

DEPTH-WISE PROFILING OF HEAVY METAL SPECIATION IN THE SEDIMENTS OF SUNDERBAN WETLAND-A PILOT STUDY

A DISSERTATION

*Submitted in partial fulfillment of the
requirements for the award of the degree*

of

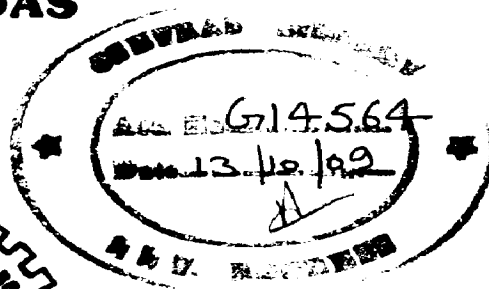
MASTER OF TECHNOLOGY

in

ADVANCED CHEMICAL ANALYSIS

By

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CANDIDATE'S DECLARATION

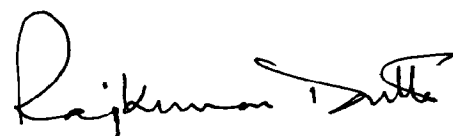
I here by certify that the work which is being presented in the dissertation in the entitled "***DEPTH-WISE PROFILING OF HEAVY METAL SPECIATION IN THE SEDIMENTS OF SUNDERBAN WETLAND – A PILOT STUDY***" for the award of the degree of Master of Technology submitted to the Indian institute of Technology Roorkee is an authentic record of my won work carried out by me during the period from August 2008 to June 2009 under the supervision of ***Dr. R.K. Dutta*** at the ***Department of Chemistry IIT Roorkee***.

The matter embodied in this dissertation has not been submitted by me for the award of any other degree.

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This is to certify that the above statement is correct to the best of my knowledge.



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ABSTRACT

Core sediment samples from three sampling sites of Sunderban Wetland region, e.g., Lot 8 S1; Jharkhali S2 and Ghusighata S3 are studied by SEM-EDAX, EPMA for major composition and point analysis of selected mineral grain. Total metal analysis of heavy metals like Cr, Mn, Co, Ni, Cu, Zn, Cd and Pb are measured by AAS method. The variations in the concentration of these heavy metals are observed along the depth of the sediment core suggesting changes in their input due to natural and anthropogenic activities.

Metal speciation has been done using sequential extraction method. Most of the heavy metals are found to be immobilized in silicate matrix and to some extent as oxides. In addition, these heavy metals are found in other forms e.g., ion-exchangeable and carbonates. The pilot study to evaluate the feasibility of using sequential extraction method to isolate the heavy metals into various chemical forms has been successfully accomplished. Other imaging techniques like SEM-EDAX and EPMA are found to be excellent supplementary methods to obtain useful data like shape, morphology and texture of the sediments, major composition (in this study it has been primarily silicate matrix) etc. In addition, EPMA is also found to be a good method for estimating minor elements, e.g., Mg, Ca, Ti, Mn.

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Chapter 1

Introduction

1.1 General:

Sediments, particularly from estuaries and lakes, are major sinks for these contaminants in aquatic environments and their study is an important step in mapping possible pollution sources and exposure pathways. The Sunderban harbors the world's largest mangrove forest together with associated flora and fauna. This coastal environment suffers from environmental degradation due to rapid human settlement, tourist activities, deforestation and increasing agricultural and aquacultural practices. A pronounced ecological change is evolving in this delta due to huge discharges of untreated domestic and industrial effluents carried by tributary rivers as well as the disposal of contaminated mud from harbour dredging and resulting from the rapid emergence of the Haldia Port Complex, a major oil disembarkment terminal in eastern India. The Sunderban delta has become susceptible to chemical pollutants such as heavy metals, organochlorine pesticides, polychlorinated biphenyls and polycyclic aromatic hydrocarbons which may have changed the estuary's geochemistry and affected the local coastal environment [1-4]. Sediments provide a temporal indication of the aquatic environment condition and act as major reservoir for metals [5] though some sediment can also act as a source of contaminants [6,7]. In fact, sediments are considered as the sink of environmental pollution which accumulates many heavy metals mainly due to the following procedures: adsorption, desorption, precipitation, diffusion processes, chemical reactions, biological activity and a combination of those phenomena. It should be noted here that due to several conditions like physical disturbances, changes in pH or redox potential, these heavy metals in the sediments may be released into the overlaying water column [8]. This is one of the means by which the water system gets contaminated with heavy metals which renders toxicity to the aquatic system. Determination of heavy metals concentration in sediments has become a common means of assessing the extent of anthropogenic input which impact on a certain area.

1.2 Estuary:

An estuary is an area where river meets the sea. It is a semi-enclosed coastal body of water having one side covered by the sea and it is below the sea level. Usually one or more rivers converge into it where fresh river water is blocked from streaming into the open ocean by one or more of the following features: surrounding mainland, peninsulas, barrier islands, or fringing salt marshes. An estuary is mainly affected by the flooding of convergent rivers and other anthropogenic activities. It is also affected by the sea such as tides, waves leading to

influx of saline water. The estuary bodies are formed due to the mixing of nutrient-laden fresh water from the land and salt water and offer a unique environment that can sustain abundance of different kinds of life – flora and fauna, viz., vast sea grass meadows, mangrove fringes and many kinds of fish and shellfish that live in these systems. As a result these regions are associated with high biological activities forming an ecosystem that contains more life per square inch than the richest Midwest farm [9].

It is well known that rivers often carry tremendous amounts of sediment, which when emptied into the oceans, construct distinctive patterns in the underlying sediments. In an estuary, the deposition of sediments is greatly influenced by tidal currents and ocean waves. Even climate is a factor in how the sands and muds settle into distinct patterns. During seasonal storms, erosion is increased leading to water heavily laden with a wide variety of sediments. Unlike deltas, in which the finer sediments are often carried far out to sea, the estuary is bordered on the deeper ocean side by heavy sands while clays and muds are dropped at river mouths. As tidal forces work the sediments by tumbling and rolling them, the lighter and finer particles are left near the river mouth. The build-up of coarse-grained (larger particle size) sands at the estuary edge often makes a barrier at the outer edge of the estuary that contains the bulk of the fine sediment and diluted water. The sediment structures in these ridges are defined as longitudinal or oblique bars. The structures in the upper reaches of the estuary are described as asymmetric and longitudinal bars become point bars similar to those observed in rivers. A dendritic (tree-shaped) pattern of channels occurs in these finer, flat lying sediments. The greatest force at work shaping and changing the estuary comes from moving water. Daily tidal fluctuation brings saltier water into the estuary and pushes medium-grained sands into the main body of the tidal flats. On the ocean side of the estuary, the sand bars are penetrated by channels through which the flow of water into and out of the estuary is restrained. These containment structures close the general water and sediment circulation paths around the main body of the estuary. Water and sediment flow is greatly restricted and additional build-up of medium and fine-grained sediments occurs. Water is forced to leave the estuary by these well-defined channels [10].

The tidal or exit channels of the estuary can be dangerous places for some life forms. As low tide occurs, the ebb of general sea level reverses the oceanic flow into the estuary. Water laden sediments release their burden and contribute to the general volume of water leaving the flats. The force of water exiting through the channels becomes great. The velocity of the water can reach dangerously high levels [11].

The estuaries are safe places for many creatures such as crabs and other crustaceans to lay their eggs. The hatched larvae live in the estuary until they are ready to join the zoo-plankton community of the larger sea. The swift release of water from the tidal flats helps the floating larvae to jet far out into the ocean where they spend the next phase of their lives. If not for the tidal currents, the larvae would have to live dangerously exposed to shore birds and other marine carnivores. The estuarine water channels help them grow and gain a slight advantage for survival that would not be found in typical shore lines.

When high tide returns, the encroaching water brings oxygen to the anaerobic (without oxygen) sediments. The water also brings marine organic food for mud dwelling inhabitants. The sediments are refreshed with salt water and another cycle of replenishment occurs. During stormy seasons, this effect can be exaggerated and actually quite harmful to inhabitants as sediments are churned and redeposit. However, this continuous recycling of sediments and resources keeps the estuary healthy and flourishing. Because the water plays such a physical and active role near the outer borders of the estuary, the inner regions of the estuary are more protected. By containing the general flow of water to the channels, the finer sediments, such as silt and clay, are left relatively undisturbed near the river mouths. They build up into areas of fine muds and contribute to the distinctive tidal flats. Organic debris is carried along by the rivers as they carve through valleys and plains of the terrestrial environment. This lightweight material comes to rest in the tidal flat as the velocity of the water is drastically reduced in the tidal flats. The decay and spreading of organic material throughout the flats makes them rich in nutrients. Subsequently, clams and other burrowing animals thrive in the rich sediments of the upper estuary. In turn, birds are lured to this feast where they are able to rear young on the nearby shore. These life forms are relatively protected because the muds make it difficult for heavier predators to walk out into the estuary with any stealth. There are even places where the muds act as a sort of quicksand and can be very dangerous.

Estuaries are fragile environments that are becoming increasingly threatened. They are being geologically altered as sediments are trapped upstream by dams. Diversion of water and sediments by agriculture is also reducing flow to the estuary. As a direct result, the life forms that rely on the dynamics of the estuary are decreasing in numbers. Many people are realizing the importance of estuarine environments and the important role they play in both marine and terrestrial ecosystems.

Estuaries are valuable to society in many ways. It provides habitat for a unique collection of plants and animals, which have both aesthetic and commercial value. They provide a sheltered access to open sea. As a consequence, many cities are located on estuaries to take advantage of commercial shipping and recreational boating opportunities. These estuaries not only support local fish and shellfish populations that are harvested commercially and recreationally, but these waters also serve as spawning and nursery grounds for populations that are harvested offshore. Also, due to their proximity to the ocean, they have been traditionally used as convenient disposal sites for urban storm water runoff and sewage. Because the rivers that feed them are often used for this same purpose, estuaries can become severely affected by municipal and industrial waste. Estuaries are coastal regions where salt water from the ocean mixes with fresh water from rivers, rainfall, and upland runoff. This dynamic combination of physical and biological factors produces an ecosystem unrivalled in productivity and a focal point for oceanic and inland commerce. Such characteristics are often cited to describe estuaries as the “cradles of civilization.” Indeed, for thousands of years great civilizations have emerged and flourished in estuaries such as those of the Tigris-Euphrates, Nile, and Yangtze River deltas. Today estuaries are the foundation for many modern cities, such as London, Cairo, Kolkata, and Shanghai. However, one of the most expansive and productive estuaries in the world is located in the United States at the interface of the Mississippi River and the Gulf of Mexico [12].

1.3 Importance of Mangrove Wetland

The mangrove wetlands found along the coastal zone could act as barrier against cyclones. It helps in protecting coastal erosion and also provides good nursery ground for a number of commercially important aquatic organisms. Mangrove ecosystems are diverse communities growing in the intertidal zones of tropical and subtropical coastal rivers, estuaries and bays [13]. The sediments in such areas have a large capacity to retain heavy metals from tidal waters, fresh water rivers and storm water runoff, and they often act as sinks for heavy metals [14-16].

Mangrove sediments are mainly found to be in reduced condition, i.e., anaerobic in nature. They are also found in areas rich in sulfides and in regions where organic matter contents are usually high. These conditions favour the retention of waterborne heavy metals in bottom sediment [17], and the subsequent oxidation of sulphides between tides allows metal mobilization and bioavailability. Down core variations in sediment cores reflect the geochemical history of a given region, including any anthropogenic impact [18]. Vertical profiles of pollutant species in sediment cores have been commonly used as “pollution records” over the last decades [19].

The studies on heavy metal of sediment cores over the last few decades have shown to be an excellent tool for establishing the effects of anthropogenic and natural processes occurring at the depositional environments. It was found that the diagenetic reactions were important near the sediment–water interface which responded to the changes in the redox conditions and thereby affecting metal concentrations in vertical sediment profiles [20, 21].

A number of recent studies have used sediment profiles to describe the contamination history of different environments. For instance [22] studied mercury contaminated sediment cores in the lagoon of Aveiro in Portugal and observing enriched layers in the surface and background concentrations in the bottom, calculated the sedimentation rates; while Silva-Filho et al. [23] evaluated atmospheric contributions to metal concentrations in undisturbed cores. Due to their stability within the sedimentary column, most of the contaminants leave their fingerprint in sediments i.e. no or insignificant post-depositional mobility is allowed. In particular, due to their high rates of sediment accretion and stabilized vegetated nature, these

wetlands may be highly efficient recorders of environmental changes and, therefore, have been extensively used to reconstruct coastal changes [24, 25].

The impact of anthropogenic activities on marine environment is known to affect estuarine and deltaic systems in various ways that leads to pollution of the marine environment through rivers. For example, organic compounds and toxic heavy metals were measured in the water column, biota, and sediments of Bohai sea, China [26,27]. In coastal zones, metals can enter surface waters principally through atmospheric deposition, industrial effluent discharge, and streams. When metals enter marine environments, they are adsorbed onto the sediments of adjacent shelf regions. Marine sediments act as scavengers of trace metals and often provide an excellent record of human impact [28-29].

1.4 Toxic Heavy Metals:

Specific gravity is a measure of density of a given amount of a solid substance when it is compared to an equal amount of water. The specific gravity of water is 1 at 4°C (39°F). Chemical elements having specific gravity that is at least 5 times the specific gravity of water are called as heavy metals. Some well-known toxic metallic elements with a specific gravity that is 5 or more times that of water are arsenic, 5.7; cadmium, 8.65; iron, 7.9; lead, 11.34; and mercury, 13.546 [30].

Our body takes heavy metals, but it becomes toxic when they are not metabolized by the body and accumulate in the soft tissues. Heavy metals may enter the human body through food, water, air, or absorption through the skin.

People are usually exposed to heavy metals by many routes, e.g., metal contaminated water, agriculture, manufacturing industries, pharmaceutical industries and products, or residential settings. Industrial exposure accounts for a common route of exposure for adults. Ingestion is the most common route of exposure in children [31]. Children may develop toxic levels from the normal hand-to-mouth activity who come in contact with contaminated soil or by accidentally ingestion of non-food materials e.g., paint chips etc [32].

The heavy metals like Cu, Fe, Zn, and Mn, are biologically essential for normal growth and development [33-35]. However, these metals work positively only in a certain concentration range, beyond which it is toxic. On the other hand, metals such as Cd, Pb and Hg have no known use in physiological processes [36], but when present in excess have ecological

significance due to their toxicity and cumulative behavior and constitute a potential hazard for humans and other mammals.

1.5 Sources of Heavy Metals in Estuary:

Heavy metals and other metals and minerals are released from chemical compounds through industrial activities. When these heavy metals come in contact with water the levels of heavy metals in aquatic environment increase and have been of much concern during the last decades due to the adverse effects of some metals on living organisms in food chains and human health.

Sediments have high metal retaining capacity and transport of metal is a very slow process in sediments. So in sediments the concentration of metal is very high. Sediments have the capacity for accumulating trace metals and other contaminants over time and serve to time integrated assessment of contamination that has occurred in water body. From the analysis of bottom sediment we can get the knowledge about the source of the heavy metal also the extent of contamination in aquatic environment.

Of the many pollutants found in coastal and estuarine sediments, heavy metals are amongst the most persistent because they cannot be destroyed or broken down. Therefore, they are useful as markers of environmental change [41]. Combination of inorganic material (i.e. clay minerals; Fe and Mn oxyhydroxides) and organic matter (detritic or alive) and sand are considered as the suspended particulate matter. These particles, due to their high surface area and the carrier nature of oxides and oxyhydroxides, are the main heavy metals carriers in aquatic systems, playing an important role of in the heavy metal transport from the continents to coastal areas [42-44].

In estuaries metal concentration in suspended particulate matter is generally orders of magnitude higher than in the dissolved form and so the fate of these elements is very closely related to the suspended particulate matter (SPM) internal cycle. The composition and variability of the SPM in estuaries are affected by many complex geochemical processes (e.g. precipitation and flocculation, desorption-adsorption) and by physical processes (e.g. river flow, tidal energy, currents).

1.6 Heavy Metal Transport:

Aquatic organisms play an important role in the heavy metal transport. These organisms accumulate heavy metals from high concentration area and move to another place or estuarine region which is the best place for these organism to grow and increase the concentration of heavy metals in that region.

