HEAVY METAL DISTRIBUTION IN DIFFERENT PARTS OF FISHES AND SEDIMENTS OF SUNDERBAN WETLAND

A DISSERTATION

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

MASTER OF TECHNOLOGY

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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 ***"HEAVY METAL DISTRIBUTION IN DIFFERENT PARTS OF FISHES AND SEDIMENTS OF SUNDARBAN WETLAND"*** 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 2009 to June 2010 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.

Date: June, 30-2010 **(VIJAY KHANDELWAL)**

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

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ABSRACT

The release of pollution into the environment is a persistent problem in every country. Pollution sources include manufacturing, fertilization, electricity generation, waste water treatment and mining operations. Biomonitoring is one way to qualitatively and quantitatively investigate the changes in the environment due to pollution by anthropogenic sources.

Sediment and fish samples from the different sites of Sundarban wetland were analyzed to find out the distribution of heavy metals like Cr, Mn, Fe, Co, Ni, Cu, Zn, Cd and Pb by ICP-MS. Further metal speciation has been done using sequential extraction. The sediment samples to check in which form metal is associated with the silica matrix or present in sediment *viz* ion-exchangeable, oxides, carbonates or sulphide using AAS.

Comparison between sediment & fish sample result can provide the idea of bioavailability of heavy metals which indirectly is the measure of propagation of toxic or essential metal in biosystem from sediment.

Analysis of metal in fish samples is also plays a important role for an idea of contamination in biological systems. Analysis of parts of fish has great significance in order to find out the accumulation of different toxic metals in different part of body while analysis of whole fish let us know which species is more sensitive to which metal.

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Chapter 1:

Introduction

1.1 Purpose of Study:-

Pollution and its effects on the environment have been studied increasingly over the past decades as public awareness and government mandates demand solutions to the problems created by industries. The release of pollution into the environment is a persistent problem in every country. Pollution sources include manufacturing, fertilization, electricity generation, waste water treatment and mining operations.[1,2]

The main problem associated with the heavy metal contamination is that when the metal enters in the ecosystem (irrespective of source of contamination) it doesn't leave the system or in other words it transfers from one form to another and recycles ultimately via soil, sediment, plant, aquatic life and human. Approximately in all countries in the world, people ingest sea and/or aquatic form of life as their food which might be the potential source of contamination. Human body in general doesn't have any specific mechanism to get rid of these metal toxicity and are deposited in the tissues or organs where they cause minimum harm to person.

Biomonitoring is one way to qualitatively and quantitatively investigate the changes in the environment due to pollution by anthropogenic sources. Indicators, such as mud-dwelling macroinvertebrates, fish, algae, and some aquatic plants, are involved in the biomonitoring process.[3]

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.

Heavy metal analysis of the sediments and biota samples of Sundarban 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.[4-7] 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.

1.2 The Area of Study & It's Significance:-

1.2.1 The Sundarban:

Sundarbans is the largest single block of tidal halophytic mangrove forest in the world.[11] The name *Sundarban* can be literally translated as "beautiful jungle" or "beautiful forest" in the Bengali language (*Sundar*, "beautiful" and *ban*, "forest" or "jungle"). The name may have been derived from the *Sundari* trees that are found in Sundarbans in large numbers.[11]

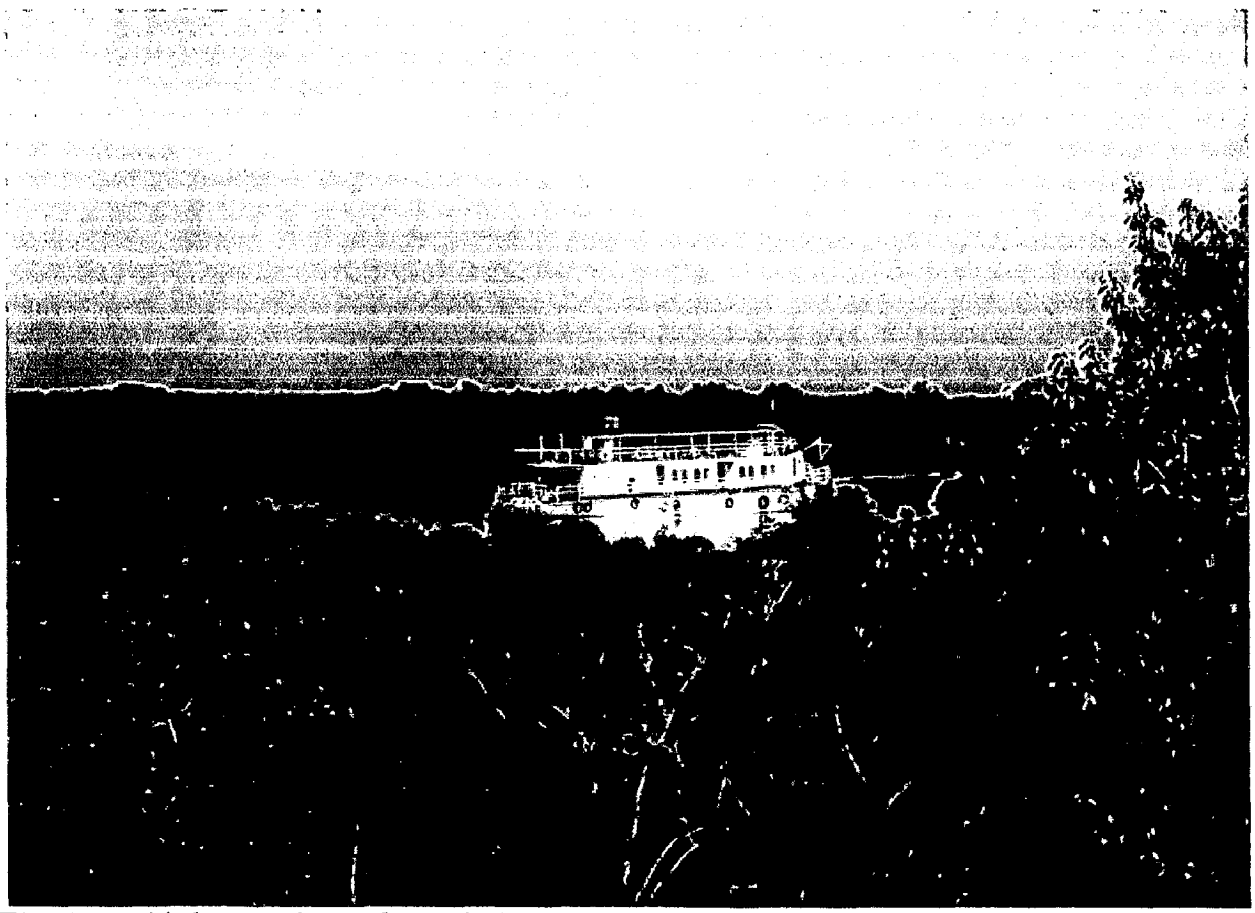

Fig 1: A bird eye view of Sundarban

The forest lies at the feet of the Ganges and is spread across areas of Bangladesh and West Bengal, India, forming the seaward fringe of the delta. The seasonally-flooded Sundarbans freshwater swamp forests lie inland from the mangrove forests **(Fig. 1)**. The forest covers **10,000** km^2 of which about 6,000 are in Bangladesh.[12] It became inscribed as a UNESCO world heritage site in 1997, but while the Bangladeshi and Indian portions constitute the same continuous ecotope, these are separately listed in the UNESCO world heritage list as the Sundarban and **Sundarban National Park**, respectively.[13,14]

1.2.2 Physiography & Ecoregions:

The mangrove dominated Ganges Delta 'The Sundarbans' is a complex ecosystem comprising one of the three largest single tract of mangrove forests of the world, the larger part (62%) is situated in the southwest corner of Bangladesh. To the south the forest meets the Bay of Bengal; to the east it is bordered by the Baleswar River and to the north there is a sharp interface with intensively cultivated land. The natural drainage in the upstream areas, other than the main river channels, is everywhere impeded by extensive embankments and polders **(Fig. 2)**. Rivers in the Sundarbans are meeting places of salt water and freshwater. Thus, it is a region of transition between the freshwater of the rivers originating from the Ganges and the saline water of the Bay of Bengal[15]

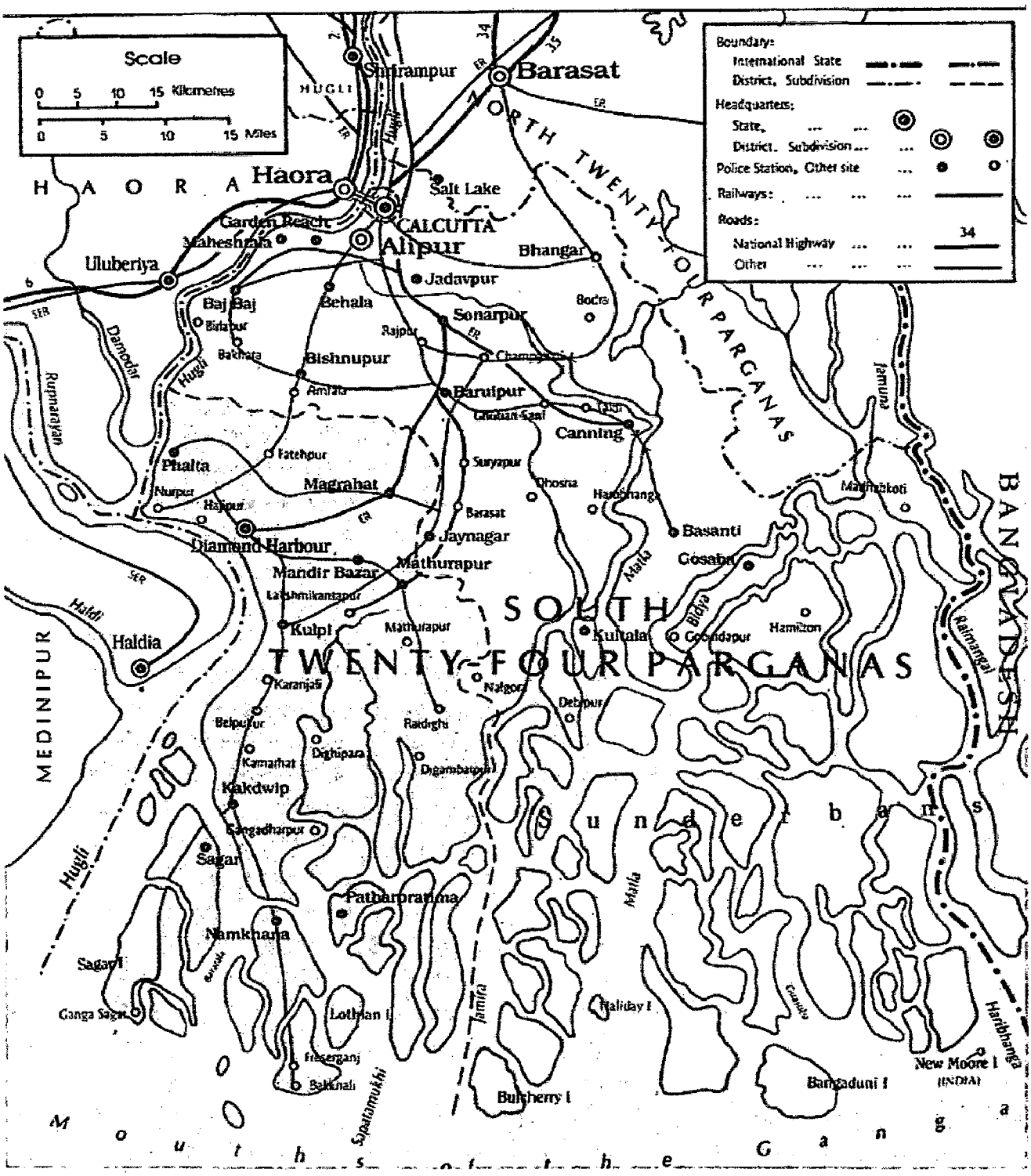

Fig. 2: Satellite image shows the forest in the protected area. The Sundarbans appears deep green, surrounded to the north by a landscape of agricultural lands, which appear lighter green, towns, which appear tan, and streams, which are blue

The Sundarbans along the Bay of Bengal has evolved over the millennia through natural deposition of upstream sediments accompanied by intertidal segregation. The physiography is dominated by deltaic formations that include innumerable drainage lines associated with surface and subaqueous levees, splays and tidal flats **(Fig. 3)**. There are also marginal marshes above mean tide level, tidal sandbars and islands with their networks of tidal channels, subaqueous distal bars and proto-delta clays and silt sediments. The Sundarbans' floor varies from 0.9 m to 2.11 m above sea level.[16]

Fig 3: Mudflat and clouds in Sundarbans

Sundarbans features two ecoregions -"Sundarbans freshwater swamp forests" (IM0162) and Sundarbans mangroves (IM1406).[17, 18]

The freshwater ecoregion is an area where the water is only slightly brackish and becomes quite fresh during the rainy season, when the freshwater plumes from the Ganges and Brahmaputra rivers push the intruding salt water out and also bring a deposit of silt. It covers an area of the vast Ganges-Brahmaputra Delta, extending from India's West Bengal state into western Bangladesh. The Sundarbans freshwater swamp forests lie between the upland Lower Gangetic plains moist deciduous forests and the brackish-water Sundarbans mangroves bordering the Bay of Bengal.[18, 19]

1.2.3 Flora & Fauna:

Biotic factors here play a significant role in physical coastal evolution and for wildlife a variety of habitats have developed including beaches, estuaries, permanent and semi-permanent swamps, tidal flats, tidal creeks, coastal dunes, back dunes and levees. The mangrove vegetation itself assists in the formation of new landmass and the intertidal vegetation plays an important role in swamp morphology **(Fig. 4)**.

Fig. 4: A View of Sundari trees & Swamp in Sundarbans, Bengal

The Sundarbans flora is characterized by the abundance of *Heritiera fomes*, *Excoecaria agallocha*, *Ceriops decandra* and *Sonneratia apetala* with total 245 genera and 334 plant species were recorded by David Prain.[20] Since Prain's report there have been considerable changes in the status of various mangrove species and taxonomic revision of the man-grove flora.[21] However, very little exploration of the botanical nature of the Sundarbans has been made to keep up with these changes. Whilst most of the mangroves in other parts of the world are characterized by members of the Rhizophoraceae, Avicenneaceae or Laganculariaceae, the mangroves of Bangladesh are dominated by the Sterculiaceae and Euphorbiaceae.[19]

The Sundarbans provide a unique ecosystem and a rich wildlife habitat. The Sundarbans were home to approximately 500 Bengal tigers (*Panthera tigris*) in 2004[12], one of the largest single populations of tigers. In addition to the endangered tiger, there are several other threatened mammal species, such as the capped langur (*Semnopithecus pileatus*), smooth-coated otter (*Lutrogale perspicillata*), Oriental small-clawed otter (*Aonyx cinerea*), and great Indian civet (*Viverra zibetha*). The ecoregion also contains the leopard (*Panthera pardus*) and several smaller predators such as the jungle cat (*Felis chaus*), fishing cat (*Prionailurus viverrinus*), and leopard cat (*Prionailurus bengalensis*).[18]

Recent studies revealed that the Bangladesh Sundarbans support diverse biological resources including at least 120 species of commercially important fishes, 270 species of birds, 42 species of mammals, 35 reptiles and eight amphibian species. This represents a significant proportion of the species present in Bangladesh (i.e. about 30% of the reptiles, 37% the birds and 34% of the mammals) and includes a large number of species which are now extinct elsewhere in the country.[22] Two amphibians, 14 reptiles, 25 aves and five mammals are presently endangered.[23] The Sundarbans is an important wintering area for migrant water birds[24] and is an area suitable for watching and studying avifauna.[25]

Apart from the Royal Bengal Tiger; Fishing Cats, Macaques, Wild Boar, Common Grey Mongoose, Fox, Jungle Cat, Flying Fox, Pangolin, Chital, are also found in

abundance in the Sundarbans. The river terrapin (*Batagur baska*), Indian flap-shelled turtle (*Lissemys punctata*), peacock soft-shelled turtle (*Trionyx hurum*), yellow monitor (*Varanus flavescens*), water monitor (*Varanus salvator*), Indian python (*Python molurus*) and the Bengal tiger (*Panthera tigris*) are some of the resident species.

