size fractions very coarse, coarse and medium sand (2.0 mm - 250 μm), fine sand and very fine sand (250 μm - 53 μm), and silt and clay (< 53 μm). Different chemical parameters (pH, cation exchange capacity (CEC), calcium carbonate ($CaCO_3$), total carbon (TC)) were measured in the dried sediment samples.

Collected seawater samples were filtered through 0.45 μm filter paper. Solvent extraction procedure was carried out for different time intervals (1 to 30 minutes) to know the equilibration time. Solvent extraction was carried out at different pH (2.0 ± 0.01 to 9.0 ± 0.02) to know the extraction efficiency of trace metals at different pH. For determination of trace metals in sediment samples microwave acid digestion procedure was adopted. In order to evaluate the percentage recovery of the digestion procedure, equivalent amounts of reference materials IAEA-405 (estuarine sediment) and IAEA-433 (marine sediment) were also digested following similar procedure. Metals of interest that were analyzed in both reference materials have shown good agreement to reference sheet values. Biota samples were segregated into two parts, edible and whole body biota. Both edible and whole body biota samples were acid digested separately and trace metals were analyzed using Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES).

CHAPTER 3: DISTRIBUTION OF TRACE METALS IN DIFFERENT COMPARTMENTS OF MARINE ECOSYSTEM

This chapter emphasizes on the distribution of trace metals in different matrices (seawater, sediment and biota) of marine ecosystem. Sea water samples were solvent extracted for matrix removal and pre-concentration of trace metals. Trace metals were analyzed using ICP-AES and Differential Pulse Anodic Stripping Voltammetry (DPASV). Trace metal concentrations in the seawater samples were observed to be higher in locations which are in the eastern side of the Thane Creek and in the eastern side are mostly industrial area. Concentrations of the trace metals in the sediment samples were observed to be higher in the vertex of the creek compared to the open sea. Mn, Cu, Zn, Pb, Co, Cd, and Ba shows higher concentration in sediment samples collected from different locations across the eastern bank of the creek. The distributions of Cr, V, Ni show a slightly different picture, which were found to be in higher concentrations in the western bank of the creek. These anomalous distributions may be attributed to urbanized pollutants along with the thermal power plant contribution and may be due to variation of different physico-chemical parameters of sediment samples in the western bank of the creek. Collected biota samples were cleaned properly with double distilled water. Biota samples were segregated into two parts, one is edible and other is whole body biota. Most of the trace metal concentrations were observed to be higher in benthic biota samples compare to aquatic biota.

Various geochemical indices that determine the degree of contamination such as enrichment factor (EF), geo-accumulation index (I_{geo}), contamination factor (CF) and pollution load index (PLI) were used here.

CHAPTER 4: DEPENDENCY OF TRACE METAL CONCENTRATIONS IN SEDIMENT ON PHYSICO-CHEMICAL PARAMETERS

This chapter describes the trace metal concentrations dependency on physico-chemical parameters in creek sediment. Most of the sampling locations are basically predominated with fine sand and very fine sand in varying percentages. The percentage of silt & clay is very low in comparison with other two fractions. The distribution of very coarse, coarse and medium sand and silt & clay are found to be higher than that of fine sand and very fine sand fractions. pH varies in the range of 7.9 to 8.3 with an average value of 8.2 ± 0.12. Large variation was not observed in $CaCO_3$ concentration in different sampling locations. TC content is quite uniformly distributed throughout the locations. But, the CEC shows smaller variation at the different locations in comparison to other chemical parameters.

One to one correlation matrix between trace metal concentrations and different physico-chemical parameters of grab sediment samples reveal the positive correlation between fine sand and very fine sand, silt and clay and total carbon with trace metals. This shows the important role of fine sand and very fine sand, silt and clay and total carbon in controlling spatial distribution of sedimentary metal concentrations. Factors like pH, CEC and $CaCO_3$ found to have lesser impact in deciding metal concentration. Results from the correlation matrix, which indicate that metal concentration is positively correlated with fine sand and very fine sand fraction, silt and clay and total carbon could be slightly misleading. Multiple regression equations suggest that metal concentrations have a positive correlation with only total carbon content but have a negative relation with very coarse, coarse and medium sand, fine sand and very fine sand and silt and clay. The results highlight the prime role of total carbon as carriers of sedimentary metals and its contribution in deciding spatial distribution of trace metals in the sediment.

CHAPTER 5: BINDING BEHAVIOR OF TRACE METALS IN MARINE ECOSYSTEM

In this chapter it is discussed about the binding behavior of trace metals in sediment samples. At present, it is widely recognized that the distribution, mobility and bioavailability of trace metals in the environment depends not only on their total concentration but also on the association form in the solid phase to which they are bound. To understand the binding behavior of trace metals in the sediment samples, sequential extraction procedure was carried out. In the sequential extraction procedure, the extraction was carried out in four steps. All the studied metals were having highest percentage of fraction in the residual fraction indicating metals are mostly bound strongly with crystal lattice of the sediment samples. Lowest percentage of fraction was observed in the acid extractable/exchangeable fraction for most of the studied metals exception observed for Mn and Cd.

CHAPTER 6: TRANSFER FACTOR/ CONCENTRATION RATIO AND INTAKE OF TRACE METALS THROUGH CONSUMPTION OF BIOTA

This chapter describes the intake and associated hazard of trace metals in human beings through consumption of biota. In this chapter it is also discussed about the transfer factor/ concentration ratio of trace metals from seawater to biota. There has been growing interest in determining trace metal levels in the marine environment and attention was drawn to the measurement of contamination levels in public food supplies, particularly different types of biota. The aquatic system near the industrialized areas contain large amount of trace metals, which have marked ecological significance due to their toxicity, persistence and bio-accumulation.

Bioaccumulation potential in aquatic biota is typically expressed using ratios of chemical concentrations in organism tissue (typically whole body) relative to chemical exposure concentrations, such as bioconcentration factors (BCFs) or transfer factor (TF) or concentration ratios (CR). Past reviews of metal BCFs for aquatic biota, which account for water-only exposures, have shown that BCFs are often highly variable.

Trace metal concentrations were determined in the edible part of biota to assess the hazards in human beingss on their consumption. It is observed that the trace metals in edible parts of biota in Thane creek, Mumbai, India were below the hazardous levels.

Transfer factor/ concentration ratios were calculated with the help of metals concentrations in seawater and whole body biota. Transfer factor/ concentration ratio of trace metals in different types of biota samples was observed to be different. The order of transfer factor/ concentration ratio values in the different types of biota samples are Lizard fish < shrimps < prawns < crabs for most of the trace metals.

CHAPTER 7: SUMMARY AND CONCLUSIONS

This chapter summarizes the results and overall conclusions of the present study. Trace metals though occur at extremely low concentrations in seawater, are accumulated by marine organisms and concentrations in their body tissue can be hundreds of times greater than seawater. Human beings consume different marine organisms like biota, can lead potential health problem. Estimation of trace metals in seawater is a difficult task because of the very low concentrations of trace metals and high salt content. Instruments capable of trace metal analysis in ppb range needs matrix separation prior to analysis. Accordingly, the separation and pre-concentration of trace metals is usually involved in the analytical procedures used for the determination of trace metals in seawater. Equilibration time and pH have great importance in the solvent extraction procedure for determination of trace metals in seawater.

The summery and few major conclusions drawn from the study are given below.

- Solvent extraction procedure was optimized for determination of trace metals in seawater samples in terms of equilibration time and pH. Equilibration time for determination of trace metals in seawater samples using solvent extraction procedure was found to be within 20 minutes. Highest solvent extraction efficiency was observed at pH near 6 for most of the trace metals.

- Concentrations of few trace metals like Pb, Cu, Cd, U etc were observed ranging from 3.0 to 9.44 ng/mL, 12.7 to 28.6 ng/mL, 0.32 to 1.21 ng/mL, 2.78 to 3.62 ng/mL respectively in the seawater samples collected across Thane Creek area, Mumbai, India.

- In the sediment samples concentrations of few trace metals like Pb, Cu, Cd, U etc were observed ranging from from 19.8 to 23.6 µg/g, 76 to 160 µg/g, 86 to 352 ng/g, 9.0 to 12.7 µg/g respectively.

- Trace metal concentrations decrease from vertex of the creek to the open sea. This is because of the increased contamination by the effluent discharged from several industries situated on the eastern bank of Thane creek. The diversified industries along the banks of the creek, includes chemical, textile, pharmaceutical, engineering and major fertilizer complex, release through their effluents, high levels of nitrates and phosphates into the creek. The scarcity of back water near the very vertex of the creek decrease the dispersion probability of these contaminants to the sea.

- Total trace metal concentrations in sediment samples do not reflect pollution behaviour in the creek due to trace metals. Sequential extraction was carried out to understand the binding behaviour of trace metals in the sediment samples. By this experiment it was observed that although concentration of trace metals in sediment samples were higher but bioavailable fraction was less for most of the trace metals except Mn and Cd.

- Trace metal concentrations dependency with respect to different physico-chemical parameters in sediment samples were also studied. Trace metal concentrations increase as particle size of sediment samples decrease. In the same time with decreasing particle size total carbon content increase in the sediment samples. Metal concentrations increase as particle size decrease in the sediment samples because of higher total carbon content in the lesser particle size sediment samples.

- Trace metal concentrations were studied in benthic and aquatic biota in edible part. Trace metal concentrations in edible part of biota samples were used to find out hazard in human beings for consumption of trace metals through biota. Trace metal concentrations in benthic biota were observed to be higher compare to aquatic biota except for Mn and Cd. Consumption of biota as such is not hazardous from Thane Creek area, Mumbai, India for trace metals like Cu, Zn, Mn, Cr, Ni, Co, Cd etc.

- In order to find out transfer factor/ concentration ratios, trace metal concentrations were studied in the whole body of aquatic and benthic biota. Transfer factor/ concentration ratios of trace metals (Cr, Ni, Pb, Cd, Zn, Cu, Mn, Fe, U etc.) in different biota samples were observed in the range 11-334, 14-394, 17-483 and 31-548 for lizard fish, shrimps, prawns and crab respectively.

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1. **Sukanta Maity**, S. K. Sahu and G. G. Pandit, (2014). "Distribution of uranium and thorium in fractionated sediment samples obtained from different locations across Thane Creek area (Mumbai, India)". Journal of Radioanalytical and Nuclear Chemistry, 302, 1363-1370.
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1. **Sukanta Maity**, D. K. Chaudhary, S. K. Sahu, G. G. Pandit and V. D. Puranik. Pb, Cd and Cu distribution in different size fractionated sediment by using differential pulse anodic stripping voltammetry. In the proceedings of fifth triennial international conference on advances and recent trends in electrochemistry (ELAC-2013), January 16-20, 2013, p 419-422.
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CHAPTER-1

Introduction

1.1. General introduction on marine pollution

Various contaminants released into the sea may significantly affect marine ecology, and in extreme cases, may lead to the destruction of whole ecosystems. The contaminants of major concern are sewage, nutrients, metallic compounds, substances disrupting endocrine functions, persistent organic pollutants and petroleum hydrocarbons [1-2]. Continuous anthropogenic input of pollutants into the aquatic environment constitutes a potential threat to natural ecosystems because of direct effect on the water quality as well as aquatic organisms. Since majority of pollutants entering into the marine environment are chemical in nature, the investigation of the release of chemicals is a topic of current interest [3]. Some contaminants such as trace metals are bio-accumulated and some are biomagnified in food chains and thereby become risks to top predators, including humans. Because the residence times of metals on the biosphere are long even the transport of small amounts through biota is of eco-toxicological importance. Marine organisms such as fish [4-5] and oyster [6] have been implicated as accumulators of trace metals. Metal body loads of aquatic biota are therefore often measured and used to evaluate ecological risks and potential sub-lethal effects [7]. Trace metals pose a serious threat to human health, living organisms and natural ecosystems because of their toxicity, persistence and bioaccumulation characteristics [8]. Trace metals in natural waters and their corresponding sediments have become a significant topic of concern for scientists and engineers in various fields associated with water quality, as well as a concern of the general public. Direct toxicity to man and aquatic life and indirect toxicity through accumulations of metals in the aquatic food chain are the focus of this concern.

Many trace metal ions are known to be toxic or carcinogenic to humans [9]. Trace metals can contribute to degradation of marine ecosystems by reducing species diversity and abundance and through accumulation of metals in living organisms and food chains [10] (Fig. 1.1). The availbale information indicates that though the open ocean suffers from some contamination and ecological damage, they are still relatively in a healthy state as compared to some coastal areas. It is the waters nearest to the shore, particularly in estuaries and semi-enclosed seas and bays bordering highly industrialised and urbanized zones that have suffered ecological degredation over the past few decades [1-2].

The outbreak of hitherto unknown neurological illness among the inhabitants living around Minamata bay, Japan was reported in 1953. The victims suffered from a weakening of muscles, loss of vision, impairment of cerebral functions and eventual paralysis and death due to Hg poisoning. This disease became known as the Minamata disease. A second outbreak of the disease was reported in Japan during 1964-1965 mainly among fishermen and their families living near Niigata. Several other alarming incidents of Hg poisoning have since been reported from other countries.

Contamination of aquatic environment by Cd is less widespread than by Hg. During 1947 an unusual and painful disease, subsequently became known as 'itai-itai', was reported among the villagers on the bank of the Jintsu River in Japan. Contamination of the river water by Cd was found to be the culprit and source of Cd was traced to a Zn mine situated some 50 km upstream from the affected villages. It is estimated that at least 100 deaths occurred due to the disease until 1965. An interesting episode of mass mortality of fish resulting from Cu poisoning occurred at the coast of Holland. Several kilograms of $CuSO_4$ buried under sand were identified as the source of Cu to the coastal water. In this area the seawater contained a Cu concentration of several hundred µg/L; the normal concentration of Cu in seawater being 1-3 µg/L [11].

The analysis of seawater and sediment samples does not allow for the estimation of the quality of the metal which is biologically available [12]. Biota take up and accumulate trace metals whether essential or not, all of which have the potential to cause toxic effects; the body concentrations of accumulated heavy metals show enormous variability across metals and aquatic organism [13]. Biota will take up trace metals into the body from solution through permeable body surface and from gut and that uptake of metals from diet [14], or by biosorption [15].

The activity of trace metals in aquatic systems and their impact on aquatic life vary depending upon the metal species. Of major importance in this regard is the ability of metals to associate with other dissolved and suspended components. Most significant among these associations is the interaction between metals and organic compounds in water and sediment. These organic species, which may originate naturally from process such as vegetative decay or result from pollution through organic discharge from municipal and industrial sources, have a remarkable affinity and capacity to bind to metals. This phenomenon would naturally alter the reactivity of metals in the aquatic environment.

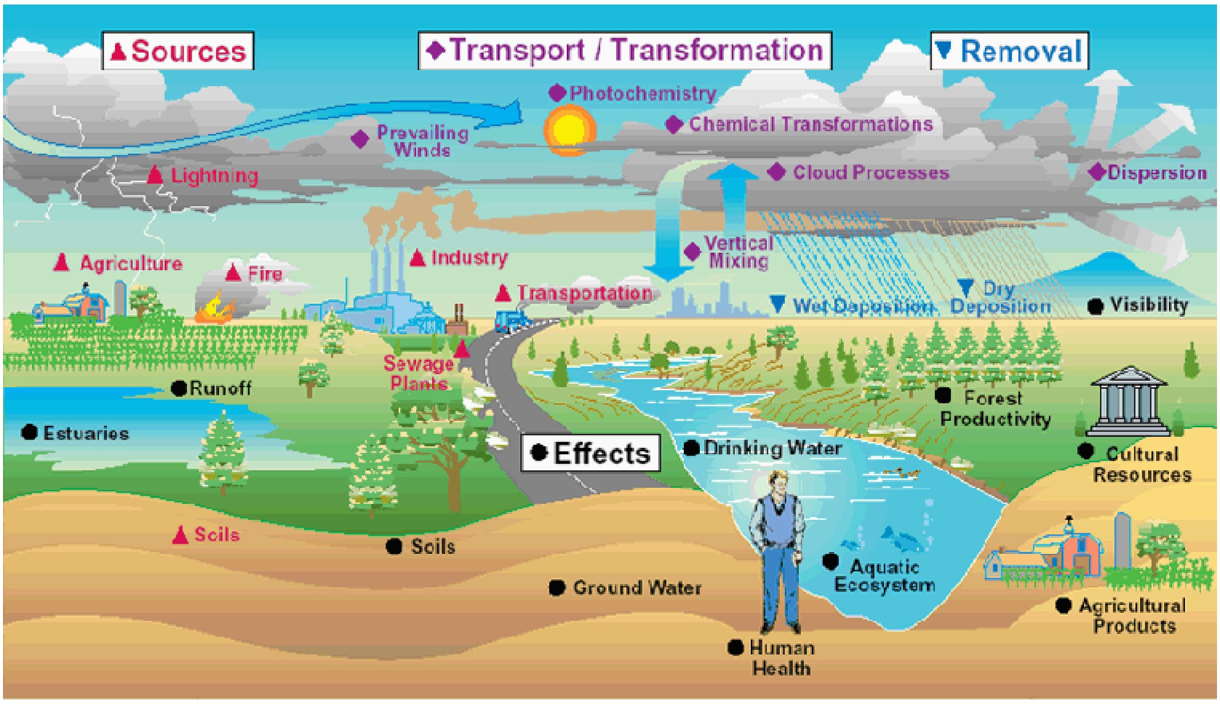


Fig. 1.1. Environmental Pollution (Google image)

1. 2. Trace metals

The urban population issues arise from failure to provide the basic amenities to urban population. The issues such as massive industrialization, rural-urban migrations, energy scarcity, waste disposal concerns and transport related problems are soaring up.

In the current world, it is very difficult to survive without chemicals. The human race is completely surrounded by chemical materials. A few elements are referred as trace metals. "Trace metals" are chemical elements are those metals which are having concentration less than 100 ppm in any substance.

USEPA gives a list of trace metals as arsenic, mercury, cadmium, chromium, copper, nickel, cobalt, lead, zinc etc. Owing to their high thermal and electrical conductivity, high density, high melting and boiling points, ductility, malleability, and other distinctly useful properties, trace metals find extensive use in human civilization. Millions of tonnes of such trace metals are used for domestic and industrial purposes and thus dispersed into environment.

In the view of environmental pollution, metals may be broadly classified into two categories namely essential and non essential (Fig. 1.2).

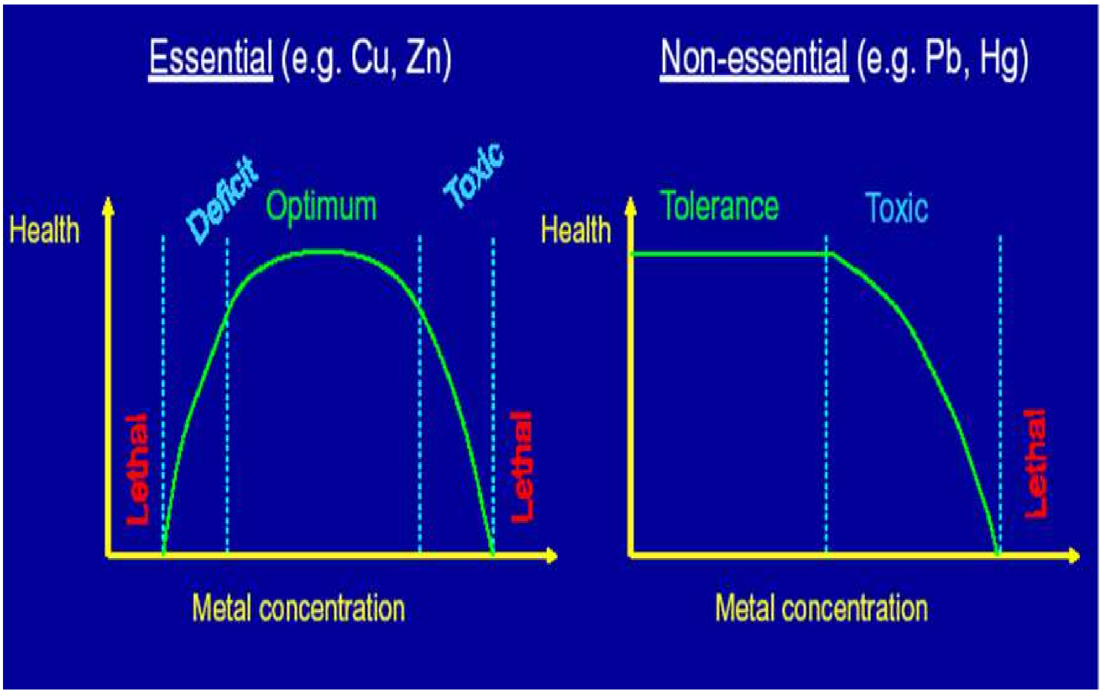


Fig.1.2. Response in human health due to essential and non essential metals [16]

1.2.1. Essential Metals

In small quantities, certain trace metals are nutritionally essential for a healthy life. Some of these are iron, copper, manganese, and zinc. Such trace metals are considered to be useful to living organisms when made available in trace quantities as they serve some useful biological functions. However all the trace metals, when present in excessive quantities have been found to be toxic for animal and vegetal life. In fact, the toxicity of all the essential trace metals follow a general trend: under supply leads to deficiency, optimum supply helps in healthy growth and over supply leads to toxicity and even eventual death of the organism. Essential metals or some form of them, are commonly found naturally in foodstuffs and in commercially available multivitamin products (International Occupational Safety and Health Information Centre 1999). Diagnostic medical applications include direct injection of gallium during radiological procedures, dosing with chromium in parenteral nutrition mixtures, and the use of lead as a radiation shield around x-ray equipment. Trace metals are also common in industrial applications such as in the manufacture of pesticides, batteries, alloys, electroplated metal parts, textile dyes, steel, and so forth (International Occupational Safety and Health Information Centre, 1999). Many of these products are in our homes and actually add to our quality of life when properly used.

The physiological role of some of the trace metals is shown in the table 1.1. Hence it is clear that these have a specific function in metabolism.

Table 1.1. Physiological role of some of the essential trace metals in plants and animals [17]

Element	Physiological Function
Copper	Activator of several enzymes
Chromium	Phosphoglytamase
Cobalt	Constituent of Vitamin B12
Manganese	Activator of pyruvate carboxylase and arginase in liver
Molybdenum	Found in metalloflavin enzymes and xanthine in liver
Zinc	Activator of several mammalian enzymes

1.2.2. Non essential metals

Trace metals become toxic when they are not metabolized by the body and accumulate in the soft tissues. Trace metals may enter the human body through food, water, air, or absorption through the skin when they come in contact with humans in agriculture and in manufacturing, pharmaceutical, industrial, or residential settings. Industrial exposure accounts for a common route of exposure for adults. Toxic metals are widely used in industry, agriculture and food processing. They are in our air, water and food supply. They are often overlooked as a cause or contributor to chronic illness and are not addressed by medical, nutritional or other health practitioners. Non essential metals are potentially toxic and relatively accessible that has attracted more attention from the point of view of environmental pollution and public health (Fig. 1.3).

The adverse effects of trace metals toxicity in biological systems may result from the following reasons:

a) Interaction of the metal with protein leading to denaturation

b) Interaction with DNA causing mutations

c) Effect on cell membranes

d) Effect on regulatory enzymes

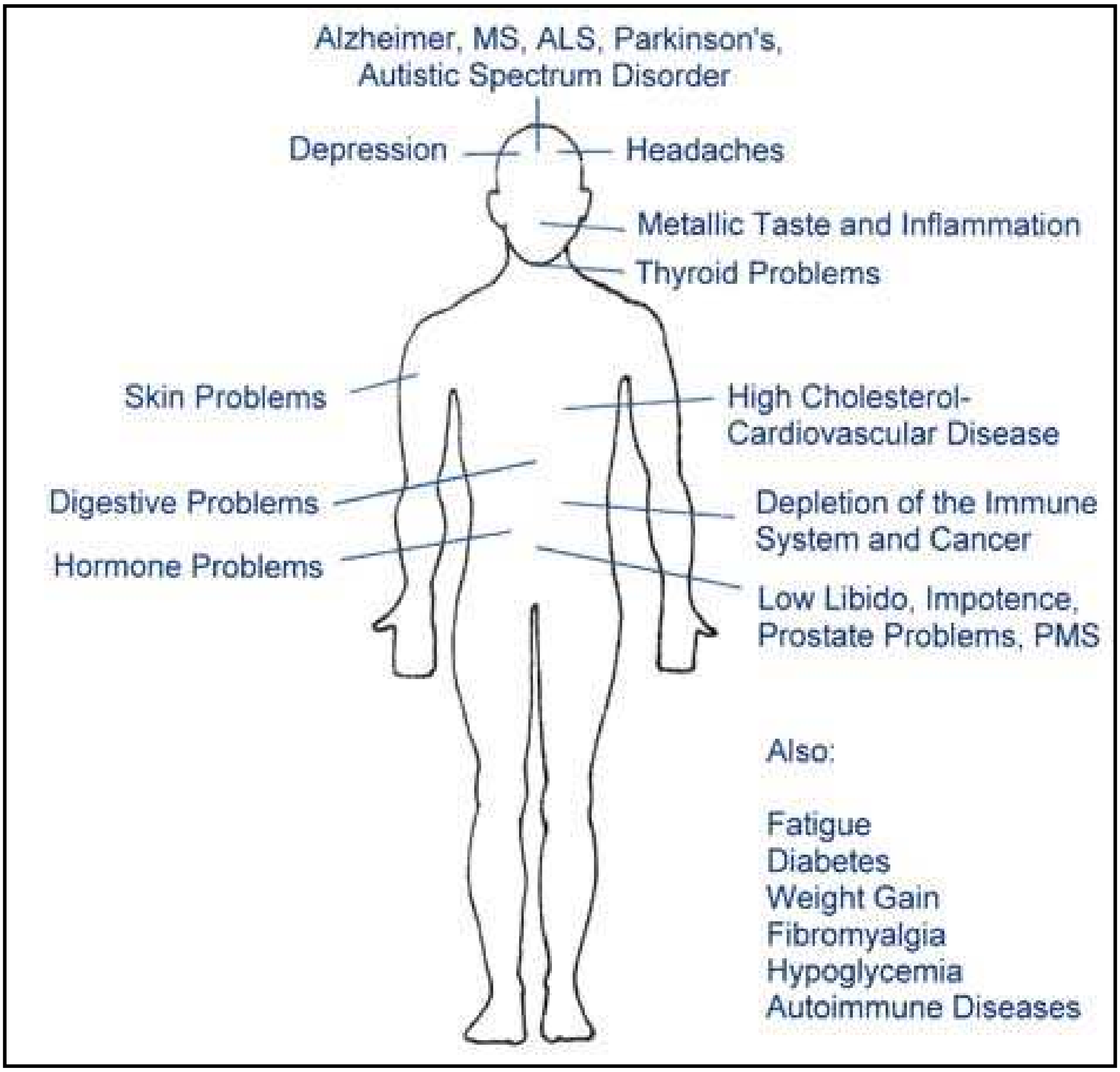


Fig.1.3. General effects of trace toxic metals on humans (Google image)

The extent of trace metal toxicity in mammalian systems is influenced by the following factors:

- Electrochemical character of the metal
- State of oxidation of the metal

- Solubility, stability and reactivity of the metal or its compound or its complex under existing conditions
- The rate of transport of the trace metal compound and its distribution and retention in the body tissues
- Ability of the trace metal to chelate with the various ligands in the body tissues and the stability of the metal chelates thus formed
- Rate of absorption of the metal in the body as against the rate of clearance
- Efficiency of the enzymatic and homeostatic mechanism which controls the absorption, distribution, retention and excretion of the trace metal ions or compounds or complexes.

Different types of diseases observed either in deficiency or excess of trace metals consumption in human body (Table 1.2).

Table 1.2. Diseases due to deficiency or **excess** of trace metals in human body [17]

Metal	**Diseases**	
	Deficiency	**Excess**
Cadmium	-	Itai Itai
Chromium	Aging,Atherosclerosis, Diabetes Mellitus	Bronchial asthma, Skin allergies
Cobalt	Pernicious Anemia	Beer drinker's disease, Polycythemia
Copper	Menke's Kinky hair disease, Trichopoliodystrophy	Grape garden sprayer's disease, Indian childhood Cirrhosis, Schizophrenia, Wilson's disease
Iron	Microcytic hypochromic anemia	Haemochromatosis, Porphyria cutaneatarda
Lead	-	Lead encephalopathy, Lead palsy, learning disability, mental retardation
Uranium	-	Radiogenic and chemically toxic
Nickel	-	Dermatitis, Pneumonia
Zinc	Aerodermatitis enteropathica, Hypogonadism	-

1.3 Sources of trace metals in marine ecosystem

Trace metals are introduced to coastal and marine environment as a result of natural processes and human activities including sewage and industrial effluents, brine discharges, coastal modifications and oil pollution (Fig. 1.4). Major natural sources of trace metals are rock weathering, degassing, and emissions from terrestrial and submarine volcanoes and dissolution from marine sediments. Trace metals are considered a major anthropogenic contaminant in coastal and marine environments worldwide [18]. The dominant inputs of most of trace metals to coastal areas are through rivers and land runoff but for few elements such as Hg, As and Pb the atmospheric route is also important, particularly in the open ocean, although even for these elements local discharges and rivers often dominate coastal input. The important anthropogenic sources of metals like Cr, Mn, Co, Ni, Cu, Zn, Pb, Cd, Hg and As to coastal and inshore water are industrial processing of ores and metals; ferrous and nonferrous metal industries including metal plating; industries producing both organic and inorganic chemicals; use of metal and metal components; leaching of metals from solid waste; and offshore dumping of domestic sewage, sludge and industrial wastes [19].

1.3.1 Discharges of trace metals in the environment from different industries

Although the toxic metals are present in trace levels in earth's crust, anthropogenic activities such as industrial processing and use of metals, alloys, and metallic compounds disperse them into the environment, thus adding up to their natural background levels (Table 1.3).

Table 1.3. Trace metals found in the effluents from some major industries [17]

Industry	**Heavy metals present in effluents**
Chlor-alkali	Pb, Cd, Cu, Zn, Cr, Hg, Se
Electroplating	Cu, Zn, Cr, Ni
Leather	Cr
Batteries	Pb, Cd, Hg
Paints and dyes	Pb, Cd, Cu, Cr, Hg, Se
Textiles	Cr
Paper and pulp	Hg
Petroleum refining	Pb, Cd, Cu, Zn, Cr
Fertilizers	Pb, Cd, Cu, Zn, Cr, Mn, As, Hg
Pesticides	Pb, Se, As, Hg
Vehicles	Pb, Cd, Zn, Cr, Hg, Se,
Pharmaceuticals	As, Hg
Mining and Metallurgy	Cu, Cd, Zn, Cr, As, Hg, Se
Explosives	Pb, Cu, As, Hg

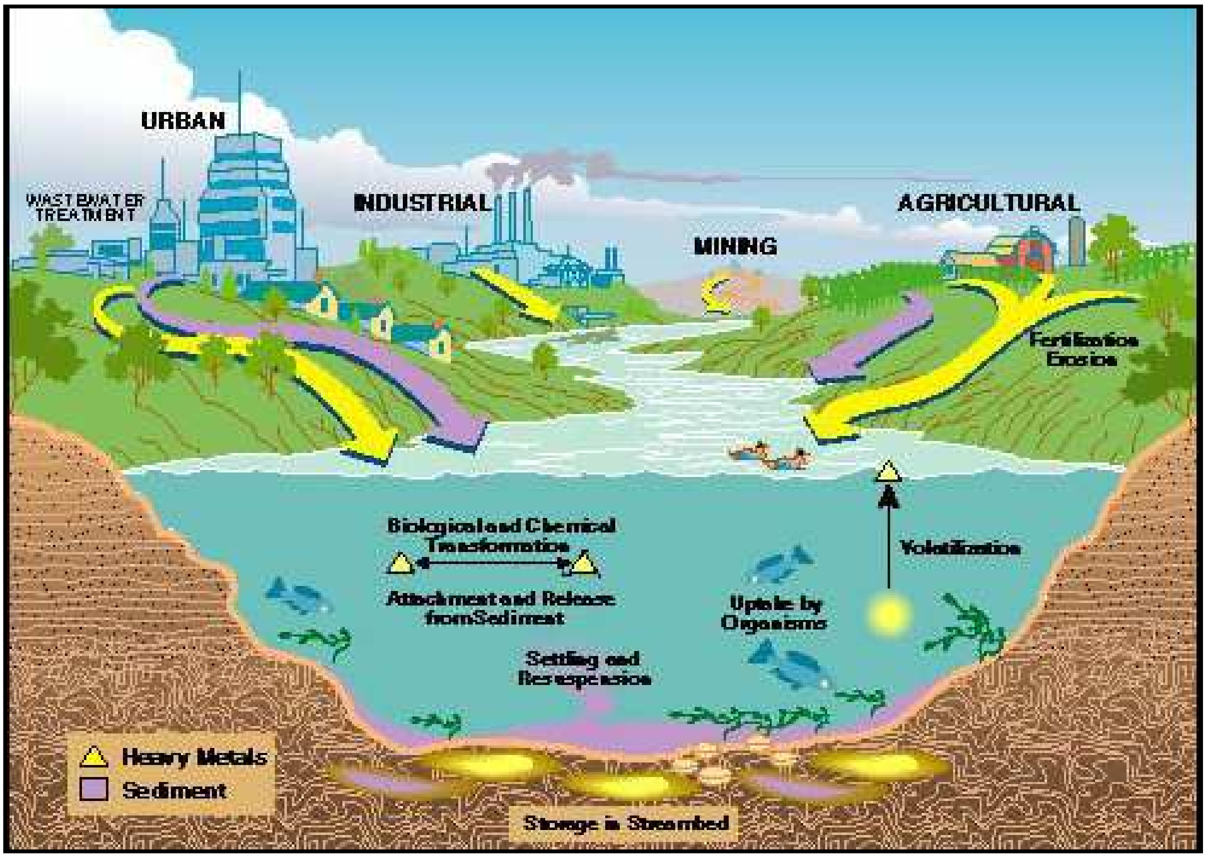


Fig.1.4. Trace metals pathway in marine environment (Google image)

1.3.2. Trace metals in seawater

The growing concern over the potential contamination of aquatic ecosystem has gained momentum in recent years and became a priority issue. River, fresh water lakes and marine coastal area are historical sites of civilization. Among other contaminants, trace metals in seawater have received particular attention due to increase in population, large-scale industrialization and newer methods of farming as a way of assessing the early impact of human activities on the marine environment. Metals can exist in seawater at least in four different forms namely in true solution, as colloidal particles, adsorbed on other colloidal particles, and as a part of living organisms. Their dispersion in seawater is affected by properties such as their volatility, tendency to form insoluble complexes with inorganic and organic compounds, and their ability to form either soluble complex with ions common in seawater or oxides or sulphides of low solubility [20]. Trace metals dissolved in seawater are present at very low concentrations and contamination during sampling, storage and analysis often gives erroneous results. In fact much of the data on metals dissolved in seawater pertaining to seventies and earlier was considered to be too high [21].

