BIOAMPLIFICATION OF SELECTED HEAVY METALS IN AN ECOSYSTEM

A THESIS

submitted in fulfilment of the
requirements for the award of the degree
of
DOCTOR OF PHILOSOPHY

by

ASHESH KUMAR

DEPARTMENT OF BIOSCIENCES AND BIOTECHNOLOGY
UNIVERSITY OF ROORKEE
ROORKEE-247 667 (INDIA)

APRIL, 1993

CANDIDATE'S DECLARATION

I hereby certify that the work which is being presented in the thesis entitled **BIOAMPLIFICATION OF SELECTED HEAVY METALS IN AN ECOSYSTEM** in fulfilment of the requirement for the award of the degree of **Doctor of Philosophy** and submitted in the **Department of Biosciences & Biotechnology** of the University is an authentic record of my own work carried out during a period from March 1989 to April 1993 under the supervision of **Dr. R.P. Mathur.**

The matter presented in this thesis has not been submitted by me for the award of any other degree of this or any other University.

Ashesh Kum

ASHESH KUMAR

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

C. B. Sharma 16.4.93

Professor & Head
Deptt. of Biosciences and
Biotechnology
University of Roorkee
Roorkee

Rmathur 12.4.93

Dr. R.P. Mathur
Professor
Department of Civil Engineering
University of Roorkee
Roorkee-247 667

The Ph.D. Viva-Voce examination of . . . Ashesh kumar . . . . .
Research Scholar, has been held on . . . Aug 23.1993 . . . . . .

Rmathur 23/8.93

Signature of Supervisor(s)

C. B. Sharma 23/8/93

Signature of H.O.D.

Signature of External Examiner

Dedicated to my Evercaring

Grand ma" Ma ji "

and

to my loving brother

ABSTRACT

Bioconcentration of toxic metals and the factors that govern the uptake by organisms in an aquatic system both qualitatively and quantitatively has been a subject of scientific research. Rapid industrialization and concomitant exploitation of nature has resulted in the prevalence of trace metals in natural waters. Aquatic organisms take up heavy metals from their aquatic environment and accumulate them. The incidence of Minimata and Itai-Itai diseases have focussed the attention of scientific community on the dangers of metal pollution, bioconcentration and bioamplification through a food chain.

Bioconcentration of Heavy Metal ions (HMs) is affected by an array of physico-chemical and biological factors. Existing literature reveals that most of the bioconcentration studies have been carried out as monitoring of body burdens of hazardous trace metals. Casual references are also available wherein the factors affecting the uptake and the levels of biomagnification have been worked out. Scientific research is required to evaluate kinetics of uptake and to define site specific levels in tissues and cells, more so for environmentally sensitive metals.

In the present investigation bioconcentration of Cd, Pb, and Zn along with impact of physico-chemical conditions on bioconcentration have been studied in a natural riverine ecosystem, Hindon and Kalinadi. In the past forty years there have been rapid industrialization in the Hindon basin. Most of the industrial wastes generated unfortunately gain access into the river along with domestic wastes without any specific treatment. Though the river system has an important geographical location, there is a lack of data on spatial and temporal variations of

water quality parameters. Six representative stations were, therefore, selected to study the bioconcentration of metals in the biota.

The physico-chemical characteristics of water in river Hindon and Kalinadi provide baseline information on the pollution of riversby various industrial and anthropogenic activities. Concentrations of Cd, Pb, and Zn in water, plankton and fish were highly elevated. Bioconcentration of HMs in plankton and fish was variable with abiotic environment of the stations. An attempt to correlate the bioconcentration of HMs with physico-chemical parameters of water revealed that pH, dissolved organic carbon (DOC), water hardness, alkalinity, and metal content collectively influence the bioconcentration of Cd, Pb and Zn in the aquatic environment.

The approach was extended further and a Water Quality Index (WQI) was formulated. Important determinants ofbioconcentration were employed in the formulation of the index. Weightages were assigned to pH, DOC, total hardness, alkalinity and metal in complexed form. Concomitant decrease in bioconcentration factors (BCFs) of Cd, Pb, and Zn in plankton and fish with an increase in index values corroborates the suitability of the index and define a relationship between pollution levels and BCFs of Cd, Pb, and Zn.

Further, a conceptual model of persistent Cd, Pb, and Zn in different trophic status of the riverine ecosystem was developed. Levels of Cd, Pb, and Zn concentration in water, plankton and fish exemplify bioamplification (higher concentration of HMs at high trophic level) at selected stations for Cd (in winter season), Pb (in postmonsoon season), and in all the observations for Zn. Biodiminution was found to be prominent at selected stations in postmonsoon and summer seasons (for Cd) and in winter season (for Pb).

Laboratory studies show that Cd and Pb bioconcentration is a function of time and sublethal metal concentration in the exposure medium. Measurements of Cd and Pb contained in individual organs expound that bioconcentration of HMs is site specific. High concentrations of Cd in gills and liver and of Pb in gills and muscles havebeen reported. Hourly and daily variations of uptake of Cd and Pb in fish tissue (gills, liver and muscles) have also been worked out. Bioconcentration kinetics of both Cd and Pb was found to comprise of an initial rapid phase and a later slow phase responsible for net accumulation.

An attempt has also been made to scan Cd and Pb in gills, liver, and muscle tissues of fish exposed to sublethal Cd and Pb by histochemical and micro-analytical techniques. Modified Silver Sulphide Technique (SST) was employed to localize the exogenous trace metals. Transmission Electron Microscopy (TEM) of gills, liver and muscle tissues show metal inclusions in gill head and gill lamellae regions. Metal inclusions were also cited in liver and muscle cells. Presence of Cd and Pb was corroborated by Electron Probe X-ray Microanalysis (EPMA) of thin sections of fish tissue.

A gist of conclusions of the present investigation reveals compartmentation and bioamplification of Cd, Pb, and Zn in an aquatic ecosystem. Selected physico-chemical parameters were found to moderate the bioconcentration of Cd, Pb, and Zn. Further, the accumulated metals have been localized, scanned and quantified in fish tissue (at cellular level) using TEM and EPMA.

ACKNOWLEDGEMENTS

I wish to accolade the suggestions and contributions forwarded to this scientific piece of work.

All above them, my gratitude and respect to Dr. R.P. Mathur, Professor & Coordinator, Center of Environmental Engineering, Department of Civil Engineering, University of Roorkee, for his constant encouragement, moral support, unbounded affection, adroitness and perception. I remain indebted to him for his keen interest and perpetual encouragement offered all through the persuance of this work.

I would also like to take the opportunity to thank all the faculty members of the Department of Biosciences & Biotechnology, University of Roorkee, especially Dr. C.B. Sharma, Professor & Head and Dr. B. M. J. Pereira.

Special appreciation is due to Dr. Indu Mehrotra, Reader, Department of Civil Engineering,for her moral support, encouragement and suggestions.

It is great pleasure to acknowledge the help and useful discussions offered by Dr. C. Venkobachar, Professor, and Dr. Leela Iyengar, Scientific Officer, Indian Institute of Technology, Kanpur.

My appreciations are due to the Department of Anatomy, All India Institute of Medical Sciences, New Delhi, for providing facilities for Transmission Electron Microscopy. Help rendered by Dr. G.F.X. David, Associate Professor, is thankfully acknowledged.

Facilities extended by University Scientific Instrumentation Center (USIC), University of Roorkee, especially the help given by Shri Saini in metal analysis, Shri Ashokan in EPMA analysis and Shri Jeol in electron microscopy is thankfully acknowledged.

Contribution of Council of Scientific and Industrial Research, New Delhi, is acknowledged for the financial support in carrying out the research work and critical evaluation of work progress.

Sincere thanks are due to Dr. Nalini Pant, Scientist at NEERI, Nagpur, and Mr. A. B. Pant, Senior Research Fellow at CDRI, Lucknow, for arranging the reprints of research papers at the hour of need.

The completion of this work was not possible without the facilities extended by Center of Environmental Engineering, The cooperation of Sri T. A. Siddiqui and Sri Dinanath is highly appreciated.

I owe to my senior colleagues in the Center of Environmental Engineering, Dr. S.N. Kumar, Dr. Sangeeta Thapar, Dr. Ranu Gadh, and Dr. M. M. Assidi for their erudite suggestions and pleasant association.

Memories of pleasant company with my friend Sanjeev now Scientist in Nuclear Power Corporation, Bombay, would be the lasting asset of my life.

Mere words would not be enough to express my feelings for my friends who supported me with a continued understanding and unending patience. I cherish Vidula, Pramila, Manju, Khurana, Indu (DO), Bharat, and Arvind.

I am also thankful to Mr. A.K. Gaur, Technician, CAD center, for his efforts made in the final formatting and Mr. Vinod Kumar for meticulous typing of the thesis.

I duly acknowledge the help of Sri M. C. Vaish for setting the micrographs and figures with perfection. A special word for Mr. Shakti for the excellent xerox of the thesis.

Last but not the least I am thankful to my sisters Seema, Shalini, Shikha, Richa and Archana for their continuous encouragement.

Ashesh Kumar

(ASHESH KUMAR)

LIST OF PUBLICATIONS

1. Kumar, A. and Mathur, R.P., Bioaccumulation of lead in a laboratory fish, -presented in International Conference on Environmental Planning and Management(ICOEM-90), organised by the Department of CivilEngineering, University of Roorkee, India (1990).

2. Kumar, A. and Mathur, R.P., Bioaccumulation kinetics and organ distribution of lead in a fresh water teleost, Colisa fasciatus, Environ. Technol., 12, 731-735 (1991).

3. Mathur, R.P. and Kumar, A., In situ compartmentation and biomagnification of cadmium and zinc, Hindon river basin, India,-presented in International Conference on Environ Metrics organised by Civil Engineering Department, University of Wisconsin, Madison, USA (1992).

4. Kumar, A. and Mathur, R.P., Quantification and speciation profile of cadmium and zinc in natural waters,-presented in International Conference on Environ Metrics organised by Civil Engineering Department, University of Wisconsin, Madison, USA (1992).

5. Kumar, A. and Mathur, R.P., Bioaccumulation of nickel and chromium in plankton and fish from a riverine ecosystem, -presented in International Conference on Environmental Planning and Management(ICOEM-92), organised by the Department of Civil Engineering, Tarbiat Modarees University, Tehran, Iran (1992).

CONTENTS

LIST OF TABLES

LIST OF EPMA

LIST OF FIGURES

LIST OF ELECTRON MICROGRAPHS

CHAPTER 1

GENERAL INTRODUCTION AND HISTORICAL PERSPECTIVE

1.1 PRELIMINARY

It looks like a sparkling blue and white jewel . . . . . Laced with slowly swirling veils of white . . . . . Like a small pearl in a thick black sea of mystery,"

Edgar Mitchell radioed back effusively to Houston, when he flew to the moon onboard Apollo 14, wayback in 1971. Twenty years later, a dull dirty earth filled with dark, swirling clouds of carbon and sulphur oxides and the filth in the water might have greeted him. A rapid pace of industrialization, coupled with uncontrolled exploitation of nature, has caused dumping of industrial by-products, hazardous chemicals and nuclear wastes, deforestation, and the pollution of river basins, lakes and seas in the recent past. In his quest for wealth and comforts, man has ignored the laws of nature and thus disturbed the natural cycle. In nature, each living organism has its own environment which is affected

by the changes in the natural cycle. Thus, anything released into the environment which degrades it can be expressed in its real terms as "matter in the wrong place or environmental pollution". The pollutants adversely alter the environment by changing the growth rate of species, interfere with the food chain and affect health, comforts, amenities or property values of the people. Pollutants are mostly introduced into the environment in significant amounts in the form of sewage, accidental discharge, as by-products of manufacturing processes or by other human activities.

1.2 ENVIRONMENTAL POLLUTION: AN OVERVIEW OF INDIAN STATE

The environmental pollution problems of India are massive. The country has a fast growing population, nearly 40% of which depends upon natures gift of land, water and forests for food, fuel and shelter. They put tremendous pressure on the natural resources. The general indifference of the industrial sector on the aspects of environmental safety, the low environmental literacy, a gross underevaluation of the economic and ecological aspects of biological diversity are some of the other root causes.

Further, India has several highly polluting industries such as sugar, pesticides, drugs, dyes and metal plating. Although, the problems related to environmental pollution were realised nearly a century ago, real awakening started in mid 1960s. As by now many countries have consolidated and defined previous legislations and introduced new laws which endeavour to deal with the environment as a whole. India is also trying to adopt waste and pollution control measures. The country has formulated a regulatory framework for controlling pollution with the enactment of Water (Prevention and Control of Pollution) Act 1974, The Water (Prevention and Control of Pollution) Cess Act 1977 (Central Board for the Prevention and the Control of Water Pollution, 1980). For the prevention and control of water pollution, the Bureau of Industrial Standards (BIS) has prescribed standards for the discharge of effluents. Similar guidelines have also been issued by Central and State Pollution Control Boards. In addition, BIS has

put up warning levels on products containing harmful ingredients and award ECO mark on environmentally safer products. Despite a plethora of environmental laws, it has been realised that there is a need to widen the range of regulatory instruments and supplement them with economic instruments (Ravi, 1992).

Water is a unique chemical essential for our survival. Its pollution is a major problem. In India 70 percent of the water sources are polluted by human and industrial wastes. A recent survey of the environment in 1992 revealed that all the 14 major river systems in India have become giant sewers for the country's urban population. The river systems of the country account for nearly 80 percent of the population in their river basins. Barely 7 percent of the 3,000 odd towns and cities in the country treat their domestic sewage. The rest dump them either into the river or directly into the seas. Of the over 4,000 water polluting large industrial units, only half have installed pollution control equipments. However, more than a million small units have not even begun to think in terms of controlling pollution (Purie, 1992).

1.3 METAL POLLUTION IN AQUATIC SYSTEMS

A wide spectrum of natural and anthropogenic sources release hazardous toxic metals into the environment. Natural water ultimately receives the wastes generated by industrial, agricultural and domestic activities either by point source dumping or indirectly by rain, by surface water run off and ground water leaching. In the environment, heavy metals are not removed by natural processes of decomposition. The fate of heavy metals thus, assumes great significance. Ecosystems, to varying degrees, are adaptable and wastes may be assimilated without serious implications to endemic biota. However, excessive uncontrolled release of toxic metals is unacceptable mainly because of inevitable environmental danger and their potential threat to human health. When levels of HMs reach in excess of the assimilative capacity of receiving waters, they affect the survival, reproduction, growth and movement of organisms.

Our present knowledge of the behaviour of toxic heavy metals and their effect on aquatic communities is not sufficient and therefore, the qualitative and quantitative evaluation of the response is fraught with difficulties.

Fig 1.1 presents a diagramatic presentation of possible ways by which the metals are introduced in the aquatic ecosystems (Forstner and Wittmann, 1983 and the references cited therein). The earth's crust contains over sixty metal elements. Weathering releases these metals into the rivers. Traces of some of these HMs are essential to plant and animal life. It is only during the past few hundred years that man has significantly altered the concentration of these metals in the environment by excessive mining, burning coal and other fossil fuels, by the discharge of various industrial wastes containing metals, by dumping solid wastes containing metal salts and by the use of certain unwanted chemicals in the aquatic ecosystems.

1.4 ECOTOXICOLOGY

Ecosystems are environmental units comprising of the living organisms and the abiotic components in a given volume of space (habitat) and basic structure (Odum, 1972). The transfer of food energy from the source in plants through a series of organisms with repeated stages of eating and being eaten is known as a food chain. In an heirarchial system man sits at the top of the food pyramid. Harm done to any part of the system by toxic wastes have repercussions on human welfare. Human activities alter the global and regional cycles of trace elements. The world-wide quantitative assessment of air, water, and soil indicates that contamination of fresh water resources and accumulation of toxic metals in the food chain are accelerating (McKinney and Rogers, 1992).

Wayback in 1968, an International Conference on Environment (Stockholm Conference) was organised by the United Nations. Most of the present day International environmental activities began with the preparations of the conference. International Council of Scientific Unions (ICSU) formed a Scientific Committee on Problems

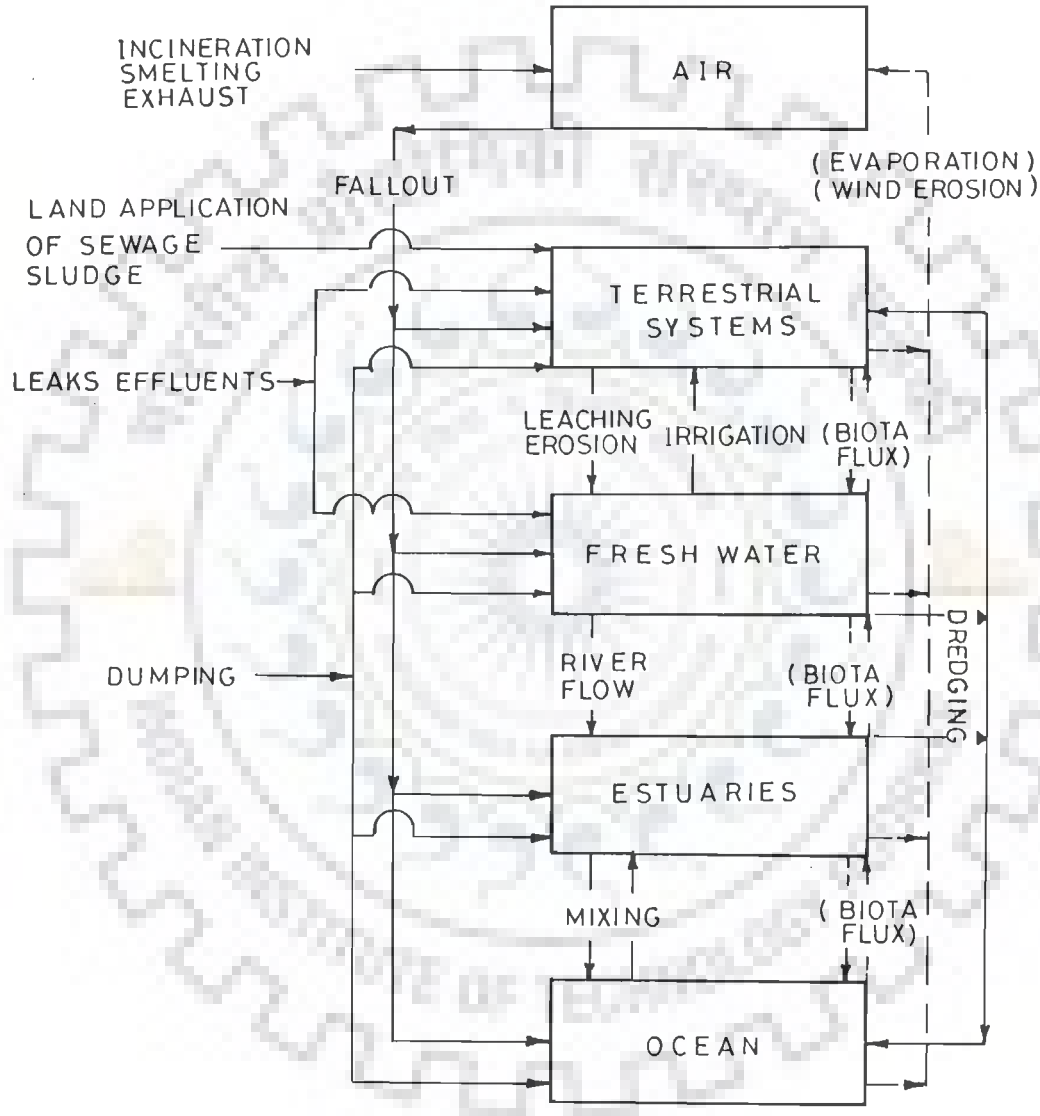


Fig 1.1 Routes of Metal Pollutants in the Environment

of the Environment (SCOPE). SCOPE project- 4 was on Ecotoxicology. It was concerned with the toxic effects of chemical and physical agents on living organisms especially on populations and communities within defined ecosystems (Butler, 1978).

Concern over metal pollution was brought into sharp focus when dramatic intricacies of metal pollution were expounded in Japan with a series of incidents involving fatal poisoning of human beings with mercury and cadmium (Kobayshi, 1971; Takeuchi, 1972). Wastes containing Hg salts polluted the shores of Minimata Bay and the consumption of contaminated fish and shell fish caused popularly known Minimata disease. Further, effluent from a zinc mine rich in cadmium resulted in a painful skeletal deformity (Itai-Itai disease) in villages on the Jintsu river (Kobayshi, 1971). Substantial problems were also recognised in Sweden (Jernelov et al., 1975) and in United States (Carter, 1977) where the aquatic ecosystems received metal discharges. These ailments focussed the dangers of metal pollution, bioconcentration and biomagnification through the food chain.

Scientific research continued since then and even today approximately 20 metal contaminants are under study or review by various United States Environmental Protection Agency (USEPA) programs for their increased human exposure and health risk. However, the details about the origin, mechanism and route of exposure of these metals and the risks they pose, remain sketchy (McKinney and Rogers, 1992). To address these data gaps and pertinent research needs, a two day workshop was held in July, 1990 at USEPA's Environmental Research Center (Research Triangle Park, NC). It focussed on issues of bioavailability and disposal kinetics of trace metals including Cd, Pb, and Zn. The major topic was the availability of metal specific input parameters and organ tissue partitioning properties and the need to develop physiologically based pharmaco - kinetic models for assessing risk from exposure to metal compounds (McKinney and Rogers, 1992).

1.5 POLLUTION AND TOXICITY OF CADMIUM, LEAD, AND ZINC

Metals, in general, whether they are toxic heavy metals or essential, when administered in excess act on the living system through their ligand binding property, thereby, interacting with either simple molecules like vitamins, coenzymes, amino acids and other metabolites or macromolecules like proteins and nucleic acids.

Today, the pollution and toxicity of Cd, Pb, and Zn is a topic of concern. Cd among them is a relatively rare element and constitutes less than 0.5 mg/kg of earth's crust (Nriagu, 1980a). It is being increasingly used in industries and culminating a sharp increase in environmental contamination. Cd is known to be acutely and chronically toxic to plants and animals (Giesy and Weiner, 1977). It has a high affinity for sulpha-hydryl groups leading to increased lipid solubility and toxicity. Binding of Cd to such groups in enzymes effect various biochemical and physiological functions (Moore and Ramamoorthy, 1984). Cd has also been implicated as a possible carcinogen and mutagen. Its exposure is being correlated with cardiovascular diseases, renal dysfunction and hypertension (Perry et al., 1976).

Lead is another environmental menace. It gets mobilized from earth's crust by natural weathering. Anthropogenic sources of contamination are mainly by its uses in storage batteries, metal products, chemicals and pigments. Alkyl lead is used on a large scale as an antiknock agent in petrol. The main routes of the entry of lead into the environment and its transfer between different environmental compartments are illustrated in Fig 1.2.

Exposure of Pb results in adverse effects on animals and human body. It is a non-essential trace element and no biological function has yet been ascribed to it. Its biochemistry, metabolism and toxicity has been studied extensively as it has been under intensive toxicological research during past decades. Pb is a neurotoxin and results in irritability, lassitude and impaired functioning of the central nervous system

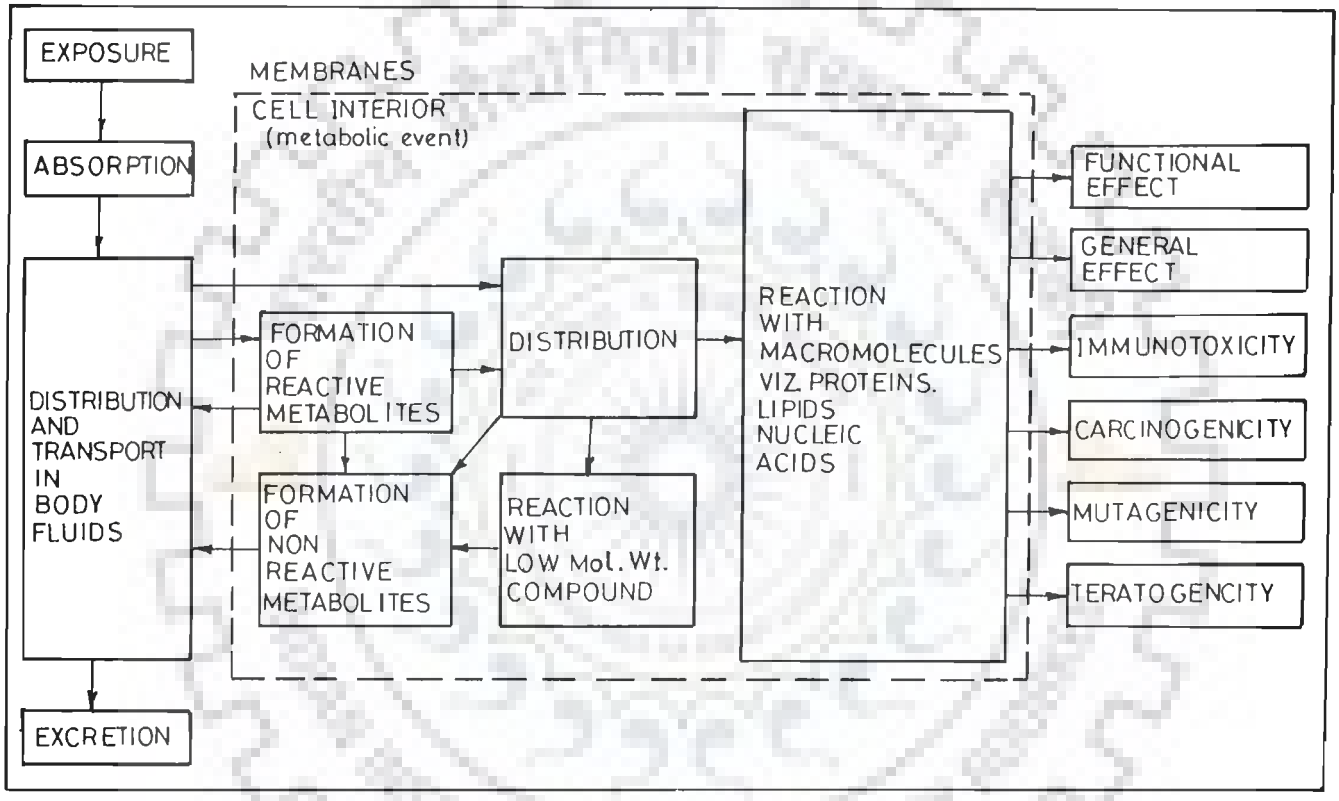


Fig 1.2 Interaction of Toxicants with Biological Systems

(Jaworski, 1979). In fish, chronic exposure produces a characteristic response of black finning and spinal curvature (Davies et al., 1976).

Zn is an essential element. More than twenty different Zn metallo-enzymes have been identified so far and include carbonic anhydrase, alkaline phosphatase and alcohol dehydrogenase. Zn plays a vital role in the biosynthesis of nucleic acids, RNA polymerase and DNA polymerases. Zn is also involved in the healing processes of tissues in the body and in a number of other physiological processes including hormone metabolism, immune response and stabilization of ribosomes and membranes (Moore and Ramamoorthy, 1984).

Despite being an essential element toxicity of Zn arises from its synergistic or antagonistic interaction with other heavy metals, particularly its homologue Cd (Moore and Ramamoorthy, 1984). In fish, treatment with Zn results in substantial gill damage. Changes result in decrease of oxygen consumption and ion transport ability across the gill, increase in hypoxia, opercular amplitude and ventilation frequency. Other physical and biochemical changes include increase in the product of lactic acid and pyruvic acid thereby, decreasing blood pH and dysfunction of kidney tissue (Nriagu, 1980b).

To epitomise it could be stated that hazards associated with exposure to metals vary. Cd, Pb, and Zn are among the metals of great concern regarding their bioavailabilities and fate in the aquatic environment (Mckinney and Rogers, 1992). It has been recognised by USEPA that bioavailabilities of the complexes and their dispersion kinetics in biological systems differ markedly from those of uncomplexed metals and this alone presents a major problem in the determination of the toxico-kinetic properties of environmentally relevant forms of metals. The bioavailability studies which attempt to define the factors that control uptake of a metal by the body qualitatively and quantitatively has become an important scientific research issue (Mckinney and Rogers, 1992).

1.6 BIOLOGICAL DEFENCE MECHANISMS

Organisms have some defence mechanisms to combat the environmental stress of the toxic contaminants (Chatterjee, 1988) (Fig 1.3). These defence mechanisms may be

- physical or anatomic (primarily function as barriers to the entry of toxic metal ions into the body or a tissue/cell),
- physiological (the prevention of absorption of HMs with additional capacity of specific agents to a variety of metal ions), and
- biochemical (act at the cellular level to prevent or repair dysfunctions caused by Cd, Pb, and Zn).

Efforts have also been made to understand the biochemical basis of resistance to the metal toxicity in biological organisms. Several strategies for resistance to metal ion have been identified (Wood and Wang, 1983) and these include:

- the development of energy driven efflux pumps that keep toxic elements low in the interior of the cell,
- the oxidation (AsO_3^{--} to AsO_4^{---}) or reduction (Hg^{++} to Hg^0) which enzymati cally and intracellularly convert a toxic form of an element to a less toxic form,
- the biosynthesis of intracellular polymers that serve as traps for the removal of metal ions from solution. Such traps have been described for Cd,Ca,Ni, and Cu,
- the binding of metal ions to cell surfaces,
- biomethylation and transport through cell membranes by diffusion controlled processes, and
- precipitation of insoluble metal complexes (e.g., metal sulfides and metal oxides).

Williams (1983) have recognised the factors important to the transport and partitioning of metals and have stated that excessive element uptake could result

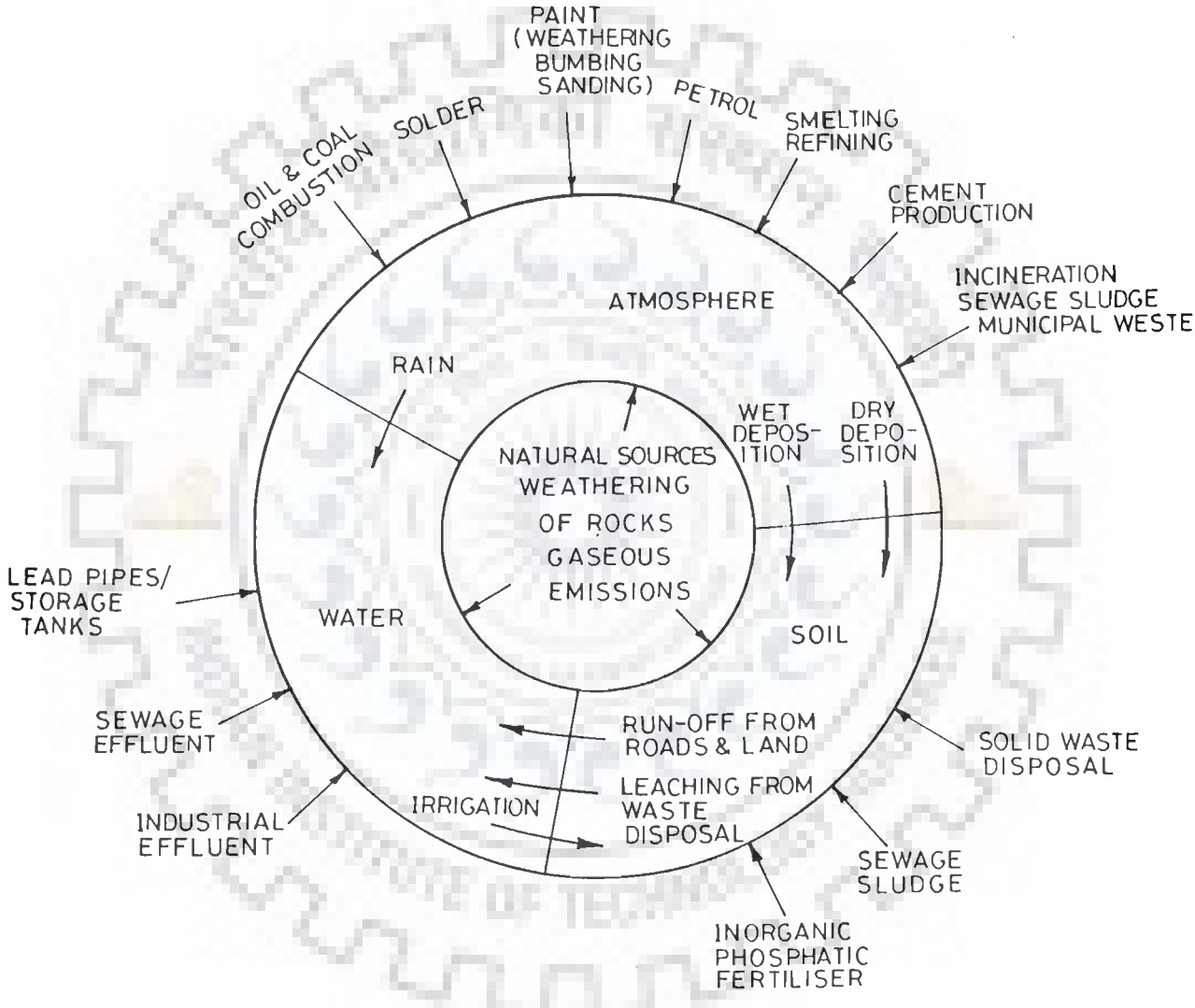


Fig 1.3 Pathways of Lead in the Environment

with the reversal of :

- excessive competition from other metal ions with similar chemical properties (Ca^{++} and Cd^{++}),
- inadequate synthesis of carrier molecules by the cell,
- low availability, and
- excessive excretion of metal ions by the cell or failure of the energy driven uptake system.

Precipitation of insoluble metal complexes have been reported to occur through the activities of membrane associated sulfate reductases (Seigal, 1983) or through the biosynthesis of oxidising agents such as H_2O_2. The reduction of sulfate to sulfide and the diffusion of H_2O_2 provide highly reactive means by which metals can be complexed or precipitated (Wood and Wang, 1983). Metals thus, toxic otherwise are rendered innocuous and result in an intracellular deposition and accumulation. The specific mechanisms by which the metals are taken up are essentially unknown but in many cases these uptake in unicellular organisms have been shown to be genetically controlled (Nakajima et al.,1981).

1.7 BIOCONCENTRATION OF HEAVY METAL IONS AND THEIR TROPHIC TRANSFER

Considerable advances have been made in recent years in the understanding of the phenomenon of bioaccumulation and a major body of information is available. In a number of cases the increased concentration of contaminants has been reported and cited as evidence for bioconcentration (Spehar et al., 1978; Vinikour et al., 1980; Crist et al., 1981; Harding and Whitton, 1981; Hardy et al., 1984; Travis and Arms, 1988; Timmermans et al., 1989)

Important studies reported on the metal burdens of biota in Indian rivers include

a study on Yamuna river, a major riverine system of Northern India. Ajmal et al. (1985) reported the distribution of Cd, Pb, and Zn in plants and fish of Yamuna river from Delhi to Allahabad, India. The results have shown wide variations in the heavy metal levels from one sampling station to the other.

In other significant studies, reported so far, Phillips (1977; 1978) found higher concentrations of Cd, Pb, Zn, and Fe in Mytilus edulis. Hardy et al. (1984) studied the trophic transfer of Cd in a phytoplankton - oyster-mouse food chain. They reported that the phytoplankton grown in a continuous system containing $CdCl_2$ and the isotope ^{109}Cd, accumulate 70% of the total supplied cadmium. Further, it was observed that about 59% of the accumulated Cd by oysters come from the phytoplankton food source and 41% from Cd dissolved in water. The step wise transfer of Cd concentration with increasing trophic level has also been observed in an agricultural food chain by Brams et al. (1989). They observed that low level contamination of a sandy soil with Cd and Pb at 0.1 to 9.0 and 3.0 to 54.0 mg/kg soil induced a significant toxicant accumulation in Sorghum sudanense (0.5 - 5.0 and 0.2 - 1.5 mg/kg dry biomass of Cd and Pb, respectively). Consumption of hay resulted in a significant but relatively diminutive accumulation of Cd and Pb in Capra hirus. Fresh tissues accumulated 0.028 - 0.002 and 0.090 - 0.017 mg/kg Cd and Pb, respectively. The assessment of food chain biomagnification by relatively low level concentrations of Cd and Pb had shown that toxicants move through the food chain eliciting positive and significant responses in top biomass tissues (Brams et al., 1989).

1.8 FACTORS INFLUENCING BIOCONCENTRATION

In the natural environment, an array of physico-chemical and biological factors moderate the phenomenon of bioconcentration. A review by Forstner and Wittmann (1983) of the physico-chemical factors determining metal accumulation by living organisms

in aquatic ecosystems include, water hardness, pH value, salinity, organic metal ion interaction and complexation. They categorically defined that it is the physico-chemical environment that deserves closer examination if the mechanisms governing the accumulation of metals by aquatic biota are to be understood. Studies on interrelations between metal uptake in natural aquatic ecosystems with various chemical parameters thus, are of interest in determining only in empirical sense, factors that can be correlated with bioaccumulation of individual trace metals.

One of the most important factors that determines the biological availability of a metal in a system is its binding to other environmental constituents. If a metal is wholly or partially removed by its binding, a decrease in or complete disappearance of bioconcentration may result. Presence of complexing agents usually result in a decrease in accumulation although it is not always the case (Poldoski, 1979).

In soil, metals can be bound strongly by organic chemicals such as humic and fulvic acids. Humic acids are especially important and it has been stated that practically every aspect of chemistry of heavy metals in soils, sediments and natural waters is related in some way to the formation of complexes with humic substances (Benes et al., 1976).

pH is another important factor and has a considerable effect on the availability of metals and thus, on bioconcentration in a given environment. In general, with acid pH, metals exist as free ionic cations but with an alkaline pH, the ionic cations precipitate as insoluble hydroxides or oxides. Thus, low pH generally increases the availability of metal ions whereas, high pH decreases it. A reduction in pH leads to greater uptake of metals (Hart and Scaife, 1977). However, metal binding at cell surfaces is recognised to be dependent on the availability of unprotonated membrane sites. Thus, the prediction that low pH will be correlated with higher body burdens may not be appropriate for all elements or organisms (Sakaguchi et al., 1979).

Water hardness has also been reported to have a considerable effect on biocon-

centration. In general, increase in water hardness decreases the uptake of metal ions (Kinkade and Erdman, 1975). Especially for Cd, inverse relationship between its uptake and the level of Ca present in water have been reported in plankton (Sakaguchi et al., 1979) and fish (Michibata, 1981). The biological activity of HMs is markedly affected by the presence of other ions (as Mg and Ca) (Wright, 1977). Anions are also able to reduce bioconcentration by precipitation of soluble metal in water. Phosphates, thiosulfates, carbonates and biocarbonates form precipitate with heavy metals and render the HMs less bioavailable depending on their concentration and the pH.

Existing literature show that most studies dealing with metals in natural waters tend to concentrate on total metal concentration with relatively little attention being paid to the effect of metal forms on bioconcentration. This is not surprising and is probably due to the uncertainity of the validity of sensitive and specific analytical techniques applicable to complex system. In recent years, there is an increasing awareness that the environmental impact of heavy metals depends to a significant extent on their physico-chemical forms (Florence and Batley, 1980). Total metal concentration indicates the extent of contamination but gives little insight about their potential bioavailabilities (Sterritt and Lester, 1980; Laxen and Harrison, 1981).

Data concerning other environmental factors affecting bioconcentration and the kinetics of uptake of HMs in aquatic ecosystems is rather fragmentary. An increase in temperature, generally, results in greater uptake in many organisms (Hart and Scaife, 1977). Bioaccumulation of Cd have been reported to have increased with increasing temperature because of increased metabolic activity (Nakajima et al., 1981). However, there are several reports that show no significant change in either concentration or uptake with increase in temperature (Jakim et al., 1977).

1.9 LOCALIZATION AND X-RAY MICROANALYSIS OF METALS

Scattering, absorption and emission studies (broadly classified as structural, dynamic, energetic and analytical informations) provide information on the intracellular traps of metal ions, their concentrations at tissue and cell level, surface bioconcentration and cellular localization. The best techniques for determining structure or the coordinates of a biological system are microscopy and X-ray micro analysis (Erasmus, 1978; Hayat, 1980). Light microscopy gives structural information directly and non invasively. The limitation of the approach is that the resolution is not sufficient to study cellular inclusions as well as their surface adsorption sites. Electron microscopy can achieve higher resolution and is a powerful tool for the study of ultra structures because it combines higher resolution with high magnification (Gabriel, 1982).

Literature on methods of localization of HMs addressed studies in recent years that corroborate presence of HMs at cellular level. Techniques for combined Transmission Electron Microscopy (TEM) and X-ray microanalysis now allow improved qualitative and quantitative analyses of the intracellular distribution of metals (Timm, 1962; Danscher, 1981c; Danscher, 1982; Prosi, 1983; Linton et al., 1985; Lormee et al., 1989; Marigomez et al., 1990).

In recent years histological techniques have become increasingly sophisticated incoporating a whole variety of specialities with corresponding advances in histology. The most appealing aspect of light and electron microscopy is their visual resolution of cellular morphology. Localization of cations including heavy metal ions and other molecules in biological specimens have been achieved with unique specificity involving autoradiography (Seidman et al., 1986), fluorescent techniques (Beesley, 1989) and autometallography (Danscher, 1981a; 1982; 1991).

Autoradiography has been widely used to detect the uptake of organic molecules

in biological specimens. However, it is still plaqued with some technical difficulties since the days of its inception (Rogers, 1967). The development in coating techniques for light and electron autoradiography are still under way (Kornhauser et al., 1992). Auto-metallography has been reported as a specific method for the demonstration of trace amounts of selected heavy metals in biological specimens at the ultrastructural level. The method has been proved to be a powerful tool for the demonstration of metals in different tissues with a wider range of possible applications in experimental biology and pathology (Danscher and Norgaard, 1985).

Electron Probe X-ray Microanalysis (EPMA) is another powerful technique to characterize the specific sites in organisms regarding their chemical composition, nature and concentration of toxicants present (Erasmus, 1978; Hayat, 1980). Low dose electron microscopy of metal loaded samples reveal depositions of HMs, if present on the surface or within the cytoplasm, when EPMA is employed. It deciphers the distinctive binding sites that could be asymmetrically arranged in outer membranes. X-ray dispersive analysis have thus, been used to determine incipient concentration of toxic metals after exposure of organisms to metal ions (Rachlin et al., 1984). Linton et al. (1985) compared laser and ion microprobe detection sensitivity, especially for Pb, in biological microanalysis and referred that microprobe detection is a very competetive technique available for Pb detection. Prosi (1983) investigated the pathways of metal uptake and their final storage for Cd, Pb, and Cu in limnic isopods. He reported identification of Cd and Pb at subcellular level using X-ray microanalysis and histochemical methods. Cd peaks were detected by electron microscopy and microanalysis as electron dense deposits consisting of cadmium-oxine complexes in endothelial cells of cornea of pregnant rats (Yoshizuka et al., 1990). Further, various tissues of common winkles (Littorina littorea) experimentally exposed to cadmium chloride were examined using light and electron microscopy and their elemental composition was detected by X-ray microanaly-

sis and histochemistry (Marigomez et al., 1990). They reported traces of Cd in the cytoplasm of excretory cells. X-ray microanalysis revealed that concretions of basophilic cells were the sites for Cd inclusions. Low amounts of Cd were also found in the granules of epithelial mantle cells rich in sulfur (Marigomez et al., 1990).

In another important X-ray microanalytical study, Vandeputte et al. (1990) studied Pb distribution in collagen in Pb induced soft tissue calcification. They reported Ca and Pb in the electron dense collagen bundles.