1.3 Heavy Metal toxicity & their source:-

1.3.1 Introduction to heavy metals:

A heavy metal is a member of an ill-defined subset of elements that exhibit metallic properties, which would mainly include the transition metals, some metalloids, lanthanides, and actinides. Many different definitions have been proposed—some based on density, some on atomic number or atomic weight, and some on chemical properties or toxicity.[26] Heavy metal can include elements lighter than carbon and can exclude some of the heaviest metals. I general 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.[27]

Some of the heavy metals are dangerous to health or to the environment (e.g. Hg, Cd, As, Pb, Cr), some may cause corrosion (e.g. Zn, Pb), some are harmful in other ways (e.g. As may pollute catalysts). Within the European community the 13 elements of highest concern are As, Cd, Co, Cr, Cu, Hg, Mn, Ni, Pb, Sn, and Tl, the emissions of which are regulated in waste incinerators. Some of these elements are actually necessary for humans in minute amounts (Co, Cu, Cr, Mn, Ni),[28-30] 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[31] while others are carcinogenic or toxic, affecting, among others, the central nervous system (Mn, Hg, Pb, As), the kidneys or liver (Hg, Pb, Cd, Cu) or skin, bones, or teeth (Ni, Cd, Cu, Cr).[32]

1.3.2 Sources of Heavy Metal contamination:

Heavy metal pollution can arise from many sources but most commonly arises from the purification of metals, e.g., the smelting of copper and the preparation of nuclear fuels. Electroplating is the primary source of chromium and cadmium.[33] Through precipitation of their compounds or by ion exchange into soils and muds, heavy metal pollutants can localize and lay dormant. Unlike organic pollutants, heavy metals do not decay and thus pose a different kind of challenge for remediation.

1.3.3 Sources of Heavy Metals in Sundarban:

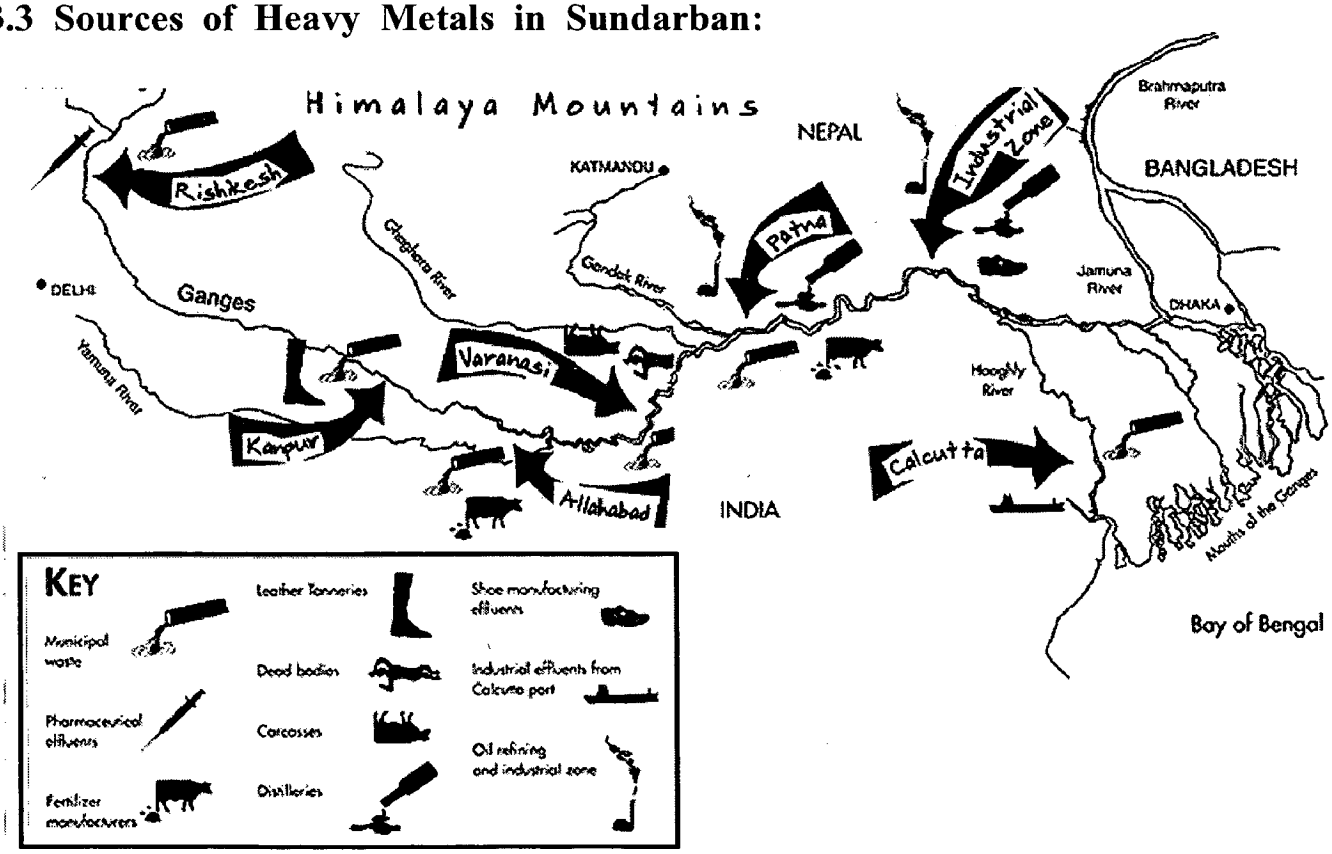

Fig. 5: Different sources of Heavy Metals which contribute to the Sundarban directly or indirectly

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.[34] Human polluting activities or physical processes also play a great role in increasing the label 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.[35]

Heavy metals occur naturally in the ecosystem with large variations in concentration. In modern times, anthropogenic sources of heavy metals, i.e. pollution, have been introduced to the ecosystem. Waste-derived fuels are especially prone to contain heavy metals so they should be a central concern in a consideration of their use.[36]

With a mean annual flow of $5.9\times10^{11}m^3yr^{-1}$ and sediment load of $1600x10^{12}g.yr^{-1}$ the Ganges river ranks second and third, respectively, in terms of water flow and sediment load among the world's rivers.[37] 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.[38] 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.[38,39]

The main sources of heavy metal in the Sundarban 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.[40] **(Fig. 5)**. 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.[41] 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 along with 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.

2.1 Sampling:-

2.1.1 The Study Site:

Different samples of sediments and fishes (as whole fish of different species and different organs of a fish) were collected from the different sites of Sundarban area during the post monsoon season and monsoon season of 2009. Sediments are then dried and powdered with a mash size of 50mm. Fish samples are dried in a frozen state with the help of liquid nitrogen.

2.1.2 Sediment samples:

Table 1: Sampling description of sediment samples collected from 6 locations from sundarban delta region, during July 2009.

Sample No.	Sample Name
1.	GHU/07/09
2.	CAN/07/09
3.	KMK/07/09
4.	CHE/07/09
5.	CAN/07/09
6.	MAY/07/09

2.1.3 Fish Organ & Fish samples:

Sample 1 (*Sanguinolaria acuminata*) Visceral Mass
Sample 2 (*Sanguinolaria acuminata)* Gills
Sample 3 *(Sanguinolaria acuminata)* Mantle
Sample 4 *(Sanguinolaria acuminata)* Podium
Sample 5 *(Sanguinolaria acuminata)* Siphon
Sample 6 *(Sanguinolaria acuminata)* Adductor Muscle
Sample 7 *(Sanguinolaria acuminata)* Shell
Sample 9 *(Sanguinolaria acuminata)* Sediment
Sample 10 Fish *(Scatophagus argus)*
Sample 11 Fish *(Protonibae diacanthus)*
Sample 12 Fish *(Rhinomugil corsula)*
Sample 13 Fish *(Cyanoglossus sp.)*

2.2 Reagents and Chemicals:-

2.2.1 For Total metal analysis of Sediments:

1. Nitric Acid (HNO_3), Analytical Grade, E. Merck,
2. Hydrogen Floride (HF), Analytical Grade, E. Merck,
3. Perchloric Acid ($HClO_4$), Analytical Grade, E. Merck,

2.2.2 For Sequential analysis of Sediments:

1. Ammonium Acetate (CH_3COONH_4), Analytical Grade, E. Merck,
2. Acetic Acid Glacial (CH_3COOH), Analytical Grade, E. Merck,
3. Hhydroxylammonium Chloride ($OHNH_4Cl$), Analytical Grade, E. Merck,
4. Hydrochloric Acid (HCl), Analytical Grade, E. Merck
5. Sodium Hydro-oxide (NaOH), Analytical Grade, E. Merck
6. HNO_3 & HF as in section **2.2.1.1 & 2.2.1.2**

2.2.3 For analysis of Fish samples:

1. Nitric Acid (HNO_3), Analytical Grade, E. Merck,
2. Hydrogen Peroxide (H_2O_2), Analytical Grade, E. Merck,

2.3 Analytical Methods:-

2.3.1 Atomic Absorption Spectroscopy (AAS):

Principle is based up on the fact that atoms of different elements absorb characteristic wavelengths of light by analyzing a sample to see if it contains a particular element means using light from that element. For example with lead, a lamp containing lead emits light from excited lead atoms that produce the right mix of wavelengths to be absorbed by any lead atoms from the sample.

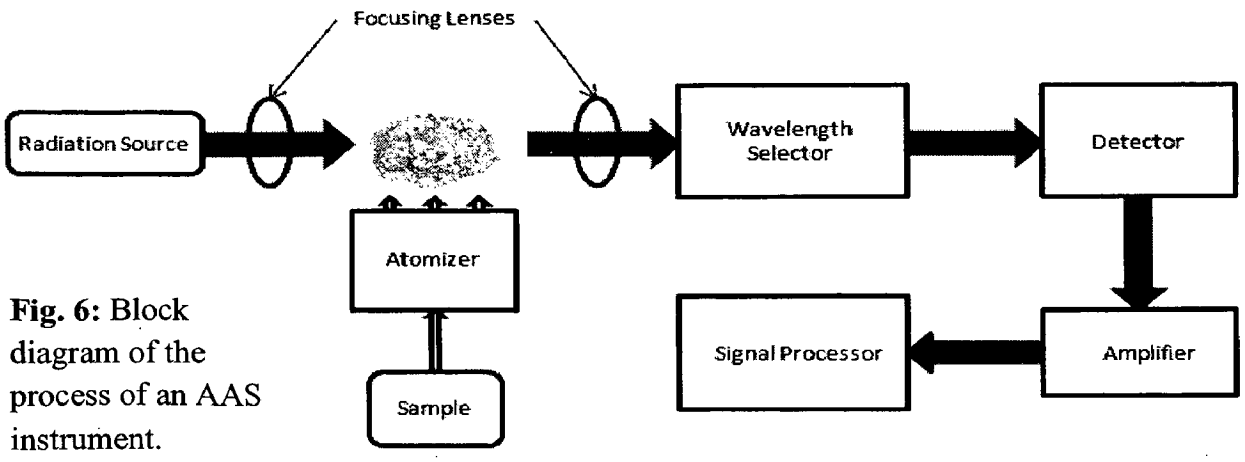

Fig. 6: Block diagram of the process of an AAS instrument.

In AAS, the sample is atomized– *i.e.* converted from ground state free atoms into the vapour state – and a beam of electromagnetic radiation emitted from excited lead atoms is passed through the vaporized sample. Some of the radiation is absorbed by the lead atoms in the sample. The greater the number of atoms there is in the vapour, the more radiation is absorbed. The amount of light absorbed is proportional to the number of lead atoms. **(Fig. 6 & 7)**

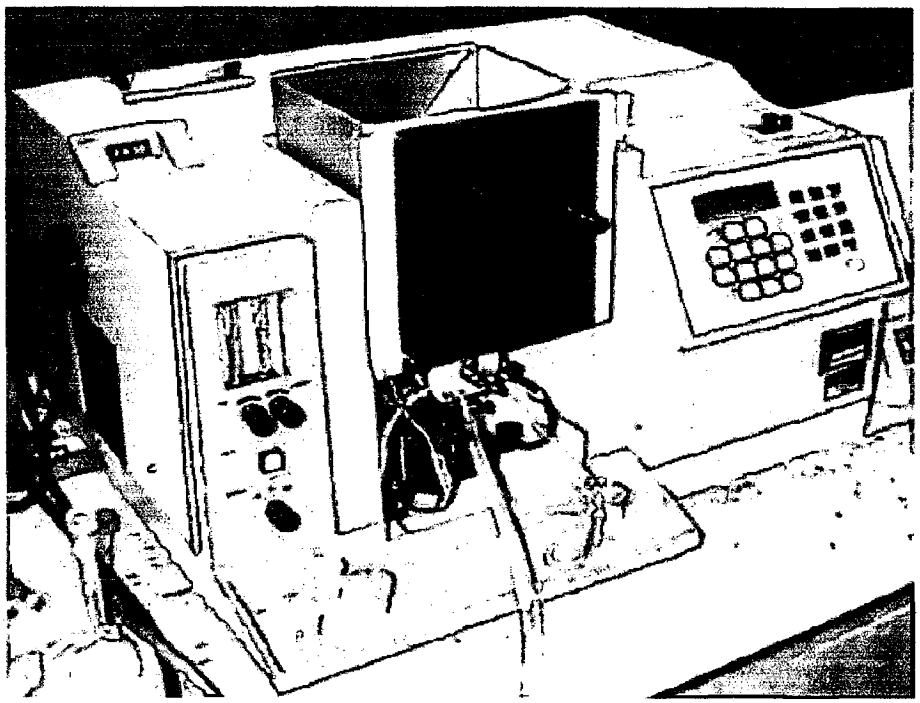

Fig. 7: An AAS Instrument.

A calibration curve is constructed by running several samples of known lead concentration under the same conditions. The amount by the standard absorbs is compared with the calibration curve and this enables the calculation of the lead concentration in the unknown sample. Consequently an atomic absorption spectrometer needs the following three components: a light source; a sample cell to produce gaseous atoms; and a means of measuring the specific light absorbed.

2.3.1.2 The light source

The common source of light is a 'hollow cathode lamp' **(Fig. 8).** This contains a tungsten anode and a cylindrical hollow cathode made of the element to be determined.