1.3.3. Trace metals in sediment

Metals discharged into near shore areas of marine environments are likely to be scavenged by particles and removed to the sediments. The sediments, therefore, become large repositories of trace metals. In aquatic environment depending on the environmental conditions sediments can act either as a sink or a source for trace metals. Trace metals in marine sediment can originate from several sources. In inshore and coastal areas the major sources of metals is lithogenous associated with weathering products from the source rock. Hydrogenous formations that include precipitation products and metals adsorbed on particles formed due to physico-chemical changes in water also contribute to the metal burden in marine sediments [22]. Anthropogenically introduced metals are ultimately transferred to the bed sediments [23-24]. Hence the environmental geochemistry requires knowledge of the naturally occurring metals in order to assess man's environmental impact. Although sediment analysis do not represent the extent of toxicity, they are useful to assess the burden of anthropogenic component over and above the lithogenic background and also in some instances, trace the sources of pollution long after input has taken place [25-27]. San Francisco Bay, California, one of the largest estuaries on the west coast of America is an example of how anthropogenic activities changed the concentrations of potentially toxic metals [28-29]. Changing environmental conditions, such as the presence of organic chelators may render the remobilization of metals from sediments [30-35]. Trace metals in bottom sediments are unevenly distributed due to bedding processes [36]. Sediment contamination by trace metals in marine areas has received increased global attention by investigators [37-52].

Trace metals may be immobilised within the stream sediments and thus could be involved in absorption, co-precipitation, and complex formation [53-54]. Sometimes they are co-adsorbed with other elements as oxides, hydroxides of Fe, Mn, or may occur in particulate form [55-56]. Trace metal concentrations in stream sediment compartments can be used to reveal the history and intensity of local and regional pollution [57].

Geochemical characteristics of the sediments can be used to infer the sources of pollution and the weathering trends [58-59]. Various studies have demonstrated that sediment from coastal areas is greatly contaminated by trace metals; therefore, the evaluation of distribution of elements in sediment is useful to assess pollution in the marine environment [27, 60]. In the sediment, metals accumulate through complex physical and chemical adsorption mechanisms depending on the nature of the sediment matrix and the properties of the adsorbed compounds. They are not removed from water by self-purification, but accumulate in suspended particulates and sediment, and then enter the food web via passing to higher consumer. They are always present in aquatic ecosystem and redistribute only among different components. Several physico-chemical parameters, such as pH, oxidative–reductive potential, dissolved oxygen, organic and inorganic carbon content, and the presence in water phase of some anions and cations influence the adsorption process [61-62].

Although sediments are sink, trace metals may re-enter the water column under certain environmental conditions. Thus, the sediment acts as a buffer capable of keeping the metal concentration in water and biota above the background levels long after the input of metals is stopped [63-67]. Mn, Fe and Al play a crucial role in the removal of metal ions from seawater as well as their behavior in sediments. The oxyhydroxides of Mn and Fe have a high adsorption capacity and large surface area and can absorb cations such as Cr^{3+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Hg^{2+} and Cd^{2+} [20, 68].

The concentration of trace metals in seafloor substratum tends to vary depending on factors such as sediment grain size, quartz and feldspar content and organic carbon etc. Different normalization techniques such as Enrichment Factor (EF), Geoaccumulation Index (I_{geo}) and sedimentary flux have been employed to massess the extent of metal accumulation in sediment over the lithogenic background [69-70].

1.3.4. Trace metals in biota

Many contaminants such as trace metals (also referred as heavy metals), though occur at extremely low concentrations in seawater, are accumulated by marine organisms and concentrations in their body tissue can be hundreds of times greater than the levels in seawater [22]. Thus, consumption of contaminated fish can be harmful; the well documented examples being Hg, Cd and Cu poisoning [71]. Simkiss and Taylor (1989) [72] have discussed the pathways of metal accumulation by aquatic organisms and suggested six possible types of uptake. The most common is by a passive process of transfer from seawater down to concentration gradient in the tissues. In some cases uptake may also occur through ion pumps because of energy dependency. For many metals including Cd, Cu and Zn, the free metal ion is the most bioavailable form of the element [73-74]. In case of metals such as Hg and As which can be in marine environment, the biological uptake is likely to occur in the alkylated form [75]. Autotrophic organisms probably accumulate trace metals directly from seawater. Hence, environmental parameters such as temperature, salinity, light and pH; and chemical factors such as speciation and complexation of the metal of considerable significance. The presence of several potentially toxic trace metals can also affect their preferential uptake [76].

1.4. Trace Metals in Arabian Sea and Bay of Bengal

The Bay receives effluents from several industries established in the Chembur- Thane-Belapur belt and untreated domestic waste water through several point sources. However, the contribution of Cr, Co, Ni, Cu, Zn, Cd, Pb and P to the anthropogenic loads by the industries and sewage has not been quantified. This information was necessary to assess relative contribution to metals in sediment by these 2 major polluting sources.

The past investigations on Al, Cr, Mn, Fe, Co, Ni, Cu, Zn, Cd, Pb and P in the Mumbai-Bassein region were confined to the period when environmental awareness in India was in infancy where by the industrial effluents entered the Bay without significant treatment. The scenario changed in early 1980s and industries were compelled to treat the effluent to the prescribed norms in a phased manner. The fluxes of metals and P through domestic wastewater, on the contrary would have gone-up due to increase in sewage throughput to the Bay from 2.5×10^5 m^3/d in 1978 to 3.16×10^6 m^3/d post 2000. Hence, it was considered worth while to revisit the Bay and freshly assess the metal burden in sediment with respect to the changed scenarios of anthropogenic fluxes of Cr, Co, Ni, Cu, Zn, Cd, Pb and P.

Taking the advantage of sedimentation rate reported for the Bay [77-78] through ^{210}Pb dating it was possible to compare the reported sedimentation rates with the signature of anthropogenic Cr, Co, Ni, Cu, Zn, Cd, Pb and P particularly in the zone of release of the effluent from industries and assess the extent of enrichment.

The Bay received high lithogenic flux estimated at $8.4x10^5$ m^3 annually through land runoff during monsoon [79] that is spread throughout the Bay under high tidal excursion and turbulence [77]. Hence, it was interesting to quantify Al, Cr, Mn, Fe, Co, Ni, Cu, Zn, Cd, Pb and P content in soil and rock in the catchment and assess the influence of lithogenic flux of presumably low content of trace metals, on the Al, Cr, Mn, Fe, Co, Ni, Cu, Zn, Cd, Pb and P burden in sediments of the subtidal and intertidal zones of the Bay which could resolve seasonal changes if any.

Based on the distribution of selected metals in sediment cores from the Bay it has been suggested that the anthropogenic trace metals probably associated with the effluent streams either settled in the effluent drains prior to their entry in the Bay or the Bay was a passive link between the sources and the sink with much of the load transferred to the shelf [80, 77]. With lack of data on industrial loads of metals to the Bay and high and variable natural levels of these metals in the sediment this issue had remained unresolved. If trace metals were transported to the sea their signature would be seen in sediments outside the Bay mouth. By estimating the sedimentation rate of a core of known metal profile, it would be also possible to establish the baseline metals in marine sediments of the region as well as the probable period of its excess accumulation in sediments.

It has been established that the bioavailability of trace metals in sediments depends on several factors [81]. To estimate the quantum of bioavailable metal in the sediment, surface and selected core sections from the Bay were considered for investigating the extent of bioavailability of metals through sequential leaching.

The worldwide reports indicate relatively wide variations in metal concentrations in seawater from different oceanic areas. According to Tappin et al. (1995) [82] the factors governing the distribution of trace metals in the marine environment are boundary inputs, particle-water exchanges and advection and mixing with the basin. A strong correlation between organic and trace metal rich particles may indicate the organic matter effectively forms complexes with trace metals [83].

The abundance, geochemical and mineralogical composition as well as distribution pattern of metals in the Arabian Sea and the Bay of Bengal depend on the biogeography, hydrography and topography of brackish waters that transports these inputs to these seas. Intercomparison of results from different geographical sites is however difficult because of different methods and materials used for sampling, pre-treatment and final analysis used by various workers. This has also been acknowledged for othe rmarine areas [84].

Published information on the concentration of dissolved metals in the Arabian Sea and the Bay of Bengal is rather limited and confined to only a few selected areas. These results are compiled in table 1.4. The results reveal wide variation in the levels of dissolved metals, particularly in estuaries and nearshore coastal areas. The data on concentration of dissolved Cr are available only for Mumbai Harbour (20 μg/L) and Kolak estuary (ND-102 μg/L) which received industrial effluents. High concentrations of trace metals such as Zn in the Kochi Backwaters, Ulhas estuary and Mahim Creek have been attributed to localized anthropogenic inputs [85-88].

Table 1.4. Concentration of dissolved trace metals (µg/L) in seawater

Location	**Cr**	**Mn**	**Fe**	**Co**	**Ni**	**Cu**	**Zn**	**Cd**	**Pb**	**Reference**
Mandovi Zuari (Goa)	-	-	-	-	-	2.0 - 63		-	-	[89]
Mandovi-Zuari estuary	-	6 - 102	-	-	ND	3.0 - 8.0	6.0 - 42	-	-	[90]
Mandovi-Zuari mouth	-	16 - 25	-	-	-	3.0 - 72	3.0 - 12	-	-	
Goa	-	42	19	0.8	5	4	16	-	-	[91]
Velsao Bay	-	13 - 16	27 - 56	-	ND	6.0 - 8.0	11.0-16.0	-	ND	[92]
Cochin Back waters	-	-	-	-	-	0.8 - 7.0	0.5 - 11.3	-	-	[93]
Periyar River	-	-	0.8 - 1.2	-	0.2	0.8 - 10.0	24 - 65	0.2	8	[88]
Cochin estuary			0.2 - 0.8		0.3 - 0.6	2.2 - 22.2	105 - 385	1.8- 4.2		
Karwar	-	2.3-10.2	9 - 26.2	-	-	0.8 - 4.2	3.1 - 20.1	-	-	[94]
Thal	-	-	-	ND	ND	7	15	-	3	[91]
Mumbai Harbour	20	8	3	0.5	0.5	16	27	0.1	4	[80]
Mumbai Harbour	-	8	3	0.5	0.5	6	27	0.1	4	[91]
Thane Creek	-	5-169	8.0-35	ND-1.5	ND-9.5	10.0-66	17-121	-	-	[95]
Thane Creek	-	16	16	0.5	2.9	19	21	-	-	[91]
ThaneCreekoff	-	5	20	ND	ND	9	20	-	-	
Thane Creek/Bombay Harbour	-	-	7.0-29	ND-0.5	ND-1.8	8.0-15	15-28	-	4.0-12	[96]

Location	Cr	Mn	Fe	Co	Ni	Cu	Zn	Cd	Pb	Reference
ThaneC reek	-	2.4-17.5	27.0-147.2	1.0-6.7	3.3-12.3	5.8-21.5	21.7-132	1.1-1.8	7.8-27.2	[97]
Thane Creek	-	3.3-99.5	16.5-40.3	2.5-4.9	6.8-14.3	5.2-15.6	38.7-93.6	1.3-2.8	19.2-25.1	[87]
Mahim coastal	-	-	17-44	0.9-0.9	3.0-6.0	12.0-15.0	103-121	-	-	[86]
Mahim Bay	-	-	14-16	1.0-2.0	4.0-10.0	10.0-16.0	40-75	-	-	
Mahim Creek	-	-	14-186	0.6-1.0	3.0-12.0	2.0-5.0	23-112	-	-	
Mahim coastal	-	4-169	-	ND-1.5	ND-9.5	10.0-66	17-121	-	-	[95]
Ulhas estuary	-	-	0.2-0.6	-	-	-	10-1000	-	-	[85]
Ulhas River	-	4.5-10.8	27.5-34.4	3.2-4.8	14.5-18.4	7.9-15.5	18.7-76.0	1.4-2.1	20.4-29.8	[87]
Tarapur	-	8	17	2	1	25	57	-	-	[91]
Kolak estuary	-	-	2.0-30	-		1.0-8.0	3.0-19	-	-	
Kolak estuary	ND-102	3.5-106	-	ND-17.3	ND-22.6	1.2-17.2	1.9-34.6	-	-	[98]
Auranga	-	-	8.0-14	-		3.0-6.0	8.0-15.0	-	1.0-2.0	[91]
Arabiansea	-	-	7.2-66.9	ND-6.6	ND-11.5	2.5-7.9	5.5-42.4	-	-	[99]
Northern Indian Ocean	-	-	-	-	-	-	-	0.04-0.26	0.04-0.5	[100]
Bay of Bengal	-	3.33	3.06	0.67	4.81	3.94	18.31	-	-	[101]
Gulf of Aqaba, Saudi Arabia	0.47-3.25	0.1-0.56	2.8-42.1	.07-.39	-	1.1-12.7	1.1-6.0	0.005-0.069	0.02-0.45	[102]

The other trace metals which have been investigated in some specific areas and not included in table 1.4 are Hg and As. The concentrations of Hg are distinctly high in Ulhas estuary [87, 103], Thane Creek-Mumbai harbor (herein after referred as Bay) [104, 87], Rishikulya estuary [105-107] and nearshore waters of Karwar [108] and have been attributed to its release through the effluent of chlor-alkali industries.

Only a few studies have been reported on the depth distribution of trace metals in the Northern Indian Ocean. The near surface Cd concentrations varied from 0.04 to 0.26 µg/L with an overall average of 0.15 µg/L. The average of intermediate and deep Cd enriched waters was 0.34 µg/L [109]. As in all other oceans, the Pb concentrations were high in the surface waters of the Indian Ocean (0.04 – 0.5 µg/L). Nakatsuka et al., (2007) [110] have reported concentrations of Ni (3.0 µg/L), Cu (3.5 µg/L), Zn (9.5 µg/L), Cd (0.17 µg/L) and Pb (3.0 µg/L) in the open waters of the Indian Ocean. Robin et al., (2012) [111] have reported concentrations of Cu (0.62-3.46 ng/mL), Cd (0.12 – 0.17 ng/mL) and Pb (1.78 – 1.99 ng/mL) in the seawater samples of Arabian sea along the south west coast of India.

Investigations on the concentration of metals in marine organisms of the Northern Indian Ocean are limited to only a few reports (Table 1.5). The past and recent data indicate general absence of bioaccumulation in the organisms studied except in localized areas receiving anthropogenic metals. The reported values of Cr, Co and Ni in organisms even in areas receiving industrial effluents are low. The concentrations of Cu and Zn are significantly high in mollusks. High levels of Cu observed in lobster, crab etc is perhaps due to the storage of Cu in the liver and presence of haemocyanin in the blood. The limited data available from Mumbai region indicated variation of Cu in the range 0.88-34 µg/g (dry wt) and for Zn 9.4-83.5 µg/g [112]. In tissues of marine organisms Cr concentration usually varied in 0.1 – 2 µg/g (dry wt) reported in the tissue of fish from the Ulhas estuary was probably the reflection of anthropogenically added Cr in the estuary [113].

Table 1.5. Concentrations of trace metals (µg/g, dry wt) in biota

Location	Cr	Mn	Fe	Co	Ni	Cu	Zn	Cd	Pb	Reference
Mandovi and Zuari Off	-	4.0-26.0	-	-	-	2.0-29	8.0-48	-	-	[90]
Mandovi and Zuari	-	3.0-18	-	-	-	251-728	446-2800	-	-	
Mandovi and Zuari	-	15-21	-	-	-	22-48	22-70	-	-	
Mandovi (P. Malabarica)	-	-	105.7	-	-	13.5	36.6	3.8	30.3	[114]
Velsao Bay (Molluscs)	-	10.0-62	362-820	-	0.5-4	280-362	1300-3000	-	1.5-12	[92]
Mumbai Harbour (Sewari)	2.7	49900	1500	3.5	10.8	11.1	132	2.8	-	[80]
Thane Creek (fish)	-	-	-	-	0.3-1.2	1.2-4.8	20.7-45.3	0.03-0.18	0.03-0.1	[115]
Bassein Creek (fish)	-	-	-	-	0.4-0.9	0.5-8.3	11.4-63.1	0.02-0.4	0.03-0.1	
Mumbai Harbour (fish)	0.78	-	-	-	0.68	0.31	8.36	0.02	0.08	[116]
Ulhas estuary	4.5	2.3	63	4.5	0.3	5.9	11.6	0.5	11.6	[113]

Location	Cr	Mn	Fe	Co	Ni	Cu	Zn	Cd	Pb	Reference
Northern Indian Ocean	-	ND-10.0	-	ND-3.8	ND-0.9	ND-4.5	ND-21	ND-2.0	0.06-6.02	[100]
Damodar River (Gangatributary)	5962	147-819	0.3-3.02	-	3.0-28	11.0-64	15-66	-	-	[116]
Northern Indian Ocean	-	3.0-7.0	-	ND-4.0	0.06-3.0	0.7-24.0	8.0-31.0	0.002-5.99	ND-0.17	[100]
Northern Indian Ocean	-	-	-	-	-	45	-	1.36	1	
Kenya (Oyster)	-	-	-	-	-	-	-	2.3-9.1	-	
Kenya(Scallop)	-	-	-	-	-	-	-	2.2-4.5	-	
Mombasa(Fish muscles)	-	-	-	-	-	-	-	0.1-0.9	-	
Mombasa(Fish scales)	-	-	-	-	-	-	-	0.8-0.21	-	

Cd in the range 0.04-40 µg/g (wet wt) has been reported [118] for marine organisms of the Andaman Sea. Cd in the muscles of common food fishes off the coast of Bangladesh was in the range of 0.04-0.13 µg/g (dry wt) [100]. Cd was non-detectable in fish muscles from the state of Malacca, while it was present (2.08-10.58 µg/g; dry wt) in shellfishes. The maximum permissible limit of Pb in the fishery products for human consumption, as set by FAO/WHO, lies in the range 1.0-2.0 µg/g (wet wt). Wide ranges of highest concentrations of Pb were found in the livers and kidneys of some of the Northern Indian Ocean fishes. This indicates its greater affinity for fat solubility.

Table 1.6 indicates that the region has been, by and large, free from contamination by toxic metals though localized areas of enhanced levels of metals in sediment have been reported. The concentrations of Co are generally below 60 µg/g and those of Ni are less than 100 µg/g in the coastal and estuarine sediments [86, 96]. Concentrations of Cu exceeding 130 µg/g in some locations are probably due to anthropogenic transfer [86-87].

The Hg in the sediment along Ulhas estuary decreased exponentially in the seaward direction with high concentrations (2.50-38.45 µg/g) confined to the inner 30 km segment and baseline (0.1 µg/g) had not attained even at 25 km off the estuary mouth [101, 119]. High mercury concentration (49.19 µg/g) in the bottom section of a core from the effluent receiving site from Thane Creek has been shown to coincide with the comissioning of the chlor-alkali industry. Based on the concentration of Hg in the sediments, depth of contamination, area of the region and sediment density total excess Hg in sediment has been estimated at 107.5 t for the Bay, 30 t for the Ulhas estuary and 77 t for the coastal area of Mumbai upto a distance of about 25 km from the shoreline [119].

Table 1.6. Concentration of trace metals (µg/g, dry wt) (except Fe in %) in marine sediment

Location	Cr	Mn	Fe	Co	Ni	Cu	Zn	Cd	Pb	Reference
Zuari estuary	-	-	-	-	-	-	-	-	-	[120]
Cochin Backwaters	-	72-256	-	10.0-38	22-83	17-47	102-348	-	-	[121]
Vembanad Lake	-	183-387	-	37-49	28-41	21-60	-	-	-	[122]
Bombay Harbour Bay	-	746-1318	7.0-8.0	-	-	162-276	153-292	-	-	[112]
Mumbai Harbour	124	1140	8.6	50	111	105	155	10	48	[80]
Bombay Harbour	-	719-1059	6.7-8.1	35-51	112-146	105-142	127-206	-	20-48	[96]
Thane Creek	-	-	-	-	-	-	-	-	3.0-4.95	[123]
Thane Creek	-	-	-	-	-	231	169	1.2-2.0	31	[124]
Thane Creek	29-63	210-1274		34-59	91-130	91-240	114-273	1.7-6.1	54-143	[87]
Mahim Bay	41-123	456-1132	-	19-35	49-82	71-150	48-345	1.5-4.5	15-41	[86]
Mahim Creek	31-154	430-1002	-	20-36	38-67	70-327	75-799	2.4-8.2	13-41	
Mahim upstreem	31-950	300-996	-	10.0-90	30-183	100-3000	44-6740	1.6-14	20-510	
Mahim coastal	29-200	422-1036	-	11.0-30	35-61	93-123	135-291	1.6-604	22-32	
Mahim coastal	-	660-1059	-	43-59	89-175	105-198	110-308	0.5-3.3	25-42	[95]

Location	Cr	Mn	Fe	Co	Ni	Cu	Zn	Cd	Pb	Reference
Ulhas estuary	-	-	-	-	-	-	-	-	1.69-5.10	[123]
Ulhas estuary	30-51	195-1024		31-61	70-133	61-150	96-183	1.7-5.3	30-68	[87]
Ulhas estuary	64-104	139-285	5.4-6.9	35-47	65-91	77-109	74-187	1.0-2.9	28-41	[112]
Tapi estuary	-	1125	7.6	-	70	128	125	-	-	[125]
Arabian Sea	-	1034-1212	-	-	66-87	99-130	109-204	-	-	[125]
Cauvery estuary	229	1310	3.4	-	379	33	75	-	38	[126]
Ganges estuary	98	732	4.2	-	49	44	151	-	32	
Krishna estuary	174	6978	10	-	149	69	1482	-	4	
Godavari estuary	128	1294	7.6	-	91	119	-	-	5	
Bay of Bengal	84	529	3.9	-	64	26	-	-	-	
Ganges estuary	147	1765	4	19	47	55	105	0.58	22	[127]
Ionian Sea, Southern Italy	86-103	893-2826	2.6-3.6	-	48-61	42-52	87-129	-	45-75	[128]

To assess the uptake in the food chain and by biota in general, knowledge on the environmental parameters ruling trace metal speciation, availability and bio-incorporation is indispensable in order to deduce a potential relation between environmental concentrations and the fraction bio-accumulated. Information on the fixation capacity, pH, carbonate content, size fractions, cation exchange capacity, and organic content of the sediment and subsequent mass transport of contaminants is needed before taking decisions on the treatment of trace-metal sediment contamination. The bioavailability of heavy metals in soils is directly related to their partitioning between sediment and sediment solution.

1.5 Present Study

The thesis describes the discharges of trace metals from different industries situated nearby Thane Creek area, Mumbai, India and their concentration distribution in different compartments (seawater, sediment and biota) of the marine ecosystem. Methodology was optimized for determination of trace metals in different matrices of marine environment. In the thesis it is also discussed about the dependency of trace metal concentrations on some physico-chemical parameters in sediment samples. Total trace metals concentration does not reflect exact toxicity in sediment samples, so binding behavior of trace metals in sediment samples were studied using sequential extraction procedure. Transfer factor/ concentration ratio of trace metals from seawater to biota was also studied. Intake of trace metals due to consumption of biota by human beings was also studied to understand associated hazard introduced into human beings. The overall work has been carried out by the following outline as represented in the fig. 1.5.

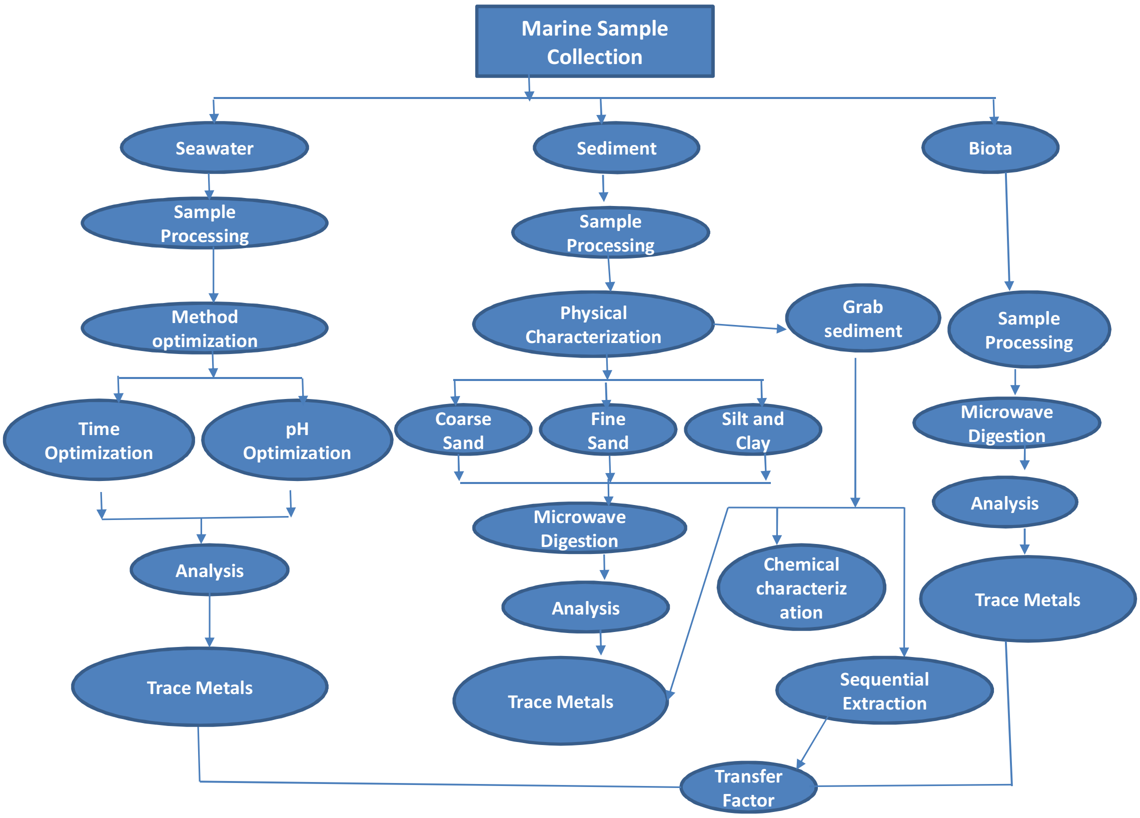


Fig. 1.5. Study framework

CHAPTER-2

Materials and Methods

This chapter mainly discusses the details on the sampling site along with various industries around the sampling sites. Chemical processing procedures used for different chemical parameters as well as trace metals extraction from collected different creek samples (seawater, sediment and biota) are also explained. Details on different analytical techniques used for various chemical species analyses are provided along with other details such as sensitivity, wavelength used for chemical analyses.

2.1. Study area

Thane Creek separates the island city from the mainland on the east. The Thane-Belapur stretch is about 20 km long and 2 km wide with an average area of 2650 ha. Thane Creek, which is adjacent to Mumbai harbor bay, is a triangular mass of brackish water which widens out and opens to the Arabian Sea in the south. Along the east and west sides of the creek, many industrial units have come up. Thane Creek is the ultimate recipient of all the liquid discharges from these industries. The Trans-Thane Creek Industrial area houses a number of major, medium and small scale industrial units largely involved in the manufacture, storage and use of chemicals, petrochemicals, pharmaceuticals and fine chemical products, pesticide formulation etc. Of the 1800 odd industries registered in the area, nearly 50 could be termed as major and the rest classified as small and medium scale. The effluent discharges both treated and untreated are left into the creek. The discharges from the eastern side of the city are dominated by the sewerage and industrial effluents. Industrial complexes, including the textile mills of South and Central Mumbai, the petrochemical, fertilizer and thermal plants at Chembur, the pharmaceutical and chemical complexes at Vikhroli, Bhandup and Mulund, the engineering and metallurgical units of Thane-Belapur region surround the creek and discharge their toxic effluents into it. The Mumbai harbor in the west and the new Nava Seva port in the east, handling more than 30 million tones/y, additionally contribute to the pollutants to the creek by way of leakage and spill. Being landlocked with very few freshwater inlets and a large amount of sewerage and industrial effluent discharges, the creek is relatively stagnant. In addition, atmospheric fallout from the chimneys, stack and vehicle exhaust also contributes to the pollutants load in the creek. Hence Thane Creek is a receptor for various pollutants from number of sources and a good fraction of them get immobilized into sediments [129]. The bay is subjected to semidiurnal tides and there exists asymmetry in both their periods and range. The water movements are mainly governed by the tidal actions. During the south-west monsoon period there is also an influx of approximately. 1.0 - 1.5x10^6 m^3 of fresh water from the river per tidal cycle, affecting the circulation pattern considerably. The Ulhas water flows into the Thane Creek only when in high flood during monsoon months June--September, rainfall; 250 cm/year. In the rainy season, i.e. between June and September, the contribution of river water to the creek is at its maximum while in summer it is at its minimum. During monsoon period salinity drops down to 4%. During the rest of the year subjected to ingress of water from the Arabian Sea the salinity is maintained in accordance with the ingress of seawater to a maximum of 38% [130]. Fourteen sampling locations were selected for sample collection located at Trans-Thane Creek area (TTC), shown in Fig. 2.1. The deatails of sampling locations with latitude and longitude is represented in table 2.1.

Fig.2.1. Sampling Locations (with industries)

Table 2.1. Details of sampling locations with latitude and longitude.

Sampling Location	Latitude	Longitude
L1/Near Sector 12, Ghansoli	19° 6'50.06"N	72°58'36.40"E
L2/Near Sector 10A, Vashi	19° 4'47.18"N	72°58'53.64"E
L3/Near Vashi Railway Station	19° 3'19.04"N	72°59'28.17"E
L4/Near Sector 18, Nerul	19° 1'46.48"N	72°59'56.96"E
L5/Near Sector 58, Seawoods	19° 0'16.62"N	72°59'51.30"E
L6/Near Targhar	18°59'38.13"N	73° 1'28.92"E
L7/Near Sector 10B, Ulwe	18°58'37.92"N	73° 0'30.55"E
L8/Near Maheshwar Temple	18°58'20.22"N	72°58'25.73"E
L9/Near JNPT	18°58'45.38"N	72°56'55.37"E
L10/Near Dockyard Road	18°59'25.00"N	72°51'51.00"E
L11/Near Mahul Village	18°59'33.40"N	72°54'0.90"E
L12/Near BARC, Trombay	19° 0'10.76"N	72°56'2.91"E
L13/Near Cheeta Camp	19° 1'43.72"N	72°57'38.23"E
L14/Near Deonar Dumping Ground	19° 4'23.00"N	72°57'35.04"E

2.2. Sample Collection

Fourteen seawater and grab sediment samples were collected from different locations across Thane Creek area, Mumbai, India (Fig. 2.1). Seawater samples were collected in 5 L plastic container previously cleaned through electronic grade nitric acid and double distilled water. Collected sediment samples were kept in polyethylene bags previously treated with one molar solution of hydrochloric acid and rinsed with distilled water to prevent contamination. Biota samples (Lizard fish, shrimp, prawn and crab) were collected at different points of the creek. The collected seawater, sediment and biota samples were brought into laboratory by portable cooler boxes covered with ice bags at 0 - 4 °C.

2.3. Initial Sample processing

2.3.1. Seawater

Seawater samples were filtered through 0.45 µm filter paper. In the filtered sweater samples 1 mL electronic grade nitric acid was added. Then the filtered acidified samples were used for solvent extraction for the determination of trace metals in seawater.

2.3.2. Sediment

Sediment samples were freeze dried and unwanted organisms were physically removed.

2.3.3. Biota

Biota samples were washed under the tap water to remove the adhering sediment particles from the body of the organism. This was then cleaned with distilled water and dried at room temperature. Biota samples were separated in two parts like edible parts and whole body biota. Then edible part and whole body biota samples were freeze dried and then stored in deep fridge till analysis.