1.10 PERSPECTIVE OF PRESENT INVESTIGATION

In the present investigation bioconcentration of Cd, Pb, and Zn along with the impacts of physico-chemical conditions on bioconcentration have been studied in a riverine ecosystem. In the past forty years there has been rapid industrialization in the Hindon basin. The catchment area of Hindon river basin covers a large population of the Northern region of India. In the basin, most of the industrial wastes generated, unfortunately gain access into the river along with domestic wastes without any specific treatment. Though, the river system has an important geographical location, there is lack of data on spatial and temporal variations of water quality parameters. Six representative stations were, therefore, selected to study the physico-chemical parameters and the bioconcentration of Cd, Pb, and Zn in biota. The impacts of physico-chemical environment on bioconcentration (in four seasons of the year) have been evaluated.

Further, to understand the bioconcentration kinetics of Cd and Pb, a common fresh water teleost, Colisa fasciatus was selected for laboratory studies. Site specific bioconcentation was evaluated at tissue and cell level.

For the convenience and clarity of presentation the subject matter of the thesis has been presented in the following chapters :

1. **Introduction and historical perspective**
2. **Experimental methodology**
3. **Bioamplification of cadmium, lead, and zinc in riverine ecosystem**
4. **Bioconcentration of cadmium and lead in fish tissue**
5. **Transmission electron microscopy and X-ray microanalysis**
6. **Conclusions**

Chapter 1 presents the background of environmental pollution with special reference to metal pollution in the riverine ecosystem. The bioconcentration potential of Cd, Pb, and Zn and their possible bioamplification in a food chain is highlighted. The pertinent literature on bioconcentration of HMs and the factors influencing bioconcentration, bioavailability including the physico-chemical characteristics of water and metal speciation is collated and presented. Light and electron microscopic approach for the localization of HMs in animal tissues as well as the quantitation of Cd,Pb, and Zn by electron probe X-Ray microanalysis has been discussed. Based on available information the objectives of the work embodied in the thesis have been identified.

Chapter 2 gives the details of methodology and instruments used. Metal analysis was carried out using Inductively Coupled Plasma - Atomic Emission Spectrometer (PLASMALAB, 8440). The ultra- thin sections (70-80 nm) of gills, liver and muscles of Colisa fasciatus were prepared using ultra- microtome (Reichert Jung, Ultra- cut E). Electron microscopic studies were carried out on Transmission Electron Microscope (Philips, CM 10) and the site specific X-ray microanalysis of thin sections of fish tissue were performed on Electron Probe X-Ray Microanalyser (Jeol, JXA 8600 M).

Chapter 3 contains the results of the physico-chemical characteristics of water

in the river Hindon and Kalinadi alongwith the description of the topography, lithology, climatic features, industrial activities and land use. The results presented provide baseline information on the pollution of river by various industrial and anthropogenic activities. Metal concentrations in water and Al/metal ratio in sediments show the state of pollution in the study area. Concentrations of Cd, Pb, and Zn were evaluated in the ambient environment, plankton and whole body fish tissue at representative stations to determine biomagnification levels in the food chain. Plankton and fish show highly elevated concentrations of Cd, Pb, and Zn as compared to the ambient environment. Based on the information bioconcentration in the different compartments of the aquatic ecosystem has been worked out.

The studies conducted reveal bioamplification (higher concentrations of HMs at higher trophic level fish tissue) for Cd (in winters), Pb (in postmonsoon season) and in all the four seasons of the year for Zn . However, biodiminution was found to be prominent at selected sites in postmonsoon and summers (for Cd) and in winters (for Pb). Bioconcentration of HMs in plankton and fish was variable with physico-chemical environment affecting the process. An attempt to cerrelate the bioconcentration of HMs with physico- chemical parameters of water exemplifies that the correlation coefficients of pH, DOC, water hardness, alkalinity with bioconcentration are highly variable (negative and positive) for different metals (Cd,Pb, and Zn) and trophic levels (phytoplankton, zooplankton and fish tissue). A multivariate analysis of the chemical analysis data show that physico-chemical parameters collectively influence the phenomenon of bioconcentration in the natural environment.

The approach was extended and a Water Quality Index (WQI) was formulated. Important determinants of bioconcentration were employed in the formulation of the index. Weightages were assigned to pH, DOC, total hardness, alkalinity and metal in complexed form. Concomitant decrease in Bioconcentration Factors (BCFs) of Cd,

Pb, and Zn in plankton and fish with an increase in index values corroborates the suitability of the index and define a relationship between pollution levels and BCFs.

Chapter 4 contains the results of laboratory studies on Cd and Pb bioconcentration kinetics in Colisa fasciatus. Fish were exposed to test water with Cd (0.20, 0.50, and 1.00 mg/l) and Pb (1.00, 2.00 and 5.00 mg/l) in laboratory aquaria. Variability of Cd and Pb in fish tissue (gills, liver and muscles) was evaluated as a function of time and concentration of HMs in the exposure medium. Measurements of Cd and Pb contained in individual organs expound that the phenomenon of bioconcentration of HMs is site specific and the metal toxicants are accumulated at particular sites. High concentrations of Cd in gills and liver and of Pb in gills and muscles is reported. Bioconcentration kinetics of both Cd and Pb comprise an initial rapid phase and a later slow phase responsible for net accumulation in fish tissue.

Chapter 5 contains the details of electron microscopic studies and electron probe X-Ray microanalysis of specific sites for the localization and quantitation of Cd and Pb in gills, liver and muscles of the laboratory fish. The relevant literature on electron microscopy and X-Ray microanalysis of HMs was reviewed and Silver Sulphide Technique (SST) was employed for the localization of HMs. Cd and Pb have been localized in gill head and lamellae regions. Liver and muscle cells have also been scanned for the presence of Cd and Pb.

Electron Probe X-ray Microanalysis (EPMA) of the outer layer of gill head region (comprising of epithelial cells and mast cells), basement membrane of primary and secondary lamellae, and in the center of secondary lamellae (comprising of pillar cells and mast cells), outer membrane and center of liver plate and muscle cells show concentrations of Cd and Pb at selected sites. Studies thus corroborate the localization of Cd and Pb at these specific sites.

A gist of conclusions of the study has been presented in Chapter 6. The results

show the compartmentation and bioamplification of Cd, Pb, and Zn in an aquatic ecosystem. The physico-chemical parameters collectively moderate the bioconcentration of Cd, Pb and Zn. Accumulated metals have been localized, scanned and quantified at specific cellular level using TEM and EPMA.

In the end the author wishes to mention that utmost care has been taken in compiling the literature on bioconcentration and X- ray microanalysis and to acknowledge the contributions. However, if there are any omissions they are purely inadvertant. Furthermore, in presenting the work care has been taken to minimize repetitions nevertheless, at times, it has not been feasible to avoid them.

CHAPTER 2

EXPERIMENTAL METHODOLOGY

2.1 PRELIMINARY

Earlier studies conducted on the riverine systems in North-western India have shown that river Hindon and Kalinadi that receive discharges from heavy metal based industries are suitable for studies on metal uptake and consequent bioaccumulation.

2.2 FIELD STUDIES ON BIOCONCENTRATION AND BIOAMPLIFICATION

To establish the temporal and spatial variations of physico-chemical parameters of water and to establish the levels of metals in environment plankton and fish tissue, an elaborate experimental protocol was followed:

2.3 SAMPLING STATIONS

Six sampling stations were selected on the basis of a reconnaissance with accessibility and representativeness as the criteria. The stations were designated as S1 to S6. The details of sampling locations and activitites around the area are discussed in Chapter 3.

2.4 SAMPLING SCHEDULE AND COLLECTION TECHNIQUE

Integrated samples of water, sediments, plankton, and a teleost fish, Colisa fasciatus, were collected in four seasons viz., September, 1990 (Postmonsoon), January,1991 (Winter), May, 1991 (Summer), and August, 1991 (Monsoon) from all the six stations. For the determination of total metal, water samples were collected in PVC acidified bottles (Duncan and Harrison, 1981). The collected water samples were immediately acidified with concentrated HNO_3 to reduce the pH of the sample below 2.0. Separate samples were collected in PVC bottles for analysis of other physico-chemical parameters. The samples were filtered through 0.45 micron millipore filter and kept in pretreated PVC containers at low temperature for the analysis of dissolved (soluble) metal content.

The sediment samples were collected from shallow water about twenty meters away from the bank with the help of Eckman dredge. Samples were collected in polyethylene bags, stored at 4 ^{0}C and dried at 105 ^{0}C in an air oven (Tessier et al., 1979).

Plankton samples were collected using warranted genuine quality standard test sieves of specific pore size at least 15 cm below the surface. Samples were precon-centrated and total community was transferred to a clean polyethylene bottle.

Same size specimens of teleost fish, Colisa fasciatus, were collected with the help of local fishermen of the area.

2.5 SAMPLE PRETREATMENT AND ANALYSIS

Physico-chemical parameters in the water samples were evaluated according to the methods specified in Standard Methods for Analysis of Water and Wastewater (AWWA, APHA, 1980). Determination of pH and conductivity was done at the spot with the help of a portable kit. A summary of the techniques employed for the analysis of selected parameters is given in Table 2.1.

Total and Soluble Metal in Water

100 ml of water sample was digested with concentrated HNO_3, cooled and filtered through Whatman 42 filter paper. The volume was made upto 100 ml with distilled water and stored. The metals were determined using Inductively Coupled Plasma-Atomic Emission Spectrometer (ICP-AES).

Samples of water filtered through 0.45 micron millipore filter paper were employed for the analysis of dissolved (soluble) metal content.

Total Metal in Sediments

For the determination of total metal content of Al, Na, K, Ca, Mg, Fe, Mn, Cd, Pb, and Zn in sediment, the samples were digested with HF-$HClO_4$ and the method given by Tessier et al. (1979) was adopted for the extraction of metals from sediments.

Metal Analysis in Plankton

Hundred liters of water was filtered through a plankton net of bolting silk No.25 (size 55 μm) for plankton separation. The total community was transferred to a clean polyethylene bottle and the final volume was made up to 100 ml with distilled water. Samples were sorted according to pore size of nets of two different sizes (55 μm-425 μm; mesh size 36 and 425 μm- 4.75 mm, mesh size 4). Based on their size,

Table 2.1 Summary of Analytical Methods

Parameter	Units	Technique
pH	- -	pH metry (CENTURY portable water analyser)
Conductivity	mmhos/cm	Conductimetry (CENTURY portable water analyser)
Total dissolved solids	mg/l	Gravimetry
Alkalinity	mg/l $CaCO_3$	Titrimetry (Acid-base)
Total hardness	mg/l $CaCO_3$	Titrimetry (Complexiметric)
Dissolved Oxygen	mg/l	Titrimetry (Winkler's method)
Dissolved organic carbon	mg/l	Colorimetry (Skalar analyser)
Chloride	mg/l	Titrimetry (Argentometric)
Nitrate	mg/l	Colorimetry (Skalar analyser)
Sulfate	mg/l	Gravimetry
Trace metals	µg/l or mg/l	Inductively Coupled Plasma - Atomic Emission Spectrometry (ICP-AES)

samples were designated as phytoplankton and zooplankton (Butler, 1978 and the references cited therein).

Plankton samples were dried at 105 ^{0}C and weighed. For the total metal analysis in phytoplankton and zooplankton, samples were digested under HNO_3 -H_2O_2 flux (Fayed et al., 1983). Metal concentrations were determined using ICP-AES and reported on dry weight basis (µm metal/gm dry weight).

Metal Analysis in Fish Tissue

For the total metal analysis, the teleost fish were randomly selected, rinsed with distilled water and sacrificed. The fish were skinned and the samples of hind part of fish tissue were exorcised, oven dried at 105 ^{0}C and weighed (Roth and Hornung, 1977). Tissue samples were finally digested with a flux of HNO_3 -H_2O_2 (Borg et al., 1981). Determinations of Cd, Pb, and Zn in the samples were carried out using ICP-AES and concentrations are reported on a dry weight basis (metal µg /gm).

2.6 LABORATORY STUDIES ON BIOCONCENTRATION

The experimental work was carried out in glass aquaria, designated as experimental Set I, Set II and Set III, initially charged with sublethal concentrations of Cd(II) [(Cd as $Cd(NO_3)_2$] and Pb (II) [(Pb as $Pb(NO_3)_2$]. The experimental fish were acclimatized in tap water (30 days) and exposed to 0.20, 0.50, 1.00 mg/l Cd and 1.00, 2.00 and 5.00 mg/l Pb, in the three sets, respectively. A total of 50 specimens were exposed to experimental conditions in each set.

Sampling Frequency and Analysis

Fish and test water (tap water charged with Cd or Pb) were drawn (initially eight hours (three times), and then every fifth day) for analysis. Fish tissue (gills, liver

and muscles) samples were prepared (Roth and Hornung, 1977) and digested with a flux of HNO_3 -H_2O_2 (Borg et al., 1981) for cadmium and with concentrated HNO_3 and $HClO_4$ (Hamelink, 1977) for lead. Metal concentrations were determined using ICP-AES and reported on dry weight basis.

To recognise possible effects induced by the physico-chemistry of water on the bioavailability of Cd and Pb, test water samples were analysed for total and soluble metal content, pH, conductivity, dissolved organic carbon, and water hardness as per Standard Methods (AWWA, APHA, 1980).

Experimental work was carried out at 24 $\pm 2^0$C with a variation of two degrees. The experiments were conducted in triplicate and the reported values are average of at least two values. In the different experiments, blanks (control) were run and the corrections applied wherever necessary.

2.7 LOCALIZATION OF CADMIUM AND LEAD IN FISH TISSUE

The test fish, were exposed to sublethal concentrations of cadmium (1.00 mg/l) and lead (5.00 mg/l) for 30 days in glass aquaria under simulated conditions. The fish tissue (gills, liver and muscles), were exorcised and samples were prepared for light and electron microscopic studies (Bancroft and Stevens, 1977).

2.8 TEM and EPMA

Localization of cadmium and lead in fish tissue was attempted in gills, liver and muscles at cellular level by modified Silver Sulphide Technique (Danscher, 1981a). Precipitates of cadmium and lead sulfides were viewed on Transmission Electron Microscope at specific cellular sites of gills, liver and muscle samples at various magnifications. For EPMA, the samples of fish tissue (0.5 μm thick) were fixed on

glass slides, developed in Danscher developer and were coated with carbon.

The preparation of the specimen involved the following steps :

i. Fixation
ii. Dehydration
iii. Embedding
iv. Microtomy and Ultra-microtomy
v. Developing and Staining
vi. Viewing

i. Fixation

Fish tissue (gills, liver and muscles) were exorcised and exposed to 0.1% Na_2S in 0.1 M phosphate buffer (pH 7.4) for 10 minutes. Tissues were fixed later with 1% gluteraldehyde in 0.1 M phosphate buffer (pH 7.4) for 90 minutes. Tissue samples were rinsed in phosphate buffer (0.1M, pH 7.4) for 5 minutes.

ii. Dehydration

Tissues were dehydrated in acetone step wise in a concentration series given below.

Wash	Duration (minutes)
30% (v/v) acetone	15
50% (v/v) acetone	10
70% (v/v) acetone	10
90% (v/v) acetone	10
100% (v/v) acetone	10
100% (v/v) acetone	15

The dehydration was carried out at room temperature in capped plastic vials.

iii. Embedding

Epoxy resin (araldite embedding medium) with the following composition was

used as media :

Component	Amount (gm.)
Araldite Cy_{212} (Epoxy monomer)	29.0
Dodecyl succinic anhydride (Hardner)	24.0
2,4,6 tridimethylaminophenol [DMP30 (d_4O_{64})] (Accelerator)	0.5

The process was carried out in a fume cupboard. The components were mixed and allowed to stand for 10 minutes. It facilitated the air bubbles to escape resulting in a homogeneous media. The embedding medium was added to the vials for block preparation. Polymerization was carried out at 65 ^{0}C for 48 h. The blocks were removed and allowed to cool at room temperature.

iv. Microtomy and Ultra-microtomy

Blocks were trimmed to produce a flat pyramid for microtomy. Thin sections (0.5µm) were cut for light microscopy and were also fixed on glass slides for EPMA.

Ultra-thin sections (70-90 nm) were raised on copper grids for developing and staining for the visualization of Cd or Pb by modified SST.

v. Developing and Staining

Ultra-thin sections were developed in complete darkness for 10 minutes in silver nitrate / hydroquinone medium (Danscher, 1981a). 100 ml of medium contained :

60 ml gum arabic (20% in distt. water)
10 ml citrate buffer (pH 3.5)
15 ml hydroquinone (0.85 gm. in 15 ml)
15 ml silver nitrate (0.11 gm. in 15 ml)

Grids were rinsed in distilled water to free the excess developer and stained

with uranyl acetate for 10 minutes. The stain was prepared as :

Uranyl acetate	Solid p.a.
Distt. water	100 ml

Solution was stored for 24 h, centrifuged and the supernatent was stored at–4 ^{0}C

The grids were finally washed to free the excess stain by dipping them in water and the excess of water was removed from the surface of the grid.

vi. Viewing

The TEM of the specimen were taken on a TEM fitted with a 35 mm camera.

Cadmium and lead were quantified at specific sites of gills, liver and muscle cells. Results of metal ion concentrations in gills, liver and muscle at specific sites of EPMA analysis are presented in weight percentage. Other specific conditions include - Current - 1.00 x 10^{-10} ampere; Voltage - 10 kV; Probe Diameter - 0.1 and 5.0 microns.

Standards supplied by SPI Supplies Division of Structure Probe Inc., Canada were used for X- ray microanalysis. The metals present in layout of standard mounts were :

Metals	Percentage
Cd	100.00%
Pb	88.60%
S	13.74%
Ca	21.73%
Mg	13.18%
P	18.50%
Zn	0.20%
Na	3.01%
K	4.18%
Fe	6.32%

2.9 EQUIPMENTS USED

The metal concentrations in the treated samples were determined by Inductively Coupled Plasma - Atomic Emission Spectrometer (model Plasmalab 8440 with Labtam 3000 series Computer, Australia).

pH measurements were made on a digital pH meter DPH 500 (Global make). Conductivity was measured by the CENTURY portable water analyser.

Nitrate and dissolved organic carbon (DOC) in water samples were analysed on Segmental Flow Analyser (model, SKALAR 20/40).

TEM of fish tissue was carried out on TEM, Phillips CM 10 model (magnification-upto X450,000, Resolution - 0.3 n.m).

Blocks of plastic resin embedded specimen were processed using Block Trimmer Reichert TM-60; and Glass Knife Maker. Ultra-microtome-Reichert OMU was used for processing the tissue sections of 0.5 micron for light microscopy. Ultra-thin sections of 70-90 nm thickness were raised on copper grids with ultra - microtome - Reichert Jung Ultra-Cut-E.

Electron Probe X-Ray Microanalyzer (EPMA, model JXA 8600M, Jeol, Japan) was used for quantitation of metals at selected sites in fish tissue (gills, liver and muscles). Carbon coating of thin sections (0.5 μm) of fish tissue samples for EPMA analysis was done using Jeol, JEE-4 vaccum-evaporator, Jeol. Ltd., Japan.

2.10 MATERIALS USED

The chemicals used in the experiments were of analytical purity. The stock solutions of the metal ions were standardised by the usual complexiметric titrations. For bioconcentration studies in the laboratory, glass aquaria were used as test chambers.

CHAPTER 3

BIOAMPLIFICATION OF CADMIUM, LEAD, AND ZINC IN RIVERINE ECOSYSTEM

3.1 PRELIMINARY

Rapid industrialization and population explosion has led to the production of large volume of liquid wastes. These wastes find their way to a nearby water resource. The normal usage of water, for irrigation, fish catch and a source of water supply for drinking purposes thus, gets impaired. With pollution in the stream, biota gets exposed to the toxicants. The high trophic level biota, especially fish, acquires specific toxic ingredients and that may render the flesh unfit for human consumption. Sensitive species deliberately leave the polluted areas and others succumb to the onslaught and die. Those which are able to survive pickup many toxic materials (Verma et al., 1980).

3.2 ECOSYSTEM STUDY

A study on riverine ecosystem is important as natural ecosystems have properties and behaviours that are not deducible solely from the organisms that comprise them. Ecosystem level phenomenon, like trophic level transfer and cycling of nutrients result from interactions among ecosystem component and can be best examined in an intact ecosystem (Heath, 1979; Giddings, 1981).

The importance of different trophic groups in ecosystem effects, is emphasized by Elmgram et al. (1980). Interactions in an ecosystem may amplify, dampen or even reverse the direct effect of environmental factors of interest, giving rise to ecosystem effects that could not be predicted from single species laboratory studies (Adams et al., 1982).

A problem with simulated studies on bioconcentration is that they deal with the ecosystem as if they are merely a collection of species exposed to single pure compound under constant conditions. However, in natural conditions many interactive processes modify the bioavailability of a toxicant (Buikema et al., 1982). In an ecosystem, while energy decreases and becomes more dispersed at each step in the food chain, it has been observed that some substances become more concentrated with each link in a process and is known as biological magnification (Odum, 1972). Thus, the amount of the poisonous substance in water or soil may be extremely small and certainly harmless but by the time it reaches the end of the food chain, concentrations may become high enough to cause sickness and death in higher trophic level.

In the riverine ecosystem, HMs are partitioned among three major compartments viz., water, sediment and biota. Interactions among them and the uptake by organisms are influenced by a number of characteristics of biota and its physical environment encounters the indirect influence of other compartments.

In the riverine ecosystem, bottom sediments also play an important role as trace metals become the part of water-sediment system. Their distribution is controlled by a dynamic set of physico-chemical interactions and equilibria. Sediments reflect the current quality of the water system and are used to detect the presence of contaminants that remain insoluble when discharged in water. Sediments act as traps for numerous compounds thus, allowing control of pollution of the overlying water to some extent. However, sediments remain less variable as compared to the water in equilibrium (Forstner and Salomons, 1980; Forstner and Wittmann, 1983). The metal solubility is principally controlled by the pH, type of ligands and the chelating agents present in the system. When a pollutant comes in contact with the ground, most of it gets absorbed or binds chemically to soil or sediment particles (Groot and Allersma, 1975). Complicated chemical and biological reactions take place which have been a subject of research with few generalizations (Wood, 1974).

A relationship between trace metal levels in biological organisms and sediment characteristics does not require the main route of entry of trace metals via ingestion of particular metals. However, such relationships could also be explained by adsorption reactions (Wood, 1974; Bolin, 1976).

A compendium of the physico-chemical characterstics of water affecting the phenomenon of bioconcentration and bioconcentration factor of HMs in plankton and fish have already been mentioned in Chapter 1. Bioconcentration of metals in a food chain is largely dependent on the labile form of the metal species. The lability of metal species in turn is conditioned by the physico-chemical characteristics of water.

When biological factors are considered in the bioconcentration studies, it is reported that different rates of bioconcentration of HMs may occur even among the related species of the same environment. Biotic factors like ventilation rate, concentra-

tion and synthesis of metal binding proteins, and internal transport are important. Bioconcentration studies in a natural ecosystem are encountered not only with the switching over from the population to the community but the biotic and abiotic interactions also make the interpretations more challenging.

3.3 SYSTEM UNDER PRESENT INVESTIGATION

Topography of the Area

Regional geology of the area shows that Hindon basin is a part of Gangetic plain which has been divided in three belts (Taylor, 1959; Krishnan, 1968). The geological succession of the region with surface lithology is given in Table 3.1.

River Basin and Land Use

The study area covers river Hindon and Kalinadi at selected representative stations. The river Hindon originates from Shivalik hill ranges north of Saharanpur. It traverses north to south in the Ganga-Yamuna basin towards Ghaziabad. The river has a large water stretch and in its course it receives large amounts of waste loads from domestic and industrial sources. The river finally meets Yamuna near Okhla (New Delhi). Hindon river covers four districts of western Uttar Pradesh, viz., Saharanpur, Muzaffarnagar, Meerut and Ghaziabad. Various industries located in the area viz., pulp and paper, textile mills, sugar mills, distilleries, electroplating, metal hardening, chemical and rubber works pour their wastes into the river. The basin is densely populated and is bound by the tributaries of Hindon, Kali and the river Yamuna . The catchment of the basin is about 500 square kilometers.

Kalinadi also originates from Shivalik hills and traverses down to Muzaffarnagar. It receives agricultural, domestic and industrial wastes during its course to Atoli where it finally meets river Hindon (Fig 3.1).

Table 3.1 Geological Succession in Ganges Plain (Taylor, 1959)

Geological Time	Plain and Foot Hills of the Area	Lithology
Recent	Bhabhar Deposit	Alluvial fan deposit essentially consisting of sand and gravel beds with cobbles and boulders.
	Terai Deposit	Clay, sandy clay and sand with gravels and pebbles.
	Gangetic Alluvial	Sand, silt, clay and kankar with gravel belts.

Table 3.2 List of Sampling Stations alongwith Relevant Details

Sampling Stations	Relative Distance	Latitude	Longitude	Activities around The Station
S1	0.0	29° 29'	77° 41'	AR
S2	27.0	29° 14'	77° 34'	AR, DE, IE
S3	-	29° 14'	77° 32'	AR, DE, IE
S4	29.5	29° 13'	77° 32'	AR, DE, IE
S5	88.5	28° 41'	77° 24'	CB, DE, IE, WR
S6	99.0	28° 40'	77° 24'	DE, IE

Note : AR - Agriculture Run off ;
CB - Community Bathing ; IE - Point / Non-Point Industrial Effluents ;
DE - Domestic Effluents ; WR - Water Regulation ,

Climatic Features

The climate of the area is moderate to subtropical monsoon type. The meteorological parameters viz., precipitation, temperature, pressure, wind velocity and relative humidity show well marked seasonal variations. May and June are the hottest months with the peak temperature in plains rising upto 45 ^{0}C. December and January are the coldest months with temperature falling as low as 1 - 2 ^{0}C. The period from July to September is characterized by monsoon with almost ninety percent of the total precipitation. The average rainfall is about 1200 mm per year. The maximum sunshine is recorded during May (11.4 h/day) and minimum in August (5.1 h/day) (Verma et al., 1980).

Sampling Stations

Six sampling stations were selected on the basis of reconnaissance with accessibility and representativeness as the criteria. The stations are Muzaffarnagar (S1), Binoli (S2), Barnava (S3), Atoli (S4), Ghaziabad bridge (S5) and Ghaziabad (S6). Sampling stations were designated as S1 to S6. The catchment area of river Hindon and Kalinadi along with the locations of sampling stations is shown in Fig 3.1.

S1 (Muzaffarnagar)

It is the first sampling station on Kalinadi. There is no well marked industrial activity upstream of the station. However, isolated agricultural practices are discernible in the area. The station gives the baseline information. The river enters Muzaffarnagar city at this station.

S2 (Binoli)

This station is located on Kalinadi, downstream of Muzaffarnagar drain which brings the wastes of Muzaffarnagar town including Mansoorpur sugar mill and distillery.

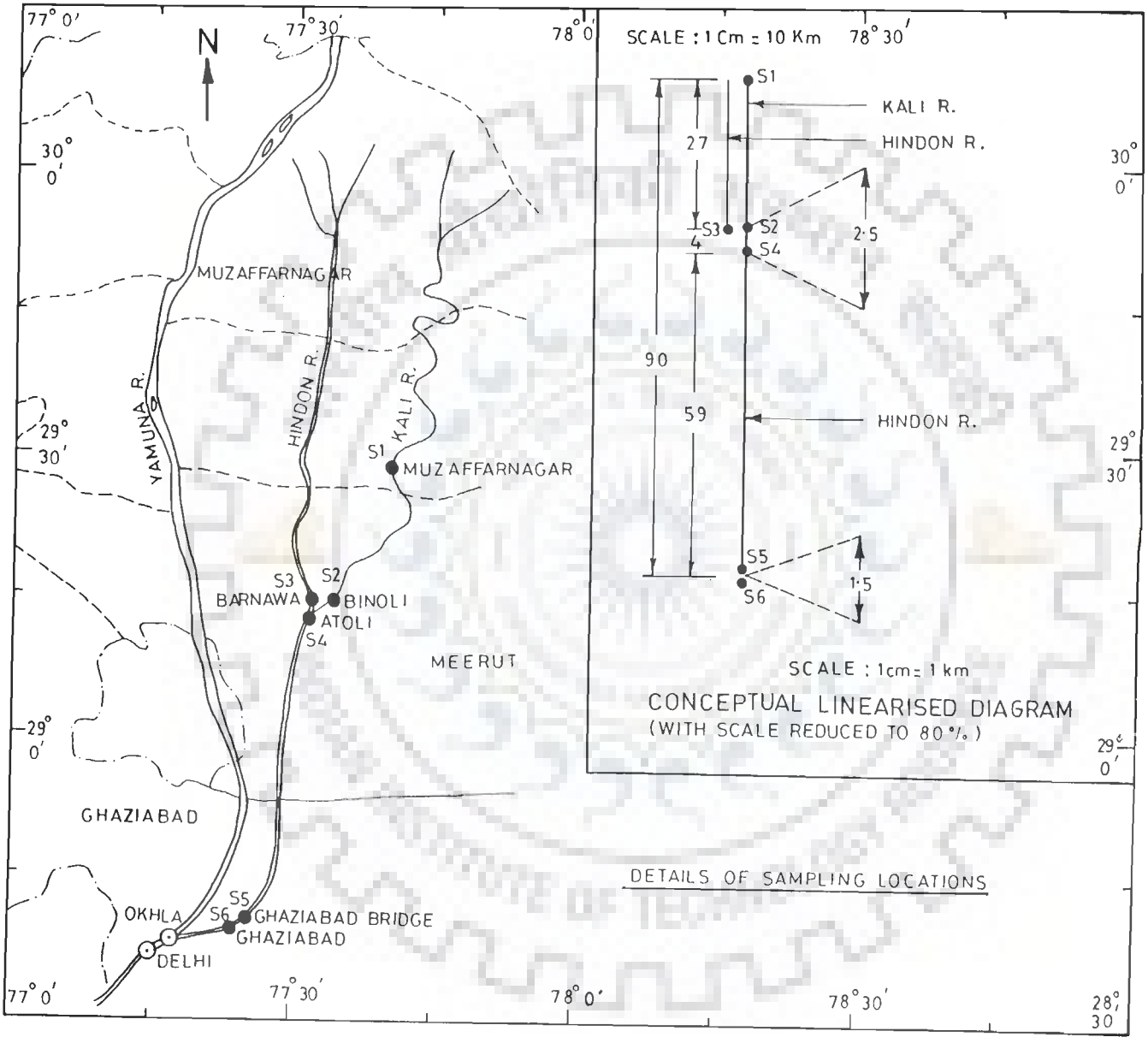


Fig 3.1 Catchment Area of River Hindon and Kalinadi

S3 (Barnava)

It is the first sampling station on river Hindon. The river receives industrial wastes from Saharanpur district upstream of sampling station.

S4 (Atoli)

This station is located on river Hindon near the confluence of Kalinadi. The station is characterised by the confluence of Kalinadi which drains industrial wastes of Muzaffarnagar town.

S5 (Ghaziabad bridge)

This station is located downstream of Atoli on the river Hindon. The river receives wastes from the drains and industries of Meerut city. Wastes from Mohan Meakins distillery meets river Hindon upstream of the station.

S6 (Ghaziabad)

Downstream on river Hindon, station S6 was chosen to study the stabilised concentrations of pollutants, especially HMs in biota, before the river Hindon meets river Yamuna at Okhla.

The geographical details and predominant anthropogenic and industrial activities at and around the sampling stations are presented in Table 3.2.

3.4 NEED FOR FURTHER STUDY

The literature on spatial and temporal variations of physico-chemical parameters and pollution loads of river Hindon and Kalinadi is rather scanty. Verma et al. (1980) have published a document on the pollution studies of river Hindon in relation to fish and fisheries. Recently, few reports on pollution of the upper Hindon basin have appeared in the literature and reveal a grave situation (Patel et al., 1985; Reena et al., 1985; Singhal et al., 1987). Reena et al .(1985) have described the characteristics of waste effluents

from paper mills and electroplating units getting into upper Hindon. Singhal et al. (1987) have studied the effects of industrial effluents on the water quality of river Hindon in Saharanpur.

These reports do not document heavy metal concentrations in the river. A study on water quality analysis of Hindon river water for potable purposes has revealed that the river water contains toxic metals. Lead was reported to be present more than 1.00 ppm at some locations in Ghaziabad district (Center of Environmental Engg., C.E.D. report, 1990).

Reports available on bioamplification in the literature reveal biotransfer of some heavy metals along the food chain with greater concentrations in herbivores and primary, secondary and top carnivores. Comprehensive reviews of metal contamination and accumulation in the aquatic environment (Phillips, 1980; Forstner and Wittmann, 1983) are available and have identified few studies which demonstrate biotransfer of heavy metals. Arsenic has been shown to increase through a food chain in a contaminated area near west Greenland (Bohn, 1975). Higher concentrations of As in fish species (88.4 µg/gm), in prawns (62.9-8.2 µg/mg), musscles (14.1-16.2 µg/gm), and zooplankton (6.0 µg/gm) are reported (Bohn, 1975).

The stepwise transfer of cadmium with increasing trophic level has been observed in agricultural food chain (Brams et al., 1989). They observed that low level contamination of soil with Cd and Pb at 0.01 - 9.00 and 3.00 - 54.00 mg/kg induced a significant toxicant accumulation in a food chain. Relatively low levels of Cd and Pb in soil have been shown to move through the food chain eliciting positive and significant response in top biomass tissues.

Biotransfer factors for six metals including Cd, Pb, Hg, As, Cr and Ni were calculated by Stevens (1992) for three bovine tissues (muscle, liver and kidney). Stevens

(1992) reported that of the metals studied, Cd exhibited the greatest bioaccumulative potential (in liver and kidney).

In India, although the importance of metal toxicity, their speciation and bioconcentration has been realised, the subject has not really taken off. It has now been perceived that studies on body burdens of HMs, the impacts of physico-chemical environment on bioconcentration and the behaviour of metals are important.

Scientific information is thus, needed to exemplify the fate of persistent Cd and Pb in the aquatic ecosystem, that has not been thoroughly examined. In view of this a project was planned to study the food chain enrichment of Cd, Pb, and Zn in a riverine ecosystem of Hindon and Kalinadi. The study was conducted during 1990-1991 by collecting water, bed sediments, plankton and fish samples from six preselected stations.

3.5 RESULTS AND DISCUSSION

The state of physico-chemical environment of water in the area under study, in general, is governed by the following factors :

- anthropogenic activities,
- geological formations of the catchment area,
- effect of tributaries, and
- climate regime from the point of view of wet months necessitating run off and dry months with ground water inflows in the rivers.

Accuracy of Data

The results of chemical analysis data of water were subjected to accuracy checks before utilising them for interpretations by electrical conductance and ionic balance methods (Standard Methods, AWWA, APHA, 1980).

Electrical Conductance Method

In this method, correlation of conductivity and total dissolved solids is established. In most natural waters, conductivity and total dissolved solids range in the order of 0.55 to 0.70, depending upon the chemical composition. In the present study, these ratios have been found in this range for nearly 80% of the samples.

Ionic Balance Method

The ionic balance method is theoretical and is based on the fact that in any water sample, the sum of total anions and total cations, expressed in meq/l, should be equal. But when measured experimentally, the sum are seldom equal because of random variations and errors of measurement. This discrimination increases with the ionic concentration. The differences should fall between the acceptable limits of $\pm$ 5%. The plots of (Σanion -Σcation x 100/Σanion +Σcation) values for different samples are shown in Fig 3.2. Nearly 60% values fall within the acceptable limits and the rest within $\pm$ 8% (Fig 3.2).

For the representation of chemical analysis data, several methods are availale viz., Collins' Bar Diagram, Shift Polygons, Pie Diagrams, Hill Piper Trilinear Diagram, Circular Diagram, Radial Co-ordinate Diagrams, Bico-ordinate Systems, Water Quality Profiles and Longitudinal Variation Diagrams (Standard Methods, AWWA, 1980).

The Piper's Trilinear Diagram is one of the most frequently used methods for representation of the chemical composition and classification of water samples (Piper, 1953). The advantage of this method is that data of several samples could be incorporated in one figure and the interpretation is easy.

Hill-Piper Trilinear Diagram

As shown in Fig 3.3, the Hill-Piper Trilinear Diagram comprises of three sub diagrams as follows:

- one triangular diagram for cations,

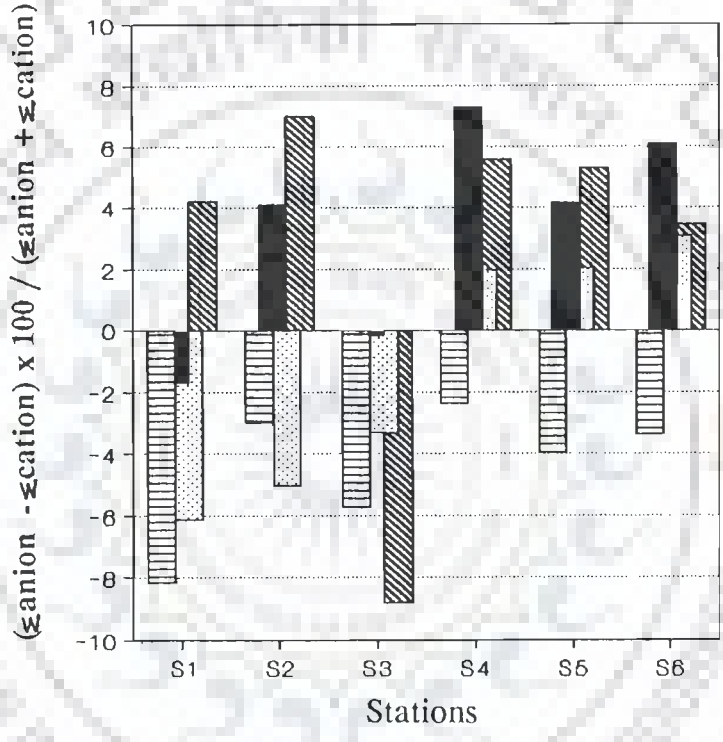


Fig 3.2 Variations in Anion-Cation Balance

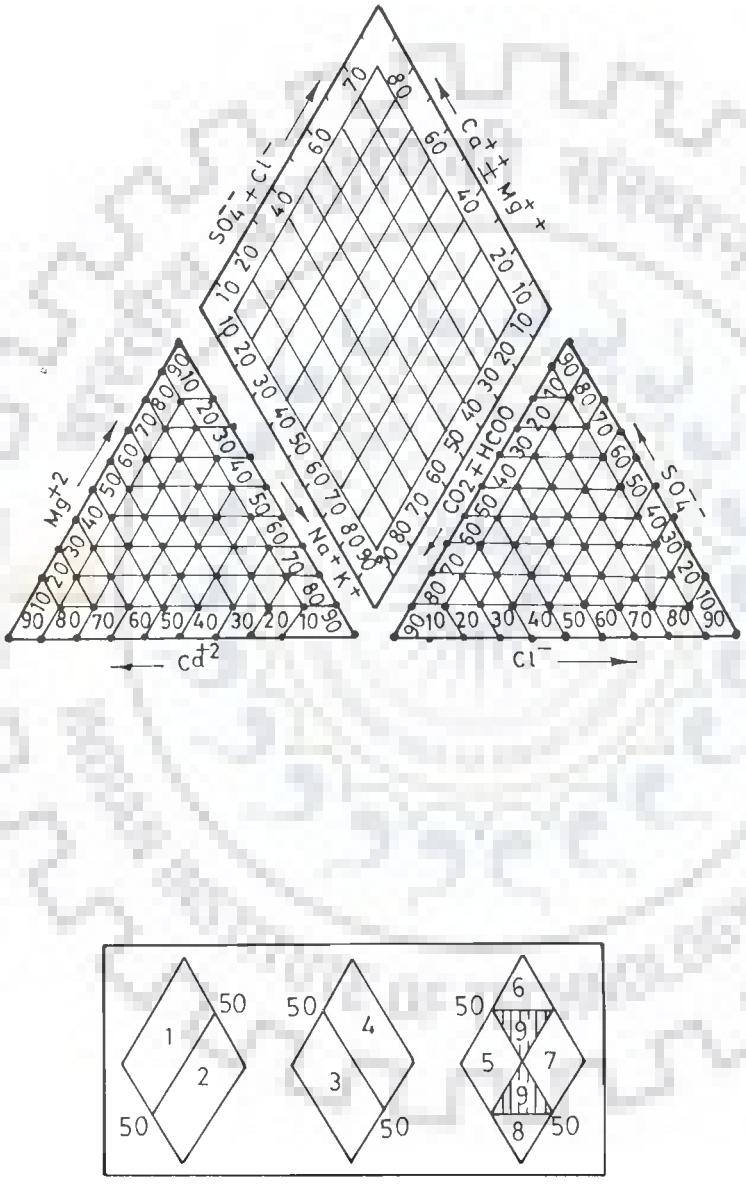


Fig 3.3 Pipers' Trilinear Diagram (After Piper,1953)

- one triangular diagram for anions, and
- a centrally located diamond shaped diagram for composite picture of cations and anions.

All the sides of the three diagrams are indicated between 0 to 100 scale. The diamond shaped field at the center is divided into different areas which represent a group of water, depending upon the major cationic-anionic composition (Fig 3.3). These nine areas describe the following characteristics of water :

Area 1 : Alkaline earth metals (Ca^{++} and Mg^{++}) dominate alkalis (Na^{+} and K^{+}).

Area 2 : Alkalis dominate alkaline earth.

Area 3 : Weak acids (carbonates and bicarbonates) dominate strong acids (chlorides and sulfates).

Area 4 : Strong acids exceed weak acids.Area 5 : Carbonate hardness exceed 50 percent i.e., the chemical properties of the water are dominated by alkaline earths with weak acids.

Area 6 : Non carbonate hardness exceed 50 percent i.e., the chemical properties are dominated by alkaline earthswith strong acids.

Area 7 : Non carbonate alkalinity exceeds 50 percent i.e., the chemical properties are dominated by alkalis and strong acids.

Area 8 : Carbonate alkalinity exceeds 50 percent i.e., the chemical properties of water are dominated by alkalis and weak acids.

Area 9 : It shows an intermittent category or mixed type of water in which no single cation or anion pair exceeds 50 %.

To plot the trilinear diagram the percentage meq/l of various cations and anions were first calculated. The mean percentage meq/l values of Ca, Mg, Na, and K were plotted on the left hand side as a single point according to conventional trilinear

coordinates. The mean percentage meq/l of bicarbonates, carbonates, chlorides and sulfates were plotted on the right side as a single point. The position of these two points in the triangular diagrams were extended into the diamond shaped field which gives the overall chemical characteristics of the water sample.

The Piper's trilinear diagram plotted for water samples collected from the study area at different locations is illustrated in Fig 3.4. The figure shows that water at S1, S3 and S4 stations was dominated by alkaline earth and weak acids, whereas, water at S2 and S6 stations was dominated by alkalis and strong acids. In other words, chlorides and sulfates with bicarbonates and carbonates are the predominant ionic species at these locations. However, the chemical characteristics of water at S5 station shows a mixed character.