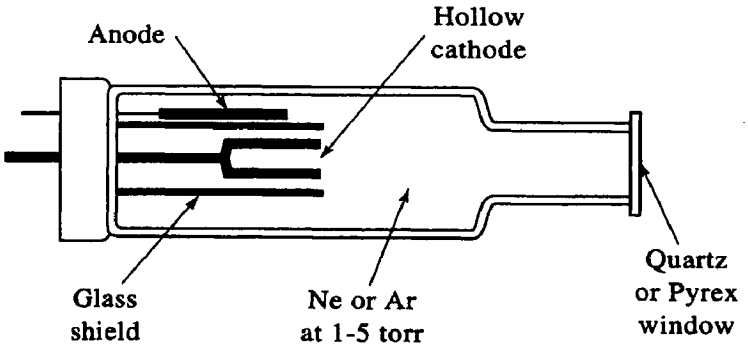

Fig. 8: Hollow cathode lamp & It's different parts.

These are sealed in a glass tube filled with an inert gas – *eg* neon or argon – at a pressure of between 1 Nm^{-2} and 5 Nm^{-2}. The ionization of some gas atoms occurs by applying a potential difference of about 300–400 V between the anode and the cathode. These gaseous ions bombard the cathode and eject metal atoms from the cathode in a process called ***sputtering***. Some sputtered atoms are in excited states and emit radiation characteristic of the metal as they fall back to the ground state **(Fig. 9).**

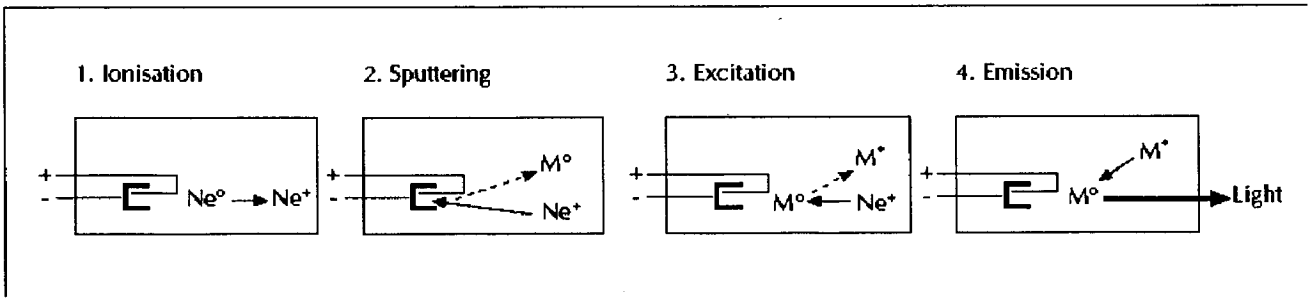

Fig. 9: Mechanism of production of radiation beam in a hollow cathode lamp.

The shape of the cathode concentrates the radiation into a beam which passes through a quartz window, and the shape of the lamp is such that most of the sputtered atoms are redeposited on the cathode. A typical atomic absorption instrument holds

several lamps each for a different element. The lamps are housed in a rotating turret so that the correct lamp can be quickly selected

2.3.1.3 The optical system and detector

A monochromator is used to select the specific wavelength of light – *ie* spectral line – which is absorbed by the sample, and to exclude other wavelengths. The selection of the specific light allows the determination of the selected element in the presence of others. The light selected by the monochromator is directed onto a detector that is typically a photomultiplier tube. This produces an electrical signal proportional to the light intensity **(Fig. 8)**

2.3.1.4 Double beam spectrometers

Modern spectrometers incorporate a beam splitter so that one part of the beam passes through the sample cell and the other is the reference **(Fig. 10)**. The intensity of the light source may not stay constant during an analysis. If only a single beam is used to pass through the atom cell, a blank reading containing no anaiyte (substance to be analysed) would have to be taken first, setting the absorbance at zero.

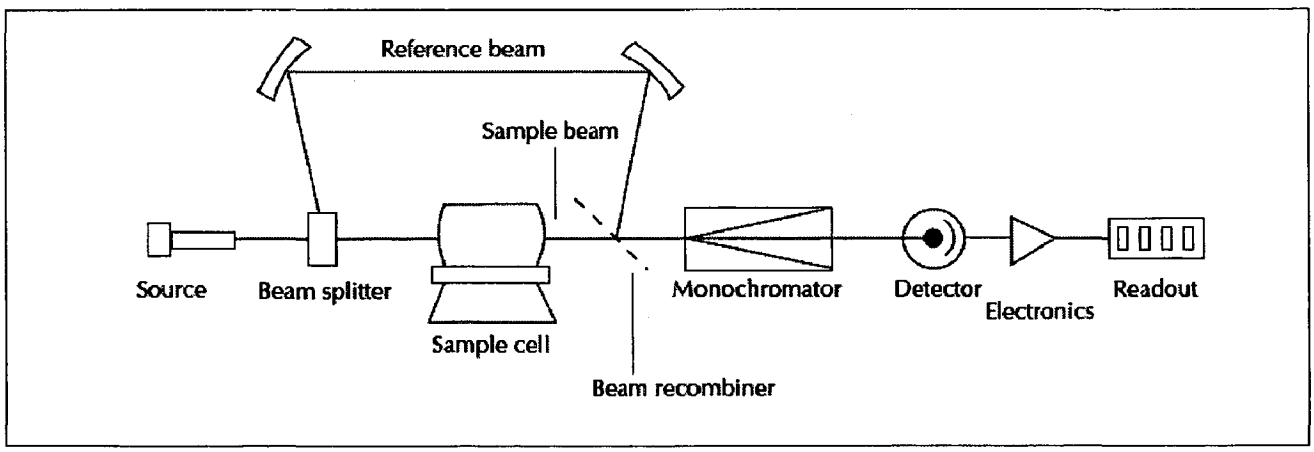

Fig. 10: A modified double beam spectrometer

If the intensity of the source changes by the time the sample is put in place, the measurement will be inaccurate. In the double beam instrument there is a constant monitoring between the reference beam and the light source. To ensure that the spectrum does not suffer from loss of sensitivity, the beam splitter is designed so that as high a proportion as possible of the energy of the lamp beam passes through the sample.

2.3.1.5 Nebulizer & Flame

Suck up liquid sample at a controlled rate & create a fine aerosol for introduction into the flame and finally mix the aerosol and fuel and oxidant thoroughly for introduction into the flame.

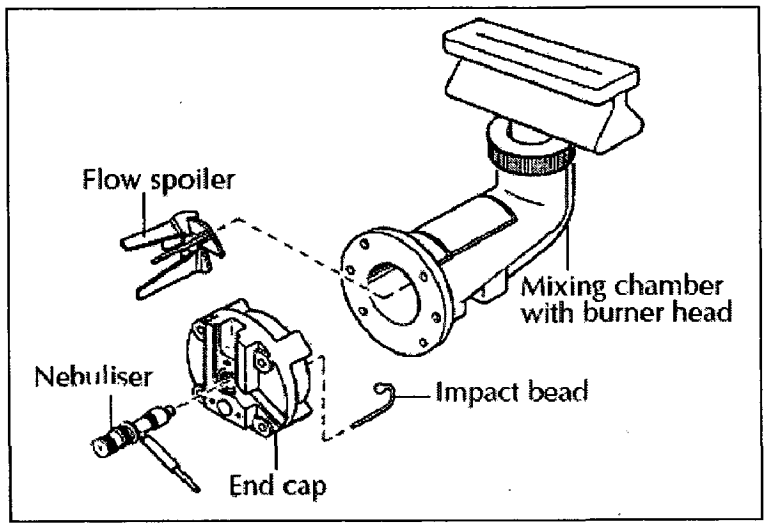

Fig. 11: Nebulization unit of an AAS Instrument

The nebulizer chamber thoroughly mixes acetylene (the fuel) and oxidant (air or nitrous oxide), and by doing so, creates a negative pressure at the end of the small diameter, plastic nebulizer tube. This negative pressure acts to suck ("uptake") liquid sample up the tube and into the nebulizer chamber, a process called aspiration.**(Fig. 11)** A small glass impact bead and/or a fixed impeller inside the chamber creates a heterogeneous mixture of gases (fuel + oxidant) and suspended aerosol (finely dispersed sample). This mixture flows immediately into the burner head where it burns as a smooth, laminar flame evenly distributed along a narrow slot in the well-machined metal

burner head. Liquid sample not flowing into the flame collects on the bottom of the nebulizer chamber and flows by gravity through a waste tube to a glass waste container. For some elements that form refractory oxides (molecules hard to break down in the flame) nitrous oxide (N_2O) needs to be used instead of air (78% N_2 + 21% O_2) for the oxidant. In that case, a slightly different burner head with a shorter burner slot length is used.

The **Flame** Destroy any analyte ions and breakdown complexes & create atoms (the elemental form) of the element of interest Fe^0, Cu^0, Zn^0, etc.

2.3.1.6 The Monochromator and PMT

Tuned to a specific wavelength and with a specified slit width chosen, the monochromator isolates the hollow cathode lamp's analytical line. Since the basis for the AAS process is atomic ABSORPTION, the monochromator seeks to only allow the light not absorbed by the analyte atoms in the flame to reach the PMT. That is, before an analyte is aspirated, a measured signal is generated by the PMT as light from the HCL passes through the flame. When analyte atoms are present in the flame--while the sample is aspirated--some of that light is absorbed by those atoms (remember it is not the ionic but elemental form that absorbs). This causes a decrease in PMT signal that is proportional to the amount of analyte. This last is true inside the linear range for that element using that slit and that analytical line. The signal is therefore a decrease in measure light: atomic **absorption** spectroscopy.

2.3.1.7 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. **(Fig. 12)**

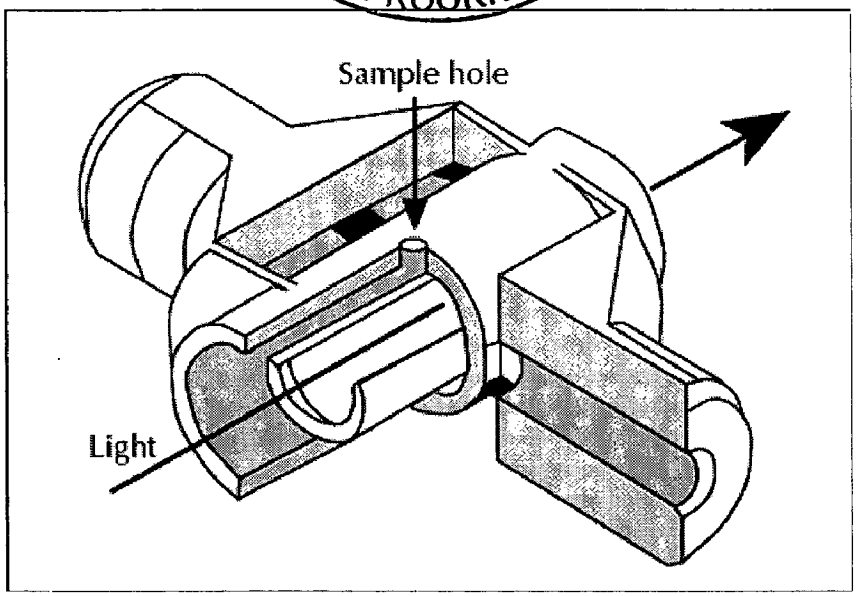

Fig. 12: An overview of a Graphite Furnace in an AAS instrument

The amount of light energy absorbed increases as the concentration of the selected element increases. Flame AA can only analyze solutions, but graphite furnace can accept very small absolute quantities of solution, slurry or solid samples.

2.3.1.8 Interferences and matrix modification

Other chemicals that are present in the sample may affect the atomization process. For example, in flame atomic absorption, phosphate ions may react with calcium ions to form calcium pyrophosphate. This does not dissociate in the flame and therefore results in a low reading for calcium. This problem is avoided by adding different reagents to the sample that may react with the phosphate to give a more volatile compound that is dissociated easily. Lanthanum nitrate solution is added to samples containing calcium to tie up the phosphate and to allow the calcium to be atomized, making the calcium absorbance independent of the amount of phosphate. With electrothermal atomization, chemical modifiers can be added which react with an interfering substance in the sample to make it more volatile than the analyte compound. This volatile component vaporizes at a relatively low temperature and is removed during the low and medium temperature stages of electrothermal atomization.

2.3.2 Inductively Coupled Plasma- Mass Spectrometry (ICP-MS):-

2.3.2.1 Principle

ICP-MS is a type of mass spectrometry that is highly sensitive and capable of the determination of a range of metals and several non-metals at concentrations below one part in 10^{12}. It is based on coupling together an inductively coupled plasma as a method of producing ions with a mass spectrometer as a method of separating and detecting the ions. **(Fig. 13)** ICP-MS is also capable of monitoring isotopic speciation for the ions of choice.

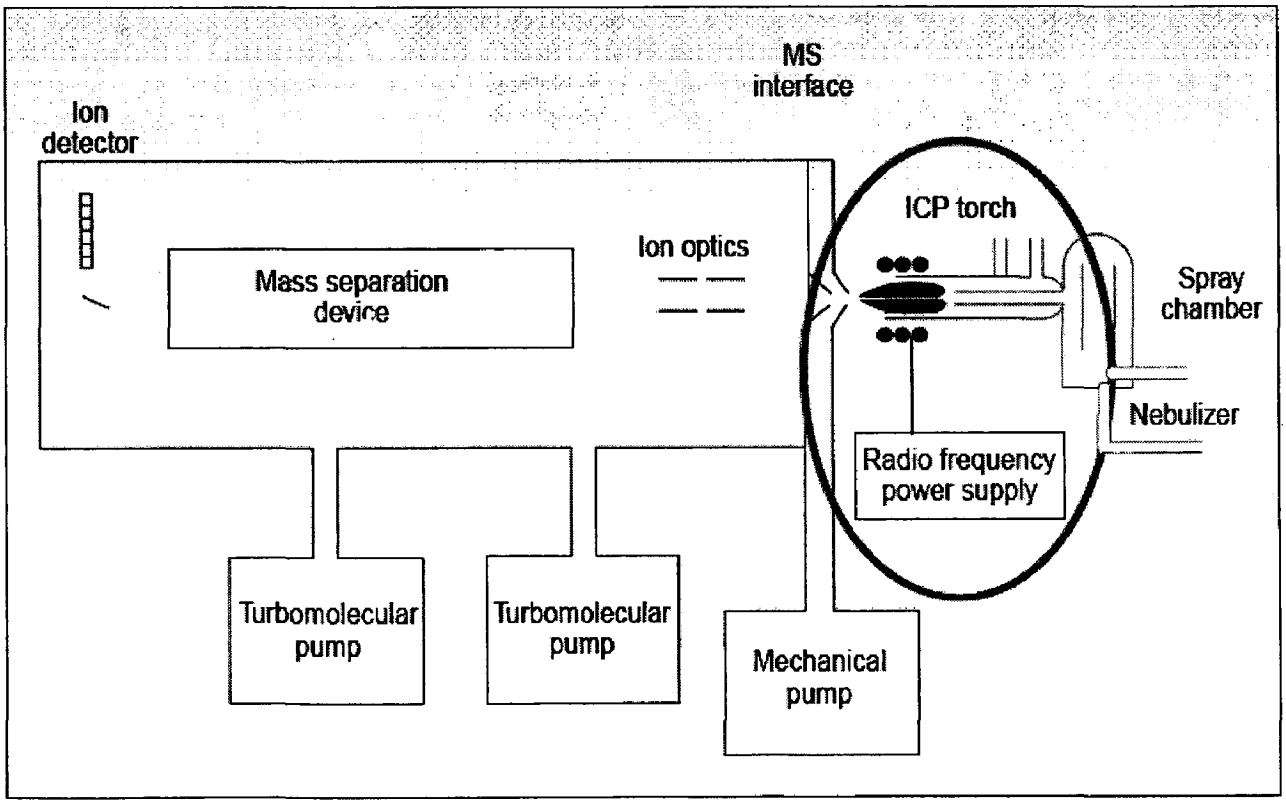

Fig. 13: Schematic of an ICP-MS system showing the location of the plasma torch and radio frequency (RF) power supply

The Plasma is inductively coupled which contains a sufficient concentration of ions and electrons to make the gas electrically conductive. The plasmas used in spectrochemical analysis are essentially electrically neutral, with each positive charge on an ion balanced by a free electron. In these plasmas the positive ions are almost all singly-charged and there are few negative ions, so there are nearly equal amounts of ions and electrons in each unit volume of plasma.