2.4. Physico-chemical characterization of different matrices of the marine ecosystem

2.4.1. Determination of pH in sweater samples

pH of the filtered seawater samples were determined using a pH meter (DPH-500) which was calibrated using standard buffer (pH 4, 7 & 9) before testing.

2.4.2. Size segregation of sediment samples

Dried sediment samples were sieved below 2 mm using an electromagnetic sieve shaker (EMS 8, Electrolab, Navi Mumbai, India). The fraction of < 2 mm (grab sediment) is sieved in the size fractions 250 μm and 53 μm to receive the three size fractions very coarse, coarse and medium sand (2.0 mm - 250 μm), fine sand and very fine sand (250 μm - 53 μm), and silt and clay (< 53 μm).

2.4.3. Determination of pH in sediment samples

Thirty grams of sediment (< 2 mm) were taken into a 100 mL beaker and 75 mL of double distilled water was added. The suspension was stirred for a few minutes and covered with a watch glass. The suspension was allowed to stand for one hour with occasional stirring with glass rod. The pH of the supernatant water was estimated using a pH meter (DPH-500, Metsar Technologies Pvt. Ltd., Hyderabad, India).

2.4.4. Determination of $CaCO_3$ in sediment samples [131]

Sediment samples (5 g, < 2 mm) were taken into a 150 mL beaker and 100 mL of 1 mol/L electronic grade HCl was added. The suspension was covered with watch glass and kept one hour with intermittent vigorous stirring with glass rod. A volume of 20 mL of the supernatant liquid was taken in a conical flask, 6 - 8 drops of a solution of bromothymol blue were added, and the aqueous phase was titrated against 1 mol/L NaOH (Merck Life Science Private Limited, Mumbai, India) solution to get the concentration of calcium carbonate (CaCO3).

2.4.5. Determination of Cation Exchange Capacity (CEC) in sediment samples

CEC were obtained as follows. One gram of sediment was taken into a 100 mL beaker, 18 mL of 1 mol/L CH_3COONH_4 (Merck Life Science Private Limited, Mumbai, India) were added, and kept for an hour under occasional stirring with glass rod. The suspension was allowed to settle and filtered, and the residue was washed 3 times with 1 mol/L CH_3COONH_4 followed by 10 mL absolute alcohol. The volume was reduced to 10 mL by evaporation. Na^+, K^+, Ca^{2+} and

Mg^{2+} were analyzed by atomic absorption spectrometry (AAS) (GBC Avanta, Melbourne, Australia) and the sum of the four cations was taken to give the CEC value.

2.4.6. Determination of Total Carbon (TC) in sediment samples

Approximately 0.5 g of sediment sample (< 2 mm) was accurately weighed and taken into a nonmetallic ceramic container and TC was determined using a carbon analyzer (TOC-VCSH, Shimadzu, Kyoto, Japan).

2.5. Standardization of solvent extraction procedure for determination of trace metals in seawater

Before extraction procedure started all the apparatus which were used for the extraction procedure cleaned properly by electronic grade nitric acid, and then thoroughly washed using double distilled water. Filtered acidified seawater (400 mL) was placed in a separating funnel and pH was adjusted to the required pH with acetic acid ammonia buffer. 4 mL of 1 % Ammonium Pyrolidine Dithio Carbamate (APDC) and 20 mL of Methyl IsoButyl Ketone (MIBK) were added into it. MIBK is preferred because of its efficiency to extract wide range dithio carbamate-metal chelates [132-134]. The samples were shaken for 20 minutes and allowed to stand for 20 minutes for phase separation. The lower seawater phase was drained and the organic phase was transferred in a separating funnel. About 20 mL of 4 (N) HNO_3 was added and shaken for 20 minutes for acid-back extraction. After phase separation, the lower aqueous phase was transferred in 50 mL beaker and evaporated near to dryness and finally volume was prepared in 0.25% HNO_3 medium in 25 mL volumetric flask after filtration and trace metals (Co, Ni, Cr, Ba, V, Zn, Mn and Fe) were analysed using Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES) (Horiba Jobin Yvon, Grenoble, France) and (Pb, Cu, Cd and

U) in Differential Pulse Anodic Stripping Voltammetry (DPASV) (Metrohm multifunction instrument model 693 VA Processor equipped with a 663 VA stand (Herisau Switzerland)).

Solvent extraction procedure was carried out for different time intervals (1 – 30 minutes) to know the equilibration time of solvent extraction procedure.

Solvent extraction procedure was also carried out at different pH (2 ± 0.01 to 9 ± 0.02) to standardize the solvent extraction procedure for the determination of trace metals using ICP-AES and DPASV. After solvent extraction procedure at different pH it is clearly observed that percentage recovery of different trace metals are extremely different at different pH. For percentage recovery determination, spike study was carried out (known concentration of trace metals were added in the seawater before solvent extraction). Simultaneously another solvent extraction procedure was carried out at different pH (2 ± 0.01 to 9 ± 0.02) without spiking in the seawater to know the blank.

After standardization of solvent extraction procedure at different pH, for the determination of trace metals solvent extraction procedure was carried out at the particular pH for the respective metals.

2.6. Microwave acid digestion for determination of trace metals in sediment samples

Sediment samples (< 2 mm) were digested in a microwave digestion system (Multiwave PRO) for the estimation of distribution of trace metals. One gram of sediment sample, 2 mL electronic grade concentrated hydrofluoric acid (HF) (Merck Life Science Private Limited, Mumbai, India) and 8 mL electronic grade concentrated nitric acid (HNO_3) (Merck Life Science Private Limited, Mumbai, India) were taken in a Teflon container and acid digested using microwave digestion system. The digested extracts were made to 25 mL using 0.25% electronic grade HNO_3 for trace metals except for As and Hg. One gram of sediment sample and 10 mL electronic grade HNO_3 were taken in Teflon container and acid digested using microwave digestion system for As and Hg. As and Hg extracts were prepared using 30% electronic grade HCl. In order to evaluate the percentage recovery of the digestion procedure, equivalent amounts of reference materials IAEA-405 (estuarine sediment) and IAEA-433 (marine sediment) were also digested following similar procedure. Trace metals (Ba, Ni, Co, Zn, Cr, V, Mn, Fe, U and Th) were analyzed in ICP-AES, whereas Pb, Cd, Cu in DPASV in sediment samples. Volatile elements (As and Hg) were determined using Hydride Generation Atomic Absorption Spectrometry (HG-AAS) in sediment samples.

2.7. Microwave acid digestion for determination of trace metals in edible and whole body biota samples

The freeze dried biota samples both edible and whole body biota were ground to a fine powder separately using mixer grinder. 2–3 g of the accurately weighed powder was treated with concentrated HNO_3 (Merck Life Science Private Limited, Mumbai, India) and $HClO_4$ (Merck Life Science Private Limited, Mumbai, India) mixture in 2:1 ratio in a microwave digestion system (Multiwave PRO). The microwave program used as described elsewhere [134]. After digestion the solution was evaporated nearly to dryness and the required volume was made using 0.25% HNO_3 for determination of the non-volatile elements. As and Hg extracts were prepared using 30% electronic grade HCl. Trace metals (Ba, Ni, Co, Zn, Cr, V, Mn, Fe, U and Th) were analyzed in ICP-AES, whereas Pb, Cd, Cu in DPASV in sediment samples. Volatile elements (As and Hg) were determined using Hydride Generation Atomic Absorption Spectrometry (HG-AAS) in sediment samples.

2.8. Instrumentation

Various instruments were used for the chemical characterization and determination of trace metals in different compartment (seawater, sediment and biota) of marine ecosystem.

2.8.1. Carbon Analyzer

Carbon analyzer (Fig. 2.3) has been adopted in various fields as an accurate quick indicator of total organic content. Carbon analyzer has been used for different aspects as follows:

✓ Study and monitoring of organic contamination of rivers, lakes, dams, and other water in the natural water cycle.

✓ Water quality control of industrial effluent and sewage plant effluent and operation management for effluent processing.

✓ Process control of the organic content in water at all types of factory.

✓ Environmental and biotechnology research and R&D at test organizations.

✓ Study and monitoring of the degree of contamination of soil, sludge, and sediments.

✓ Control of purified or recycled water for the semiconductor, pharmaceutical, water-washing system, or nuclear power industries.

2.8.1.1. Working Principle

The key point about a TOC analyzer is whether it can efficiently oxidize hard to-decompose insoluble and macromolecular organic compounds, not just the easily decomposed, low molecular weight, organic compounds. The 680°C combustion catalytic oxidation method, that was developed by Shimadzu and subsequently spread round the world, can efficiently analyze all organic compounds. In presence of platinum based catalyst at high temperature (680^0

C) total carbon in the sample is combusted and the produced carbon di oxide is detected by Non Dispersive Infra Red detector. This gives the total carbon present in the sample (Fig. 2.2).

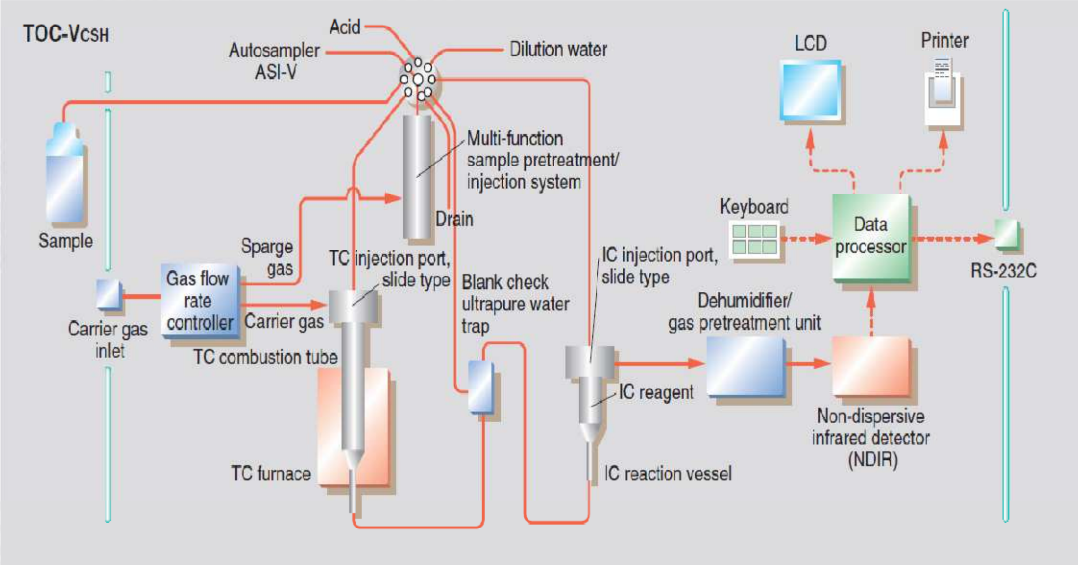


Fig.2.2. Measurement flow line diagram of carbon analyzer (Google image)

Fig.2.3. Picture of carbon analyzer (Carbon analyzer manual)

2.8.2. Microwave Digester

Microwave digestion unit, Multiwave PRO, was adopted for the digestion of sediment and biota samples shown in fig. 2.4. Multiwave PRO is a versatile and modular microwave reaction system for sample preparation and synthesis. The instrument supports various rotor types for fast and complete closed vessel digestion and extraction of organic and inorganic samples under high pressure and temperature as well as for high throughput method development, parallel optimization and multigram synthesis of valuable compounds in pharmaceutical, biomedical and other organic-chemical synthesis laboratories. Two standard magnetrons of 850 W deliver up to 1500 W microwave power in an unpulsed mode over the full power range. The sophisticated software prevents thermal overshoots and the design of the microwave applicator provides utmost field density with a high homogeneity, which allows efficient heating. The applied microwave energy is continuously controlled by optional wireless sensors for internal temperature and vessel pressure and an IR sensor to monitor the temperature of all vessels permanently. Three different temperature sensors are permanently controlling the magnetrons, the exhaust unit and the dissipated exhaust air. Together with the constant cooling during operation, the instrument provides maximum safety.

The operating parameters of microwave digestion system (Multiwave PRO) are represented in Fig. 2.5.

Fig.2.4. Picture of a microwave digester (microwave digester manual)

Fig.2.5. Operating parameters of microwave digestion system (Multiwave pro)

2.8.3. Atomic absorption spectrometry (AAS)

AAS is one of the oldest analytical techniques that were used to quantify the trace and major metals in various environmental matrices such as soil, water, biological samples, sediment and particulate matter [135]. This analytical technique has got good selectivity leading lower elemental interferences during metal analysis. Following section gives the details of the instrument and the experimental parameters that were set for metal analyses in the present study.

In the present study, GBC make AAS system (model Avanta-∑) was used for the elemental analysis. The AAS picture in our laboratory is shown in Fig.2.6.

Fig.2.6. Picture of a flame atomic absorption spectrometer (EMAS laboratory)

FAAS is a suitable technique for determining metals at lower part per million (ppm) concentration levels with good precision for many elements [136]. FAAS offers air-acetylene and/or nitrous oxide flame atomizer. FAAS technique provides fast analysis of 2-5s per sample, with very good precision (repeatability), moderate interferences that can be easily corrected, and relatively low cost [137]. Using this system, elements such as Na, K, Ca, Mg, As and Hg were analyzed. As and Hg were analyzed using hydride generation atomic absorption spectrometry technique.

2.8.3.1. Hydride generation Atomic Absorption spectrometer

The hydride generation technique which makes use of a separation of the analyte element from the matrix by conversion to its volatile hydride offers a route to the trace analysis of several important elements such as As, Se, Sb, Bi, Pb, etc., which have specific problems when analysed by conventional methods. Volatile hydrides are generated by using reducing agents and the corresponding chemical reactions are given below.

$$BH_4^- + H^+ + 3\ H_2O \rightarrow H_3BO_3 + 8\ H^+$$

$$H_3AsO_3 + 3BH_4^- + 3H^+ \rightarrow AsH_3 + 3BH_3 + 3\,H_2O$$

$$BH_3 + H_2O \rightarrow H_3BO_3 + 3H_2$$

The reducing reagent $NaBH_4$ was proved to be exceptionally reliable reagent for the conversion of the element to its volatile form. The hydride generation procedure can also be used for differential determination of As (III) and As (V), based on the fact that As (III) reacts with tetrahydroborate at a higher pH than As (V). Fig.2.10 shows the schematic diagram of hydride generation technique used in the As and Hg analysis in the present study.

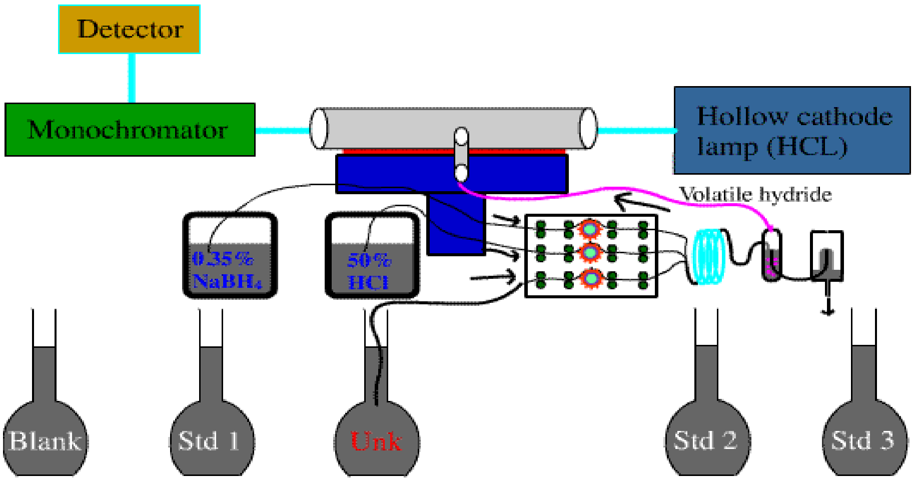


Fig.2.7. Schematic diagram of Hydride generation atomic absorption spectrometer setup (Google image)

General instrumental parameters that are fixed for the elemental analysis in the present study are given below (Table 2.2). These values were arrived by testing many times the Cu standard in flame AAS. Similarly for As and Hg also these parameters were fixed by analyzing As and Hg standards seperately.

Table 2.2. Instrumental parameters that are set in AAS for elemental analysis.

Parameter	**value**
Fuel flow	2 L/min.
Oxidant flow	10 L/min.
Work head height	8.8 mm
Burner angle	0.3^0
Work head center	0.4 mm

Mainly As and Hg were analyzed using HG-AAS along with few metals of interest for the inter comparison of elemental concentrations with ICP-AES. Before the analysis analytical line was selected that has got good sensitivity. Different analytical parameters used for the chemical analyses of elements of interest are summarized in Table 2.3. Calibration of element of interest was done using set of three standards prepared from single stock solution standard.

Table 2.3. Element specific parameters that are used in AAS system during quantitative analysis

Element	Wave length (nm)	Slit width (nm)	Sensitivity (µg/ml)	Lamp current (mA)
Na	589.6	0.5	0.008	5.0
K	769.9	0.5	0.024	6.0
Ca	422.7	0.5	0.02	10
Mg	285.2	0.5	0.003	3.0
Hg	253.7	0.5	0.02 ng/mL	3.0
As	193.7	1.0	0.01 ng/mL	8.0

2.8.4. Inductively coupled plasma atomic emission spectrometer (ICP-AES)

ICP-AES is widely used for the chemical analysis for wide range of elements viz. alkali, alkaline metals, transition metals, rare earths as well as some non-metals [138-140] in ppb level. The instrument was helpful in quantifying the elemental concentrations in different environmental matrices and also in various other materials that are synthesized in materials research. The instrument was proved to have multiple advantages such as multi element analysis over wide range of concentrations, low chemical and matrix interferences. Especially, the instrument was widely used for the elemental analyses in environmental samples all over the world [141-146].

In the present study, Horiba make Jobin-Yvon (model ULTIMA 2) ICP-AES system (Fig. 2.8) was used for the trace metal analysis. Different elements such as Ba, Ni, Co, Zn, Cr, V, Mn, Fe, U and Th were analyzed in ICP-AES. Different instrumental parameters that are used for the elemental analysis are given in Table 2.4.

Fig.2.8. Overview of a Basic Inductively Coupled Plasma—Atomic EmissionSpectrometry (ICP-AES) (EMAS laboratory)

Table 2.3. Different parameters of ICP-AES that are set for trace metal analysis

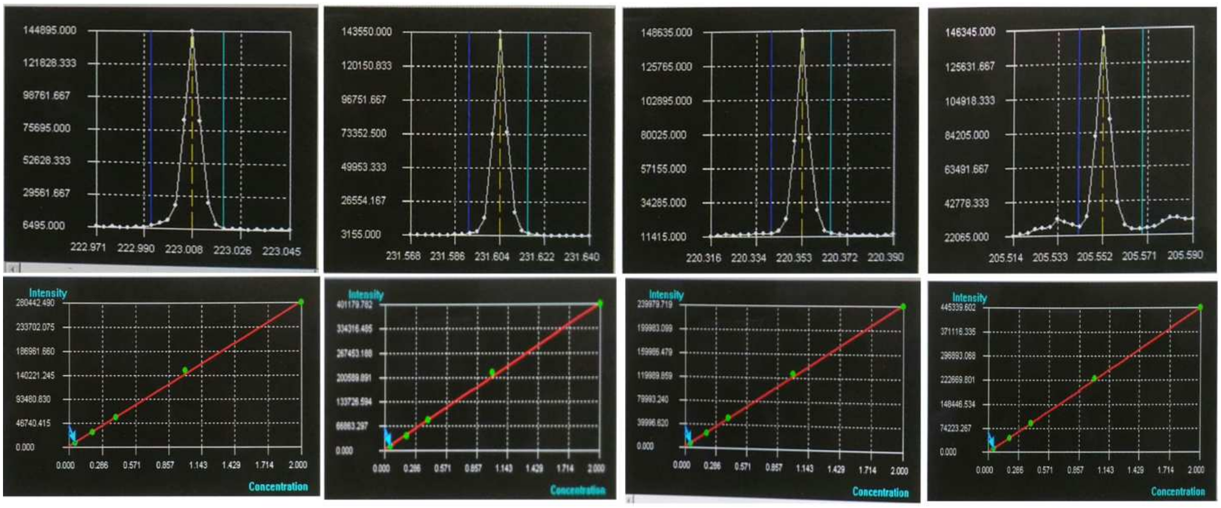
S.No	Parameter	value		Parameter	value
1	Nebulizer type	Concentric	5	Scanning mode Sweeps/reading Readings/replicate Replicates Measuring points Integration time Gain of PMT Increments of grating	Peak hopping 20 1 3 9 0.5 sec. 100 0.002 nm
2	Plasma gas	High purity Argon	6	Sampling parameters Sample flush time Sample flush pump speed Read delay Read delay pump speed Wash time Wash pump speed Mixing coil length(cm)	 35s 24rpm 65s 20rpm 60s 24rpm 100
3	Spray chamber	Teflon	7	Gas flow rates Plasma Nebulizer Auxiliary	 12 $\mathrm{Lmin^{-1}}$ 1$\mathrm{Lmin^{-1}}$ 1$\mathrm{Lmin^{-1}}$
4	Torch injector	Quartz	8	RF power	1000W

Prior to calibration the instrument was run for peak search and peak profiling of all the elements of interest. In this process for each element different emission line were monitored to select best emission line that has maximum sensitivity without any interference from abundant element. In the process the peak shape was also monitored that has to be used in the background correction for the elements of interest. After monitoring many emission lines, method was developed using software that could facilitate the analysis of multiple elements as mentioned above. The best emission lines selected for elemental analysis in the present study are summarized in table 2.5.

Table 2.5. Elements and their sensitive emission lines selected for trace metal analysis

Element	Wave length (nm)
Ba	455.403
Ni	231.604
Co	238.892
Zn	213.856
Cr	205.552
V	290.882
Mn	257.610
Fe	217.809
U	385.465
Th	283.73

In the present study, ICP-AES was calibrated for each metal using set of three to five standards (VHG labs make). The calibration curve was checked frequently by injecting the known standard solution. Field blanks were analysed and the data was subsequently blank corrected to determine accurate results of chemical species. Fig.2.9 shows the peaks of few metals in interest along with their calibration curves observed in the present study.



Cu **Ni** **Pb** **V**

Fig.2.9. Characteristic emission intensity peaks and calibration curves of metals in ICP-AES

2.8.5. Differential Potential Anodic Stripping Voltammetry technique

Voltammetric methods are applicable to the determination of a wide variety of both organic and inorganic species [147-148]. In voltammetry we apply a time-dependent potential excitation signal to the working electrode—changing its potential relative to the fixed potential of the reference electrode—and measure the current that flows between the working and auxiliary electrodes. The auxiliary electrode is generally a platinum wire, and the reference electrode is usually a SCE or Ag/AgCl electrode. We call the resulting plot of current versus applied potential a voltammogram**,** and it is the electrochemical equivalent of a spectrum in spectroscopy, providing quantitative and qualitative information about the species involved in the oxidation or reduction reaction.

Among different voltammetry techniques Differential pulse voltammetry is a widely employed technique for quantitative analysis. It provides superior detection limits as compared to linear sweep and cyclic voltammetry, and allows resolution of more closely spaced electrode processes. In this technique, the potential vs. time program which is applied to the electrode consists of a linearly increasing ramp upon which arises of small amplitude (5 to 100 mV) pulses are superimposed, as indicated in Fig. 2.15. The duration of the pulses is approximately 50 ms. The current is sampled just prior to the application of each pulse (at t_1), and again very near the end of the pulse (t_2). The difference between these two values, $\Delta i = i(t_2) - i(t_1)$, is then plotted against the ramp potential as indicated in Fig. 2.10.

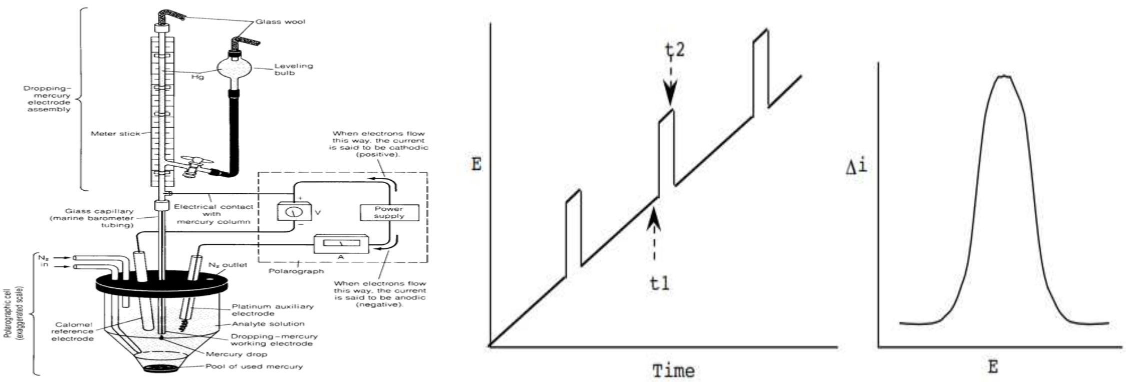


Fig.2.10. Differential pulse that applied to working electrode in voltammetry (Google image)

The improved detection limits of differential pulse voltammetry over conventional voltammetric methods are due to superior discrimination against charging current and impurity Faradaic currents. The capacitance of the electrode/solution interface is a function of the potential applied to the electrode. The magnitude of the charging current will thus also be dependent on the potential. This results in sloping baselines that can obscure the analytical information in conventional voltammetry. In differential pulse voltammetry, the difference in current at two very similar potentials is determined. Because of the similarity in potentials, the charging current contributions will be very nearly equalas well, and are effectively subtracted out. Also, any Faradaic current due to impurities will be subtracted out, provided the potential is not near the half-wave potential for the impurity.

In the present study, Metrohm make Autolab Potentiostat instrument (Model 693 VA Processor equipped with a 663 VA stand) was used for the Pb, Cd and Cu analysis. The calibration of the instrument was carried out three mixed standards of Pb, Cd and Cu. Before calibration of the instrument, different parameters such as purging time, deposition time, equilibrium time and pulse parameters were optimized to achieve best sensitivity and good peak shape. The details of optimized analytical parameters in voltammetry technique are given in table 2.6. Fig.2.11. shows the instrumental setup in our laboratory along with analytical peak of Pb, Cd and Cu obtained using optimized parameters in DPASV mode.

Table 2.6. Different analytical parameters that is set during the analysis of Pb, Cd, and Cu in DPASV

Parameters	**Value chosen**	**Parameters**	**Value chosen**
Initial N_2 purge time	60 sec.	End potential	0.17 V
Deposition time	60 sec.	Pulse amplitude	0.05 V
Equilibration time	10 sec.	Pulse time	0.04 Sec
Deposition potential	-0.8V	Voltage step	0.005951V
Starting potential	-0.8 V	Voltage step time	0.1 Sec.

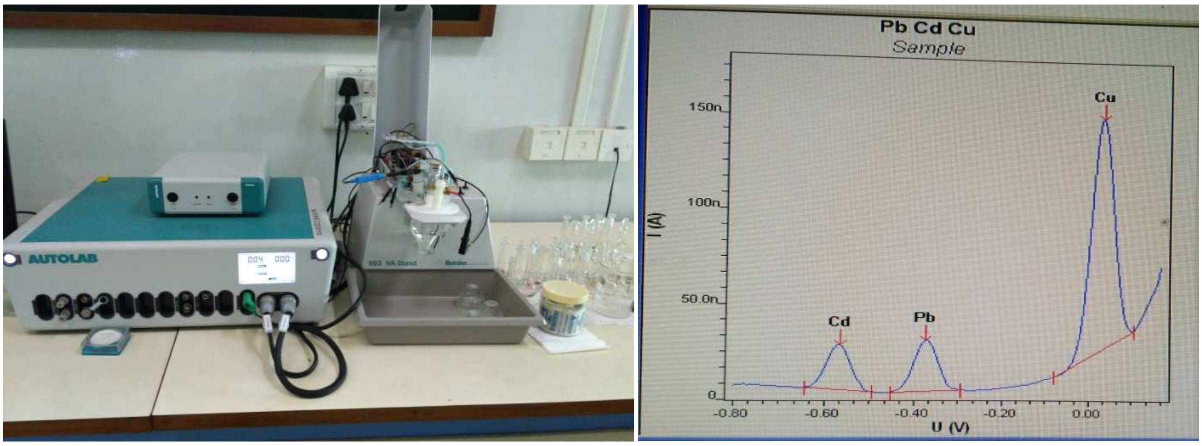


Fig.2.11. Metrohm make voltammetry system and voltammogram of selected metals (EMAS laboratory).

CHAPTER-3

Distribution of Trace Metals in Different Compartment of Marine Ecosystem

3.1. Trace metals in seawater

Trace metals are introduced to the marine environment by domestic and industrial activities as anthropogenic pollutants. Many contaminants though occur at extremely low concentration in seawater, are accumulated by marine organisms and concentrations in their body tissue can be hundreds of times greater than seawater. As human being consume different marine organisms like biota, can lead potential health problem. However, for sea-water, the high salt content and low levels of trace metals invariably preclude direct analysis. Hence the analysis of seawater by instrumental techniques generally requires a preliminary matrix separation. Several methods are adopted to pre-concentrate the trace metals and to separate the salt matrix from seawater prior to trace metal determination. Techniques such as solvent extraction [149], ion exchange [150] and carrier precipitation [151] have been extensively used to achieve selective removal of most interfering ions and to pre-concentrate for further lowering of detection limits. The method based on metal complexation with ammonium pyrolidine dithicarbamate (APDC) and extraction into MIBK [152] is widely adopted with some modification even today by several researchers. Sometimes di-ethylammonium diethyldithiocarbamate (DDDC) was added in combination with APDC to improve complex stability and broaden the effective working range [149]. Determination of distribution of trace metals in seawater is very much important. Solvent extraction procedure was optimized in terms of equilibration time and pH. Trace metals were determined in the seawater samples collected from fourteen different locations across Thane Creek area, Mumbai, India.

3.1.1. Equilibration time in the solvent extraction procedure

Equilibration time is one of the most important parameter in the solvent extraction procedure to understand the highest extraction efficiency in which time. Equilibration time was optimized for trace metals (Co, Ni, Cr, Cu, Pb, Ba, V, Cd, U, Zn, Mn and Fe) in sweater sample and represented in the following figures 3.1a, 3.1b, 3.1c, 3.1d, 3.1e, 3.1f, 3.1g, 3.1h, 3.1i, 3.1j, 3.1k and 3.1l respectively. Optimized equilibration time for trace metals like Co, Ni, Cr, Cu, Pb, Ba, V, Cd, U, Zn, Mn and Fe were observed to be 13, 19, 10, 13, 13, 10, 13, 10, 10, 10, 13 and 13 minutes respectively. By getting the equilibration time for trace metals, solvent extraction procedure was carried out in 20 minutes as within 20 minutes all the studied trace metals are coming into equilibrium in the solvent extraction procedure.