Trends in Physico-Chemical Characteristics of River Water in the Study Area

Trends in the physico-chemical parameters of water samples of river Hindon and Kalinadi including the concentration of trace metals are discussed in the following section:

The pH values of the river water at different sampling stations with their seasonal variations are presented in Fig 3.5. The values predominantly lie in alkaline range. Normally, the prevailing condition of the pH does not necessarily favour the metals to remain in their ionic form and they are expected to be hydrolysed. The pH range thus favours high concentrations of heavy metals in the particulate form, either adsorbed on the surface of the biota or in the sediments. Seasonal variation in pH shows that after postmonsoon season there is a decreasing trend (Fig 3.5). It reflects that the system gets stabilised with addition of alkaline washouts during the postmonsoon season. The other reason may be that some acidic effluents of anthropogenic acitivities are added to the system. The values in summers are usually high ranging from 7.2 to 8.4 at various stations. In general, pH at S2 and S6 were high. This may be attributed to the presence of higher

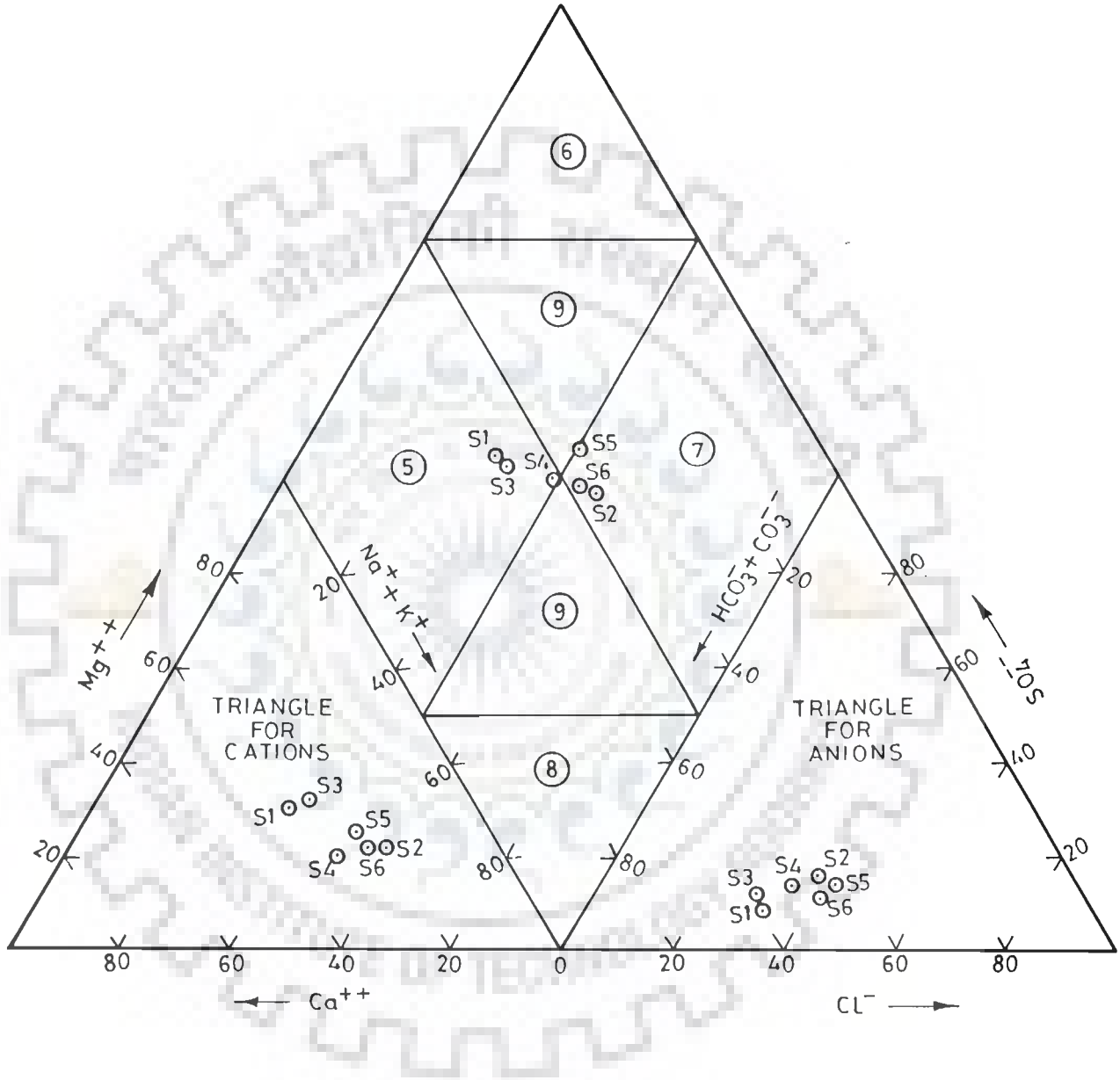


Fig 3.4 Hill Piper Trilinear Diagram

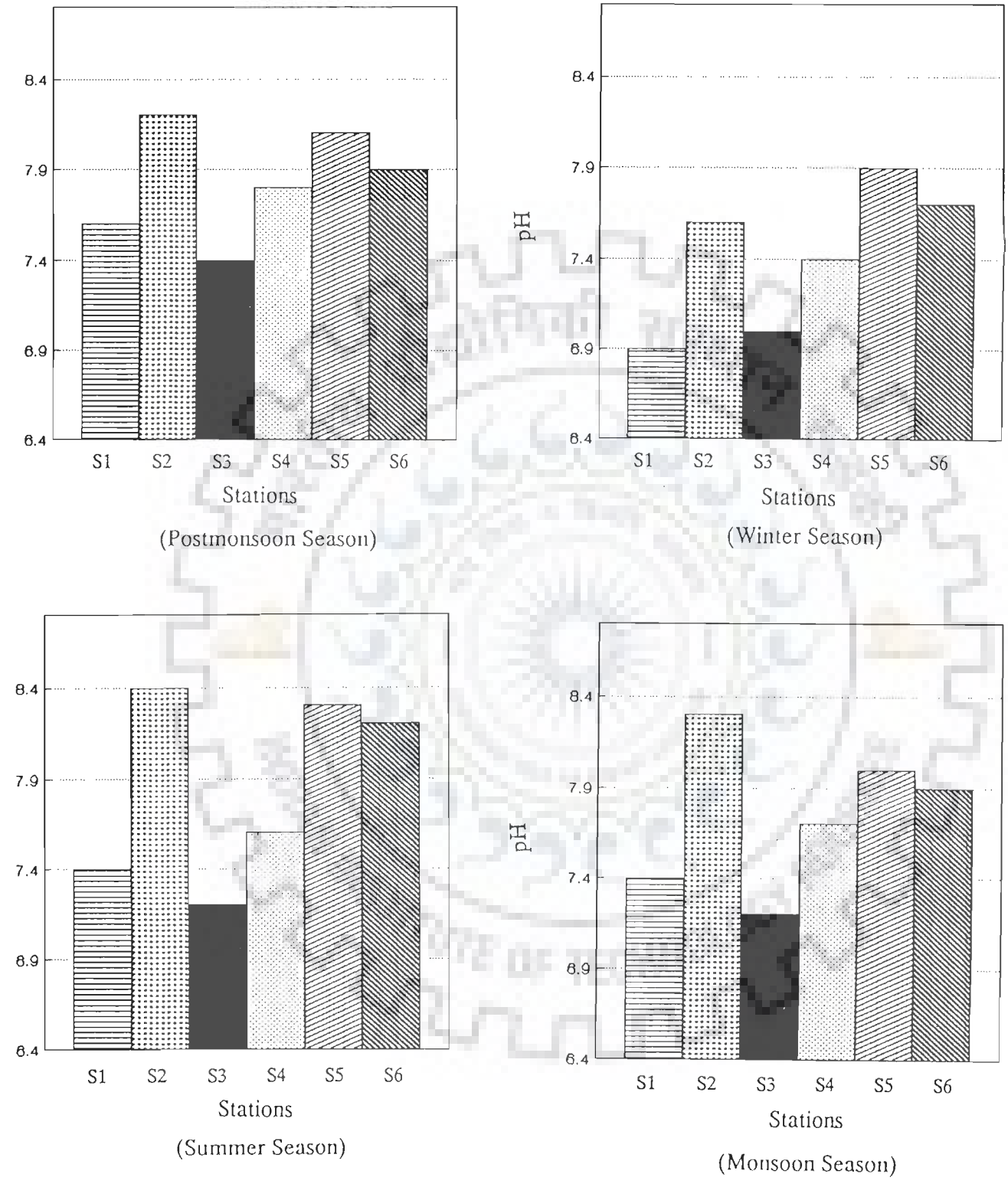


Fig 3.5 Downstream Variation of pH

concentrations of organics as is evident from higher values of DOC at these stations.

Fig 3.6 & 3.7 illustrate the trends of conductivity and Total Dissolved Solids (TDS) in the study area, reflecting in general, the changes in chemical characteristics of river water. Conductivity reflects the mineral/ionic status of the system. As shown in Fig 3.6 & 3.7 the conductance varies. Variations in TDS shows a similar trend. The similarity in the trends of two values reflects on the accuracy of the data.

Fig 3.8 & 3.9 represent the variation in Dissolved Oxygen (DO) and nitrates. DO is one of the significant variables which ascribes the pollution level of a natural water. DO values in the study area vary both spatially and temporally. The impact of addition of point and non-point industrial effluents in addition to the climatic activity factor results in a decrease in DO downstream (Fig 3.8). DO, in general, was low with further deviation at S2. The comparatively low values at this station show the impact of industrial discharges upstream.

The variations in the concentrations of carbonates and bicarbonates are shown in Fig 3.10 & 3.11. S5 and S6 stations have low concentrations of bicarbonates in the postmonsoon season. This may be due to dilution by surface runoff. In winter season and thereafter the values were high probably due to the fact that river in this lean season is basically fed with ground water.

Trends in the variations of total hardness and chloride content are shown in Figs 3.12 & 3.13. It is reported that load of industrial discharges downstream results in an increased water hardness and chloride content in the lower stretch of the river. Seasonal variations of total hardness and chloride concentrations show, in general, lower values in postmonsoon and reflect the dilution by surface runoff during the postmonsoon season.

Variations of dissolved organic carbon (DOC) are presented in Fig 3.14. DOC

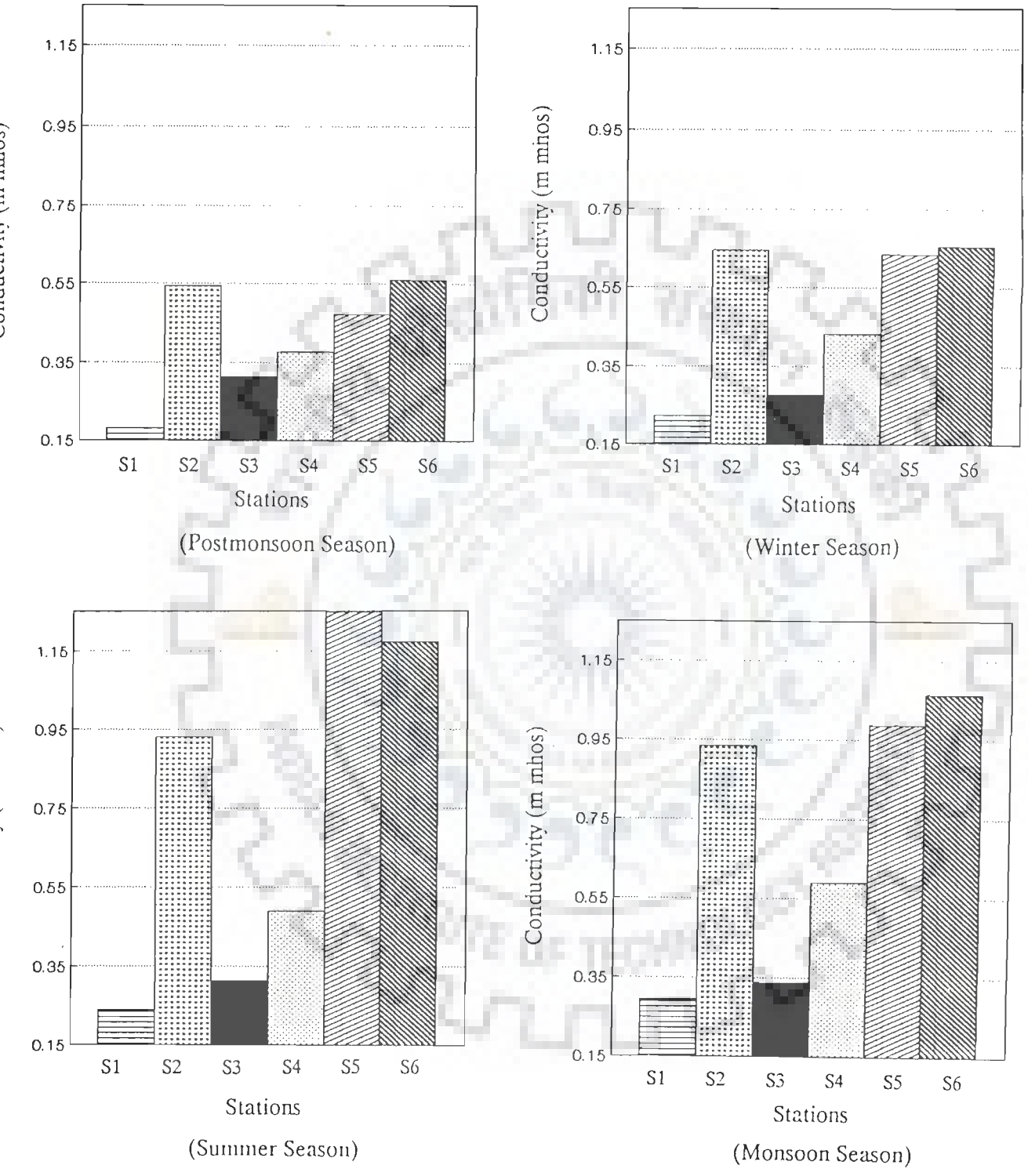


Fig 3.6 Downstream Variation of Conductivity

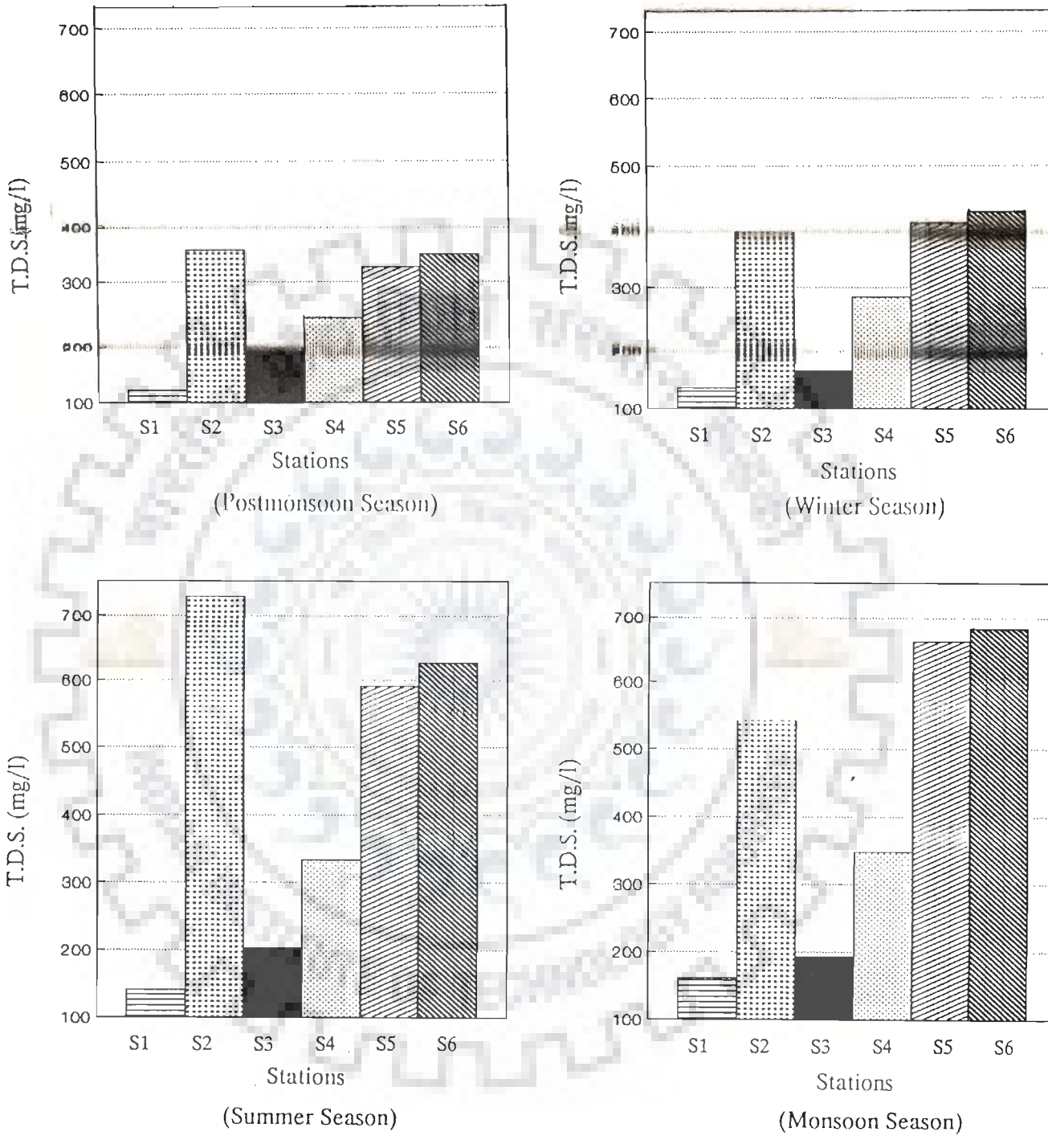


Fig 3.7 Downstream Variation of Total Dissolved Solids (TDS)

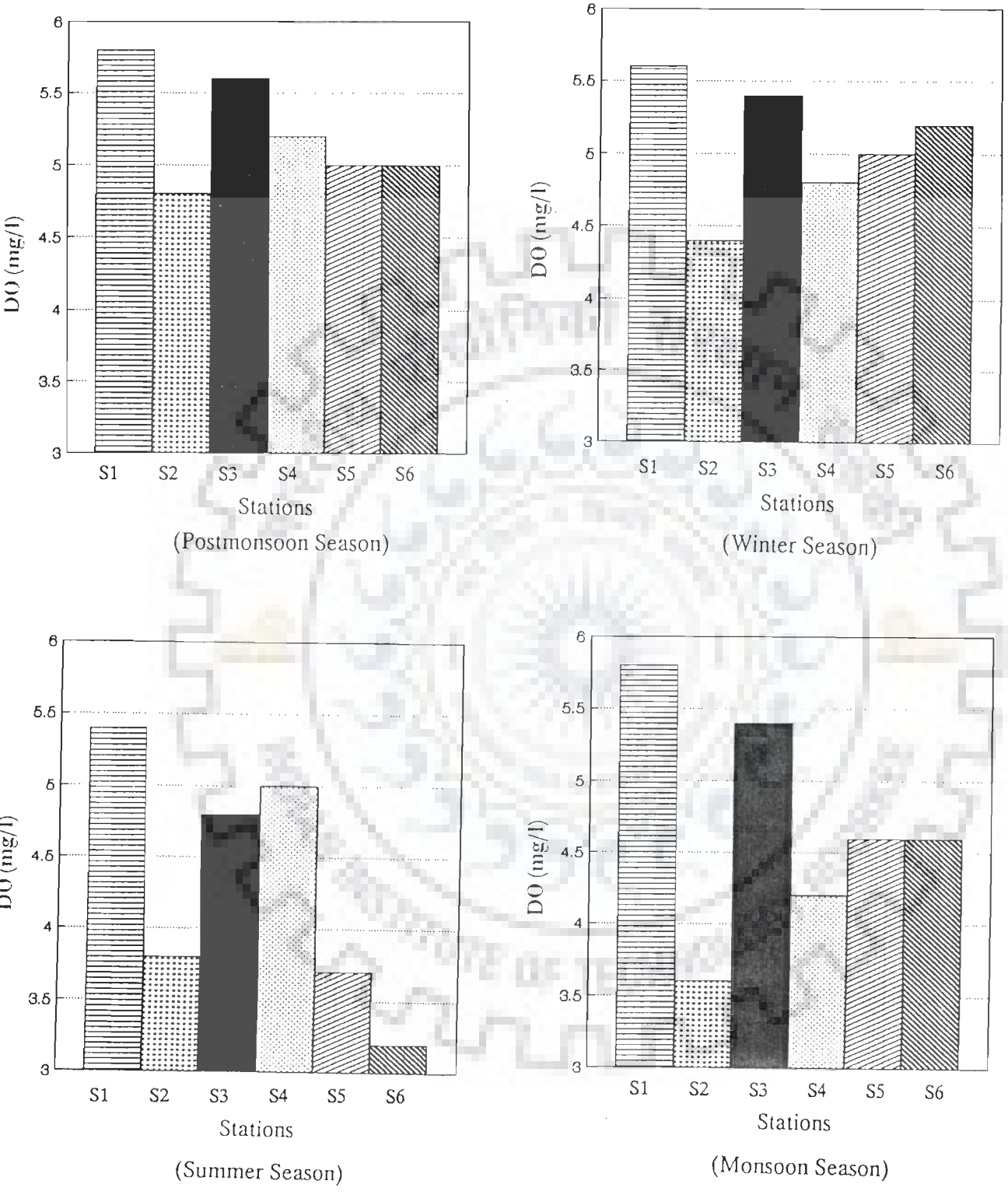


Fig 3.8 Downstream Variation of Dissolved Oxygen (DO)

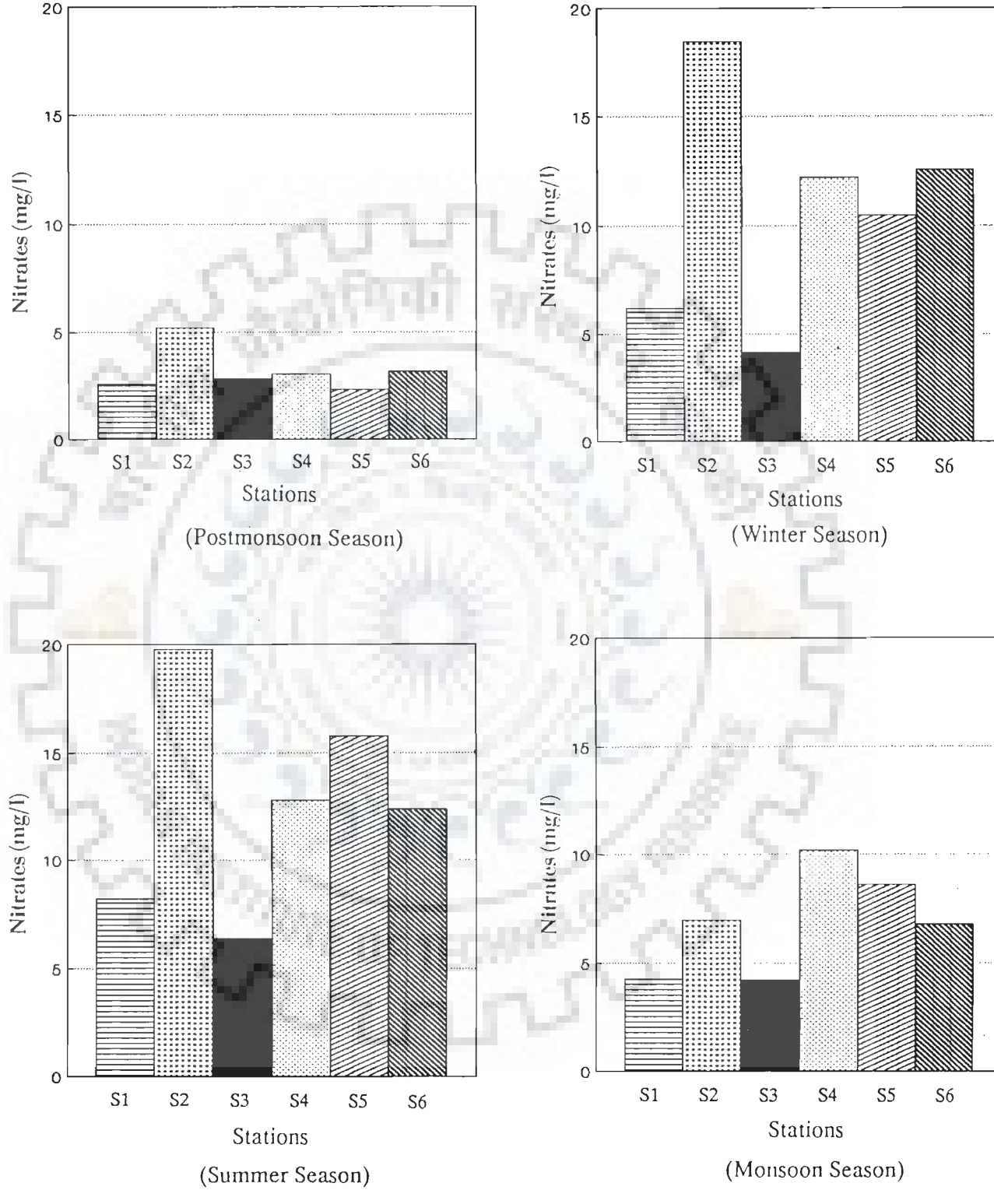


Fig 3.9 Downstream Variation of Nitrates

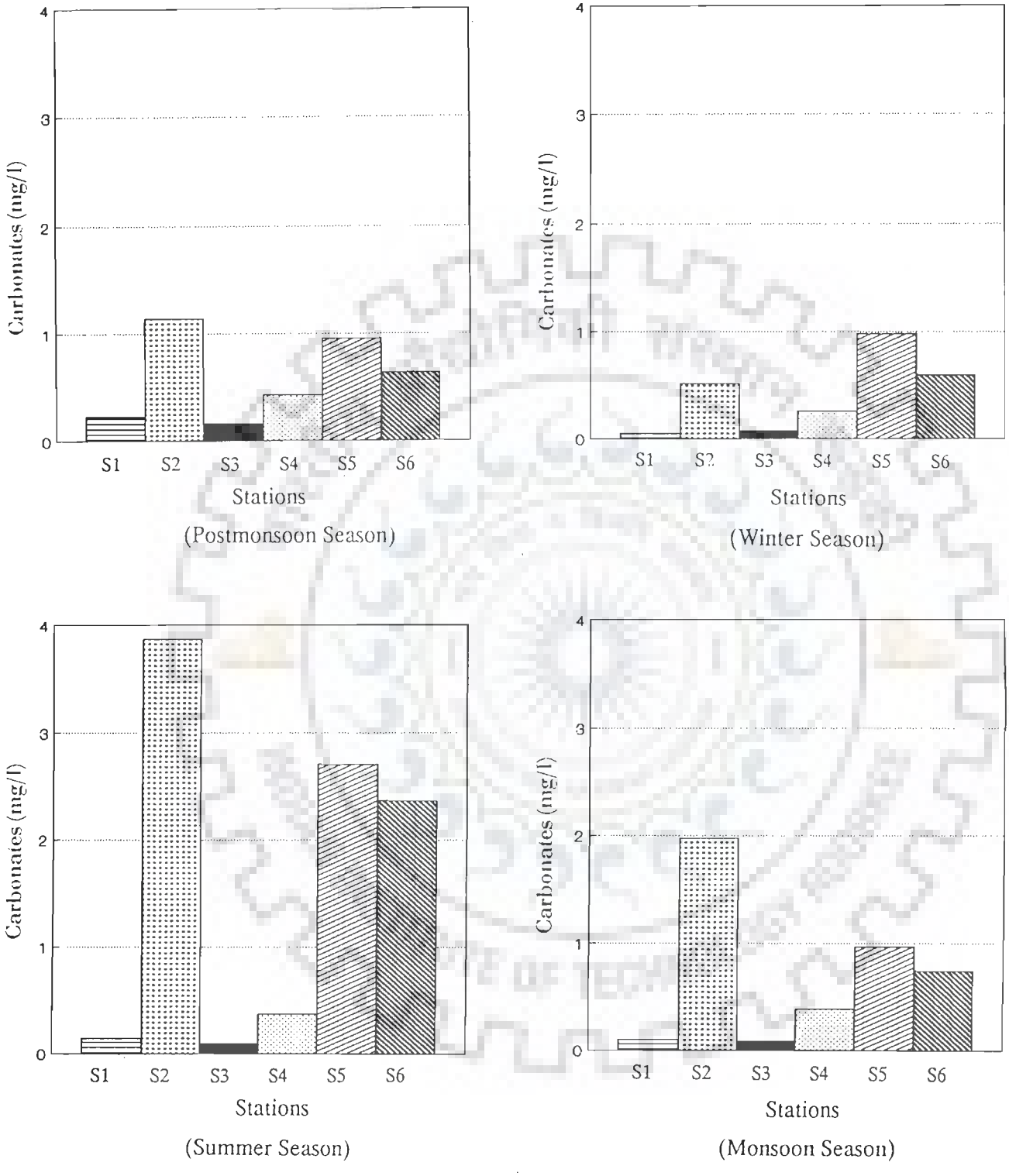


Fig 3.10 Downstream Variation of Carbonates

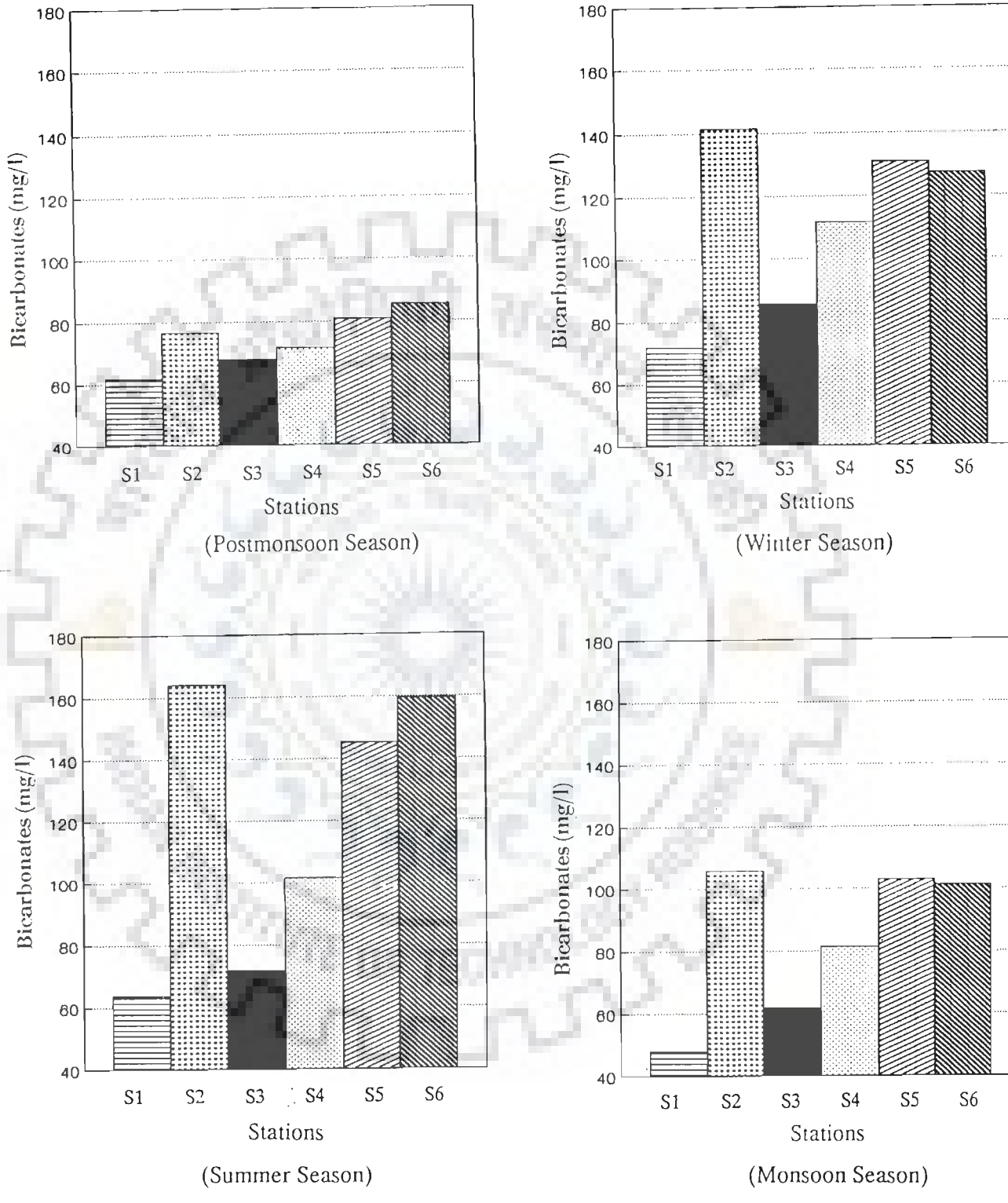


Fig 3.11 Downstream Variation of Bicarbonates

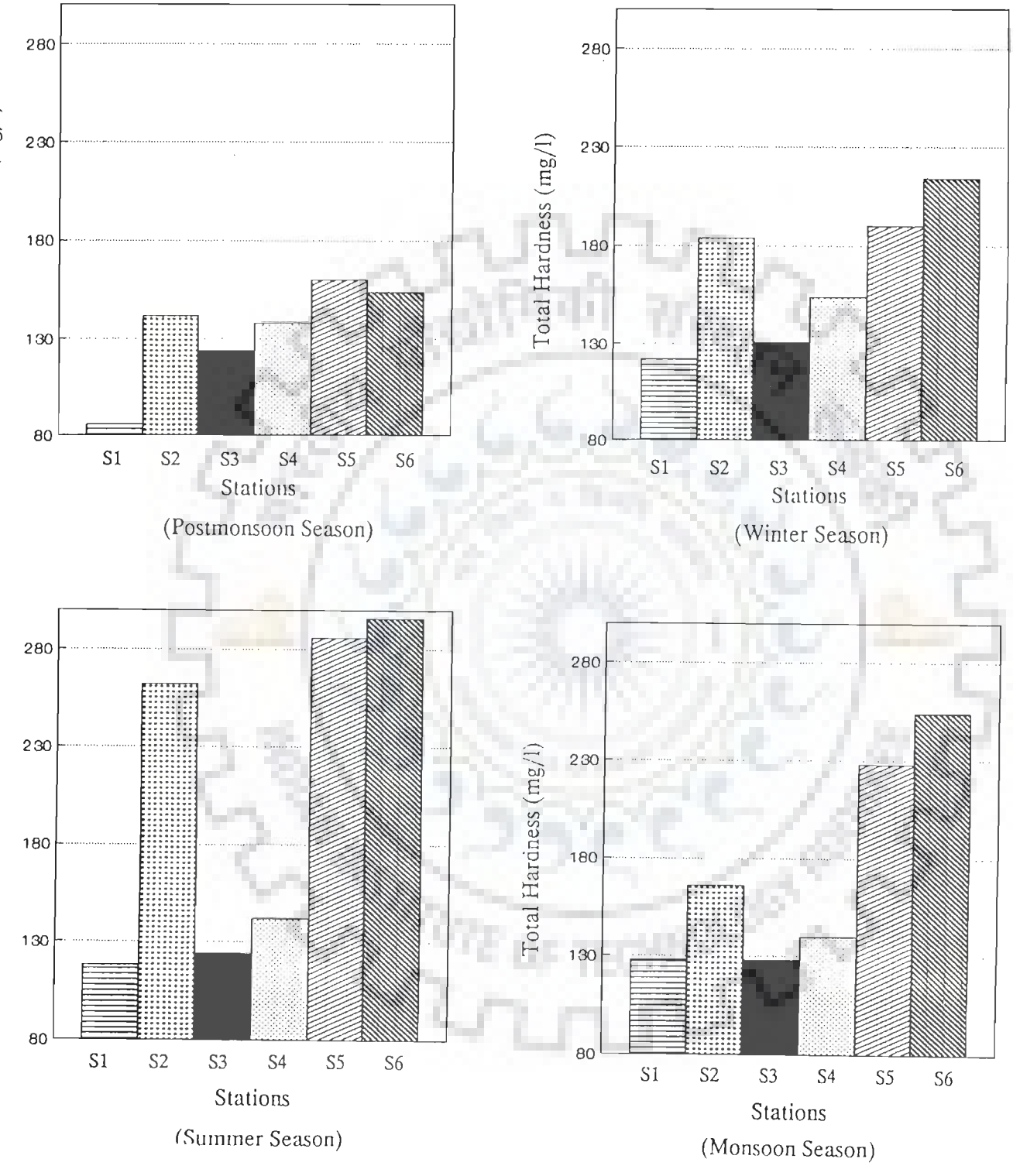


Fig 3.12 Downstream Variation of Hardness

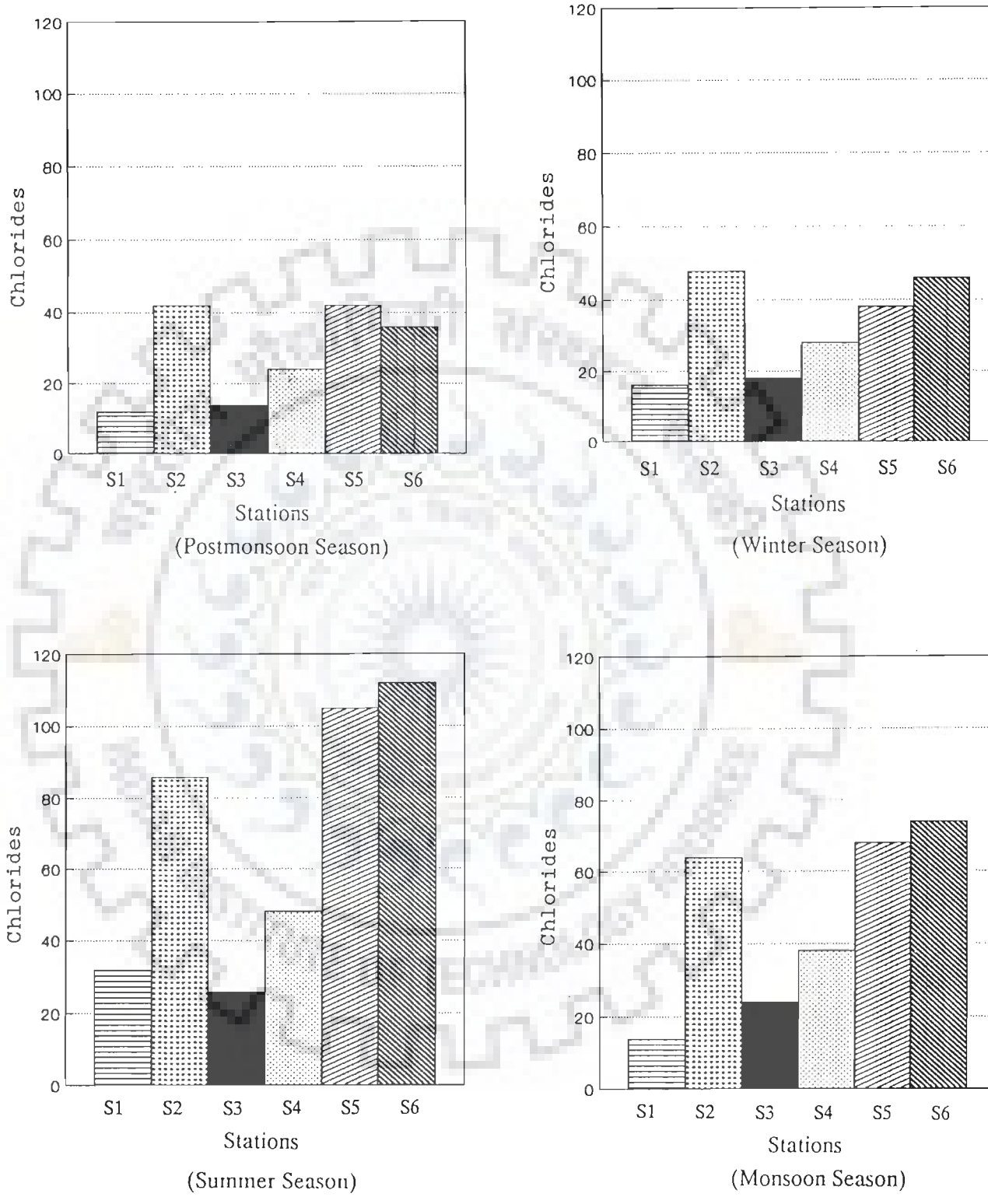


Fig 3.13 Downstream Variation of Chlorides

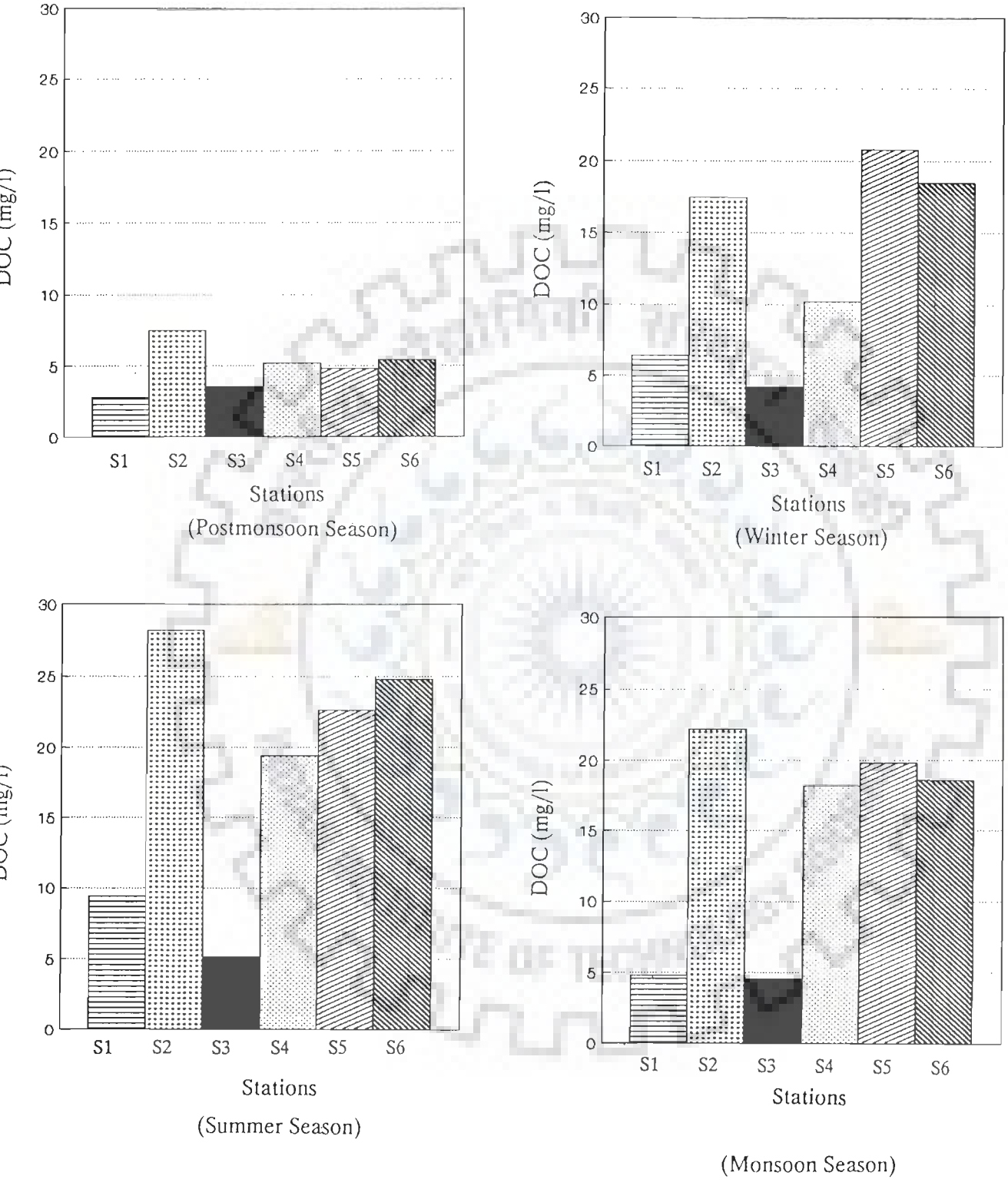


Fig 3.14 Downstream Variation of Dissolved Organic Carbon (DOC)

signifies the pollution load of an aquatic ecosystem and it is one of the most important factor that determines the bioavailability of metal ions. In the present study high concentrations of DOC were recorded in the lower stretch of the river due to the predominance of domestic and industrial activities in the area. Seasonal variations show lower values of DOC in postmonsoon season. Higher values of DOC in summer season demonstrate the impact of seasonal variations as well as increased domestic and industrial activities during the period.