Human polluting activities or physical processes also play a great role in increasing the level of heavy metals in estuarine region. These enter into the marine environment from rivers and affect the estuarine systems where they are quite often deposited. Heavy metals, pesticides and other 'particle reactive' toxic substances can be absorbed from the water column onto surfaces of fine grained sediment particles and move thereafter with the sediments.

1.7 Source of heavy metal for Sunderban Estuary:

With a mean annual flow of $5.9 \times 10^{11} \text{m}^3 \text{yr}^{-1}$ and sediment load of $1600 \times 10^{12} \text{g} \cdot \text{yr}^{-1}$ the Ganges river ranks second and third, respectively, in terms of water flow and sediment load among the world's rivers. Considering the enormous sediment transport by Ganges to the Bay of Bengal, a study was conducted on the size distribution and mineral characteristics of the suspended sediments of the Ganges river and is reported here. The sediments are mostly medium to coarse silt and are poorly sorted. Mica dominates among the clay minerals, followed by chlorite, vermiculite, kaolinite, and smectite. Due to differences in geology, smectite becomes a major clay mineral in downstream rivers. At Calcutta, the clay mineral transport in millions of tons per year is 18,464, 8000, and 2147, for mica, smectite, and chlorite, respectively.

The main sources of heavy metal in the Sunderban estuarine are the range of industries, namely, basically leather, jute, paper and pulp, medicine, oil and paints, sugar etc in Kolkata and adjacent locations across which the river Ganges flows. The wastes of these industries are emitted into the river. The waste of these industries containing toxic heavy metals like Pb, Cr and Cd, say from a paint industry is one of the major contributors for polluting the soil and water resources. Motor driven transport system is used in nearby areas which is also a factor for the increased concentration of heavy metals. In this case, Pb is the main toxic metal that is released by the vehicles. As a result the river water becomes contaminated with toxic heavy metals. In addition to this, agricultural land is another source of heavy metals alongwith organic pollutants due to excessive use of pesticides. Waste water from residential

houses also contributes to emission of some heavy metals to Ganga water. These heavy metals are carried from the upstream and get deposited in the estuarine region.

1.8 Background of This Study:

Trace metal pollution in the marine environment has long been recognized as a matter of serious environmental concern [45]. Hence, the knowledge of trace elements levels and distribution in the marine environment, leads to a better understanding of their behavior in aquatic systems and allows the detection of sources of pollution [46]. Heavy metals, in particular, are likely to insinuate potentially hazardous conditions to estuarine and marine organisms, due to their persistence in the environment and toxicity above threshold levels. They may be subdivided into two categories: (i) transition metals (e.g., cobalt, copper, iron, manganese, etc) which are essential to metabolism at low concentration but may be toxic at high concentrations and (ii) metalloids (e.g., arsenic, cadmium, lead, mercury, selenium, tin) which generally are not required for metabolic function but are toxic at low concentrations.

Due to their environmental persistence, biogeochemical recycling and ecological risks, heavy metals are of particular concern worldwide [47,48]. Metal contaminated environments may release metals to surrounding waters in three ways: (a) by desorption from suspended particles upon contact with seawater, (b) by desorption from bottom sediments and (c) by diffusion from interstitial water subsequent to diagenetic alteration of sediments [49]. However, if the equilibrium between marine sediments and the overlying water body is broken, marine sediments would transfer most pollutants into seawater [50]. As metal concentrations in marine sediments increase, more heavy metals will return to water bodies via chemical and biological processes [51].

The spatial distribution of heavy metals in marine sediments is of major importance in clarifying the pollution history of aquatic systems [52,53] The process is impacted by natural and anthropogenic factors, such as climate, parent rock weathering, industrial and domestic wastewater, aquaculture, and agriculture [54,55]. It is essential to distinguish between natural and human impacts on heavy metals in marine sediments. Eventually, geoaccumulation index, enrichment factor and factor analysis have been applied to indicate the degree of contamination by heavy metals from lithogenic and anthropogenic sources [52, 55-57].

The present study has been undertaken in the Sunderban delta of West Bengal, northeast India at the northernmost interface of the Bay of Bengal. It is formed by the alluvial and tidal network systems of Hugli, Saptamukhi, Jamira, Matla and Gosaba rivers, all of which have funnel shaped openings to the south before meeting the Bay of Bengal. The other tidal distributory drainage network includes, among others, the Bidya and Bidyadhari rivers, which are connected to the major river systems by interlacing creeks. The lower delta plain exhibits a fascinating network of channels and interchannel areas with dynamically changing tidal shoals and islands of variable dimensions and shapes [58]. The seaface is characterized by macro to mesotidal amplitude and moderate to moderately high wave climate. The wave-tidal energy wanes out farther inland toward north.

1.9 Aim and Scope of the Present Study:

Heavy metal analysis of the sediments and biota samples of Sunderban wetland has gained scientific importance in the last decade as it is identified a World Heritage Site due to unique collection of endangered species of flora and fauna. A few studies have been reported featuring total metal analysis in sediments collected from various locations of this area (1-4). The reported studies dealt with analysing heavy metals like Cr, Mn, Fe, Co, Cu, Zn, As, Cd, Hg, Pb and in mostly AAS and ICPMS were used for analysis (1a, 3a). However, no imaging studies are reported by which individual particles can be analysed and one may be able to study in details about possible preference of any trace heavy metals in a mineral phase, which can be accomplished by EPMA, provided the metals are present above the detection limits. In addition to this, it may give a new dimension to such studies if it is possible to “make out” the fractions of these heavy metals present as different species of geochemical and biological importances.

Scope for Sequential Extraction.

Sediment is the sink of environmental pollution and it is rich with many heavy metals brought by the rivers. Natural sediments are complex mixture of various phases, namely, residues of weathering and erosion such as clays and other alumino-silicates and iron and aluminium oxyhydroxides and sulfides, and substances produced by biological activity, both organic (living microorganisms, biological detritus and humic substances) and inorganic (carbonates, phosphates and silica) [62]. In the present study a five step sequential extraction method was followed to quantify metal concentration in various fraction and to know the effect of these metals on the environment. In addition, the total concentration of the heavy metals in the sediment was determined.

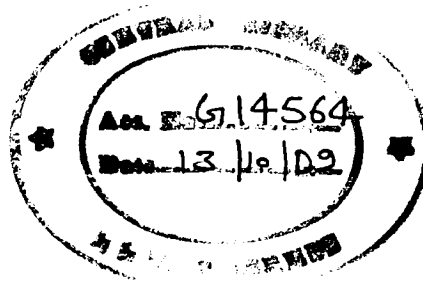
The five fractions are:

1. Exchangeable (adsorbed and exchanged),
2. Bound to Carbonates,
3. Bound to Iron and Manganese Oxides,
4. Bound to Organic Matter (humic substances), and
5. Residual (Primary and Secondary Minerals).

Metals present in the ion exchange form shows the bioavailability of the metal. if the concentration in this form is high then the metal is harmful to the environment. If the metal is

present in the residue fraction then that will not affect the environment. This means using sequential extraction method we can know the fraction of the total metal which biologically available to the environment.

Before taking up such speciation studies (by chemically isolating the metal species) in a larger scale, here we have undertaken a pilot study on a smaller scale to assess the feasibilities of such speciation studies.



Chapter 2

Materials and Methods

2.1 Study Area

The Hugli estuary ($88^{\circ} 00' - 89^{\circ} 28' E$ and $21^{\circ} 00' - 22^{\circ} 30' N$), which supports the world's largest magnificent mangrove block, the Sunderbans, is a typical and unique ecosystem of the Indian subcontinent and has been recognized as a World Heritage Site, covering approximately an area of 4267 km^2 (Fig. 1). It is mostly populated, low-lying and highly vulnerable coastal environment where silting of rivers has become a common problem. The deltaic tidal stretch receives heavy discharge of industrial effluents to the tune of 430 million liter per day from various industries situated on both the banks of Hugli River. Tide is semi-diurnal with distinct diurnal variability and belongs to macrotidal regime (tidal range up to 6 m) [1]. The predominant wind direction reverses from southwest in the rainy season to northeast in the dry season. These two factors produce strong flushing of the coastal areas, effectively dispersing pollutants.

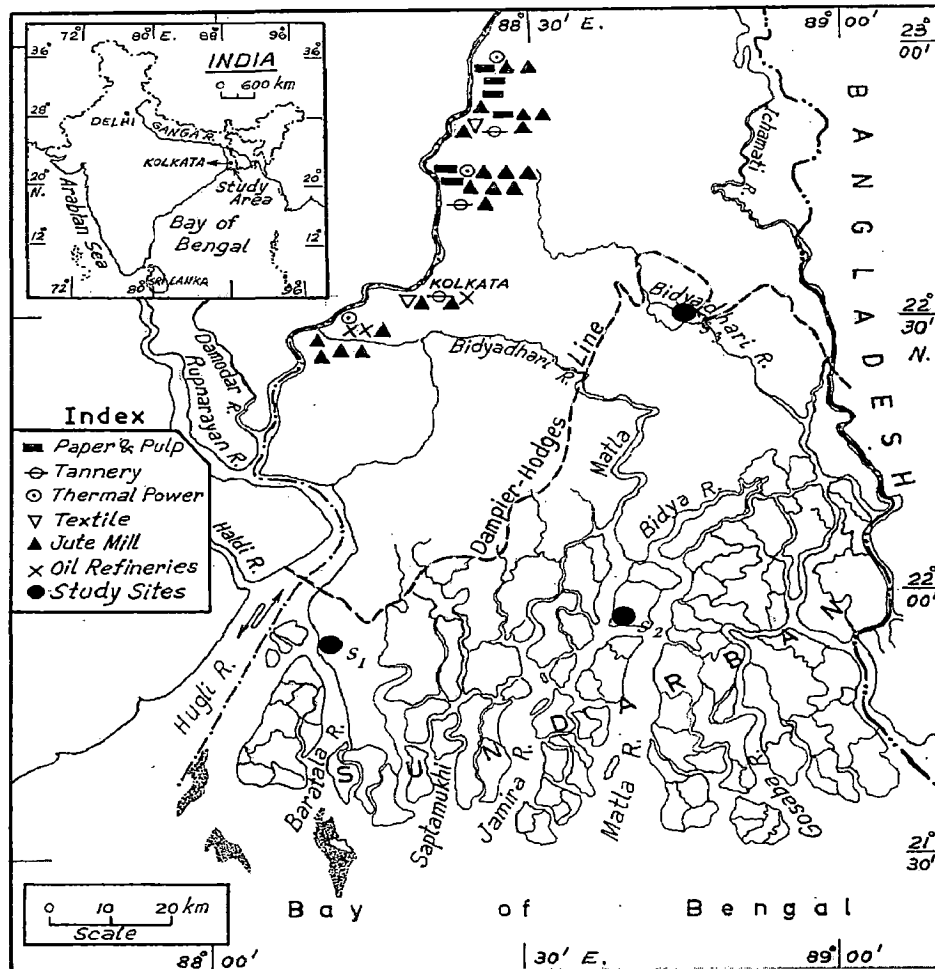


Fig. 1: Map of Sundarban showing locations of sampling stations. S₁ Lot8, S₂ Jharkhali, S₃ Gushighata

This macrotidal estuary together with the luxuriant Sunderban mangrove complex forms the genetically diverse ecosystems. This river system feeds the major rural, agricultural, urban and industrial areas and flows into the Bay of Bengal. The coastal environment has been undergoing the rapid urban expansion and subsequent socio-economic development and has put tremendous pressure on the local environment. A significant ecological change is pronounced in Hugli estuarine environment due to huge discharges of domestic and industrial wastes along with the rapid emergence of Haldia port complex, a major oil disembarkment terminal in eastern India. The estuary has become vulnerable to chemical pollutants such as heavy metals, organochlorine pesticides, petroleum-derived hydrocarbons, chlorinated hydrocarbons etc., which may have changed the estuary's geochemistry and affected the quality of the local coastal environment [1-4].

Coastal sediments in the vicinity of the mouth of the Hugli estuary is a complex mixture of various components originating from both the Hugli river and tidal and longshore sediments from the Bay of Bengal. A substantial portion of the sediments is transported landward by waves and tides and is deposited along the river course and tidal flats of the estuary. The river borne sediments carried downstream mix with the tidal sediments to form the tidal bars and tidal islands at the funnel mouth of the estuary. Hugli River collects and transports natural weathering products as well as potentially harmful elements derived from anthropogenic sources. [60] calculated that at Kolkata (former Calcutta) the Ganges annually supplies 411×10^6 t (i.e., 328×10^6 t sediment + 83×10^6 t solute load) of total load of the Hugli estuary.

Tropical cyclones also affect the coastal zone 3-4 times per year. The climate is humid (up to 96%) and the seasons are pronounced with four-month duration. Premonsoon (March - June) is the dry season, the monsoon (July - October) is accompanied by heavy rainfall (annual average precipitation 1600 mm) and the winter season (November - February) is characterized by low temperature and lower precipitation. The studied stations belong to different tidal environments; have different wave energy fluxes and distances from the sea (Bay of Bengal) and diverse human interference with a variable degree of exposure to heavy metallic contamination. The estuarine stretch, from Diamond Harbor to Gangasagar is a wide divergent one with eroding banks, submerged flats and unstable mid channel bars. The main estuary is identified as positive estuary in the mixohaline region (Pantalu, 1966). Three sampling sites (**Lot 8 S1; Jharkhali S2 and Ghusighata S3**) have been chosen for analyses of heavy metals in the Sundarban wetland. The widths of the mudflats range from 200 - 300 m under typical tropical macrotidal conditions.

The sample description is given in Table 1. The station Lot 8 (S1), is situated at the eastern bank of Muriganga River, the bifurcating distributory channel of the Hugli estuary, about 40 km upstream from its mouth.

Sample Description.

1. S1/June'07/0-4
2. S1/June'07/4-8
3. S1/june'07/8-12
4. S1/June'07/12-16
5. S1/June'07/16-20
6. S1/June'07/20-24
7. S1/June'07/24-28
8. S1/June'07/28-32
9. S2/June'07/0-4
10. S2/June'07/4-8
11. S2/June'07/8-12
12. S2/June'07/12-16
13. S2/June'07/16-20
14. S2/June'07/20-24
15. S2/June'07/24-28
16. S3/June'07/0-4
17. S3/June'07/4-8
18. S3/June'07/8-12
19. S3/june'07/12-16
20. S3/June'07/16-20
21. S3/June'07/20-24
22. S3/june'07/24-28
23. S3/June'07/28-32

Table 1: Showing sampling description. Samples were collected during June 2007. The information of the depth of the collected sediment is given in italics.

Mudflat deposits characterize the station where bioturbation and tidal currents play a major role in sediment suspension-resuspension cycles. Wave energy is subordinate. Sedimentation in the station is characterized by dominant clay supposed to be deposited from the 'turbidity maxima' of the Hugli estuary. This station receives wave impacts from south summer wind from the seaward side and, has been given protection at places by constructing brick pavements.

Station S2 (Jharkhali): The mudflat of the Jharkhali area occurs in a cove at the juncture of the eastern bank of Matla river and northern bank of the Bidya river, a major distributory of the Matla. The sediments are silty clay with an admixture of 7-10% of sand. Organic Carbon ranges from 0.66- 0.87% with the average value of 0.78%.

Station S3 (Ghusighata): The mudflat of the Ghusighata station occurs by the side of the north-south trending Karati river, a tidal channel upstream of the Matla river in its south and Bidyadhari river in its northwest. The sediments are dominantly silty (average 62%) with some admixture of silt (average 28 %) and sand (average 10 %). The organic carbon content is highest in this station ranging from 0.91 to 1.40 % with the average value of 1.23%. The sediments are generally neutral to slightly acidic. It is fed by the huge discharge of the sewage sludge through the sewage disposal canal of the Kolkata Metropolitan city.