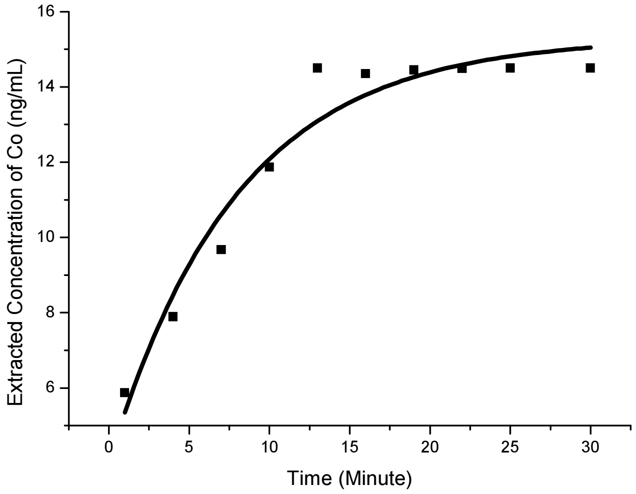


Fig.3.1a. Equilibration time for Co in the extraction procedure

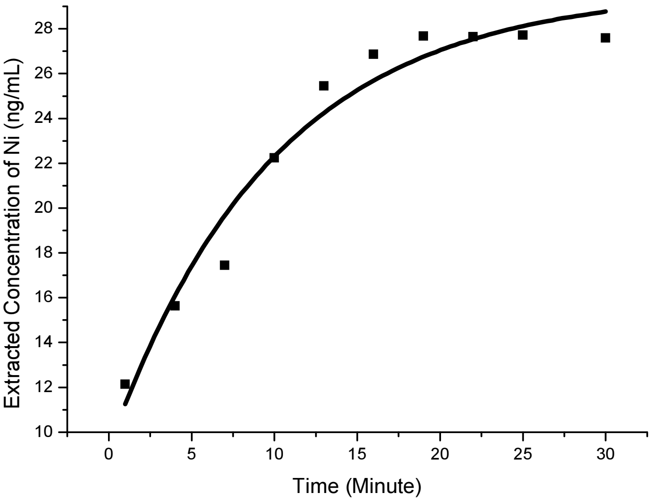


Fig.3.1b. Equilibration time for Ni in the extraction procedure

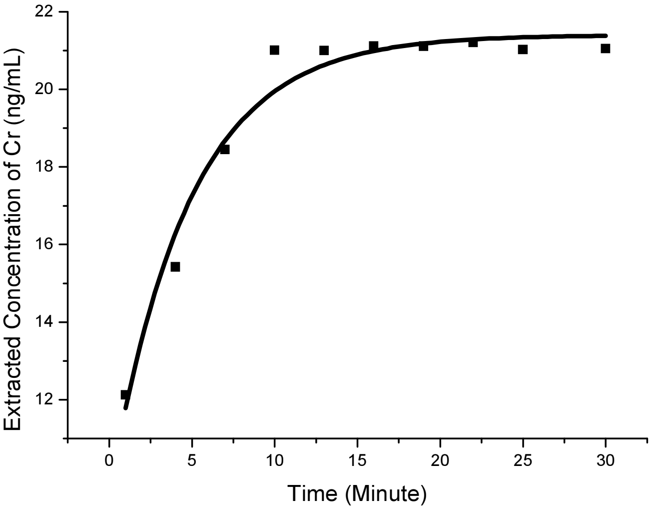


Fig.3.1c. Equilibration time for Cr in the extraction procedure

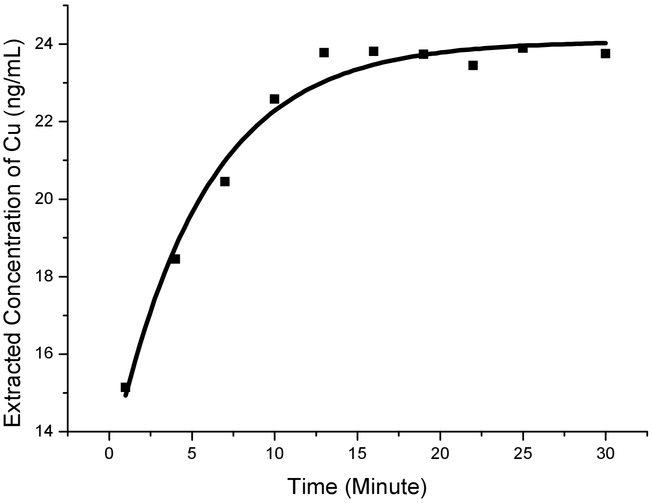


Fig.3.1d. Equilibration time for Cu in the extraction procedure

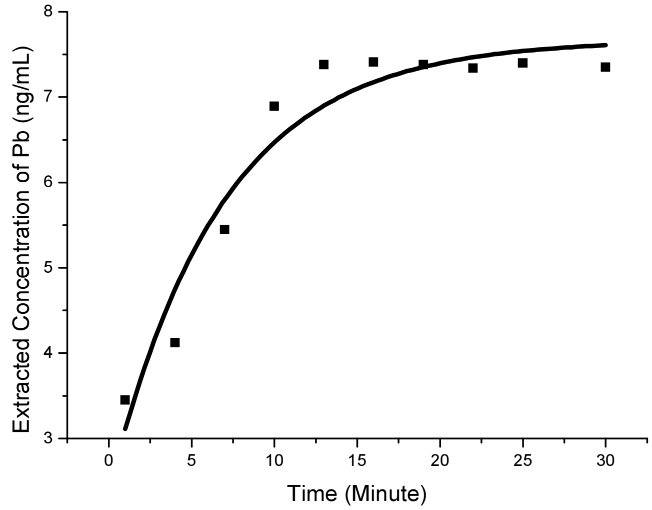


Fig.3.1e. Equilibration time for Pb in the extraction procedure

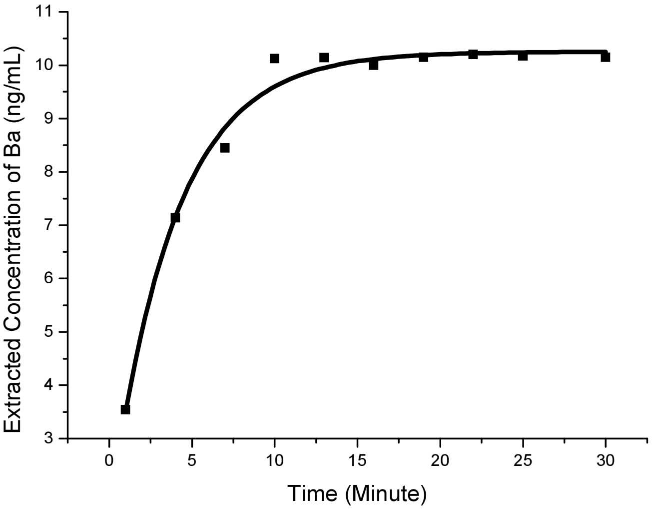


Fig.3.1f. Equilibration time for Ba in the extraction procedure

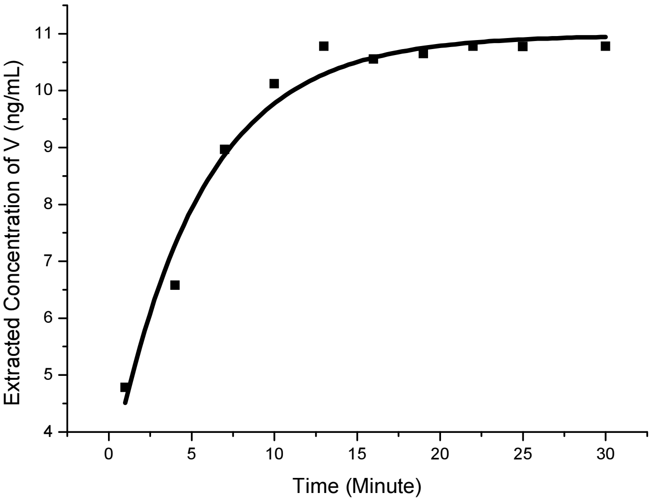


Fig.3.1g. Equilibration time for V in the extraction procedure

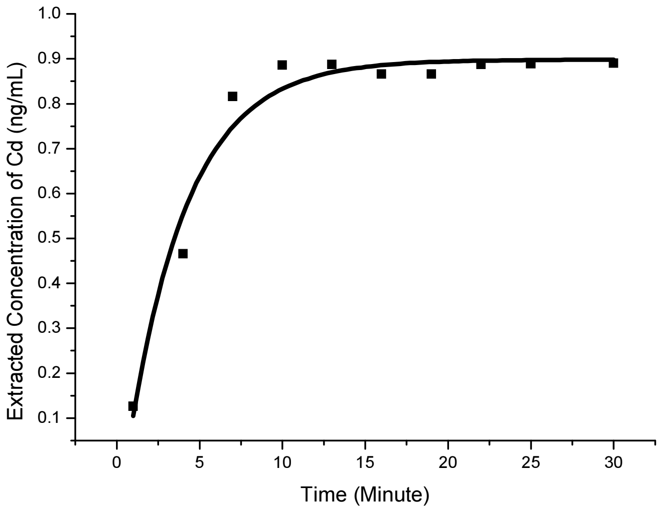


Fig.3.1h. Equilibration time for Cd in the extraction procedure

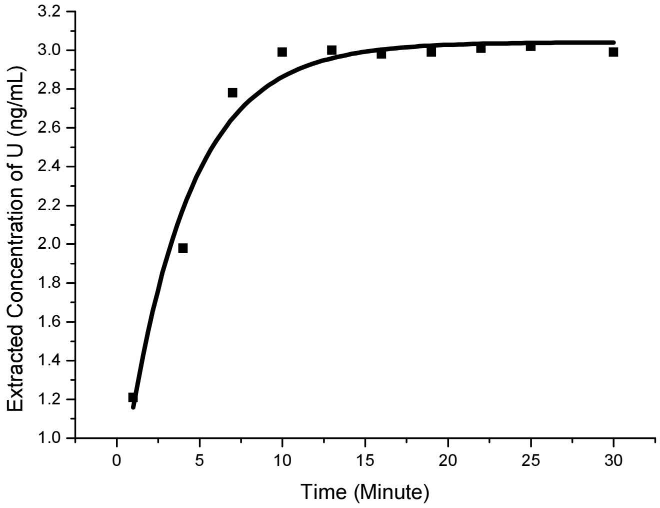


Fig.3.1i. Equilibration time for U in the extraction procedure

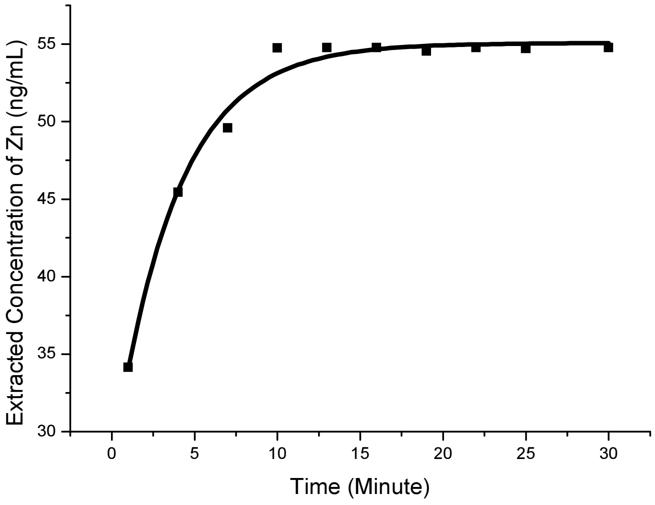


Fig.3.1j. Equilibration time for Zn in the extraction procedure

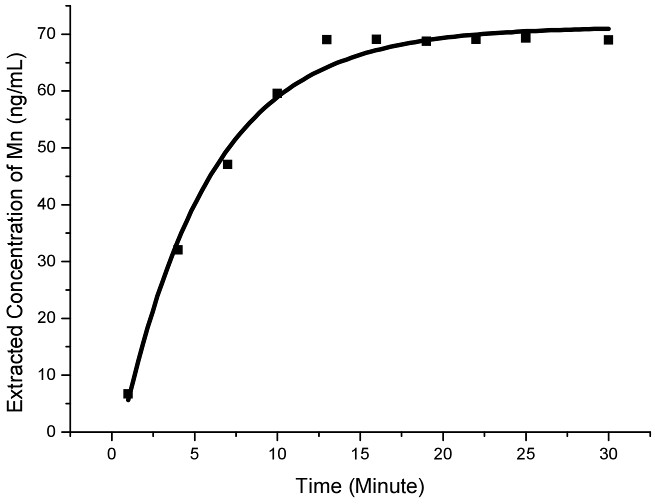


Fig.3.1k. Equilibration time for Mn in the extraction procedure

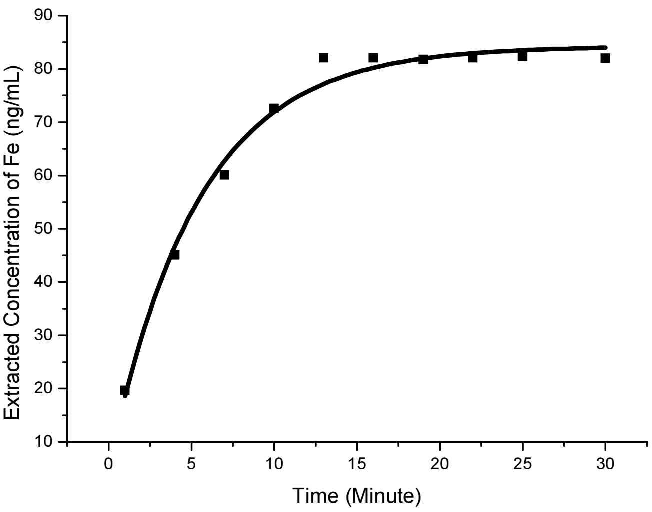


Fig.3.11. Equilibration time for Fe in the extraction procedure

3.1.2. Recovery of trace metals in the solvent extraction at different pH

Solvent extraction procedure was carried out in different pH to understand the extraction efficiency with variation of pH. Solvent extraction procedure was carried out at different pH (2 ± 0.01 to 9 ± 0.02) for 20 minutes to standardize the solvent extraction procedure for the determination of trace metals using ICP-AES and DPASV. After solvent extraction procedure at different pH it is clearly observed that percentage recovery of trace metals are extremely different at different pH and represented in table 3.1. For percentage recovery determination spike study was carried out (known concentration of trace metals) were added in the seawater before solvent extraction). Simultaneously another solvent extraction procedure was carried out at different pH (2 ± 0.01 to 9 ± 0.02) without spiking in the seawater to know the blank. It is observed that extraction efficiency for trace metals are different at different pH in the solvent extraction procedure represented in table 3.1. From the experimental results it is observed highest solvent extraction efficiency reached at pH near 6 for most of the trace metals in the solvent extraction procedure except for U and Ni.

Table 3.1. Recovery of trace metals (%) in seawater in the solvent extraction procedure at different pH

Element	pH							
	2.0 ± 0.02	3.0 ± 0.02	4.0±0.03	5.0± .01	6.0± .02	7.0±0.03	8.0±0.02	9.0±0.02
Co	65 ± 3	76 ± 3	87 ± 3	96 ± 3	99 ± 3	85 ± 3	76 ± 3	56 ± 3
Ni	20 ± 2	58 ± 3	63 ± 2	99 ± 2	93 ± 4	74 ± 3	71 ± 2	50 ± 2
Cr	72 ± 3	50 ± 2	65 ± 2	83 ± 2	98 ± 3	70 ± 3	63 ± 3	69 ± 3
Cu	66 ± 3	85 ± 5	73 ± 4	78 ± 5	100 ± 5	63 ± 3	73 ± 4	93 ± 4
Pb	73 ± 3	87 ± 3	80 ± 4	85 ± 3	97 ± 4	65 ± 2	83 ± 3	70 ± 2
Ba	68 ± 4	78 ± 3	87 ± 4	95 ± 5	98 ± 4	90 ±3	78 ± 4	71 ± 2
Cd	45 ± 3	39 ± 2	20 ± 1	63 ± 2	98 ± 4	33 ± 2	73 ± 3	19 ± 2
U	75 ± 3	98 ± 2	92 ± 2	88 ± 3	86 ± 2	56 ± 4	48 ± 4	52 ± 3
Zn	85 ± 4	73 ± 2	50 ± 2	72 ± 3	99 ± 4	87 ± 2	88 ± 3	86 ± 2
Mn	63 ± 2	73 ± 3	75 ± 4	85 ± 4	100 ± 5	83 ± 2	69 ± 2	68 ± 2
Fe	43 ± 2	73 ± 3	75 ± 4	80 ± 4	100 ± 5	63 ± 2	59 ± 2	48 ± 2

3.1.3 Trace metal concentrations in seawater across Thane Creek area, Mumbai, India

After standardization of solvent extraction procedure at different pH, for the determination of trace metals, solvent extraction procedure was carried out at the particular pH for the respective metals. After solvent extraction and finally acid digested solution preparation trace metals were determined by ICP-AES and DPASV. Concentration of Co, Ni, Cr, Cu, Pb, Ba, V, Cd, U, Zn, Mn and Fe varies from 6.1 ± 0.1 to 16.1 ± 0.2 ng/mL, 12.9 ± 0.1 to 28.6 ± 0.3 ng/mL, 12.7 ± 0.1 to 27.2 ± 0.4 ng/mL, 12.8 ± 0.1 to 28.6 ± 0.3 ng/mL, 3.0 ± 0.0 to 9.4 ± 0.1 ng/mL, 5.6 ± 0.1 to 10.6 ± 0.1 ng/mL, 8.3 ± 0.1 to 14.5 ± 0.2 ng/mL, 0.32 ± 0.01 to 1.21 ± 0.02 ng/mL, 2.68 ± 0.05 to 3.54 ± 0.09 ng/mL, 38.6 ± 0.4 to 58.1 ± 0.8 ng/mL, 44.6 ± 0.5 to 71.8 ± 0.9 ng/mL and 55.1 ± 0.6 to 93.9 ± 1.0 ng/mL respectively in seawater samples (fig. 3.2a, 3.2b, 3.2c, 3.2d) collected from different locations across Thane Creek area, Mumbai, India.

From the experimental results it is clearly observed that concentration of Co, Pb, Cu, Cd, Ba, Zn, Mn and Fe are higher in locations 1, 2, 3 and 4 compare to other locations. Industries are situated in the eastern bank of the Thane creek and that may be the reason of getting higher concentrations of the previously mentioned metals in these locations. Higher concentrations are observed for Ni, Cr and V in the locations 12, 13 and 14. These anomalous distributions may be attributed to urbanized pollutants in the western bank of the creek. U concentrations were randomly varying in different sampling locations.

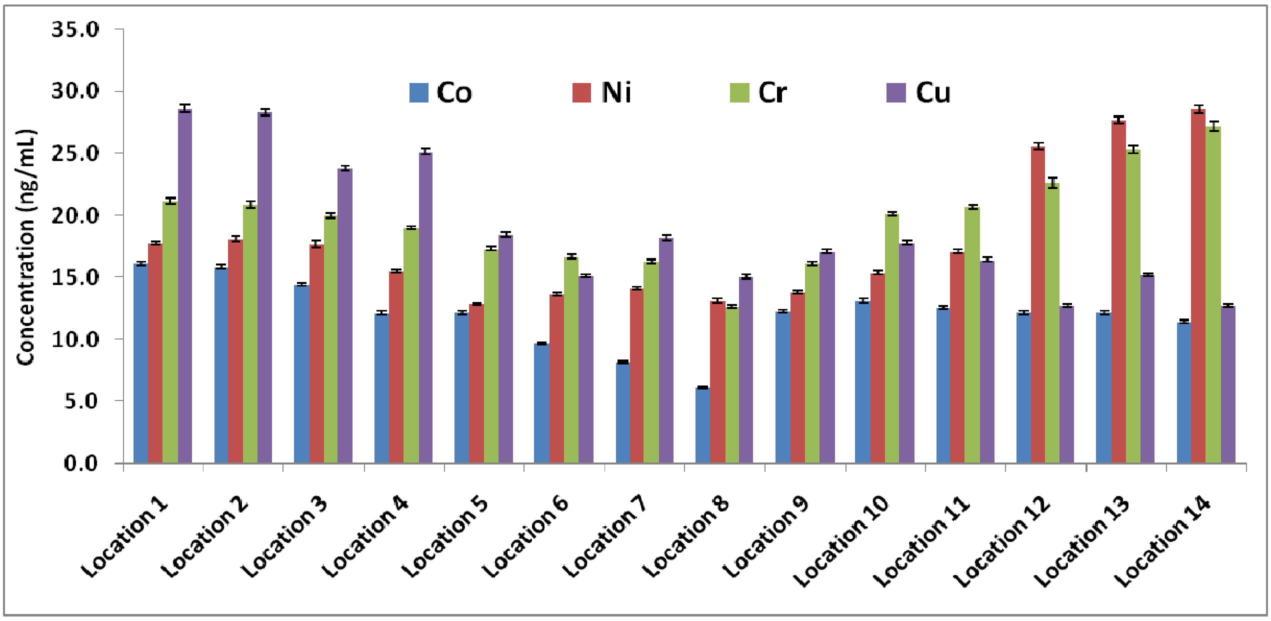


Fig.3.2a. Co, Ni, Cr and Cu concentrations in seawater samples across Thane Creek area, Mumbai, India

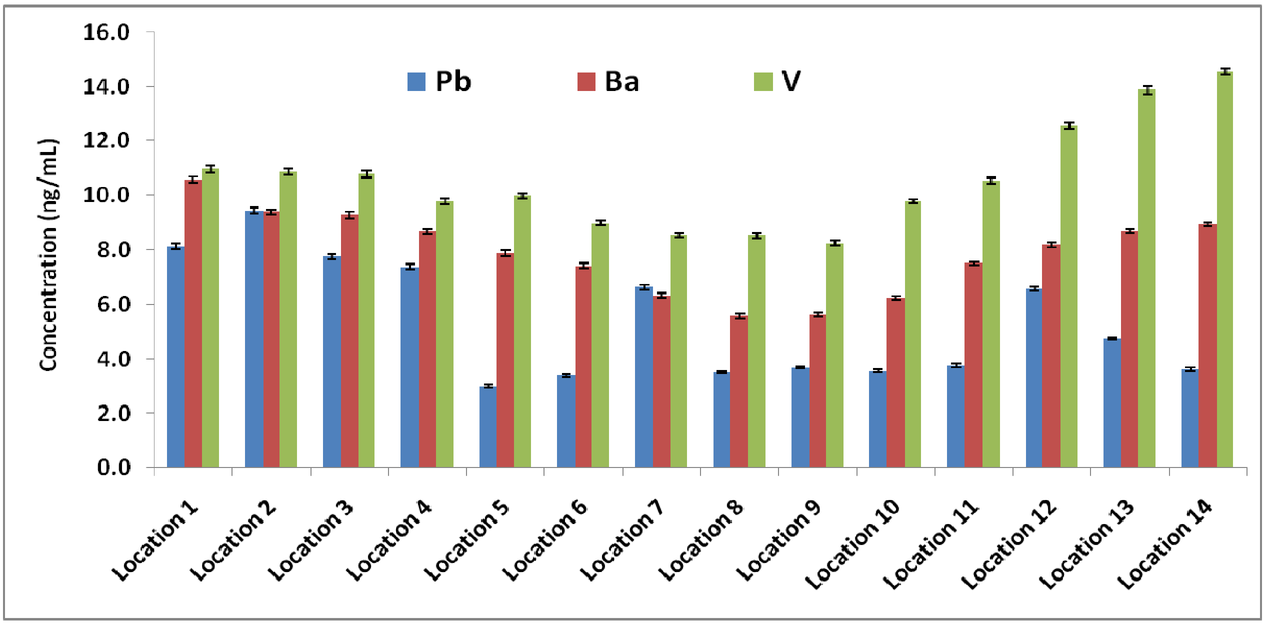


Fig.3.2b. Pb, Ba and V concentrations in seawater samples across Thane Creek area, Mumbai, India

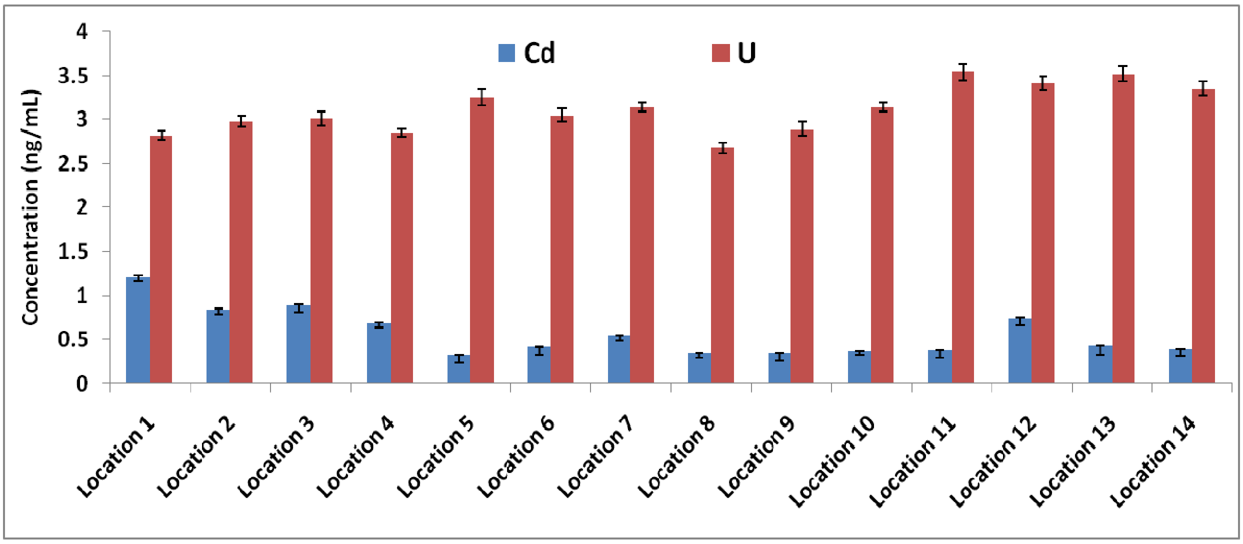


Fig.3.2c. Cd and U concentrations in seawater samples across Thane Creek area, Mumbai, India

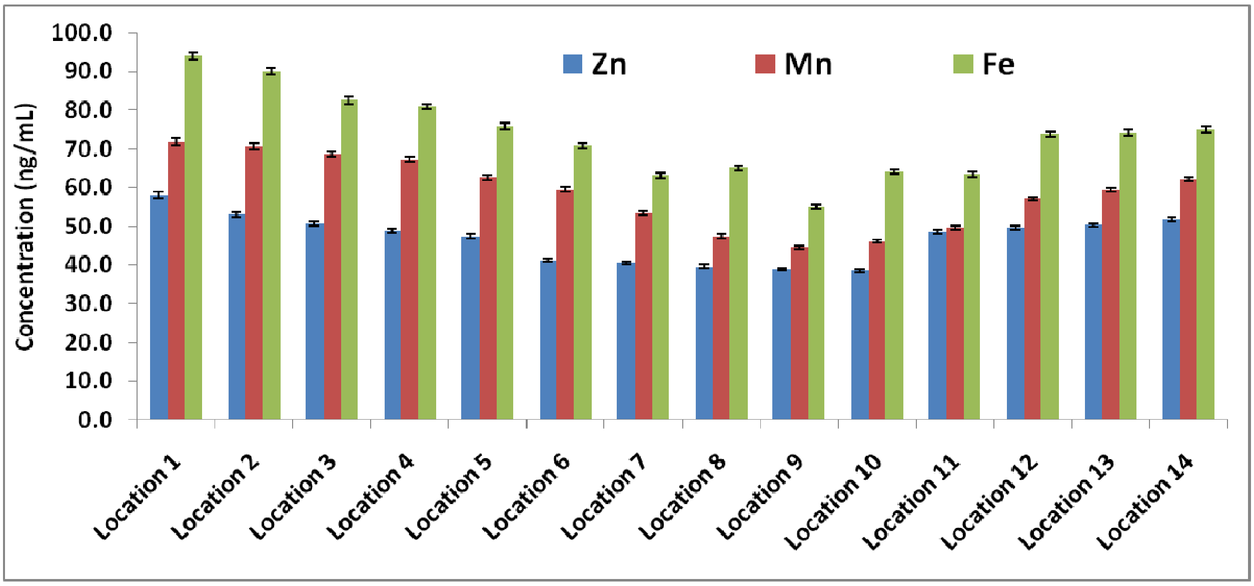


Fig.3.2d. Zn, Mn and Fe concentrations in seawater samples across Thane Creek area, Mumbai, India

3.2. Trace metals in sediment

Metal contamination of sediments can directly affect the seawater quality, resulting in uptake by low level organisms, bioaccumulation through food chains, and ultimately potential consequences to human health [153]. Trace metals are not to be constantly fixed in marine sediments; sediment-bound metals can be released by re-suspension, redox reactions, and under specific conditions, biodegradation of the sorptive substance [154-157]. Distribution and accumulation of trace metals in sediments depend on source rock composition, grain size, metal characteristics, biochemical situation of the environment and physical transport [158-160].

Spatial distribution of trace metals in sediment samples collected from fourteen different locations are shown in figures 3.3a, 3.3b, 3.3c and 3.3d. Concentration of Ba, Ni, Pb, Co, Cu, Zn, Cr, V, Mn, As, Cd, Hg, Al, Fe, U and Th varies from 20.5 ± 1.1 to 56.5 ± 1.6 µg/g, 20.1 ± 1.4 to 34.8 ± 1.8 µg/g, 19.8 ± 1.3 to 23.6 ± 1.5 µg/g, 16.5 ± 1.3 to 23.0 ± 1.5 µg/g, 76.0 ± 2.5 to 160.1 ± 4.1 µg/g, 69.8 ± 2.1 to 122.5 ± 3.1 µg/g, 91.8 ± 2.9 to 157.5 ± 4.2 µg/g, 52.5 ± 2.0 to 115.3 ± 3.3 µg/g, 433 ± 21 to 1112 ± 45 µg/g, 6090 ± 300 to 8780 ± 480 ng/g, 86 ± 7 to 352 ± 18 ng/g, 41 ± 4 to 315 ± 17 ng/g, 55.0 ± 2.4 to 62.7 ± 2.8 mg/g, 37.7 ± 1.7 to 58.1 ± 2.6 mg/g, 9.0 ± 0.3 to 12.7 ± 0.6 µg/g and 20.0 ± 0.9 to 29.4 ± 1.0 µg/g respectively in sediment samples collected from different locations across Thane Creek area, Mumbai, India. At location 1, Fe, Mn, Cu, Zn, Pb, Co, Cd, Hg, As and Ba shows highest concentration among all the locations. It is seen that these concentrations roughly decrease as we move farther away from the location 1. This is because of the increased contamination by the effluent discharged from several industries situated on the eastern bank of Thane creek. The diversified industries along the banks of the creek, includes chemical, textile, pharmaceutical, engineering and major fertilizer complex, release through their effluents, high levels of nitrates and phosphates into the creek [161]. The scarcity of back water in location 1 (as it is situated in very vertex of the creek) during high tide decrease the dispersion probability of these contaminants to the sea. The distributions of Cr, V, Ni show a slightly different picture, (Fig. 3.3a and 3.3b) which were found to be in higher concentrations in the locations 13 and 14. Cr shows highest in concentration at location 13 while V and Ni are highest at location 14. These anomalous distributions may be attributed to urbanized pollutants along with the thermal power plant contribution and may be due to variation of different physico-chemical parameters of sediment samples. The analyzed trace metal concentrations are within the range of previous study [162] except for As. Al, U and Th concentrations vary randomly from location to locations as there was no anthropogenic source present either bank of the Thane Creek.