Profiles of sulfate variations are included in Fig 3.15. Sulfate concentrations increase in the lower stretch and show higher concentrations at all stations in summer as compared to the postmonsoon season (Fig 3.15).

Cadmium, Lead and Zinc in River Water

The concentrations of Cd, Pb, and Zn in the river stretch under study show that both natural and anthropogenic sources contribute to the heavy metals. Variations in Cd concentration (total and soluble) presented as bar diaigrams are shown in Fig 3.16. Cd concentrations were high at S1 & S2 stations. Concentrations were 132 µg/l and 146 µg/l as compared to 62 (S3), 86 (S4), 92 (S5) and 98 µg/l (S6) in summer season. Spatial and temporal variations in Cd concentrations show that soluble Cd represent nearly 60 - 80 % of the total Cd.

Fig 3.17 shows the concentration of total and soluble Pb. Pb concentrations at S1 and S2 stations were low as compared to other stations. Pb increases downstream in the lower stretch of the river. Pb concentrations were significantly high at S3, S4, S5, and S6 stations. At S3 station , 1.04 mg/l Pb was present in summer season. It depicts intense industrial activity upstream the station. Industrial activities downstream of S4 station add further load to river water. Highest Pb concentration recorded during the investigations was 1.44 mg/l at S6 station in summers. Prominent seasonal variations are

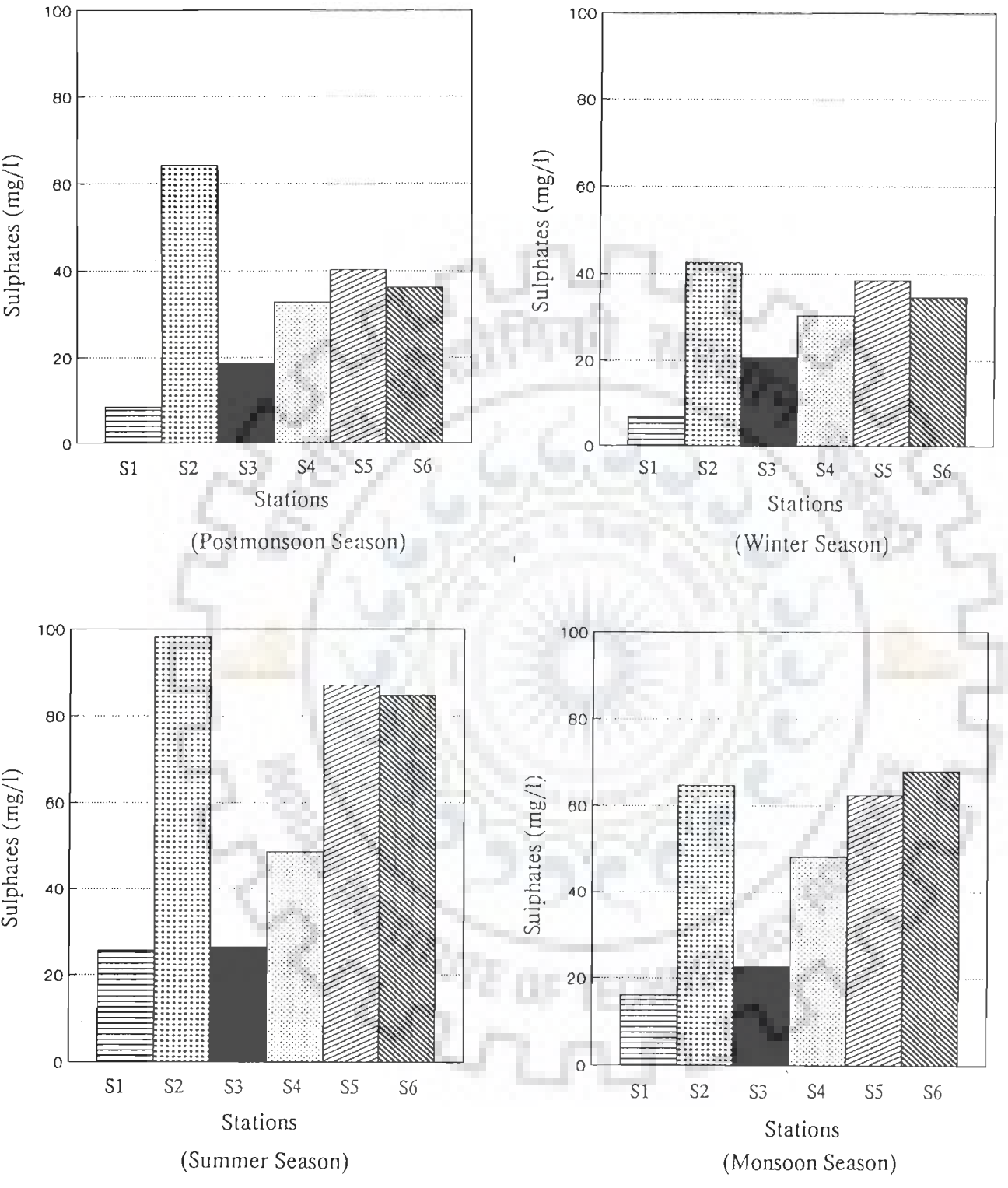


Fig 3.15 Downstream Variation of Sulfates

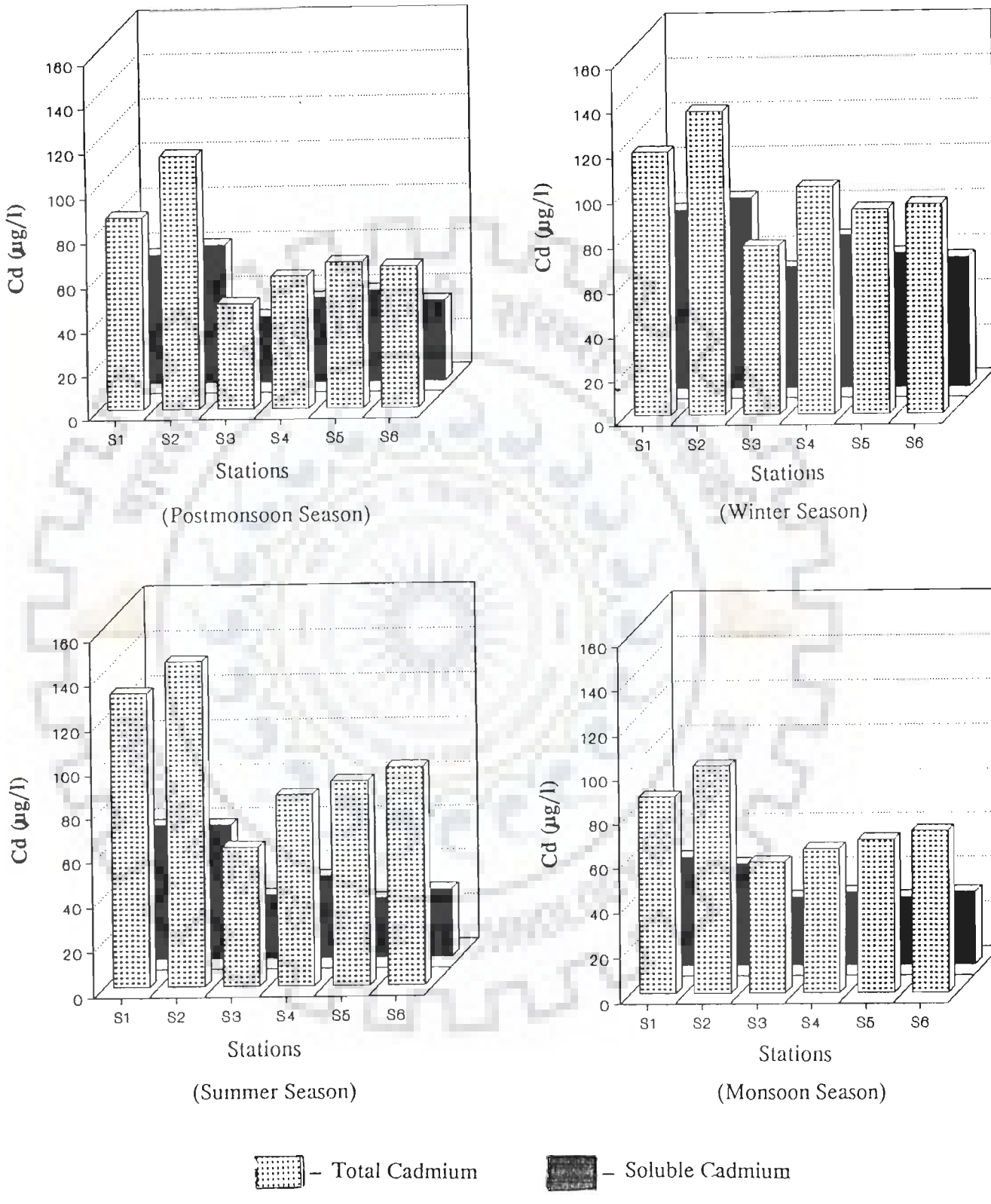


Fig 3.16 Variation of Total and Soluble Cadmium Content

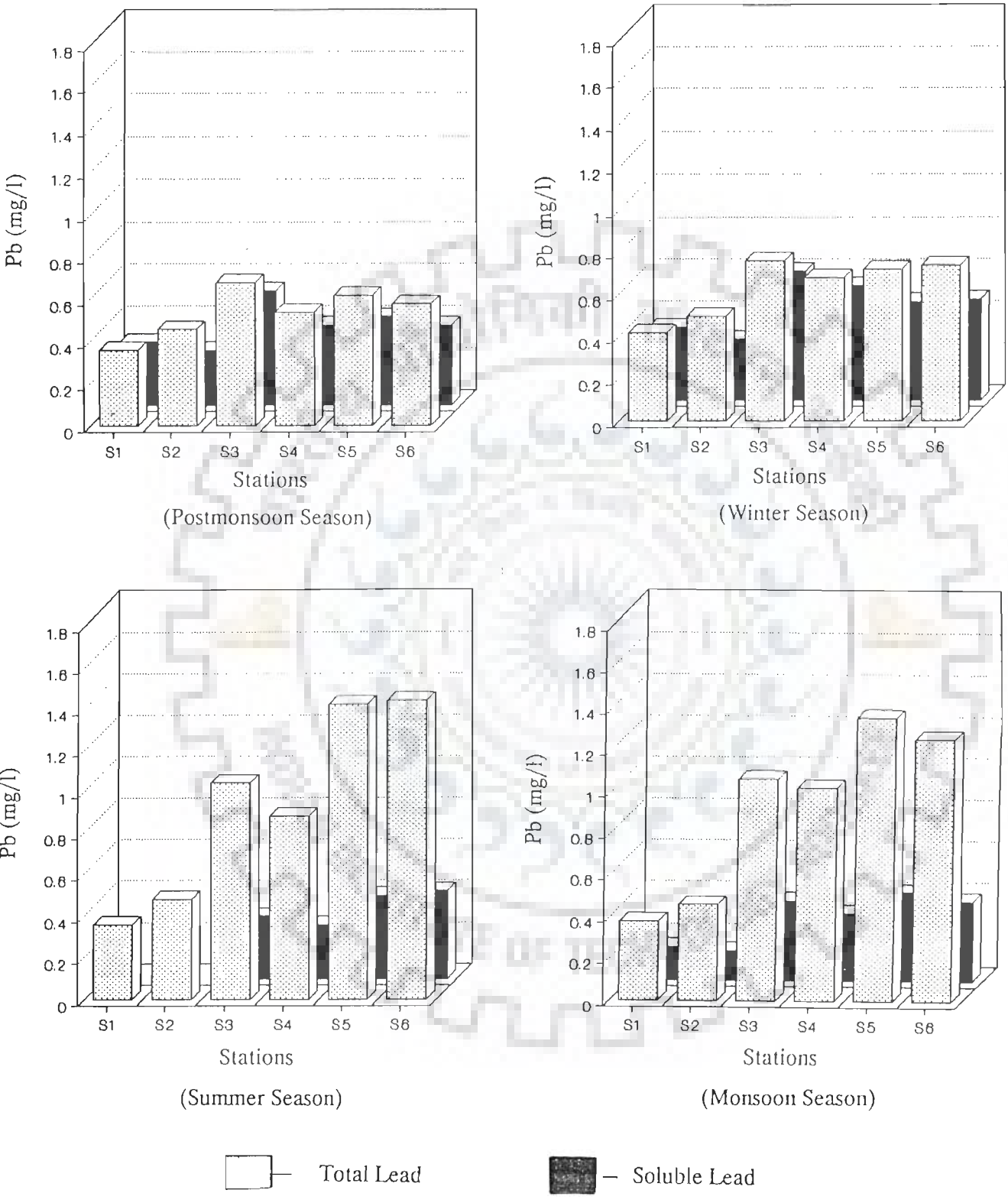


Fig 3.17 Variation of Total and Soluble Lead Content

characterised by low Pb concentrations in postmonsoon season. This may be because of surface runoff dilution in the monsoon season. Trends in soluble and total Pb concentrations show that soluble Pb was high in winters, whereas, in summers most of the Pb was in particulate form. Pb was in particulate form at S1 and S2 stations in summers and no soluble species were present (Fig 3.17).

Trends in the concentrations of Zn with their spatial and temporal variations are presented in Fig 3.18. Sharp seasonal variations were observed. Concentrations of Zn were high in postmonsoon season and may be due to variations in surface runoff and other climatic activities including soil erosion. During the study sharp spatial variations were observed in Zn concentrations. During summers, low concentrations of Zn were present and may be because Zn gets into the sediment phase due to alkaline nature of water. It is evidenced by increase in Zn concentrations in bed sediments during summers. Variations in soluble Zn show that in winter and monsoon seasons nearly 50% of total Zn is present as soluble fraction, whereas, in summers only 20 - 25% of total Zn constitute the soluble fraction (Fig 3.18).

A perusal of trends in concentrations of Cd, Pb, and Zn in the river water thus, show that both natural and anthropogenic activities contribute to HMs. Important among the natural sources are the weathering of rocks, precipitation and surface runoff, whereas, significant anthropogenic sources include industrial effluents and agricultural runoff. It is observed that these factors alter the concentrations of metals to varying degrees depending upon the intensity and quantum of load.

Variations of Heavy Metals in River Bed Sediments

Total metal concentrations of Al, Na, K, Ca, Mg, Fe, and Mn in bed sediments are presented in Table 3.3. Determination of metals in sediments is important as concentrations of heavy metals in sediments and their characteristics indicate the state of environment (Forstner and Wittmann, 1983). They determine distribution and fate of

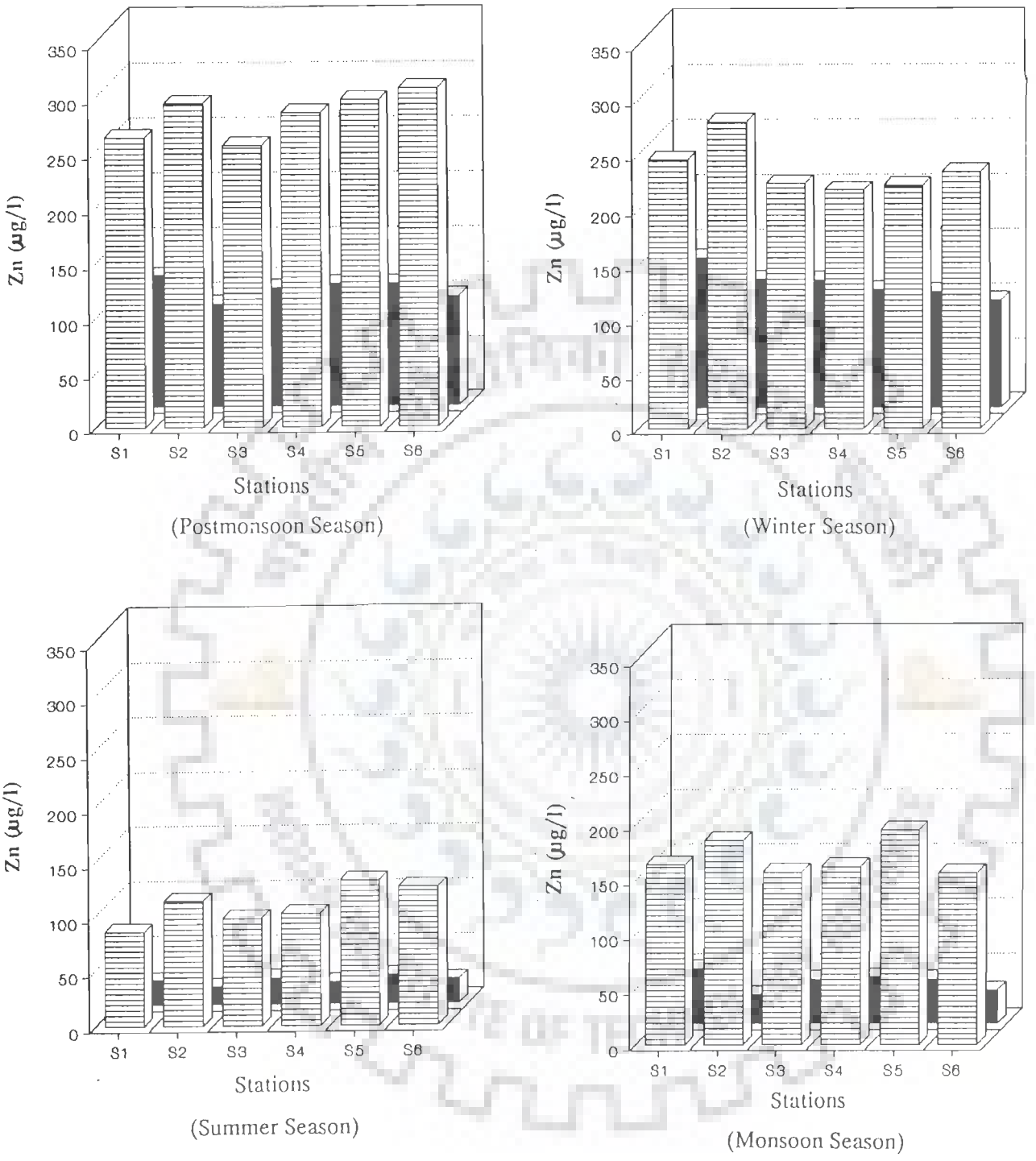


Fig 3.18 Variation of Total and Soluble Zinc Content

Table 3.3 Total Metal in River Bed Sediments (unit mg/gm)

Metals	Stations & Seasons														
	S 1					S 2					S 3				
	P.M.	W	P.W.	S	M	P.M.	W	P.W.	S	M	P.M.	W	P.W.	S	M
Al	24.30	28.60	34.10	32.50	29.90	68.40	64.10	60.60	61.80	63.70	36.50	38.60	43.50	42.60	40.30
Na	7.30	8.50	14.30	9.40	9.90	12.60	14.60	16.40	15.20	14.70	9.10	8.70	9.30	10.30	9.30
K	4.80	3.30	6.40	3.90	4.60	10.80	8.50	8.60	7.50	8.90	5.60	3.90	4.80	5.80	5.00
Ca	14.30	10.40	12.90	14.40	13.00	23.70	18.60	16.20	18.30	19.20	11.60	8.80	12.40	14.40	11.80
Mg	5.90	4.10	3.30	4.90	4.50	8.90	6.20	5.80	6.10	6.80	4.00	3.90	4.80	5.30	4.50
Fe	16.40	14.20	8.30	8.00	11.80	29.30	24.60	22.10	18.40	23.60	12.50	9.70	19.30	14.20	13.90
Mn	0.19	0.20	0.11	0.13	0.16	0.35	0.41	0.16	0.22	0.29	0.27	0.20	0.15	0.21	0.21

Contd. ....

Metals	Stations & Seasons														
	S 4					S 5					S 6				
	P.M.	W	P.W.	S	M	P.M.	W	P.W.	S	M	P.M.	W	P.W.	S	M
Al	44.20	52.80	48.80	49.50	48.80	26.30	36.50	32.50	30.60	31.50	30.90	42.60	36.50	34.30	36.10
Na	8.90	9.80	10.50	9.90	9.80	8.60	10.40	8.50	10.00	9.40	7.10	11.50	13.80	11.60	12.00
K	4.30	4.30	7.50	5.50	5.90	4.40	4.50	4.30	5.20	4.60	4.00	4.80	5.60	4.80	4.70
Ca	20.40	16.30	9.70	11.30	14.50	22.60	16.90	11.20	12.50	15.80	15.20	15.20	10.30	11.90	13.10
Mg	6.80	6.00	4.40	3.20	5.10	66.00	7.90	4.30	5.00	5.90	7.70	8.10	3.40	4.10	5.80
Fe	18.90	15.10	18.40	16.90	17.30	12.70	10.50	17.60	12.60	13.30	13.90	10.90	12.40	14.20	12.80
Mn	0.19	0.15	0.15	0.29	0.19	0.19	0.17	0.14	0.18	0.17	0.18	0.17	0.17	0.20	0.18

Note:- P.M. - Postmonsoon , W - Winter , S - Summer , M - Monsoon

several pollutants in the ecosystem, act as principal transport vehicle and define the site of accumulation or release. Partition of HMs at sediment-water phase is significant in variations of HMs in the ambient water.

Concentrations of HMs in the samples show that Al is the most abundant element followed by Fe, Ca, Na, Mg, K, and Mn (evidenced by their mean concentrations in all four seasons of the year). Relatively high percentage of Al in sediments may be due to immense weathering processes in which most of the elements are removed from the sediments leaving Al as residue (Ramesh, 1983). Na, K, Ca, Mg, Fe and Mn are present at varying concentrations, however, no significant spatial variations in their concentrations were observed.

Figure 3.19 shows the seasonal and spatial variations in the concentrations of Cd, Pb, and Zn in bed sediments. Cd shows prominent spatial variations. The concentrations of Cd at S3 - S6 were low as compared to S1 and S2 stations. The variations of Pb in bed sediments are also shown in Fig 3.19. Variations in Pb show an increasing trend downstream from S3 to S6 stations. Variations of Zn in bed sediments show low concentrations of Zn in bed sediments in postmonsoon and winter seasons at all the stations as compared to summer and monsoon seasons (Fig 3.19). The seasonal variations thus, show prominent variations of Zn. Sedimentation and industrial effluents probably add to the load of Zn and results in high Zn concentrations in summer season at all the stations. Spatial variations were not prominent. However, in general, higher concentrations of Zn were observed in the lower stretch of the river (Fig 3.19).

Metal Enrichments in Bed Sediments

The non-extractable or detrital components represent, generally, the immobile fraction of metal associated with bed sediments (Gadh, 1991). Taking Cd, Pb, and Zn as a typical reactive component and aluminium (Al) as detrital component, metal/Al ratio

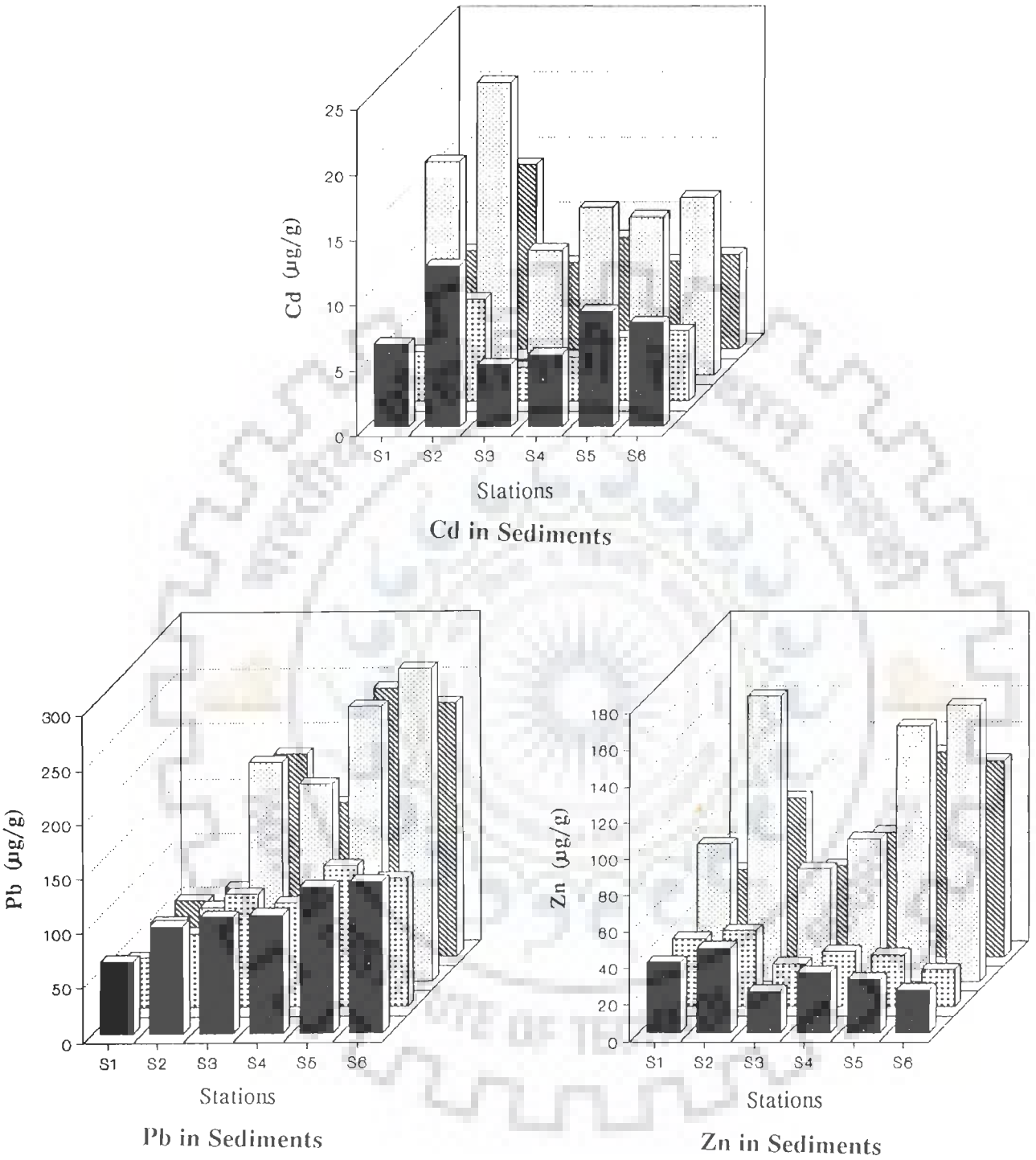


Fig 3.19 Variation of Cadmium, Lead and Zinc content

could be calculated and used to illustrate the dynamic behaviour of Cd, Pb, and Zn in a river system (Gadh, 1991). In the sediment elements as well as metals exist together in relative proportions to each other. These ratios are dependant on large number of processes in a geochemical cycle including weathering, transportation and deposition. Ratios of trace metals to conservative elements reveal the geochemical imbalance due to elevated trace metal concentrations normally attributed to anthropogenic activities. Table 3.4 shows the trace metal ratio of Cd, Pb, and Zn within the sediment samples. The metal pair ratios reflect a few enrichments; Cd at S1, S5 and S6 stations in summers; Pb at S3 station in monsoon season and S5 & S6 stations in summer season; Zn at S6 station in monsoon season and S5 & S6 stations in summer season.

Another method of indicating metal enrichment in sediments is the comparison of metal concentration in the sediments with the average shale value (Turekian and Wedepohl,1961) or by method of comparing their average values with those reported in earlier literature on some of the important rivers of the world (Mathis and Cummings, 1973; Reddy, 1978; Sakai et al., 1986) and Indian rivers (Sitaswad, 1984; Ajmal et al., 1987; Saikia, 1987; Subramaniam et al., 1987). A comparison of average values of Cd, Pb, and Zn in the bed sediments of Kalinadi (average of S1 and S2 stations) and river Hindon (average of S3, S4, S5, and S6 stations) is presented in Table 3.5. Values of Cd, Pb and Zn in Kalinadi and river Hindon show that metal load in river basin is high. Comparison of values with average shale value corroborate high concentrations of Cd, Pb, and Zn in the Hindon river basin.

Bioconcentration of Cadmium, Lead and Zinc

The results presented herein on body burdens of Cd, Pb, and Zn in the biota of the river have been discussed in terms of bioconcentration (BC) of Cd, Pb, and Zn in plankton (phyto and zoo) and bioconcentration factor (BCF). Bioconcentration

Table 3.4 Metal Pair Ratio (M/Al) For Cd , Pb , and Zn in River Bed Sediments

Stations	Metal Pair Ratio											
	$Cd/Al \times 10^{-4}$				$Pb/Al \times 10^{-3}$				$Zn/Al \times 10^{-3}$			
	P.M.	W	S	M	P.M.	W	S	M	P.M.	W	S	M
S1	2.64	1.32	5.04	2.23	2.72	1.61	1.68	1.53	1.59	1.32	2.34	1.41
S2	1.81	1.22	3.62	2.34	1.43	1.06	1.11	1.06	0.68	0.66	2.54	1.45
S3	1.31	0.68	2.25	1.54	2.90	2.70	4.75	4.28	0.61	0.61	1.46	1.15
S4	1.27	0.65	2.58	1.76	2.45	1.82	3.69	2.91	0.74	0.58	1.59	1.40
S5	3.39	1.33	3.94	2.09	5.10	3.56	8.26	7.59	1.10	0.79	4.59	3.47
S6	2.58	1.27	3.94	2.00	4.49	2.78	8.36	6.36	0.75	0.48	4.43	2.96

Note:- P.M. - Postmonsoon , W - Winter , S - Summer , M - Monsoon

Table 3.5 Concentrations of Heavy Metals in Sediments of Different Rivers of the World and Average Shale Value (units -μg/gm)

River	Cd	Pb	Zn	Reference
Genesse (U.S.A.)	-	40.0	69.0	Reddy, 1978
Toyohira (Japan)	0.2	24.0	152.0	Sakai et al., 1986
Illinois (U.S.A.)	2.0	28.0	81.0	Mathis & Cummings, 1973
Ganga (India)	2.6	25.6	36.1	Saikia, 1987
Ganga (India)	-	25.0	46.0	Subramaniam et al., 1987
Yamuna (India)	3.8	243.7	88.8	Gadh, 1991
Yamuna (India)	-	57.0	123.0	Sitaswad, 1984
Average shale	0.3	20.0	95.0	Turekian & Wedepohl, 1961
Kalinadi	11.4	64.8	66.7	Present study
Hindon	7.6	166.7	61.5	Present study

is reported on a dry weight basis (µg metal (Cd, Pb or Zn)/ gm plankton or fish tissue) and the BCF as:

$$BCF = \frac{\text{Equlibrium tissue concentration of HMs (Cd, Pb, or Zn)}}{\text{Concentration of metal in the ambient water}}$$

Results show that the concentrations of Cd, Pb, and Zn present in phytoplankton, zooplankton and fish were highly elevated as compared to their values in the ambient environment. Plankton and fish selectively sequester toxic metals present in the system and concentrate them to overcome the toxic effects by selectively precipitating/complexing them inside their body in an innocuous form. The bioconcentration values are reported in Table 3.6-3.14. Plankton and fish show variation in their uptake capabilities and in sequestering the toxicants from the aquatic environment.

Bioconcentration of Cadmium

The bioconcentration levels of Cd are detailed in Table 3.6-3.8. In phytoplankton Cd concentrations varied between 2.9-16.4 µg/gm, with the highest value at station S1 in winter season. During monsoon, a value of 2.9 µg/gm was observed at station S5. Seasonal variations had marked effect on Cd bioconcentration. In zooplankton Cd concentrations varied from Nil-18.6 µg/gm, the highest being at S2 station in summer season. In whole body fish tissue the concentration varied from 4.1-30.2 µg/gm. The significant value of 30.2 µg/gm was at S2 station in the winter season.

Bioconcentration of Lead

Spatial and temporal variations in bioconcentration of Pb in plankton and fish are reported in Table 3.9-3.11. Pb concentrations in phytoplankton varied between 6.2-46.4 µg/gm. Marked seasonal and temporal variations were noticed. Higher concentrations were observed in winters with the maximum concentration of 46.4 µg/gm at S3

Table 3.6 Bioconcentration (BC) and Bioconcentration Factor(BCF) of Cadmium in Phytoplankton

Stations	Seasons							
	Postmonsoon		Winter		Summer		Monsoon	
	BC	BCF	BC	BCF	BC	BCF	BC	BCF
S1	12.3	141.4	16.4	139.0	5.8	43.9	10.5	119.3
S2	12.4	108.8	16.2	119.1	3.7	25.3	8.6	84.3
S3	8.2	170.8	12.8	168.4	9.4	151.6	3.6	67.2
S4	6.4	106.7	8.6	84.3	N.A.	N.A.	4.5	70.3
S5	10.6	160.6	N.A.	N.A.	2.9	31.5	1.9	27.4
S6	11.9	185.9	13.0	138.1	4.0	40.4	3.2	44.4

Table 3.7 Bioconcentration (BC) and Bioconcentration Factor(BCF) of Cadmium in Zooplankton

Stations	Seasons							
	Postmonsoon		Winter		Summer		Monsoon	
	BC	BCF	BC	BCF	BC	BCF	BC	BCF
S1	2.5	28.7	4.8	40.7	14.2	107.6	10.8	135.0
S2	0.0	-	2.3	17.2	18.2	127.4	14.2	139.2
S3	0.0	-	5.3	69.7	4.2	67.7	6.8	117.2
S4	0.0	-	0.0	-	N.A.	-	2.7	41.9
S5	0.0	-	-	-	10.3	111.9	11.4	167.6
S6	1.4	21.9	1.9	20.0	12.2	124.9	8.7	120.6

Table 3.8 Bioconcentration (BC) and Bioconcentration Factor(BCF) of Cadmium in Whole Body Fish Tissue

Stations	Seasons							
	Postmonsoon		Winter		Summer		Monsoon	
	BC	BCF	BC	BCF	BC	BCF	BC	BCF
S1	7.9	90.8	26.4	223.7	12.3	93.2	8.1	92.0
S2	10.1	88.6	30.2	222.1	N.A.	-	14.9	146.6
S3	5.4	112.5	14.1	185.5	5.7	91.9	6.4	110.3
S4	3.8	63.3	9.9	97.1	N.A.	-	18.6	290.6
S5	4.1	62.1	N.A.	-	18.6	202.2	N.A.	-
S6	4.9	76.6	10.3	109.6	N.A.	-	10.2	141.7

Note : N.A. - Not Analysed ; BC Units - µg/gm

Table 3.9 Bioconcentration (BC) and Bioconcentration Factor(BCF) of Lead in Phytoplankton

Stations	Seasons							
	Postmonsoon		Winter		Summer		Monsoon	
	BC	BCF	BC	BCF	BC	BCF	BC	BCF
S1	16.8	46.7	38.2	91.0	6.8	18.9	12.8	33.7
S2	7.8	17.0	30.8	61.6	4.2	8.7	8.6	18.7
S3	18.6	27.4	46.4	61.3	8.6	8.3	18.8	17.7
S4	14.2	26.3	38.8	57.0	N.A.	-	6.2	6.1
S5	10.8	17.4	N.A.	-	8.4	5.9	15.4	11.3
S6	12.4	21.4	44.5	60.1	6.2	4.3	18.6	14.8

Table 3.10 Bioconcentration (BC) and Bioconcentration Factor(BCF) of Lead in Zooplankton

Stations	Seasons							
	Postmonsoon		Winter		Summer		Monsoon	
	BC	BCF	BC	BCF	BC	BCF	BC	BCF
S1	14.2	39.4	8.8	21.0	7.4	20.6	8.6	22.6
S2	8.6	18.7	6.2	12.4	6.8	14.2	9.8	21.3
S3	10.4	15.3	4.6	6.1	16.2	15.6	16.6	15.7
S4	4.9	9.1	10.2	14.2	N.A.	-	26.4	25.8
S5	6.6	10.6	N.A.	-	24.6	17.3	16.2	11.9
S6	7.8	13.4	5.4	7.3	26.4	18.3	12.4	9.8

Table 3.11 Bioconcentration (BC) and Bioconcentration Factor(BCF) of Lead in Whole Body Fish Tissue

Stations	Seasons							
	Postmonsoon		Winter		Summer		Monsoon	
	BC	BCF	BC	BCF	BC	BCF	BC	BCF
S1	14.6	40.6	26.1	62.1	12.1	33.6	23.7	62.4
S2	20.2	43.9	28.1	56.2	N.A.	-	32.6	70.9
S3	20.4	30.0	24.5	32.2	48.6	46.7	19.6	19.2
S4	28.4	52.6	22.8	4.1	N.A.	-	8.4	6.2
S5	16.2	26.1	N.A.	-	24.6	17.3	N.A.	-
S6	11.6	20.0	21.4	28.9	N.A.	-	4.6	3.6

Note : N.A. - Not Analysed ; BC Units - µg/gm

Table 3.12 Bioconcentration (BC) and Bioconcentration Factor(BCF) of Zinc in Phytoplankton

Stations	Seasons							
	Postmonsoon		Winter		Summer		Monsoon	
	BC	BCF	BC	BCF	BC	BCF	BC	BCF
S1	54.6	210.0	42.6	170.4	28.9	317.8	22.6	141.3
S2	38.8	129.3	88.9	317.5	32.4	294.5	16.4	86.3
S3	46.2	177.7	68.8	312.7	14.6	146.0	18.2	113.7
S4	86.6	298.6	78.4	356.4	N.A.	-	20.8	130.0
S5	104.2	347.3	N.A.	-	38.4	295.4	18.2	95.8
S6	76.8	247.7	114.5	497.8	40.2	309.2	26.5	165.6

Table 3.13 Bioconcentration (BC) and Bioconcentration Factor(BCF) of Zinc in Zooplankton

Stations	Seasons							
	Postmonsoon		Winter		Summer		Monsoon	
	BC	BCF	BC	BCF	BC	BCF	BC	BCF
S1	46.8	180.0	56.4	225.6	88.4	982.2	38.2	238.8
S2	42.4	141.3	32.4	115.7	42.0	381.8	26.8	141.1
S3	28.6	110.0	48.0	218.2	96.8	968.0	30.4	190.0
S4	64.8	223.5	86.3	392.3	N.A.	-	22.6	141.3
S5	54.8	182.6	N.A.	-	78.4	603.1	36.4	191.6
S6	72.2	232.9	46.2	200.9	54.0	415.4	20.8	130.0

Table 3.14 Bioconcentration (BC) and Bioconcentration Factor(BCF) of Zinc in Whole Body Fish Tissue

Stations	Seasons							
	Postmonsoon		Winter		Summer		Monsoon	
	BC	BCF	BC	BCF	BC	BCF	BC	BCF
S1	294.2	1131.5	362.8	1451.2	186.4	2071.1	214.6	1341.3
S2	304.2	1014.0	318.2	1136.4	N.A.	-	256.2	1348.4
S3	318.4	1224.6	412.6	1875.5	158.6	1586.0	168.2	1051.3
S4	186.6	643.4	386.4	1756.4	N.A.	-	189.8	998.9
S5	248.6	828.7	N.A.	-	104.8	806.2	N.A.	-
S6	188.8	641.3	248.8	1081.7	N.A.	-	224.2	1041.3

Note : N.A. - Not Analysed ; BC Units µg/gm

station. Spatial variations show higher concentrations at S3- S6 stations than at S1 and S2 stations. The concentration in zooplankton was 4.6-26.4 µg/gm. The highest concentration was present at S6 station in summer season. Spatial variation of Pb in zooplankton was not prominent, however, at S3-S6 stations Pb concentrations were high as compared to S1 and S2 stations. Pb concentrations in whole body fish tissue were 4.6-48.6 µg/gm. Highest concentration was observed at S3 station in summer season.

In general, Pb concentrations in plankton and fish were variable and site specific. It shows that physico-chemical environment have impacts on bioconcentration resulting in wide variations in metal concentrations in biota in the ecosystem.

Bioconcentration of Zinc

Table 3.12-3.14 present the levels of Zn in biota. Levels of Zn in phytoplankton and zooplankton were invariably high as compared to the ambient water. Concentration in phytoplankton varied between 14.6 µg/gm and 114.5 µg/gm and 20.8-96.8 µg/gm dry wt. in zooplankton. In phytoplankton, Zn was high in postmonsoon and winter seasons, whereas, in zooplankton high concentrations were observed in summer season.

Bioconcentration of Zn in whole body fish tissue also shows wide variations. In winters Zn concentration upto 412.6 µg/gm was recorded at S3 station. Variations recorded in the Zn levels were 104.8-412.6 µg/gm. Seasonal variations exemplify high concentrations of Zn in postmonsoon and winter seasons as compared to monsoon and summer seasons.

Reports corroborating the result presented herein are on records (Demon et al., 1980; Koli et al., 1980; Fayed et al., 1983; Rachlin et al., 1984; Salles and Bailey, 1984; Ajmal et al., 1985).

In his field studies, Roth and Hornung (1977) studied the Mediterranean coastal area and reported heavy metal concentration in water, sediments, algae and fish. In

chlorophyta, the bioconcentration of Cd, Pb, and Zn was reported as 0.9, 1.9 and 218 µg/gm, respectively (dry weight basis). Rhodophycean algae was reported to contain Pb at 22.2 µg/gm in Haifa beach. Concentration of Cd, Pb, and Zn were also reported in fish Sardinella aurita , Epinephelus aeneus and Siganus riulatus as 0.60, 0.60, 1.60, 0.10, 0.04, 33.00 and 0.20, 0.30, 25.80 µg/gm (dry weight), respectively. The highest concentration of Zn was reported in Sardinella aurita (61-84 µg/gm) (Roth and Hornung, 1977).

Koli et al. (1980) have reported Cd in shellfish species of Atlantic coast of south Carolina. Oysters were reported to contain 0.189 ppm Cd whereas boneyfish contained 0.010 ppm Cd.

Saltes and Bailey (1984) reported upto 1000 and 395 µg/gm Zinc in gills and liver of rainbow trout (Salmo gairdneri) and brook trout (Salvelinus fontinalis) from Spokane river. No consistent difference in tissue concentration between rainbow trout and brook trout was observed by them.

Saiki and May (1988) analyzed the whole body samples of blue gills (Lepomis macrochirus) and common carp (Cyprinus carpio) from San Joaquin and Merced River. They reported elevated concentrations of Cd and Pb in fish exposed to agriculture water. Cd levels were 0.14 and 0.27 µg/ gm in bluegills and common carp, respectively. While the Pb concentrations were 0.26 and 2.3 mg/gm dry weight, respectively.