An inductively coupled plasma (ICP) for spectrometry is sustained in a torch that consists of three concentric tubes, usually made of quartz. The end of this torch is placed inside an induction coil supplied with a radio-frequency electric current. **(Fig. 14)**

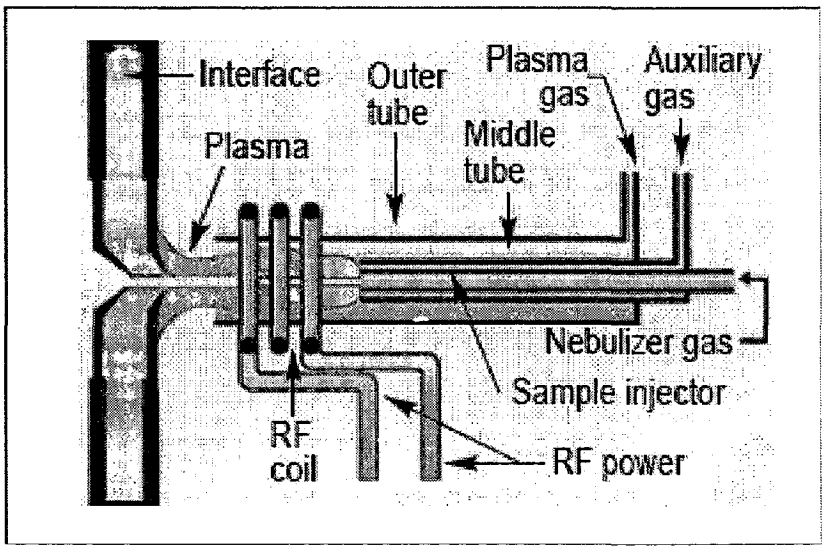

Fig. 14: Detailed view of a plasma torch and RF coil relative to the ICP-MS interface

A flow of argon gas (usually 14 to 18 liters per minute) is introduced between the two outermost tubes of the torch and an electrical spark is applied for a short time to introduce free electrons into the gas stream. These electrons interact with the radio-frequency magnetic field of the induction coil and are accelerated first in one direction, then the other, as the field changes at high frequency (usually 27.12 million cycles per second). The accelerated electrons collide with argon atoms, and sometimes a collision causes an argon atom to part with one of its electrons. The released electron is in turn accelerated by the rapidly-changing magnetic field. The process continues until the rate

of release of new electrons in collisions is balanced by the rate of recombination of electrons with argon ions (atoms that have lost an electron). This produces a 'fireball' that consists mostly of argon atoms with a rather small fraction of free electrons and argon ions. The temperature of the plasma is very high, of the order of 10,000 K.

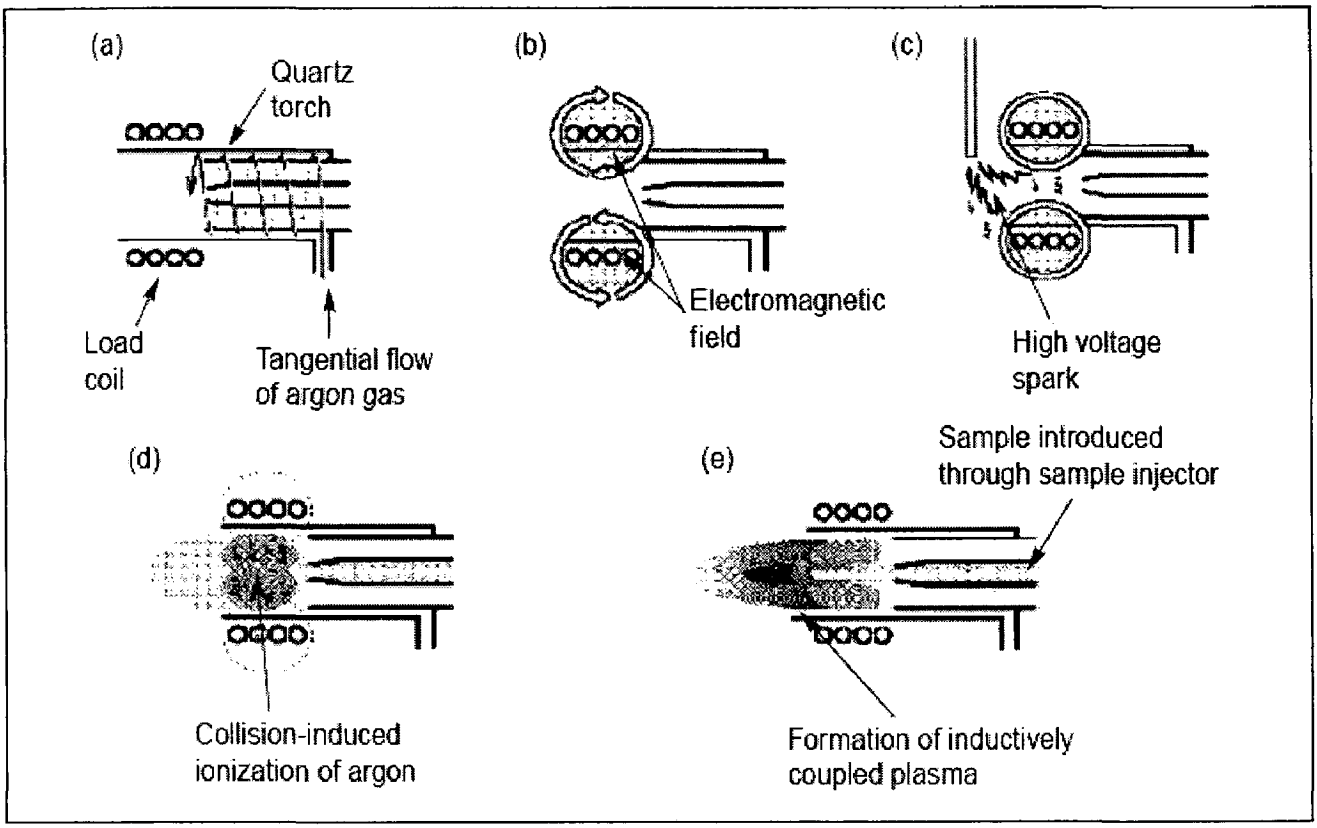

Fig. 15: Schematic of an ICP torch and load coil showing the Plasma formation.

(a) A tangential flow of Ar gas is passed between the outer and middle tube of the quartz torch.

(b) RF power is applied to the load coil, producing an intense electromagnetic field.

(c) A high-voltage spark produces free electrons.

(d) Free electrons are accelerated by the RF field, causing collisions and ionization of Ar gas.

(e) The ICP is formed at the open end of the quartz torch. The sample is introduced into the plasma via the sample injector

The ICP can be retained in the quartz torch because the flow of gas between the two outermost tubes keeps the plasma away from the walls of the torch. A second flow of argon (around 1 liter per minute) is usually introduced between the central tube and the intermediate tube to keep the plasma away from the end of the central tube. A third flow (again usually around 1 liter per minute) of gas is introduced into the central tube of the torch. This gas flow passes through the centre of the plasma, where it forms a channel that is cooler than the surrounding plasma but still much hotter than a chemical flame. Samples to be analyzed are introduced into this central channel, usually as a mist of liquid formed by passing the liquid sample into a nebulizer.

As a droplet of nebulized sample enters the central channel of the ICP, it evaporates and any solids that were dissolved in the liquid vaporize and then break down into atoms. At the temperatures prevailing in the plasma a significant proportion of the atoms of many chemical elements are ionized, each atom losing its most loosely-bound electron to form a singly charged ion.

2.3.2.2 Mass spectrometry

For coupling to mass spectrometry, the ions from the plasma are extracted through a series of cones into a mass spectrometer, usually a quadrupole. The ions are separated on the basis of their mass-to-charge ratio and a detector receives an ion signal proportional to the concentration.

The concentration of a sample can be determined through calibration with certified reference material such as single or multi-element reference standards. ICP-MS also lends itself to quantitative determinations through Isotope Dilution, a single point method based on an isotopically enriched standard.

Other mass analyzers coupled to ICP systems include double focusing magnetic-electrostatic sector systems with both single and multiple collector, as well as time of flight systems (both axial and orthogonal accelerators have been used).

2.3.2.3 Sample introduction

The first step in analysis is the introduction of the sample. This has been achieved in ICP-MS through a variety of means, the most common method is the use of a *nebulizer*. This is a device which converts liquids into an aerosol, and that aerosol can then be swept into the plasma to create the ions. Nebulizers work best with simple liquid samples (i.e. solutions). However, there have been instances of their use with more complex materials like a slurry. Many varieties of nebulizers have been coupled to ICP-MS, including pneumatic, cross-flow, Babington, ultrasonic, and desolvating types. The aerosol generated is often treated to limit it to only smallest droplets, commonly by means of a double pass or cyclonic spray chamber. Use of autosamplers makes this easier and faster.

Less commonly, the laser ablation has been used as a means of sample introduction. In this method, a laser is focused on the sample and creates a plume of ablated material which can be swept into the plasma. This is particularly useful for solid samples, though can be difficult to create standards for leading the challenges in quantitative analysis.

Other methods of sample introduction are also utilized. Electrothermal vaporization (ETV) and in torch vaporization (ITV) use hot surfaces (graphite or metal, generally) to vaporize samples for introduction. These can use very small amounts of liquids, solids, or slurries. Other methods like vapor generation are also known.

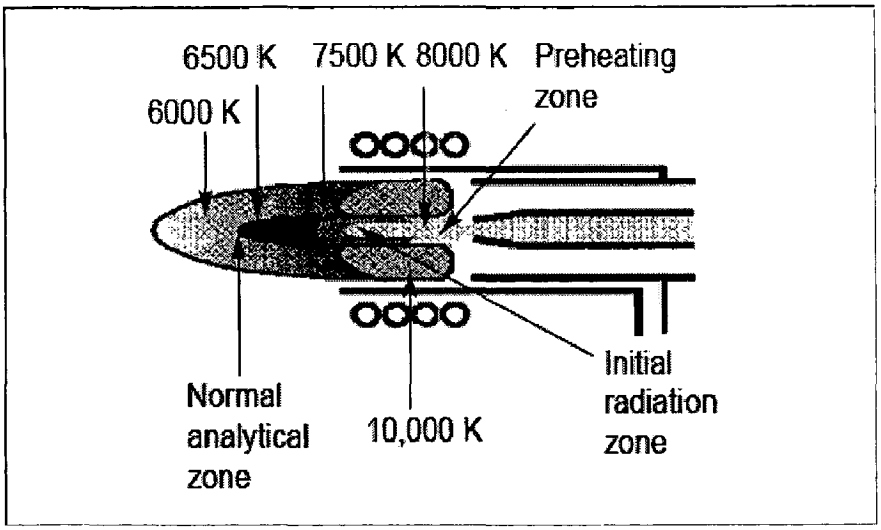

Fig. 16: Different temperature zones in the plasma

2.3.2.4 Transfer of ions into vacuum

The carrier gas (usually argon or occasionally helium) is sent through the central channel and into the very hot plasma. The sample is then exposed to radio frequency which converts the gas into a plasma. The high temperature of the plasma is sufficient to cause a very large portion of the sample to form ions.

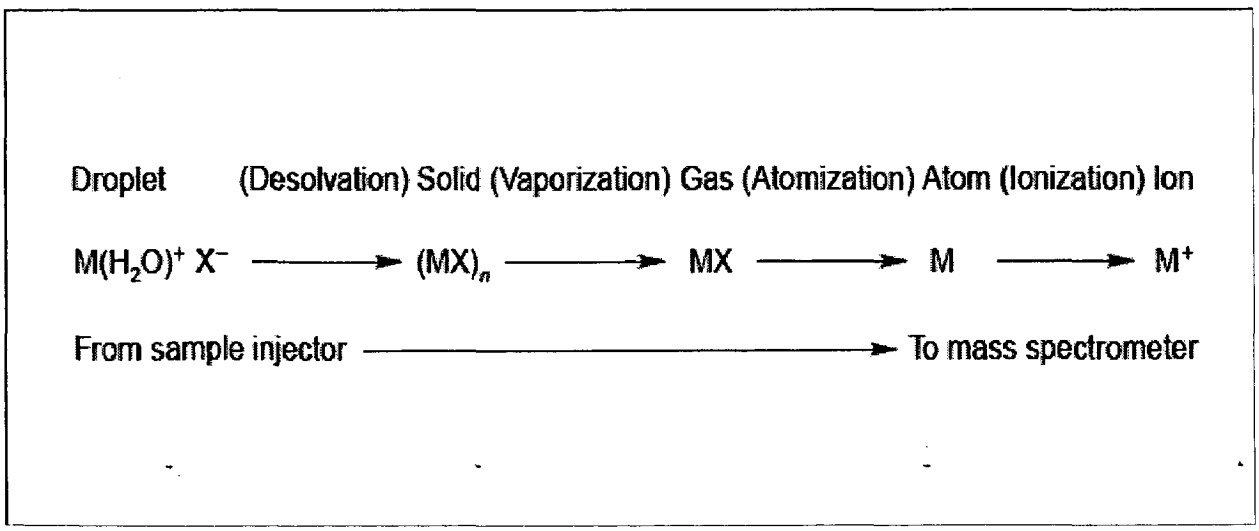

Fig. 17: Mechanism of conversion of a droplet to a positive ion in the ICP.

This fraction of ionization can approach 100% for some elements (e.g. sodium), but this is dependent on the ionization potential. A fraction of the formed ions passes through a ~1mm hole (sampler cone) and then a ~0.4mm hole (skimmer cone). The purpose of which is to allow a vacuum that is required by the mass spectrometer.

The vacuum is created and maintained by a series of pumps. The first stage is usually based on a roughing pump, most commonly a standard rotary vane pump. This removes most of the gas and typically reaches a pressure of around 133 Pa. Later stages have their vacuum generated by more powerful vacuum systems, most often turbomolecular pumps. Older instruments may have used oil diffusion pumps for high vacuum regions.

2.3.2.5 Advantage of Argon

Making the plasma from argon, instead of other gases, has several advantages. First, argon is abundant (in the atmosphere, as a result of the radioactive decay of potassium) and therefore cheaper than other noble gases. Argon also has a higher first ionization potential than all other elements except He, F, and Ne. Because of this high ionization energy, the reaction ($Ar^+ + e^- \rightarrow Ar$) is more energetically favorable than the reaction ($M^+ + e^- \rightarrow M$). This ensures that the sample remains ionized (as M^+) so that the mass spectrometer can detect it.