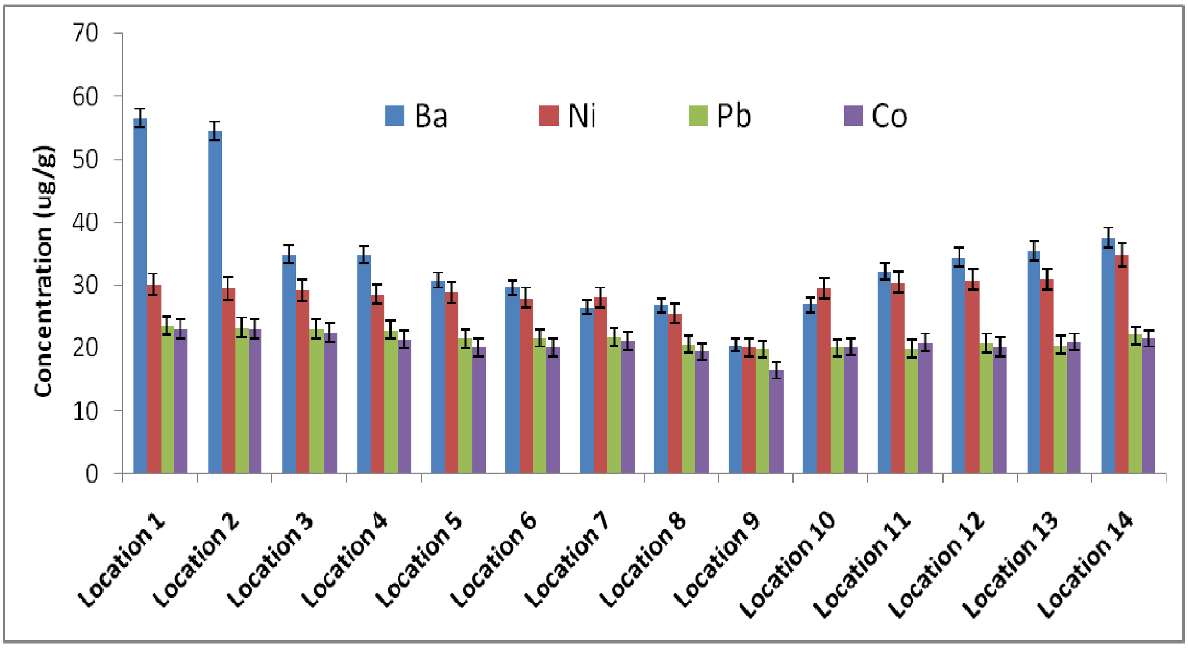


Fig.3.3a. Ba, Ni, Pb and Co concentrations in sediment samples across Thane Creek area, Mumbai, India

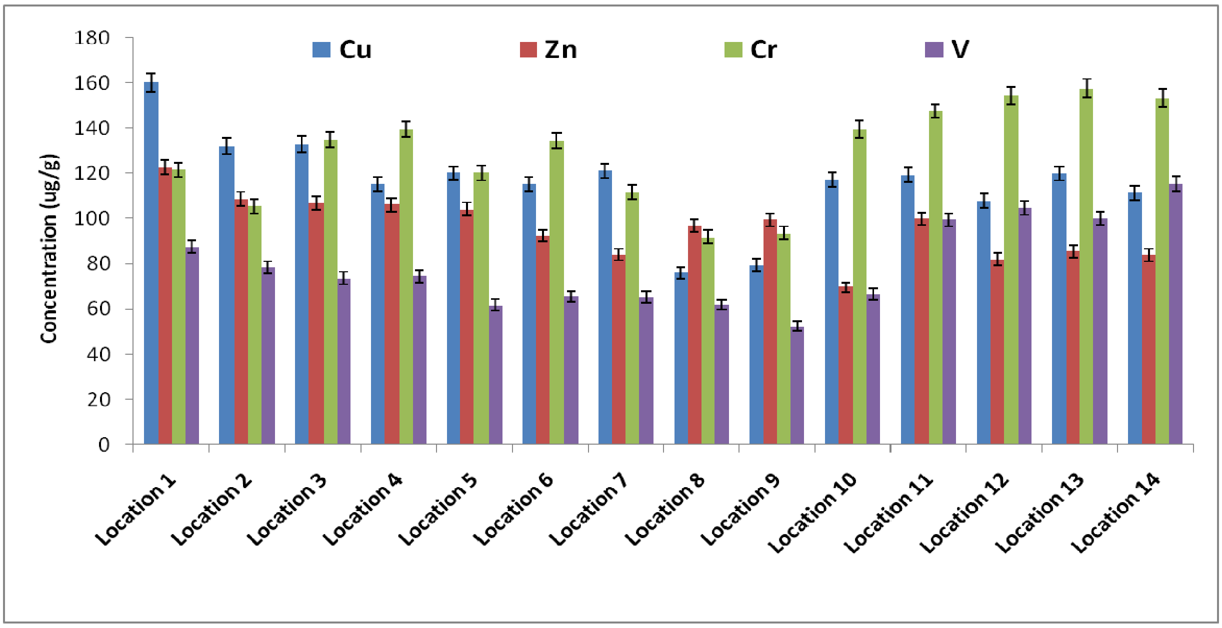


Fig.3.3b. Cu, Zn, Cr and V concentrations in sediment samples across Thane Creek area, Mumbai, India

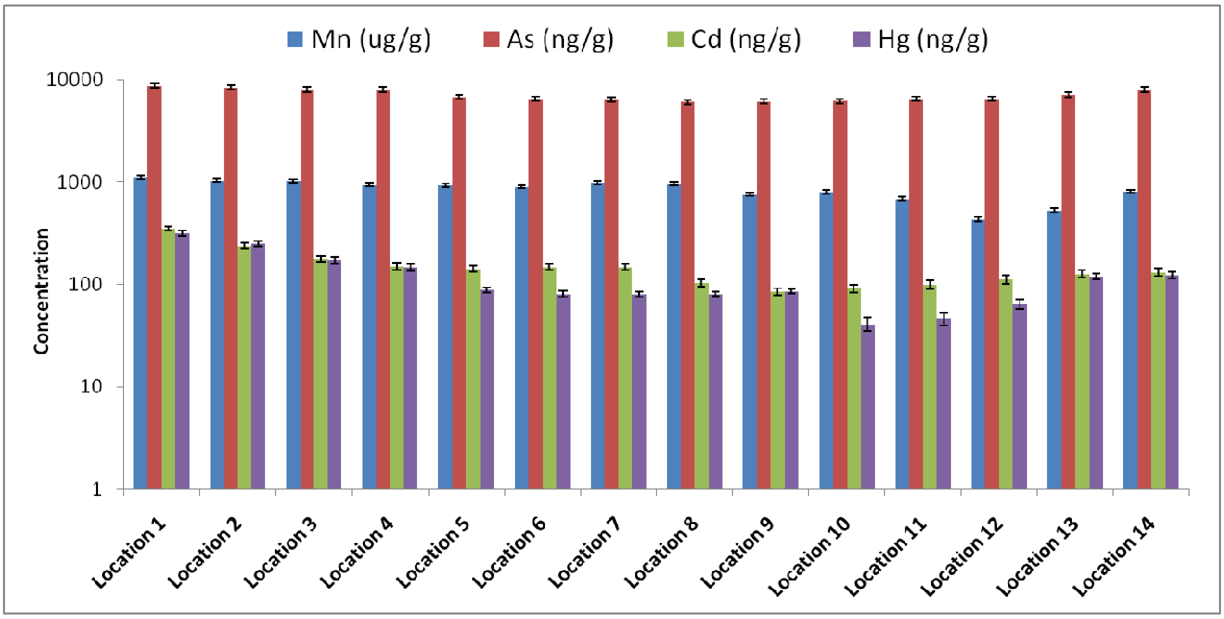


Fig.3.3c. Mn, As, Cd and Hg concentrations in sediment samples across Thane Creek area, Mumbai, India

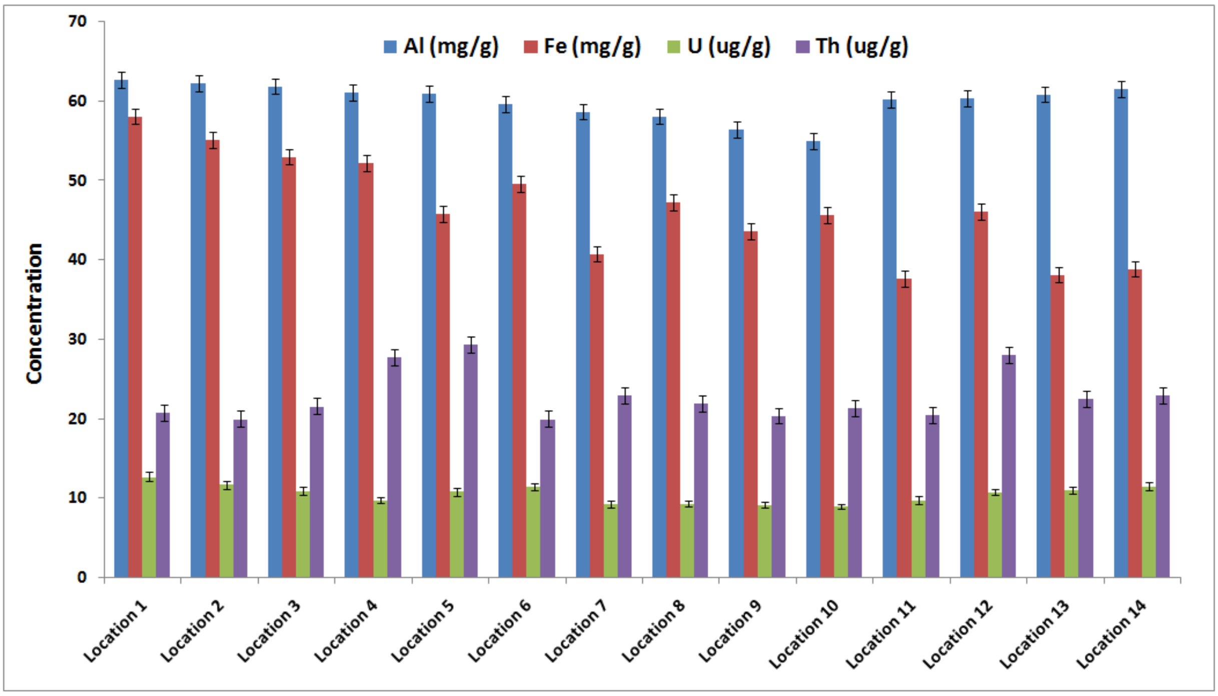


Fig.3.3d. Al, Fe, U and Th concentrations in sediment samples across Thane Creek area, Mumbai, India

3.2.1. Quality control and quality assurance

IAEA-405 (estuarine sediment) and IAEA-433 (marine sediment) reference materials were acid digested in the similar procedure as for sediment samples from Thane Creek and trace metals were analysed using HG-AAS, ICP-AES and DPASVand represented in table 3.2 . Metals of interest that were analysed in both reference materials shown good agreement to reference sheet values.

Table 3.2. Measured and certified values of trace metals in IAEA-405 and IAEA-433 reference materials

Metal	IAEA-405 recommended value (µg/g)	Observed value (µg/g)	IAEA-433 recommended value (µg/g)	Observed value (µg/g)
Fe	37400	37058	40800	39700
Zn	279	260.78	101	98.5
Mn	495	482	316	310
Ni	32.5	33.25	39.4	38.8
Co	13.7	13.4	12.9	12.5
Cr	84	82.25	136	132
Pb	74.8	73.9	26.0	25.4
Cu	47.7	47.0	30.8	30.2
Cd	0.73	0.72	0.153	0.151
Ba	-	250	268	259
V	95	93.4	160	158
As	23.6	23.0	18.9	18.5
Hg	0.81	0.79	0.168	0.161
U	3.01	2.83	2.45	2.28
Th	14.3	13.16	9.78	8.80

3.2.2. Geochemical indices calculation for trace metals in sediment

There are various **geochemical** indices which determine the degree of contamination in creek system due to trace metals. Some of them are enrichment factor (EF), geo-accumulation index (I_{geo}), contamination factor (CF), and pollution load index (PLI). Tiwari et al., (2013) [163] investigated the vertical concentration profiles of sixteen elements in core sediment samples, namely Na, Mg, Al, K, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Br, Sr, and Pb using energy dispersive X-ray fluorescence (EDXRF) technique in sediment samples collected across Thane Creek area, Mumbai. To check the anthropogenic input various indices (enrichmentfactor, contamination factor, index of geoaccumulation and pollution load index) were calculated and compared. Background concentration values of trace metals for calculation of different geochemical indices have been presented in table 3.3.

Table 3.3. Background concentration values of trace metals for calculation of different geochemical indices:

Element (µg/g)	**Al**	**Zn**	**Mn**	**Ni**	**Co**	**Cr**	**Pb**	**Cu**	**Cd**	**Ba**	**V**	**As**	**Hg**
Background Concentration (µg/g)	81529.0	67.0	774.0	47.0	17.3	92.0	17.0	28.0	0.09	628.0	97.0	4.8	0.05

3.2.2.1. Enrichment factor (EF)

Enrichment factor can be used to evaluate the metal contamination in the studied sediment in more comprehensive way. This method normalizes the measured trace metal concentration with respect to a reference metal such as Fe or Al [164]. Fe and Al usually have relatively high natural concentrations, and are therefore not expected to be substantially enriched from anthropogenic sources in estuarine sediments [165]. Currently, Al is the most frequently used geochemical normalizer in estuarine and coastal sediments [166]. Enrichment factor (EF), an appropriate tool to determine sedimentary metals' source produced by anthropogenic events or natural origin [167-168], normalizes metal concentrations according to the sediment texture properties. Enrichment factor was calculated using the formula:

$$EF = (\frac{M_{sample}}{Al_{sample}}) / \frac{M_{average\ shale}}{Al_{average\ shale}} \qquad (2)$$

M_{sample} is the concentration of the examined metal in the examined sediment, Al_{sample} represents concentration of the reference metal (Al) in the examined sediment, $M_{average}$ is the concentration of the examined metal in the average shale and $Al_{average}$ is the concentration of the reference metal in the average shale [169]. Five contamination categories are generally recognized on the basis of the enrichment factor: EF<2, depletion to minimal enrichment; $2 \leq EF < 5$, moderate enrichment; $5 \leq EF < 20$, significant enrichment; $20 \leq EF < 40$, very high enrichment; and EF > 40, extremely high enrichment [60].

3.2.2.2. Geo-accumulation Index (I_{geo})

The geo-accumulation index (I_{geo}) is a quantitative measure of the metal pollution in aquatic sediments. To characterize the level of pollution in each sampling point, I_{geo} values were calculated using the following mathematical formula [170]:

$$I_{geo} = log_2[\frac{C_n}{1.5\ B_n}] \tag{3}$$

Where, C_n is the measured concentration of metal N in the samples, and B_n is either directly measured in texturally equivalent uncontaminated soils or taken from the literature. It is distinct from EF because of the factor 1.5 is introduced to include possible variations of the background values that are due to lithogenic variations. In addition, EF does not take into account the nature and genesis of the matrix, which play a crucial role in metal contamination [87]. The Müller Index of Geoaccumulation, I_{geo}, indicates the level of contamination found in various sediments and soils. I_{geo} consists of seven grades ranging from unpolluted to very seriously polluted [171]. The contamination level may be classified in a scale ranging from 1 to 6 (unpolluted ($I_{geo} \leq 0$), unpolluted to moderately polluted ($0 < I_{geo} < 1$), moderately polluted

($1<I_{geo}<2$), moderately to strongly polluted ($2<I_{geo}<3$), strongly polluted ($3<I_{geo}<4$), strongly to very strongly polluted ($4<I_{geo}<5$), and very strongly polluted ($I_{geo}>5$)) [172].

3.2.2.3. Contamination Factor (CF)

The level of contamination of sediment by a metal is often expressed in terms of a contamination factor calculated as follows:

$$CF = \left({}^{Metal\ content\ in\ the\ sediment}\!/_{Background\ level\ of\ metal} \right) \tag{4}$$

Where, $CF<1$ refers to low contamination, $1\leq CF\leq 3$ means moderate contamination, $3\leq CF\leq 6$ indicates considerable contamination, and $CF>6$ indicates very high contamination [173].

3.2.2.4. Pollution load index (PLI)

The extent of pollution by trace metals has been assessed by employing the method based on Pollution load index (PLI) developed by Thomilson et al., 1980 [174] and the relation is shown below:

$$PLI = \sqrt[n]{(CF_1 \times CF_2 \times CF_3 \times \ldots\ldots..\times CF_n)} \tag{5}$$

Where, CF = contamination factor and n = number of metals. PLI provides a simple, comparative means for assessing a site or estuarine quality — a value of zero indicates perfection, a value of one indicates only baseline levels of pollutants present and values above one would indicate progressive deterioration of the site and estuarine quality [175].

3.2.3. Pollution load in Thane Creek area due to trace metals burden

Different geochemical indices were calculated using the following metals like Co, Ni, Cr, Cu, Pb, Ba, V, Cd, Zn, Mn, As and Hg. The values of EF for different metals were represented in table 3.3. Mn, Ni, Co, Pb, Ba and V show depletion to minimal enrichment for all the sampling locations. Significant enrichment observed for Cu in all the sampling locations except location 8 and 9. Hg shows significant enrichment at locations 1 and 2 and for Cd at location 1.

Table 3.4. Enrichment factor (EF) values for different metals in sediment across Thane Creek area, Mumbai, India

	L 1	L 2	L 3	L 4	L 5	L 6	L 7	L 8	L 9	L 10	L 11	L 12	L 13	L 14
Zn	2.4	2.1	2.1	2.1	2.1	1.9	1.7	2.0	2.1	1.5	2.0	1.7	1.7	1.7
Mn	1.9	1.8	1.8	1.6	1.6	1.6	1.8	1.7	1.4	1.5	1.2	0.8	0.9	1.4
Ni	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.6	0.9	0.9	0.9	0.9	1.0
Co	1.7	1.7	1.7	1.7	1.6	1.6	1.7	1.6	1.4	1.7	1.6	1.6	1.6	1.6
Cr	1.7	1.5	1.9	2.0	1.8	2.0	1.7	1.4	1.5	2.3	2.2	2.3	2.3	2.2
Pb	1.8	1.8	1.8	1.8	1.7	1.7	1.8	1.7	1.7	1.7	1.6	1.7	1.6	1.7
Cu	7.4	6.2	6.3	5.5	5.7	5.6	6.0	3.8	4.1	6.2	5.8	5.2	5.7	5.3
Cd	5.1	3.5	2.6	2.2	2.1	2.3	2.3	1.6	1.4	1.5	1.5	1.7	1.9	1.9
Ba	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
V	1.2	1.1	1.0	1.0	0.9	0.9	0.9	0.9	0.8	1.0	1.4	1.5	1.4	1.6
As	2.4	2.3	2.2	2.2	1.9	1.9	1.9	1.8	1.8	1.9	1.8	1.8	2.0	2.2
Hg	8.2	6.6	4.5	3.9	2.4	2.2	2.2	2.3	2.5	1.2	1.3	1.7	3.2	3.3

The calculated I_{geo} values, based on the concentration of metals in upper continental crust, are presented in table 3.4. It is evident that I_{geo} values for Mn, Ni, Co, Pb, Ba and V fall in class ‘0’ in all the sampling locations. Unpolluted to moderately polluted scenario occurs for Cd. I_{geo} values of Hg are less than 2 in all locations except location 1. I_{geo} value of Hg is more than 2 at location 1 indicates moderate to strong pollution.

Table 3.5. Geoaccumulationindex (I_{geo}) values for different metals in sediment across Thane Creek area, Mumbai, India

	L 1	L 2	L 3	L 4	L 5	L 6	L 7	L 8	L 9	L 10	L 11	L 12	L 13	L 14
Zn	0.3	0.1	0.1	0.1	0.1	-0.1	-0.3	-0.1	-0.0	-0.5	-0.0	-0.3	-0.2	-0.3
Mn	-0.1	-0.2	-0.2	-0.3	-0.3	-0.4	-0.2	-0.3	-0.6	-0.5	-0.8	-1.4	-1.1	-0.5
Ni	-1.2	-1.3	-1.3	-1.3	-1.3	-1.3	-1.3	-1.5	-1.8	-1.3	-1.2	-1.2	-1.2	-1.0
Co	-0.2	-0.2	-0.2	-0.3	-0.4	-0.4	-0.3	-0.4	-0.7	-0.4	-0.3	-0.4	-0.3	-0.3
Cr	-0.2	-0.4	-0.0	0.0	-0.2	-0.0	-0.3	-0.6	-0.6	0.0	0.1	0.2	0.2	0.2
Pb	-0.1	-0.1	-0.2	-0.2	-0.3	-0.2	-0.2	-0.3	-0.4	-0.4	-0.4	-0.3	-0.3	-0.2
Cu	1.9	1.7	1.7	1.5	1.5	1.5	1.5	0.9	0.9	1.5	1.5	1.4	1.5	1.4
Cd	1.4	0.8	0.4	0.2	0.1	0.1	0.1	-0.4	-0.7	-0.6	-0.4	-0.3	-0.1	-0.0
Ba	-4.0	-4.1	-4.8	-4.8	-5.0	-5.0	-5.2	-5.1	-5.5	-5.1	-4.9	-4.8	-4.7	-4.7
V	-0.7	-0.9	-1.0	-1.0	-1.2	-1.2	-1.2	-1.2	-1.5	-1.1	-0.6	-0.5	-0.5	-0.3
As	0.3	0.2	0.2	0.2	-0.1	-0.2	-0.2	-0.2	-0.2	-0.2	-0.2	-0.2	-0.0	0.2
Hg	2.1	1.7	1.2	1.0	0.2	0.1	0.1	0.1	0.2	-0.9	-0.7	-0.2	0.7	0.7

The CF values of metals were represented in table 3.5 for all sediment samples. All sediment samples were found in a level of low contamination with respect to Ni and Ba concentrations. Mn, Co and V were found in low to moderate contamination levels. Moderate contamination levels were found for the metals like Zn, Cr, Pb and As. Hg shows low to considerable contamination in the locations 2 to 14, but a value of 6.3 at location 1 indicates very high contamination. Most of the industrial pollutants are discharged in the close proximity of location 1.

Table 3.6. Contamination factor (CF) values for different metals in sediment across Thane Creek area, Mumbai, India

	L 1	L 2	L 3	L 4	L 5	L 6	L 7	L 8	L 9	L 10	L 11	L 12	L 13	L 14
Zn	1.8	1.6	1.6	1.6	1.6	1.4	1.3	1.4	1.5	1.0	1.5	1.2	1.3	1.3
Mn	1.4	1.3	1.3	1.2	1.2	1.2	1.3	1.2	1.0	1.0	0.9	0.6	0.7	1.0
Ni	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.5	0.4	0.6	0.7	0.7	0.7	0.7
Co	1.3	1.3	1.3	1.2	1.2	1.2	1.2	1.1	1.0	1.2	1.2	1.2	1.2	1.2
Cr	1.3	1.2	1.5	1.5	1.3	1.5	1.2	1.0	1.0	1.5	1.6	1.7	1.7	1.7
Pb	1.4	1.4	1.4	1.4	1.3	1.3	1.3	1.2	1.2	1.2	1.2	1.2	1.2	1.3
Cu	5.7	4.7	4.8	4.1	4.3	4.1	4.3	2.7	2.8	4.2	4.3	3.9	4.3	4.0
Cd	3.9	2.7	2.0	17	1.6	1.7	1.7	1.2	1.0	1.0	1.1	1.2	1.4	1.5
Ba	0.1	0.1	0.1	0.1	0.1	0.1	0.04	0.04	0.03	0.04	0.1	0.1	0.1	0.1
V	1.0	0.8	0.8	0.8	0.6	0.7	0.7	0.6	0.5	0.7	1.0	1.1	1.0	1.2
As	1.8	1.8	1.7	1.7	1.4	1.4	1.3	1.3	1.3	1.3	1.4	1.4	1.5	1.7
Hg	6.3	5.0	3.5	3.0	1.8	1.6	1.6	1.6	1.7	0.8	0.9	1.3	2.4	2.5

Values of PLI were reported in table 3.6 in all the sampling locations. The PLI values vary from 0.9 to 1.5. Maximum PLI value was observed in the location 1 and minimum in the location 9. PLI value decreases from location 1 to location 9, which shows that the pollutants are being diluted with distance from their discharge points. Decreased dispersion due to the lesser availability of back water in location 1 (as it is situated in very vertex of the creek) and its adjacent area during high tide also can be the reason for high PLI values. The values of PLI less than 1 shows that there is no significant anthropogenic contribution to the grab sediment samples in the locations 7, 8, 9, 10, 11 and 12. The PLI value 1.5 shows that there is significant anthropogenic contribution to the grab sediment sample in the location 1 only.

Table 3.7. Pollution load index (PLI) values in sediment across Thane Creek area, Mumbai, India.

	L 1	L 2	L 3	L 4	L 5	L 6	L 7	L 8	L 9	L 10	L 11	L 12	L 13	L 14
PLI	1.5	1.1	1.1	1.1	1.0	1.0	1.0	1.0	0.9	0.9	1.0	1.0	1.0	1.1

3.2.4. Sediment Quality Guidelines

To predict detrimental biological effects and conservation of the organisms living inside or near the polluted sediments, the method of Sediment Quality Guidelines (SQGs) was applied. Mostly, SQGs used for marine sediments are established based on Sediment Quality Guidelines from the American NOAA Organization. These guidelines are composed of Effect Range Guidelines derived from a series of chemical and biological information collected from the coastal areas of Northern America and a combination of experimental and field data obtained by different biological and chemical methods [176]. The concentrations of chemicals which result in 10% and 50% of adverse biological effects are called as Effect range-low (ERL) and Effect-range-medium (ERM), respectively.

Based on SQGs, trace metal contents (Zn, Ni, Pb, Cd and As) were lower than the determined limits of ERL and ERM in most of the sampling locations in this study, implicating non-harmful biological impacts of these metals on living organisms. Hg is having non-harmful biological impacts on living organisms in most of the sampling locations except location 1 to 3 where there could be occasional or frequent adverse biological impacts. Sediment samples in all the sampling locations with occasionally or frequently adverse biological impacts observed due to Cr and Cu.

3.3. Trace metals in biota

There has been growing interest in determining trace metal levels in the marine environment and attention was drawn to the measurement of contamination levels in public food supplies, particularly fish [177, 9, 178]. The ingestion of food is an obvious means of exposure to metals, not only because many metals are natural components of foodstuffs but also because of environmental contamination and contamination during processing [179-180]. The aquatic system near the industrialized areas contain large amount of trace metals, which have marked ecological significance due to their toxicity, persistence and bio-accumulation. It is being realised that infinitesimally small concentration of many trace metals have a strong influence on biological productivity of the oceans. Metal toxicity is important as they adversely affect the biota. Direct uptake from the sediment particles can be an important additional source of sediment bound contaminants for sediment feeding organisms. Trace metals have a unique property of accumulation over a period of time along a food chain. Metals that are deposited in the aquatic environment may accumulate in the food chain and cause ecological damage and also poses threat to human health [181-183]. These health concerns are quite considerable. For example, cancer, damage to the nervous system has all been documented in humans as a result of metal consumption [184]. The US Environmental Protection Agency conducted a national study of accumulated toxins documenting this concern [185]. Fish species are often the top consumers in aquatic ecosystems [186] and thus metal concentrations in fish can act as an environmental indicator of the state of the environment [187-188].

3.3.1. Trace metals in whole body biota

Different types of biota samples (Lizard fish, shrimps, prawns and crabs) (Fig. 3.4) were collected from different locations across Thane Creek area, Mumbai India. Trace metal concentrations were determined in whole body biota as well as edible part of biota. Trace metal concentrations in the whole body biota are represented in figures 3.5a, 3.5b, 3.5c and table 3.8. Cu, Zn, Mn, Fe, Cr, Ni, Co, Ba, V, As, Cd, Hg, Pb, U and Th concentrations vary from 0.86 ± 0.06 to 7.5 ± 0.3 µg/g, 7.5 ± 0.3 to 16.5 ± 0.5 µg/g, 1.5 ± 0.0 to 15.2 ± 0.4 µg/g, 24.6 ± 0.7 to 40.2 ± 1.0 µg/g, 0.36 ± 0.01 to 0.84 ± 0.03 µg/g, 0.21 ± 0.01 to 0.73 ± 0.02 µg/g, 0.11 ± 0.01 to 0.36 ± 0.01 µg/g, 0.74 ± 0.02 to 0.99 ± 0.03 µg/g, 0.21 ± 0.01 to 0.35 ± 0.01 µg/g, 22.3 ± 0.9 to 28.5 ± 1.1 ng/g, 73.6 ± 2.0 to 112.6 ± 3.1 ng/g, 20.9 ± 0.8 to 66.9 ± 1.5 ng/g, 87.7 ± 2.1 to 189.6 ± 5.1 ng/g, 42.6 ± 1.1 to 97.8 ± 2.9 ng/g and 24.6 ± 0.8 to 32.6 ± 1.0 ng/g respectively in the whole body of different types of biota samples collected from different locations across Thane Creek area, Mumbai, India. It is clearly observed that most of the trace metal concentrations in the biota samples increase in the following order Lizard fish<Shrimps<Prawns<Crabs except for Mn and Cd. This concentration trend observed may be due to Lizard fish consumes its food mostly from the aqueous medium, shrimps and prawns consume their food from both aqueous and sediment medium and crab consumes its food mostly from sediment medium. Concentrations of trace metals are higher in the sediment samples compare to seawater samples. Concentrations of Mn and Cd were observed higher in the lizard fish samples compare to shrimps and prawns that may be because of higher bioavailability of Mn and Cd compare to other trace metals. This can be further explained by studying the binding behaviour of trace metals in the sediment samples.

Lizard fish

Shrimps

Prawns

Crabs

Fig.3.4. Biota samples collected across Thane Creek area, Mumbai, India

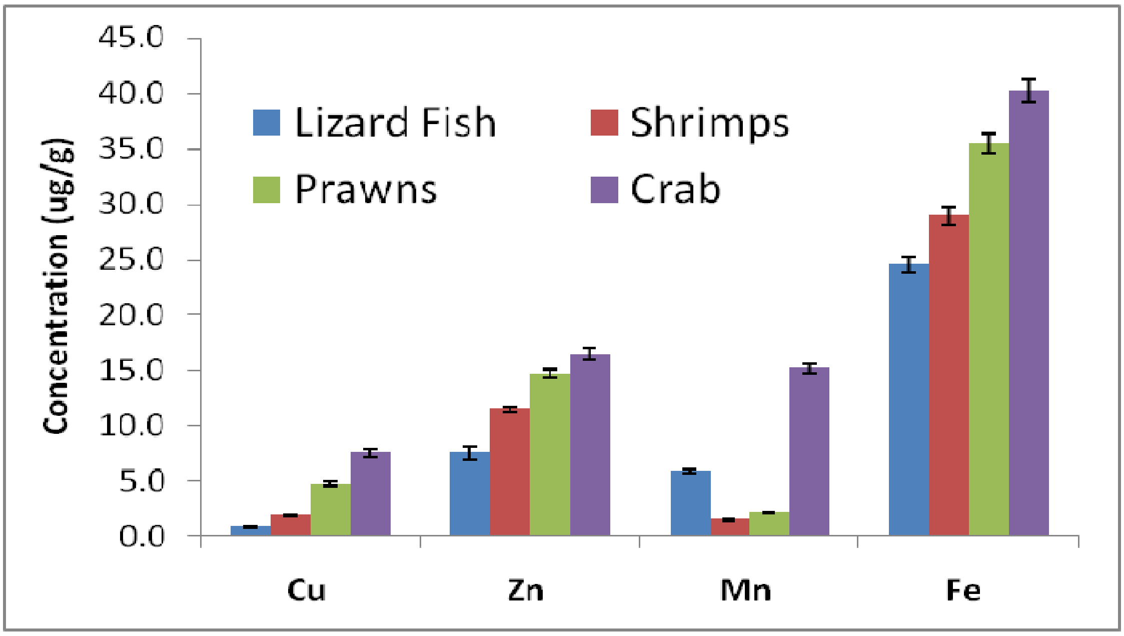


Fig.3.5a. Cu, Zn, Mn and Fe concentrations (wet weight basis) in the whole body of biota samples across Thane Creek area, Mumbai, India

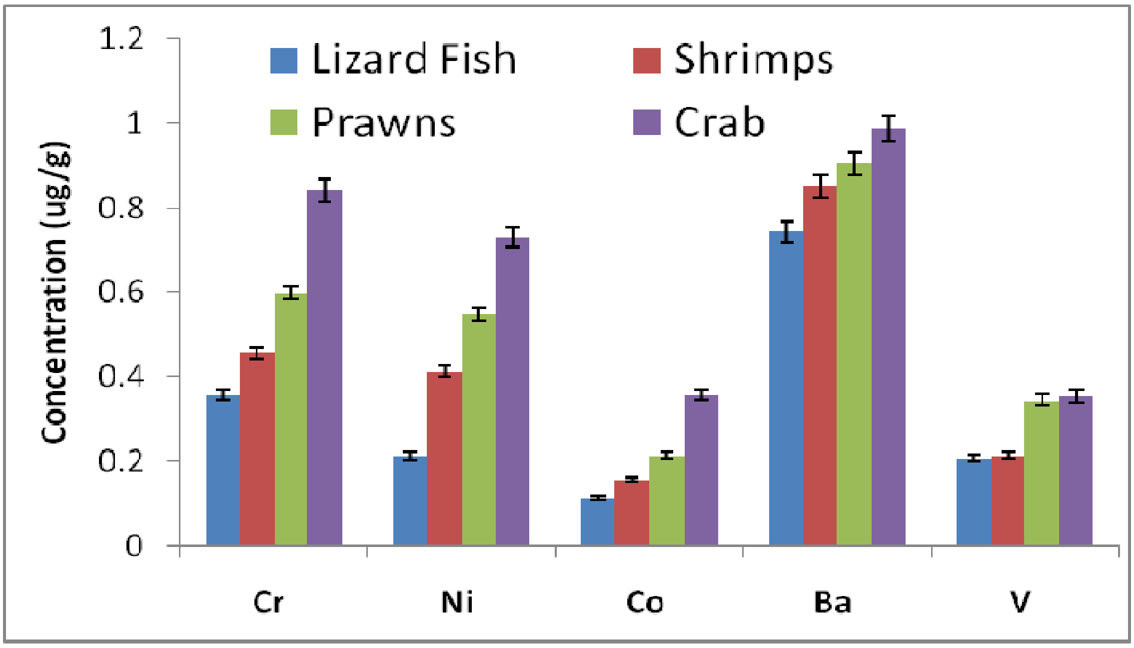


Fig.3.5b. Cr, Ni, Co, Ba and V concentrations (wet weight basis) in the whole body of biota samples across Thane Creek area, Mumbai, India

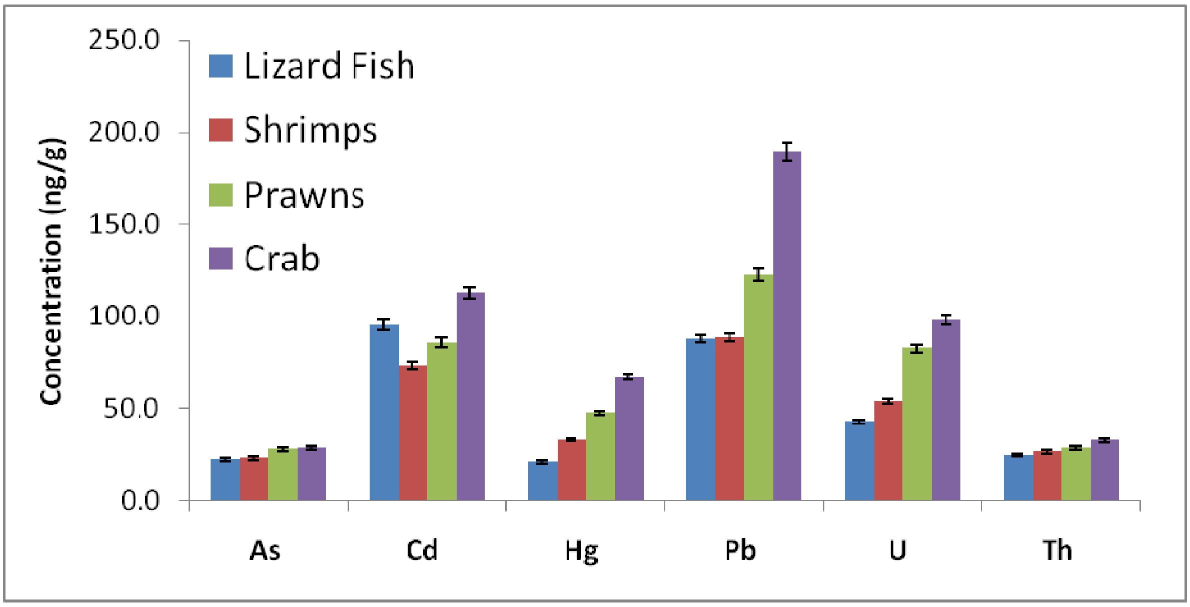


Fig.3.5c. As, Cd, Hg, Pb, U and Th concentrations (wet weight basis) in the whole body of biota samples across Thane Creek area, Mumbai, India

Table 3.8. Trace metal concentrations in the whole body biota (wet weight basis):

Element	**Lizard Fish**	**Shrimps**	**Prawns**	**Crab**
Cu (µg/g)	0.9 ± 0.04	1.8 ± 0.1	4.7 ± 0.2	7.5 ± 0.3
Zn (µg/g)	7.5 ± 0.5	11.5 ± 0.4	14.7 ± 0.4	16.5 ± 0.5
Mn (µg/g)	5.9 ± 0.2	1.5 ± 0.04	2.1 ± 0.1	15.2 ± 0.4
Fe (µg/g)	24.6 ± 0.7	28.9 ± 0.8	35.5 ± 0.9	40.2 ± 1.0
Cr (µg/g)	0.36 ± 0.01	0.46 ± 0.01	0.60 ± 0.02	0.84 ± 0.02
Ni (µg/g)	0.21 ± 0.01	0.41 ± 0.01	0.55 ± 0.02	0.73 ± 0.02
Co (µg/g)	0.11 ± 0.01	0.16 ± 0.01	0.21 ± 0.01	0.36 ± 0.01
Ba (µg/g)	0.74 ± 0.02	0.85 ± 0.03	0.90 ± 0.03	0.99 ± 0.03
V (µg/g)	0.21 ± 0.01	0.21 ± 0.01	0.35 ± 0.01	0.35 ± 0.01
As (ng/g)	22.3 ± 0.9	23.1 ± 0.9	27.9 ± 1.0	28.5 ± 1.1
Cd (ng/g)	95.2 ± 2.9	73.6 ± 2.0	85.7 ± 2.6	112.6 ± 3.1
Hg (ng/g)	20.9 ± 0.8	32.9 ± 0.8	47.6 ± 1.1	66.9 ± 1.5
Pb (ng/g)	87.7 ± 2.1	88.6 ± 2.2	122.6 ± 3.4	189.6 ± 5.1
U (ng/g)	42.6 ± 1.1	53.7 ± 1.2	82.6 ± 2.1	97.8 ± 2.9
Th (ng/g)	24.6 ± 0.8	26.6 ± 0.9	28.5 ± 0.9	32.6 ± 1.0

3.3.2. Trace metals in edible part of biota

In edible part of biota samples trace metal concentrations are represented in figures 3.6a, 3.6b, 3.6c and table 3.9. Similar type concentration trend observed in the edible part of biota and whole body biota samples. But concentrations of trace metals in the edible part of biota samples were observed lower compare to whole body biota. That means bioaccumulation of trace metals in the non edible part of biota samples were higher compare to edible part of biota. Cu, Zn, Mn, Fe, Cr, Ni, Co, Ba, V, As, Cd, Hg, Pb, U and Th concentrations vary from 0.76 ± 0.02 to 6.5 ± 0.2 µg/g, 6.5 ± 0.2 to 13.5 ± 0.3 µg/g, 1.3 ± 0.0 to 14.2 ± 0.3 µg/g, 20.6 ± 0.5 to 35.2 ± 0.7 µg/g, 0.31 ± 0.01 to 0.74 ± 0.04 µg/g, 0.18 ± 0.01 to 0.63 ± 0.03 µg/g, 0.09 ± 0.00 to 0.31 ± 0.02 µg/g, 0.64 ± 0.02 to 0.89 ± 0.04 µg/g, 0.18 ± 0.01 to 0.31 ± 0.01 µg/g, 20.1 ± 0.5 to 25.1 ± 6 ng/g, 53.6 ± 1.4 to 100.6 ± 2.5 ng/g, 19.0 ± 0.5 to 55.8 ± 1.1 ng/g, 79.7 ± 1.5 to 169.6 ± 3.8 ng/g, 32.6 ± 0.7 to 85.8 ± 0.8 ng/g and 18.6 ± 0.4 to 25.6 ± 0.5 ng/g respectively in the edible part of different types of biota samples collected from different locations across Thane Creek area, Mumbai, India.