The information available on bioconcentration of HMs in fresh water organisms in the riverine ecosystems from Indian sources is rather scanty. Ajmal et al. (1985) have reported distribution of Cd, Pb, and Zn in plant and fish of Yamuna river from Delhi to Allahabad. They reported concentrations of Cd, Pb and Zn in plants (Eicchornia crassipes 0.02 - 0.12, 4.80 - 30.20 and 22.10- 356.50 mg/gm for Cd, Pb and Zn, respectiely). In Heteropneustes fossilis the concentrations of Cd, Pb, and Zn were 0.0 - 0.40, 1.40 - 12.80

and 101.80 - 364.80 µg/gm, respectively. Ajmal et al. (1983) have also reported elevated levels of Cd, Pb and Zn in fish and submerged plants of Ganga river.

A general comparison of trace metal bioconcentration in different species of plankton and fish corroborates the findings on bioconcentration of Cd, Pb, and Zn reported in Hindon riverine ecosystem. Study suggests that bioconcentration is influenced by the physico-chemical environment. Several investigators (Weiner et al., 1984; Lowe et al.,1985; Schmitt and Finger, 1987) have reported that species of fish, variations in foraging behaviours and microhabitat biota affect the rate of uptake.

Impacts of Physico-Chemical Environment on Bioconcentration

Physico-chemical environment strongly influence the phenomenon of bioconcentration of HMs in plankton and fish (Ajmal et al. 1982; Forstner and Wittmann,1983; Hobe, 1987). In the present study an attempt has been made to correlate the bioconcentration in phytoplankton, zooplankton and fish with the physico-chemical characteristics of water (pH, DOC, hardness, alkalinity and conductivity). Table 3.15 presents the correlation coefficients of bioconcentration with individual physico-chemical parameters of water. A perusal of data expounds no established relationship between bioconcentration and pH, DOC, hardness, alkalinity or conductivity, individually. Correlation coefficients are highly variable (negative or positive) for the same parameter and bioconcentration at different trophic levels or for a specific metal (Cd, Pb or Zn) (Table 3.15).

Results on the impact of physico-chemical environment on bioconcentrations and the variability of individual parameter on bioavailability of metal ions in the system presented herein have been supported by many investigators (Forstner, 1977; Skowronski, 1986; Verbost et al., 1987; Prahalad and Seenayya, 1988; Spry and Wood, 1989).

pH of the water has a considerable effect on the bioavailability of metals. In general, at low pH, metals exist as free ionic cations (principal bioavailable species), but at higher

Table 3.15 Correlation Coefficients of Bioconcentration with Physico-Chemical Parameters of Water

Trophic Level & Metals	Correlation Coefficients (r)				
	Water Quality Parameters				
	pH	DOC	Alkalinity	Hardness	Conductivity
For **Cd**					
Phytoplankton	0.36	0.37	- 0.39	- 0.47	- 0.46
Zooplankton	- 0.37	- 0.22	0.25	0.26	0.26
Fish	0.41	0.37	0.25	- 0.10	- 0.14
For **Pb**					
Phytoplankton	- 0.79	- 0.68	- 0.61	- 0.50	- 0.58
Zooplankton	- 0.10	- 0.14	- 0.14	- 0.35	- 0.28
Fish	0.22	0.28	- 0.20	- 0.42	- 0.33
For **Zn**					
Phytoplankton	0.56	0.68	0.66	0.68	0.68
Zooplankton	- 0.47	- 0.46	- 0.56	- 0.36	- 0.44
Fish	- 0.36	- 0.30	- 0.32	- 0.57	- 0.49

pH, the ionic cations precipitate as insoluble hydroxides or oxides. Existing literature shows that a reduction in pH leads to greater uptake of metals (Hart and Scaife, 1977).

Skowronski (1986) reported that high pH decreases the uptake of Cd in Stichococcus bacillaris. Cd precipitation occurs above pH 7.5 and thus results into the diminution of bioconcentration at higher pH. Brumbaugh and Kane (1985) showed that solubility generally increases inversely with pH and the soluble Cd is absorbed by plants and accumulated in herbivores and predators. However, metal binding at cell surfaces is recognised to be dependent on the availability of unprotonated membrane sites (Sakaguchi et al., 1979). Thus, the prediction that low pH will be correlated with higher body burdens may not be appropriate for all elements or all organisms and it would be important to determine the effects of pH as an important determinant of bioconcentration of toxic metals in aquatic organisms.

Presence of complexing agents usually results in a decrease in accumulation although it is not always the case (Poldoski, 1979). Sedlacek et al. (1983) reported that accumulation and toxicity of cadmium to green algae Selanastrum capricornutum, grown in a medium devoid of chelating agents in the laboratory was diminished by aquatic humus (Humic acids, Pyrophosphate (P_2O_7), NTA, EDTA and EBDP. However, on the other hand, DDC stimulates the uptake of metals so that uptake is greater than for comparable concentrations of free metal ions (Poldoski, 1979).

The observations, thus, support that the organic matter present in the system is one of the important determinants of bioavailability of metal ion and need a close observation. The other important determinants of chemical character of water influencing the bioconcentration include water hardness, metals in sediments, conductivity and other ions.

Metal bioconcentration in aquatic organisms is reported to be hardness dependent (Kinkade and Erdman, 1975). It is reported that bioavailability of HMs is markedly affected by the presence of Ca and Mg (Wright, 1977). Demon et al. (1980) reported that

presence of high concentrations of Ca affects trace element uptake and results in a decrease in uptake of Cd and Zn by algae Scendesmus pannonicus. It is also reported that waterborne Ca is an important determinant of branchial permeability in the rainbow trout, Salmo gairdneri (McWilliams, 1983) and restricts access of transported ions to their carriers or channels (McDonald, 1983). Both Cd or Zn and Ca being divalent cations could conceivably compete the same transport mechanisms and effect uptake in vivo (Spry and Wood, 1985; Verbost, 1987). This increased water hardness results into competetive interactions between Ca and other toxic divalent ions at membranes and effect their uptake (Spear, 1981; Pagenkopf, 1983).

Besides Ca and Mg other ions e.g., phosphate, carbonate or sulfates can form precipitates with HMs depending on their concentrations and the pH of the medium and effect the uptake of HMs in aquatic organisms (Gadd and Griffiths, 1978).

In the present study, the approach to find out important determinants of chemical characters of water influencing bioconcentration of Cd, Pb, and Zn is extended and multivariate analysis (Principal Component Analysis) has been used with the following objectives:

- to determine variables at different locations contributing significantly to bioconcentration, and
- to evaluate the validity of sampling network and frequency.

Principal Component Analysis

The purpose of Principal Component Analysis (PCA), in general, is to interpret the structure within the variance-covariance matrix of a multivariate data collection. The technique uses extraction of an eigen value and an eigen vector from the matrix of correlations. PCA is commonly regarded as a deep and mysterious methodology of great complexity. Analysts are sharply divided on the topic, both as to the validity and utility

of the technique. Nevertheless, it is one of the most widely used procedures and extends a beguiling promise to experiments faced with multicomplex data. Sometimes it confirms the temporal and spatial variations of the parameters besides providing additional information.

The following terms are used in Principal Component Analysis:

(i) **Mean** is the arithmatic average of sample population and is expressed as :

$$x = i = 1 \sum^{n} \frac{x_i}{n}$$

x_i = Random sample from normal distance whose mean is x^2

(ii) **Variance** is defined as the average squared deviation of all possible deviations from the population mean and can be expressed mathematically as,

$$S^2 = i=1 \sum^{n} \frac{(x_i - x)2}{n}$$

and the square root of variance is termed as the **standard deviation** and is expressed as,

$$S = \left[i=1 \sum^{n} \frac{(x_i - x)^2}{n} \right] -2$$

(iii) **Covariance**

The variables measured on the same observational unit tend to change together in the same manner. Because of their dependency, some measure of their mutual interaction is needed. This measure is termed as covariance which, in other words, is the joint variance of two variables about their common mean.

(iv) **Correlation Coefficients**

For determining the degree of interaction between variables, correlation coefficient is used. It is the ratio of the covariance of two variables to the product of their deviations and can be expressed as,

$$r_{ij} = \frac{COV_{ij}}{S_i S_j}$$

where

r_{ij} is the correlation coefficient of the variable

i, j and S_i and S_j are standard deviations of the variables i and j and COV_{ij} is the variance of these variables.

Correlation coefficient is a unitless number whose value ranges from +1 to -1. The positive sign indicates a direct relation between the two variables, whereas, the negative sign indicates that the two variables are inversely correlated.

(v) Eigen values and Eigen vectors

PCA mainly consists of finding of the Principal Components that are nothing more than the eigen vectors of a variance-covariance matrix. Eigen values are the length of various axes of the m-dimentional ellipsoide and eigen vectors are the orientation of the axes of the ellipsoide. PCA is mainly concerned with the finding of these axes and measuring their magnitude. If m-variables of the data are taken, m x m matrix of variance-covariance can be computed.

(vi) **Principal Components**

Principal components are the eigen vectors of variance-covariance matrix. If a number of variables are measured on a set of samples a linear transformation of these variables will yield new variables. These new variables are called principal components

and are independent of each other and account as much total variation as possible. This process of computation is called Principal Component Analysis (PCA).

Procedure Followed

Data of variables of 4 observations (seasonal variations) at 6 locations was organised in two different matrices, typed as input files. The first type of matrix contains the data of i variables of m observations at n locations. The variables included were pH, conductivity, TDS, nitrates, DO, bicarbonates, carbonates, total hardness, chlorides, DOC, sulfates, total and soluble metal (Cd, Pb, and Zn) in water and metal (Cd, Pb, and Zn) in sediments. The input files of two stations (S1 & S2) are presented in Appendix A.

The second type of matrix included the data of i variables at n locations during mth observation and the input files for two seasonal observations (postmonsoon and winter seasons) are presented in Appendix B.

The principal components were computed on PC-AT 286 using modified program on Principal Compenent Analysis (Davis, 1973). The flow chart of the program is presented in Figure 3.20 and the listing is given in Appendix C.

The principal components contributing upto 90% were considered for further interpretations. In general, the variables that constitute the major Principal Components were identified from the eigen vectors by fixing a cut off limit. This limit is subjective and as such no clear guidelines are available in the literature to decide this limit. In the present study, the cut off limit of eigen vectors was kept as 0.2500 and the variables yielding the eigen vectors above this limit were only considered.

Interpretations of PCA

The important variables contributing the principal components at different locations are presented in Table 3.16. In all the cases it was observed that the first three

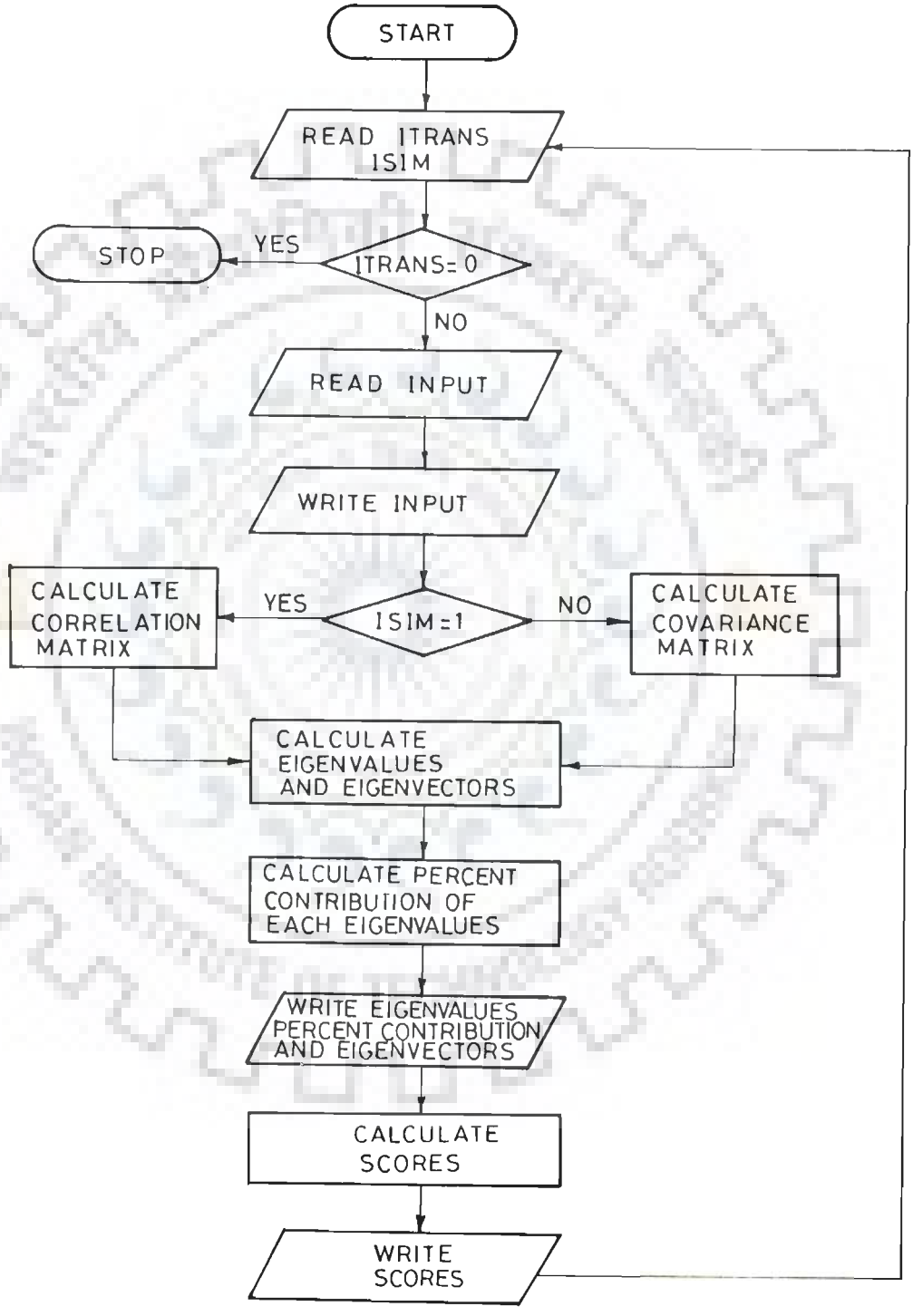


Fig 3.20 Principal Component Analysis (PCA)

Table 3.16 First Three Principal Components Contributing to the Chemical Character of Water

n & m	PCA 1	PCA 2	PCA 3	Comulative PCA
At nth location				
Station S1	32.8%	25.7%	20.5%	79.0%
Station S2	33.9%	29.9%	22.5%	86.3%
Station S3	41.7%	32.7%	20.0%	94.4%
Station S4	38.7%	31.8%	29.2%	99.6%
Station S5	34.2%	31.8%	23.6%	89.6%
Station S6	31.1%	29.5%	21.3%	81.6%
In mth observation				
Postmonsoon	34.8%	33.2%	20.5%	88.5%
Winter	38.6%	31.3%	20.1%	90.0%
Summer	32.5%	26.2%	19.0%	77.7%
Monsoon	33.5%	33.2%	24.0%	90.7%

Note : Cumulative PCA = PCA 1 + PCA 2 + PCA 3

principal components contribute more than 80% . The variables constituting upto 3rd principal component are termed as significant.

Table 3.17 & 3.18 show that pH, conductivity, TDS, total hardness, DOC, bicarbonates, carbonates, total and soluble metal in water are among the significant parameters. The results presented on eigen vectors contributing significantly to principal components thus, expound that natural aquatic systems react in a unique way with chemical pollution. Therefore, except for screening purposes, it may not be sufficient to describe the environmental bioconcentration by a single parameter or a single chemical character of water as a determinant of bioconcentration in the natural environment but summary parameters should be used to assess the risk of bioconcentration potentials in the natural aquatic ecosystems.

Thus, to epitomise it could be stated that in the natural riverine ecosystem it would not be sufficient to describe the bioconcentration by a single parameter but summary parameters would assess the bioconcentration of toxic metal ions in a better way.

Water Quality Indices

Water quality indices, are formulated as a numerical scale to represent the gradations in water quality levels. An index, in general, is a comparison of a quantity to a scientific or arbitrary standard or to a pre-specified base (Lohani, 1984).

The concept of water quality indices was started way back in 1848 when attempts were made to correlate the levels of water purity with the occurrence of certain biological organisms. A concept of classification of a water body, based on the use of a numerical scale to represent gradations of quality was pioneered by Horton in 1965. The Horton's index provided basis for the development of other indices in later years.

Water Quality Indices (WQI) provide tangible information about the percentage of pollution or purity of waters by avoiding huge amount of data. WQI also facilitate a

Table 3.17 Significant Parameters Contributing to First Three Principal Components in Various Seasons

Parameters	Seasons											
	Postmonsoon Season			Winter Season			Srmmer Season			Monsoon Season		
	PCA1	PCA2	PCA3	PCA1	PCA2	PCA3	PCA1	PCA2	PCA3	PCA1	PCA2	PCA3
pH	*			*	*	*	*			*		*
Conductivity	*					*	*					
TDS	*				*	*	*					
Nitrates	*	*					*					
DO							*				*	
Bicarbonates		*		*			*	*			*	
Carbonates	*					*						*
Total Hardness	*			*								
Chlorides									*	*		
DOC				*			*		*			
Sulfates												
Total Cd in Water								*			*	
Soluble Cd in Water									*			
Total Pb in Water									*		*	
Soluble Pb in Water		*										
Total Zn in Water		*	*								*	
Soluble Zn in Water			*		*				*		*	
Total Cd in Sediments											*	*
Total Pb in Sediments												
Total Zn in Sediments												

Note : * - Significant Parameter ; PCA1 , PCA2 , PCA3 - Principal Components.

Table 3.18 Significant Parameters at Various Stations Contributing to First Three Principal Components

Parameters	Stations								
	Station S 1			Station S 2			Station S 3		
	PCA1	PCA2	PCA3	PCA1	PCA2	PCA3	PCA1	PCA2	PCA3
pH	*			*		*		*	
Conductivity				*				*	
TDS	*			*				*	
Nitrates	*			*					
DO				*	*		*		
Bicarbonates							*		
Carbonates									
Total Hardness					*		*		
Chlorides			*	*					
DOC			*	*				*	
Sulfates									
Total Cd in Water									
Soluble Cd in Water									
Total Pb in Water			*		*				
Soluble Pb in Water						*			
Total Zn in Water									*
Soluble Zn in Water									
Total Cd in Sediments									
Total Pb in Sediments									
Total Zn in Sediments									

Contd. . . . . .

Parameters	Stations								
	Station S 4			Station S5			Station S 6		
	PCA1	PCA2	PCA3	PCA1	PCA2	PCA3	PCA1	PCA2	PCA3
pH		*	*	*			*		
Conductivity		*		*					
TDS		*		*					
Nitrates	*			*					
DO	*			*					
Bicarbonates	*			*					
Carbonates						*			
Total Hardness		*			*				
Chlorides									
DOC	*			*			*		
Sulfates	*								
Total Cd in Water									
Soluble Cd in Water					*				
Total Pb in Water							*		
Soluble Pb in Water									
Total Zn in Water									*
Soluble Zn in Water									
Total Cd in Sediments	*		*			*			*
Total Pb in Sediments					*	*			*
Total Zn in Sediments									

Note : * - Significant Parameter ; PCA1 , PCA2 , PCA3 - Principal Components.

better system for quality monitoring. Ott (1978) identified six basic uses of WQI as :

- resource allocation,
- ranking of the location by comparing the environmental conditions at different locations or geographical areas,
- standard enforcement i.e., to determine the extent to which the standard criteria is met,
- trend analysis i.e., to determine the changes in environmental quality that has occurred over a period,
- public information, and
- specific research (indices may be applied as a means for reducing large quantity of data to a form that gives insight to the research).

Thus, the indices are concise and objective tools to analyse the trends of water quality. Ott (1978) suggested a classification of WQI in a generalized way:

(i) General WQI are based on the assumption that water quality is a general attribute of surface waters.

(ii) Specific use WQI are developed with respect to a specific use of water body viz., irrigation, bathing and drinking.

(iii) Planning WQI are generated for management purposes for decision making.

(iv) Statistical approaches are mainly based on either factor analysis or non parameteric multivariate transforms.

Table 3.19 illustrates the mathematical characteristics of some WQI reported in literature. The principle behind the index calculation, the main formulations and the flexibility to include and exclude any parameter is presented in Table 3.20. The variables included in WQI are presented in Table 3.21.

HORTON'S INDEX

Based on rating of water quality on comparative basis, Horton proposed the

Table 3.19 Mathematical Characteristics of Some Water Quality Indices

Index	Subindices	Aggregation Function	Comments
Horton	Segmented Linear	Weighted sum multiplied by two dichotomous terms	Eclipsing Region
NSF WQI	Implicit Nonlinear	Weighted sum	Eclipsing Region
Landwehr et al	Implicit Nonlinear	Weighted product	Nonlinear
McDuffie and Haney	Linear	Wighted sum	Eclipsing Region
Prati et al	Segmented Nonlinear	Weighted sum (Arithmetic mean)	Eclipsing Region
Dinius	Nonlinear (Linear,Power)	Weighted sum	Eclipsing Region
Dee et al	Implicit Nonlinear	Weighted sum	Eclipsing Region

Table 3.20 Formulations and the Parameter Flexibility in the Reported General Water Quality Indices

Index	Formulation	Principle	Parameter Flexibility
Horton's	$QI = \frac{\sum_{i=1}^{n} Wi\ Ii}{\sum_{i=1}^{n} Wi}$	Rating & Weightages based on auther's judgement	Any parameter can be included or excluded
NSF WQI	$WQIa = \sum_{i=1}^{n} Wi\ Ii$	Rating & Weightages based on experts opinion poll	Parameters are fixed
Pratis's implicit Index of Pollution	$I = 1/13 \sum_{i=1}^{13} Ii$	Explicit Mathematical function for sub index values have been developed on the basis of auther's own judgement on severity of pollution	Parameters are fixed
McDuffie's River Pollution Index (RPI)	$Ii = 10[X/XN]i$	Sub-indices were calculated by dividing the parmaeter value with a control value (standard) and multiplying with a factor to put the index value in a scale	Any paramater can be included or excluded
	$RPI = \frac{10}{n+1} \sum_{i=1}^{n} Ii$		
Dinius Social Accounting System	$I = [1/21] \sum_{i=1}^{21} WiIi$	Subindex functions and weightages taken were based on the auther's evaluation of the importance of each pollutant variable	Any parameter can be included or excluced

Table 3.21 Variables Used in Water Quality Indices

Variables	Water Quality Indices					
	Horton	NSF	Prati et al	McDuffie et al	Dinius	Dee et al
Physical						
pH	*	*	*		*	*
Temperature	*	*		*	*	*
Conductivity	*			*	*	
Turbidity		*				*
Dissolved Solids						*
Suspended Solids			*			
Total Solids		*				
Color					*	
Other						*
Chemical						
D.O	*	*	*	*	*	*
C.O.D.			*	*		
B.O.D.		*	*	*	*	*
Alkalinity	*				*	
Hardness					*	
Chlorides	*		*		*	
Sulfates						
Phosphates		*		*		
Fluorides						
Nitrogen						
Ammonical			*			
Nitrite						
Nitrate		*	*			
Other				*		*
Oil and Grease						
Phenol						
ABS	*					
CCE	*		*			
Iron			*			
Manganese			*			
Others			*	*		*
Biological						
Fecal coliform		*			*	*
Total coliform	*			*	*	

first formal WQI. In Horton's index ten variables were included with the following selection criteria:

- the number of variables should be limited to avoid the index becoming bulky,
- the variables should be of significance, and
- the variables should reflect the availability of the data.

The variables included in Horton's index with their respective weightage and break points of rating scale for Ohio River Sanitation Commission's Data is presented in Table 3.22 and 3.23.

Horton's WQI uses a linear sum aggregation function. It consists of the weighted sum of the sub-indices divided by the sum of the weights and multiplied by two coefficients M1 and M2. The formula used was:

$$WQI = \frac{\sum_{i=1}^{n} Wi\ Ii}{\sum_{i=1}^{n} Wi}$$

The break points of rating scale and the weightage assigned to the variables were based on the author's judgement.

In India, Bhargava (1983) developed two specific use indices for Ganga water. Singhal et al. (1987) modified the rating procedure and the weights with inclusion of few more parameters in their modified Horton's Index calculated for Hindon river. They considered pH, conductivity, DO, chemical oxygen demand, biochemical oxygen demand, ammonical nitrogen, nitrate nitrogen, nitrite nitrogen, phosphates, chlorides, sulfates, cadmium, lead and chromium. They fixed the rating scale from 100 to 400. The coefficients of temperature were eliminated as they were less sensitive in the study area. The formulation used was as follows:

$$WQI = \frac{\sum_{i=1}^{n} Wi\ Qi}{\sum_{i=1}^{n} Wi}$$

TABLE 3.22 Weightages for Horton's Water Quality Index (Horton, 1965)

Parameters	Weightages
Dissolved Oxygen	4.0
Sewage Treatment (per % population served)	4.0
pH	4.0
Coliforms	2.0
Specific conductance	1.0
Carbon chloroform extract	1.0
Alakalinity	1.0
Chloride	1.0

Table 3.23 Breakpoints for Horton's Water Quality Index (Horton, 1965)

I	D.O. (%)	Coli-form (MPN/ 100ml)	CCL_4 Ext-ract (0.001)	pH	Specific Cond. (μ m)	Alkali-nity (mg/l)	Chlor-ides (mg/l)	Sewage Treat	Coefficients
100	>7 0	<1000	0-100	6-8	0-750	20-100	0-100	95-100	If temp >
80	50-70	1000-5000	100-200	5-6; 8-9	750-1500	5-20; 100-200	100-175	80-95	critical value
60	30-50	5000-10000	200-300	4-5; 9-10	1500-2500	0-5; >200	175-200	70-80	M1 = 1/2 otherwise
30	10-30	10000-20000	300-400					60-70	M1 = 1
0	<10	>20000	>400	> 4	>2500	Acid	>250	<50	If obvious pollution is present M2 = 1/2 otherwise M2 = 1

where,

Q_i = Rating values of ith parameter

W_i = Assigned weightage of ith parameter

n = Number of parameters included

The rating value of ith parameter was computed as

$$Q_i = 300\ [(X-X_1)/(X_2-X_1)]x400$$

where,

X_2 = concentration of ith parameter corresponding to 400 on rating scale

X_1 = concentration of ith parameter corresponding to 100 on rating scale

X = observed concentration of ith parameter

The weightages of parameters were fixed on the basis of available literature, the experience of author and the maximum observed value of the particular parameter.

A perusal of the information thus, reveals that no index among the reported WQI is suitable for defining bioconcentration/ bioconcentration factor of Cd, Pb, and Zn in plankton and whole body fish tissue. However, it was realised that for specific use WQI could be formulated by providing suitable weightages to the important chemical determinants of bioconcentration.

MODIFIED HORTON'S WQI AND BIOCONCENTRATION FACTOR

A WQI was formulated considering important chemical determinants of river water contributing to the bioconcentration factors of Cd, Pb, and Zn in plankton and whole body fish. The parameters selected with their corresponding weightages are given in Table 3.24. The rating scales of the parameters were so chosen that each parameter was assigned a rating value corresponding to the observed concentration of the parameter. Rating scale

Table 3.24 Parameters and their Weightages in Modified Harton's Index

Parameters	Weightages
pH	2.0
Conductivity	2.0
Dissolved Organic Carbon	3.0
Water Hardness	2.0
Alkalinity	2.0
Metal Content as (Total - Soluble / Total)	4.0

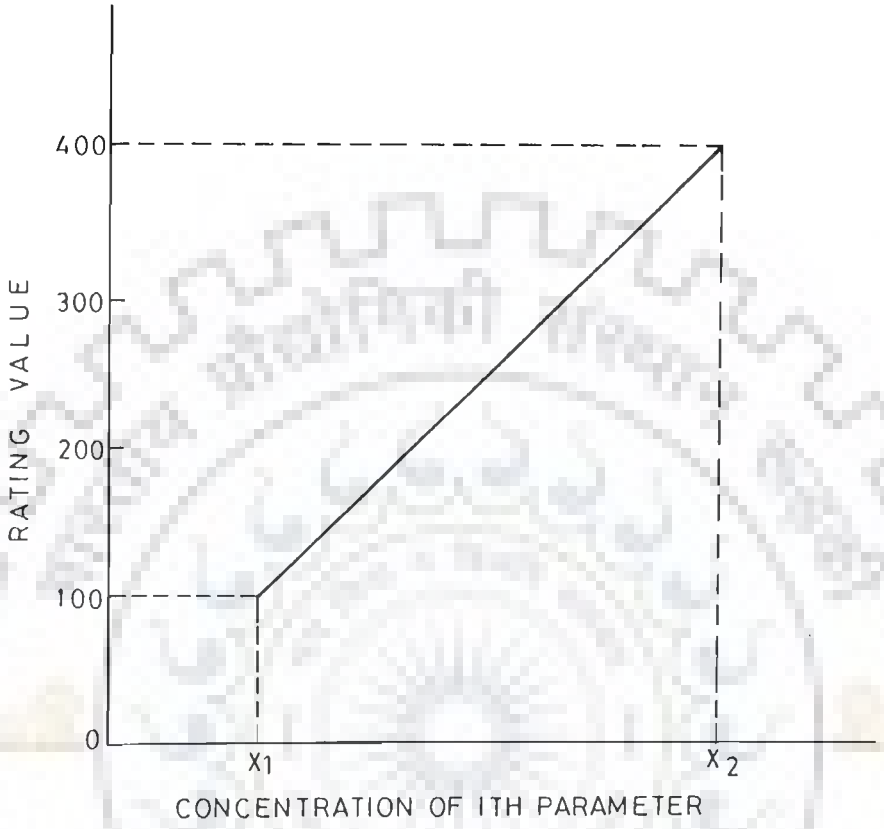
was expressed in the range of 100 to 400. A graphical representation of the rating equation is given in Fig 3.21.

Calculated index values observed during the study are presented in Table 3.25. A perusal of data shows variations in the values with a similar trend in spatial variations. Correlation of the calculated index values with that of bioconcentration factors (BCFs) of Cd, Pb, and Zn in plankton (phyto and zoo), and whole body fish with calculated WQI of river water show that WQI is inversely correlated with BCFs of Cd, Pb, and Zn. It connotes that increasing pollution renders the toxicants (Cd, Pb, and Zn) biologically unavailable and their bioavailability decreases.

Food Chain Biotransfer of Cadmium, Lead and Zinc in Riverine Ecosystem

Bioconcentration levels of cadmium, lead and zinc in phytoplankton, zooplankton and fish were used to examine the potential biotransfer of HMs in aquatic food chain in a riverine ecosystem. Studies were found in the scientific literature that addressed disposition of toxic metals in the aquatic (Wong and Cheung, 1985), agricultural (Stevens, 1992) and terrestrial food chains (Brams et al. 1989; Stevens, 1992). The phenomenon of bioamplification is prominently mentioned to occur in aquatic ecosystems for persistent organic pollutants (Evans et al., 1991; Stevens, 1992 and the references cited therein) and for selected heavy metals (Marinucci and Bartha, 1982; Wong and Cheung, 1985; Timmermans et al., 1989).

Wong and Cheung (1985) reported biomagnification of selected HMs in an aquatic ecosystem. They studied a two level food chain to find out possible biotransfer of heavy metals. It was reported that when fresh water shrimp, Macrobrachium hainanense as a primary consumer was fed on unicellular green alga, Chlorella pyrenoidosa, grown on Pb, Cu, Zn, and Mn waste materials, high contents of Pb, Cu, and Mn in the flesh were present. Further, Pb was found to be significantly correlated with that of Pb in



MATHEMATICAL EXPRESSION

$$\text{Rating Value } Q = 300\left(\frac{x - x_1}{x_2 - x_1}\right) + 100$$

x_1 = The minimum concentration limit of ith parameter
x_2 = The maximum concentration limit of ith parameter
x = Observed value of the ith parameter

If $x \leqslant x_1$, $Q = 100$
$x > x_2$, $Q = 400$

Fig 3.21 Graphical Representation of Rating Equation

Table 3.25 Water Quality Index of River Water at Various Stations (with Cd, Pb, and Zn)

Stations	Metal included in WQI	WQI Values Seasons			
		Postmonsoon	Winter	Summer	Monsoon
S1	Cd	149.8	129.2	199.6	162.8
	Pb	142.6	123.4	230.2	124.9
	Zn	162.9	122.8	209.1	180.9
S2	Cd	221.4	253.4	360.4	301.8
	Pb	215.2	258.8	382.6	302.7
	Zn	234.3	262.4	383.3	329.6
S3	Cd	154.2	130.1	103.4	157.9
	Pb	142.0	132.7	177.0	167.6
	Zn	162.8	136.9	185.1	181.3
S4	Cd	177.4	182.9	250.8	234.6
	Pb	174.0	179.8	194.2	192.3
	Zn	193.0	187.2	265.2	254.2
S5	Cd	198.8	234.1	376.1	296.8
	Pb	197.3	235.8	350.2	297.4
	Zn	216.0	234.2	364.8	312.6
S6	Cd	207.1	243.2	374.6	284.8
	Pb	195.8	246.2	354.1	286.1
	Zn	220.0	254.2	375.2	304.2

algae used as food. Phenomenon of biomagnification for selected heavy metals in a littoral food chain have also been reported by Timmermans et al. (1989). They determined Cd, Pb, Zn, and Cu concentrations in 15 species of fresh water macroinvertebrates. They reported that the biomagnification process in which predators had a higher concentration than their prey prominently existed for Zn. It was also reported that feeding habits, proximity to the sediment and physico-chemical factors appeared to be the determining factors for trace metal concentrations at high trophic level. They concluded that various explanations for the differences in pollutant residues include trophic level, body weight, physiologic equipment and abiotic factors.

Based on the findings of present investigation, a conceptual model of persistent Cd, Pb, and Zn in the different trophic status of the reverine ecosystem was developed (Fig 3.22-3.25). The model is composed of three dimensions: Metal concentrations (Cd, Pb or Zn) in the ambient environment (water); level of Cd, Pb or Zn in plankton (indicated by mean concentration in phytoplankton and zooplankton) and bioconcentration of Cd, Pb or Zn in fish tissue. Data presented in Table 3.6 to 3.14 was reorganised to give bioconcentration at low trophic level biota (mean values of phytoplankton and zooplankton plotted as bioconcentration in plankton) and at higher trophic level biota (bioconcentration in whole body fish tissue).

In Fig 3.22, variations of Cd concentration are plotted in postmonsoon, winter, summer and monsoon seasons employing data of all the six sampling stations. In postmonsoon season, concentrations of Cd in fish tissue were diminutive when compared with concentration of Cd in plankton. In winters, however, Cd concentrations were high at higher trophic level biota compared to plankton and water. In monsoon season dimensions in plankton and fish tissue follow same pattern as in winter season.

Fig 3.23 incorporates the variations of Pb concentrations in water, plankton, and fish tissue in various seasons. In postmonsoon and monsoon season, concentrations

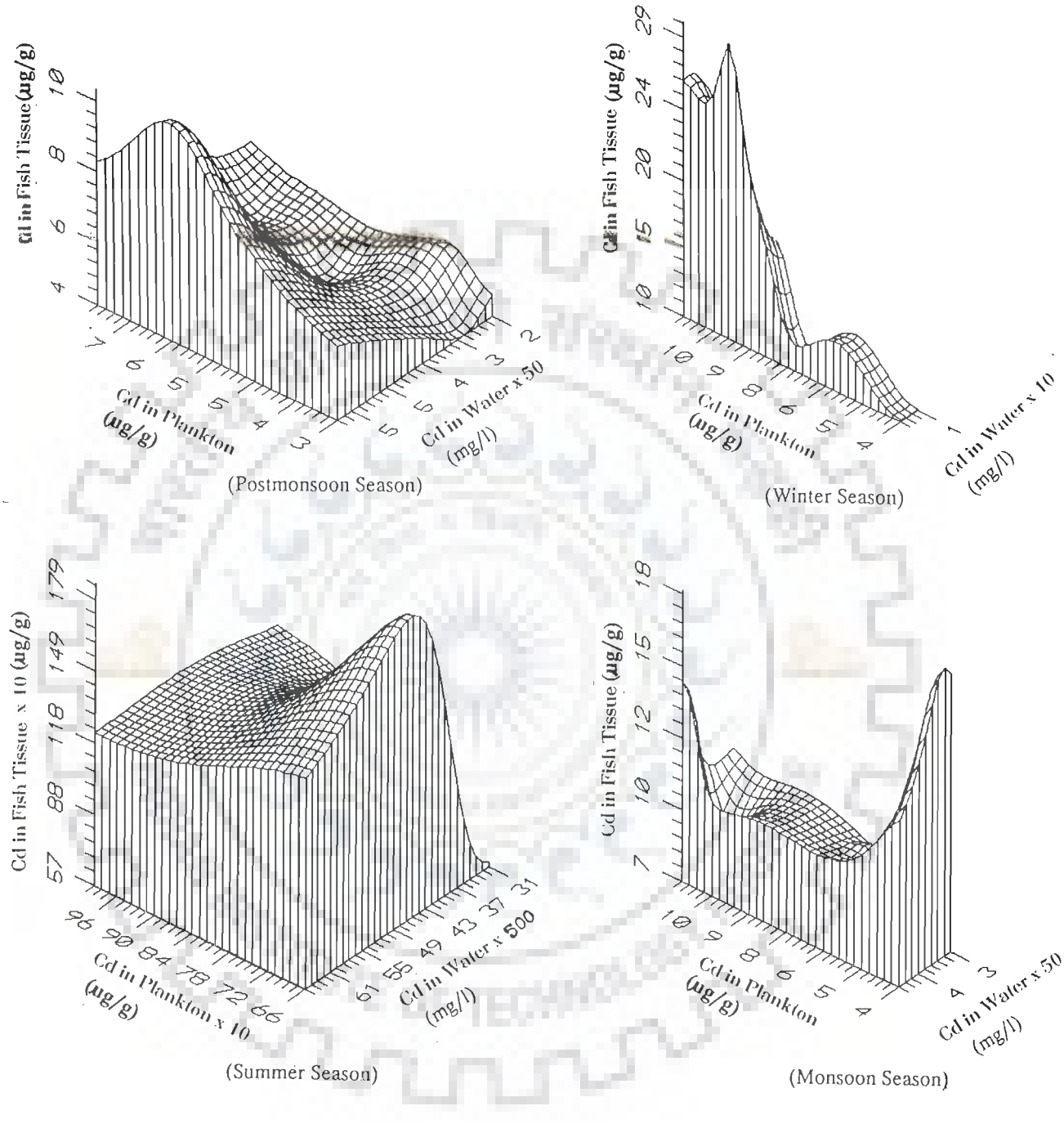


Fig. 3.22 Bioamplification of Cadmium in Riverine Eosystem (Seasonal variations)

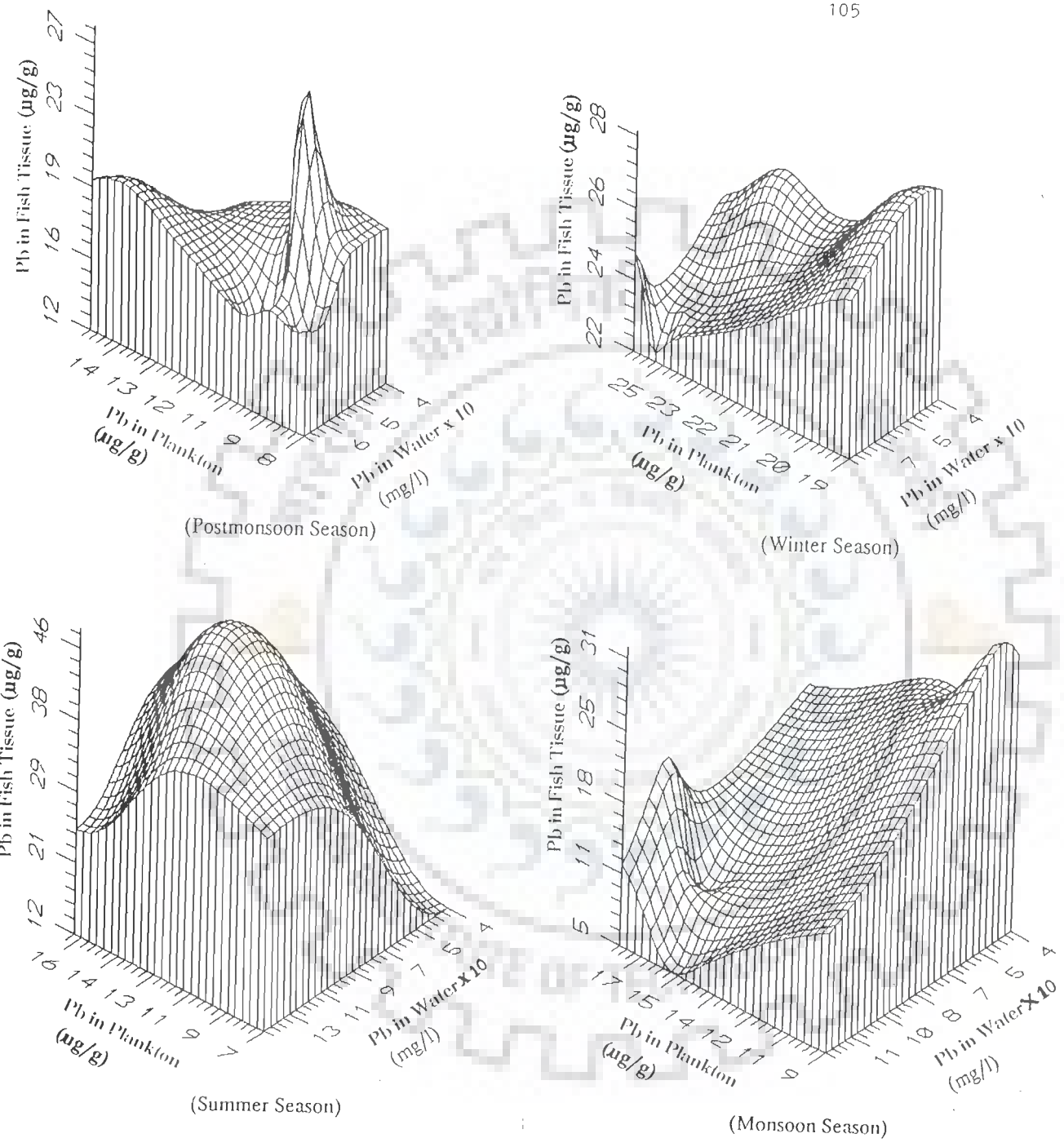


Fig. 3.23 Bioamplification of Lead in Riverine Ecosystem (Seasonal variations)