Argon can be purchased for use with the ICP-MS in either a refrigerated liquid or a gas form. However it is important to note that whichever form of argon purchased, it should have a guaranteed purity of 99.9% Argon at a minimum. It is important to determine which type of argon will be best suited for your specific situation. Liquid argon is typically cheaper and can be stored in a greater quantity as opposed to the gas form, which is more expensive and takes up more tank space. If your instrument will be in an environment where it will get infrequent-use, then buying argon in the gas state will be most appropriate as it will be more than enough to suit smaller run times and will remain stable for longer periods of time, whereas liquid argon will suffer loss to the environment due to venting of the tank when stored over extended time frames.

However if your ICP-MS will be used routinely and will be on and running for eight or more hours each day for several days a week, then going with liquid argon will be the most suitable. If there are to be multiple ICP-MS instruments running for long periods of time, then it will most likely be beneficial for the laboratory to install a bulk or micro bulk argon tank which will be maintained by a gas supply company, thus eliminating the need to change out tanks frequently as well as minimizing loss of argon that is left over in each used tank as well as down time for tank changeover.

2.4 Sample Preparation:-

2.4.1 Washing of the glasswares:

All the glassware's was first cleaned with soap solution. Then it was kept in (0.5%) nitric acid for around 24 hours then dipped in distilled water for 1hr and again washed with distilled water. Then these are washed with Millipore water for 3-4 times and apparatus was then dried in an oven.

2.4.2 For Total Metal analysis of Sediment:[8]

The sediment samples were digested in three stages as follows:

1. 1gm of accurately weighed sample was taken in a pre-cleaned Teflon beaker to which 10mL (70%) of concentrated HNO_3 was added & was digested for overnight at room temperature.
2. After digestion, the acid treated sample was heated at 100-110°C with random shaking till brown fumes ceased to emit and white fumes started emitting.
3. The sample was then cooled and 10 mL of 40% HF was added.
4. The sample was then digested in HF at 120-130°C for 2.5-3 hrs.
5. After cooling the HF digest, 5mL of $HClO_4$ was added and carefully heated at 120-130°C for 2-2.5 hrs.
6. Finally the digested sample was cooled, filtered and made up to 25 mL as stock samples solution for element analysis.
7. A blank solution was prepared in the same manner as described above.

The elemental analyses were carried out by AAS and ICPMS and the concentration of individual element was determined in (ppm or μg/g) as

$$\frac{[(\text{AAS/ICPMS data of sample}) \times (\text{Dilution Factor}) \times (\text{F.V.})] - \text{Blank Data} \times \text{F.V.}}{\text{Amount of sample taken}}$$

Where F.V. stands for final volume and dilution factor was applicable when the stock solution was diluted to a fixed volume so that the corresponding element which was analysed was within the working concentration range.

2.4.3 For Sequential metal analysis of Sedimants:[9]

2.4.3.1 Step I

Exchangeable metal :- 2.5 g of soil sample was treated with 45 mL of 1M ammonium acetate at pH 5 with acetic acid under stirring for 24 h at room temperature. The resulting suspension was then centrifuged at 3000 rpm for 20 min. The supernatant liquid was separated and diluted to 100 mL with deionized water for analysing the metals of interest.

2.4.3.2 Step II

Metals associated with iron oxide and manganese oxides:- The residual solid of the previous step was treated with 22.5 mL of 1M hydroxylammonium chloride and 22.5 mL of acetic acid (25%). After 24 h of stirring at room temperature a solid–liquid separation was performed by centrifugation as before and the metal-bearing solution (supernatant part) was diluted (to 100 mL) and analysed.

2.4.3.3 Step III

Metals weakly bound to organic matter:- The residual solid of the previous step was treated with 12.5 mL of 0.1M HCl and stirred for 24 h at room temperature. As in previous steps, a solid–liquid separation was performed and the solution diluted to 25 mL was analysed for metal concentrations.

2.4.4 Sample preparation method for total metal analysis of fish samples:[10]

1. A known amount of sample (0.5 -1.0g) was accurately weighed in a clean 100mL beaker.
2. About 15-20 mL of concentrated HNO_3 was added to the beaker depending up on the quantity of sample.
3. After a whole night digestion, the acid treated sample was heated at 120-140°C till brown fumes seizes to escape.
4. The digest was cooled and to it 2-5 mL of H_2O_2 was added if the solution obtained in previous step was turbid.
5. Finally the solution was cooled and the final volume was made up to 10mL if the sample taken is less then 1g, otherwise the volume was made up to 25mL.
6. The concentration of the metal was determined as described in section **2.4.2**

The elemental analyses were carried out by AAS and ICPMS and the concentration of individual element was determined in (ppm or μg/g) as

$$\frac{[(\text{AAS/ICPMS data of sample}) \times (\text{Dilution Factor}) \times (\text{F.V.})] - \text{Blank Data} \times \text{F.V.}}{\text{Amount of sample taken}}$$

Where F.V. stands for final volume and dilution factor was applicable when the stock solution was diluted to a fixed volume so that the corresponding element which was analysed was within the working concentration range.

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2.4.3.3 Step III

Metals weakly bound to organic matter:- The residual solid of the previous step was treated with 12.5 mL of 0.1M HCl and stirred for 24 h at room temperature. As in previous steps, a solid–liquid separation was performed and the solution diluted to 25 mL was analysed for metal concentrations.

2.4.3.4 Step IV

Metals strongly bound to organic matter:- The residual solid of the previous step was treated with 12.5 mL of NaOH 0.5 M under stirring for 24 h at room temperature. For soil samples with large organic content this treatment was repeated until a clear solution was obtained.

All the solutions separated from the solids were then dried by an IR lamp at 60^0C and then digested by using 4 mL of HNO_3 (65%) and 2 mL HF (40%) in a microwave oven (250 W, 1 min; 0 W, 2 min; 250 W, 5 min; 400 W, 5 min; 600 W, 5 min). The acid solution is then diluted to 25 mL and analysed for metals.

2.4.3.5 Step V

Bound to sulphide phase:- The residual solid of the previous step (step IV) was added to 12.5 mL of 8M HNO_3 and digested for 3 h at 80^0C. The solution is then diluted to 25 mL and analysed.

2.4.3.6 Step VI

Residual:- The residual solid of the fifth step is finally digested with 4 mL of an oxidizing mixture (HNO_3:HCl = 3:1) and 6 mL HF in a Teflon recipient put in a microwave oven (800 W, 4 min; 400 W, 4 min; 800 W, 4 min; 20 min of ventilation) and diluted to 100 ml by deionized water.

Final concentration (In ppm or μg/g) as :-

$$\frac{[(\text{AAS/ICP Data of sample}) \times (\text{Dilution Factor}) \times (\text{F.V.})] - \text{Blank Data} \times \text{F.V.}}{\text{Amt. of sample taken}}$$

2.4.4 Sample preparation method for total metal analysis of fish samples:[10]

1. A known amount of sample (0.5 -1.0g) was accurately weighed in a clean 100mL beaker.
2. About 15-20 mL of concentrated HNO_3 was added to the beaker depending up on the quantity of sample.
3. After a whole night digestion, the acid treated sample was heated at 120-140°C till brown fumes seizes to escape.
4. The digest was cooled and to it 2-5 mL of H_2O_2 was added if the solution obtained in previous step was turbid.
5. Finally the solution was cooled and the final volume was made up to 10mL if the sample taken is less then 1g, otherwise the volume was made up to 25mL.
6. The concentration of the metal was determined as described in section **2.4.2**

3.1 Total Metal analysis of Sediments:-

The total metal contents of Cr, Mn, Fe, Co, Ni, Cu, Zn, Pb and Cd is shown for the all six sediment samples in **Table 2** as mean and the standard deviation for the triplicate results along with the blank in same conditions. It can be observed from table 2, that mean Cr is highest in 1 & lowest in 6, with exact order as 1 > 5 > 4 > 3 > 2 > 6. The mean Co is highest in 4 and lowest for 6 ranging the value from 27.85-16.18 for 4 & 6 respectively and in between for rest of the samples.

The range of concentration for Ni (79.02 – 35.52 ppm), Zn (23.41 – 16.51 ppm) and Cd (1.44 – 0.86 ppm) is also found higher then the prescribed one. It is further noted that the minimum Pb is highest in 6 (37.41 ppm). In this regard, it may be substantiated that the sample 1 is high in most of the metals which may be attributed to the discharge of nearby industrial effluents. Though the Mn values are apparently different and very with wide range (due to large variation in Mn containing minerals) restricted us to comment on the variations of Mn concentration in different samples.

Table 2: Concentration of heavy metals in different sediment samples namely 1-6

	Sample 1		Sample 2		Sample 3		Sample 4		Sample 5		sample 6	
nent	Mean ppm	S D	Mean ppm	S D	Mean ppm	S D	Mean ppm	S D	Mean ppm	S D	Mean ppm	S D
	119.52	8.19	21.60	7.95	33.35	5.03	56.32	4.26	66.55	4.31	15.32	3.2
n	94.16	3.71	84.11	9.05	264.37	33.64	132.72	4.47	79.11	33.57	109.29	5.7
	20154.25	968.90	25330.82	832.05	30672.57	505.54	20448.55	268.49	19952.47	53.17	17013.65	537.9
	20.05	2.69	17.20	15.25	20.73	2.90	27.85	1.83	22.98	4.07	16.18	2.7
	35.52	4.74	79.02	0.52	58.95	1.04	65.41	3.55	64.23	4.50	46.01	6.8
	30.05	4.86	32.85	1.73	36.15	3.03	27.34	4.25	29.96	0.13	15.14	1.6
	20.34	0.33	19.67	0.28	23.41	2.05	16.51	0.31	18.46	0.81	18.1	2.4
	1.05	0.24	0.86	0.05	1.2	0.05	1.44	0.18	1.22	0.39	1.07	0.1
	29.25	5.05	13.85	2.04	16.6	4.97	24.02	1.28	23.27	2.22	37.41	3.8

Chromium:

It was found to be low in sample 6, while it was very high in sample 1, which could be attributed to the effluents of nearby leather industries. The Cr levels in sample 2 and sample 3 were nearly similar to that of sample 1. The Cr levels in sample 4 and

5 were also found to be on higher side, which was more or less average of sample 1 and sample 6.

Manganese:

The manganese levels were higher for sample 3 and 4, while the others were less than 100 ppm.

Iron:

The iron level was also high in sample 3 as found for manganese. The Fe levels were of 2-3% levels.

Cobalt:

The Co levels were found to be invariant for all the sampling sites.

Nickel:

The maximum level of Ni was measured in sample 2, which was considered to be high for sediment samples. The Ni levels of samples of 3, 4 and 5 were also high though lower than sample 2. The Ni levels of sample 1 and 6 were of lower levels.

Copper:

Level of copper is approximately constant for all samples but it is relatively low for 6^{th} one with the highest value for sample 3.

Zinc:

The zinc levels were also found to be invariant as measured for Co.

Cadmium:

The Cadmuim levels for samples 1 – 6 were nearly constant and its level in sediment was not very high. It appears the source of Cd was mostly from natural source.

Lead:

While the lead was found to be high for sample 6, moderate for sample 1, 4 and 5. The Pb contents in the sediments of sample 2 and 3 were very low. Since Pb is mostly accumulated due to anthropogenic sources like petrol or diesels from steamer of motorized boat which are common in the sampling sites of sample 6.