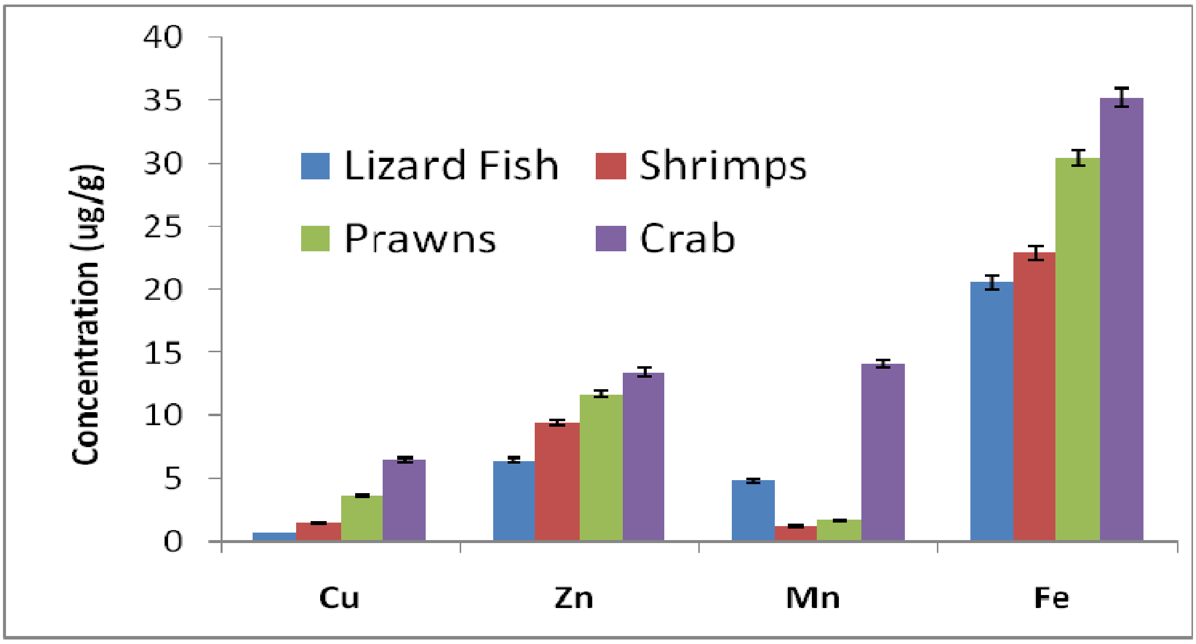


Fig.3.6a. Cu, Zn, Mn and Fe concentrations (wet weight basis) in the edible part of biota samples across Thane Creek area, Mumbai, India

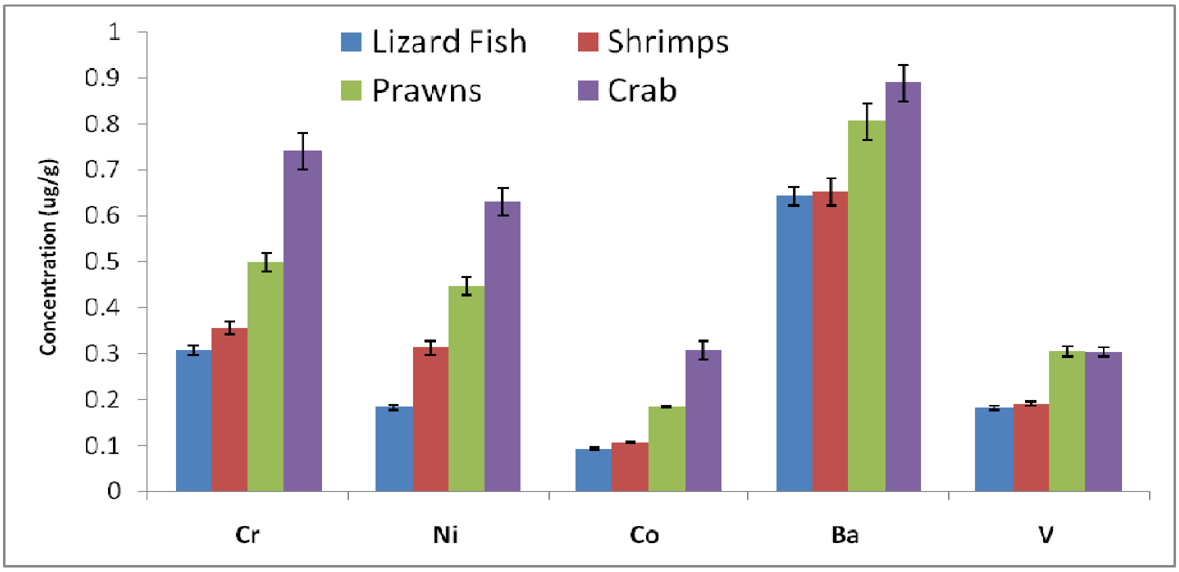


Fig.3.6b. Cr, Ni, Co, Ba and V concentrations (wet weight basis) in the edible part of biota samples across Thane Creek area, Mumbai, India

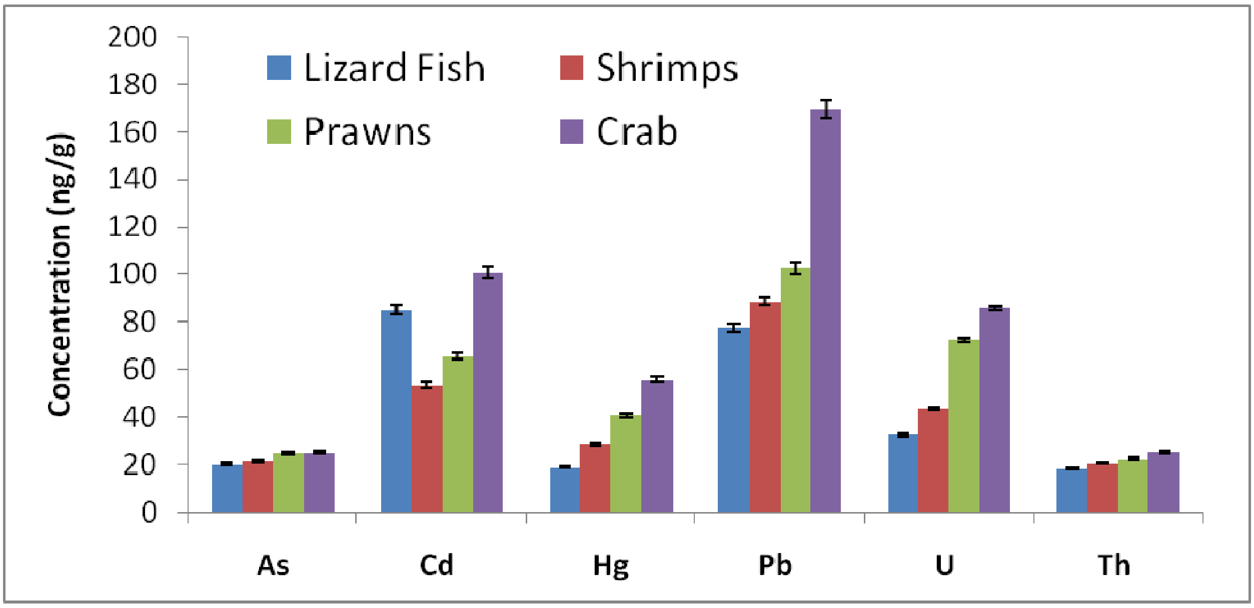


Fig.3.6c. As, Cd, Hg, Pb, U and Th concentrations (wet weight basis) in the edible part of biota samples across Thane Creek area, Mumbai, India

Table 3.9. Trace metal concentrations in the edible part of biota (wet weight basis):

Element	**Lizard Fish**	**Shrimps**	**Prawns**	**Crab**
Cu (µg/g)	0.8 ± 0.02	1.5 ± 0.04	3.7 ± 0.1	6.5 ± 0.2
Zn (µg/g)	6.5 ± 0.2	9.5 ± 0.3	11.7 ± 0.3	13.5 ± 0.3
Mn (µg/g)	4.9 ± 0.2	1.3 ± 0.03	1.7 ± 0.1	14.2 ± 0.3
Fe (µg/g)	20.6 ± 0.5	22.9 ± 0.6	30.5 ± 0.6	35.2 ± 0.7
Cr (µg/g)	0.31 ± 0.01	0.36 ± 0.02	0.50 ± 0.02	0.74 ± 0.04
Ni (µg/g)	0.18 ± 0.01	0.31 ± 0.02	0.45 ± 0.02	0.63 ± 0.03
Co (µg/g)	0.09 ± 0.002	0.11 ± 0.003	0.18 ± 0.003	0.31 ± 0.02
Ba (µg/g)	0.64 ± 0.02	0.65 ± 0.03	0.80 ± 0.04	0.89 ± 0.04
V (µg/g)	0.18 ± 0.01	0.19 ± 0.01	0.31 ± 0.01	0.30 ± 0.01
As (ng/g)	20.1 ± 0.5	21.5 ± 0.5	24.8 ± 0.6	25.1 ± 0.6
Cd (ng/g)	85.2 ± 2.1	53.6 ± 1.4	65.7 ± 1.6	100.6 ± 2.5
Hg (ng/g)	19.0 ± 0.5	28.7 ± 0.6	40.9 ± 0.9	55.8 ± 1.1
Pb (ng/g)	77.7 ± 1.5	88.6 ± 1.6	102.6 ± 2.6	169.6 ± 3.8
U (ng/g)	32.6 ± 0.7	43.7 ± 0.7	72.6 ± 0.9	85.8 ± 0.9
Th (ng/g)	18.6 ± 0.4	20.6 ± 0.4	22.5 ± 0.5	25.6 ± 0.5

CHAPTER-4

Dependency of Trace metal concentrations in Sediment on Physico-Chemical Parameters

4.1. Physico-chemical Characterization of Sediment samples

4.1.1. Particle size distribution of sediment samples

Dried sediment samples were size segregated in three size fractions namely very coarse, coarse and medium sand (2.0 mm - 250 μm), fine sand and very fine sand (250 μm - 53 μm), and silt and clay (< 53 μm) to understand the physical characteristics of sediment samples. All the locations, except 8, 10 and 14 are basically predominated with fine sand and very fine sand in varying percentages (Figure 4.1). Whereas, the major grain size fraction is the very coarse, coarse and medium sand for locations 8, 10 and 14. The percentage of silt & clay is very low in comparison with other two fractions. The distribution of very coarse, coarse and medium sand and silt & clay are found to be higher than that of fine sand and very fine sand fractions. Large variations were observed in very coarse, coarse and medium sand and silt & clay fractions (15 to 55 % (w/w) with a mean value of 36 ± 11 % and 3 to 18 % (w/w) with a mean value of 10 ± 4 % respectively). The percentage of fine sand and very fine sand is relatively high in all locations and it varies from 40 and 67 % (w/w) with the mean value of 55 ± 8 %.

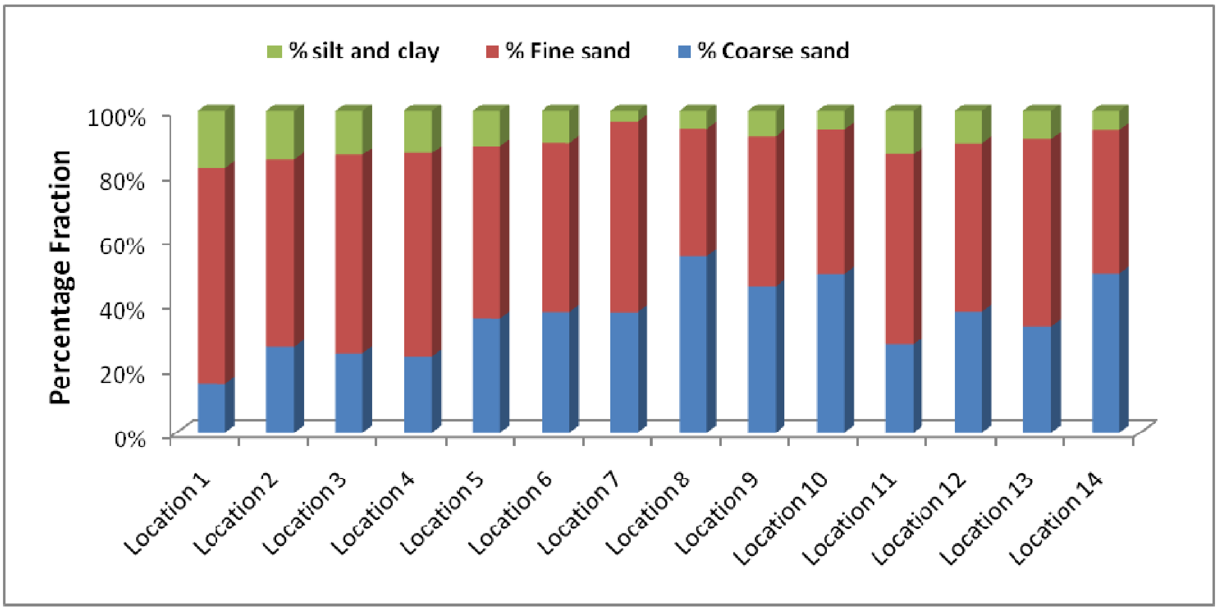


Fig.4.1. Size fractionation of sediment samples across Thane Creek area, Mumbai, India

4.1.2. pH in sediment samples

pH of the sediment samples vary from 7.90 ± 0.01 to 8.33 ± 0.03 with an average value of 8.2 ± 0.12 in the different sampling locations represented in figure 4.2.

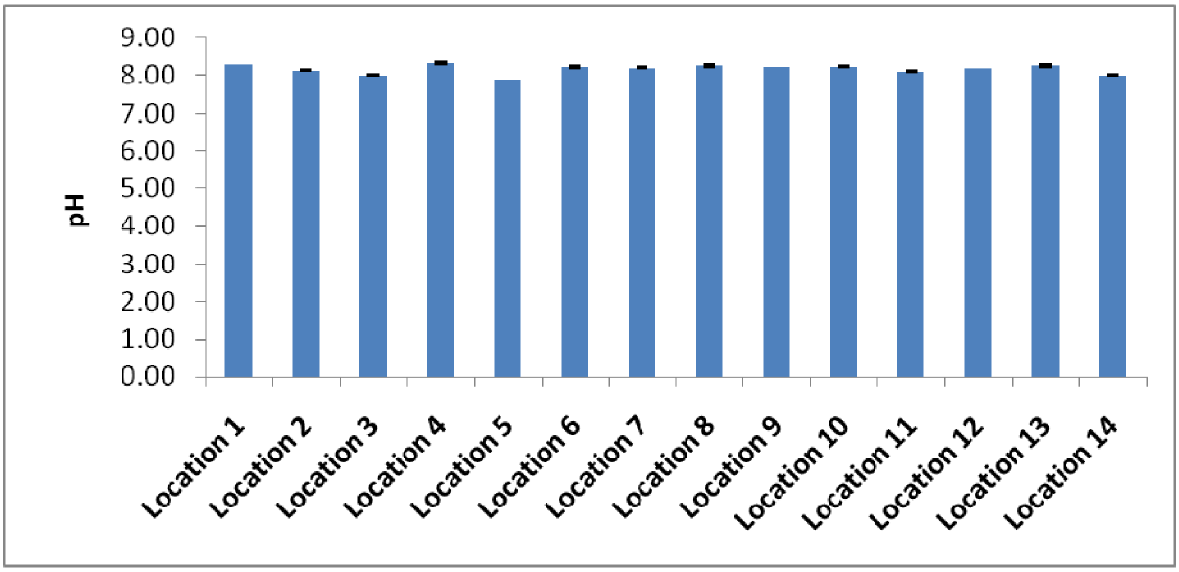


Fig.4.2. pH of sediment samples across Thane Creek area, Mumbai, India

4.1.3. $CaCO_3$ in sediment samples

Moderate variation was not observed in $CaCO_3$ concentration ranging from 19.4 ± 0.2 to 23.3 ± 0.3 % with an average value of 21.1 ± 1.1 in different sampling locations represented in figure 4.3.

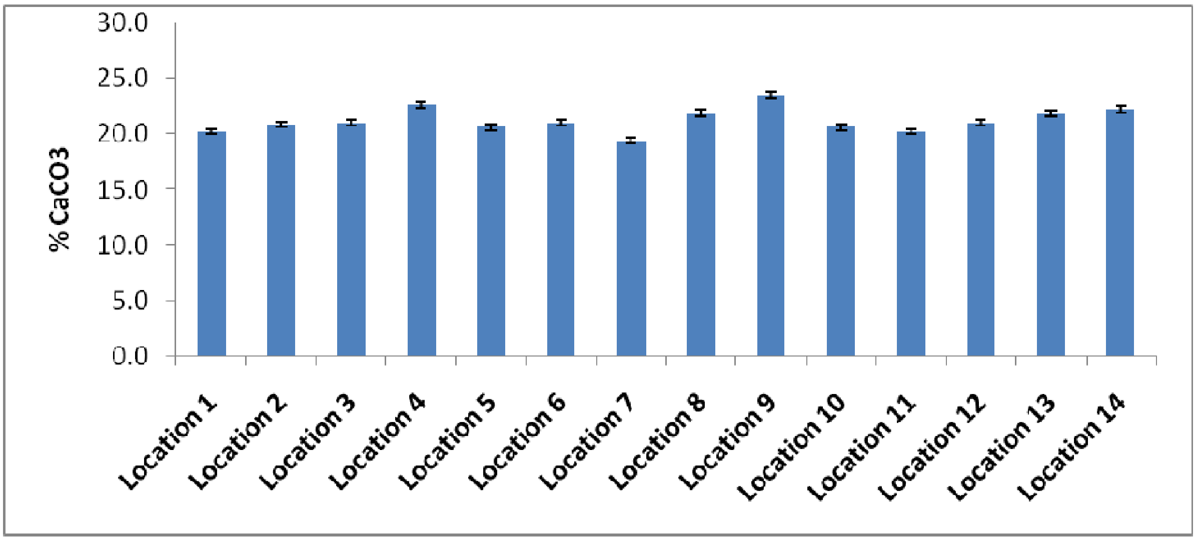


Fig.4.3. $CaCO_3$ of sediment samples across Thane Creek area, Mumbai, India

4.1.4. CEC in sediment samples

CEC also shows smaller variation ranging from 104 ± 2 to 144 ± 2 miliequivalent/100g with an average value of 127 ± 11 miliequivalent/100g at the different locations represented in figure 4.4.

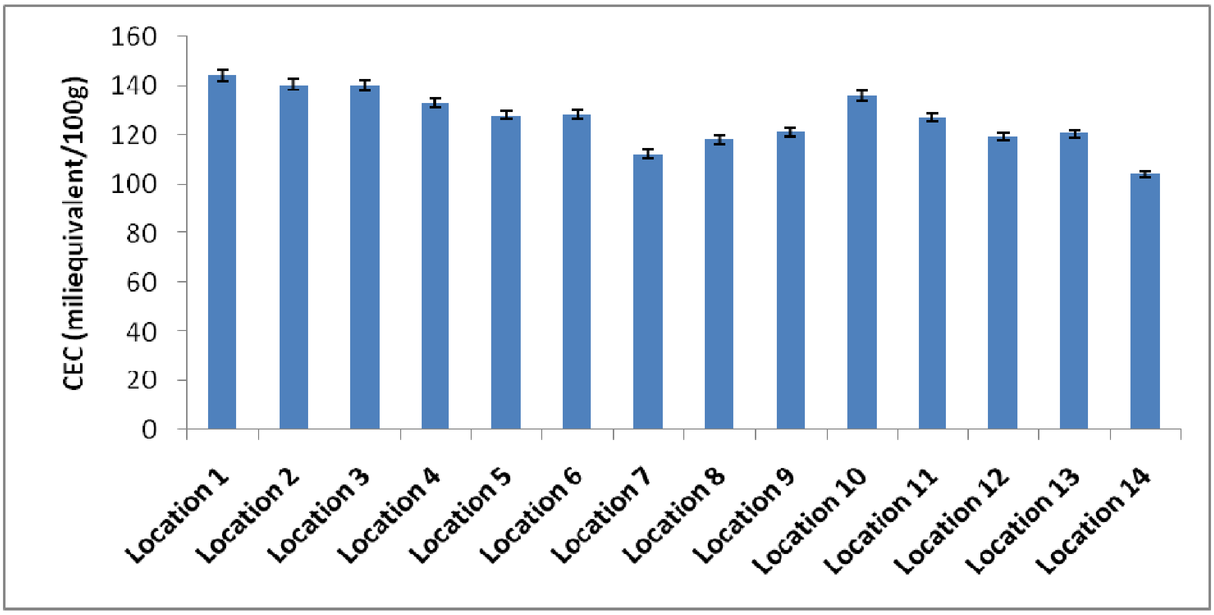


Fig.4.4. CEC of sediment samples across Thane Creek area, Mumbai, India

4.1.5. TC in sediment samples

TC content are quite uniformly distributed ranging from 2.33 ± 0.02 to 3.12 ± 0.04 % with an average value of 2.67 ± 0.24 % throughout the locations represented in figure 4.5.

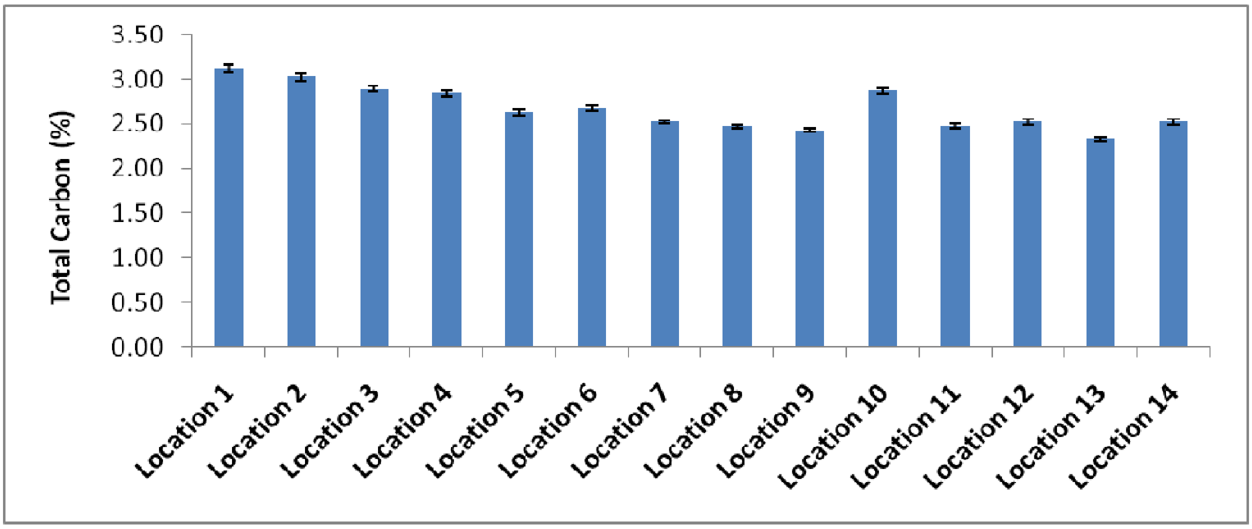


Fig.4.5. TC of sediment samples across Thane Creek area, Mumbai, India

The major factors affecting spatial variation of trace metals in the sediment are total organic matter (TOM) and the grain size [189-191]. The fine grains, presenting the higher surface to volume ratio and ionic absorption power, are more capable in the absorption of contaminated organic and inorganic materials [192-193]. Generally, fine-grained sediments with their increased amount of organic matter tend to be more contaminated than coarse-grained sediments [194].

4.2. Correlation study between different physico-chemical parameters and trace metals concentration in sediment samples across Thane Creek area

One to one correlation matrix between trace metal concentrations and different physico-chemical parameters of grab sediments **is** shown in table 4.1. Negative relationship is found between the very coarse, coarse and medium sand and sedimentary metal contents. A positive correlation was found between fine sand and very fine sand, silt and clay and total carbon with metals concentrations. This shows the important role of fine sand and very fine sand, silt and clay and total carbon in controlling spatial distribution of sedimentary metal concentrations. Factors like pH, CEC and $CaCO_3$ found to have lesser impact in deciding metal concentration. These results indicate either simultaneous entry of metals to aqueous environments bound to silt & clay and total carbon or their similar sources. Results from the correlation matrix (table 4.1), which indicate that metal concentration is positively correlated with fine sand and very fine sand fraction, silt and clay and total carbon could be slightly misleading. Multiple regression analysis reveals good correlation between concentration of metals like As, Ba, Cd, Co, Cu, Hg, Mn, Pb and Zn to different sediment parameters like very coarse, coarse and medium sand, fine sand and very fine sand , silt and clay and total carbon (table 4.2). The total carbon content is higher in the silt and clay fraction compared to the fine sand and very fine sand and very coarse, coarse and medium sand fraction. Multiple regression equations suggest that metal concentrations have a positive correlation with only total carbon content but have a negative relation with very coarse, coarse and medium sand, fine sand and very fine sand and silt and clay. The results highlight the prime role of total carbon as carriers of sedimentary metals and its contribution in deciding spatial distribution of trace metals in the sediment. There were no significant correlations observed between U and Th concentrations and different physico-chemical parameters of sediment samples. This is may be because of there was no anthropogenic origin of U and Th in the sediment samples across Thane creek area. Fernandes and Nayak (2014) [195] investigated the concentration, distribution and speciation of metals (Fe, Mn, Cu, Pb, Co, Zn and Cr) in sediments from Thane Creek area, Mumbai region. They found good association of metals with grain size and organic matter content in sediment samples. Risk Assessment Code suggested low risk to the aquatic environment.

Table 4.1. Correlation matrix of metals and different physicochemical parameters in grab sediment samples across Thane Creek area, Mumbai, India

Parameters	As	Ba	CaCO3	Cd	CEC	Co	Very coarse, coarse and medium sand	Cr	Cu	Fine sand and very fine sand	Hg	Mn	Ni	Pb	pH	Silt and clay	Total Carbon	V	Zn	Fe	U	Th
As	1.0																					
Ba	0.9	1.0																				
CaCO3	0.0	-0.3	1.0																			
Cd	0.8	0.9	-0.4	1.0																		
CEC	0.5	0.5	-0.3	0.6	1.0																	
Co	0.8	0.8	-0.5	0.7	0.4	1.0																
Very coarse, coarse and medium sand	-0.7	-0.7	0.3	-0.7	-0.7	-0.7	1.0															
Cr	0.2	0.1	-0.1	-0.1	-0.1	0.3	-0.2	1.0														
Cu	0.7	0.8	-0.6	0.8	0.6	0.8	-0.8	0.3	1.0													
Fine sand and very fine sand	0.6	0.6	-0.4	0.6	0.5	0.6	-1.0	0.2	0.8	1.0												
Hg	0.9	0.9	-0.1	0.9	0.6	0.7	-0.7	-0.2	0.7	0.6	1.0											
Mn	0.5	0.3	-0.2	0.6	0.5	0.4	-0.3	-0.6	0.4	0.3	0.6	1.0										
Ni	0.5	0.5	-0.4	0.2	-0.1	0.7	-0.2	0.8	0.6	0.2	0.2	-0.2	1.0									
Pb	0.9	0.7	-0.2	0.8	0.5	0.8	-0.6	-0.1	0.7	0.6	0.8	0.7	0.3	1.0								
pH	-0.1	0.0	0.2	0.1	0.1	-0.1	0.0	-0.1	-0.1	0.1	0.1	-0.1	-0.3	-0.1	1.0							
Silt and clay	0.7	0.7	-0.2	0.7	0.8	0.5	-0.9	0.1	0.7	0.7	0.7	0.3	0.2	0.6	0.0	1.0						
Total Carbon	0.7	0.7	-0.3	0.8	0.8	0.6	-0.6	-0.1	0.7	0.5	0.7	0.7	0.2	0.7	0.1	0.7	1.0					
V	0.4	0.4	0.0	0.1	-0.3	0.4	-0.2	0.8	0.3	0.2	0.1	-0.5	0.8	0.1	-0.1	0.2	-0.1	1.0				
Zn	0.6	0.6	0.0	0.7	0.6	0.4	-0.7	-0.4	0.4	0.6	0.7	0.6	-0.2	0.6	-0.1	0.8	0.5	-0.2	1.0			
Fe	0.6	0.6	-0.1	0.7	0.8	0.4	-0.5	-0.3	0.4	0.4	0.7	0.6	-0.1	0.7	0.2	0.7	0.9	-0.3	0.7	1.0		
U	0.1	0.1	0.1	0.1	0.1	-.1	0.3	0.3	0.1	-0.4	0.1	-0.1	0.3	0	0.0	-0.1	0.3	0.1	-0.3	0.1	1.0	
Th	0.0	0.0	0.0	-0.1	-0.2	0.0	0.3	0.3	0.0	-0.2	-0.2	-0.1	0.4	0.1	-0.3	-0.2	0.0	0.2	-0.3	-0.1	0.4	1.0

Table 4.2. Multiple regression equations for metals with correlation coefficient values

Multiple regression equation	Correlation Coefficient (R^2)
As = 666.133 - 6.66745*Very coarse, coarse and medium sand - 6.61534*Fine sand and very fine sand - 6.66819*Silt and Clay + 1.90602*Total Carbon	66.9
Ba = 6494.8 - 65.282*Very coarse, coarse and medium sand - 64.9577*Fine sand and very fine sand - 64.7488*Silt and Clay + 17.4812*Total Carbon	67.9
Cd = 63.2701 - 0.638328*Very coarse, coarse and medium sand - 0.633357*Fine sand and very fine sand - 0.641727*Silt and Clay + 0.185717*Total Carbon	82.5
Co = 453.777 - 4.48283*Very coarse, coarse and medium sand - 4.35797*Fine sand and very fine sand - 4.58267*Silt and Clay + 3.59902*Total Carbon	58.6
Cu = 5967.95 - 60.5819*Very coarse, coarse and medium sand - 58.6399*Fine sand and very fine sand - 61.7777*Silt and Clay + 42.9079*Total Carbon	77.7
Hg = 78.8698 - 0.794551*Very coarse, coarse and medium sand - 0.79009*Fine sand and very fine sand - 0.796081*Silt and Clay + 0.18814*Total Carbon	79.7
Mn = 55536.0 - 566.201*Very coarse, coarse and medium sand - 561.329*Fine sand and very fine sand - 587.383*Silt and Clay + 712.423*Total Carbon	51.3
Pb = 1162.31 - 11.5573*Very coarse, coarse and medium sand - 11.4488*Fine sand and very fine sand - 11.7032*Silt and Clay + 4.05151*Total Carbon	77.9
Zn = 5358.19 - 52.9671*Very coarse, coarse and medium sand - 52.7985*Fine sand and very fine sand - 50.6895*Silt and Clay + 1.42205*Total Carbon	67.9

CHAPTER-5

Binding Behavior of Trace Metals in Marine Ecosystem

5.1. Metal speciation in environmental matrices

Generally, trace metals accumulate in the sediment [196-197]. However, there has been considerable concern about trace metals in recent years [198], because of their toxicities, persistence, abiotic degradation, and bioaccumulation in the environment [199]. When water environment conditions change, trace metals in the sediments can be released into the overlying water, deteriorating the quality of the water [200-201]. It is therefore imperative that trace metal pollution in sediments is evaluated.