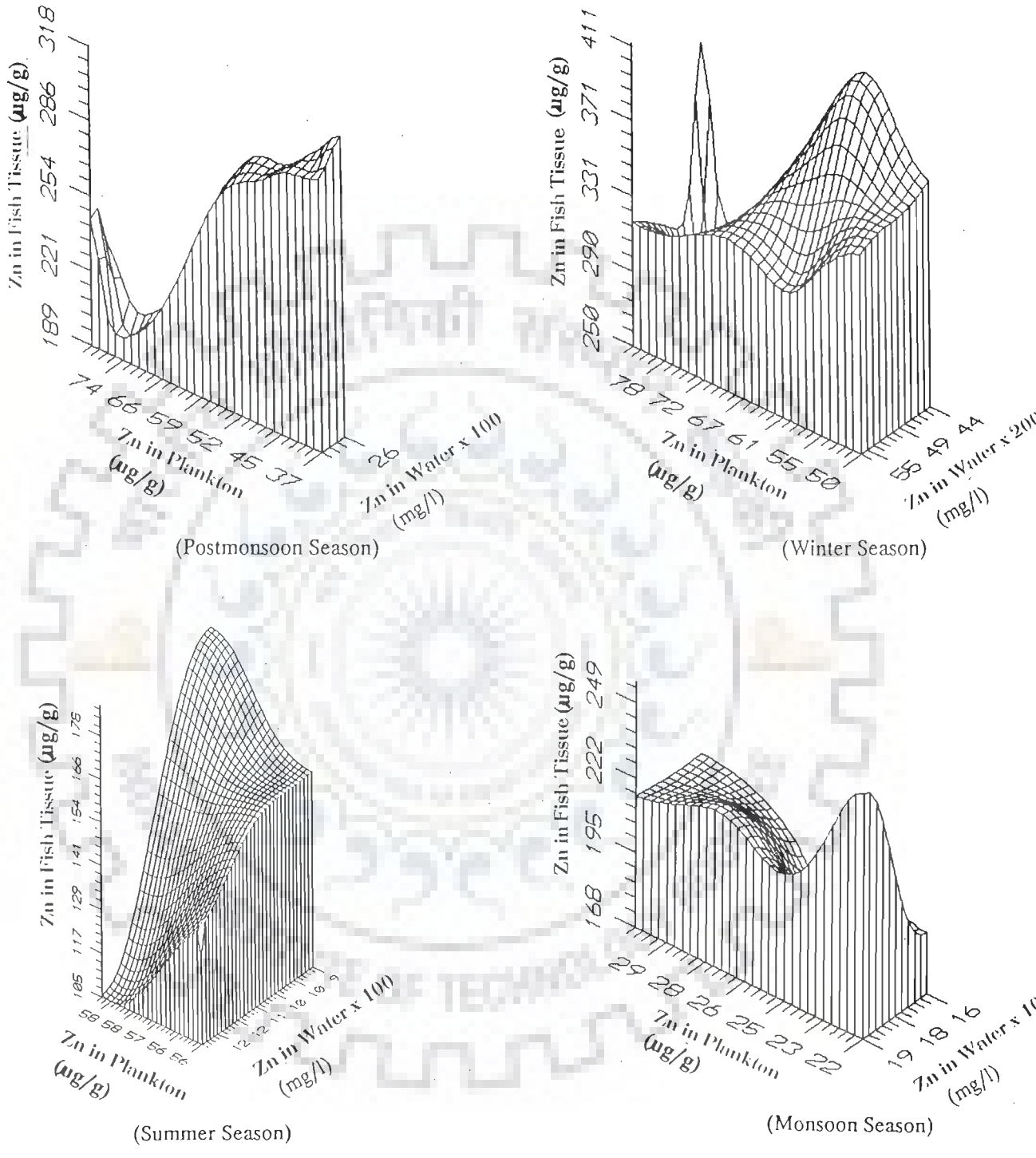


Fig. 3.24 Bioamplification of Zinc in Riverine Ecosystem (Seasonal variations)

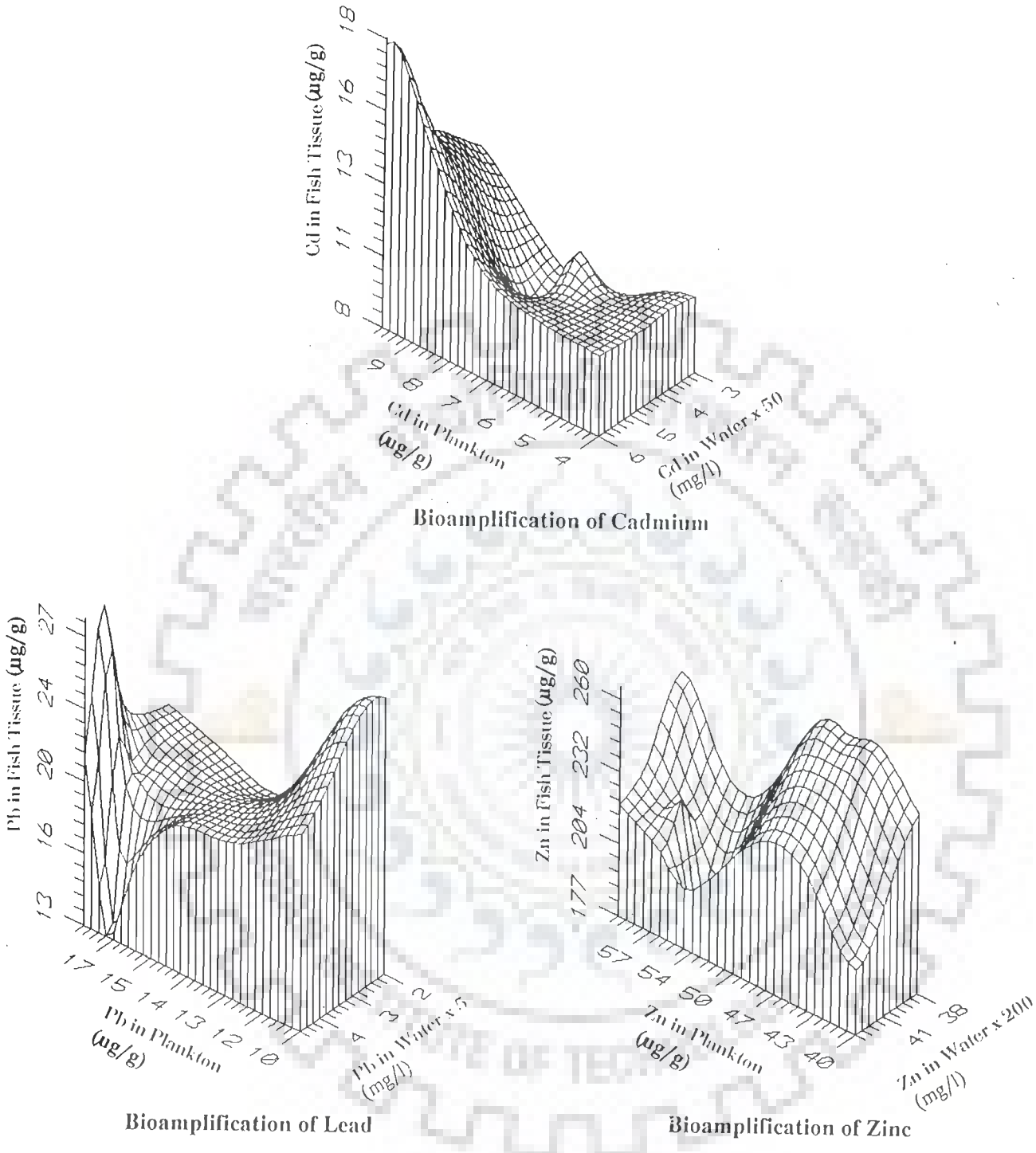


Fig. 3.25 Bioamplification of Cadmium, Lead and Zinc in an Ecosystem

of Pb in fish were high as compared to plankton except at S1 station in postmonsoon and S4 station in monsoon season. Biodiminution was, however, prominent in winters (Fig 3.23) when Pb concentrations in plankton were high as compared to fish tissue.

Fig 3.24 are the plots of concentrations of Zn in water, plankton and fish tissue. Bioconcentration in all the seasons of the year was high in fish when the values were compared with plankton. Variations in Zn concentrations among fish tissue and plankton and water were significant.

Barring seasonal variations, data of all observations was also plotted collectively for Cd, Pb, and Zn and is presented in Fig 3.25. It is exemplified and represented figuratively that even with varying concentrations of HMs (Cd, Pb or Zn) in the ambient environment (water), high concentration of toxic metals are present at higher trophic level biota and thus the phenomenon of bioamplification (Bryan, 1979) has been manifested. A variety of terms have been used to describe the process as ecological biomagnification (Metcalfe et al., 1976) or bioaccumulation in the food chain (Goldbach et al., 1976). Investigations, thus,report the ability of some chemicals to move through the food chain resulting in higher and higher concentrations at each trophic level in an ecosystem.

CHAPTER 4

BIOCONCENTRATION OF CADMIUM AND LEAD IN FISH TISSUE

4.1 PRELIMINARY

The interaction between pollutants and the components of environment is considered a pre-requisite for pollution control programmes and in establishing environmental quality criteria. With the increase in metal discharge from industrial activities, the interaction of metal toxicants with aquatic organisms in an ecosystem have assumed great importance. When a chemical is discharged into the environment, it distributes itself into different phases and an equilibrium is reached depending on the properties of the chemical. Water to biota transfer is critical because it is principally concerned with the adverse effects on life processes. Organisms possess mechanisms by which toxic metal ions can be taken up. Uptake mechanisms operate at high concentrations of HMs in the environment and result in accumulation. Uptake of some toxicants in the tissue of aquatic

organisms at concentrations many times higher than ambient water is known as Bioconcentration or Bioaccumulation (Kenaga, 1972). It is an additional response and is important in hazard evaluation strategies.

4.2 IMPORTANCE OF BIOCONCENTRATION STUDIES

Studies were carried out to suggest guidelines for assessing the bioconcentration ability of organisms and its effects in an ecosystem. Bioaccumulation, under standard conditions, help in defining the toxicity of a particular chemical to biological systems. The phenomenon of bioconcentration assumes importance when the acute toxicity of a chemical is low and the physiological effects go unnoticed until the chronic effects become evident. Due to the insidious nature of the effects, corrective action may not take hold to alleviate the situation before irreparable damage has been done. For this prior knowledge of the bioconcentration potentials of new and existing chemicals is desired. The United States Toxic Substances Control Act (1976) and the scheme for the Hazard Assessment of Chemicals have initiated regulation for registration of new chemicals before commercial use. The evaluation of bioaccumulation capacity of a chemical and the prediction of this property has become an important part of the evaluation schemes (Kenaga and Goring, 1980). The importance of bioconcentration has also been recognised by United States Environmental Protection Agency (USEPA). The ability of a chemical to build up in the environment has become one of the proposed criteria that USEPA is using in establishing toxic pollutant effluent standards (Kenaga, 1972; Kenaga and Goring, 1980).

4.3 BIOCONCENTRATION PROCESSES

In retrospect, increased attention has been paid to define the ability of toxicants to bioaccumulate. However, due to the complexity of the living systems, the results obtained

do not necessarily explain observations in the organisms that result with several biochemical mechanisms. An understanding of the mechanisms by which organisms accumulate metals expound that several processes collectively moderate the phenomenon of bioconcentration. Biosorption is a non-directed physico-chemical complexation reaction between dissolved metal species and charged cellular components and the precipitation results into initial bioconcentration.

The uptake of metal in microorganisms involve metabolically mediated uptake such as ion transport systems and energy expenditure. It is reported that metabolically mediated accumulation results into intracellular concentration. However, accumulation at the cell surface is strongly influenced by physico-chemical characteristics of water (Forstner and Wittmann, 1983).

Reports on the bioconcentration of HMs in aquatic animals postulate that heavy metal content, generally, originates from two routes. Free ions and simple compounds dissolved in water are taken up directly through the epithelium of the skin, gills and alimentary canal, while, other being accumulated in food are incorporated by nutrition. Several workers have agreed that the uptake of metals from food is an important route in the transfer of HMs in the environment (Phillips, 1977; 1978; Forstner and Wittmann, 1983).

Lurdes et al. (1989) have postulated that living cells adsorb metal ions on their surfaces and also take them up internally by binding and pumping them across the cellular membranes. Binding of HMs to the surface is the first step of the bioconcentration mechanism. The cells have a number of complexing sites encompassing physical and chemical characteristics (Goncalves and Corceicao, 1989) and this is due to the different complexing sites of the cell that result in reactions on the surface and formation of aggregates (Buffle et al., 1987).

4.4 METAL TOXICANTS RELATED TO FISH AND FISHERIES

The dangerous substances directive include Cd and Pb on the basis of their toxicity, persistance, carcinogenicity and bioaccumulation. Cd is included in List I, referred as black list substances, whereas, Pb is included in grey list (List II) (Butler, 1978 and the references cited therein). Limit values for effluents and environmental standards for Cd are 200 and 3.00 mg/l (dissolved), respectively (annual average concentrations in EC directives). Further, for Pb a limit of 10 and 60 mg/l is established for the protection of salmonid and other fresh water fish, respectively (Water hardness 50-100 mg/l). However, there appears to be no well accepted standards for various metals in the food that are considered safe for human consumption (Butler, 1978).

Organisms have been used to define the poisonous effects of a toxicant under controlled conditions. In static test exposures, animals are exposed to a substance for the duration of the test (Sprague, 1969). Sublethal tests provide information on the effect of various concentrations of toxicant on the survival, growth and bioconcentration of toxicant in an organism. In acute toxicity tests Maximum Acceptable Toxic Concentration (MATC) is an estimate of safe concentration. MATC is emperically determined as the highest exposure concentration that does not result in significant harm to the test organism in terms of survival. It is interpolated as the geometric mean of the lowest concentration having an effect and the lowest concentration having no effect. Chronic toxicity of a substance is measured as LC_{50} (median lethal concentration) i.e., concentration which is calculated to cause the mortality of 50% of a test population.

Toxicity of Cadmium to Fresh Water Fish

Existing reports on the toxicity of cadmium to fresh water fish (Nriagu, 1980a) show that salmonid species are more sensitive to Cd than non salmonid species. The lowest reported adverse effect concentration that resulted from a 4 day exposure of Salmo gairdneri

is 0.00092 mg/l. It caused an increased rate of bacterial infection of the exposed fish (Knittel, 1980). The adverse effect concentrations for salmonid species appear to have a stable minimum of 0.0002 mg/l in exposures upto 700 days. However, no such minimum is discernible for non-salmonid species (Nriagu, 1980a). A compendium of the review on toxicity of cadmium to fresh water is presented in Table 4.1.

It is reported that the water hardness, pH, temperature and chemical form of the cadmium are important determinants of toxicity on fresh water fish. Water hardness plays an important role in determining the toxicity to adults e.g., 48 h LC_{50} for adult rainbow trout increased from 0.09 to 3.70 mg/l as the hardness increased from 20 - 320 mg/l (Calamari et al., 1980). In general, for all species of fish, increasing water hardness (mg/l $CaCO_3$) is associated with reduced toxicity.

Toxicity of Lead to Fresh Water Fish

The acute toxicity data for fresh water fish shows that 96 h LC50 for total lead generally falls within the range of 0.5 - 10 mg/l. However, in hard waters (> 350 mg/l $CaCO_3$) the LC_{50} exceeds 400 mg/l. Chronic exposure of fish to Pb produces characteristic response of black finning and spiral curvature (Hodson et al., 1982). It is reported that in soft water Salmo gairdneri developed black tail at 0.1 mg/l after 40 days exposure, whereas, it took 570 days exposure at much higher concentration of 0.20 mg/l to produce the same effect in non-salmonid species (Davies et al., 1976). A compendium of relevant literature is presented in Table 4.2.

Bioindicators of Cadmium and Lead pollution

The increased degradation of the environment requires urgent action on pollution control and calls for setting up network monitoring programmes using bioindicators (Burton, 1986; Kapusta et al., 1990). Biological monitoring uses living organisms as sensors to detect changes in an effluent or waste that may endanger aquatic life (Rand and Petrocelli,

Table 4.1 Toxicity of Cadmium to Fresh Water Fish.

Species	System	Water Hardness (mg/l)	pH	Temp. (°C)	Chemical form	Metal Conc.	Effect	Referance
Salmo gairdneri	F	82	7.8	10.0	$CdCl_2$	0.031(O)T	5 Days LC_{50}	Majewski and Giles, 1981
Anguilla rostrata	S	55	8.0	28.0	Cd^{++}	1.10(N)	2 Days LC_{50}	Rehwoldt et al., 1972
Cypyrinus carpio	S	55	8.0	28.0	Cd^{++}	0.03(N)	2 Days LC_{50}	Rehwoldt et al., 1972
Channa punctatus	S	-	-	-	$Cd(NO_3)_2$	0.05(N)	1 to 35 Enzzyme changes	Dubale and Punta, 1981
Lepomis macrochirus	S	18	7.4-7.7	22.2	$CdCl_2$	4.00(O)T	1 Day LC_{50}	Bishop and McIntosh, 1981
Poecillia reticulata	F	200	-	24.0	$CdCl_2$	33.0(O)T	1 Day LC_{50}	Canton and Slooff, 1982

Note :- S - Static System ; F - Flow Through System ; (O)T - Observed Total ; N - Nominal.

Table 4.2 Toxicity of Lead to Fresh Water Fish.

Species	System	Water Hardness (mg/l)	pH	Temp. (°C)	Chemical form	Metal Conc.	Effect	Referance
Salmo gairdneri	S	353	8.2	14.0	$Pb(NO_3)_2$	1.32 (O)T	4 Day LC_{50}	Davies et al., 1976
Salmo salar	S	11	6.3	10.0	$Pb(NO_3)_2$	0.10 (O)T	40 Day Black finning lordo scoliosis	Grande and Anderson, 1983
Catostomus commersoni	-	34	-	-	-	0.235	60 Day No effects, growth reduced	Sauter et al., 1976
Pimephales promelas	-	20	-	-	-	2.4 (O)T	4 Days LC_{50}	Hodson et al., 1980

Note :- S - Static System ; (O)T - Observed Total

1985). It is reported that some organisms have the ability to concentrate and retain certain persistent substances. Salmo gairdneri (Verbost et al., 1987), Colisa fasciatus (Kumar and Mathur,1991),and Mytilus edulis (Phillips, 1976) are reported to be good indicators of metal pollution. In North America, the Asiatic clams appeared as a suitable organism. It is reported that the selection of bioindicators facilitate studies and help in defining the bioconcentration potentials of heavy metals (Doherty, 1990).

A particular way of monitoring exposures involve determinations of toxicant concentrations in animal tissues. Such measurements provide valuable information on the toxicity of a chemical. Studies show that liver and gills of fresh water fish have been predominantly used as bioindicator organs (Marigomez and Ireland, 1989).

4.5 BIOCONCENTRATION : NEED FOR FURTHER STUDIES

Exposed to the sublethal concentrations of a toxicant, bioindicator organisms could be used to define -

- possibility and extent of bioconcentration,
- kinetics of accumulation,
- effect of receiving system on bioconcentration, and
- effect of short term episodic spills of toxicants measured as short term bioconcentration studies.

Various studies have confirmed the utility of defining the bioconcentration levels. The compounds that exhibit bioaccumulation include aromatic hydrocarbons, chlorinated aromatic hydrocarbons, chlorinated aliphatic hydrocarbons, their substitutes and a variety of other substances (Connell, 1988; Murphy and Lutenske, 1990). However, it is noteworthy that bioaccumulation is a wider phenomenon and there are many aspects that are not understood.

Hardy and Roesijadi (1982) proposed bioaccumulation kinetics and organ distribution of Ni in the marine clam (Protothaca staminea). Concentration of Ni in the whole body clams was 20.1 ng/gm dry weight after the entire 48 h exposure (BCF = 4.0). Organ distribution showed that gills accumulated 139.6 ng Ni/gm at 8.18×10^{-10} gm Ni/l in the test solution. Linear relationship for accumulation was reported as Y1 = 20.5 + B1t (r2 = 0.99). For Cu and Ni in sandy beach molluscs (Donax serra and Bullia rhodostoma), accumulation factor (CF) was 2.1, 3.8 and 1.6, 3.2, respectively exposed to 20 µg/l metal (both Cu or Ni) for three weeks. The rates of Cu or Ni accumulation for both the molluscs species were 0.04, 0.04 and 0.03, 0.02, respectively, in D. serra and B. rhodostoma. Accumulation of metals in molluscs was shown to be related to the average metal concentration in the surrounding water.

Cooper (1983) investigated Hg accumulations in fishes of Lahontan reservoir. Hg concentrations in muscle tissues collected from 11 species ranged from 0.11 mg/kg in white bass (Morone chrysops) to 9.52 mg/kg in striped bass (Morone saxatilis). It was reported that out of 53 muscle tissues analysed, 68% exceeded the action level considered safe by FDA. Crayfish (Pacifastacus sp.) and California seagull (Larus californicus) were also reported to exhibit elevated metal concentrations.

Zinc bioaccumulation has been reported in rainbow trout (Salmo gairdneri) and brook trout (Salvelinus fontinalis) using gills and liver as bioindicator organs. Average zinc concentrations reported in gills and liver tissues are 1000 and 395 µg/gm. A high correlation (r = 0.74) between metal burdens in liver and gill tissues was reported (Saltes and Bailey, 1984).

Variability of Al have been investigated by Brumbaugh and Kane (1985). Concentrations of 58.0, 1.5, < 1.0 and 3.9 are reported in liver, gills, kidney and whole body samples of small mouth bass (Micropterus dolomieui). Liver is reported as bio-indicator

organ containing highly elevated levels of Al.

In a laboratory study, crayfish (Cambarus bartoni) exposed for 4 weeks to Cu and Ni have been reported to accumulate Cu in hepatopancreas and in viscera. Highest Ni concentration were detected in both at 0.8 mg Ni/kg. Exoskeleton and gills were found to accumulate relatively low Ni at 0.2 and 0.4 mg/kg, respectively (Alikhan et al., 1990).

Baudin and Fritsch (1989) in order to assure an accurate evaluation of the relative importance of food and water as ^{60}Co sources to the carp, carried out a study on a fresh water fish, Cyprinus carpio, under simulated conditions. They reported that the fish accumulated 94 + 12 and 417 + 195 Bq/gm ^{60}Co accounting nearly 25% and 75% from food and water after 63 days of exposure. Organ distribution of ^{60}Co was also studied. Greatest fraction of residual radiocobalt resided in digestive tract that accounted for 20-25% of the total ^{60}Co body burden.

In light of the above information, a study on bioconcentration, kinetics of accumulation and tissue distribution of cadmium and lead in a fresh water teleost, Colisa fasciatus was undertaken in laboratory aquaria. Colisa fasciatus was selected as test organism as they meet the pre-requisites suggested as desirable (Butler, 1971; Hanna and Muir, 1990).

In the laboratory aquaria, organisms were exposed to sublethal concentrations of Cd and Pb -

- to determine bioconcentration of Cd and Pb in fish tissue,
- to identify bioindicator organs for Cd and Pb in Colisa fasciatus and sites of accumulation,
- to evaluate the variability of bioconcentration with time,
- to determine bioconcentration of Cd and Pb in fish tissue exposed to sublethal concentrations of Cd and Pb in the exposure medium,
- to determine kinetics of accumulation (changes in the rate of

accumulation as a function of time), and

- to determine bioconcentration factors (BCFs) of Cd and Pb in fish tissue in short term exposures.

4.6 RESULTS AND DISCUSSION

The results presented herein have been discussed in terms of bioconcentration, kinetics of accumulation, bioconcentration factor (BCF), and variability of the physico-chemical characteristics of test water including total and soluble Cd or Pb in test water. Bioconcentration factor was calculated as:

$$BCF = \frac{\text{Equilibrium tissue concentration of Cd or Pb}}{\text{Concentration of Cd or Pb in test water}}$$

Bioconcentration and Organ Distribution of Cd in Fish Tissue

Bioconcentration and organ distribution of Cd in gills, liver and muscles of fish were recorded every eight hours (three times), initially for 24 h and later every fifth day. Cadmium appeared in gills and liver in the first observation after 8 h. In the initial 24 h, gills and liver accumulated 5.86, and 4.78, 19.30 and 13.70, 14.80, 13.10 µg/gm Cd in Set I, II and III, respectively. Muscle tissue showed relatively low Cd concentration (0.93, 2.48 and 3.10 µg/gm) in the initial 24 h of exposure of Cd in the three sets, respectively. Initial concentration of Cd in muscle tissue was recorded after 16 h in all the experimental sets (Fig 4.1).

Concentrations of Cd in gills, liver and muscles increased with time and bioconcentration was recorded upto 7.30, 5.24, 2.05 (Set I), 20.60, 21.30, 5.90 (Set II) and 26.90, 23.00, 7.70 µg/gm Cd (Set III), in gills, liver and muscles, respectively. Bioconcentration in first 24 h exposure appears to diminish and a static phase results after 25 days (Fig 4.1).

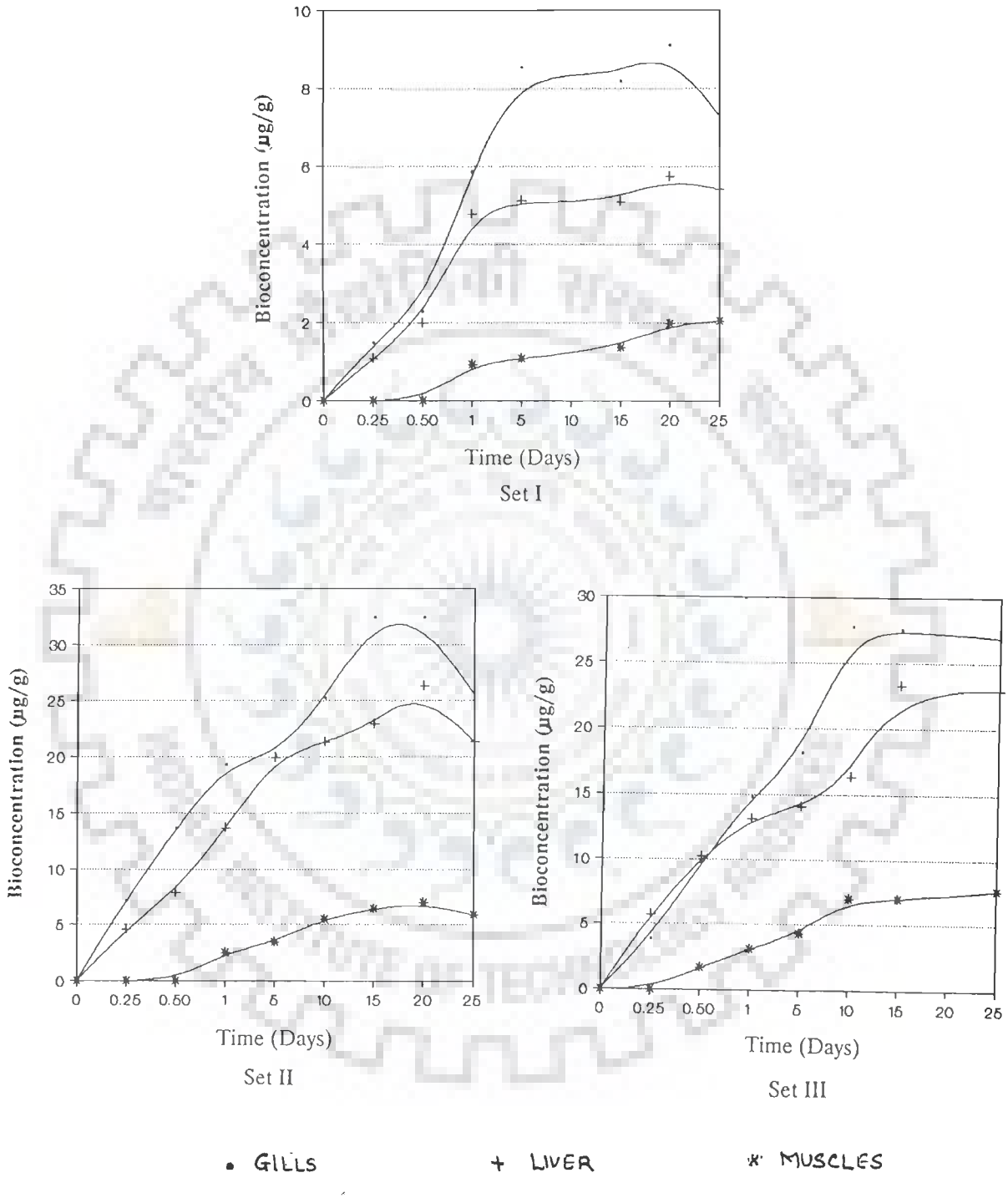


Fig 4.1 Bioconcentration of Cadmium in Fish Tissue

Bioconcentration and organ distribution of Cd presented herein show that bioconcentration of Cd is a function of time and organ tissue. Bioconcentration increases with time and Cd is accumulated at specific sites (gills, liver or muscles). It was noted that the concentration of Cd in the exposure medium affect the extent of uptake. In general, bioconcentration in fish tissue in Set I was lower (exposed to 200 µg/l) than Set II (Cd - 500 µg/l).

Kinetics of Cd Accumulation in Fish Tissue

Based on the experimental observations, the rate kinetics of the process was worked out for each set. Data of bioconcentration in the tissue in first 24 hours (n=4) and for 25 days experiment (n=6) was employed. Hourly and daily variations in the rate of accumulation have been worked out and the results are incorporated in Table 4.3. Details of sorption, retention and organ distribution show an initial uptake rate of 0.230 and 0.191 ug/gm/h in gills and liver, respectively in the initial 24 h exposure (Set I). Variations in uptake rates (0.805, 0.555 µg/gm/h (Set II), 0.628, 0.549 µg/gm/h (Set III) were observed in gills and liver, respectively (Table 4.3).

Rate changes of accumulation have also been worked out for 25 days experiment in fish tissue exposed to 0.20, 0.50, 1.00 mg/l Cd. Daily variation in the rate of accumulation are 0.231, 0.168 and 0.076 µg/gm/day in gills, liver and muscles, respectively (Set I). Daily variations in accumulation rate have also been calculated for Set II & Set III and are presented in Table 4.3. A perusal of data show that uptake is high initially and slackens later resulting in a slow phase responsible for net accumulation.

Bioconcentration factors (BCFs) in fish tissue have also been worked out and are presented in Table 4.4. Perusal of the data shows that fish tissue accumulate Cd to highly elevated concentrations as compared to the ambient environment. BCFs were 104.0, 86.0, and 24.0 in gills, liver and muscles after 25 days of exposure to an initial Cd concentration

Table 4.3 Bioconcentration Kinetics of Cadmium in Fish Tissue

Experiment	Time	Statistical Constants	Fish Tissue		
			Gills	Liver	Muscles
Set I	24 Hours	m_1	0.230	0.191	-
		C	-0.352	-0.325	-
		r	0.909	0.925	-
	25 Days	m_2	0.231	0.168	0.076
		C	3.608	2.053	0.316
		r	0.407	0.535	0.888
Set II	24 Hours	m_1	0.805	0.555	-
		C	0.386	-0.122	-
		r	0.997	0.990	-
	25 Days	m_2	0.983	0.728	0.227
		C	10.333	9.534	1.839
		r	0.583	0.526	0.669
Set III	24 Hours	m_1	0.628	0.549	-
		C	-0.439	0.690	-
		r	0.994	0.979	-
	25 Days	m_2	0.947	0.848	0.276
		C	9.665	6.011	2.162
		r	0.583	0.740	0.699

Note : m_1 , m_2 - slope(rate of bioconcentration), m_1 - per hour, m_2 - per day ;
C - intercept ; r - regression coefficient.

Table 4.4 BCF of Cd in Fish Tissue as a Function of Time and Cd Concentration

	Set I			Set II			Set III		
Time	G	L	M	G	L	M	G	L	M
0 Hour	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
8 Hours	8.8	6.4	-	16.4	10.4	-	4.3	6.4	-
16 Hours	14.4	12.6	-	32.8	18.9	-	12.0	12.6	-
24 Hours	36.6	28.6	5.8	48.2	34.2	6.2	18.0	16.0	3.8
5 Days	54.0	32.4	6.9	54.6	54.2	9.4	26.0	20.8	6.2
10 Days	-	-	-	76.0	64.1	16.6	38.8	22.8	9.8
15 Days	62.0	38.6	10.4	104.6	74.0	20.8	40.2	34.0	10.2
20 Days	68.0	42.8	14.8	114.2	92.6	24.8	-	-	-
25 Days	64.0	46.0	18.0	104.0	86.0	24.0	42.2	36.0	12.0

Note: G - Gills ; L - Liver ; M - Muscles

of 500 µg/l (Set II). BCF showed an increase with time. It increased to 64.0, 46.0 and 18.0 in 25 days as compared to 36.6, 28.6 and 5.8 in 24 h in gills, liver and muscles, respectively (Set I). In general, BCF was high in Set II (Cd- 500 µg/l) than Set I (Cd- 200 µg/l).

Bioconcentration and Organ Distribution of Lead in Fish Tissue

Bioconcentration and organ distribution of Pb in fish tissue (gills, liver and muscles) exposed to sublethal concentrations of Pb (1.00, 2.00 and 5.00 mg/l) have been investigated and the results are shown in Fig 4.2. Bioconcentration in fish tissue is shown to be a site specific response of bioindicator organs. Concentrations of Pb observed in the initial 24 h exposure in gills, liver and muscles were 2.70, 0.20 and 0.90 µg/gm respectively when the fish was exposed to Pb (1 mg/l) (Set I). Concentration of Pb in fish tissues increased with time and test water concentrations of Pb (Set II & Set III). High concentrations of Pb were detected in fish tissues (gills, liver and muscles) exposed to 5.00 mg/l Pb and were 18.0, 5.6 and 17.2 µg/gm, respectively. A general trend of bioconcentration shows an initial rapid phase (24 h) and a later slow phase responsible for net accumulation (Fig 4.2).

Kinetics of Pb Accumulation in Fish Tissue

Rate kinetics of accumulation, worked out for Pb in each set, is presented in Table 4.5. Initial 24 h exposure measurements were employed to determine hourly variations in uptake rate while the data of static exposure of 25 days have been utilized to calculate daily variations in accumulation of Pb in fish tissues. Hourly and daily variations in the rate of accumulation with increasing concentrations of Pb in exposure medium (1.00, 2.00 and 5.00 mg/l) were calculated and incorporated in Table 4.5. Hourly variations in the uptake rate are 0.106, 0.013 and 0.073 µg/gm/h in gills, liver, and muscle tissues, respectively (Set I, 24 h experiment). Rate of Pb uptake worked out for 25 days exposure (1.00 mg/l Pb) gills, liver, muscles are 0.145, 0.022 and 0.181 µg/gm/day. Perusal of the data presented in Table

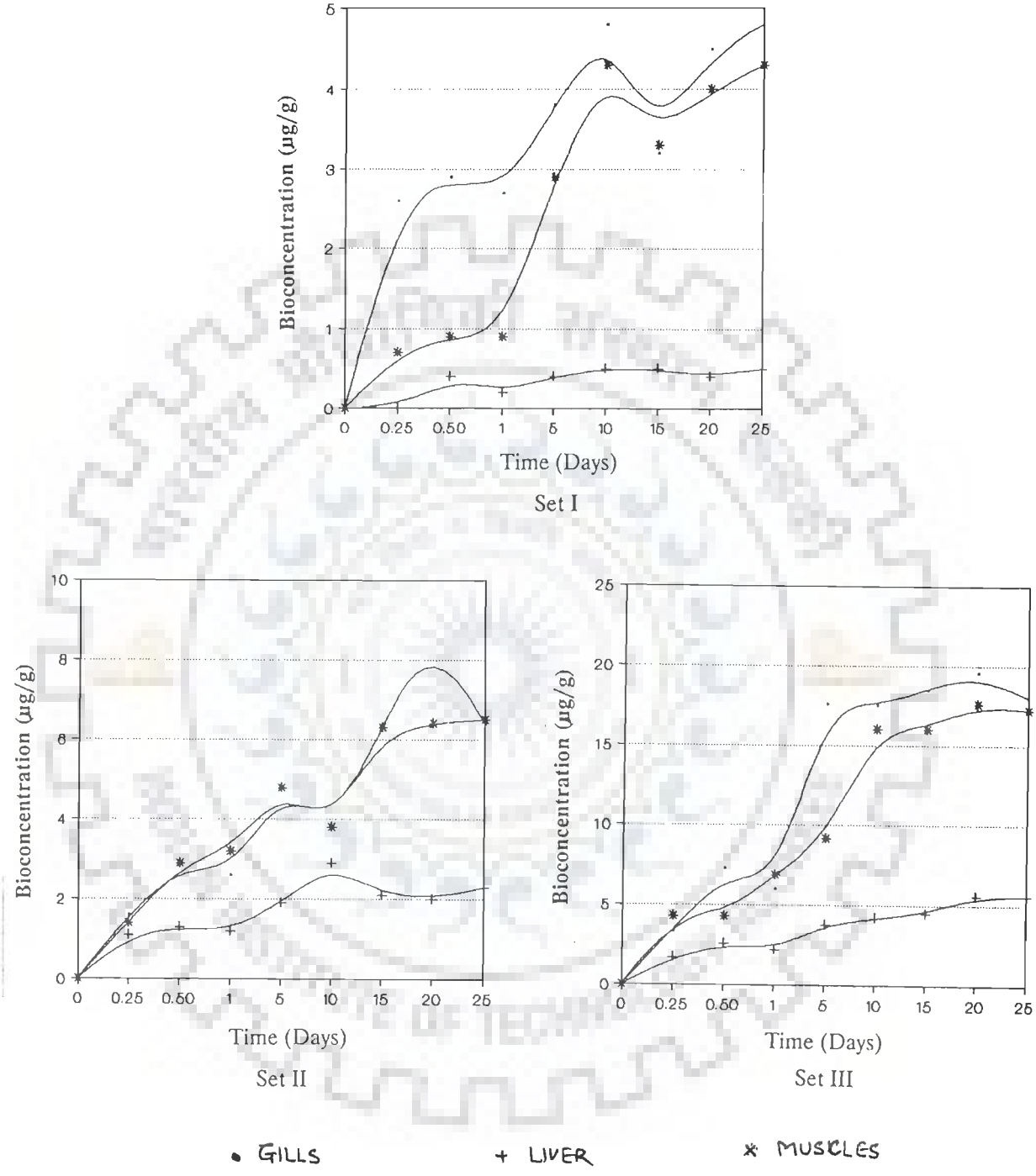


Fig 4.2 Bioconcentration of Lead in Fish Tissue

4.5 shows an initial high rate change (uptake) that diminishes with time.

Hourly and daily variations have also been worked out for Set II and Set III and are included in Table 4.5. Accumulation follows the same pattern with increase in concentration of Pb in exposure medium. Evidenced by the increasing value of m (changes in the rate of accumulation), it appears that Pb uptake increases with an increase in Pb concentration in test water (Set I,II & III) (Table 4.5).

Bioconcentration factors (BCFs) of Pb for gills, liver and muscle tissues have also been worked out and are presented in Table 4.6. BCF was observed to be a function of time. Exposure of experimental fish to 1.00 mg/l Pb resulted in a BCF of 8.0, 0.9 and 7.1 in gills, liver and muscle, respectively and represent them to be the major sites of Pb bioconcentration. BCF does not increase with an increase in Pb concentrations of exposure medium (Set II (2.00 mg/l) and Set III (5.00 mg/l)). For 25 days exposure, BCF in gills liver and muscles were 4.9, 1.8 and 5.0 (Set II) and 4.5, 1.4 and 4.3 (Set III), (Table 4.6).

Comparison of Bioconcentration in Colisa fasciatus with other Bioindicator Organisms

A comparison of the bioconcentration, bioconcentration factor (BCF), kinetics of accumulation of Cd and Pb worked out in the present study with other studies have been reviewed.

Borg et al. (1981) reported Cd concentrations in perch (Perca fluviatilis), white fish and pike (Esox lucius). The concentrations were 4.00, 0.51, 0.16 in three fish species, respectively. Results of the present study are in conformation to earlier observations that tissue Cd concentration increases with increased Cd concentrations in test water for Donax serra and Bullia rhodostoma (Watling and Watling, 1983). Cd accumulations were 2.1 and 11.4 µg/gm (w/w) exposed to 100 µg/l Cd in the two species, respectively. Rate of Cd accumulation (µg/gm accumulation/day) was more in B. rhodostoma (0.3 µg/gm/

Table 4.5 Bioconcentration Kinetics of Lead in Fish Tissue

Experiment	Time	Statistical Constants	Fish Tissue		
			Gills	Liver	Muscles
Set I	24 Hours	m_1	0.106	0.013	0.073
		C	0.794	- 0.016	- 0.011
		r	0.790	0.810	0.970
	25 Days	m_2	0.145	0.022	0.181
		C	1.726	0.141	1.228
		r	0.740	0.800	0.810
Set II	24 Hours	m_1	0.115	0.050	0.130
		C	0.369	0.278	0.272
		r	0.910	0.870	0.960
	25 Days	m_2	0.269	0.106	0.267
		C	1.632	0.762	1.718
		r	0.840	0.740	0.830
Set III	24 Hours	m_1	0.279	0.089	0.256
		C	0.837	0.475	0.753
		r	0.870	0.850	0.930
	25 Days	m_2	0.553	0.242	0.831
		C	8.193	1.228	3.350
		r	0.690	0.890	0.910

Note : m_1 , m_2 - slope(rate of bioconcentration), m_1 - per hour, m_2 - per day ;
C - intercept ; r - regression coefficient.

Table 4.6 BCF of Pb in Fish Tissue as a Function of Time and Pb Concentration

Time	Set I			Set II			Set III		
	G	L	M	G	L	M	G	L	M
0 Hour	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
8 Hours	2.9	0.0	0.8	0.9	0.6	0.8	0.8	0.4	1.0
16 Hours	3.4	0.4	1.0	1.8	0.8	1.8	1.7	0.6	1.0
24 Hours	3.4	0.3	1.1	1.7	0.8	2.1	1.4	0.5	1.6
5 Days	5.0	0.5	3.9	3.1	1.2	3.2	4.2	0.9	2.2
10 Days	6.9	0.7	6.1	2.8	2.1	4.2	4.2	1.0	3.8
15 Days	5.3	0.8	5.7	4.5	1.5	3.7	4.5	1.1	3.9
20 Days	7.2	0.7	6.5	6.8	1.9	4.9	4.9	1.4	4.4
25 Days	8.0	0.9	7.1	4.9	1.8	5.0	4.5	1.4	4.3

Note: G - Gills ; L - Liver ; M - Muscles

day) than that of D.serra (0.1 µg/gm/day). BCF of Cd and Pb are reported to be 1.9 and 16.2 (D. serra) and 1.8 and 13.3 (B. rhodostoma), respectively. Rates of Pb accumulations were 0.03 and 0.05 µg/gm/day in D. serra and B. rhodostoma.

Stripp et al. (1990) reported concentration of Cd and Pb in the muscle, liver and kidney tissues of fish white suckers (Catostomus commersoni) and yellow perch (Perca flavescens) in Darts lake. Concentrations were 0.20 ± 0.10, 0.35 ± 0.13, 0.45 ± 0.23 (for Cd) and 0.24 ± 0.13, 0.53 ± 0.22, 0.29 ± 0.18 (for Pb) in muscle, liver and kidney tissues, respectively, in yellow perch. The concentrations in white suckers were 0.26 ± 0.05, 0.91 ± 0.18, 1.50 ± 0.70 (for Cd) and 0.56 ± 0.11, 1.30 ± 0.50, 0.89 ± 0.49 (for Pb) in muscle, liver and kidney tissues, respectively.

Accumulation of Cd was studied for a homogeneous experimental population of Littorina littorea at two experimental concentrations of Cd for various exposure times (Marigomez and Ireland, 1990). The individuals were subjected to Cd exposure of 0.50 and 1.25 mg/l for 27 days. Results indicate that metal concentrations in the operculum have a high correlation with external Cd concentration and concentration x time interaction ($r=0.7148$ $P<0.01$). They reported that Cd concentrations in soft tissues were highly variable. However, metal concentration in shell was shown to correlate well ($r=0.826$ $P<0.01$) with environmental levels of Cd. BCF was reported as 43, 17 and 47 in kidney, gills and digestive glands of Littorina littorea exposed to 1.25 mg Cd/l for 27 days. The rates of accumulation were 10.4 µg/gm/day and 22.8 µg/gm/day (dry wt) when exposed to 0.50 and 1.25 mg/l Cd for a period of 27 days.