These all results can also be observed in graphs in following pages

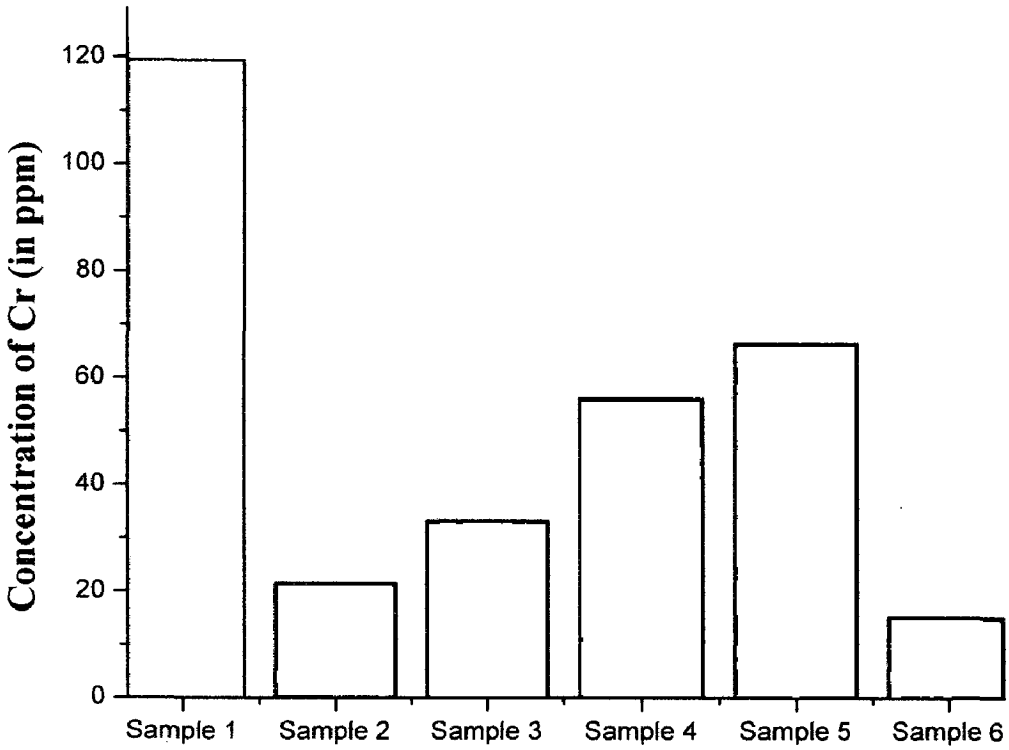

Fig. 18: Concentration of Chromium in different samples

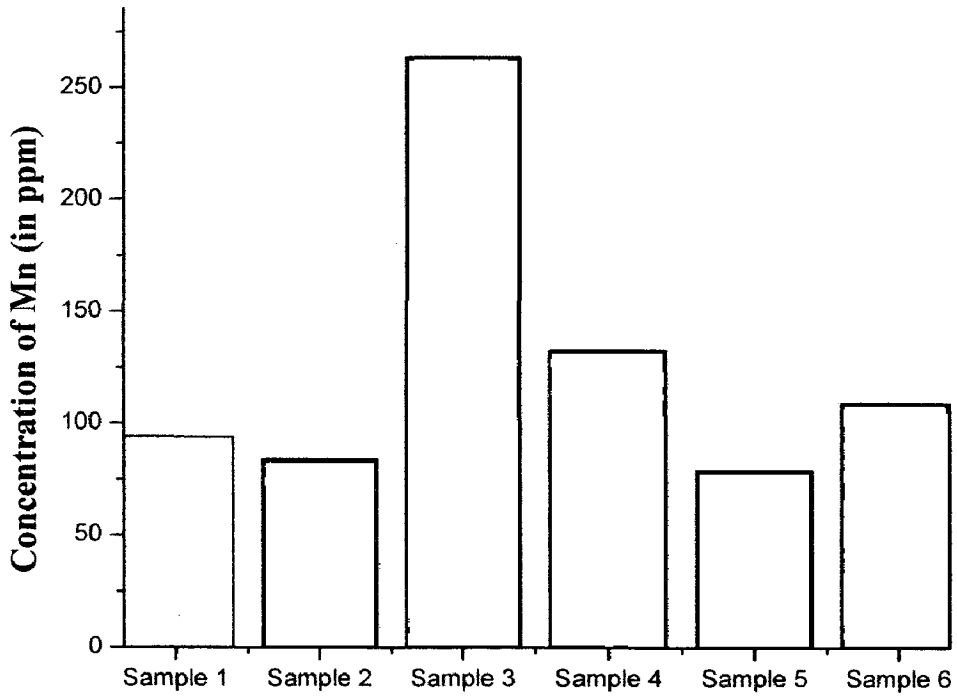

Fig. 19: Concentration of Manganese in different samples

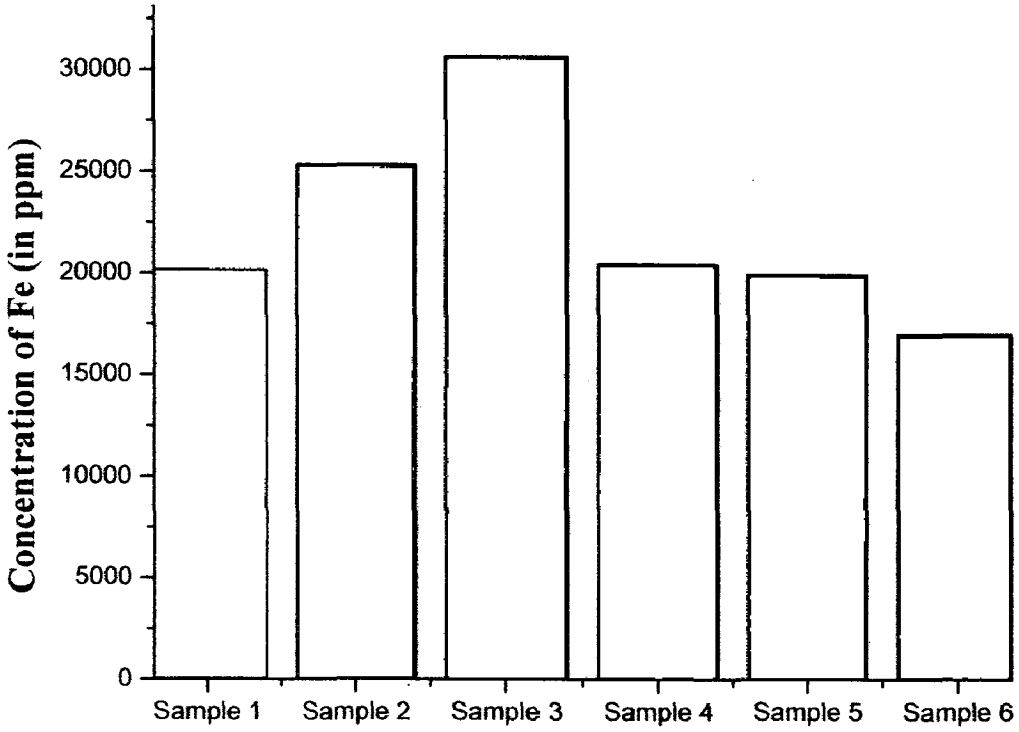

Fig. 20: Concentration of Iron in different samples

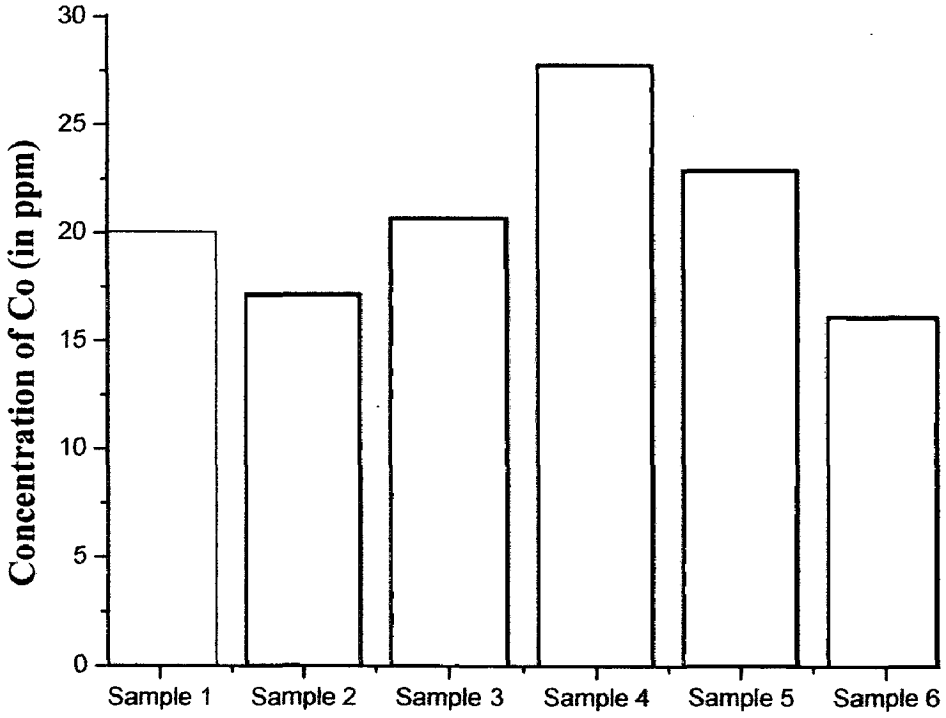

Fig. 21: Concentration of Cobalt in different samples

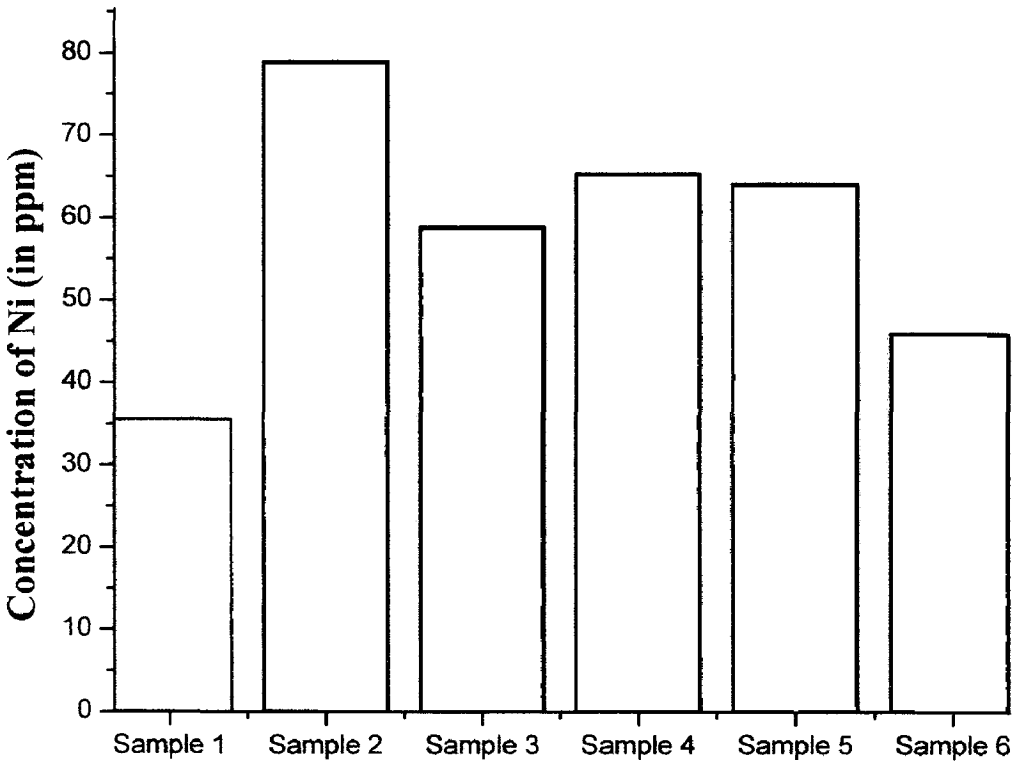

Fig. 22: Concentration of Nickel in different samples

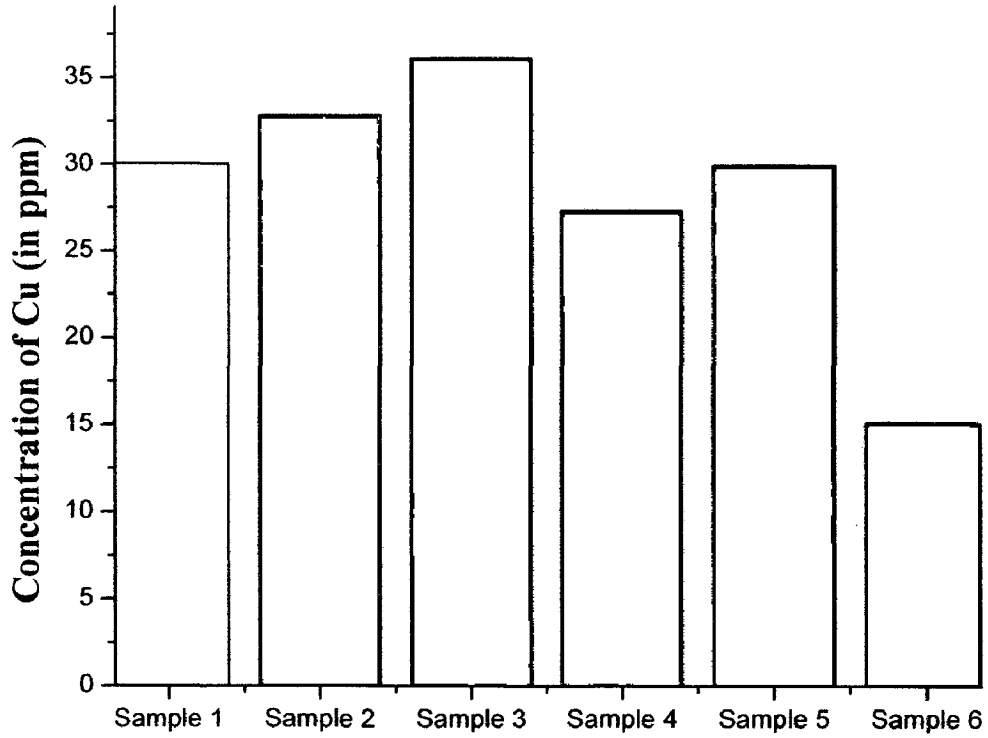

Fig. 23: Concentration of Copper in different samples

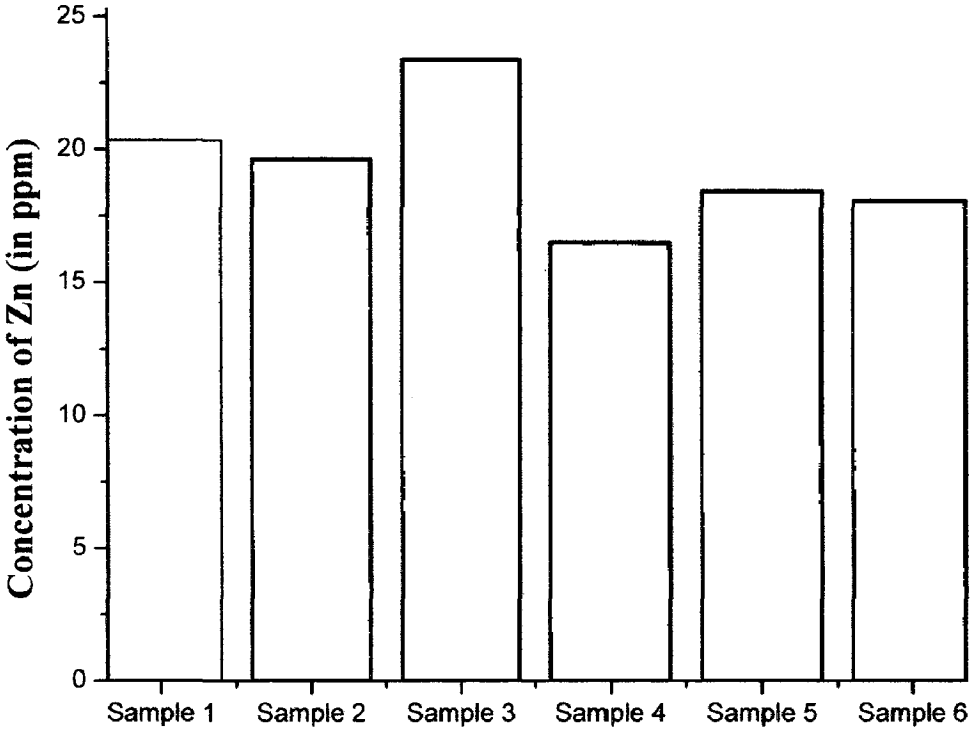

Fig. 24: Concentration of Zinc in different samples

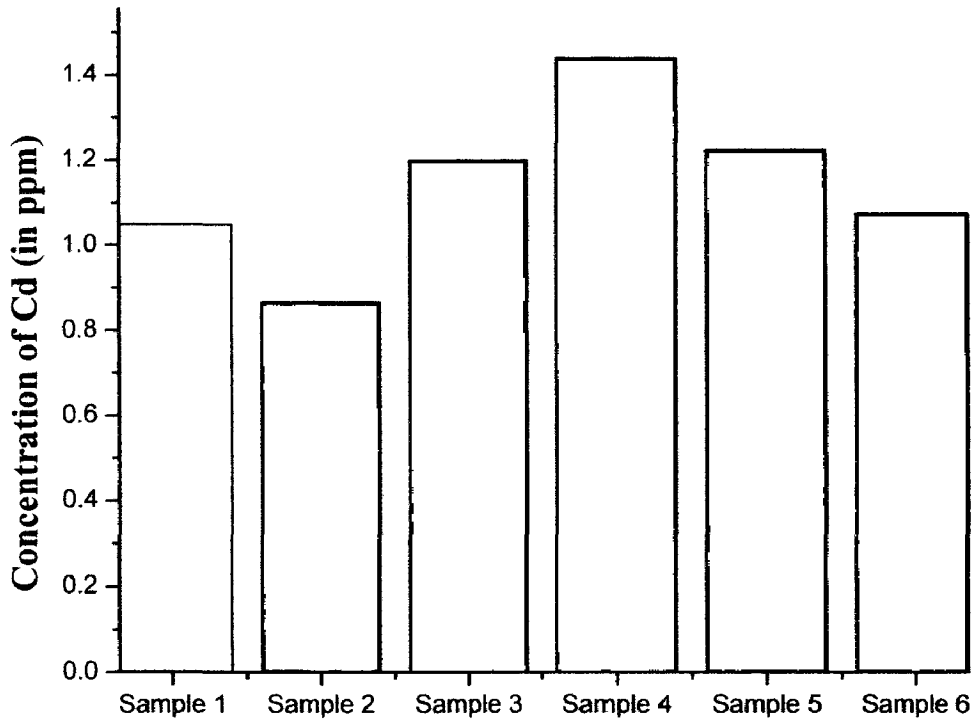

Fig. 25: Concentration of Cadmium in different samples

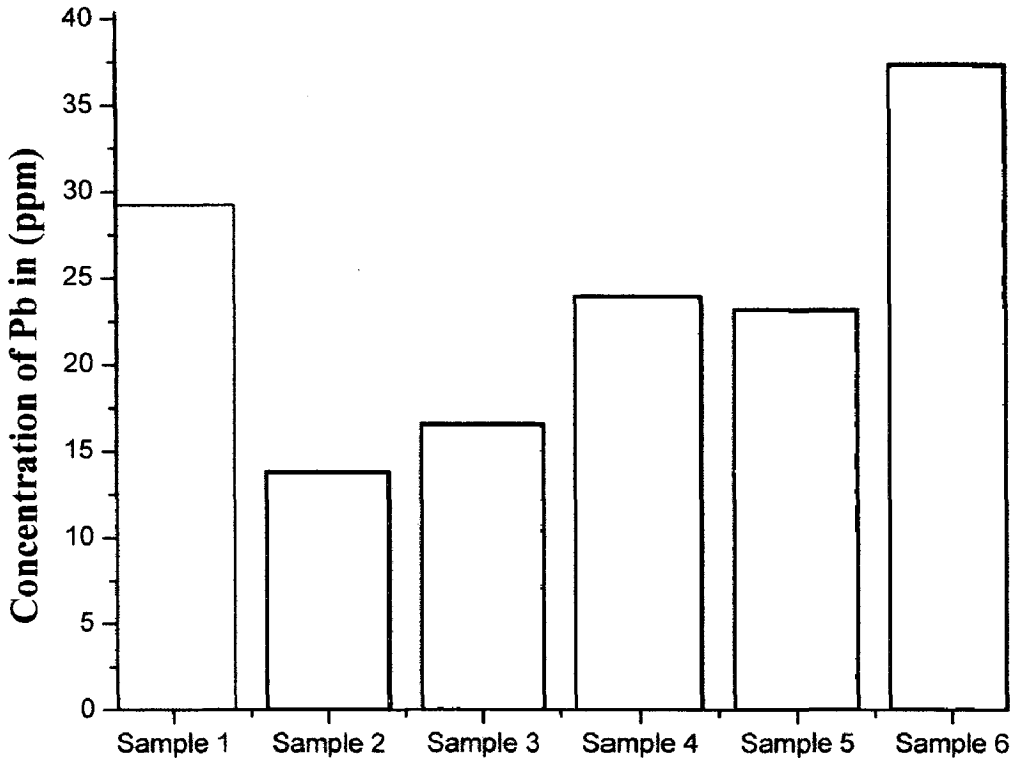

Fig. 26: Concentration of Lead in different samples

3.2 Sequential Metal analysis of Sediments:-

Chromium: Table 3: Result of sequential metal extraction for Chromium; values are concentration given here is in ppm (μg/g)

Metal Cr	Step 1	Step 2	Step 3	Step 4	Step 5	Residue	Sum	Total
Sample 1	26.796	6.070	0	23.582	0	34.348	90.798	119.525
Sample 2	0	0	0	0	0	17.474	17.474	21.608
Sample 3	2.853	3.178	0	0	0	18.598	24.630	33.358
Sample 4	8.676	3.461	0	13.123	0	21.893	47.154	56.325
Sample 5	13.927	3.084	0	12.184	0	26.242	55.440	66.558
Sample 6	0	0	0	0	0	11.908	11.908	15.325

As it is clear from the **Table 3** that Cr is mostly present in the form of exchangeable, oxide and strongly bound organic form and it also be noted that weekly

bound organic fraction and sulphide is completely missing. In sample 2 and 6 the concentration is too low to detect from AAS.

Manganese: **Table 4:** Result of sequential metal extraction for Manganese; values are concentration given here is in ppm (μg/g)

Metal Mn	Step 1	Step 2	Step 3	Step 4	Step 5	Residue	Sum	Total
Sample 1	20.646	3.44	7.250	9.838	16.198	25.886	83.261	94.166
Sample 2	11.160	4.525	5.676	12.552	15.247	23.372	72.533	84.116
Sample 3	22.130	18.147	21.194	33.391	39.746	88.474	222.986	264.375
Sample 4	9.386	7.687	8.979	14.105	16.644	52.410	109.204	132.725
Sample 5	4.046	3.461	3.785	6.209	10.894	34.766	63.164	79.116
Sample 6	9.390	7.699	8.992	14.130	16.658	37.554	94.429	109.291

Manganese is present in all forms in all samples but mostly it is concentrated as exchangeable fraction and strongly bound organic matter. The concentration is highest for sample 4.

Iron: **Table 5:** Result of sequential metal extraction for Iron; values are concentration given here is in ppm (μg/g)

Metal Fe	Step 1	Step 2	Step 3	Step 4	Step 5	Residue	Sum	Total
Sample 1	2523.933	1833.573	1477.546	2320.960	2770.987	6168.373	17095.370	20154.220
Sample 2	4399:213	2345.920	1680.106	2639.199	3150.880	7014.187	21229.510	25330.830
Sample 3	2655.680	2177.760	2543.360	3995.040	4769.630	10616.960	26758.430	30672.580
Sample 4	1770.453	1451.839	1695.573	2663.360	3178.733	7077.973	17838.930	20448.550
Sample 5	1628.643	1335.520	1499.786	2450.437	2925.173	6511.680	16411.150	19952.480
Sample 6	1451.573	1190.346	1390.240	2183.787	2607.146	5803.893	14626.990	17013.650

Iron has approximately similar pattern as Manganese except that it has relatively very high concentration compared to other metals at each step. The highest concentration is in sample 3 and lowest is in 6.

Cobalt: **Table 6:** Result of sequential metal extraction for Cobalt; values are concentration given here is in ppm (μg/g)

Metal Co	Step 1	Step 2	Step 3	Step 4	Step 5	Residue	Sum	Total
Sample 1	5.306	1.792	0	0	0	9.766	16.865	20.058
Sample 2	5.083	0	0	0	0	9.908	14.991	17.208
Sample 3	2.534	0	0	0	0	10.598	13.133	20.733
Sample 4	5.640	3.461	0	0	0	10.569	19.670	27.850
Sample 5	3.934	3.084	0	0	0	10.740	17.758	22.983
Sample 6	0	0	0	0	0	9.070	9.070	16.183

Cobalt is mostly present in the form of exchangeable ions and oxides. For samples 2, 3, & 6 the concentration is too low to detect. The variation is also low.

Nickel: **Table 7:** Result of sequential metal extraction for Nickel; values are concentration given here is in ppm (μg/g)

Metal Ni	Step 1	Step 2	Step 3	Step 4	Step 5	Residue	Sum	Total
Sample 1	7.023	1.726	0	5.633	0	11.265	25.648	35.525
Sample 2	18.461	3.760	0	16.834	0	24.516	63.572	79.025
Sample 3	7.925	3.570	0	15.986	0	17.953	46.156	58.950
Sample 4	10.289	4.208	0	14.880	0	21.938	55.386	65.416
Sample 5	10.399	4.208	0	18.839	0	21.938	55.386	64.233
Sample 6	4.129	2.538	0	11.351	0	16.553	34.573	46.008

Weekly bound organic matter and sulphide forms are completely missing in Nickel and there is substantial variation in data of concentration of Nickel in different samples.

Copper: **Table 8:** Result of sequential metal extraction for Copper; values are concentration given here is in ppm (μg/g)

Metal Cu	Step 1	Step 2	Step 3	Step 4	Step 5	Residue	Sum	Total
Sample 1	11.523	2.977	0	3.090	1.945	5.736	25.272	30.058
Sample 2	7.903	4.126	0	4.285	2.698	7.954	26.968	32.858
Sample 3	5.117	3.305	0	3.430	2.160	6.370	20.384	36.158
Sample 4	3.146	2.032	0	2.109	1.326	7.914	16.529	27.341
Sample 5	5.226	3.374	0	3.504	2.204	6.854	21.164	29.966
Sample 6	1.828	1.180	0	1.225	0.770	2.278	7.278	15.141

In copper weekly bound organic matter is completely absent and there is substantial consistency in results for different samples for Copper except for sample 6.

Zinc: **Table 9:** Result of sequential metal extraction for Zinc; values are concentration given here is in ppm (μg/g)

Metal Zn	Step 1	Step 2	Step 3	Step 4	Step 5	Residue	Sum	Total
Sample 1	4.660	1.474	0	2.396	1.945	5.736	16.212	20.341