Trace metal concentrations in sediment, soil and groundwater is one of the largest threats to environmental quality and human health. It is often stated that sediment is the most important reservoir or sink of metals and other pollutants [202]. Toxic trace metals released into aquatic systems are generally bound to particulate matter, which eventually settles and becomes incorporated into sediments. However, some of the sediment-bound metals may remobilize and be released back to waters with a change of environmental conditions, and impose adverse effects on living organisms. Besides the physical, chemical and biological characteristics of the interstitial water and the sediment, the chemical partitioning of trace metals between different sediment forms is very important in determining the bio-availability of trace metals [203].

Total trace metal concentrations are often used to assess trace metal pollution in sediments, but the total concentration poorly reflects the mobility and bioavailability of the trace metals [204, 81]. In general, trace metal properties in environmental media depend on the form in which each species is bound coupled with the reactivity of that species, rather than on the total trace metal concentrations [205], so there is considerable interest in understanding metal speciation in sediments and other environmental media.

The European Community Bureau of Reference sequential extraction procedure (BCR-SEP), which involves using a series of selective extractants, separating the trace metals into four forms, has been used as an evaluative tool [206]. The BCR-SEP had been widely used to investigate trace metal speciation in various environmental media, such as soil [207], sewage sludge [208], fresh water sediments [209], salt water sediments [210], and roadside dust [81], because it is easy to perform and provides a realistic estimate of trace metal contamination in the environment. Using sequential extraction procedures (SEPs) has allowed indicators to be developed that comprehensively reflect trace metal pollution. For example, the risk assessment code (RAC) has been used to evaluate environmental risks from trace metals and to estimate the potential damage they could cause to sediment-dwelling organisms [204].

The mobility of metals in each fraction is determined by its specific form binding and coupled reactivity [81]. The metal speciation accompanying its mobility will change when external metals are introduced [211]. Consequently, the metal mobility is different in each case due to the differences of pollution levels in sediments from the residential, the mixed, and the agricultural areas. Metals in 1st fraction are considered to be the most mobile [197], and this fraction is often assumed to predominantly reflect anthropogenic inputs. The reducible fraction (2nd fraction) is usually also seen as a mobile phase [204]. The oxidizable fraction (3rd fraction) is also classified as a mobile phase [197, 212]. The residual fraction (4th fraction) is less available than the other fractions to aquatic organisms [197, 81, 213]. The proportion of the total heavy metal concentration found in the residual fraction can be used as an indicator of the degree of anthropogenic contamination present in sediment, higher proportions meaning less anthropogenic contamination [214].

5.2. Sequential Extraction procedure

Sequential extraction was carried out in the sediment samples collected across Thane Creek area, Mumbai, India to understand the binding behavior of trace metals in the sediment samples. Sequential extraction procedure is carried out in four steps and four fractions were generated.

5.2.1. Step 1 (FRACTION 1: Easily exchangeable and acid extractable fraction)

Dried sediment samples (2g) were taken and 0.11 (M) 80mL CH_3COOH was added to it. Then extraction was carried out in microwave system. The details of the microwave program for all four steps are represented in table 5.1. After extraction in microwave sample mixture was centrifuged at 6000 rpm. The supernatant was separated through filtration using 0.45μm filter paper. In the separated supernatant 1mL electronic grade concentrated nitric acid was added and evaporated near to dryness. Then 0.25 % electronic grade nitric acid was added and volume was made up in 25mL volumetric flask. This solution represents the fraction 1. Metals loosely bound to surfaces and edges of minerals or carbonates come into the 1st fraction. Exchangeable and acid extractable fraction of metals released easily into the solution phase using weak acid like CH_3COOH.

5.2.2. Step 2 (FRACTION 2: Fraction bound Fe- and Mn-oxide minerals)

Residue remaining in the 1st step is used in the 2nd step. In the residue 80mL of 0.5 (M) $NH_2OH.HCl$ was added and extraction was carried out in microwave. After extraction in the microwave, sample solution was prepared as following the 1st step. This solution represents the fraction 2. A reducing agent is used to convert the solid and oxidized forms of Fe and Mn to reduced and soluble species (e.g. Fe^{2+} and Mn^{2+}). Metals associated with oxides of Fe and Mn gets released to the solution phase using the reducing agent and come into the 2nd fraction.

5.2.3. Step 3 (FRACTION 3: Fraction bound to organic matter)

Residue remaining in the 2nd step is used in the 3rd step. In the residue 18mL of 30% H_2O_2 and 2mL double distilled water were added and evaporated near to dryness in boiling water bath. Temperature of the solution mixture was mentioned 80-85^0 C during this drying procedure. This procedure was carried out twice. In the nearly dried residue 80mL of 1(M) CH_3COONH_4 was added and extracted in microwave. After extraction in the microwave, sample solution was prepared as following the 1st step. This solution represents the fraction 3. In organic phase, metals bound to this phase are assumed to stay in the sediment for longer periods but may be immobilized by decomposition process. Under oxidizing conditions, degradation of organic matter can lead to a release of soluble trace metals bound to this component. An oxidizing agent is used (H_2O_2) to break organic moiety and bring metals to solution phase. CH_3COONH_4 is added to avoid metal sorption on oxidized solid surfaces.

5.2.4. Step 4 (FRACTION 4: Residual fraction)

Residue remaining in the 3rd step is used in the 4th step. In the residue 24mL 16(N) electronic grade HNO_3, 4mL 12(N) electronic grade HCl and 8mL 23(N) electronic grade HF were added and extraction was carried out in the microwave system. After microwave digestion solution was completely evaporated and 20mL 16(N) electronic grade HNO_3 was added and again completely evaporated in Teflon container to remove HF. Complete evaporation was carried out twice. After that 0.25% electronic grade nitric acid was added and filtered through

0.45µm filter paper and final volume was made in 25mL volumetric flask. This solution represents the fraction 4. Residual phase serve as a useful tool in the assessment of the long-term potential risk of heavy metal or toxic metals entering into the biosphere. Strong acid mixtures (HCl, HNO_3, HF) can destroy the sediment matrix and bring the remaining metals into solution phase. By getting all the four fractions, trace metals were analyzed using ICP-AES and DPASV.

Table 5.1. Microwave program in the four steps of sequential extraction procedure

Set 1	Time (min)	Power (W)	Sample reaction temp (T_1 ^0C)	Outside sample container temp (T_2 ^0C)
	3	900	60	50
	5	900	60	50
	10	Vent	-	-
Set 2	3	900	60	50
	5	900	60	50
	10	Vent	-	-
Set 3	3	900	60	50
	5	900	60	50
	10	Vent	-	-
Set 4	10	1000	200	180
	20	1000	220	180
	10	Vent	-	-

5.3. Trace metals in different fractions of sequential extraction procedure

Trace metals (Cu, Zn, Cr, V, Ni, Pb, Co, Mn, Cd, Ba, Fe, U and Th) were sequentially extracted to understand the binding behaviour in grab sediment samples collected from fourteen different locations across Thane Creek area, Mumbai, India. Trace metals were found in different percentage in the four sequentially extracted fractions represented in figures 5.1a, 5.1b, 5.1c, 5.1d, 5.1e, 5.1f, 5.1g, 5.1h, 5.1i, 5.1j, 5.1k, 5.1l and 5.1m. All the studied trace metals (Cu, Zn, Cr, V, Ni, Pb, Co, Mn, Cd, Ba, Fe, U and Th) in the sequential extraction procedure were found mainly associated with residual fraction in varying percentages like 62 – 62.8 %, 66.2 – 69.7 %, 58.7 – 62.1 %, 87.6 – 90.3 %, 63.1 – 66.2 %, 59.4 – 63.4 %, 72.4 – 76.8 %, 45.3 – 48.7 %, 46.6 – 49.6 %, 79.4 – 82.3 %, 68.7 – 70.8 %, 54 – 62.1 % and 58.2 – 68 % respectively in different sampling locations across Thane Creek area, Mumbai, India. The dominance of the residual fraction for the studied trace metals of the Creek is probably due to the low level of total trace metal concentrations in the sediment samples. Most of the total trace metals come from the parent material of geological origin and may exist in the residual form in the sediments. The anthropogenic sources are less important for studied trace metals in these sediments. High levels of trace metals in residual phase reveals that these metals are relatively insensitive to any change of surrounding conditions. Also, this result presents that Cr, V, Co, Cd and Ni reflect the geological characteristics [215]. Kaplan and Serkiz (2001) [216] also found similar behaviour and showed that uranium was associated primarily with the residual fraction (up to 91 %) in wetland uncontaminated sediment. From earlier observations by Martı´nez-Aguirre and Peria´n˜ez (2001) [217] showed that the distribution of thorium in sediment was found mainly in the residual fraction. Luji Bo (2015) [218] reported that most predominant fraction is residual fraction for Cr and Ni in sediment samples. According to the speciation of trace metals, residual phase of trace metals (Co, Cr, Cu, Ni, Pb, Zn, V and Cd) was dominated in sediment samples at all sampling sites [219]. Trace metals (Cu, Zn, Cr, V, Ni, Pb, Co, Ba, Fe, U and Th) contribution in the first fraction of the sequential extraction procedure was observed to be lowest in varying percentages like 1.23 – 2.45 %, 0.29 – 0.45 %, 1.21 – 2.15 %, 0.25 – 0.36 %, 2.8 – 3.9 %, 0.64 – 0.91 %, 2.1 – 3.4 %, 1.7 – 2.3 %, 2.5 – 3.9 %, 2.6 – 4.7 % and 1.5 – 4.5 % respectively in the sampling locations exception observed for Mn and Cd (17.4 -19.7 % and 27.4 – 28.5 %). First fraction of the sequential extraction procedure represent bio available fraction. Bio available fraction is higher for Mn and Cd compare to other studied metals. The bioavailable fraction demonstrates the fraction that when the right pH and redox conditions are favorable, the metal will be soluble taken up by aquatic biota which then will cause environmental toxicity [209]. The percentage of trace metals (Zn, Cr, V, Ni, Pb, Co and Fe) associated with different fractions was in the order: Residual>Fe-Mn oxide>Organic>Exchangeable and acid extractable. These results are in agreement with the several other studies [220-221]. It has been reported that Zn can be associated with Fe-Mn oxides of soils and sediments [220,222, 223]. The Zn adsorption onto these oxides has higher stability constants than onto carbonates. Fe and Mn oxides were reported to be the main carriers of Zn from the fluvial environment to the lagoonal one [220]. Association of Cu, Ba, U and Th in different fractions were observed in slightly different order like Residual> Organic >Fe-Mn oxide >Exchangeable and acid extractable. The Fe-Mn oxide fraction was the 2nd most important fraction for Mn in the studied sediment samples, followed by the exchangeable and acid extractable fraction. The organic fractions were found to be minor contributors for Mn. In the case of Mn and Cd, the exchangeable phase had a relatively high percentage (17.4 – 19.7 % and 27.4 – 28.5 % respectively). Significant high concentrations of exchangeable Mn in sediments have already been reported [224]. These authors suggested that in most natural waters Mn (II) oxidation is a much slower process than Fe (II) oxidation. Because of this, the presence of Mn in exchangeable sites is always expected in sediments. Except residual fraction, the next important phase of Cd in sediment was the exchangeable and acid extractable fraction. Ma and Rao (1997) [222] reported that the Cd association with different fractions followed the order: residual > exchangeable > carbonate > Fe-Mn oxide > organic > water soluble in soil samples.

5.4. Risk Assessment Code (RAC)

The RAC is defined as the exchangeable and carbonate fraction of a metal (the percentage of the metal found in F1 for the BCR method) in sediment samples. RAC values were interpreted following the method described by Perin *et al.* (1985) [225], who suggested that an RAC of less than 1% indicates that there is no risk to the aquatic environment from the metal, an RAC of 1–10% indicates low risk, an RAC of 11–30% indicates medium risk, and that an RAC of 31–50% indicates high risk. An RAC of more than 50% means that the sediment poses a very high risk to the aquatic environment and should be considered dangerous because the trace metals will easily be able to enter the food chain. The sediments had no risks associated with Zn, V and Pb, which had RAC values below 1 % in all of the samples, indicating that these metals mobility is very less in the creek studied. All of the samples had low risks (RAC values within 1-10%) associated with Cr, Ni, Co, Fe, Cu, Ba, U and Th. Mn and Cd shows medium risk (RAC values within11 - 30%) in all the sediment samples. The results suggested that Cd and Mn were slightly easily released by the sediments into the water in the creek, and therefore that they would be likely to enter the food chain. Similar results obtained by Nemati *et al.* (2011) [204], using sediments from Sungai Buloh (Malaysia) highly polluted by contaminants contained in industrial effluents, showed that 33% of Cd was present in the acid-soluble fraction, resulting in a high risk to the environment.

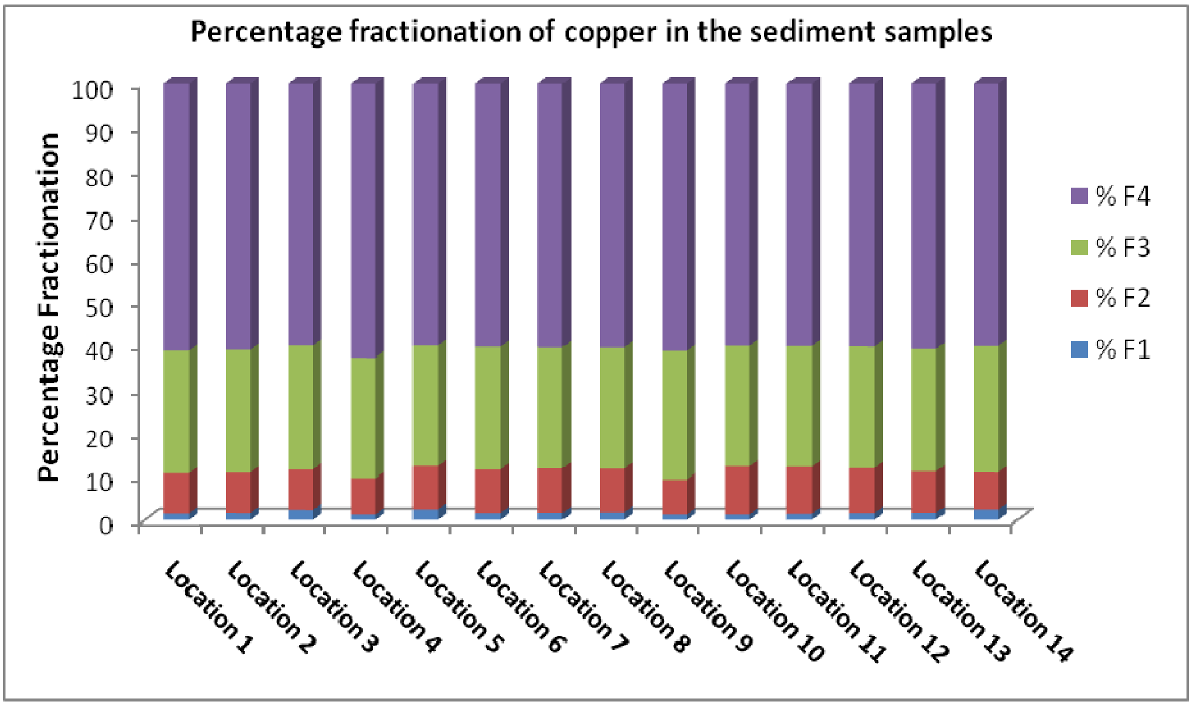


Fig.5.1a. Percentage fractionation of Cu in the sequential extraction procedure in sediment samples across Thane Creek area, Mumbai, India.

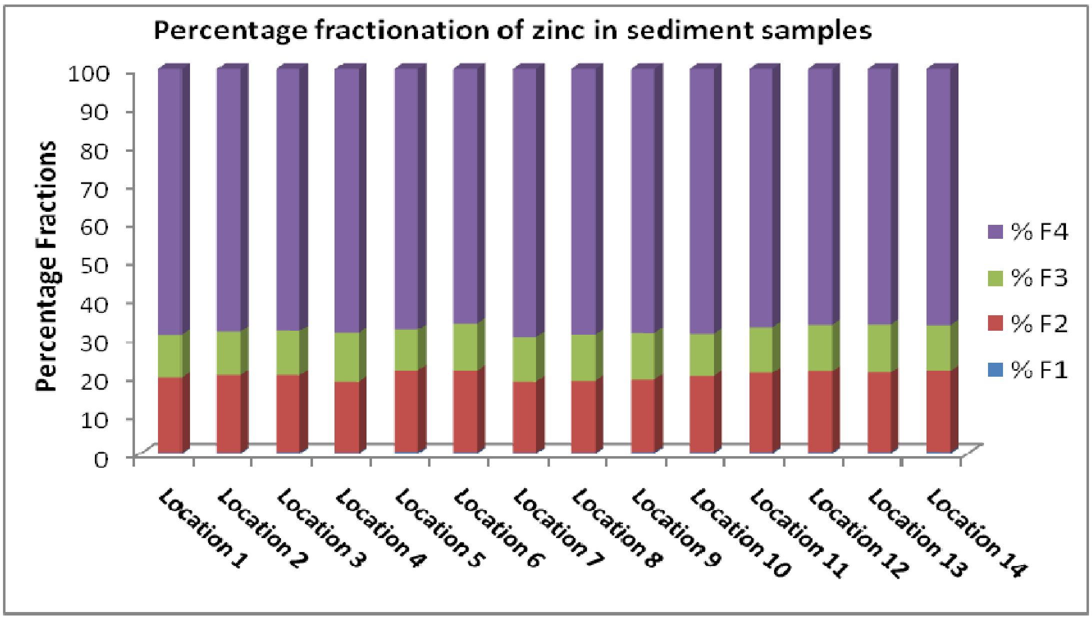


Fig.5.1b. Percentage fractionation of Zn in the sequential extraction procedure in sediment samples across Thane Creek area, Mumbai, India.

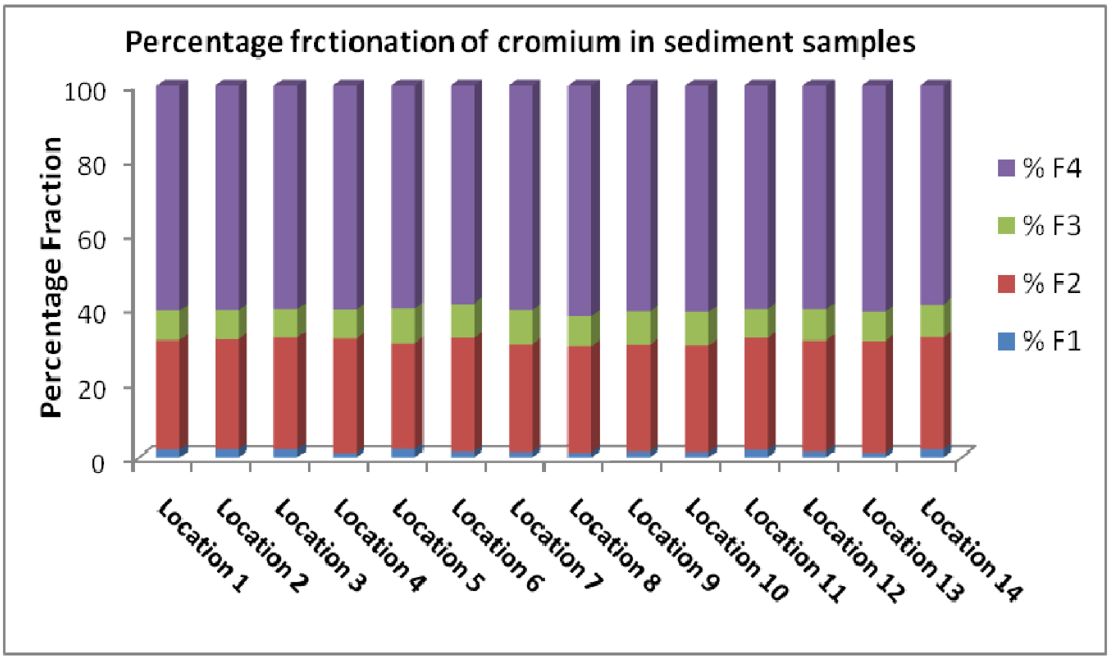


Fig.5.1c. Percentage fractionation of Cr in the sequential extraction procedure in sediment samples across Thane Creek area, Mumbai, India.

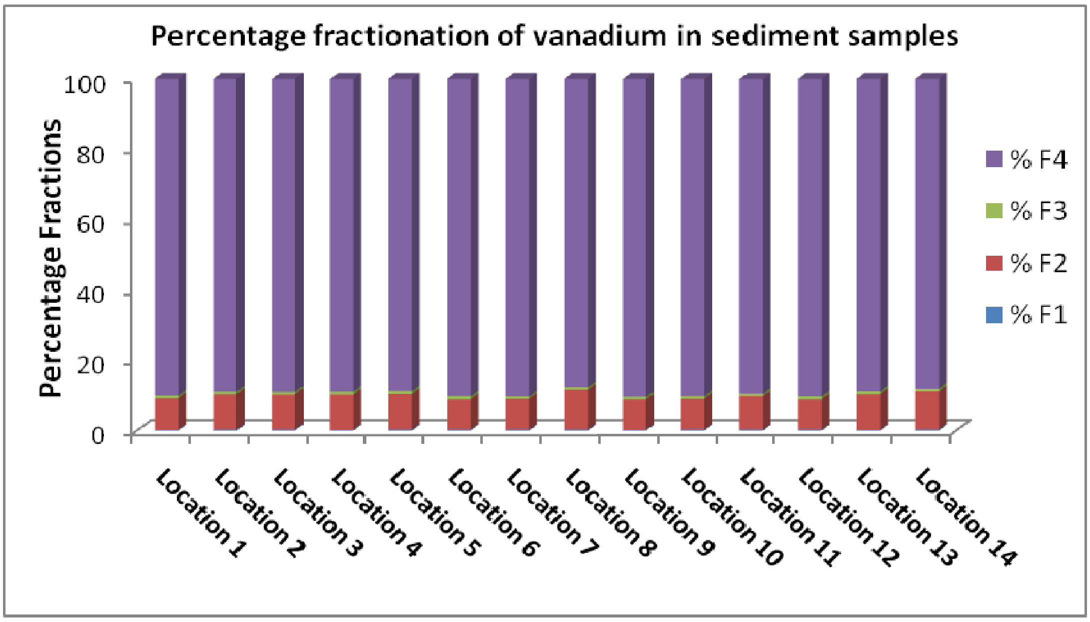


Fig.5.1d. Percentage fractionation of V in the sequential extraction procedure in sediment samples across Thane Creek area, Mumbai, India.

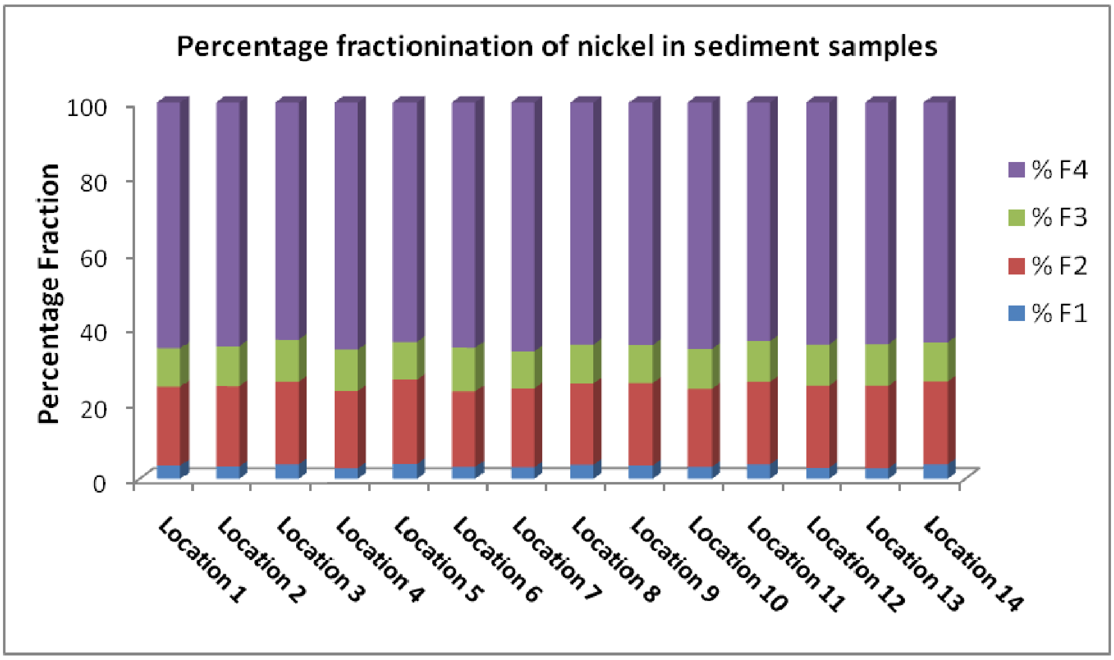


Fig.5.1e. Percentage fractionation of Ni in the sequential extraction procedure in sediment samples across Thane Creek area, Mumbai, India.

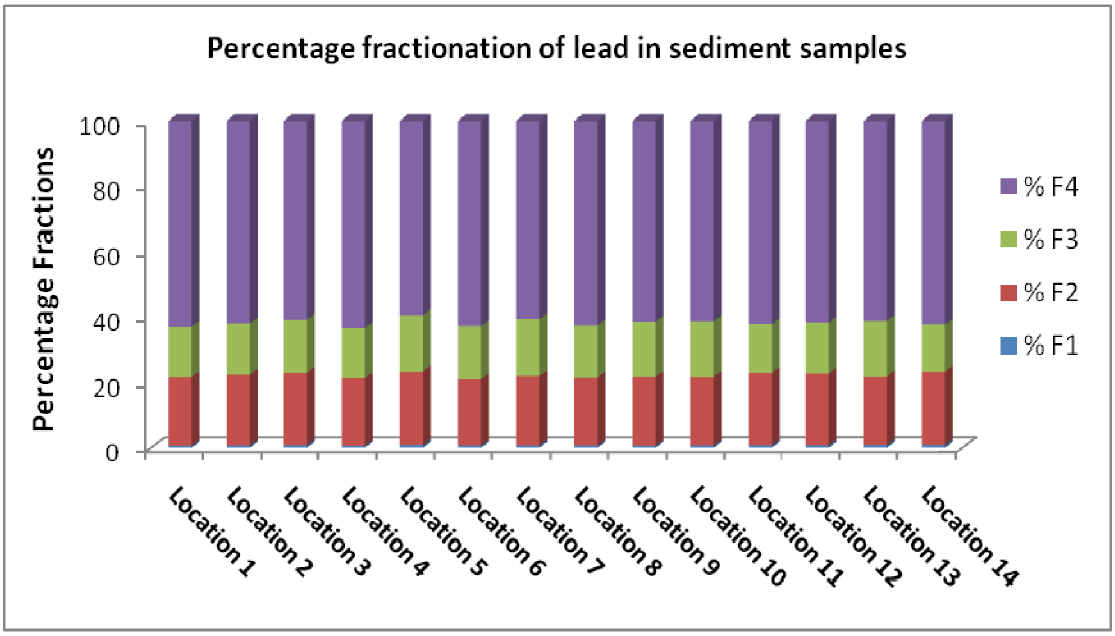


Fig.5.1f. Percentage fractionation of Pb in the sequential extraction procedure in sediment samples across Thane Creek area, Mumbai, India.

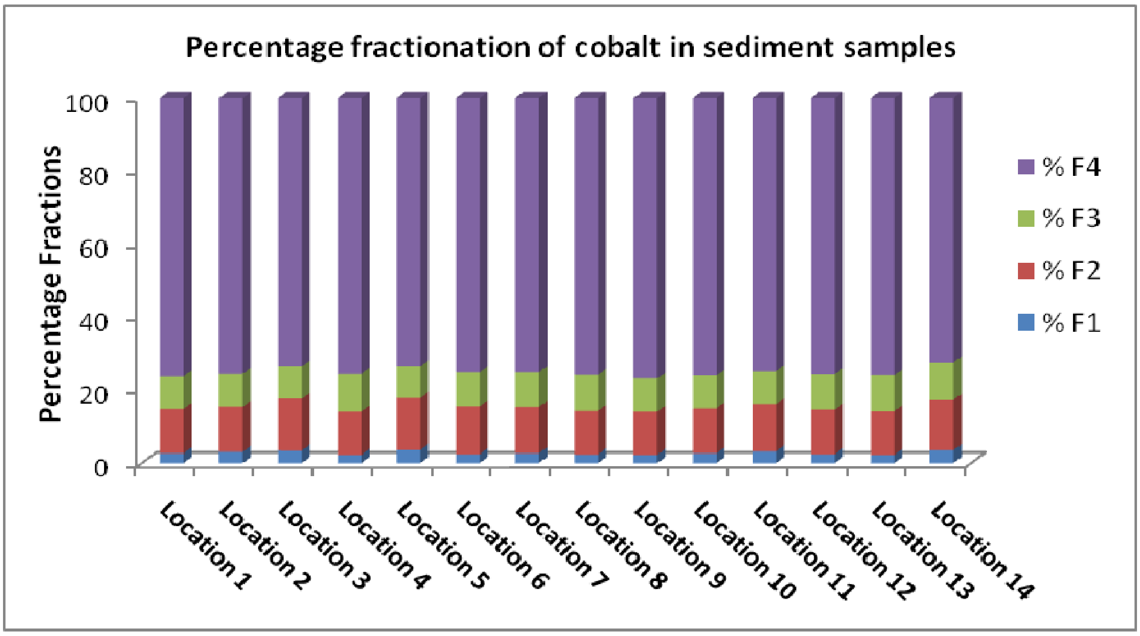


Fig.5.1g. Percentage fractionation of Co in the sequential extraction procedure in sediment samples across Thane Creek area, Mumbai, India.

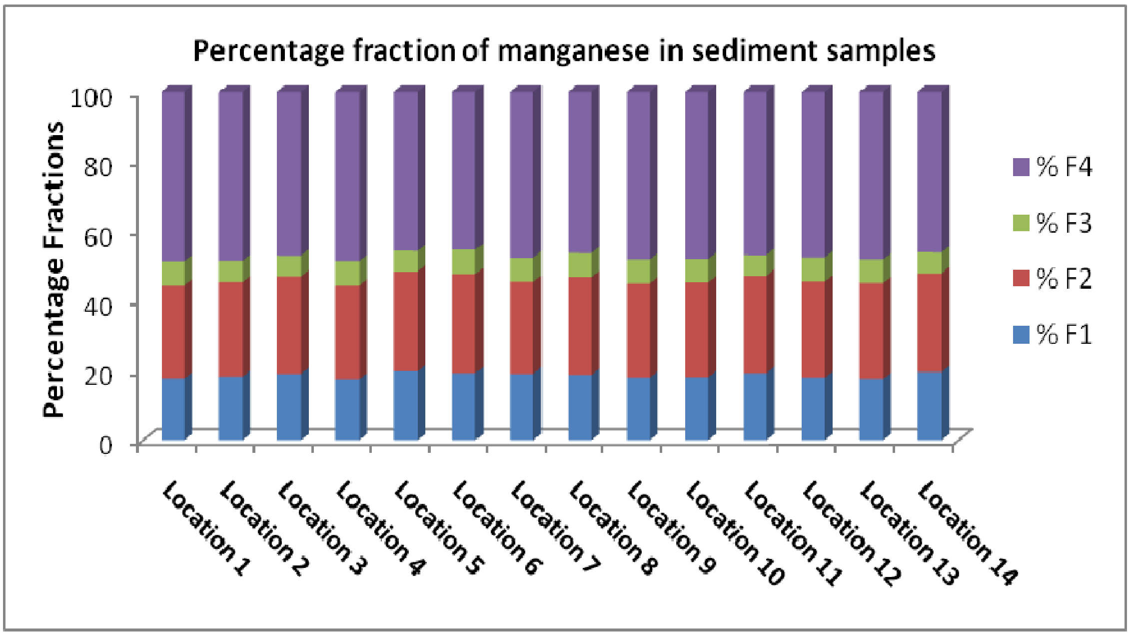


Fig.5.1h. Percentage fractionation of Mn in the sequential extraction procedure in sediment samples across Thane Creek area, Mumbai, India.