2.2 Reagents and Chemicals:

- 1) Magnesium Chloride($MgCl_2$), Analytical Grade , E. Merck
- 2) Sodium Hydroxide (NaOH), Analytical Grade , E. Merck
- 3) Oxalic Acid($C_2H_2O_4$), Analytical Grade , E. Merck
- 4) Calcium Oxalate(CaH_2O_4), Analytical Grade , E. Merck
- 5) Sodium Acetate(CH_3COONa), Analytical Grade , E. Merck
- 6) Hydrogen Fluoride(HF), Analytical Grade , E. Merck
- 7) Nitric Acid (HNO_3), Analytical Grade , E. Merck
- 8) Hydrochloric Acid (HCL), Analytical Grade , E. Merck

2.3 Sample Collection and Analysis Protocol:

Sediment cores of ~ 30 cm in length were collected by steel corers (45 cm length and 5 cm diameter) at the sampling sites as discussed above. Each core was arbitrarily divided at 4 cm fractions (sub samples) with the help of PVC spatula. All visible marine organisms and shell fragments were removed by hand. The sediment samples were then brought to laboratory in a pre-acid cleaned Tarson vessels at -20°C. The samples were air dried, powdered, sieved and stored in hermetic plastic bags until analyses for heavy metals for further analysis.

2.4 Analytical Methods.

2.4.1. Scanning Electron Microscopy (SEM)

A typical SEM instrument, showing the electron column, sample chamber, EDS detector, electronics console, and visual display monitors. The scanning electron microscope (SEM) images the sample surface by scanning it with a high-energy beam of electron. Characteristic X-rays are emitted when the electron beam removes an inner shell electron from the sample, causing a higher energy electron to fill the shell and release energy. These characteristic x-

rays are used to identify the composition and measure the abundance of elements in the sample. The electrons interact with the target atoms of the sample. This produces signals that contain information about the sample's surface topography, composition and other properties such as electrical conductivity.

In SEM, signals are produced by secondary electrons, back scattered electrons (BSE), characteristic X-rays, light (cathodoluminescence), specimen current and transmitted electrons. These electrons interact with atoms at or near the surface of the sample and produces images of the sample. In the most common or standard detection mode, secondary electron imaging or SEI, the SEM can produce very high-resolution images of a sample surface, revealing details about 1 to 5 nm in size. SEM gives a three-dimensional appearance useful for understanding the surface structure of a sample. The magnification up to x 250,000 is possible with SEM. This is about 250 times the magnification limit of the best light microscopes. SEM uses Back-scattered electrons (BSE) which are the electron beams that are reflected from the sample by elastic scattering. Along with the BSE, characteristic x-rays of the elements present in the sample are also recorded. Because the intensity of the BSE signal is strongly related to the atomic number (Z) of the specimen, BSE images can provide information about the distribution of different elements in the sample. Characteristic X-rays are emitted when the electron beam removes an inner shell electron from the sample, causing a higher energy electron to fill the shell and release energy. These characteristic x-rays are used to identify the composition and measure the abundance of elements in the sample.

The scanning electron microscope (SEM), data are collected over a selected area of the surface of the sample, and a 2-dimensional image is generated that displays spatial variations in these properties. A focused beam of high-energy electrons is used to generate a variety of signals at the surface of solid specimens. The signals coming from the electron-sample interactions give information of the sample surface. It also gives the chemical composition of the sample, crystal structure as well as the external morphology (texture) of the sample. A particular area of the sample is selected and the electron is bombarded on that area to get the information about the sample. Areas ranging from approximately 1 cm to 5 microns in width can be imaged in a scanning mode using conventional SEM techniques (magnification ranging from 20X to approximately 30,000X, spatial resolution of 50 to 100 nm). Since electron is bombarded at a particular area the SEM is useful in performing analyses of selected point locations on the sample; this approach is especially useful in qualitatively or

semi-quantitatively determining chemical compositions (using EDS), crystalline structure, and crystal orientations (using EBSD). The design and function of the SEM is very similar to the EPMA, and considerable overlap in capabilities exists between the two instruments.

Principles

An electron microscope that forms a three-dimensional image on a cathode-ray tube by moving a beam of focused electrons across an object and reading both the electrons scattered by the object and the secondary electrons produced by it.

Accelerated electrons with a few tens of KV of energies are used in SEM resulting to an equivalent amount of kinetic energy. When the electron is bombarded on the sample, the electron interacts with the sample and gets decelerated. This electron- sample interaction gives signal about the morphology, chemical composition and crystal structure. These signals include secondary electrons (that produce SEM images), backscattered electrons (BSE), diffracted backscattered electrons (EBSD that are used to determine crystal structures and orientations of minerals), photons (characteristic X-rays that are used for elemental analysis and continuum X-rays), visible light (cathodoluminescence-CL), and heat. Secondary electrons and backscattered electrons are commonly used for imaging samples: secondary electrons are most valuable for showing morphology and topography on samples and backscattered electrons are most valuable for illustrating contrasts in composition in multiphase samples (i.e. for rapid phase discrimination). X-ray generation is produced by inelastic collisions of the incident electrons with electrons in discrete orbitals (shells) of atoms in the sample. As the excited electrons return to lower energy states, they yield X-rays that are of a fixed wavelength (that is related to the difference in energy levels of electrons in different shells for a given element). Thus, characteristic X-rays are produced for each element in a mineral that is "excited" by the electron beam. SEM analysis is considered to be "non-destructive"; that is, x-rays generated by electron interactions do not lead to volume loss of the sample, so it is possible to analyze the same materials repeatedly. The X-rays are usually called K or L x-rays. The nomenclature is based on the transition of the electrons in the atoms of the samples. For example: if electron knocks a K-shell electron from an atom Z and subsequently the vacancy is filled by a L shell electron, then the characteristic X-ray that is emitted during this transition is called K- x-ray. Similarly, if the transition occurs from M shell to the vacant L shell, the corresponding X-ray will be named as L-x-ray.

The Scanning Electron Microscope (SEM) consists of an energetically well-defined, highly focused beam of electrons scanned across a sample. The electron microscope uses a LaB₆ source and is pumped using turbo and ion pumps to maintain the highest possible vacuum.

Samples Preparation for SEM-EDAX analysis:

Dried powdered samples were sprinkled on a carbon tape with double sided adhesive, and mounted on the metallic sample holder. The analysis and imaging were carried out using 20 keV electrons.

2.4.2. Electron Probe Micro Analysis(EPMA)

Principle of EPMA:

An electron probe micro-analyzer is a micro beam instrument used primarily for the *in situ* non-destructive chemical analysis of minute solid samples. An electron microprobe operates under the principle that if a solid material is bombarded by an accelerated and focused electron beam, the incident electron beam has sufficient energy to liberate both matter and energy from the sample. The electron probe micro analyzer (EPMA) utilizes both wavelength dispersive (WDS) and energy dispersive (EDS) x-ray spectrometers for measurement of the x-ray energies and intensities from samples. The instrument is capable of giving the morphological details of the sample as fine as 10 nm resolution using scanning electron microscope. The spatial resolution of x-ray microanalysis of thick specimens is limited to a volume with dimensions of approximately 1 micrometer due to electron scattering effects. Quantitative analysis is carried out by means of the NIST-developed COR and FRAMEC matrix correction procedures. X-ray spectra can be modelled and deconvoluted with NIST Desktop Spectrum Analyzer, which incorporates *ab initio* physical descriptions of x-ray generation. The instrument gives very precise result having relative standard deviation of less than 3% when samples are in the ideal form of a metallographically polished bulk solid. Standards utilized in these analyses are in the form of pure elements or simple compounds (e.g., GaP). The EPMA technique provides great versatility in the analysis of multi-element unknowns of virtually any composition. The main limitation of the technique is that it can not analyse light elements having atomic numbers less than 11. Detection limits are of the order of 100 ppm with wavelength dispersive spectrometry and 1000 ppm with energy dispersive spectrometry. Typical applications include metallurgical studies, failure analysis, and the analyses of particulates, minerals, ceramics, advanced alloys, and electronic devices.

Instrumentation:

The electron-sample interactions mainly liberate heat, but they also yield both derivative electrons and x-rays. Of most common interest in the analysis of geological materials are secondary and back-scattered electrons, which are useful for imaging a surface or obtaining an average composition of the material. X-ray generation is produced by inelastic collisions of the incident electrons with electrons in the inner shells of atoms in the sample; when an inner-shell electron is ejected from its orbit, leaving a vacancy, a higher-shell electron falls into this vacancy and must shed some energy (as an X-ray) to do so. These quantized x-rays are characteristic of the element. EPMA analysis is considered to be "non-destructive"; that is, x-rays generated by electron interactions do not lead to volume loss of the sample, so it is possible to re-analyze the same materials more than one time.

W-filament cathode referred to as a "gun." is used as the electron source. A series of electromagnetic lenses, used to condense and focus the electron beam emanating from the source. This is located in the column of the instrument and comprises the electron optics and operates in an analogous way to light optics. A sample chamber used here is a movable sample stage in X-Y-Z direction. The sample-stage is kept in a vacuum chamber to prevent gas and vapour molecules from interfering with the electron beam on its way to the sample. A light microscope allows for direct optical observation of the sample. A variety of detectors arranged around the sample chamber that are used to collect x-rays and electrons emitted from the sample. . ▼ Hide

The main feature of the EPMA instrument is the vertical electron-beam column, the detectors placed around the sample chamber block, a sample entry vacuum lock, a console to control operating conditions, screens to view control interfaces and sample output, and a computer for control of data acquisition. The EPMA includes, from left to right, an electron gun, sample chamber with detector array, control console, and monitors. Image courtesy of the University of Minnesota Electron Microprobe Lab. Details

Quantitative EPMA analysis is the most commonly used method for chemical analysis of geological materials at small scales. In most cases, EPMA is chosen where individual phases need to be analyzed (e.g., igneous and metamorphic minerals), or where the material is of small size or valuable for other reasons (e.g., experimental run product, sedimentary cement, volcanic glass, matrix of a meteorite, archaeological artifacts such as ceramic glazes and tools.

It uses wavelength dispersive spectrometers for the quantitative chemical analysis at high sensitivity. Chemical analysis of solid materials as small as 1-2 micron diameter at small spatial scales can be done by using EPMA. Spot chemical analyses can be obtained in situ, due to which small compositional variations within textural context or within chemically zoned materials can be done. Electron probes commonly have an array of imaging detectors that allow the investigator to generate images of the surface and internal compositional structures that help with analyses.

Limitations

Electron probes have the ability to analyze almost all elements, but it is incapable to detect the lightest elements (H, He and Li); as a result, for example, the "water" in hydrous minerals cannot be analyzed. Some elements generate x-rays with overlapping peak positions (by both energy and wavelength). So the resolution of the instrument should be further improved to separate these lines. Microprobe analyses are reported as oxides of elements, not as cations; therefore, cation proportions and mineral formulae must be recalculated following stoichiometric rules. EPMA cannot distinguish between the different valence states of Fe, so the ferric/ferrous ratio cannot be determined

Sample Preparation for EPMA:

Sample in the powder form is mixed with adhesive (a mixture of fevite and xylene in 1:1 ratio). The mixture is then kept in a frame to give it a shape also the sample is hardened. It is kept there for 2 days. The rough sample is then polished first with 600 mesh size carborandum powder then again with 800 mesh size powder. When the sample becomes plane; it is then mounted on a glass slide. Mounting was done using an adhesive prepared by adding 50% hardener and 50% resin. This adhesive is applied both on the sample and on the glass slide. It was kept in this manner for a few minutes and the sample was then mounted on the slide. After mounting, the sample was made thin (polished) by patrothin thin sectioning machine. The thin sample was first rubbed with 300 mesh size (coarse) carborandum powder, followed by polishing with 400 mesh size powder and finally polished with fine 800 mesh size carboradum powder. Every time the slide was being checked by a microscope to verify the fineness of the sample.

2.4.3 Atomic Absorption Spectroscopy(AAS):

Atomic-absorption (AA) spectroscopy uses the absorption of light to measure the concentration of gas-phase atoms. Since samples are usually liquids or solids, the analyte atoms or ions must be vaporized in a flame or graphite furnace. The atoms absorb ultraviolet or visible light and make transitions to higher electronic energy levels. The analyte concentration is determined from the amount of absorption. Applying the Beer-Lambert law directly in AA spectroscopy is difficult due to variations in the atomization efficiency from the sample matrix, and non uniformity of concentration and path length of analyte atoms (in graphite furnace AA). Concentration measurements are usually determined from a working curve after calibrating the instrument with standards of known concentration.

Principle:

In short, the electrons of the atoms in the atomizer can be promoted to higher orbitals for an instant by absorbing a set quantity of energy (i.e. light of a given wavelength). This amount of energy (or wavelength) is specific to a particular electron transition in a particular element, and in general, each wavelength corresponds to only one element. This gives the technique its elemental selectivity. As the quantity of energy put into the flame is known, and the quantity remaining at the other side (at the detector) can be measured, it is possible, from Beer-Lambert law, to calculate how many of these transitions took place, and thus get a signal that is proportional to the concentration of the element being measured.

Instrumentation of AAS:

The following are the different parts of the atomic absorption spectrometer :

Hollowcathodelamp:

Source of the analytical light line for the element of interest Give a constant and intense beam of that analytical line

Nebulizer:

Suck up liquid sample at a controlled rate Create a fine aerosol spray for introduction into the flame Mix the aerosol and fuel and oxidant thoroughly for introduction into the flame

Flame:

Destroy any analyte ions and breakdown complexes Create atoms of the element of interest, Fe⁰, Cu⁰, Zn⁰, etc.

Monochromator:

Isolate the analytical line photons passing through the flame
Remove scattered light of other wavelengths from the flame In doing this, only a narrow spectral line impinges on the PMT.

Photomultiplier tube:

This is the detector. The PMT determines the intensity of photons of the analytical line exiting the monochromator. The PMT is the most commonly used detector for atomic absorption spectroscopy. However, solid state detectors are now replacing conventional vacuum-type photomultipliers. High tech electronics amplify, filter, and process the electrical signal, using a series of chips and microprocessors, transmitting the result to an internal or external computer which handle all data-handling and display.

Graphite Furnace:

graphite furnace is an electrothermal atomiser system that can produce temperatures as high as 3.000°C. The heated graphite furnace provides the thermal energy to break chemical bonds within the sample held in a graphite tube, and produce free ground state atoms. Ground-state atoms then are capable of absorbing energy, in the form of light, and are elevated to an excited state. The amount of light energy absorbed increases as the concentration of the selected element increases. Flame AA can only analyse solutions, but graphite furnace can accept very small absolute quantities of solution, slurry or solid samples.

Atomizer:

In order to analyze a sample for its atomic constituents, it has to be atomized. Atomizer is the device that produces a spray of fine droplets of liquid i.e. it atomises the sample. A vertical tube connected with a horizontal tube dips into a bottle of liquid, and at one end of the horizontal tube is a nozzle, at the other a rubber bulb. When the bulb is squeezed, air rushes over the top of the vertical tube and out through the nozzle. Following Bernoulli's principle the pressure at the top of the vertical tube is reduced, allowing the liquid to rise. The air stream picks up the liquid, breaks it up into tiny drops, and carries it out of the nozzle as a spray. Scent spray, paint spray guns, and carburettors all use the principle of the atomizer.

2.4.4 Protocol for Total Metal Determination:

Around 1 gm of sample was taken in a cleaned Teflon beaker. 15 ml aquaregia was added to it. The solution was kept for overnight reaction. Next, a 10 ml of HF was treated for a few hours and heated on a hot plate to digest sample completely. During the heating the excess HF if present will vapourize. The complete digestion was achieved by heating till the brown fumes (NO_2) ceases. The solution was cooled & filtered through whatman filter paper in to a 25 ml volumetric flask. The volume was then made up to the mark with milliQ water. It may be noted that a small amount of acidified water was added to make up the final volume to avoid precipitation of metals while storing the samples for element analysis by AAS.

2.4.5 Heavy Metal speciation by Sequential Extraction

The five fractions as discussed earlier were obtained by treating the sample sequentially as the protocol given below [66]

- Fraction 1 10ml 1M MgCl_2 , pH 7 leaching for 1hr
- Fraction 2 10 ml 1M NaOAc (Sodium Acetate), pH 5 ,leaching for 1 hr
- Fraction 3 10 ml 0.2M calcium oxalate + 0.2M oxalic acid, leaching for 1 hr
- Fraction 4 10 ml NaOH, leaching for 1 hr
- Fraction 5 10 ml HF + HCl, leaching for 5 hr

Washing of the glasswares:

All the glassware's was first cleaned with soap solution. Then it was kept in nitric acid for around 1 hour then deeped in distilled water for 1hr and again washed with distilled water for 3-4 times to make the apparatus free from acid. The apparatus was then dried in an oven.