Sample 2	6.686	2.909	0	0	2.698	4.661	16.956	19.675
Sample 3	5.117	3.305	0	3.430	2.160	6.370	20.384	23.416
Sample 4	3.146	2.032	0	2.109	0	5.248	12.536	16.516
Sample 5	2.993	3.126	0	2.217	1.301	5.373	15.012	18.466
Sample 6	3.306	2.592	0	1.762	1.113	5.601	14.376	18.1

Zinc has similar pattern as Cu in different fractions but for sample 2 and sample 4 concentration is too low to detect at step 4 & 5 respectively.

Cadmium: **Table 10:** Result of sequential metal extraction for Cadmium; values are concentration given here is in ppm (μg/g)

Metal Cd	Step 1	Step 2	Step 3	Step 4	Step 5	Residue	Sum	Total
Sample 1	0	0	0	0	0	0	0	1.050
Sample 2	0	0	0	0	0	0	0	0.866
Sample 3	0	0	0	0	0	0	0	1.201
Sample 4	0	0	0	0	0	0	0	1.441
Sample 5	0	0	0	0	0	0	0	1.225
Sample 6	0	0	0	0	0	0	0	1.075

Concentration of Cadmium is too low to detect at any step.

Lead: **Table 11:** Result of sequential metal extraction for Lead; values are concentration given here is in ppm (μg/g)

Metal Pb	Step 1	Step 2	Step 3	Step 4	Step 5	Residue	Sum	Total
Sample 1	3.473	1.726	0	2.522	2.028	11.265	21.016	29.258
Sample 2	2.313	1.873	0	1.518	0	5.849	11.554	13.858
Sample 3	1.809	1.798	0	2.316	0	5.953	11.877	16.601
Sample 4	2.864	4.218	0	2.261	0	6.177	15.521	24.025
Sample 5	2.845	1.408	0	2.370	0	9.938	16.562	23.275

Sample 6	4.129	2.538	0	4.270	3.081	9.886	23.906	37.408

Weekly bound organic matter is again absent in lead and for samples 2, 3, 4, 5 the concentration is very low.

3.3 Total Metal analysis of Fish Samples:-

Whole fish as well as different parts (tissues and organs) of fishes were analysed by ICPMS method for measuring selected heavy metals and metalloids like Cu, Cr, Cd, Hg and As. Except Cu the other metals and metalloid are non essential elements, i.e., they are not required for any biochemical process and infact they are considered to be toxic. These marine fishes are supposed to be a good indicator of heavy metal bioaccumulation in sundarban wetland areas.

The metal concentration in the whole fish could be an indicator of average bioaccumulation. It was noted that the metal concentrations in the whole fish was mostly less than that of the underlying sediment, particularly those of Cr, As and Cd. Strinkingly, the fish *Cyanoglossus sp.* Was found to accumulate very high amount of Cu and Hg as compared to the other fishes. A better indicator of bioaccumulation factors in fishes could be derived from normalization of the metal concentrations in fishes with those of sediments (given in **Table 12**).

Table 12

	Cu	**Cr**	**Cd**	**Hg**	**As**
Sediment	2.32	7.72	0.18	0.23	16.27
SA	1.07	2.4	0.05	0.3	1.92
PD	1.97	3.77	0.07	0.39	1.22
RC	2.22	4.25	0.06	0.36	1.45
CS	13.6	3.8	0.11	0.39	1.15

Concentration (in μg/g) of metals and metalloids in whole fishes collected from sundarban wetland areas, where SA: *Scatophagus argus*; PD: *Protonibae dicanthus*; RC: *Rhinimogil corsula*; CS: *Cyanoglosus sp.*

Table 13:

	Cu	Cr	Cd	Hg	As
SA	0.46	0.31	0.28	1.30	0.12
PD	0.85	0.49	0.39	1.70	0.07
RC	0.96	0.55	0.33	1.57	0.09
CS	5.86	0.49	0.61	1.70	0.07

Enrichment factor or bioaccumulation factor of metals and metalloids (with respect to underlying sediments) in the fishes where SA: *Scatophagus argus*; PD: *Protonibae dicanthus*; RC: *Rhinimogil corsula*; CS: *Cyanoglosus sp.*

It may be noted that for Hg, the the bioaccumulation factors were in the range 1.3 - 1.7, while those of As was very low (0.07-0.12). Ingestion of sediments could be thought to be one of the major sources of heavy metal accumulation in fishes. The levels of Cd and Hg in the fishes were though not very high, but introduction of these fishes in the food chain of human system may cause toxicity due to their transfer from fishes to human circulatory system. However it may be worth investing the accumulation of these heavy metals in to different tissues and organs of human system.

Table 14:

	Cu	Cr	Cd	Hg	As
Sediment	2.32	7.72	0.18	0.23	16.27
Visceral mass	26.67	5.69	0.43	0.45	1.93
Gill	11.86	10.01	0.28	1.75	17.66
Mantle	9.9	5.75	0.23	0.04	12.73
Podium	5.3	4.94	0.2	0.8	1.14
Siphon	5.46	4.2	0.07	0.49	0.89
Abductor	10.96	17.86	2.42	0.36	0.96
Shell	0.7	1.25	0.05	0.05	0.54

Bioaccumulated concentrations of metals and metalloids in various parts of fishes collected from sundarban region. The concentrations of the metals and metalloid in the sediment were also measured by ICPMS

Cu is mostly accumulated in visceral mass, gill, abductor and mantle (above 10 ug/g), amongst which, the highest accumulation was found in visceral mass. Shell

constituted the minimum accumulation of Cu. Maximum Cr and Cd was accumulated in abductor and their concentrations was found to be high in terms of toxicity in food items.

The Cr levels in gill was also high (10 ug/g), while the other tissue parts constituted about 5 ug/g of Cr. The Cd concentration in visceral mass was nearly two folds higher than that of gill, podium, mantle, while the Cd concentration in siphon and shell was negligible. For Hg, maximum concentration was found in gill, followed by podium. These concentrations were high to cause toxicity. The Hg concentrations in other fractions were more or less uniformly distributed at 0.4 - 0.5 ug/g levels.

Interestingly, for As, very high concentration was measured in gill and mantle which were nearly 12-15 folds higher than the rest fractions. Further, normalization of metal concentrations in the tissues with those of sediments, it was found that, Cu levels are highly enriched (above 10) in visceral mass, high (nearly 5) in gill, mantle and abductor, and moderately high (~ 2) in podium and siphon.