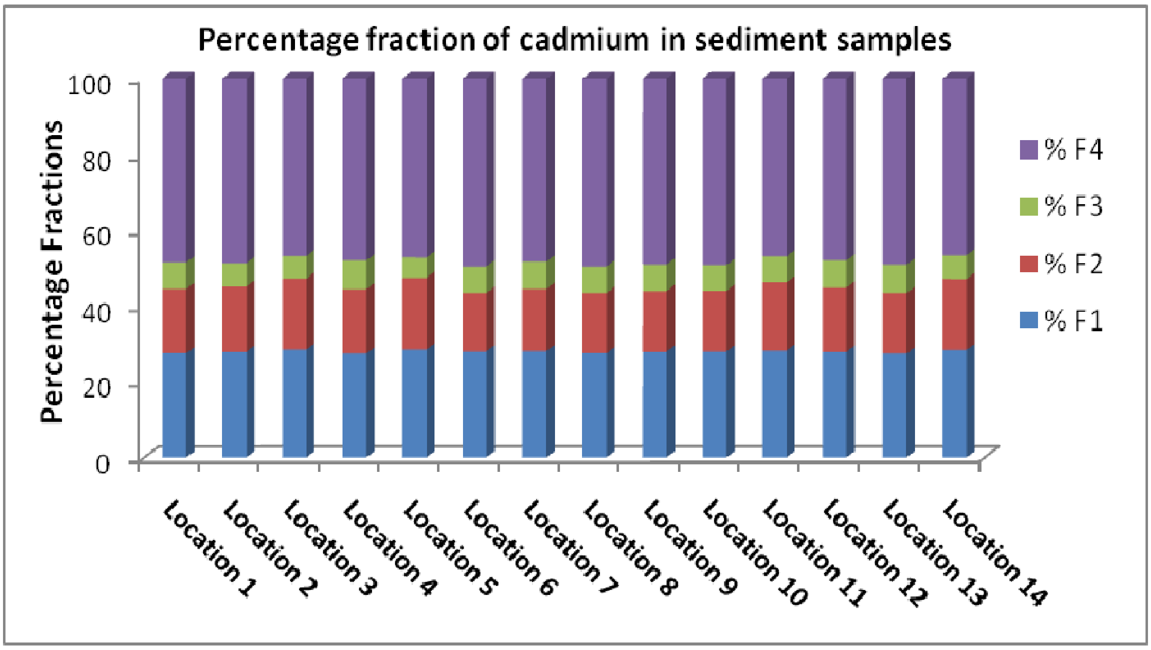


Fig.5.1i. Percentage fractionation of Cd in the sequential extraction procedure in sediment samples across Thane Creek area, Mumbai, India.

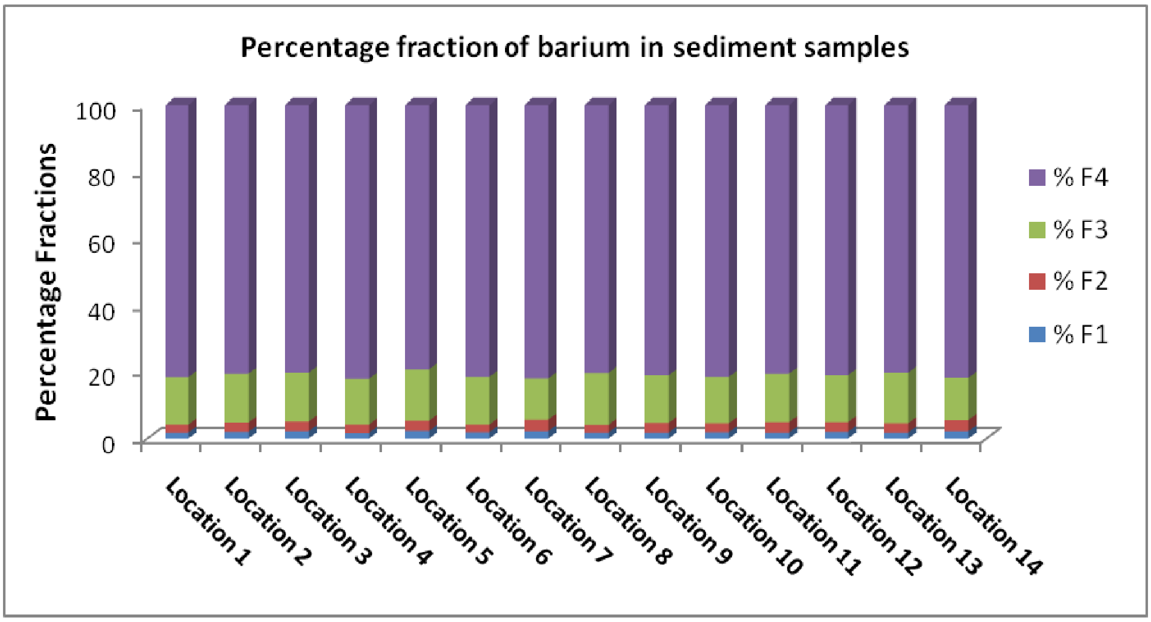


Fig.5.1j. Percentage fractionation of Ba in the sequential extraction procedure in sediment samples across Thane Creek area, Mumbai, India.

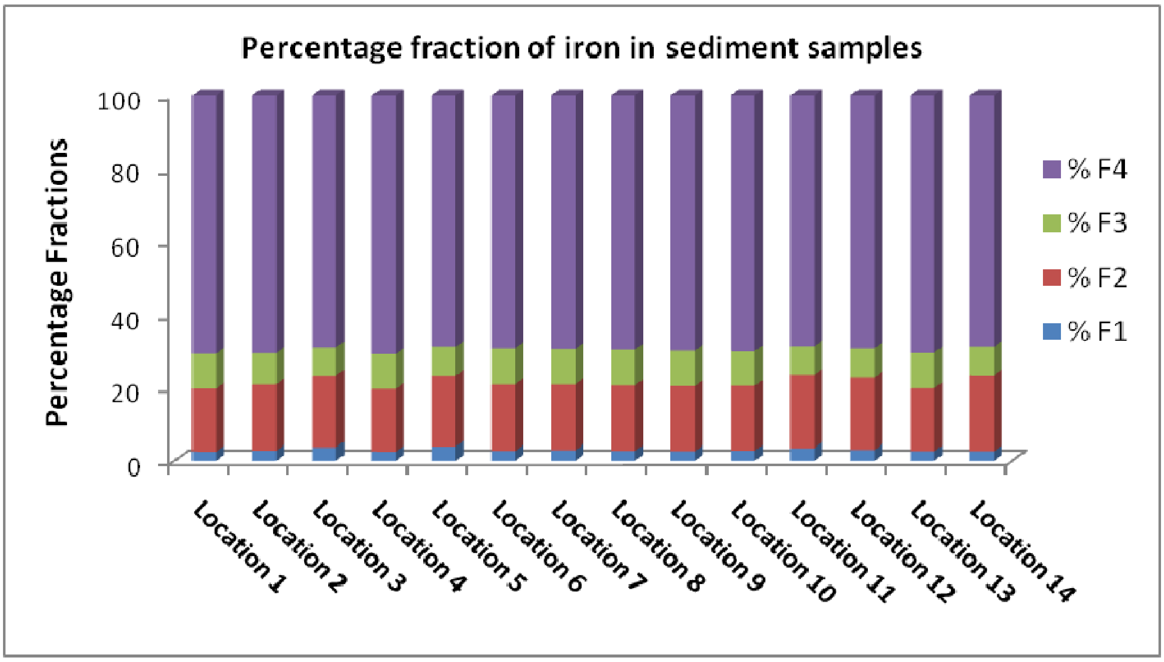


Fig.5.1k. Percentage fractionation of Fe in the sequential extraction procedure in sediment samples across Thane Creek area, Mumbai, India.

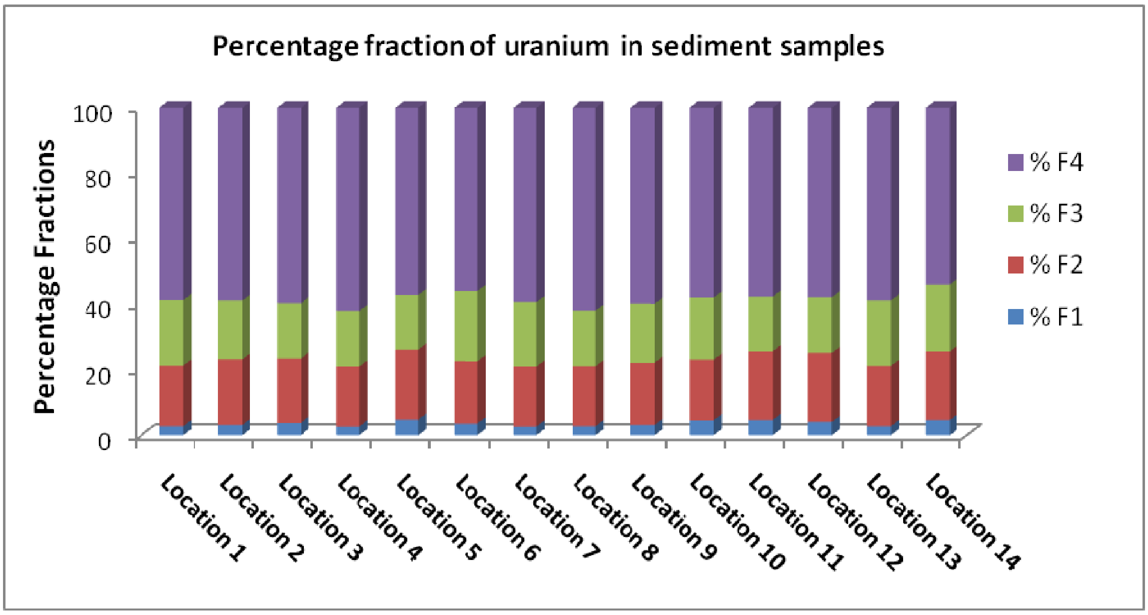


Fig.5.1l. Percentage fractionation of U in the sequential extraction procedure in sediment samples across Thane Creek area, Mumbai, India.

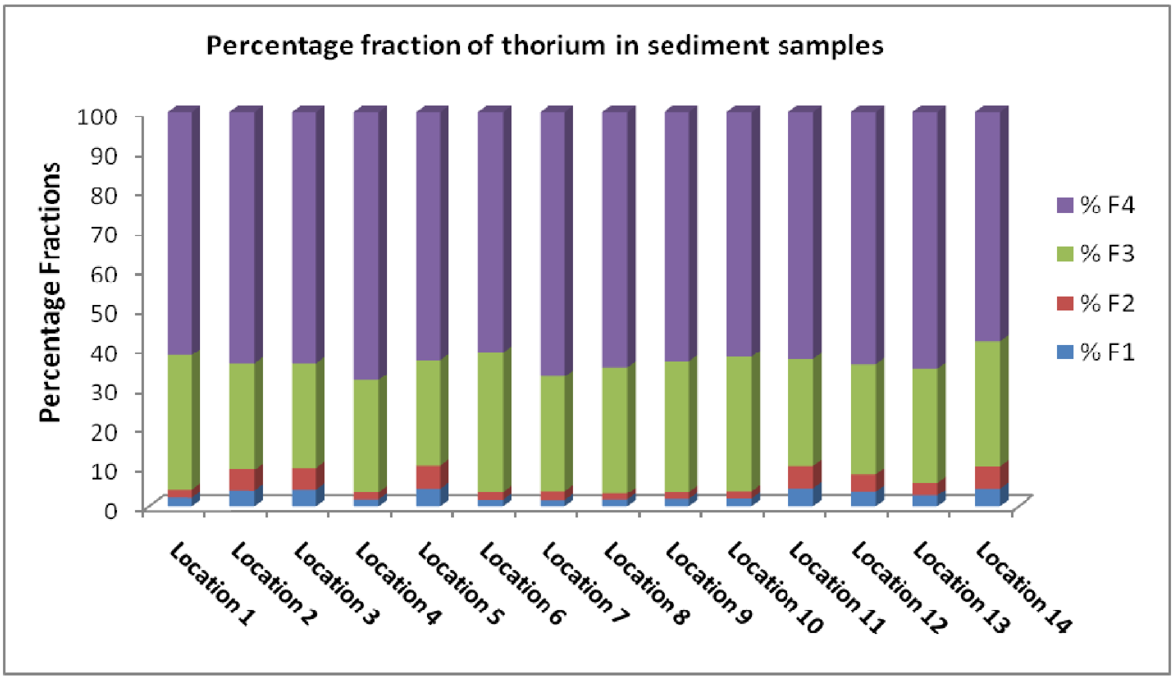


Fig.5.1m. Percentage fractionation of Th in the sequential extraction procedure in sediment samples across Thane Creek area, Mumbai, India.

CHAPTER-6

Transfer Factor/ Concentration Ratio and Intake of Trace Metals through Consumption of Biota

Natural resources occur and are embedded in the environment, and any factor(s) that result in stress to the environment therefore have direct impact on the biodiversity resources that they contain. Environmental threats to exploitable natural resources could arise from human activities or as a result of natural events [226]. Natural and anthropogenic metal inputs influence the bioavailable metal supply in aquatic systems. This bioavailable fraction cannot be detected by measuring metal concentrations in the soluble phase; they can be assessed only by determining the amount of the metal incorporated into organisms, which is the main goal in bio-monitoring [227-228]. To detect potential human impact on ecosystems, natural background concentrations of chemicals in organisms and their fluctuations have to be well established [229-230].

6.1. Transfer factor/ concentration ratio

Bioconcentration factors (BCFs) or transfer factors (TFs) or concentration ratios (CRs), as well as uptake rates derived from toxicokinetic studies have been frequently used to evaluate the potential of an organism for bioaccumulation of waterborne or particle bound metals [231]. The transfer factors (TFs) also known as concentration ratios or bioaccumulation factors, are the ratio of concentration of target element in an organism of interest to the concentration in the source medium such as seawater in marine ecosystem [232]. The chemical concentration in the organism has traditionally been expressed on a whole body basis. McGeer et al. (2003) [233] compiled metal BCF data for a variety of aquatic organisms, on a whole body basis.

6.2. Transfer factor/ concentration ratio of trace metals from seawater to biota across Thane Creek area, Mumbai, India

Transfer factors/ concentration ratios were calculated with the help of metals concentrations in seawater and whole body biota. Transfer factor/ concentration ratio of trace metals in different types of biota samples was observed to be different. Transfer factor/ concentration ratio values vary from 11 - 36, 12 – 41, 18 – 43, 16 – 35, 20 -33, 14 – 31, 45 – 399, 94 – 125, 131 – 200, 160 – 351, 25 – 259 and 335 – 548 for Co, Ni, Cr, Pb, V, U, Cu, Ba, Cd, Zn, Mn and Fe respectively for all types of studied biota samples represented in figures 6.1a and 6.1b. The order of transfer factor/ concentration ratio values in the different types of biota samples are lizard fish < shrimps < prawns < crabs for most of the trace metals. In case of Cd and Mn transfer factor/ concentration ratio values follow in different order like shrimps< prawns < lizard fish < crabs. This type of behavior observed may be because of higher bio-available fraction of Cd and Mn in the Thane creek ecosystem. This was explained in chapter 5. Higher transfer factor/ concentration ratio values were observed for Cu, Ba, Cd, Zn, Mn and Fe compare to Co, Ni, Cr, Pb, V and U in all the biota samples. It is clearly observed that crab was having highest transfer factor/ concentration ratio for all the studied trace metals in the Thane creek region as crab takes its food mostly from sediment. And concentrations of trace metals in sediment are 100 to 1000 times higher comparing to seawater in the same sampling locations.

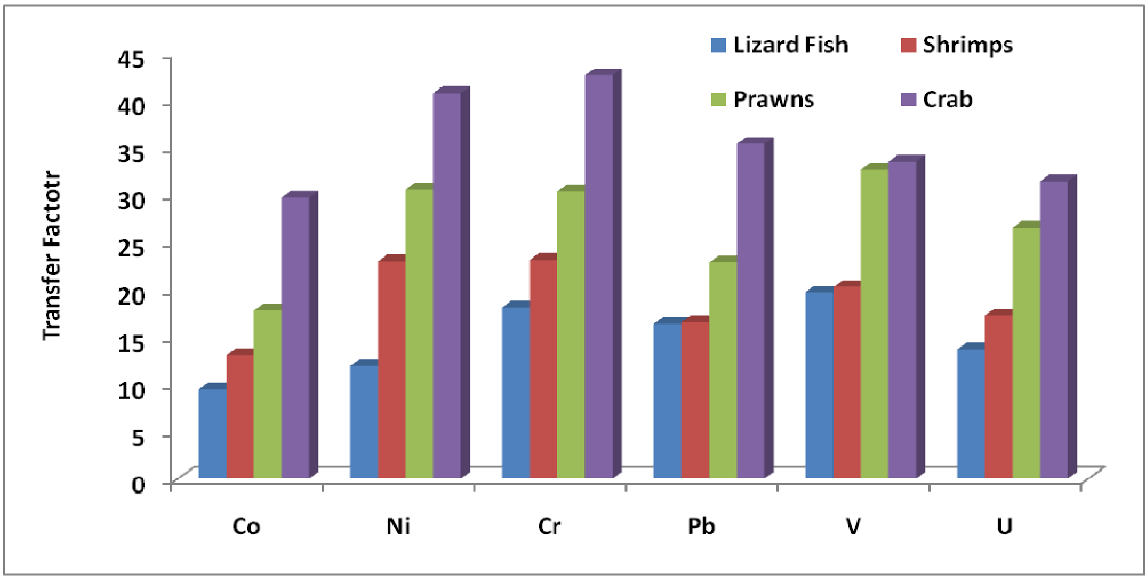


Fig.6.1a Transfer factor/ concentration ratio of Co, Ni, Cr, Pb, V and U from seawater to biota across Thane Creek area, Mumbai, India

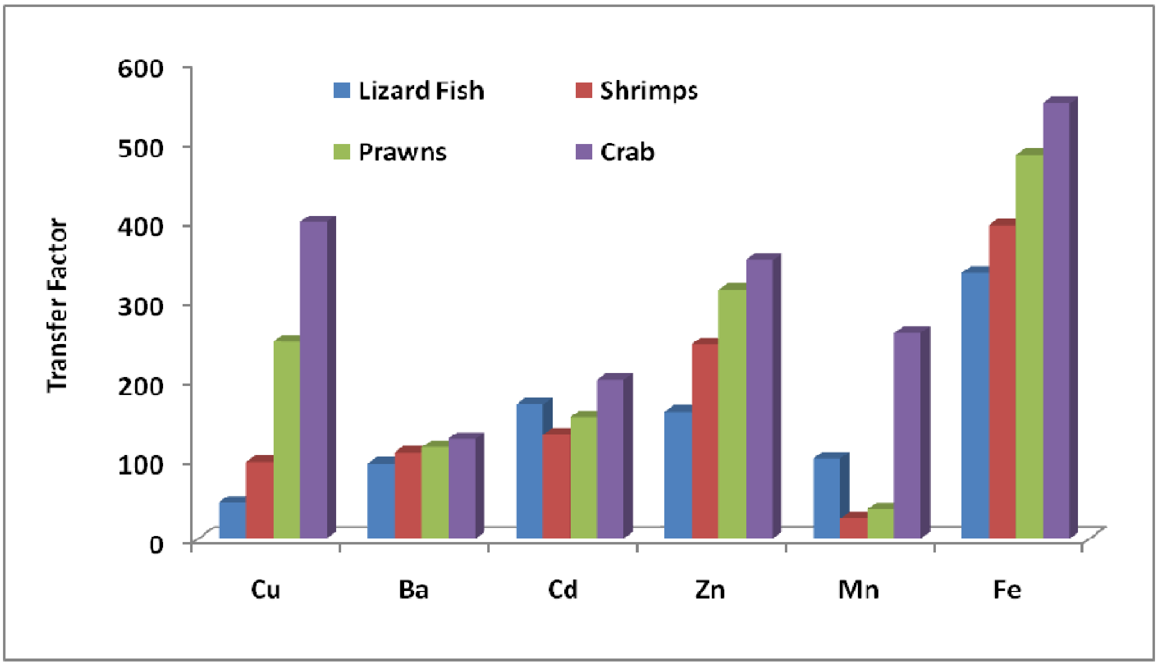


Fig.6.1b Transfer factor/ concentration ratio of Cu, Ba, Cd, Zn, Mn and Fe from seawater to biota across Thane Creek area, Mumbai, India

6.3. Health hazard due to trace metals through consumption of biota

During the last decades a rapid accumulation of trace metals into the aquatic biota has been observed which poses serious concern, because of their adverse health effects for both human and wildlife, particularly in developing countries [234-237]. Fishes are exposed to a variety of toxic elements mainly via food chain accumulation processes and may eventually become potentially harmful to humans [238-239]. In the fish, the gastrointestinal pathway accounts for more than 90% of the total element uptake [240], while gills and skin also serving as routes for metal/element intake, ultimately resulting into contamination of different body parts, including muscles [241-242].

Fish consumption is considered as an important part of a balanced diet, but the increased accumulation of trace metals into fish tissues, due to increased anthropogenic activities, has emerged as a serious public health hazard worldwide [243-244]. The metabolically active organs, liver, gills and kidneys, accumulate relatively higher quantities of trace metals than other tissues such as muscles [245-246]. However, the screening of toxic metals into muscles is of particular concern because of its dietary significance [247]. In aquatic environments, fish stand at the top of the food chain [248], and are known to be highly vulnerable to environmental contamination [249]. Fish species often exhibit different profiles of toxic elements contamination, as a result of differences in their feeding behaviors, diets, habitat preferences, genetic variation, physiology, duration of exposure, and to water quality [239,246, 250]. Generally, benthic fish species are considered more prone to environmental contamination [237, 251] as they tend to accumulate elements more than the other fish species that feed at lower trophic levels [252,245]. As a result, recent studies have shown elevated levels of toxic chemicals in fishes are associated with several physiological, morphological, histological and behavioral abnormalities, including reduction in swimming performance, gill purge, alteration in sensory reception, delayed hatching and maturation period [253,245,254].

For dose response assessment, the purpose was to derive a maximum allowable concentration (MAC) for a specific chemical in the tissue of the fish that represents the highest concentration of each toxicant that can occur in the fish without causing harm to the human beings. To this end, MACs-based on the relationships between concentrations of specific trace elements in the fish tissues and response in terms of adverse biological effects on the human beingss were estimated using the reference dose (RfD, mg kg^{-1}wet weight (w/w) d^{-1}).

6.4. Intake of trace metals through consumption of biota

The maximum allowable concentration in food based on the RfD (MACRfD) is calculated using the equation for intake of a contaminant.

$$Intake = (CF * IR * EF * ED)/(BW * AT) \tag{6}$$

Where, CF is the contaminant concentration in fish, (mg kg^{-1} ww); IR is the ingestion rate (kg d^{-1}); EF is the exposure frequency (d y^{-1}); ED is the exposure duration (y); BW is the body weight (kg); AT is the averaging time (period over which exposure is averaged in days).

When the intake of a contaminant is at the highest rate at which health risk is at an acceptably low level, then, Intake = RfD and CF = MACRfD [255].

If the hazard quotient (HQ) defined as the ratio of daily intake to the RfD value is less than 1, toxic effects are not expected to occur. The RfD is an estimated single daily chemical intake rate that appears to be without risk if ingested over a lifetime. The ingestion rates were chosen to represent average consumption rates of fish by an adult person (14 g d^{-1}) [256-257].

Trace metal concentrations were determined in the edible part of biota to find out hazard in human beings due to trace metals through consumption of biota. Intake of trace metals (Zn, Mn, Cr, Ni, Cd, As, Co, Cu and Hg) were calculated through consumption of edible part of different types of studied biota samples collected across Thane Creek area, Mumbai, India and represented in table 6.1.

Table 6.1. Intake (mg/kg/d) and reference dose (mg/kg/d) of trace metals through consumption of biota across Thane Creek area, Mumbai, India

Metals	Intake ((mg/kg/d)				Reference Dose (mg/kg/d)
	Lizard Fish	Shrimps	Prawns	Crabs	
Zn	20.3E-05	29.6E-05	36.7E-05	42.1E-05	0.3[a]
Mn	15.3E-05	4.0E-05	5.4E-05	44.3E-05	0.14[a]
Cr	0.96E-05	1.1E-05	1.6E-05	2.3E-05	0.003[a]
Ni	0.57E-05	0.98E-05	1.4E-05	2.0E-05	0.02[a]
Cd	0.27E-05	0.17E-05	0.21E-05	0.32E-05	0.001[a]
As	0.06E-05	0.07E-05	0.08E-05	0.08E-05	0.0003[a]
Co	0.29E-05	0.34E-05	0.57E-05	0.96E-05	0.06[b]
Cu	2.4E-05	4.8E-05	11.5E-05	20.5E-05	0.02[c]
Hg	0.06E-05	0.09E-05	0.18E-05	0.18E-05	0.0007[d]

- a) Obtained from the Integrated Risk Information System, USEPA (IRIS).
- b) Obtained from Ontario Ministry of the Environment and Energy (2001).
- c) Obtained from ATSDR (1995).

- d) Obtained from WHO (1996).

It is observed that intake of trace metals (Zn, Mn, Cr, Ni, Cd, As, Co, Cu and Hg) due to consumption of biota collected across Tahane Creek area, Mumbai, India are much below compare to reference dose values prescribed by different international agencies.

6.5. Hazard Quotient (HQ) and Hazard Index (HI) due to trace metals through consumption of biota

Hazard Quotient (HQ) and Hazard Index (HI) were calculated using the following equations:

$$HQ = Intake/RfD \tag{7}$$

$$HI = \sum(HQ)metals \tag{8}$$

HQ and HI were calculated represented in table 6.2 to understand the health hazard in human beings due to trace metals through consumption of studied biota across Thane Creek area, Mumbai, India. The results showed that steady consumption of biota found in this study is not hazardous. For all cases the HQ value found to be less than unity. The sum of all HQ values representing the hazard index (HI) also found to be less than unity. So the consumption of fish or biota as a whole as such is not hazardous from the given location for the reported trace metals. Results of this investigation suggest that the risks to the human beingss from consumption of biota were generally low and within safe limits.

Table 6.2. Hazard Quotient (HQ) and Hazard Index (HI) of trace metals in biota samples across Thane Creek area, Mumbai, India.

Metal	Hazard Quotient (HQ)			
	Lizard Fish	Shrimps	Prawns	Crabs
Zn	0.0007	0.0010	0.0012	0.0014
Mn	0.0011	0.0003	0.0004	0.0032
Cr	0.0032	0.0037	0.0052	0.0077
Ni	0.0003	0.0005	0.0007	0.0010
Cd	0.0027	0.0017	0.0021	0.0031
As	0.0021	0.0022	0.0026	0.0026
Co	0.0000	0.0001	0.0001	0.0002
Cu	0.0012	0.0024	0.0058	0.0102
Hg	0.0008	0.0013	0.0018	0.0025
Hazard Index (HI)	0.0121	0.0131	0.0198	0.0319

CHAPTER-7

Summary and Conclusions

This chapter summarizes the results and conclusions of the present study. The summery and few major conclusions drawn from the study are given below.

Trace metals are having great importance in the marine ecosystem. So determination of trace metals in different compartments (seawater, sediment and biota) of marine ecosystem is necessary. Thane Creek receives large concentrations of trace metals due to effluents discharged from different industries and urban effluents. Very limited information is available on trace metals pollution in the estuaries of India. This is the main motivation to carry out the present study at Thane Creek, Mumbai, India. Estimation of trace metals in seawater is a difficult task because of the very low concentrations of trace metals and high salt content. Instruments capable of trace metal analysis in ppb range needs matrix separation prior to analysis. Accordingly, the separation and pre-concentration of trace metals is usually involved in the analytical procedures used for the determination of trace metals in seawater. Solvent extraction procedure was optimized for accurate determination of trace metals in seawater samples. Sediments are considered as sink of trace metals in marine environment. So trace metal concentrations were determined in the collected sediment samples. Different geochemical indices were calculated to understand the pollution load in the Thane Creek area due to trace metals burden. Trace metal concentrations in sediment samples also depend on different physico-chemical parameters. Different physico-chemical parameters were studied in sediment samples. Correlation studies were carried out to understand the major contributing parameter for trace metal concentrations in sediment samples. Total trace metal concentrations do not give exact idea of toxicity in sediment samples. Therefore binding behaviour of trace metals in sediment samples were studied using sequential extraction procedure. Trace metals though occur at extremely low concentrations in seawater, are accumulated by marine organisms and concentrations in their body tissue can be hundreds of times greater than seawater. As human being consume different marine organisms like biota, can lead potential health problem. Trace metal concentrations were determined in whole body biota and as well as edible part of biota. Transfer factors/ concentration ratios for trace metals were determined from seawater to biota. Health hazard in human beings due to trace metals through consumption of biota across Thane Creek area, Mumbai, India were also investigated.

- Solvent extraction procedure was optimized for determination of trace metals in seawater samples in terms of equilibration time and pH. Equilibration time for determination of trace metals in seawater samples using solvent extraction procedure was found to be within 20 minutes. Highest solvent extraction efficiency was observed at pH near 6 for most of the trace metals.
- Trace metal concentrations in sediment samples decrease from vertex of the creek to the open sea. This is because of the increased contamination by the effluent discharged from several industries situated on the eastern bank of Thane creek. The diversified industries along the banks of the creek, includes chemical, textile, pharmaceutical, engineering and major fertilizer complex, release through their effluents, high levels of nitrates and phosphates into the creek. The scarcity of back water near the very vertex of the creek decrease the dispersion probability of these contaminants to the sea.

- The pollution load index (PLI) values vary from 0.9 to 1.5. Maximum PLI value was observed in the location 1 and minimum in the location 9. PLI value decreases from location 1 to location 9, which shows that the pollutants are being diluted with distance from their discharge points. The values of PLI less than 1 shows that there is no significant anthropogenic contribution to the grab sediment samples in the locations 7, 8, 9, 10, 11 and 12. The values of PLI greater than 1 show that there is significant anthropogenic contribution to the grab sediment samples in the locations 1, 2, 3, 4, 5, 6, 13 and 14.
- Based on SQGs, trace metal contents (Zn, Ni, Pb, Cd and As) were lower than the determined limits of ERL and ERM in most of the sampling locations in this study, implying non-harmful biological impacts of these metals on living organisms. Hg is having non-harmful biological impacts on living organisms in most of the sampling locations except location 1 to 3 where there could be occasional or frequent adverse biological impacts. Sediment samples in all the sampling locations with occasionally or frequently adverse biological impacts are observed due to Cr and Cu.
- Trace metal concentrations dependency with respect to different physico-chemical parameters in sediment samples were also studied. Trace metal concentrations increase as particle size of sediment samples decrease. In the same time with decreasing particle size total carbon content increase in the sediment samples. Metal concentrations increase as particle size decrease in the sediment samples because of higher total carbon content in the lesser particle size sediment samples.
- Total trace metal concentrations in sediment samples do not reflect pollution behaviour in the creek due to trace metals. Sequential extraction was carried out to understand the

binding behaviour of trace metals in the sediment samples. By this experiment it was observed that although concentration of trace metals in sediment samples were higher but bioavailable fraction was less for most of the trace metals except Mn and Cd.

- The sediments had no risks associated with Zn, V and Pb, which had RAC values below 1 % in all of the samples, indicating that these metals mobility is very less in the creek studied. All of the samples had low risks (RAC values within 1-10%) associated with Cr, Ni, Co, Fe, Cu, Ba, U and Th. Mn and Cd shows medium risk (RAC values within11 - 30%) in all the sediment samples. The results suggested that Cd and Mn were slightly easily released by the sediments into the water in the creek, and therefore that they would be likely to enter the food chain.

- Trace metal concentrations were determined in the edible part of biota to find out hazard in human beings due to trace metals through consumption of biota. Intake of trace metals (Zn, Mn, Cr, Ni, Cd, As, Co, Cu and Hg) were calculated through consumption of edible part of different types of studied biota samples collected across Thane Creek area, Mumbai, India. It is observed that intake of trace metals due to consumption of biota collected across Thane Creek area, Mumbai, India are much below compare to reference dose values prescribed by different international agencies.

- The hazard quotient (HQ) values were calculated for ingestion of biota. The results showed that steady consumption of biota found in this study is not hazardous. For all cases the HQ value found to be less than unity. The sum of all HQ values representing the hazard index (HI) also found to be less than unity. So the consumption of fish or biota as a whole as such is not hazardous from the given location for the reported trace metals.

Future perspectives

Trace metals bound in different organ of the marine organisms with different extent. By calculating transfer factors/ concentration ratios for trace metals in different organ of the marine organisms will give better idea of organ specific transfer factors/ concentration ratios for trace metals. Total trace metal conecentration does not give exact idea of toxicity in biota samples. Toxicity is different for different oxidation state of trace metals. Speciation studies of trace metals on biota will give better idea of toxicity.

Lab experiments for metal uptake during the growth of biota and also to assess the physiological requirement of quantum of metals can be carried out.

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