The experimental protocol is discussed briefly as follows:

Fraction-1 (Ion-exchangeable):

Weighted amount of the sample was taken in a 50 ml conical flask. For the first fraction 10 ml of MgCl_2 was added. The solution was leached for 1hr. the total container was transferred to a centrifuge tube and centrifuged at about 4000 rpm for ~6-7 min depending upon the sample. The supernatant liquid was transferred to the previous conical flask. The residue was dried in the oven at 80 degree centigrade for 2hr.

Fraction-2 (Carbonate):

For the 2nd fraction 10 ml of NaOAc was added to the dried residue in the centrifuge tube. The whole container was transferred to another cleaned conical flask, kept it for 1 hr leaching in a conical flask. Again the same procedure was followed and the supernatant liquid was collected in the same conical flask and named it as F-2 (2nd fraction).

Fraction-3 (Oxide):

To the residue obtained from the 2nd fraction was leached with a mixture of 10 ml 0.2M calcium oxalate and 0.2M oxalic acid, leaching for 1 hr in a cleaned conical flask. After 1 hr the solution was transferred to a centrifuge tube. The solution was centrifuged for ~10 min and the 3rd fraction was collected in a cleaned conical flask.

Fraction-4 (Organic):

Similarly the residue from the 3rd fraction was leached with 10 ml NaOH, for 1 hr. The solution was centrifuged for ~10 min and the supernatant liquid was collected in a cleaned conical flask and named as 4th fraction.

Fraction-5 (Residual):

All the above fractions were filtered through whatman filter paper (41) into a 25 ml conical flask. The volume was made up to the mark. The samples were taken in thoroughly cleaned sample vials for AAS analysis.

The fractionated samples were analysed by AAS.

3.1 Major Composition:

The major element analyses of the sediment samples were carried out using SEM-EDAX and further corroborated by point analysis by EPMA. The SEM studies show wide range of sizes of the particles (from 30 to 350 μm). It also revealed different shapes of these particles, e.g., rectangular, oval, squarish, nearly spherical etc (**Fig. 2a**), which represents the nature of the sample. The X-ray spectrum of the scanned region imaged by SEM showed strong K-X-ray peaks of O, Si, and Al (**Fig. 2b**). The K- X-ray of C is primarily from the carbon tape used for mounting samples. The atomic ratios of Si:O for a particular sample (referred to as S1:0-4 cm; which means the sample belongs to S1 sampling site and collected from the first 4 cm of the sediment core) was found to be 3.6. The samples from all the three sampling sites were analyzed by EDAX and the atomic ratio of Si:O was found to vary between 2.2 and 6.8, which primarily indicates the presence of silicates and the variation of the ratios could be due to the variation of sizes of the particles. This is in analogy to the studies of core sediments from the Hugli estuary that showed a wide range of Si/Al ratios (2.63–9.90) reflecting different mixtures of coarse and fine-grained materials [67, 68]. The X-ray spectrum also reveals signature of S –K α X-rays (**Fig. 2b**). It is however not possible to discern here if the sulphur is due to sulphide (related to anaerobic condition) or sulphate (after sulphide is oxidised). In this regard, it should be noted that the estuarine sediments are usually associated with anaerobic conditions rich in sulphides.

From the above scan region, a single grain marked in red box as given in **Fig 2a** was selected for element analysis by EDAX, which revealed very strong Si X-ray signal as compared to O signal (**Fig. 2c**). The Si:O atomic ratio in this selected grain was found to be nearly 1:2. It also showed very strong Al X-ray peak and may be suggesting aluminosilicate phase. A strong positive correlation was observed between Al and K (shown in **Fig. 3a**). It was also noted that the present Al, Si rich mineral was associated with high K content, where the K ion is most likely bound to the silicate matrix. A careful observation of EDAX measurements of the samples from the three different sampling sites indicated that K and Al are positively correlated (**Fig. 3b**) and Fe is negatively correlated with both Al and K (**Fig. 4a and 4b**).

In site 3 (S3), the silicate fraction was about 20 – 22% by weight for the first 12 cm of the sediment from the surface (shown in **Table 2**), whereas at a greater depth, the silicate fraction was higher (25-26%) by weight. The bulk Fe level was found to be higher (4.9 – 7.5

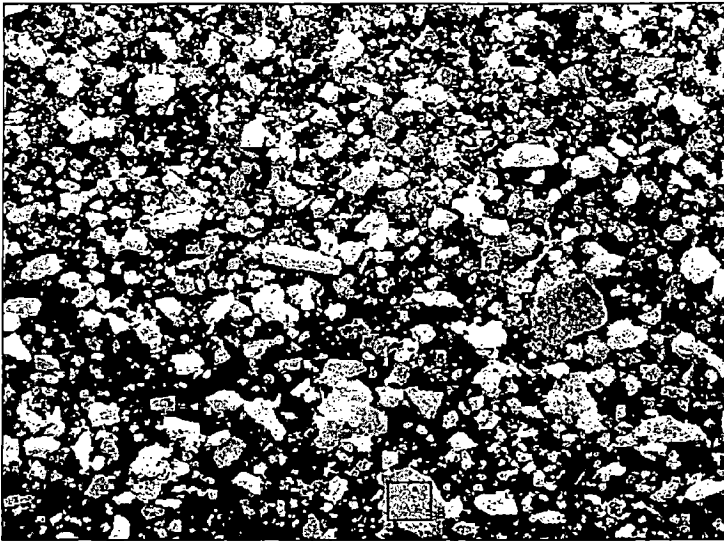


Fig. 2a: SEM image of the sediment particles of S1.

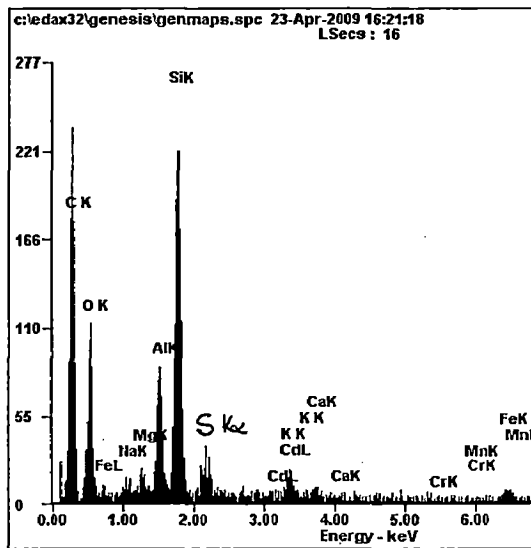


Fig. 2b: X-ray spectrum (EDAX) of the scanned area of Fig. 2a

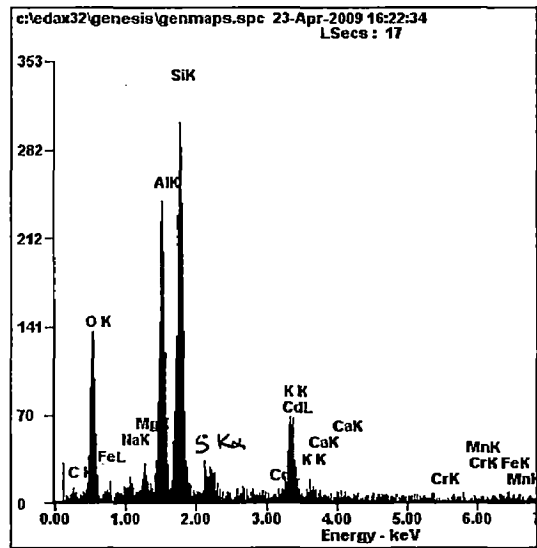


Fig. 2c: X-ray spectrum (EDAX) of the single mineral grain marked in Fig. 2a

Samples	Si	Al	K	Fe
S1: 0-4	29.0	18.1	8.6	2.0
S1: 8-12	23.6	7.2	3.1	3.9
S1: 16-20 sg	28.5	18.1	10.3	3.9
S1: 16-20	24.7	7.7	0.7	13.4
S1: 16-20	21.7	7.1	2.1	5.9
S1:16-20 sg	25.5	6.1	3.1	5.1
S2:0-4	21.7	11.8	6.1	4.1
S3 :0-4	20.2	6.7	2.6	5.9
S3: 4-8	20.5	8.1	2.9	5.9
S3-8-12	21.6	8.2	2.5	5.7
S3:16-20	24.9	10.2	3.6	7.5
S3: 20-24	18.2	6.7	2.6	4.9
S3: 24-28	25.3	9.1	3.4	6.6

(sg: single grain analysis)

Table 2: X-ray analysis of S1-S3 sediments by EDAX

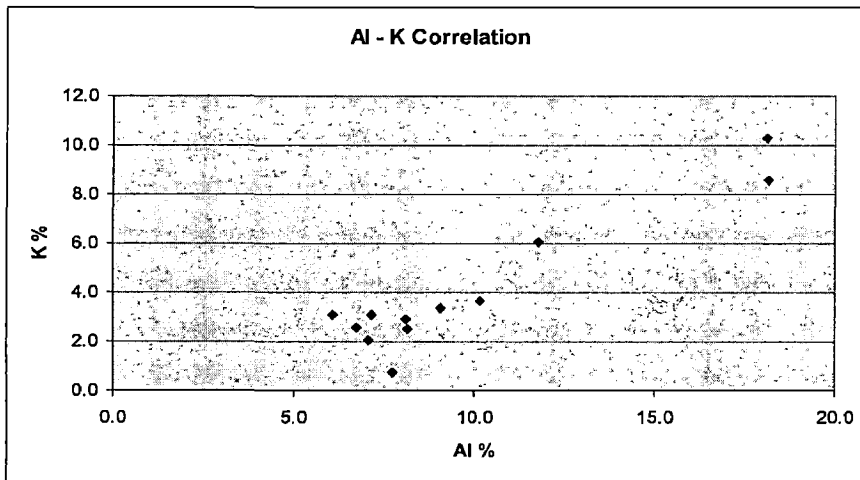


Fig. 3a: Showing Al – K positive correlation (EDAX)

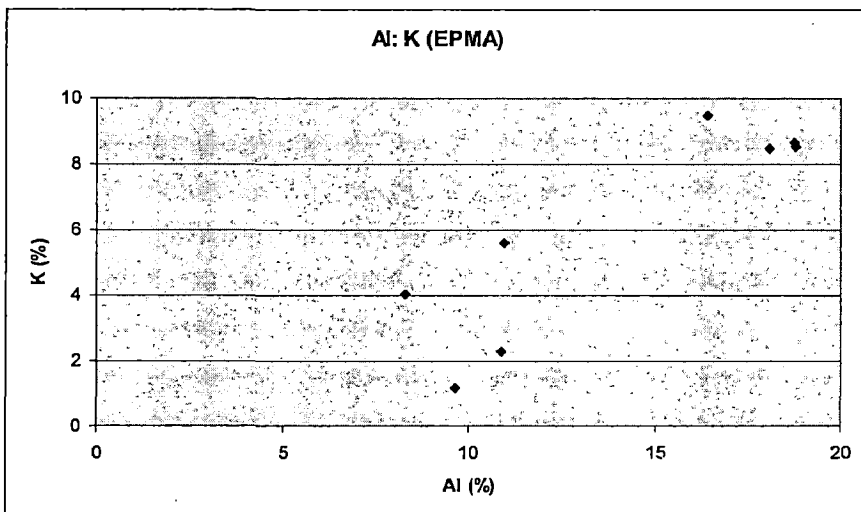


Fig. 3b: Showing Al – K positive correlation (EPMA)

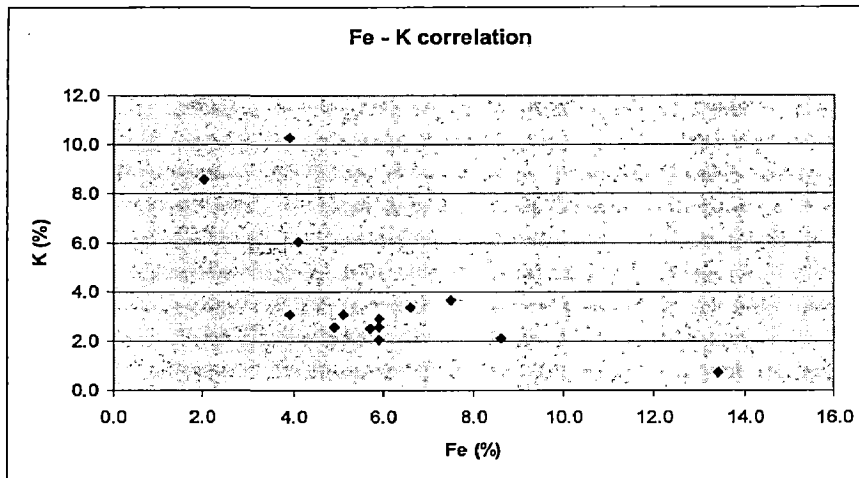


Fig. 4a: showing a negative correlation between Fe and K of sediments S1 and S3 (EDAX)

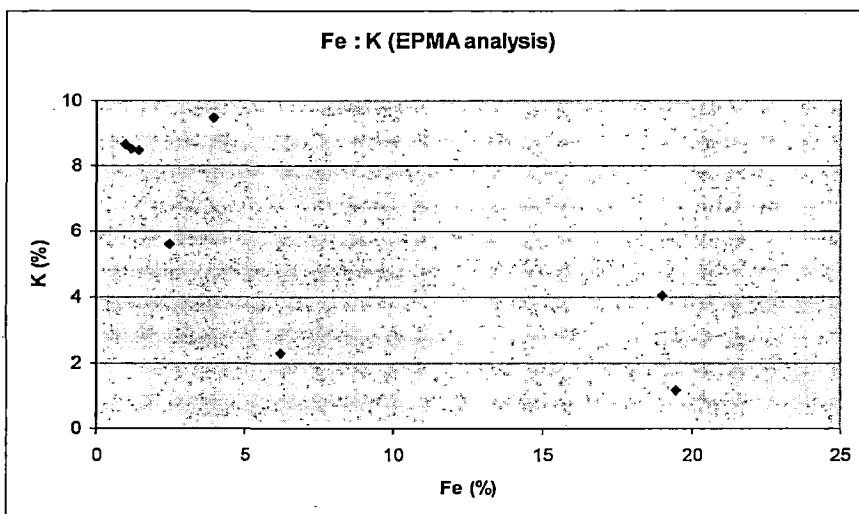


Fig. 4b: Fe - K negative correlation obtained from EPMA analysis of S1 and S2 sediment (EPMA)

%) in the samples from site 3 (S3) as compared to those from site 1 (S1), where Fe level was between 2.0 and 3.9 %. It may be noted here that there is insignificant X-ray signal for C, which clearly suggests that the C X-ray observed in the Fig. 2a was due to the exposed carbon tape, as in the single grain analysis, no carbon tape was scanned.