The uptake of heavy metals by an organism largely depends on the interaction of the various environmental factors. Physico-chemical characteristics of test water and bioavailable metal content are important determinants of uptake of metals by organisms. (Forstner and Wittmann, 1983) and therefore, evaluation of physico-chemical characteristics of test

water is important.

Variability of Total and Soluble Cd and Pb in Test Water

Variability of total and soluble Cd was recorded during the experimental period of 25 days and is presented in Table 4.7. Concentration of Cd decreases with time in all the experimental sets. Soluble Cd represent nearly 65-90% of the total Cd and varies with physico-chemical characteristics of test medium. Soluble Cd in Set I was particularly high and represented 80-90% of the total Cd (Table 4.7).

Variations in total and soluble Pb in test water in the initial 24 h and 25 days are incorporated in Table 4.8. Nearly 56-86% of total Pb was present as soluble fraction. Soluble Pb was comparatively high in Set I and Set III (76-86%) than Set II (58-68%). Concentrations of Pb decreases with duration of the experiment. Soluble Pb follows the same pattern as total Cd (Table 4.8).

Variability of Physico-Chemical Characteristics of Test Water

Variability of selected physico-chemical parameters of test water has also been examined. pH, conductivity, dissolved organic carbon (DOC), and water hardness were monitored regularly during the experiment. The variations are reported in Table 4.9 and 4.10. Perusal of data shows that test water characteristics vary with the system and increases during the experiment in all the sets. The increase in pH, water hardness, and DOC is also evidenced by the decrease in soluble metal fractions in all the sets (Table 4.7-4.8). Measurement of these physico-chemical characteristics of test water in static bioconcentration studies is important as they help in defining the bioavailability of a metal and have impacts on bioconcentration.

Table 4.7 Variability of Cadmium in the Test Water (unit µg/l)

Time	Set I		Set II		Set III	
	Cadimum					
	Total	Soluble	Total	Soluble	Total	Soluble
0 Hour	186	166	428	364	1022	745
8 Hours	168	146	438	368	894	674
16 Hours	158	136	418	340	814	590
24 Hours	160	140	400	320	820	594
5 Days	158	136	368	290	698	480
10 Days	N.A.	N.A.	332	265	716	486
15 Days	132	105	310	235	686	452
20 Days	134	86	284	210	N.A.	N.A.
25 Days	114	92	246	188	638	408

Note : N.A. - Not Analysed

Table 4.8 Variability of Lead in the test water (unit mg/l)

Time	Set I		Set II		Set III	
	Lead					
	Total	Soluble	Total	Soluble	Total	Soluble
0 Hour	0.964	0.830	1.874	1.280	4.800	4.130
8 Hours	0.902	0.758	1.799	1.220	4.324	3.680
16 Hours	0.860	0.720	1.602	1.060	4.300	3.600
24 Hours	0.801	0.640	1.523	1.000	4.285	3.500
5 Days	N.A.	N.A.	1.544	0.958	4.188	3.440
10 Days	0.701	0.550	1.368	0.862	4.180	3.300
15 Days	0.586	0.446	1.402	0.896	4.060	3.200
20 Days	0.621	0.484	1.301	0.794	4.000	3.040
25 Days	0.601	0.426	1.300	0.754	4.000	3.080

Note : N.A. - Not Analysed

Table 4.9 Variability of Some Physico-Chemical Parameters of Test Water (For Cd Bioconcentration Studies)

Time	Parameters			
	pH	DOC (mg/l)	Conductivity (mmhos)	Hardness (mg/l)
	Set I (Cd - 0.2 mg/l)			
0 Hour	6.8	4.52	0.412	114
24 Hours	6.9	6.80	0.438	120
5 Days	6.9	7.04	0.526	128
10 Days	7.1	8.94	0.564	130
15 Days	N.A.	9.58	0.586	128
20 Days	7.1	9.42	0.608	132
25 Days	7.2	10.60	0.694	134
	Set II (Cd - 0.5 mg/l)			
0 Hour	7.0	2.86	0.380	124
24 Hours	7.1	4.12	0.412	128
5 Days	N.A.	4.36	0.428	130
10 Days	7.1	6.28	0.486	132
15 Days	7.2	7.64	0.514	136
20 Days	7.2	8.14	0.542	142
25 Days	7.4	8.94	0.586	144
	Set III (Cd - 1.00 mg/l)			
0 Hour	7.3	8.38	0.418	138
24 Hours	7.4	9.24	0.618	144
5 Days	7.5	12.68	0.742	146
10 Days	7.5	14.28	0.796	144
15 Days	7.7	15.38	0.812	150
20 Days	7.8	14.26	0.802	154
25 Days	7.9	16.24	0.842	162

Note : N.A. - Not Analysed

Table 4.10 Variability of some physico-chemical parameters of test water (For Pb cioconcentration studies)

Time	Parameters			
	pH	DOC (mg/l)	Conductivity (mmhos)	Hardness (mg/l)
	Set I (Pb - 1.00 mg/l)			
0 Hour	6.9	5.26	0.380	114
24 Hours	7.0	5.48	0.412	118
5 Days	7.1	5.86	0.510	120
10 Days	7.1	6.12	0.542	124
15 Days	7.2	6.32	0.566	124
20 Days	7.3	N.A.	0.648	126
25 Days	7.3	6.34	0.680	128
	Set II (Pb - 2.00 mg/l)			
0 Hour	7.0	4.84	0.360	158
24 Hours	7.1	N.A.	N.A.	162
5 Days	7.2	5.08	0.514	164
10 Days	7.3	6.18	0.624	168
15 Days	7.3	7.12	0.648	166
20 Days	7.4	8.48	0.752	172
25 Days	7.5	8.23	0.750	178
	Set III (Pb - 5.00 mg/l)			
0 Hour	6.9	6.46	0.390	124
24 Hours	7.1	6.86	0.548	126
5 Days	7.2	7.00	0.716	128
10 Days	7.4	6.98	0.789	128
15 Days	7.6	7.18	0.868	130
20 Days	7.7	8.79	0.948	138
25 Days	7.8	7.89	0.950	136

Note : N.A. - Not Analysed

CHAPTER 5

TRANSMISSION ELECTRON MICROSCOPY AND X-RAY MICROANALYSIS

5.1 PRELIMINARY

In the past, the seeds of qualitative microscopic chemical localization planted by the morphologists have been nourished by biochemical analysts. The localization, quantitative analysis and activities of biological substances have been employed to expound the chemistry of the cells, substructures and products in both the normal and altered living state of the cells (Glick, 1985).

A variety of techniques have been developed to elucidate the structure and chemistry of the cells. Histochemical tests have been used in the localization of cellular substances to enable the identification of some organelles while the electron microscope analytical techniques permit qualitative and quantitative determinations of elemental distribution.

5.2 SPECTROSCOPY IN MICROSCOPY AND MICROANALYSIS

During the last three decades, there has been useful addition of information of new physical techniques for elemental analysis of small specimen volumes with high sensitivity. These techniques involve bombardment of specimen with material particles or electromagnetic radiation:

- to produce excitation of the elements in the specimen detected by the emission of particles or as electromagnetic radiation, and
- to obtain quantitative information about the specimen by scattering or absorption of the incident particles or byradiation.

Applications of Autometallography and the Localization of Heavy Metal Ions

Autometallography (AMG) is a histochemical process by which certain metals, metal sulfides and selenides are visualized involving a process of catalyzing the reduction of silver ions on their surfaces (Danscher, 1981a; 1991). With high specificity, traces of metals have been demonstrated in tissues and cells that had previously not been suspected of containing metals. AMG has until now been successfully used to demonstrate:

(a) Exogenous trace metals

- Gold (Roberts, 1935; Danscher, 1981b)
- Silver (Danscher, 1981c)
- Mercury (Timm, 1962; Danscher and Mollen-Madsen, 1985)
- Cadmium (George et al., 1976)

(b) Endogenous trace metals

- Zinc (Timm, 1958; Danscher, 1981a; 1982)

(c) Silver enhanced metal markers of macromolecules and enzymes

- Colloidal gold particles (Holgate et al., 1983; Danscher and Norgaard, 1985; Hacker et al., 1988; Lah et al., 1990)
- Copper sulphide accumulations associated with cuprolinic blue method (Lormee et al., 1989)
- Silver in plastic sections (Danscher, 1983)
- Silver used as histochemical indicator of lysosomal function (Rungby et al., 1990).

Literature is replete with reports on applications of AMG for the demonstration of HMs with known toxic effects. Chemically bound gold ions in tissue sections have been demonstrated autometallographically after reduction to metallic gold by ultraviolet radiation (Danscher, 1981b; 1991). Gold has also been localized by AMG in the cultures of marine macrophages (Moller- Madsen et al., 1985). Silver method (Danscher, 1981a) has been used to demonstrate metal as silver sulfide or silver selenide as an autometallographic application (Danscher, 1981c; 1991). The sulfide osmium method for ultrastructural localization of mercury was suggested by Silberberg et al. (1969). Mercury has been demonstrated by AMG in tissue from human beings and animals exposed to environmental mercury.

Electron Probe X-ray Microanalysis (EPMA) verifications of electron dense metal accumulations, identified by AMG in the ultra-thin sections, have been shown for gold (in lysosomes (Danscher, 1984), silver (when electron micrographs were compared before and after enhancement; Gherardi et al., 1984) and for mercury (by the concomitant presence of mercury and silver; Whittaker et al., 1990).

Volumetric AMG method has demonstrated that exposure of lead, primarily, affects the growth and division of neurons (Slominka et al., 1989). Use of volumetric AMG methods in the demonstration of hypertrophy of granule cells of neurons with very low doses of lead is a new application.

5.3 ANALYTICAL TECHNIQUES IN MICROANALYSIS

Advances in microanalytical techniques have contributed the development of:

- the improved high vaccum techniques and ultra high vaccum techniques,
- Conventional Transmission Microscopes (CTEM) in a Scanning Transmission Electron Microscopy mode (STEM),
- the improved electrostatic focussing electron spectrometers for Auger Electron Spectroscopy (AES) and X-ray Photoelectron Spectroscopy (XPS),

- the improved magnetic focussing electron spectrometers and their integration in standard SEM, (TEM and STEM instruments) for Electron Energy Loss Spectrometry (EELS),
- the solid state X-ray detector and associated electronics for Energy Dispersive X-ray Spectrometry (EDS),
- the electron sources of high brightness (field emission cathodes),
- the positive and negative primary ion sources for secondary ion mass spectrometry,
- high speed counters and multichannel analysers, and
- Quantitative Video Intensification Microscopy (QVIM) (Sisken et al., 1986).

All the techniques of microanalysis utilize some method of localization of the HMs for analysis. Spatial localization could be achieved by -

(i) Area localization
   - by focussing the exciting beam (microprobe method), or
   - by focussing the emitted or transmitted beam (selected area method), and

(ii) Depth localization
   - by using signals with a small excitation depth,
   - by using signals with small escape depth, or
   - by using very thin sections.

Among the methods available for microanalysis, EPMA represents the most widely used example of a microprobe technique for determining elemental composition in biological tissues (Erasmus, 1978; Hayat, 1980).

5.4 ELECTRON PROBE X-RAY MICROANALYSIS (EPMA)

Electron probe microanalysis or analytical electron microscopy is a relatively new method of analysis at ultra-structural levels. The major contribution of X-ray microanalysis to cell biology is in understanding the mechanisms that control various

cellular processes. EPMA has mainly been applied to elemental analysis using characteristic X- rays generated by the sample directly under the beam. Rapid progress in the application of X-ray microanalysis to the biological area has been made during the last few years. EPMA has been coupled with scanning, transmission and scanning transmission electron microscopy. Ion distribution in normal as well as in pathological cell systems of both plant and animal specimens has been studied using EPMA technique.

In EPMA, identification and localization of main elements of the periodic table in the biological specimen is accompanied with specificity, sensitivity and spatial resolution sufficient to permit measurements of element concentration in a single cell as well as in an organelle. X-ray microanalysis of naturally occurring elements in biological specimens could resolve 10^{-18} gm in an ultra-thin section under favourable conditions (Hall, 1975).

Quantitative X-ray Microanalysis of Thin Biological Sections

Soft biological tissue samples for EPMA fall into three basic categories:

- tissue sections thin or transparent to the electron beam,
- thick tissue sections in which the electron beam is severely attenuated, and
- solid samples infinitely thick or opaque to the electron beam.

Two basic criteria have been established to define a thin biological section for EPMA (Hayat, 1980):

- the electrons reach the far surface of the section without losing an appreciable fraction of their initial energy, and
- the X-rays generated in the sections are able to reach the surface of the section without being absorbed to an appreciable extent.

Approximately 1-2 μm sections of biological materials are usually considered thin enough to comply with the above criteria. The lower limit is appropriate for material embedded in resin. The use of thin sections of biological specimen in EPMA has two

additional advantages. First, the lateral spread of the exciting beam is greatly reduced and second, the X-ray (background) can be reduced by favourable location of the detector (Hayat, 1980).

Thin sections of soft biological materials in EPMA, that result in higher resolution and an immediate simplification of quantitative expressions (Russ, 1978) are prepared by investing the specimens with a polymerizable resin. Apart from facilitating the preparation of thin sections, the plastic matrix is useful because:

- the sections can be stained and morphologically studied by conventional microscopy in correlative studies, and
- the embedded specimen which is about 80% plastic is more homogeneous in composition and probably more stable under the electron beam(Hall & Gupta, 1974).

Morgan et al. (1978) have proposed the use of tissue whole mounting followed by air drying. Air drying is useful for:

- maintaining the chemistry of the cells, and
- for preserving the gross chemical integrity of certain discrete and highly mineralized intracellular structures.

5.5 APPLICATIONS OF EPMA

The biological applications of quantitative X-ray microanalysis range from studies on microorganisms to human materials. The available literature on cation concentrations of the membranes and the specific cells of bioindicator organisms is rather fragmentary. Reports mainly include Ca, Na, and K. Investigators have reported on the X-ray microanalysis of elements in the matrix of cnidarian nematocysts (Tardent et al., 1990), localization of putative calcium binding sites in striated muscles (Anderson and Josephson, 1983), X-ray microanalysis of sulfur containing granules in the fat body cells of homopteran insects

(Marshall, 1983), histochemical and X-ray microprobe study of spherites in Amblema plicata perplicat (Davis et al., 1982), and levels of chromosomal bound metals in dinoflagellates, Glenodinium foliaceum (Sigee and Kearns, 1981).

To elucidate the mechanism of iso-osmotic fluid, Gupta et al. (1976) carried out EPMA studies on ion - distribution in the tubular fluid secreting organisms. Na, K, and Cl concentrations were determined in Rhodnius (malphighian tubules) and Calliphora (salivary glands) (Hayat, 1980). To elucidate the mechanisms of transepithelial Na transport in frog skin, the electrolyte concentrations were determined on 1 um thick sections (Rick et al., 1978). Granular spiny and germinal cells showed low sodium and high potassium concentrations.

The measurements of cation concentrations in skeletal muscle cells is another area of application of EPMA in biological specimens. Somlyo et al. (1977a) reported the distribution of various elements in frog striated muscles. They found no chlorine in the terminal cisternae of resting muscle and reported that mitochondria partially excluded chlorine. Ca concentration of cisternae was much higher than that of the cytoplasm. In another important study on element distribution in the swim bladder muscle of the toad fish, it was exemplified by EPMA that neither chlorine nor sodium was sequestered in their sarcoplasmic reticulum in contrast to calcium (Somlyo et al., 1977b).

Limitations of the Approach

A reliable X-ray microanalysis is encountered with a number of difficulties. Several requirements have to be fulfilled for accurate quantitative analysis:

- separation of peaks from background continuum radiation and deconvolution of overlapping peaks,
- spatial resolution in dried biological specimen tissues with differing thickness, and
- preparation of thin or bulk specimen samples for EPMA and method of preparation.

Other experimental difficulties include contamination, mass loss, redistribution of elements under analysis, biological, extraneous background, instrumentation and automation of EPMA.

5.6 PRESENT STUDY

In the light of the above information, TEM and EPMA studies were carried out on thin sections of gills, liver amd muscle tissues of a fish, Colisa fasciatus. Investigations were carried out to:

- histochemically localize Cd and Pb in the tissue (gills, liver, and muscle) at cellular level,
- test applicability of SST in localization of metals (Cd and Pb) in bioindicator organs,
- scan, probe and quantify Cd and Pb at the membrane surfaces (inner and outer) and in specific cells of gills, liver and muscle tissues of fish,
- locate the type of Cd and Pb inclusions at cellular level in bioindicator organs, and
- define concentrations and distribution of major monovalent (Na, K) and divalent (Ca, Mg, Zn, Fe, S) cations at specific sites in the normal and altered living state of cells.

5.7 RESULTS AND DISCUSSION

Results presented herein on TEM and EPMA carried out on thin sections of gills, liver and muscle tissues of the fish have been discussed in terms of instruments and automation, quantitative elemental analysis, localization and quantitative analysis of Cd or Pb at specific cellular level and the variations of metabolic cations (Na, K, Ca, Mg, Fe, Zn, P, and S) with their possible redistribution in cells when exposed to sublethal Cd and Pb.

Instrumentation (EPMA, Jeol, JXA, 8600M) and Automation

EPMA, Jeol, JXA, 8600M is a fifth generation scanning type electron probe microanalyser that possesses high accuracy in analysis and is easy to operate. The instrument

is equipped with plural computers DEC LS1- 11/23 and 11/73 for coefficient control of its electron optical stage, automatic crystal change X-ray spectrometer and X-ray measurement system. Some limitations normally encountered with EPMA are overcome with the backscattered electron detector, secondary electron detector, and energy dispersive X-ray spectrometer. The application programs available for wavelength dispersive spectrometers include quantitative analysis (trace element analysis) with ZAF and Bance and Alee corrections and thus facilitate analysis of elements and the results are normally obtained as weight percentage.

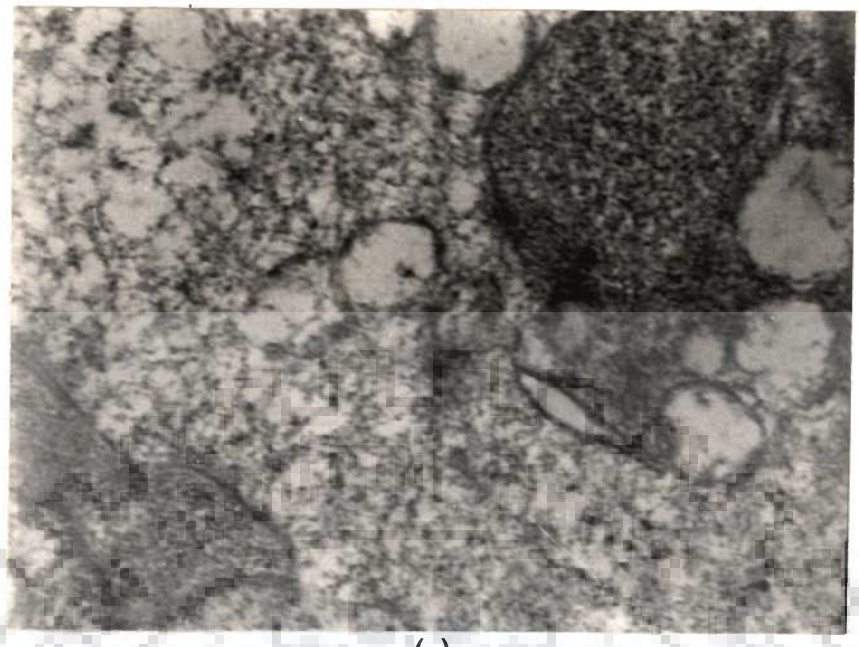
Localization of Cadmium and Lead in Fish Tissues

In Colisa fasciatus, gills have five pairs of gill arches with gill filaments on the first four pairs. The gill filaments are borne on hypobranchial, ceratobranchial and epibranchial arches. On the upper and lower surfaces of each filament, a row of closely packed leafy structures are present and are called as secondary lamellae.

Gills have two distinct regions (i) gill head region, and (ii) gill lamellae region. The head region consists of an outer layer which possesses epithelial cells. The inner side of gills head region consist of a number of small cells. Abductor muscles present in this region consists of muscle fibres. Secondary lamellae have epithelial layers, the endothelium of which consists of a series of pillar cells. Smooth muscle cells and mast cells remain present in the primary and secondary gill lamellae (Anand et al., 1976).

The transmission electron micrograph (TEM 1-4) of gill head region and gill lamellae region show silver intensified inclusions of Cd and Pb at selected sites in mast cells, smooth muscle cells and at the membranes of the epithelial cells.

A cellular view of liver (magnification 4500X) is presented in TEM 5. Cd was visualized as silver amplified electron dense inclusions inside the cells (TEM 5b). However, in liver tissue of Pb exposed fish, inclusions were not observed.



(a)

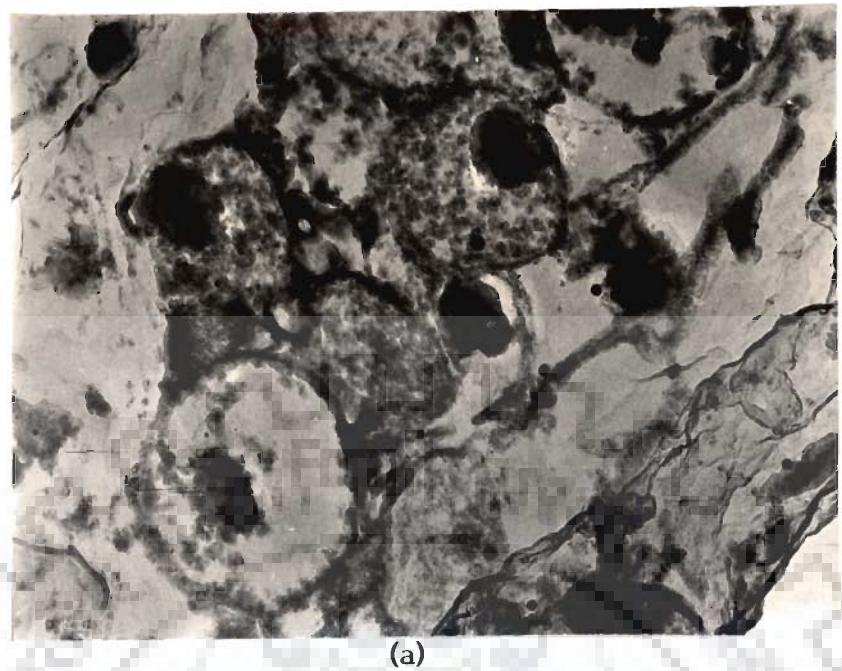
(b)

TEM1. Thin section of gills of Colisa fasciatus a. Gill head region (4500x) b. Gill lamellae region (4500x)

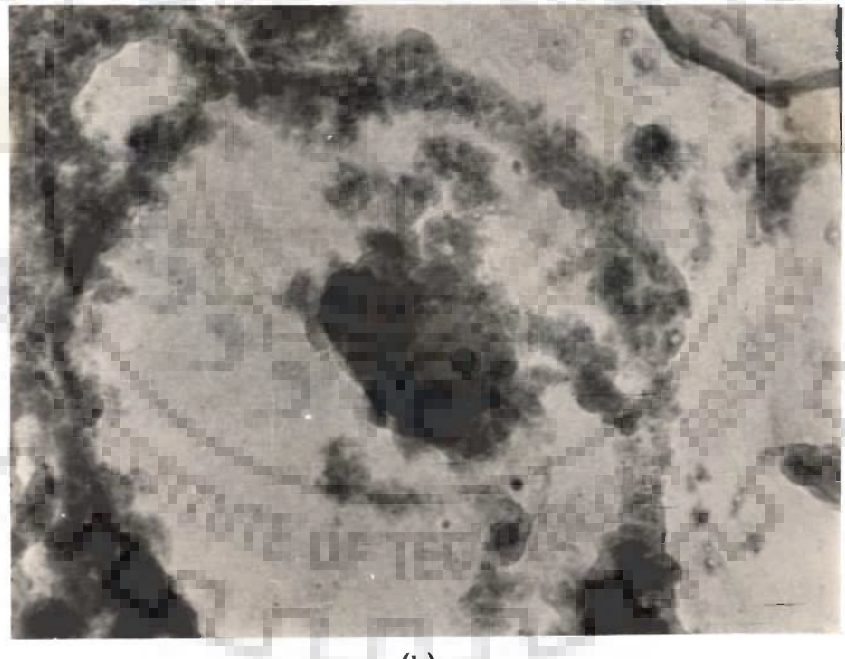
(a)

(b)

TEM2. Thin section of gills of Colisa fasciatus (Cd adsorption in gill head membrane)
a. 9600x b. 12700x



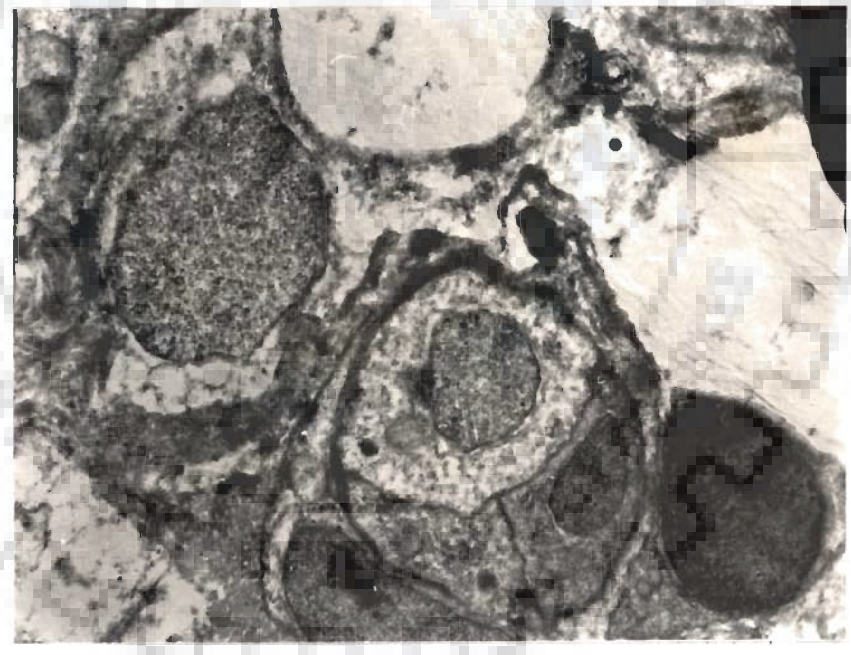
(a)



(b)

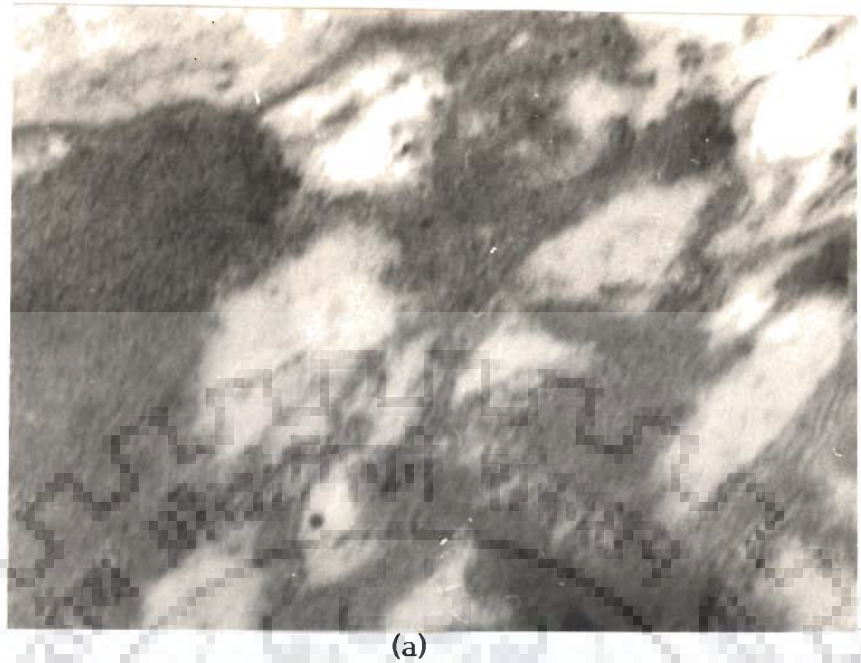
TEM3. Thin section of gills of Colisa fasciatus (Cd inclusion in gill lamellae cells)
a. 7000x b. 20,000x

(a)

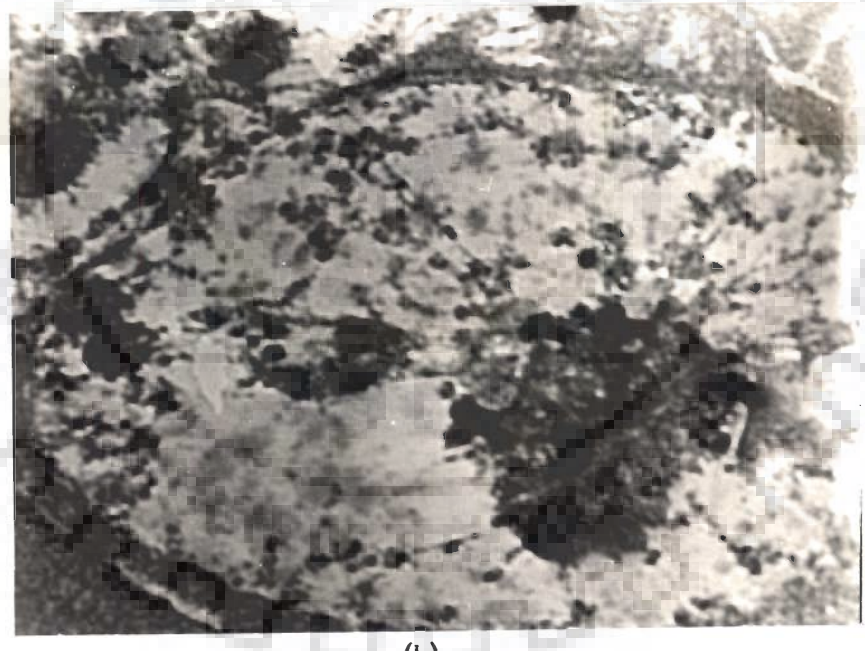


(b)

TEM4. Thin section of gills of Colisa fasciatus (Pb inclusion in gill lamellae cells) a. 4500x b. 12000x



(a)



(b)

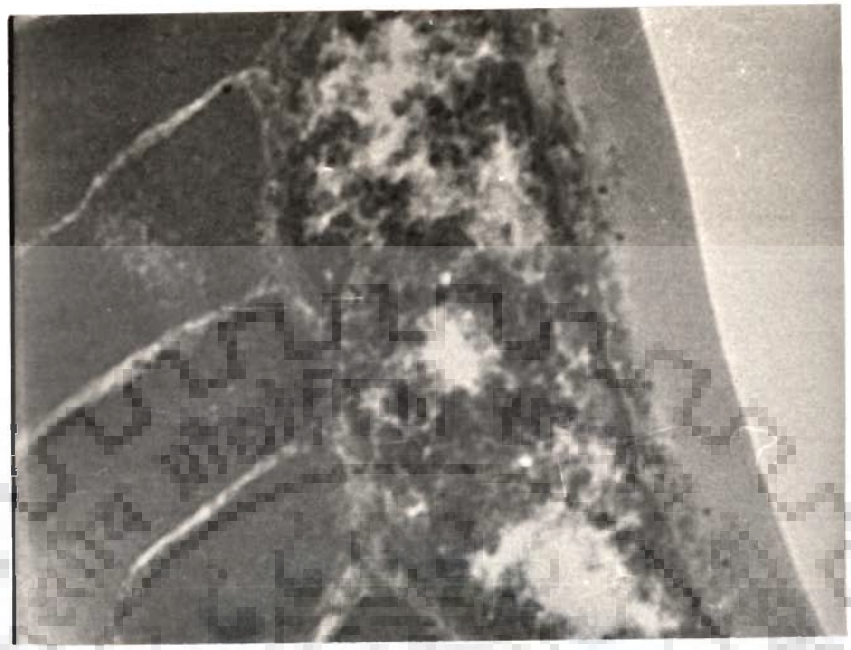
TEM5. Thin section of liver plate a. Control (4500x) b. Cd inclusion (9600x)

A cellular view of skeletal muscle cells is presented in TEM 6. In both Cd and Pb exposed fish, muscle surface shows silver amplified electron dense structures at the membrane surfaces of the cells and corroborates adsorption of Cd and Pb at the surface (TEM 6 & 7).

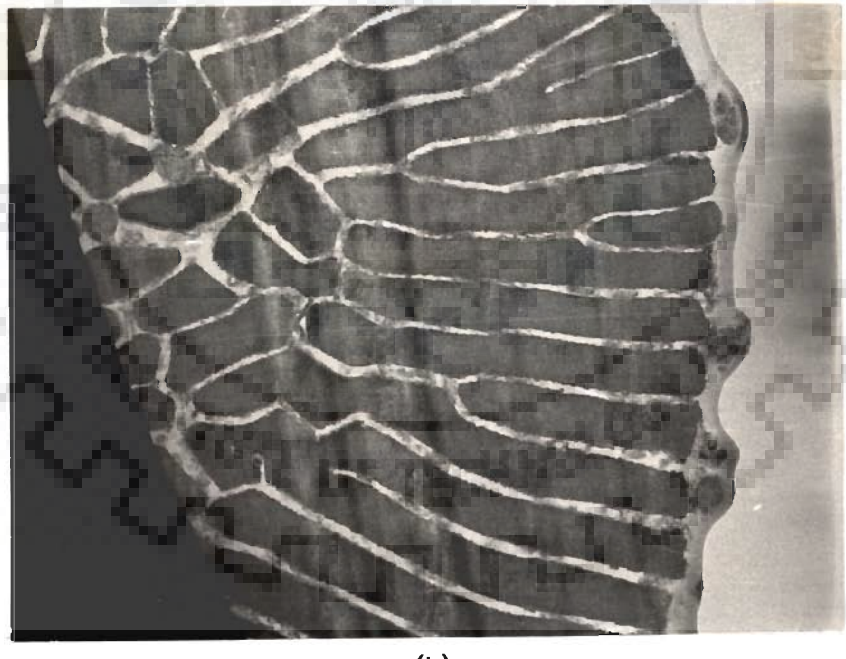
Subcellular localization of metal ions with known toxic effects have been demonstrated with AMG in earlier studies and corroborates the results of present investigation. It has been reported that SST increases the insight into the dynamics of heavy metal intoxications. SST basically employs a developer with a low pH, silver nitrate as silver ion donator and a protecting colloid, gum arabic. Developer involves the use of small amounts of hydroquinone as reduction molecules. SST results into reduced silver ion magnifications at specific sites with gum arabic reducing the autocatalytic activity in the developer itself and the catalytic activity of the zone between the developer and the surface of the section (Danscher, 1981c; 1991).

The results of the present study on localization of Cd and Pb, identified as silver magnifications, show that SST visualizes only a certain fraction of heavy metals represented by free or loosely bound metal which in the presence of Na_2S is converted into insoluble metal sulfides. However, metal that is strongly bound to organic/inorganic ligands may not be sulfidated and remains histochemically invisible.

The visualization of the fraction of Cd and Pb in fish tissue support Danscher and Norgaard (1985) that SST could be used for the demonstration of trace amounts of HMs at ultra-structural level and that the metal sulfides can be shown by the technique. In their investigation, George et al. (1976), using the precipitation reactions (sulfide for heavy metals), reported precipitation of Cd as sulfide in bivalve tissues. They also reported that precipitation reactions result in a better localization with a loss of 14% as compared to 28% when the bivalve tissues were processed without precipitant.



(a)



(b)

TEM6. Thin section of skeletal muscle of Colisa fasciatus a. Control (12700x) b. Cd adsorption (7400x)

(a)

(b)

TEM7. Thin section of skeletal muscle of Colisa fasciatus a. Cd adsorption (12700x) b. Pb adsorption (12700x)

Results are also corroborated by the findings of Seigal (1983) that excessive uptake results into bioconcentration from precipitation of insoluble metal complexes through the activities of membrane associated sulfate reductases or through the biosynthesis of oxidising agents such as H_2O_2. The reduction of sulfate to sulfide and the diffusion of H_2O_2 provide highly reactive means by which metals can be precipitated (Wood and Wang, 1983). In SST, metal sulfides are responsible for the precipitation of metallic silver and result into silver enhanced visualization.

Simpson and Dom (1983) investigated several heavy metal cations as prospective ionic tracers and reported Pb deposits with reproducible results. Pb deposits were shown in the intracellular spaces of parotid acinar cells. They advocated that ion precipitation could be applied to tracer methodology and provide a potential tool for analysis of epithelial and cellular transport including Pb as ionic tracer.

EMPA verifications of localization of Cd and Pb at specific sites discussed in section 5.6 further corroborate the presence of these toxic metal ions in fish tissues at the epithelial membranes and inside the specific cells of the tissues.

Electron Probe X-Ray Microanalysis of Fish Tissue

Cd and Pb were scanned, probed and quantified at selected sites in gills, liver and muscles in exposed Colisa fasciatus and were compared with that in normal living state of cells. The other cations detected and quantified include Na, K, Ca, Mg, S, P, Fe, and Zn. The results of quantitative analysis are presented in weight percent of the probed area.

EPMA of Gills of the Teleost Fish

In gill head region three sites were scanned. These include the outer membrane of epithelium, the inner membrane of the epithelial cells and inside the gill head region. In the gill lamellae region, the probe position was on the outer membrane of primary

lamellae, basement membrane and endothelium of secondary lamellae. The results obtained from the EPMA are presented in EPMA 1 and EPMA 2. Normal distribution of cations (S, Ca, Mg, Zn, Na, K, P, and Fe) is incorporated in EPMA 1 and EPMA 2. In EPMA 3-6 redistribution of cations (concentration variation at the same sites on the exposure of fish to sublethal concentrations of Cd and Pb) is incorporated.

Results of the cation distribution in EPMA 1 and EPMA 2 show that Cd and Pb are not present in unexposed fish gills and other cations constitute a weight percentage of 6.810, 11.286 and 6.695, respectively, at the outer surface and inner surface of epithelial cells and inside the gill head region. In the gill lamellae region, the cations scanned constitute a fraction of 12.844, 10.579 and 8.479 (weight percent of the area) at the outer membrane of primary lamellae, basement membrane and endothelium of secondary lamellae, respectively.

EPMA of the gill head region exposed to Cd shows this exogenous metal at selected sites. The concentration (average weight percent) of Cd in the gill head region of the exposed fish were 0.012, 0.028 and 0.018 at the probed sites. However, the concentrations of Cd in gill lamellae region were 0.026, 0.062 and 0.036 at the three probed sites, respectively (EPMA 3 and EPMA 4).

In the fish, exposed to Pb, the concentrations of Pb were 2.369, Nil and 0.030 in the gill head region while the concentrations in gill lamellae region were 1.516, Nil and 0.026 at the outer membrane of primary lamellae, basement mambrane and endothelium of secondary lamellae, respectively (EPMA 5 and EPMA 6).

EPMA of Liver of Teleost Fish

The selected sites scanned, probed and quantified include three sites of liver plates viz., outer surface, inner surface and inside the liver plate (including liver cells). The results obtained show that the area of three sites are represented by a weight percentage of 8.621,

EPMA 1. Analysis of Gill Head Region : Distribution of Cations

Probe Position	Outer Membrane			Inner Membrane			Inside the Gill Head		
Probe Diameter	0.1 μm			0.1 μm			5.0 μm		
Analysis	I	II	X	I	II	X	I	II	X
Metal									
Cd	-	-	-	-	-	-	-	-	-
Pb	-	-	-	-	-	-	-	-	-
S	-	0.171	0.086	0.207	-	0.104	0.427	0.005	0.216
Ca	0.829	2.381	1.605	1.360	3.884	2.622	1.356	1.744	1.550
Mg	0.540	1.500	1.020	0.976	2.436	1.706	0.890	1.394	1.142
Zn	-	0.055	0.028	0.288	-	0.144	-	0.477	0.239
Na	1.924	5.746	3.835	3.197	8.445	5.821	2.916	3.079	2.998
K	0.115	0.317	0.236	0.162	0.489	0.326	0.104	0.259	0.182
P	-	-	-	1.125	-	0.563	-	0.599	0.290
Fe	-	-	-	-	-	-	0.155	-	0.078
Total	3.408	10.17	6.810	7.315	15.254	11.286	5.848	7.557	6.695

Note :- X - Mean of I & II Analysis ; Units - Weight % of Area Probed

EPMA 2. Analysis of Gill Lamellae Region : Distribution of Cations

Probe Position	Outer Membrane			Inner Membrane			Inside the Gill Head		
Probe Diameter	0.1 μm			0.1 μm			5.0 μm		
Analysis	I	II	X	I	II	X	I	II	X
Metal									
Cd	-	-	-	-	-	-	-	-	-
Pb	-	-	-	-	-	-	-	-	-
S	0.223	0.123	0.173	0.181	0.152	0.167	0.375	0.114	0.245
Ca	3.580	3.009	3.295	2.605	2.083	2.344	1.654	2.464	2.059
Mg	1.977	2.315	2.146	1.264	1.352	1.308	1.172	1.517	1.345
Zn	0.264	-	0.132	-	-	-	-	-	-
Na	4.635	8.354	6.495	5.310	4.794	5.052	2.930	5.107	4.019
K	0.415	0.457	0.436	0.306	0.265	0.286	0.316	0.331	0.324
P	-	0.244	0.122	2.297	0.418	1.358	-	0.797	0.399
Fe	0.090	-	0.045	0.128	-	0.064	0.088	0.087	0.088
Total	11.184	14.502	12.844	12.091	9.064	10.579	6.535	10.417	8.479

Note :- X - Mean of I & II Analysis : Units - Weight % of Area Probed

EPMA 3. Analysis of Gill Head Region of Teleost Fish Exposed to Cd

Probe Position	Outer Membrane			Inner Membrane			Inside the Gill Head		
Probe Diameter	0.1 μm			0.1 μm			5.0 μm		
Analysis	I	II	X	I	II	X	I	II	X
Metal									
Cd	0.019	0.004	0.012	-	0.055	0.028	-	0.036	0.018
S	0.377	0.262	0.320	0.280	0.269	0.275	0.022	0.175	0.098
Ca	3.692	2.824	3.258	1.426	1.255	1.341	0.577	1.305	0.941
Mg	2.322	1.738	2.030	0.771	0.929	0.850	0.361	0.596	0.479
Zn	0.265	0.014	0.140	0.088	0.244	0.210	-	0.422	0.211
Na	8.555	4.182	6.369	3.117	2.121	2.619	2.077	3.281	2.679
K	0.527	0.397	0.462	0.124	0.190	0.157	0.061	0.105	0.083
P	0.332	-	0.166	-	-	-	0.211	-	0.106
Fe	0.065	-	0.033	0.067	0.126	0.097	0.082	0.053	0.068
Total	16.154	9.421	12.790	5.873	5.189	5.577	3.391	5.973	4.683

Note :- X - Mean of I & II Analysis ; Units - Weight % of Area Probed

EPMA 4. Analysis of Gill Lamellae Region of Teleost Fish Exposed to Cd

Probe Position	Outer Membrane			Inner Membrane			Inside the Gill Head		
Probe Diameter	0.1 μm			0.1 μm			5.0 μm		
Analysis	I	II	X	I	II	X	I	II	X
Metals									
Cd	0.036	0.015	0.026	0.073	0.051	0.062	0.013	0.059	0.036
S	0.111	0.115	0.113	0.041	0.051	0.046	0.369	0.090	0.230
Ca	3.489	1.553	2.521	3.523	3.533	3.528	3.012	4.274	3.643
Mg	1.935	0.954	1.445	2.112	1.753	1.933	2.005	2.472	2.239
Zn	0.046	-	0.023	0.213	-	0.166	-	0.362	0.181
Na	3.856	3.753	3.805	7.702	4.168	5.935	6.188	7.270	6.729
K	0.386	0.215	0.288	0.416	0.439	0.428	0.336	0.486	0.411
P	0.649	-	0.325	0.630	0.089	0.360	-	-	-
Fe	0.143	0.073	0.180	0.017	0.058	0.038	-	0.097	0.049
Total	10.651	6.678	8.716	14.727	10.142	12.496	11.923	15.110	13.518

Note :- X - Mean of I & II Analysis : Units - Weight % of Area Probed

EPMA 5. Analysis of Gill Head Region of Teleost Fish Exposed to Pb

Probe Position	Outer Membrane			Inner Membrane			Inside the Gill Head		
Probe Diameter	0.1 μm			0.1 μm			5.0 μm		
Analysis	I	II	$\bar{X}$	I	II	$\bar{X}$	I	II	$\bar{X}$
Metals									
Pb	0.861	3.877	2.369	-	-	-	-	0.060	0.030
S	0.179	0.206	0.193	0.059	0.024	0.042	0.102	0.134	0.118
Ca	3.514	3.675	3.595	4.044	1.589	2.817	1.948	1.754	1.851
Mg	2.067	2.055	2.061	2.393	0.599	1.492	1.032	0.950	0.991
Zn	0.013	0.023	0.018	-	0.018	0.009	-	0.018	0.009
Na	6.761	11.289	9.025	9.730	3.684	6.707	2.577	2.366	2.472
K	0.422	0.527	0.475	0.540	0.235	0.388	0.230	0.218	0.224
P	0.409	0.463	0.436	1.469	0.933	1.201	-	-	-
Fe	0.042	0.043	0.043	0.142	0.023	0.083	-	0.216	0.108
Total	14.268	22.158	18.215	18.377	7.105	12.739	5.889	5.716	5.803

Note :- $\bar{X}$ - Mean of I & II Analysis ; Units - Weight % of Area Probed

EPMA 6. Analysis of Gill Lemallae Region of Teleost Fish Exposed to Pb

Probe Position	Outer Membrane			Inner Membrane			Inside the Gill Head		
Probe Diameter	0.1 μm			0.1 μm			5.0 μm		
Analysis	I	II	X	I	II	X	I	II	X
Metals									
Pb	-	3.031	1.516	-	-	-	0.052	-	0.026
S	0.056	0.243	0.150	0.327	0.152	0.240	0.148	0.399	0.274
Ca	3.579	1.735	2.657	2.749	1.643	2.196	3.219	1.687	2.453
Mg	2.133	0.952	1.543	1.117	0.944	0.779	2.169	0.825	1.497
Zn	0.069	-	0.035	-	0.208	0.104	-	-	-
Na	9.576	4.827	7.202	3.228	2.249	2.765	9.715	2.120	5.918
K	0.480	0.256	0.368	0.276	0.195	0.236	0.409	0.246	0.327
P	1.749	1.794	1.772	0.526	-	0.263	-	-	-
Fe	0.151	-	0.076	-	0.111	0.056	0.032	0.006	0.019
Total	17.793	12.838	15.319	8.223	5.502	6.639	15.744	5.283	10.514

Note :- X - Mean of I & II Analysis ; Units - Weight % of Area Probed

11.223 and 7.257. The cation distribution is represented in EPMA 7.