On the similar ground, Cr accumulation factor was mostly low (below 0.75) for visceral mass, mantle, podium, siphon, and moderately enriched in gill and abductor. Cd is highly enriched in abductor and moderately enriched in the rest except in siphon which showed low enrichment. Mercury showed high enrichment in gill and moderately high in the rest, except for mantle where the enrichment was very low. The enrichment of As in different tissues were mostly found to be very low, except in gill

The same pattern of distribution of a metal in different samples can be seen in the following graphs. Here sample number should be noted from the section **2.1.3**

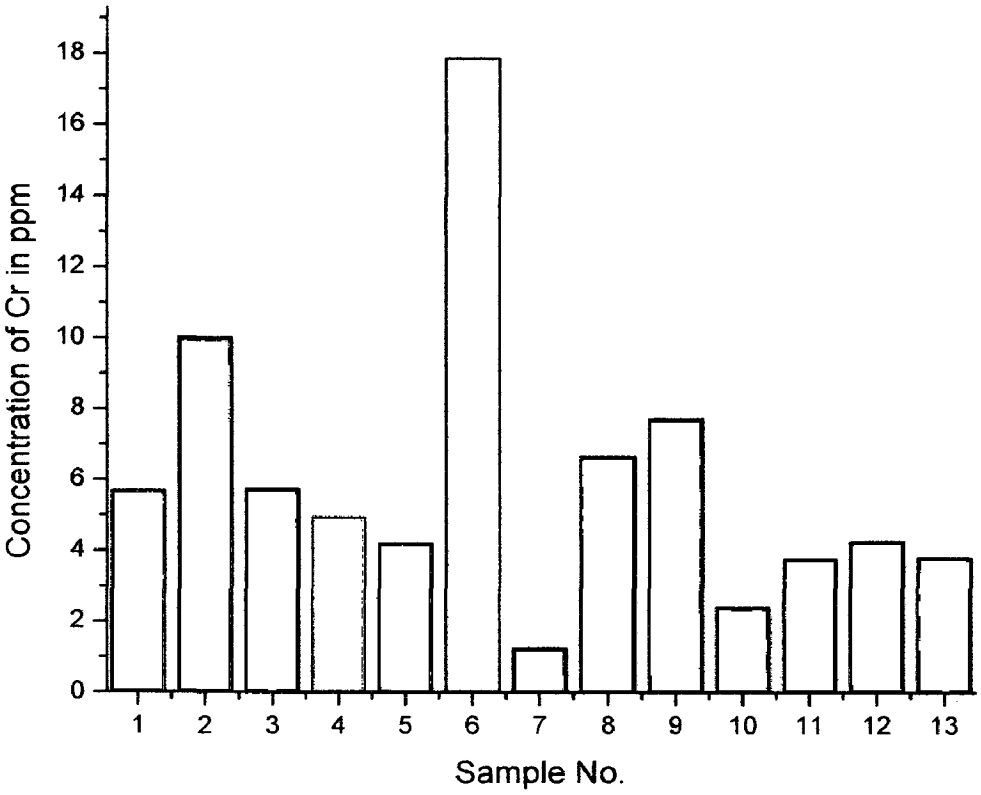

Fig. 27: Concentration of Chromium in different samples of fishes

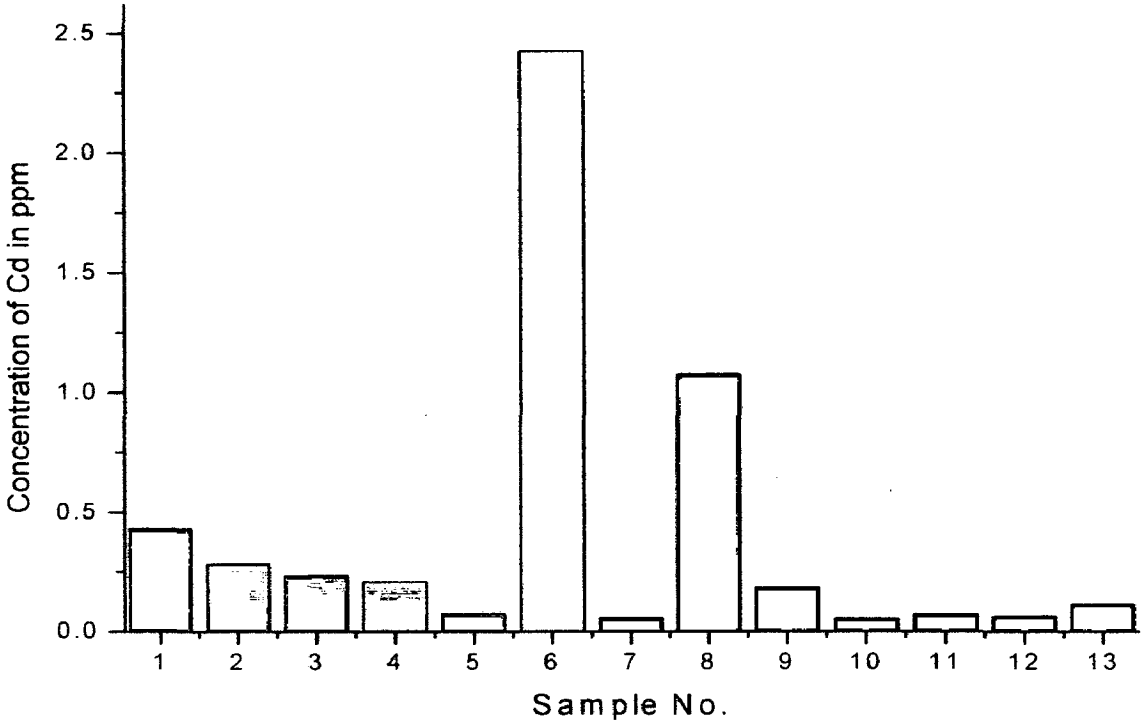

Fig. 28: Concentration of Cadmium in different samples of fishes

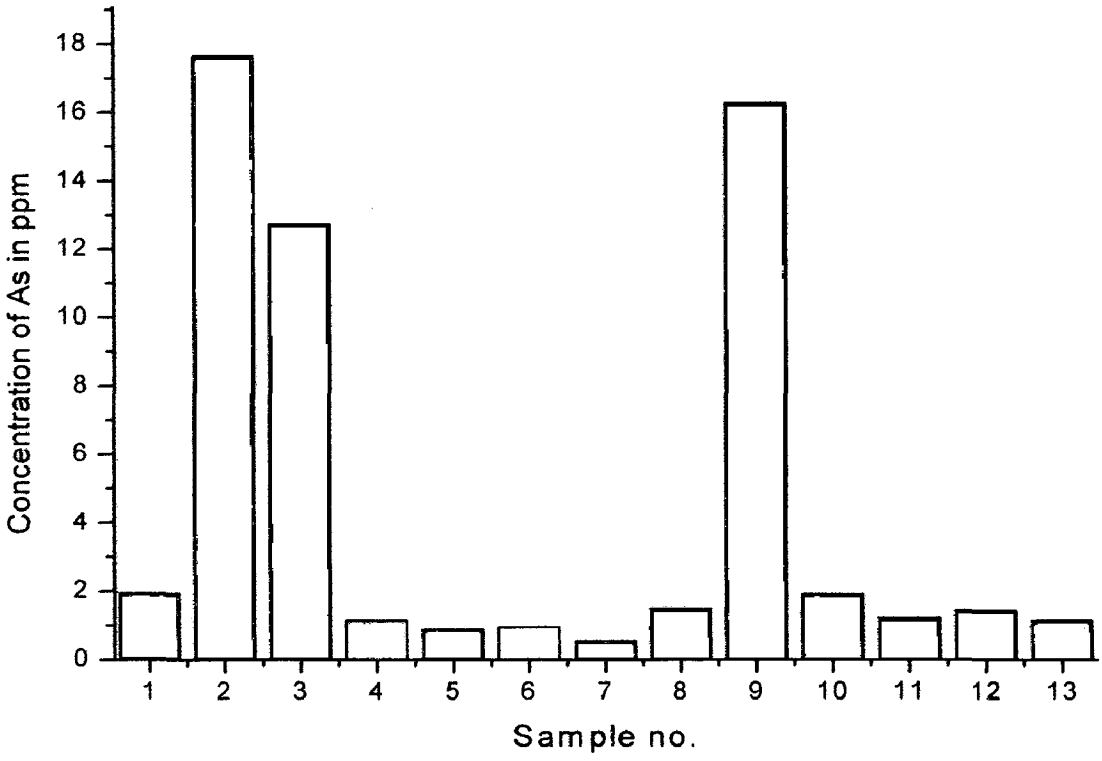

Fig. 29: Concentration of Arsenic in different samples of fishes

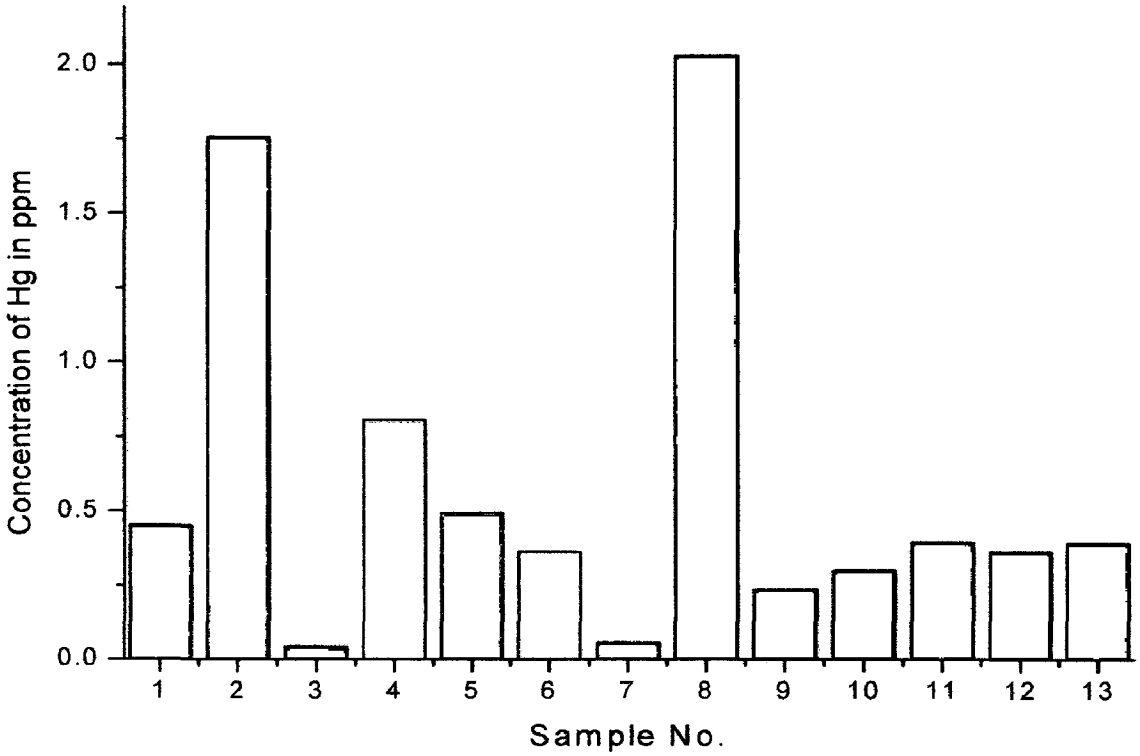

Fig. 30: Concentration of Mercury in different samples of fishes

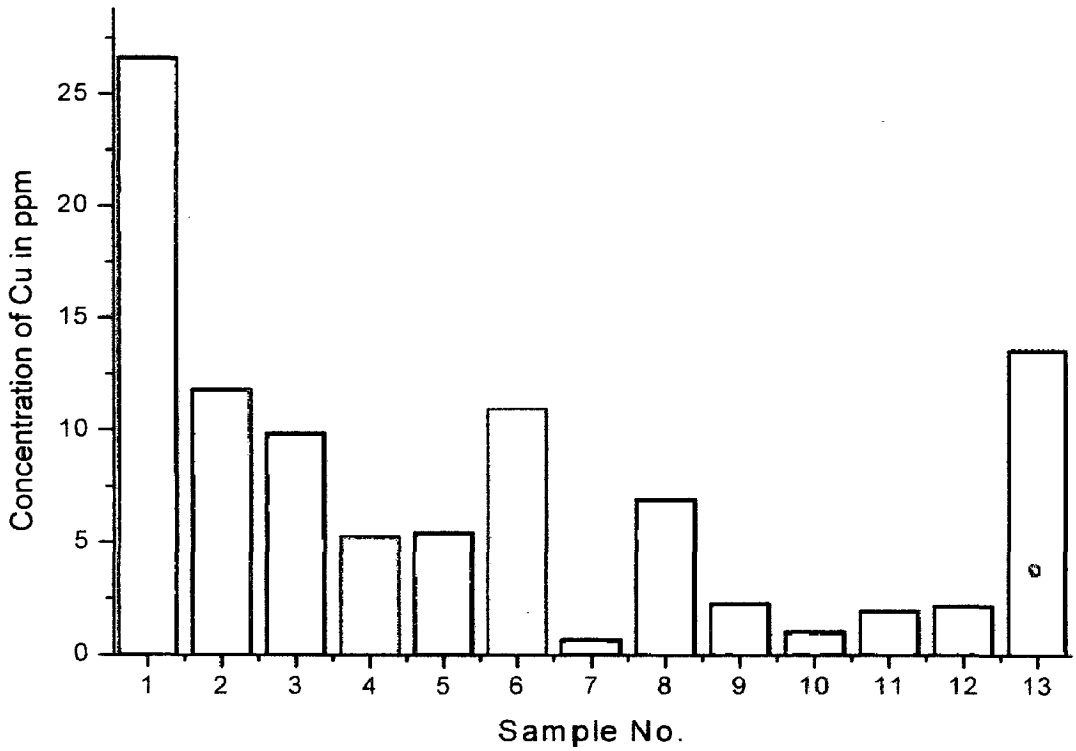

Fig. 31: Concentration of Copper in different samples of fishes

Chapter 4:

Conclusion

CONCLUSION

Collected samples of sediment & fishes are processed for total metal analysis using standard acid digestion procedure than the sample obtained in solution form is analysed by ICP-MS. On the other hand sequential extraction is carried out as per protocol and metal obtained in solution form at each step is analysed by AAS. With the help of results the following can be concluded:-

1. The study is completed successfully and the results obtained from the research work is sufficient to conclude that sediment has high amount of heavy & toxic metals which implies that the area is contaminated.

2. This study revealed the distribution pattern of nine metals in the world's largest mangrove (Sundarban) ecosystem. The metal content in sediment of the entire study stretch are too high to sustain a normal environmental condition in future.

3. In this context it may be mentioned that the environmental behavior of Pb, Co, and Zn clearly exhibited anthropogenic sources. The textural composition and organic matter of freshly deposited sediments play crucial role in the sorption and complexation of transition metals.

4. Though anthropogenic inputs persist but estuarine–marine condition (physical and chemical) in this zone prevents enrichment of metals in the sediment. The outcome of sequential extraction revealed a valuable insight into the geochemical mode of the metal retention.

5. The strong association with residual part and poor association with exchangeable portion for few metals at particular transect indicated low availability and thereby bio-availability or mobility was not in alarming level to biotic community.

6. Whereas, exchangeable fraction of few metals those are easily removed and used by organisms was present. This fraction is potentially toxic for the organisms. Also the

prominent association of few metals with the phases of organic, carbonate and Fe–Mn oxide in particular transects can be solubilized depending upon physical and chemical parameters.

7. This study focused that the variation of percentage of metals in different phases in the sundarban mangrove ecosystem indicated large heterogeneities in the distribution of metals among phases in the freshly deposited sediments and showed the difficulty of performing a geochemical evaluation in complex environments of sundarban.

8. Analysis of different organs of fish provide the idea which toxic metal deposits in which organ and where the essential might be present which provides a very useful data about nutrition value of different organs.

9. As a part of this study, it may be concluded that analysis of total metal as well as speciation of in the sediments and fishes 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.

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