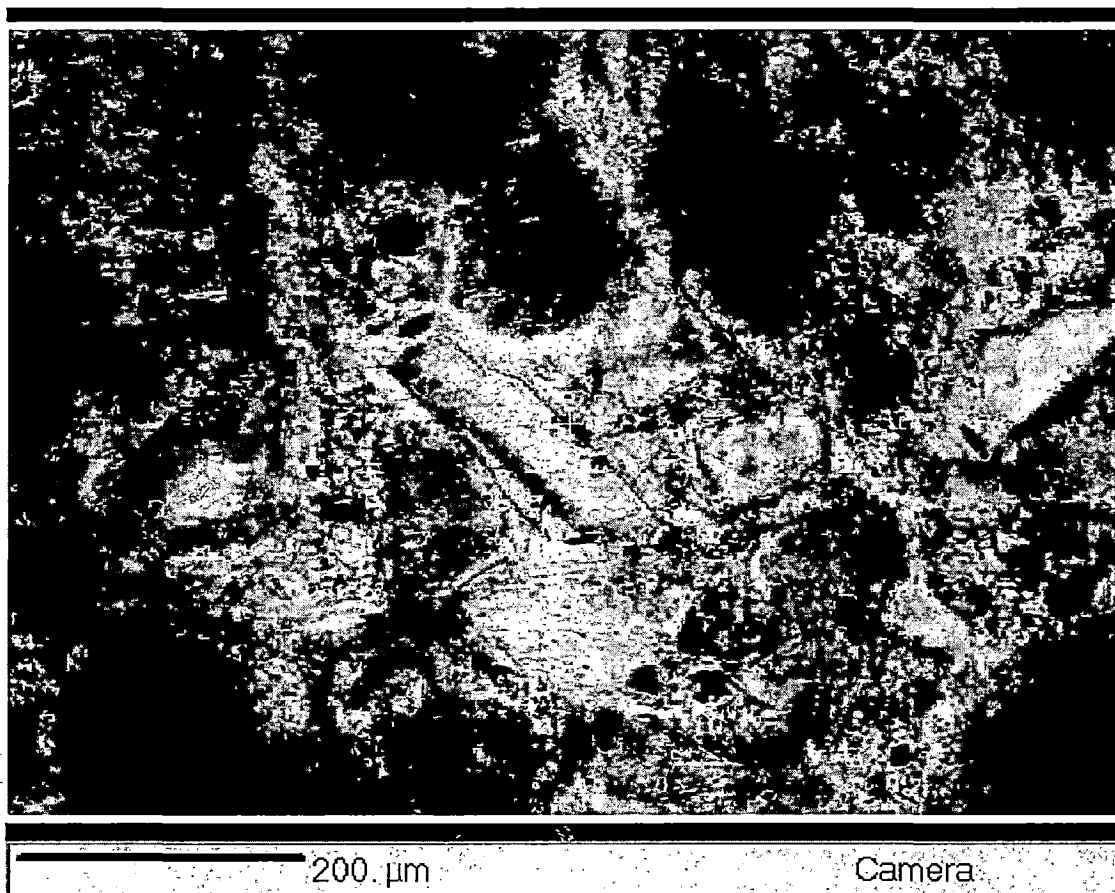


Fig. 5: EPMA image of the sample S1: 24-28 cm depth

The EPMA image of the sample S1: 24-28cm depth is shown in **Fig. 5**, that exhibits the mineral grain in the sample and the mark of the point analysis is shown there in. The Point analysis of some selected mineral grain was carried out on S1: 24-28 cm depth by EPMA, which showed Si values ranging from 17.1 % to 30.5 %, Al (8.3 % to 18.8%), K (1.2 % to 9.4 %), given in **Table 3a**, are all in good agreement with the EDAX analysis of single grain analysis and block scan analysis of the samples of S1 sites. It was also noted that the atomic ratio measured from EPMA studies also revealed silicate matrix (i.e., Si:O = 1:3.5) as shown in **Table 3b** and is a good supplement to EDAX data as discussed above. The positive correlation between Al and K, and the inverse correlation of Fe and K was also observed in the EPMA “point analysis” of the samples from site S1 (S1:24 – 28 cm depth) and for the sample from site S2 (S2: 12 – 16 cm depth) as shown in **Fig. 3b** and **4b** respectively. The EPMA analysis also revealed the concentrations of Na (0.43 %- 1.04 %), Ca (0.02 – 1.19%), Mg (0.5-7.3%), Ti (0.11 – 0.88%) and Mn (0.01 – 0.64%). It should be realized that the detection limits of these elements are of the order of 0.01% which is much better reliable as

compared to EDAX data. It was found that the most of the minor elements like Ti, Mg, Ca and Na are much lower in concentrations in the sample collected from S3 site (S3: 24 – 28 cm depth) shown in **Table 4**, as compared to that of S1: 24 – 28 cm depth. So it may be concluded that EPMA together with SEM EDAX are useful tools for characterizing the major and the minor element composition of sediments.

Na	Mg	Al	Si	K	Ca	Ti	Mn	Fe	O	Total
0.75	0.58	18.73	22.96	8.67	0.02	0.52	0.01	0.97	45.87	99.07
0.68	0.62	18.8	22.89	8.54	0.02	0.44	0.02	1.15	45.85	99.03
1.04	7.3	8.3	17.13	4.03	1.19	0.88	0.64	18.99	39.56	99.04
0.45	0.98	16.4	23.34	9.47	0.1	0.11	0.07	3.93	45.17	100.02
0.55	0.48	18.05	24.23	8.5	0.08	0.21	0.02	1.4	46.5	100.04
0.53	9.1	9.64	17.11	1.18	0.35	0.72	0.26	19.45	40.75	99.09
0.43	0.56	10.98	30.45	5.62	0.14	0.33	0	2.47	47.12	98.09
0.96	2.09	10.89	28.77	2.31	1.31	0.42	0.07	6.21	47.25	100.3

Table 3a: point analysis of different grains of sample S1: 24-28 cm depth by EPMA in wt%

Na	Mg	Al	Si	K	Ca	Ti	Mn	Fe	O	Total
0.7	0.51	14.81	17.45	4.73	0.01	0.23	0	0.37	61.19	100
0.63	0.55	14.89	17.4	4.67	0.01	0.2	0.01	0.44	61.2	100
1.06	7.08	7.25	14.39	2.44	0.69	0.44	0.27	8.02	58.35	100
0.42	0.87	13.09	17.91	5.22	0.05	0.05	0.03	1.51	60.84	100
0.5	0.42	14.14	18.24	4.59	0.04	0.09	0.01	0.53	61.43	100
0.53	8.66	8.27	14.11	0.7	0.21	0.35	0.11	8.07	58.99	100
0.4	0.49	8.7	23.19	3.08	0.07	0.15	0	0.94	62.98	100
0.89	1.82	8.54	21.69	1.25	0.7	0.18	0.03	2.36	62.54	100

Table 3b point analysis of different grains of sample S1: 24-28 cm depth by EPMA in At

Na	Mg	Al	Si	K	Ca	Ti	Mn	Fe	O	Total
0.41	7.99	13.63	12.44	0.16	0.16	0.07	0.15	24.97	39.04	99.02
0.55	8.06	13.7	12.84	0.17	0.2	0.07	0.16	23.9	39.39	99.05
0.73	0.81	17.96	23.65	8.37	-0.03	0.27	0	1.3	45.97	99.02
0.2	9.14	8.35	19.92	0.38	0.75	0.09	0.2	18.16	41.9	99.08
0.24	0.11	0.27	45.24	0.12	0.16	0.02	0.01	0.65	52.25	99.09
0.2	0.07	0.05	45.33	0.16	0.16	0.02	-0.01	0.15	51.95	98.07
0.74	1.53	5.83	35.73	1.33	1.05	0.11	0.07	3.66	49	99.07
1.36	4.9	11.08	24.99	1.69	0.79	0.15	0.1	8.71	45.32	99.09

Table 4. Point analysis of the Sample S3: 24-28 cm depth (Wt %) by EPMA

3.2 Total Metal Analysis (trace heavy metals):

The total metal contents of Cr, Mn, Co, Ni, Cu, Zn, Pb and Cd for the samples from the three locations or sites S1- S3 with their respective depths are given in **Table 5**. The means and the standard deviations of these heavy metals along the depth from the surface to inwards are given in Table 6. It can be observed from table 24b, that mean Cr is higher in S1 and S2 samples than S3 samples. The mean Co is higher in S1 and nearly similar for S2 and S3. The Ni (33.5 ppm – 42.3 ppm), Zn (55.2 – 60.7 ppm) and Cd (~ 5.4 ppm) contents are more or less of similar levels in all the three sampling locations. The Cu was found to be almost 2 folds higher in S3 (56.6 ppm) samples as compared to S1 (37.0 ppm) and S2 (34.5

Sample	Cr	Co	Ni	Cu	Zn	Pb	Cd	Mn
S1: 0 – 4	19.5	12.4	26.9	29.4	41.2	4.5	5.1	Not measured
S1: 12 – 16	71.3	32.3	35.1	32.2	61.2	11.4	5.5	154
S1: 16 – 20	46.5	56.4	35.4	45.4	69.4	11.7	5.8	476
S1: 20 – 24	21.7	11.2	32.4	36.2	46.5	13.9	5.3	318
S1: 24 – 28	43.7	26.6	34.8	46.1	73.3	15.2	5.8	295
S1: 28 – 32	39.6	25.4	36.2	32.6	65.4	26.0	6.2	214
S2: 0 – 4	63.2	22.6	32.6	32.8	70.2	11.8	6.1	152
S2: 4 – 8	56.6	14.4	36.4	27.3	40.6	16.4	6.2	161
S2: 8 – 12	26.6	16.3	39.9	38.2	53.5	26.5	5.6	122
S2: 12 – 16	29.8	16.8	43.3	34.5	58.6	14.2	5.3	225
S2: 16 – 20	23.8	15.2	37.6	32.4	55.6	12.3	5.3	346
S2: 20 – 24	27.0	17.2	42.7	34.8	54.8	9.6	5.5	377
S2: 24 – 28	27.2	17.4	30.5	41.2	53.2	11.7	5.3	268
S3: 0-4	19.4	18.5	23.0	13.6	54.5	10.4	5.2	184
S3: 4 - 8	26.4	18.8	52.7	63.5	52.3	22.3	5.5	138
S3: 8 – 12	24.4	16.6	46.7	62.4	65.8	20.8	5.7	182
S3: 12-16	21.0	17.4	50.5	51.7	58.3	22.5	5.2	198
S3: 20-24	26.4	16.99	52.8	78.6	63.3	22.5	5.5	242
S3: 24 – 28	33.5	12.2	31.6	62.2	75.4	19.2	5.6	290
S3: 28 – 32	19.7	16.6	38.7	64.5	55.5	18.2	5.3	216

Table 5: Total metal analysis of the sample from sites S1, S2, S3 with their respective depths of the core sediment.

ppm) samples. It is further noted that the minimum Pb is highest in S3 (19.4 ppm) location which is located very close to Kolkata as compared to S1 (13.8 ppm) and S2 (14.6 ppm). In this regard, it may be substantiated that the Site S3 is also higher in Ni and Cu which may be attributed to the discharge of nearby industrial effluents. Though the Mn values are apparently different for S1 – S3 samples, but high standard deviation (due to large variation in Mn containing minerals) restricted us to comment on the variations of Mn levels in the three

sites. Moreover, it could be noted that the standard deviation is large for the other heavy metals like Cr, Co (especially for S1), Ni and Cu for S3 samples. This clearly indicates that there is a definite variation of heavy metal levels along the depth of the sediment which is most likely due to environmental factors and to some extent due to industrial activities.

Samples		Cr	Co	Ni	Cu	Zn	Pb	Cd	Mn
S1 0-32cm	Mean	40.4	27.4	33.5	37.0	59.5	13.8	5.6	291.4
	Sd	18.9	16.5	3.5	7.1	12.9	7.0	0.4	122.1
S2: 0-28 cm	Mean	36.3	17.1	37.6	34.5	55.2	14.6	5.6	235.9
	Sd	16.3	2.6	4.8	4.4	8.7	5.7	0.4	99.0
S3: 0-32 cm	Mean	24.4	16.7	42.3	56.6	60.7	19.4	5.4	207.1
	Sd	5.0	2.2	11.6	20.5	8.1	4.3	0.2	48.6

Table 6: Mean and Standard deviation of the concentration of the heavy metals along the depth of the sediment.

The heavy metal distribution along the depth of the sediments of the three sampling locations S1, S2 and S3 are shown in Fig. 6a-c. It could be seen in the Fig. 6a-c, that the Cd and Ni levels in the sediments collected at different depths did not show much variation for all the three sampling sites.

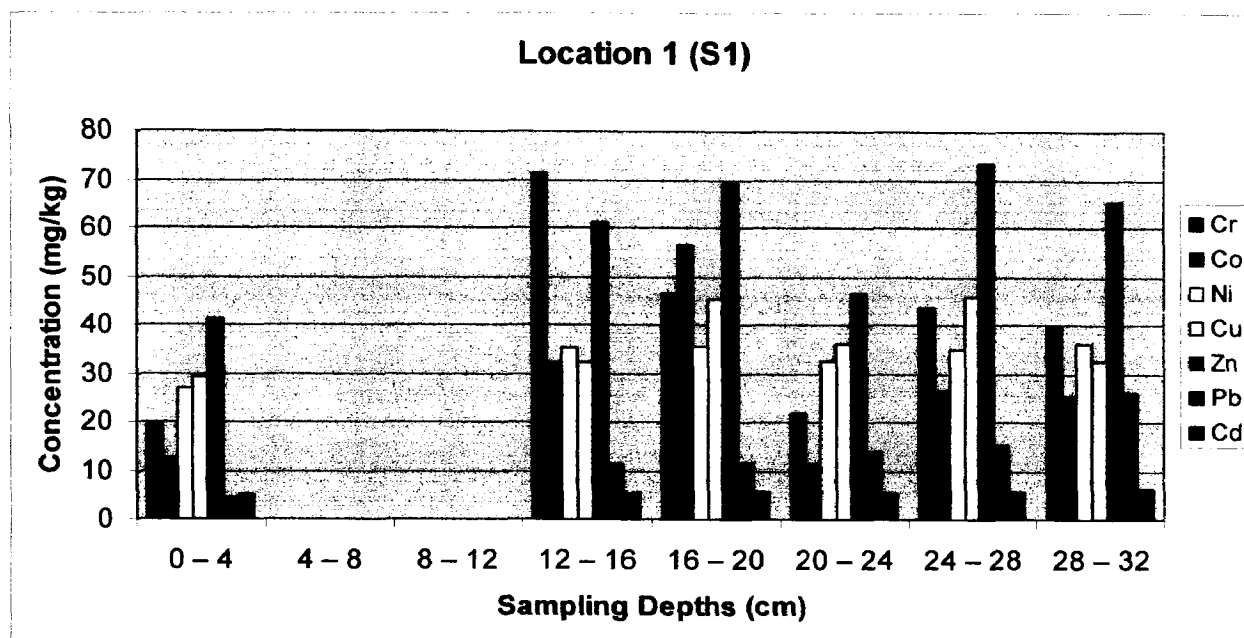


Fig. 6a: The heavy metal distribution of the sediment core divided at 4 cm each of the three sampling locations S1

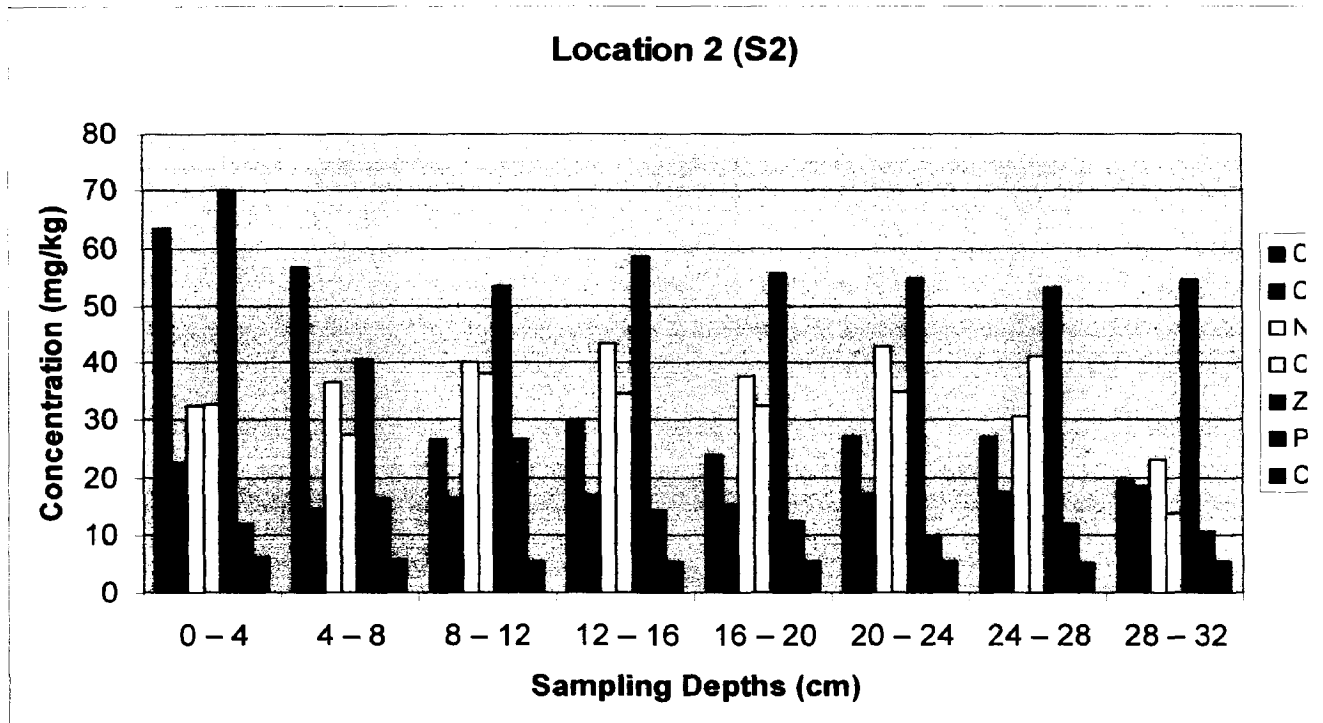


Fig. 6b: The heavy metal distribution of the sediment core divided at 4 cm each of the three sampling locations S2

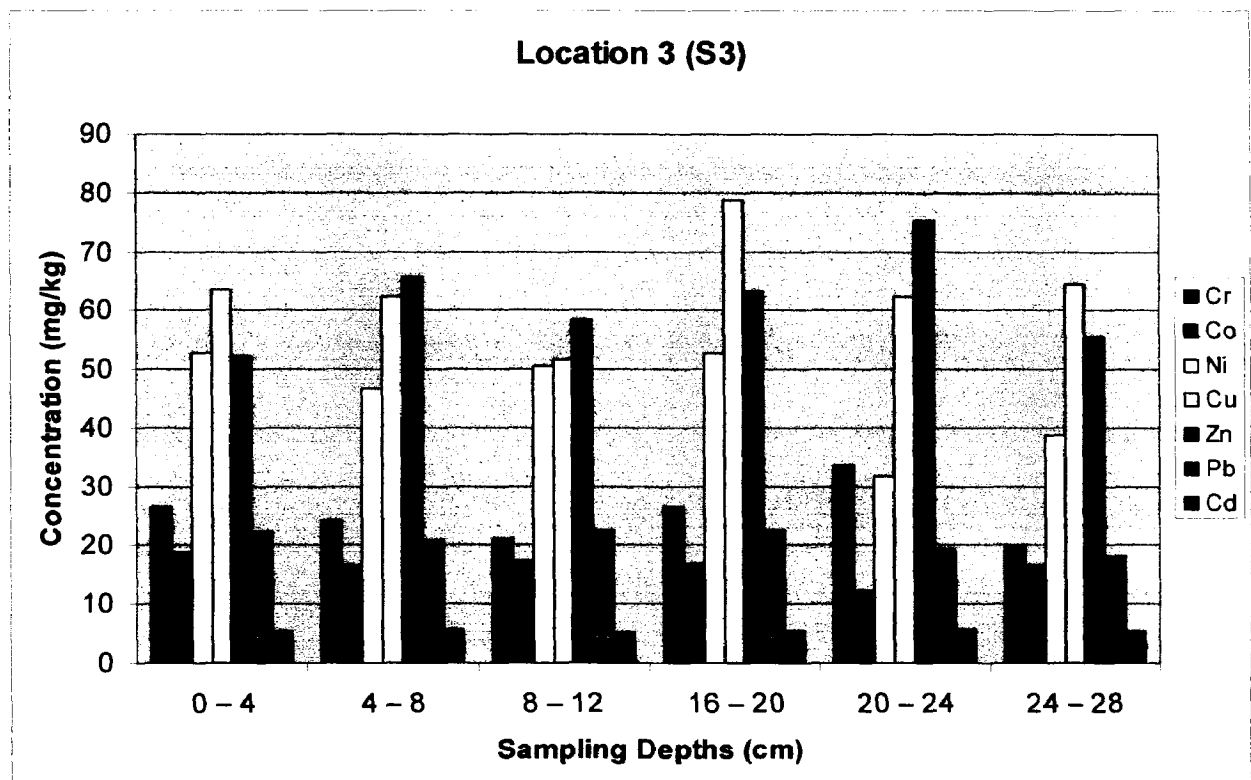


Fig. 6c: The heavy metal distribution of the sediment core divided at 4 cm each of the three sampling locations S3

The depth profiling of individual heavy metals are discussed below:

Mn:

Besides, depth-wise concentration distribution of Mn for all three locations (Fig.7a) clearly shows variation in Mn levels in different depths, higher at greater depths and it was maximum at a depth of 16 – 20 cm and also in higher amount beneath that (i.e., 20 – 24 cm) for all the three location. Except for the depth where maximum Mn was observed, for other depths, there is hardly any difference in Mn levels among the three locations.

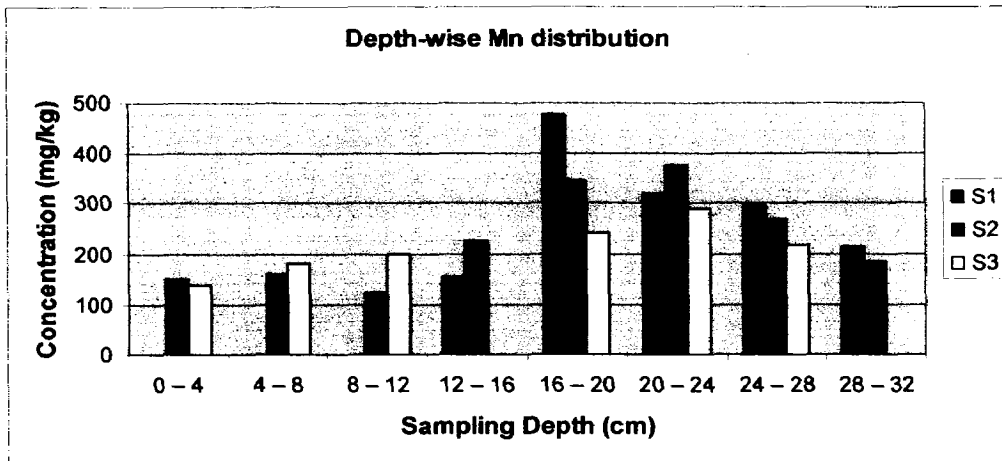


Fig. 7a: Depth-wise concentration distribution of Mn for all three sampling sites

Cr:

The Cr levels were found to be higher near the surface of the sediments from Site S1 (0- 8 cm) as shown in Fig. 17b. The Cr levels in Sediments from S3 are nearly uniform along the depth, while extremely high Cr levels were observed at the depths of 12-16 cm (71 ppm) and 16-20 cm (45 ppm) in the sediments from S1 site.

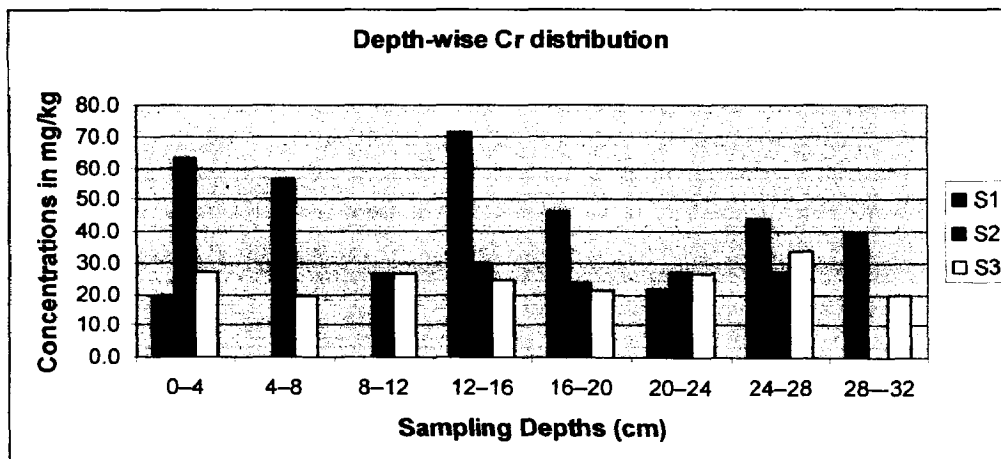


Fig. 7b: Depth-wise concentration distribution of Cr for all three sampling sites

Cu:

Higher levels of Cu were observed in the sediments of S3, especially for the sediments below 4 cm from the surface as shown in Fig. 17c. Maximum value of Cu was measured at the depth of 20 – 24 cm. It may be noted that the Cu levels at various depths of S1 and S2 were measured to be of similar levels (30 – 40 ppm)

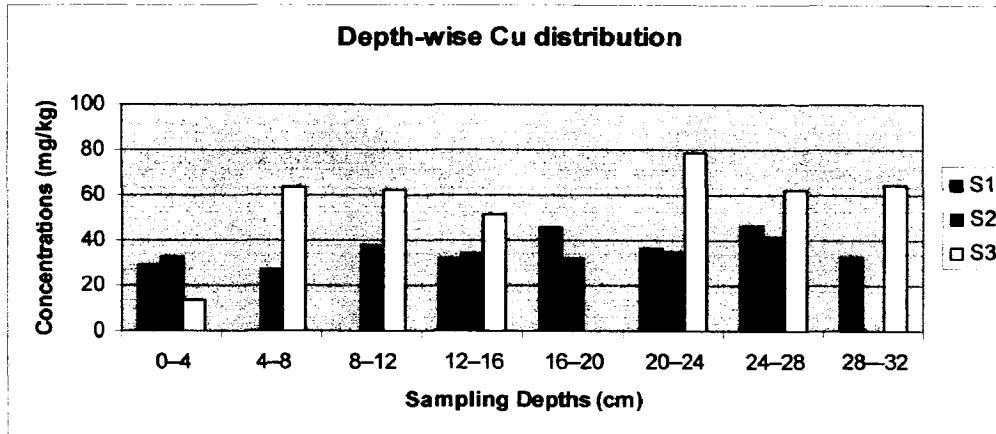


Fig. 7c: Depth-wise concentration distribution of Cu for all three sampling sites

Zn:

A marginal depth-wise distribution of Zn could be observed in the sediments from S1-S3 as shown in Fig. 17d. About 10 – 20 % higher Zn levels was measured in S1 sediments.

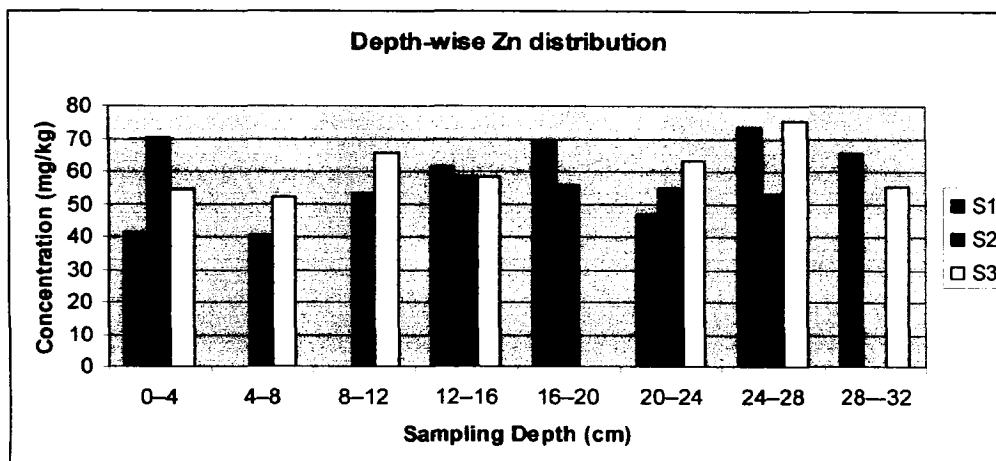


Fig. 7d: Depth-wise concentration distribution of Zn for all three sampling sites

Pb

Maximum Pb for sediments from S1 was measured at the bottom sediment (26 ppm). The Pb contents in the sediments from S3 site was found to be more or less uniform from a depth of 4 cm onwards (~ 23 ppm). On the other hand, the Pb levels for S2 sediments were found to be increasing at the shallower depths and at greater depths 12 cm onwards (~14 ppm), the Pb contents were quite low as compared to the above layers (27 ppm), as shown in Fig. 17e

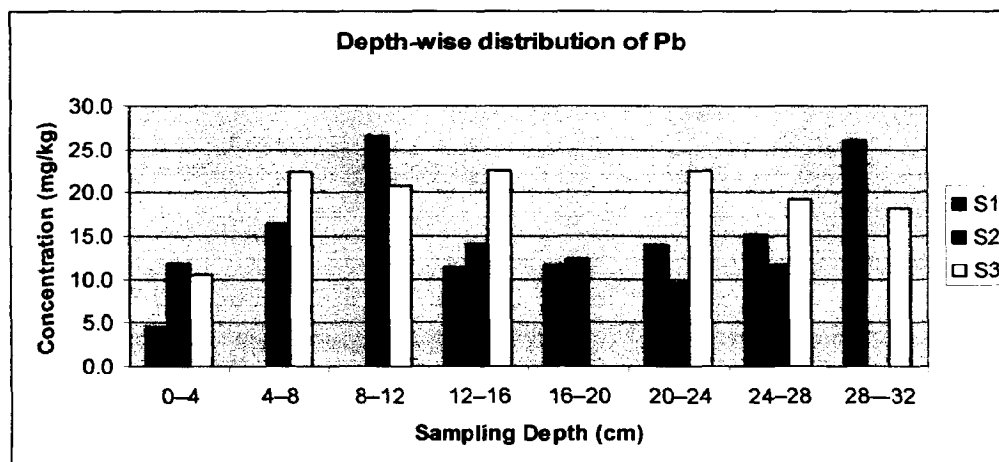


Fig. 7e: Depth-wise concentration distribution of Pb for all three sampling sites

3.3 Heavy Metal Speciation and their Depth Profiling:

The sediment samples were sequentially extracted using the extracting agents as discussed in the section 2.3.5 in order to find out the fraction of each heavy metals present as one of the following species and essentially the sum of all the fractions should lead to the value obtained in total metal contents of individual heavy metals.

1. Exchangeable (adsorbed and exchanged) (F1)
2. Bound to Carbonates, (F2)
3. Bound to Iron and Manganese Oxides, (F3)
4. Bound to Organic Matter (humic substances) (F4)
5. Residual (Primary and Secondary Minerals). (F5)

Where the F1 to F5 represents the respective fractions or metal species.

It was noted that the sum of the heavy metals present as each of the above defined fractions were mostly within 10 – 15 % of the measured total metal values. except in a few cases where the sum fraction deviated by ~ 20% and in case of Cd, it was as high as 40%

Cr

Samples	F1	F2	F3	F4	F5	Sum	Total metal
S1: 12 – 16 cm	2.1	2.2	3.4	0	51.2	58.9	71.3
S1: 16 – 20 cm	2.4	1.3	2.1	0.2	32.6	38.6	46.5
S1: 24 – 28 cm	2.7	1.6	4.5	0	30.4	39.2	43.7
S1: 28 – 32 cm	3.6	1.3	2.9	1.1	22.5	31.4	39.6
S2: 0 – 4 cm	1.5	3.3	8.8	0	35.8	49.4	63.2
S2: 4 – 8 cm	1.9	2.5	9.3	0	30.3	44	56.6
S2: 16 – 20 cm	2.1	1.6	6.5	2.8	9.4	22.4	23.8
S2: 20 – 24 cm	2.4	1.4	7.3	3.6	8.4	23.1	27
S2: 24 – 28 cm	1.8	1.8	5.5	2.8	12.4	24.3	27.2

Table 7a: Cr speciation distribution in selected sediments of S1 and S2**Mn**

Samples	F1	F2	F3	F4	F5	Sum	Total metal
S1: 12 – 16 cm	13.6	56	44	0.2	66	179.8	154
S1: 16 – 20 cm	18.4	156	176	0.2	66	416.6	476
S1: 24 – 28 cm	16.2	87	96	0.2	74	273.4	295
S1: 28 – 32 cm	13.2	59	62	1.3	58	193.5	214
S2: 0 – 4 cm	15.4	24	38	0.3	61	138.7	152
S2: 4 – 8 cm	16.2	35	40	0.4	58	149.6	161
S2: 16 – 20 cm	28.4	120	72	1.8	108	330.2	346
S2: 20 – 24 cm	32.2	158	64	0.6	96	350.8	377
S2: 24 – 28 cm	20	86	60	1.3	94	261.3	268

Table 7b: Mn speciation distribution in selected sediments of S1 and S2**Cu**

Samples	F1	F2	F3	F4	F5	Sum	Total metal
S1: 12 – 16 cm	2.4	2	7.2	1.9	14.4	27.9	32.2
S1: 16 – 20 cm	1.7	2.8	10.6	2.4	27.4	44.9	45.4
S1: 24 – 28 cm	1.1	1.2	16.8	2.6	26.8	48.5	46.1
S1: 28 – 32 cm	0.8	1.1	11.4	0.6	19.5	33.4	32.6
S2: 0 – 4 cm	1	1	12.2	0	14.2	28.4	32.8
S2: 4 – 8 cm	1.2	4.1	12.6	0	14	31.9	27.3
S2: 16 – 20 cm	1.2	3.9	12.9	0	13.5	31.5	32.4
S2: 20 – 24 cm	1.6	7.1	13.5	1	13.8	37	34.8
S2: 24 – 28 cm	2.2	7.6	15.3	1.2	18.8	45.1	41.2

Table 7c: Cu speciation distribution in selected sediments of S1 and S2**Zn**

Samples	F1	F2	F3	F4	F5	Sum	Total metal
S1: 12 – 16 cm	3.2	6.2	13.2	2.1	26.2	50.9	61.2
S1: 16 – 20 cm	3.8	7.1	15.5	2.2	30.2	58.8	69.4
S1: 24 – 28 cm	3.1	5.1	22.6	2.4	37.3	70.5	73.3
S1: 28 – 32 cm	3.1	4.8	18.2	1.7	33.2	61	65.4
S2: 0 – 4 cm	5.5	6.8	21.2	3.6	30	67.1	70.2
S2: 4 – 8 cm	3.2	8.6	19.9	3.6	32.5	67.8	40.6
S2: 16 – 20 cm	6.3	8.2	20.1	3.8	32.2	70.6	55.6
S2: 20 – 24 cm	5.6	8.2	20	4.2	35.2	73.2	54.8
S2: 24 – 28 cm	6.7	8.1	19.8	3.8	32.6	71	53.2

Table 7d: Zn speciation distribution in selected sediments of S1 and S2

Cd

Samples	F1	F2	F3	F4	F5	Sum	Total metal
S1: 12 – 16 cm	1.5	0.7	0	0	5.4	7.6	5.5
S1: 16 – 20 cm	1.2	1.1	0	0	5.9	8.2	5.8
S1: 24 – 28 cm	1.8	0.8	0	0	5.1	7.7	5.8
S1: 28 – 32 cm	1.6	0.8	0	0	5.5	7.9	6.2
S2: 0 – 4 cm	1.3	1.9	0	0	6.2	9.4	6.1
S2: 4 – 8 cm	1.2	1.4	0	0	5.5	8.1	6.2
S2: 16 – 20 cm	1.5	1.8	0	0	5.7	9	5.3
S2: 20 – 24 cm	1.4	1.8	0	0	6.2	9.4	5.5
S2: 24 – 28 cm	1.5	1.6	0	0	5.4	8.5	5.3

Table 7e: Cd speciation distribution in selected sediments of S1 and S2

Pb

Samples	F1	F2	F3	F4	F5	Sum	Total metal
S1: 12 – 16 cm	2.3	3.4	0	0	8.4	14.1	11.4
S1: 16 – 20 cm	2.3	3.6	0	0	8.6	14.5	11.7
S1: 24 – 28 cm	3.6	5.8	0	0	11.2	20.6	15.2
S1: 28 – 32 cm	5.4	9.7	0	0	14.2	29.3	26
S2: 0 – 4 cm	2.2	3.6	0	0	8.6	14.4	11.8
S2: 4 – 8 cm	3.3	4.8	0	0	10.5	18.6	16.4
S2: 16 – 20 cm	2.5	4.2	0	0	8.6	15.3	12.3
S2: 20 – 24 cm	2.1	3.6	0	0	6.8	12.5	9.6
S2: 24 – 28 cm	2.4	4.1	0	0	7.8	14.3	11.7

Table 7f: Pb speciation distribution in selected sediments of S1 and S2

deviation (**Table 7a-7f**). The deviation observed here could be due to incomplete digestion of the residual fraction. A small amount of residue (F5) was always obtained after treating the residual fraction with concentrated HNO₃, HCl and HF during the digestion process. The best results were obtained for Cu and Zn where the deviations of the sum fraction from the total metal contents were only 4-8 %. This could be due the ease with which these metals are leached out from the complicated silicate matrix.

The process employed here was found to be of poor sensitivity for quantitatively determining Cd in various fractions (or Cd species). It may be likely to be due to extremely lower Cd levels present in the sediments.

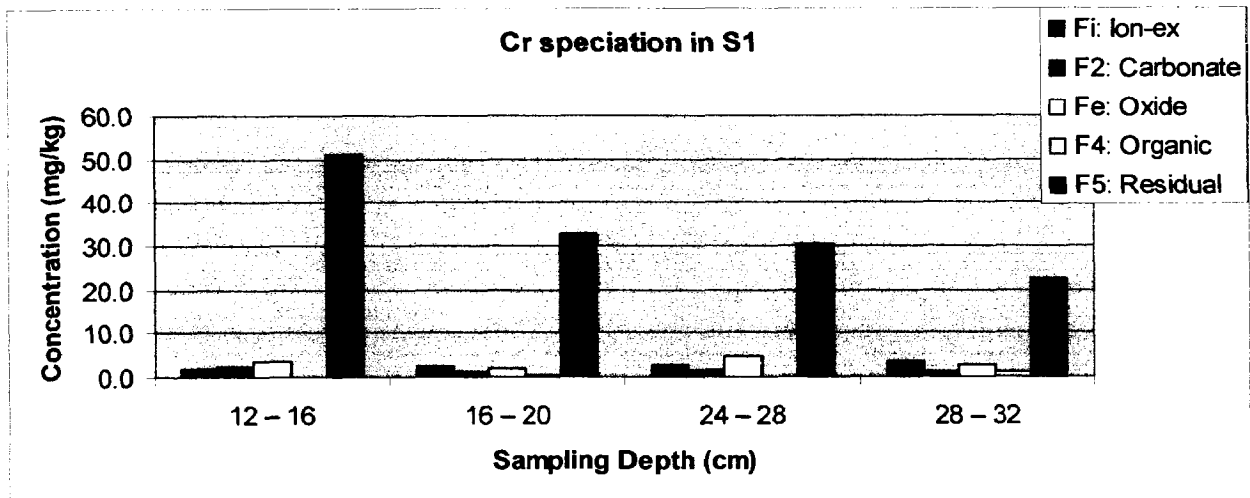


Fig. 8a: Distribution of Cr species in the sediments of various depths from Sites S1

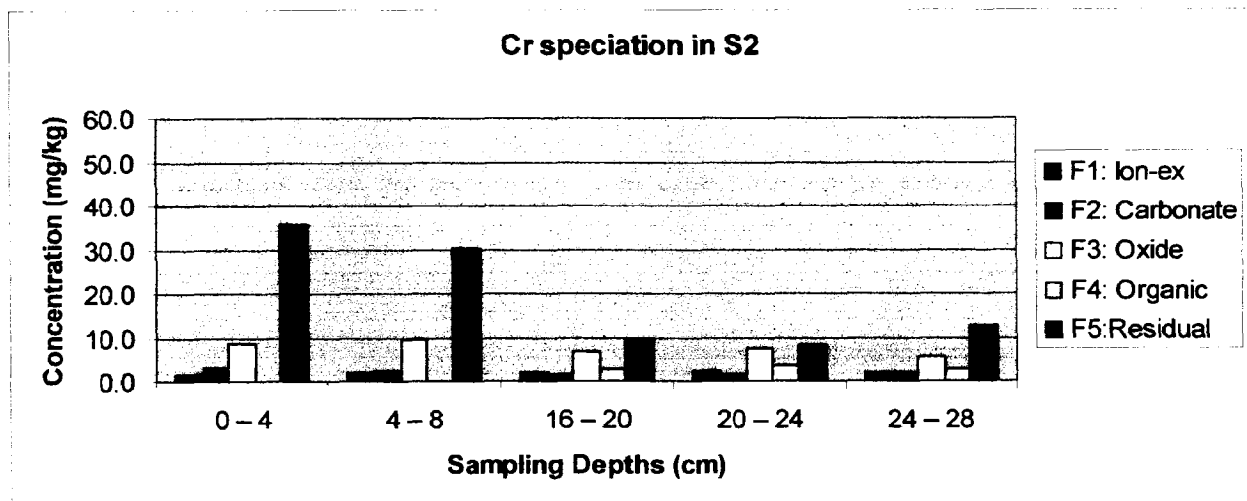


Fig. 8b: Distribution of Cr speciation in the sediments of various depths from Sites S2

The distribution of Cr species in the sediments of various depths from Sites S1 and S2 clearly suggests that Major Cr is present in the silicate matrix (residue) as shown in **Fig. 8a & 8b**. It is also noted that Cr is merely present in organic mass. In S1 site, the Cr species as exchangeable fraction, carbonates and oxides are present in very small amount (< 5 mg/kg), which is same in S2 site except for the oxide fraction (~ 10 mg/kg)

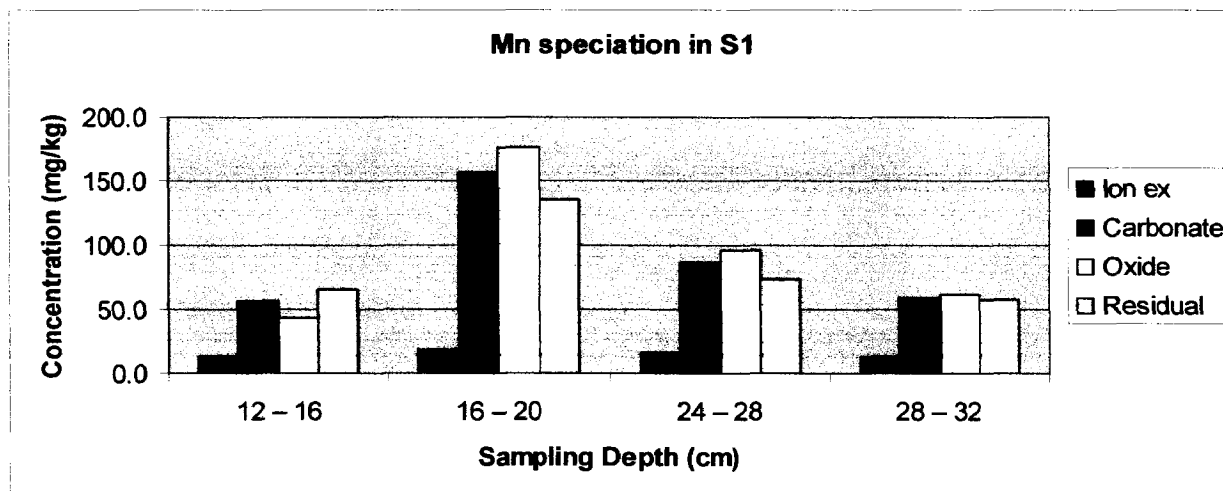


Fig. 9a: Distribution of Mn speciation in the sediments of various depths from Sites S1

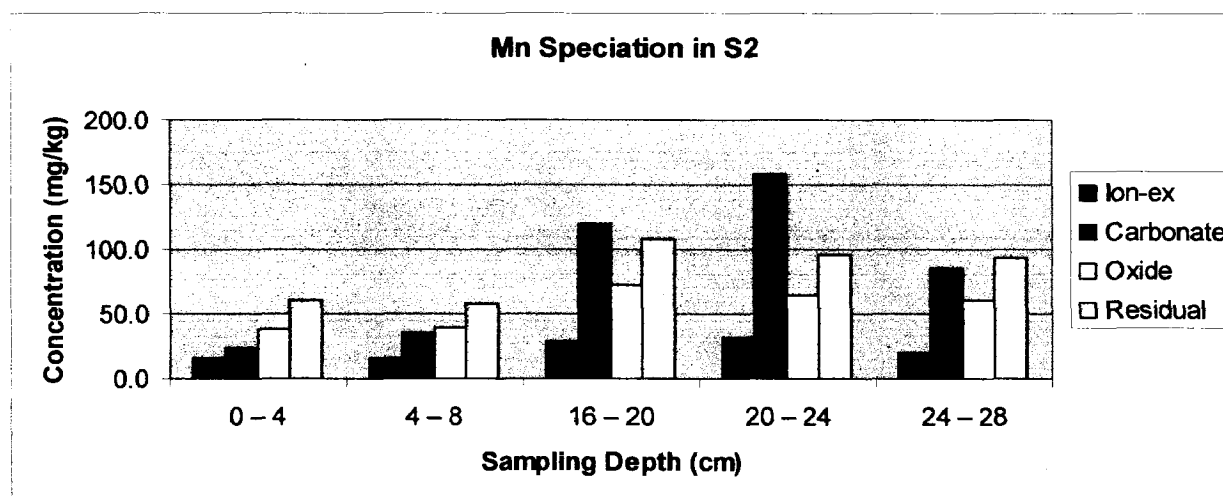


Fig. 9b: Distribution of Mn speciation in the sediments of various depths from Sites S2

The Mn is found to be present in four fractions other than organic fraction (**Fig. 9a and 9b**). At greater depths (16-24 cm deep), Mn is rich in carbonates, oxides and silicates. For Site S2, Mn as carbonates is found to be maximum at the depths of 16-24 cm and 20 – 24 cm (Shown in **Fig. 9b**). In both the sites, it is noticed that Mn species are lower in concentrations at the shallower depths of the sediment.

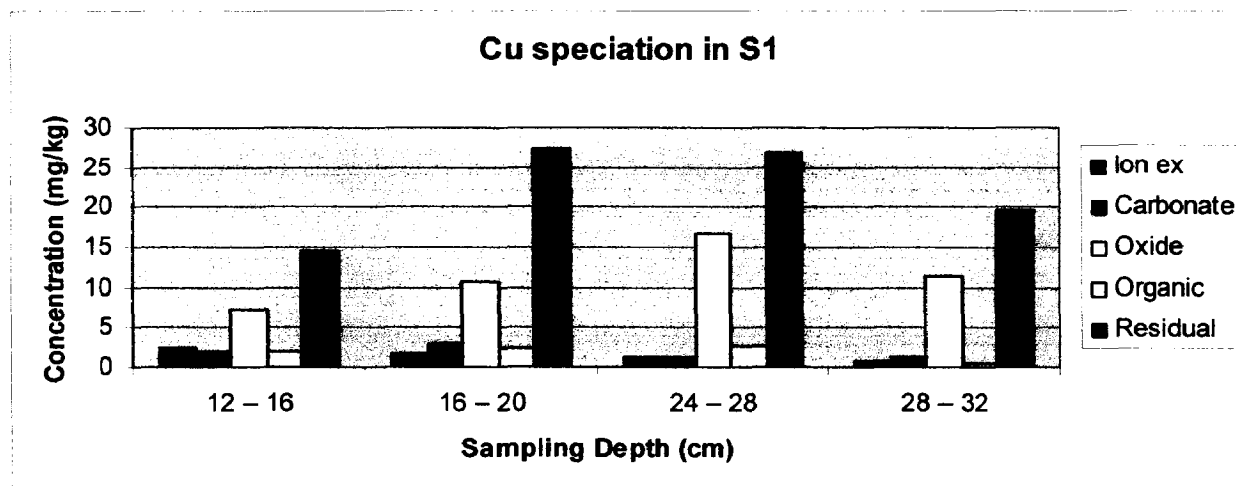


Fig. 10a: Distribution of Cu speciation in the sediments of various depths from Sites S1

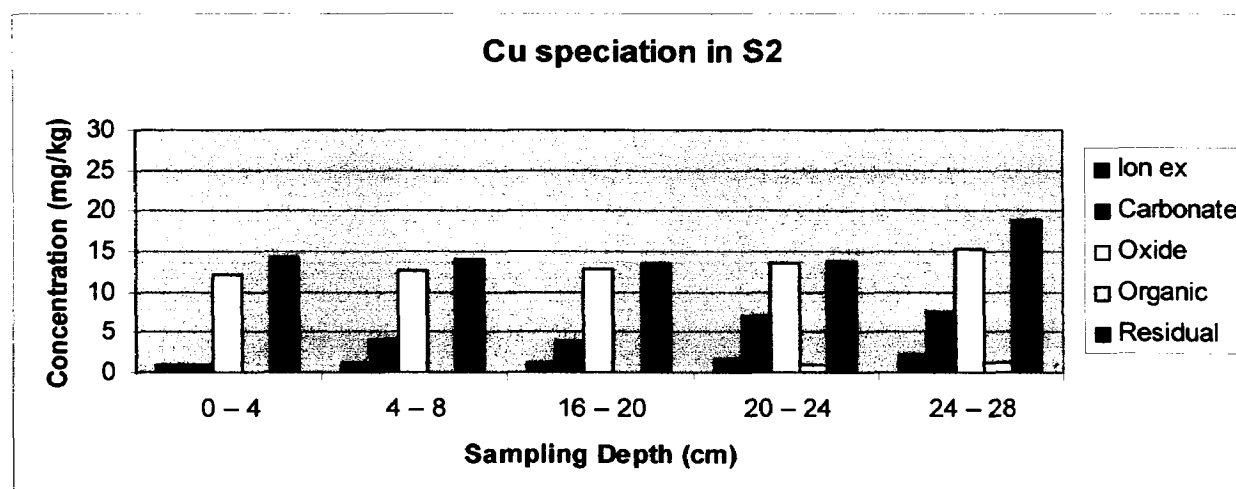


Fig. 10b: Distribution of Cu speciation in the sediments of various depths from Sites S2

Variation in Cu species is distinct in the sediments of site S1 than that in S2 (**Fig. 10a & 10b**). Mostly, Cu is present as silicates and oxides in S1 and S2. However, at greater depths of the sediments from Site S2, the Cu in carbonate fraction is measured to be about 5 mg/kg, which is about one-third of those present in silicate matrix. Copper in other fractions are extremely low (less than 2 mg/kg).

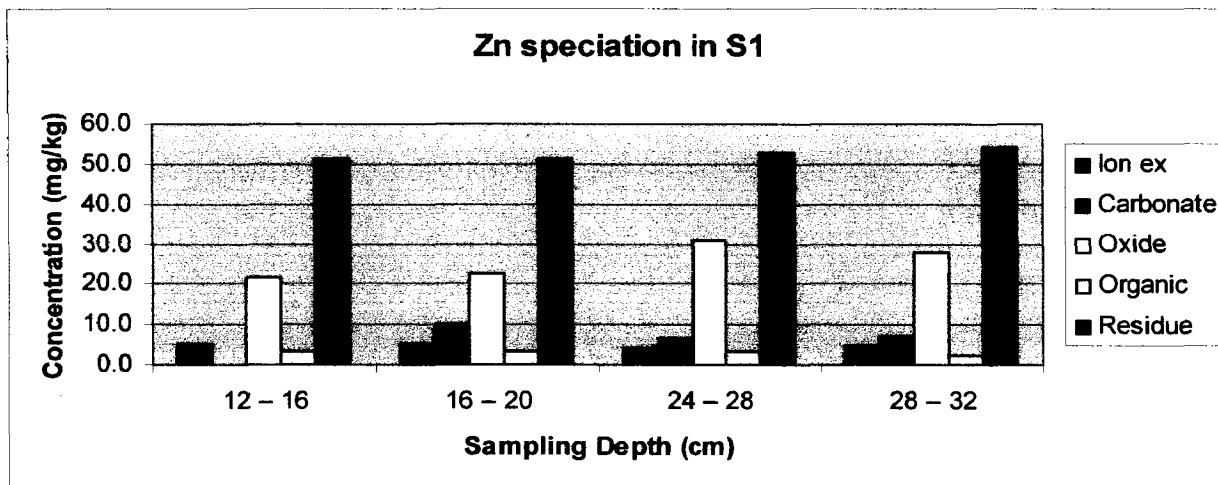


Fig. 11a: Distribution of Zn speciation in the sediments of various depths from Sites S1

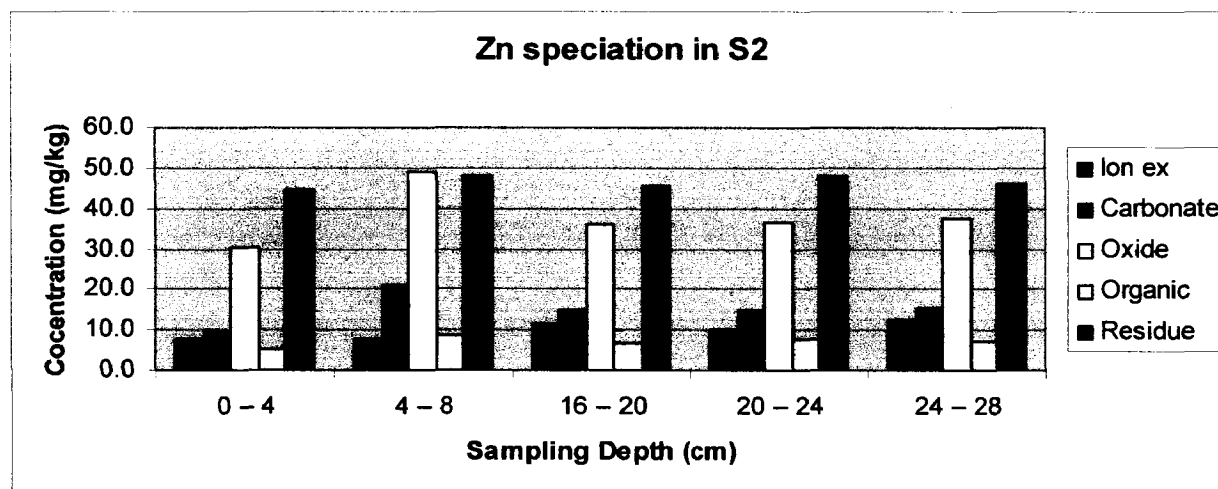


Fig. 11b: Distribution of Zn speciation in the sediments of various depths from Sites S1

The profile of the distribution of Zn species is noted to be very similar to that of Cu. Here also, Zn in S1 and S2 (Fig. 11a & 11b) are primarily present as oxides and silicates (shown in Fig. 10a and 10b). In this regard, it may be correlated with higher silicate contents measured by EDAX and EPMA for the sediments of S1 site. However, the oxide fraction of Zn is higher (30 – 49 mg/kg) in S2 as compared to that in S1 (21 – 31 mg/kg). The carbonate fraction of Zn is higher in S2 (9 – 21 mg/kg) than in S1 (lower than 10 mg/kg). It may also be noted that Zn is present in significant concentrations as ion-exchangeable (8 – 12 mg/kg) form and in organic matrix (5-9 mg/kg) especially for the sediments from S2 site.

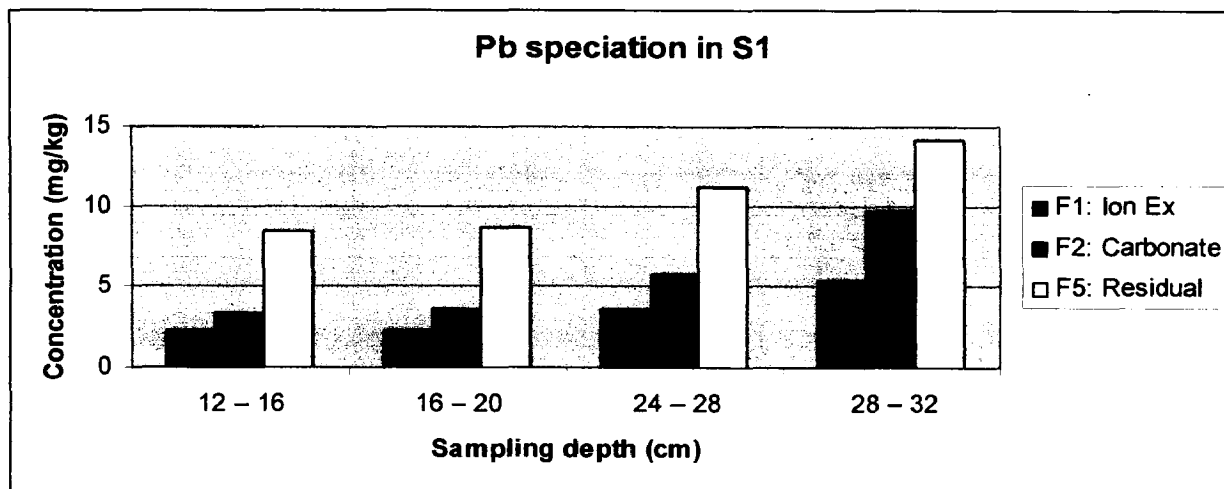


Fig. 12a: Distribution of Pb speciation in the sediments of various depths from Sites S1

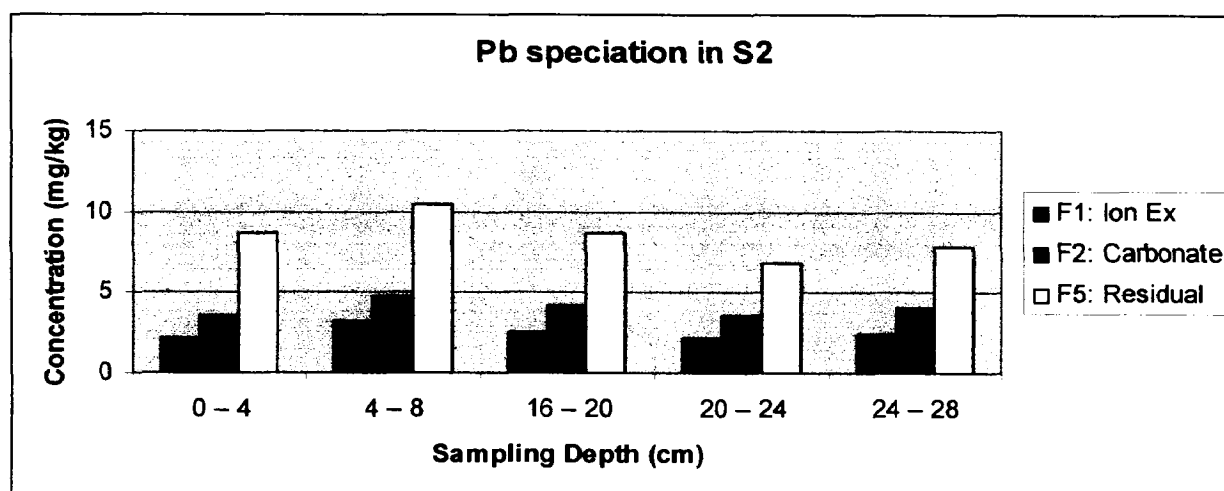


Fig. 12b: Distribution of Pb speciation in the sediments of various depths from Sites S2

Lead is mainly present as carbonates and in silicate matrix in the sediments of both S1 and S2 sites (shown in **Fig.12a & 12b**). About 2 – 5 mg/kg of Pb is also present as ion-exchangeable fraction. The sequential extraction for Pb shows that it is neither present as oxide nor in organic mass.

3.4 Conclusion:

Site S1 sediment has higher silicate composition which is also reflected on higher concentrations of the heavy metal species in silicate fraction. It appears from EPMA and EDAX studies that alumino-silicate is the major mineral phase in these sediments. Fe was found to have a negative correlation with K as well as Al and Fe was found to be mostly present as oxides and silicates (data not shown here). EPMA studies are useful to obtain several minor element compositions like, Na, Mg, Ca, Ti, Fe.

Total metal analyses of heavy metals like Cr, Mn, Co, Ni, Cu, Zn, Cd and Pb were carried out with respect to the depth of the sediment samples from three location of Sunderban Wetland area, showing variation in their levels along the depth – suggesting variation in natural and anthropogenic contributions.

From this speciation studies of the selected heavy metals (Cr, Mn, Cu, Zn and Pb), it may be concluded that ion-exchangeable species of these metals are of very low levels. It is primarily the ion exchangeable fraction of a heavy metal that is most susceptible to bioaccumulation and hence metal toxicity. Besides, the carbonate fraction may also take part in bioaccumulation. In that case, Pb and Mn are the only heavy metals which have significant contribution in these two fractions. Most of the heavy metals studied here, viz., Cu, Zn, Cr were found to be present mainly in silicate matrix and are hence immobilized as the metals in silicate matrix are not easily leachable even with strong acids, as a result they are not bioavailable.

As a part of this pilot study, it may be concluded that speciation of most heavy metals in the sediments of Sunderban Wetland regions has been accomplished. The procedure used here can be used to a bigger scale project to evaluate, understand and correlate (if any) the heavy metal species and bioaccumulation.

Figure Captions:

- Fig. 1:** Map of Sundarban showing locations of sampling stations. S₁ Lot8, S₂ Jharkhali, S₃ Gushighata
- Fig. 2a:** SEM image of the sediment particles of S1
- Fig. 2b:** X-ray spectrum (EDAX) of the scanned area of Fig. 2a
- Fig. 2c:** X-ray spectrum (EDAX) of the single mineral grain marked in Fig. 2a
- Fig. 3a:** Showing Al – K positive correlation (EDAX) of S1
- Fig. 3b:** Showing Al – K positive correlation (EPMA) of S1
- Fig. 4a:** showing a negative correlation between Fe and K of sediments S1 and S3 (EDAX)
- Fig. 4b:** Fe - K negative correlation obtained from EPMA analysis of S1 and S2 sediment (EPMA)
- Fig. 5:** EPMA image of the sample S1: 24-28 cm depth
- Fig. 6a:** The heavy metal distribution of the sediment core divided at 4 cm each of the three sampling locations S1
- Fig. 6b:** The heavy metal distribution of the sediment core divided at 4 cm each of the three sampling locations S2
- Fig. 6c:** The heavy metal distribution of the sediment core divided at 4 cm each of the three sampling locations S3
- Fig. 7a:** Depth-wise concentration distribution of Mn for all three sampling sites
- Fig. 7b:** Depth-wise concentration distribution of Cr for all three sampling sites
- Fig. 7c:** Depth-wise concentration distribution of Cu for all three sampling sites
- Fig. 7d:** Depth-wise concentration distribution of Zn for all three sampling sites
- Fig. 7e:** Depth-wise concentration distribution of Pb for all three sampling sites
- Fig. 8a:** Distribution of Cr species in the sediments of various depths from Sites S1
- Fig. 8b:** Distribution of Cr speciation in the sediments of various depths from Sites S2
- Fig. 9a:** Distribution of Mn speciation in the sediments of various depths from Sites S1
- Fig. 9b:** Distribution of Mn speciation in the sediments of various depths from Sites S2
- Fig. 10a:** Distribution of Cu speciation in the sediments of various depths from Sites S1
- Fig. 10b:** Distribution of Cu speciation in the sediments of various depths from Sites S2
- Fig. 11a:** Distribution of Zn speciation in the sediments of various depths from Sites S1
- Fig. 11b:** Distribution of Zn speciation in the sediments of various depths from Sites S1
- Fig. 12a:** Distribution of Pb speciation in the sediments of various depths from Sites S1
- Fig. 12b:** Distribution of Pb speciation in the sediments of various depths from Sites S2

Table Captions:

Table 1: Showing sampling description. Samples were collected during June 2007.

The information of the depth of the collected sediment is given in italics.

Table 2: X-ray analysis of S1-S3 sediments by EDAX

Table 3a: Point analysis of different grains of sample S1: 24-28 cm depth by EPMA in Wt%

Table 3b: Point analysis of different grains of sample S1: 24-28 cm depth by EPMA in At%

Table 4: Point analysis of the Sample S3: 24-28 cm depth (Wt %) by EPMA

Table 5: Total metal analysis of the sample from sites S1, S2, S3 with their respective depths of the core sediment.

Table 6: Mean and Standard deviation of the concentration of the heavy metals along the depth of the sediment

Table 7a: Cr speciation distribution in selected sediments of S1 and S2

Table 7b: Mn speciation distribution in selected sediments of S1 and S2

Table 7c: Cu speciation distribution in selected sediments of S1 and S2

Table 7d: Zn speciation distribution in selected sediments of S1 and S2

Table 7e: Cd speciation distribution in selected sediments of S1 and S2

Table 7f: Pb speciation distribution in selected sediments of S1 and S2

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