In the Cd exposed organisms, the concentration of Cd were recorded at all the three sites of liver and the concentrations were 0.019, 0.053 and 0.044 in three selected sites,respectively (EPMA 8).

In the Pb exposed teleost fish, EPMA of the same sites show higher concentration of Pb at the outer surface and inside the liver plate (with liver cells) than the inner surface (EPMA 9). The concentrations of Pb were 0.533, 0.197 and 0.502, respectively, with the total concentration of cations being 24.201, 16.477, and 12.868, respectively.

EPMA of Skeletal Muscle Cells of the Teleost Fish

Probe position in the skeletal muscle cells was fixed at the outer surface, inner surface and inside the muscle cells. EPMA 10 includes the results of cations present in the unexposed teleost fish. The total cation concentrations were 8.062, 8.532 and 9.150 (average weight percent of the area) at the three sites, respectively.

In the Cd exposed teleost fish, when microanalysis was carried out, Cd was present at all the three sites with concentrations 0.033, 0.049 and 0.039, respectively. The total concentration of cations (probed in the present study) were 9.272, 7.069 and 6.920, respectively, at the three probed sites (EPMA 11).

In the Pb exposed teleost fish, Pb was 0.782, 0.853 and 1.156 at the outer surface, inner surface and inside the skeletal muscle cells. The total cation concentrations (at the same sites) represent a fraction of 12.771, 10.725 and 11.076 (average weight percentage) (EPMA 12).

Results presented herein (EPMA 1 - EPMA 12) on the concentration of Cd, Pb, S, Ca, Mg, Zn, Na, K, P, and Fe at the given sites of gill head region, gill lamellae region, liver plates and skeletal muscle cells of the unexposed fish, and in the experimental fish tissues (exposed to sublethal concentrations of cadmium and lead), show that cations

EPMA 7. Analysis of Liver : Distribution of Cations in Liver Plate

Probe Position / Probe Diameter	Outer Membrane 0.1 μm				Inner Membrane 0.1 μm				Inside the Liver Plate 5.0 μm			
Analysis	I	II	III	X	I	II	III	X	I	II	III	X
Metals												
Cd	-	-	-	-	-	-	-	-	-	-	-	-
Pb	-	-	-	-	-	-	-	-	-	-	-	-
S	-	0.452	0.436	0.271	0.032	0.005	0.348	0.128	0.400	0.085	0.136	0.207
Ca	3.263	1.525	1.602	2.169	3.128	3.606	1.440	2.725	2.716	1.403	1.364	1.828
Mg	2.126	0.986	1.168	1.427	2.365	2.209	0.856	1.810	1.521	0.978	0.988	1.623
Zn	-	-	0.204	0.068	0.023	0.188	-	0.070	0.083	-	-	0.028
Na	7.502	2.381	3.043	4.309	7.979	7.210	2.260	5.816	3.648	2.250	2.325	2.741
K	0.365	0.273	0.304	0.314	0.376	0.390	0.212	0.326	0.260	2.207	0.197	0.221
P	-	-	-	-	-	-	0.646	0.215	0.442	0.592	0.767	0.600
Fe	0.111	0.022	0.058	0.063	0.217	0.040	0.142	0.133	-	-	0.028	0.009
Total	13.367	5.639	6.815	8.621	14.120	13.648	5.904	11.223	9.070	7.515	5.805	7.257

Note :- X - Mean of I , II & III Analysis ; Units - Weight % of Area Probed

EPMA 8. Analysis of Liver Plate of Teleost Fish Exposed to Cd

Probe Position / Probe Diameter	Outer Membrane 0.1 μm				Inner Membrane 0.1 μm				Inside the Liver Plate 5.0 μm			
Analysis	I	II	III	X	I	II	III	X	I	II	III	X
Metals												
Cd	0.058	-	-	0.019	0.025	0.069	0.067	0.053	0.010	-	0.121	0.044
S	0.269	0.349	0.358	0.325	0.042	0.156	0.027	0.075	0.220	0.428	0.135	0.261
Ca	3.693	3.097	1.441	2.756	2.045	4.300	1.438	2.594	3.249	1.559	1.933	2.247
Mg	1.518	1.389	0.994	1.300	1.026	2.264	1.010	1.433	1.828	1.137	1.338	1.434
Zn	-	2.225	0.222	0.149	-	0.448	0.032	0.160	0.057	0.130	0.102	0.094
Na	3.349	3.303	2.568	3.073	2.368	8.561	2.190	4.373	7.781	3.089	3.601	4.824
K	0.301	2.229	0.238	0.256	0.189	0.501	0.233	0.304	0.337	0.279	0.275	0.297
P	-	-	1.142	0.381	1.840	-	0.090	0.643	1.686	-	1.733	1.140
Fe	0.160	-	-	0.053	0.002	0.173	-	0.058	0.131	0.405	-	0.179
Total	9.348	12.592	6.963	8.312	7.537	16.472	5.087	9.693	15.299	7.027	9.238	10.520

Note :- X - Mean of I , II & III Analysis ; Units - Weight % of Area Probed

EPMA 9. Analysis of Liver Plate of Teleost Fish Exposed to Pb

Probe Position / Probe Diameter	Outer Membrane 0.1 μm				Inner Membrane 0.1 μm				Inside the Liver Plate 5.0 μm			
Analysis	I	II	III	X	I	II	III	X	I	II	III	X
Metals												
Pb	1.598	-	-	0.533	0.393	-	N.A.	0.197	1.459	0.022	0.026	0.502
S	0.057	0.061	0.109	0.076	0.079	0.192	N.A.	0.136	0.147	0.201	0.096	0.148
Ca	3.489	15.415	15.859	11.588	4.093	11.204	N.A.	7.649	3.590	3.179	3.160	3.310
Mg	1.757	1.338	1.010	1.388	1.932	1.246	N.A.	1.589	1.972	1.629	1.657	1.751
Zn	-	0.549	-	0.183	0.091	-	N.A.	0.045	-	0.028	-	0.009
Na	3.840	4.194	7.348	5.128	3.956	4.615	N.A.	4.286	4.024	8.055	7.650	6.576
K	0.402	0.359	0.367	0.376	0.395	0.436	N.A.	0.416	0.373	0.376	0.390	0.380
P	2.750	6.061	5.804	4.884	-	4.318	N.A.	2.159	-	0.327	-	0.109
Fe	0.027	0.107	-	0.045	-	-	N.A.	-	0.045	0.110	0.093	0.083
Total	13.929	28.084	30.497	24.201	10.939	22.011	N.A.	16.477	11.610	13.927	13.072	12.868

Note :- $\bar{X}$ - Mean of I , II & III Analysis ; N.A. - Not Analysed ; Units - Weight % of Area Probed

EPMA 10. Analysis of Skeletal Muscle Cells of Teleost Fish

Probe Position	Outer Membrane				Inner Membrane				Inside the Cells			
Probe Diameter	0.1 μm				0.1 μm				5.0 μm			
Analysis	I	II	III	$\bar{X}$	I	II	III	$\bar{X}$	I	II	III	$\bar{X}$
Metals												
Cd	-	-	-	-	-	-	-	-	-	-	-	-
Pb	-	-	-	-	-	-	-	-	-	-	-	-
S	0.030	0.098	0.525	0.217	0.305	0.138	0.120	0.188	0.204	0.146	0.270	0.207
Ca	3.282	1.686	1.695	2.221	2.286	2.113	1.749	2.049	2.166	2.050	2.017	2.078
Mg	1.686	1.230	1.078	1.331	1.375	1.554	1.388	1.439	1.184	1.544	1.511	1.413
Zn	0.028	-	-	0.009	0.327	0.037	0.462	0.275	-	0.173	-	0.058
Na	3.991	3.629	3.089	3.569	3.306	3.199	4.959	3.821	2.852	3.812	6.744	4.469
K	0.369	0.321	0.314	0.335	0.215	0.237	0.377	0.276	0.219	0.362	0.431	0.337
P	-	1.109	0.030	0.380	-	-	0.940	0.313	-	0.633	0.958	0.530
Fe	-	-	-	-	0.165	0.164	0.181	0.170	0.090	0.041	0.043	0.058
Total	9.386	8.073	6.731	8.062	7.979	7.442	10.176	8.531	6.715	8.761	11.974	9.150

Note :- $\bar{X}$ - Mean of I, II & III Analysis ; Units - Weight % of Area Probed

EPMA 11. Analysis of Skeletal Muscle Cells of Teleost Fish Exposed to Cd

Probe Position	Outer Membrane 0.1 μm				Inner Membrane 0.1 μm				Inside the Cells 5.0 μm			
Probe Diameter / Analysis	I	II	III	X	I	II	III	X	I	II	III	X
Metals												
Cd	0.042	0.032	0.025	0.033	-	0.027	0.119	0.049	0.058	0.040	0.018	0.039
S	0.200	0.124	0.270	0.198	0.281	0.410	0.511	0.401	0.208	0.223	0.312	0.205
Ca	2.325	1.487	2.276	2.030	1.378	1.888	2.804	2.023	1.369	1.757	1.749	1.625
Mg	1.382	1.108	1.786	1.425	0.837	0.838	1.439	1.038	1.010	1.195	1.063	1.089
Zn	-	-	0.112	0.037	0.153	-	0.551	0.235	0.452	0.436	-	0.296
Na	4.340	3.326	7.395	5.020	1.872	1.765	3.844	2.494	2.016	3.838	3.736	3.197
K	0.217	0.104	0.397	0.239	0.144	0.242	0.338	0.241	0.141	0.206	0.176	0.174
P	-	-	0.543	0.181	0.237	0.377	0.996	0.537	0.447	0.381	-	0.276
Fe	0.074	0.197	0.056	0.109	-	0.154	-	0.051	-	-	0.057	0.019
Total	8.580	6.378	12.860	9.272	4.902	5.701	10.602	7.069	5.701	8.076	7.111	6.920

Note :- $\bar{X}$ - Mean of I, II & III Analysis ; Units - Weight % of Area Probed

EPMA 12. Analysis of Skeletal Muscle Cells of Teleost Fish Exposed to Pb

Probe Position	Outer Membrane				Inner Membrane				Inside the Cells			
Probe Diameter	0.1 μm				0.1 μm				5.0 μm			
Analysis	I	II	III	X	I	II	III	X	I	II	III	X
Metals												
Pb	-	1.614	0.733	0.782	0.060	1.794	0.706	0.853	0.852	-	2.616	1.156
S	0.150	0.070	0.178	0.116	0.326	0.281	0.223	0.277	0.240	0.226	0.157	0.207
Ca	4.011	3.354	2.006	3.124	1.505	3.231	1.540	2.092	1.098	4.368	1.577	2.348
Mg	2.432	1.696	1.525	1.884	1.032	2.229	1.049	1.437	0.726	2.414	1.053	1.398
Zn	0.279	-	0.300	0.193	0.142	0.044	-	0.062	-	-	0.031	0.010
Na	10.093	4.127	3.348	5.856	4.032	9.029	3.748	5.603	3.112	9.242	2.414	4.923
K	0.494	0.403	0.297	0.398	0.199	0.405	0.247	0.284	0.153	0.437	0.187	0.259
P	1.081	-	-	0.360	-	-	0.232	0.077	-	1.992	0.243	0.745
Fe	0.105	0.068	-	0.058	-	0.108	0.012	0.040	0.089	-	-	0.030
Total	18.644	11.332	8.387	12.771	7.296	17.121	7.757	10.725	6.270	18.679	8.278	11.076

Note :- $\bar{X}$ - Mean of I , II & III Analysis ; Units - Weight % of Area Probed

quantified in the present study constitute only a fraction of total weight of the area. The distribution and concentration of cations is shown to be site specific. Varying concentrations of Cd and Pb are present in exposed fish tissue when probed as bound to the outer membrane, inner membrane of the cells and inside the cell surface. The results thus, show compartmentation of exogenous trace metals at the cellular level.

Distribution of cations at various sites at the cellular level in the tissues of control and in altered living state of cells (exposed to sublethal concentrations of cadmium or lead) show discrimination when compared with the distribution of cations at the same sites in the unexposed control and exposed tissues. However, it would be premature to state that introduction of toxic metal ions (Cd or Pb) in fish tissue replaces other cations for its accumulation.

The findings on the localization, distribution and concentration of membrane bound and other cellular cations of metabolic importance are supported by the scientific reports available in the literature (Berry et al., 1978; Birkhead et al., 1982; Hopkin and Martin, 1982a; Anselme et al., 1990). However, these studies normally report for other biological organisms.

Birkhead et al. (1982) reported intracellular localization and quantitative analysis of Pb in the tissues of Cygnus olor. They reported that the intracellular inclusions of Pb were present. X-ray microanalysis of renal granules in the study confirmed an amorphous mass of Pb. Large electron dense granules were also reported in the liver of the Pb poisoned Cygnus olor. Hutton (1980) in his microanalytical study demonstrated that 50% of the subcellular lead was associated with the nuclei of cells of Columba livia. It was shown that large amounts of Pb was present in mitochondria with marked abnormalities in the cells.

Hopkin and Martin (1982a) reported that the specimens of Onicus asellus of Cd, Pb, and Zn contaminated sites contained about 12%, 0.5% and 2.5% of Zn, Cd, and Pb (dry

weight). They reported the distribution of metals within the hepatopancreas of Onicus asellus (crustacean) by AAS, TEM, and X-ray microanalysis in the B cells of hepatopancreas. Flocculent deposits were present that contained Pb and Zn.

Woodlice from the contaminated sites have also been shown to have deposits of Zn and Pb on the membrane of the cells. It was found that the membrane of the cells were lined with a fine deposits of electron dense material in which Zn and Pb were detected by X-ray microanalysis (Hopkin and Martin, 1982b). It was also shown that Zn, Cd, and Pb concentrations in the test fraction of Onicus asellus were about 70, 5 and 65 μg/gm dry weight, respectively.

Anselme et al. (1990) reported that Pb precipitates were observed as spherical structures when 3 metal/collagen sponges were implanted subcutaneously. X-ray microanalysis of the structural localization of the electron dense granules seen in the lysosome of phagocytic cells were reported to contain Pb and were associated with calcium.

Wayback in 1974, Jundt et al., used proton induced X-ray analysis of trace elements in tissue sections (liver of the new born, thyroid carcinoma and osteosarcoma) with concentrations (in nanograms/0.5 mg of various tissue samples) of K, Ca, Fe, Zn and Pb. The concentrations were 568, 514, 619, 146 and 22 (in liver), 861, 443, 52, 15 and 9 (in thyroid carcinoma) and 573, 426, 61, 15 and 6 (in osteosarcoma) for K, Ca, Fe, Zn, and Pb, respectively. It was shown that the method of analysis (proton induced X-ray microanalysis) is a powerful tool for quantitative element analysis, uniform sensitivity and requires no chemical preparation or ashing (which leads to loss of volatile elements) (Jundt et al., 1974).

A thorough survey of the literature available shows that in the investigations carried out and reported, emphasis has been on the localization and quantitative analysis of metabolic cations such as Ca, Mg, K etc. Literature is enriched with the reports on the concentration of these cations with their analysis at various membrane surfaces of different

model systems (Yarom and Chandler, 1974; Berry et al., 1978; Sauer and Watabe 1989).

Yarom and Chandler (1974) reported quantitative assessment of Ca by X-ray microanalysis on resting frog skeletal muscles. Significant concentrations of Ca were reported in the dense parts of nucleus. Ca was demonstrated by Berry et al. (1978) in the intracellular vacuoles of the proximal renal tubule cells by an ultra-structural microanalytical study.

Hargest et al. (1985) reported Na, Mg, P, S, Ce, K and Ca concentrations in cytoplasm, extracellular matrix, matrix vesicled in the epoxy embedded tissue levels of the four zones of chick epiphyseal growth cartilage. Exceptionally high levels of Na and K (550 and 200 mmol/kg wet weight, respectively) were reported in the matrix. Within the cells, Na was reported to be higher than k (140 versus 20-34 mmol/kg wet wt). Ca and P were reported to be low in cells while the Mg was found to be undetectable. S levels were reported to be high in the matrix (400 mmol/kg wet weight).

Sauer and Watabe (1989) reported Zn accumulations in the lysosome like structures in the estuarine teleost, Fundulus heteroclitus when examined using histochemical (Timm Sulfide Silver Technique) and X-ray microanalytical techniques. Samples were dehydrated through a graded series of ethanol and embedded in Embed 81Q plastic resin via propylene oxide. Element analysis of K, Ca, Fe, and Zn were reported in the lysosome like structures. The concentrations were 14.3, 20.3, 1.0, 1.4 in control and 13.9, 15.4, 0.4 and 10.0 in the fish exposed to Zn. The element analysis for K, Ca, Fe, and Zn in the osteoblast cytoplasm of the control and Zn exposed tissues were 23.8, 17.5, Nil (control) and 21.2, 17.0, Nil and 0.5, respectively. The element analysis in these structures have a relatively high metal content in the surrounding cytoplasm.

CHAPTER 6

CONCLUSIONS

CONCLUSIONS

The preceeding chapters of this dissertation cover a study on bioamplification of Cd, Pb, and Zn in an ecosystem. Levels of Cd, Pb, and Zn in sediment, water, plankton and whole body fish tissue were recorded in a riverine ecosystem, Hindon and Kalinadi. Laboratory studies were carried out to determine the bioconcentration kinetics of Cd and Pb in fish tissue (gills, liver and muscles) exposed to the sublethal concentrations of Cd and Pb. An attempt has also been made to localize and quantify Cd and Pb in fish tissue using histochemical and X-ray microanalytical techniques. TEM and EPMA of specific sites in gills, liver and skeletal muscle cells of fish were carried out to scan, probe and quantify selected trace metals at cellular level. On the basis of experimental studies, the conclusions

arrived at are cited below:

1. River Hindon and Kalinadi have toxic pollutants including high levels of trace metals that render the system grossly polluted at some points endangering its fauna and flora.

2. Biota of river Hindon and Kalinadi have built up elevated concentrations of metal toxicants including Cd and Pb.

3. The physico-chemical characteristics of water (abiotic environment) moderate the extent of bioconcentration in an aquatic ecosystem.

4. In the riverine ecosystem, higher levels of certain persistent metal ions (Cd, Pb, and Zn) were found at high trophic status (fish tissue) and thus, bioamplification of selected heavy metals in an ecosystem is manifested.

5. A conceptual model of bioamplification of Cd, Pb, and Zn is developed defining three levels of contamination viz., water, plankton (low trophic level) and fish (high trophic level).

6. Simulated studies on bioconcentration of Cd and Pb show that bioconcentration of Cd and Pb in fish tissue is a function of time and sublethal concentration of Cd and Pb in the exposure medium.

7. Bioconcentration and bioconcentration factors (BCFs) of Cd and Pb in a teleost fish tissue (gills, liver and muscles) have been worked out under static conditions.

8. Rate of accumulation for Cd and Pb in fish tissue show an initial rapid phase and later slow phase, responsible for net accumulation.

9. Modified Sulphide Silver Technique (SST) was employed to localize Cd and Pb in gills, liver, and muscle cells of a teleost fish. Cd and Pb were silver amplified in gill head region, gill lamellae region, membranes of liver cells and muscle surface (Cd only) at

cellular level. Technique was shown to visualize the loosely bound exogenous metals.

10. Electron probe X-ray microanalysis (EPMA) of gill head region, gill lamellae region, liver plate and skeletal muscle cells at the cell membranes and inside the specific cells of the tissue show the presence of exogenous metals (Cd and Pb).

11. Cd and Pb were quantified in fish tissue at cellular level and their presence was corroborated with EPMA. Distribution of cations (S, P, Na, K, Ca, Mg, Zn and Fe) shows that these probed cations form only a fraction (weight percent) of the total area of the cells of fish tissue quantified.

12. Distribution of cations of metabolic importance at probed sites and their redistribution on exposure of fish to sublethal concentrations of Cd or Pb is presented with the remark that distribution of cations is site specific and it is still premature to state that bioconcentration of Cd and Pb replaces other divalent cations.

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APPENDIX- A

DATA FILE OF STATION S-1

```
1,2,5
7.6,0.18,118.0,2.61,5.80,61.8,0.23,86.0,12.0,2.8,8.6,87.0,58.0,
0.36,0.30,264.0,120.0,6.4,66.0,38.5
6.9,0.22,132.0,6.21,5.60,71.9,0.05,122.0,16.0,6.40,6.80,118.0,
80.0,0.42,0.35,246.0, 138.0,3.8,46.0,37.8
7.4,0.24,142.0,8.2,5.4,63.8,0.15,0.15,118.0,32.0,9.4,25.6,132.0,
60.0,0.36,0.00,86.0,22.0,16.4,54.6,76.0
7.4,0.30,162.0,14.3,5.8,47.9,0.11,128.0,14.0,4.8,16.2,88.0,48.0,
0.38,0.16,164.0,50.0,7.6,52.4,48.2
```

DATA FILE OF STATION S-2

```
1,2,5
8.2,0.54,348.0,5.21,4.80,76.9,1.14,142.0,42.0,7.5,64.2,114.0,
62.0,0.46,0.26,295.0,94.0,12.4,98.0,46.4
7.6,0.64,384.0,18.5,4.4,141.5,0.52,184.0,48.0,17.5,42.6,136.0,
85.0,0.50,0.29,280.0,118.0,7.8,68.0,42.0
8.4,0.93,728.0,19.8,3.8,164.1,3.87,262.0,86.0,28.2,98.4,146.0,
60.0,0.48,0.00,114.0,16.0,22.4,68.8,157.0
8.3,0.93,542.0,17.0,3.6,106.0,1.98,166.0,64.0,72.2,64.8,102.0,
45.0,0.46,0.14,186.0,26.0,14.2,64.2,87.6
```

APPENDIX B

DATA FILE OF POSTMONSOON SEASON

```
1,2,5
7.6,0.18,118.0,2.61,5.80,61.8,0.23,86.0,12.0,2.8,8.6,87.0,
58.0,0.36,0.30,264.0,120.0,6.4,66.0,38.5
8.2,0.54,348.0,5.21,4.80,76.9,1.14,142.0,42.0,7.5,64.2,
114.0,62.0,0.46,0.26,295.0,94.0,12.4,98.0,46.4
7.4,0.32,186.0,2.85,5.60,67.8,0.16,124.0,14.0,3.6,18.6,48.0,
30.0,0.68,0.54,257.0,108.0,4.8,106.0,22.4
7.8,0.38,246.0,3.09,5.20,71.6,0.43,138.0,24.0,5.2,32.8,60.0,
38.0,0.54,0.38,286.0,112.0,5.6,108.4,32.8
8.1,0.43,322.0,2.38,5.0,81.0,0.96,160.0,42.0,4.8,40.2,66.0,
41.0,0.62,0.42,298.0,112.0,8.92,134.2,29.0
7.9,0.56,342.0,3.21,5.0,85.4,0.64,154.0,36.0,5.4,36.2,64.0,
36.0,0.58,0.38,308,100.0,7.98,138.6,23.3
```

DATA FILE OF WINTER SEASON

```
1,2,5
6.9,0.22,132.0,6.21,5.60,71.9,0.05,122.0,16.0,6.40,6.80,118.0,80.0,
0.42,0.35,246.0,138.0,3.8,46.0,37.8
7.6,0.64,384.0,18.5,4.4,141.5,0.52,184.0,48.0,17.5,42.6,136.0,85.0,
0.50,0.29,280.0,118.0,7.8,68.0,42.0
7.0,0.28,158.0,4.2,5.4,85.9,0.08,131.0,18.0,4.2,20.8,76.0,54.0,0.76,
0.61,224.0,117.0,2.64,104.2,23.6
7.4,0.43,286.0,12.2,4.8,111.7,0.26,154.0,28.0,10.2,30.4,102.0,68.0,
0.68,0.54,218.0,108.0,3.42,96.0,30.6
7.9,0.63,415.0,10.5,5.0,131.0,0.98,190.0,38.0,20.8,38.6,92.0,60.0,
0.72,0.47,222.0,106.0,4.9,130.0,28.8
7.7,0.66,432.0,12.6,5.2,127.4,0.60,214.0,46.0,18.5,34.6,94.0,58.0,
0.74,0.48,234.0,98.0,5.4,118.4,20.4
```

APPENDIX C

```
$DEBUG
C     ************************************************************************
c     PRINCIPAL COMPONENT ANALYSIS
C     DATA SHOULD BE FORMAT FREE
C     M IS  THE NUMBER OF VARIABLRES (COLUMNS)
C     N IS THE NUMBER OF OBSERVATIONS (ROWS)
C     ************************************************************************
      DIMENSION X(20,20),FSCORE(20,20)
      DIMENSION A1(20,20),A2(20,20),A3(20,20)
      DIMENSION YXA(20,20)
      OPEN(UNIT=1,FILE='S15.DAT',STATUS='OLD')
      OPEN(UNIT=2,FILE='S15.RES',STATUS='NEW')
      MD=20
      ND=20
      MM=20
1     READ(1,*) ITRAN,ISIM,L
      IF(ITRAN.LE.0)GO TO 500
      CALL READM(X,N,M,ND,MD)
      WRITE(2,2001)
      CALL PRINTM(X,N,M,ND,MD)
      IF(ISIM.NE.1)GO TO 2
      CALL STAND (X,N,M,ND,MD)
      WRITE(2,2008)
      CALL PRINTM (X,N,M,ND,MD)
2     IF(ITRAN.NE.2) GO TO 3
      MT=N
      IF(N.GT.M)NT=N
      DO 110 I=1,MT
      DO 110 J=1,MT
      XS=X(J,I)
      X(I,J)=X(J,I)
      X(J,I)=XS
110   CONTINUE
      MT=N
      M=N
      N=MT
 3    IF(ISIM.EQ.1)CALL RCOEF(X,N,M,ND,MD,A1,MM)
      IF(ISIM.EQ.2)CALL CTHETA(X,N,M,ND,MD,A1,MM)
      WRITE(2,2002)
      CALL PRINTM(A1,M,M,MM,MM)
      IF(ISIM.NE.1) GO TO 4
      DO 111 I=1,M
      DO 111 J=1,M
      A3(I,J)=A1(I,J)
111   CONTINUE
4     CALL EIGENJ(A1,A2,M,MM)
```

```
      SUME=0.0
      DO 100 I=1,M
      A1(I,1)=A1(I,I)
      SUME=SUME+A1(I,1)
100   CONTINUE
      SUMEE=0.0
      DO 101 I=1,M
      A1(I,2)=A1(I,1)*100.0/SUME
      SUMEE=SUMEE+A1(I,1)
      A1(I,3)=SUMEE*100.0/SUME
101   CONTINUE
      WRITE(2,2003)
      CALL PRINTM(A1,M,M,MM,MM)
      WRITE(2,2004)
      CALL PRINTM(A2,M,M,MM,MM)
      DO 102 I=1,M
      DO 102 J=1,L
      A2(I,J)=A2(I,J)*SQRT(A1(J,1))
102   CONTINUE
      WRITE(2,2005)
      CALL PRINTM(A2,M,M,MM,MM)
      IF(ISIM.NE.1) GO TO 5
      DO 112 I=1,M
      DO 112 J=1,M
      DET=0.0
      DO 113 K=1,L
      DET=DET+A2(I,K)*A2(J,K)
113   CONTINUE
      A1(I,J)=DET
      A1(J,I)=DET
112   CONTINUE
      WRITE(2,2010)
      CALL PRINTM(A1,M,M,MM,MM)
      CALL SUBM(A3,A1,A3,M,M,MM,MM)
      WRITE(2,2011)
      CALL PRINTM(A3,M,M,MM,MM)
5     DO 103 I=1,L
      DO 103 J=1,L
      DET=0.0
      DO 104 K=1,M
      DET=DET+A2(K,I)*A2(K,J)
104   CONTINUE
      A3(I,J)=DET
      A3(J,I)=DET
103   CONTINUE
      DO 261 I=1,L
      YXA(I,1)=100.0
261   CONTINUE
      DO 600 I=1,L
```

```
      WRITE(2,2012)(A3(I,J),J=1,L)
600   CONTINUE
2012  FORMAT(1X,15F7.3)
      CALL MATINV(A3,L,YXA,1,DET,ID)
      WRITE(2,2014)ID,DET
2014  FORMAT(10X,I5,E16.5)
      DO 262 I=1,L
      DO 263 J=1,L
      A1(I,J)=A3(I,J)
263   CONTINUE
262   CONTINUE
      CALL MMULT(X,A3,FSCORE,N,M,L,ND,MD,MM,MM,ND,MD)
      WRITE(2,2007)
      CALL PRINTM(FSCORE,N,L,ND,MD)
      CALL VARMAX(A2,M,L,MM)
C     PRINT ROTATED MATRIX
      WRITE(2,2006)
      CALL PRINTM(A2,M,L,MM,MM)
      DO 105 I=1,L
      DO 105 J=1,L
      DET=0.
      DO 106 K=1,M
      DET=DET+A2(K,I)*A2(K,J)
106   CONTINUE
      A3(I,J)=DET
      A3(J,I)=DET
105   CONTINUE
      DO 264 I=1,L
      YXA(I,1)=100.0
264   CONTINUE
      DO 605 I=1,L
      WRITE(2,2012)(A3(I,J),J=1,L)
605   CONTINUE
      CALL MATINV(A3,L,YXA,1,DET,ID)
      WRITE(2,2014)ID,DET
      DO 265 I=1,L
      DO 266 J=1,L
      A1(I,J)=A3(I,J)
266   CONTINUE
265   CONTINUE
      CALL MMULT(A2,A1,A3,M,L,L,MM,MM,MM,MM,MM,MM)
      CALL MMULT(X,A3,FSCORE,N,M,L,ND,MD,MM,MM,ND,MD)
      WRITE(2,2009)
      CALL PRINTM(FSCORE,N,L,ND,MD)
1000  FORMAT(3I3)
2001  FORMAT(1H0,4X,'INPUT DATA MATRIX-',1X,'COLUMNS=VARIABLES
     1,ROWS=OBSERVATIONS')
```

```
2002  FORMAT(1H0,4X,'SIMILARITY MATRIX')
2003  FORMAT(1H0,4X,'COLUMN1=EIGEN VALUES',2X,'COLUMN2=PERC
     1ENT OF TRACE-',5X,'COLUMNS=CUMULATIVE PERCENT OF TRACE')
2004  FORMAT(1H0,4X,'PRINCIPAL AXIS MATRIX-',1X,'COLUMNS=EIGEN
     1VECTORS,ROWS=VARIABLES')
2005  FORMAT(1H0,4X,'FACTOR LOADINGS-',1X,'COLUMNS=FACTORS,
     1ROWS=VARIABLES')
2006  FORMAT(1H0,4X,'ROTATED FACTOR MATRIX-',1X,'COLUMNS=FACTORS,
     1ROWS=VARIABLES')
2007  FORMAT(1H0,4X,'FACTOR SCORES-',1X,'COLUMNS=VARIABLES,ROWS=
     1OBSERVATIONS')
2008  FORMAT(1H0,4X,'STANDARERDISED INPUT DATA MATRIX-',1X,'COLUMNS=
     1VARIABLES, ROWS=OBSERVATIONS')
2009  FORMAT (1H0,4X,'VARIMAX SCORES-',1X,'COLUMNS=FACTORS,ROWS
     1=OBSERVATIONS')
2010  FORMAT(1H0,4X,'REPRODUCED CORRELATION MATRIX')
2011  FORMAT(1H0,4X,'RESIDUAL CORRELATION MATRIX')
      CLOSE(UNIT=1)
500   STOP
      END
C       ***************************************************************
      SUBROUTINE VARMAX(F,M,L,M1)
C       ***************************************************************
      DIMENSION F(M1,M1),H(20)
      WRITE(2,2001)
      SQRT2=1.0/SQRT(2.0)
      XM=M
      L1=L-1
      NIT=-1
      NCM=0
      DO 100 I=1,M
      SUMH=0.0
      DO 101 J=1,L
      SUMH=SUMH+F(I,J)**2
101   CONTINUE
      H(I)=SUMH
      DO 102 J=1,L
      F(I,J)=F(I,J)/SQRT(SUMH)
102   CONTINUE
100   CONTINUE
1     TVF=0.0
      DO 103 I=1,L
      SF1=0.0
      SF2=0.0
      DO 104 J=1,M
      SF1=SF1+F(J,I)**2
      SF2=SF2+F(J,I)**4
```

```
104   CONTINUE
      TVF=TVF+(XM*SF2-SF1*SF1)/(XM*XM)
103   CONTINUE
      IF(NIT.LT.0) GO TO 2
      IF(ABS(TVF-TV1).GT.0.000001) GO TO 2
      NCM=NCM+1
      IF(NCM.GE.5)GO TO 50
2     NIT=NIT+1
      TV1=TVF
      WRITE(2,2002)NIT,TVF
      DO 105 I=1,L1
      L2=I+1
      DO 106 J=L2,L
      A=0.0
      B=0.0
      C=0.0
      D=0.0
      DO 107 K=1,M
      X=F(K,I)
      Y=F(K,J)
      U=(X+Y)*(X-Y)
      V=2.0*X*Y
      A=A+U
      B=B+V
      C=C+(U+V)*(U-V)
      D=D+2.0*U*V
107   CONTINUE
      XN=D-(2.0*A*B)/XM
      XO=C-(A*A-B*B)/XM
      XR=SQRT(XN*XN+XO*XO)
      IF(XR.LE.0.001)GO TO 106
      COS4T=XO/XR
      COS2T=SQRT(abs((1.0+COS4T)/2.0))
      COS1T=SQRT(abs((1.0+COS2T)/2.0))
      SIN1T=SQRT(abs(1.0-COS1T*COS1T))
      IF(SIN1T.LE.0.001) GO TO 106
      IF(XN.LT.0.0)SIN1T=-SIN1T
      DO 108 K=1,M
      X=F(K,I)
      Y=F(K,J)
      F(K,I)=X*COS1T+Y*SIN1T
      F(K,J)=Y*COS1T-X*SIN1T
108   CONTINUE
106   CONTINUE
105   CONTINUE
      GO TO 1
50    WRITE(2,2004)
      WRITE(2,2001)
      DO 110 I=1,M
```

```
      SUMH=0.0
      DO 111 J=1,L
      F(I,J)=F(I,J)*SQRT(H(I))
      SUMH=SUMH+F(I,J)**2
111   CONTINUE
      D=H(I)-SUMH
      WRITE(2,2003)I,H(I),SUMH,B
110   CONTINUE
      WRITE(2,2005)
      RETURN
2001  FORMAT(1H1)
2002  FORMAT(1H0,I5,3X,F15.7///)
2003  FORMAT(1X,I5,3F15.7)
2004  FORMAT(1H0,4X,'NUMBER OF VARIMAX ITERATIONS',1X,
     1'AND VARIANCE AT EACH STEP')
2005  FORMAT(1H0,4X,'COLUMN1=INITIAL COMUNALITY',2X,'COLUMN2=C
     1OMUNALIT AFTER ROTATION',/,5X,'COLUMN3=DIFFERENCE')
      END
C       ***************************************************************
C     PROGRAMME FOR PRINCIPAL COMPONENT ANALYSIS
      SUBROUTINE CTHETA(X,N,M,N1,M1,A,M2)
C       ***************************************************************
      DIMENSION X(N1,M1),A(M2,M2)
      DO 100 I=1,M
      DO 100 J=I,M
      SX1X1=0.0
      SX2X2=0.0
      SX1X2=0.0
      DO 101 K=1,N
      SX1X1=SX1X1+X(K,I)**2
      SX2X2=SX2X2+X(K,J)**2
      SX1X2=SX1X2+X(K,I)*X(K,J)
101   CONTINUE
      A(I,J)=SX1X2/SQRT(SX1X1*SX2X2)
      A(J,I)=A(I,J)
100   CONTINUE
      RETURN
      END
C       ***************************************************************
      SUBROUTINE EIGENJ(A,B,N,N1)
C       ***************************************************************
      DIMENSION A(N1,N1),B(N1,N1)
      ANORM=0.0
      DO 100 I=1,N
      DO 101 J=1,N
      IF(I-J)2,1,2
1     B(I,J)=1.
      GO TO 101
```

```
2       B(I,J)=0.0
        ANORM=ANORM+A(I,J)*A(I,J)
101     CONTINUE
100     CONTINUE
        ANORM=SQRT(ANORM)
        FNORM=ANORM*1.0E-09/FLOAT(N)
        THR=ANORM
23      THR=THR/FLOAT(N)
3       IND=0
        DO 103 I=2,N
        I1=I-1
        DO 103 J=1,I1
        IF(ABS(A(J,I))-THR)103,4,4
4       IND=1
        AL=-A(J,I)
        AM=(A(J,J)-A(I,I))/2.0
        AO=AL/SQRT(AL*AL+AM*AM)
        IF(AM)5,6,6
5       AO=-AO
6       SINX=AO/SQRT(2.0*(1.0+SQRT(1.0-AO*A0)))
        SINX2=SINX*SINX
        COSX=SQRT(1.0-SINX2)
        COSX2=COSX*COSX
        DO 104 K=1,N
        IF(K-J)7,10,7
7       IF(K-I)8,10,8
8       AT=A(K,J)
        A(K,J)=AT*COSX-A(K,I)*SINX
        A(K,I)=AT*SINX+A(K,I)*COSX
10      BT=B(K,J)
        B(K,J)=BT*COSX-B(K,I)*SINX
        B(K,I)=BT*SINX+B(K,I)*COSX
104     CONTINUE
        XT=2.0*A(J,I)*SINX*COSX
        AT=A(J,J)
        BT=A(I,I)
        A(J,J)=AT*COSX2+BT*SINX2-XT
        A(I,I)=AT*SINX2+BT*COSX2+XT
        A(J,I)=(AT-BT)*SINX*COSX+A(J,I)*(COSX2-SINX2)
        A(I,J)=A(J,I)
        DO 105 K=1,M
        A(J,K)=A(K,J)
        A(I,K)=A(K,I)
105     CONTINUE
103     CONTINUE
102     CONTINUE
        IF(IND)20,20,3
20      IF(THR-FNORM)25,25,23
```

```
25      DO 110 I=2,N
        J=I
29      IF(A(J-1,J-1)-A(J,J))30,110,110
30      AT=A(J-1,J-1)
        A(J-1,J-1)=A(J,J)
        A(J,J)=AT
        DO 111 K=1,N
        AT=B(K,J-1)
        B(K,J-1)=B(K,J)
        B(K,J)=AT
111     CONTINUE
        J=J-1
        IF(J-1)110,110,29
110     CONTINUE
        RETURN
        END
C       ***********************************************************************
        SUBROUTINE RCOEF(X,N,M,N1,M1,A,M2)
C       ***********************************************************************
        DIMENSION X(N1,M1),A(M2,M2)
        AN=N
        DO 100 I=1,M
        DO 100 J=1,M
        SX1=0.0
        SX2=0.0
        SX1X1=0.0
        SX2X2=0.0
        SX1X2=0.0
        DO 101 K=1,N
        SX1=SX1+X(K,I)
        SX2=SX2+X(K,J)
        SX1X1=SX1X1+X(K,I)**2
        SX2X2=SX2X2+X(K,J)**2
        SX1X2=SX1X2+X(K,I)*X(K,J)
101     CONTINUE
        R=(SX1X2-SX1*SX2/AN)/SQRT((SX1X1-SX1*SX1/AN)*(SX2X2-
       1SX2*SX2/AN))
        A(I,J)=R
        A(J,I)=R
100     CONTINUE
        RETURN
        END
C       ***********************************************************************
        SUBROUTINE PRINTM(A,N,M,N1,M1)
C       ***********************************************************************
        DIMENSION A(N1,M1)
        DO 100 IB=1,M,10
        IE=IB+9
```

```
      IF(IE-M)2,2,1
1     IE=M
2     WRITE(2,2000)(I,I=IB,IE)
      DO 101 J=1,N
      WRITE(2,2001)J,(A(J,K),K=IB,IE)
101   CONTINUE
100   CONTINUE
      WRITE(2,999)
999   FORMAT(1X,120('-'))
      RETURN
2000  FORMAT(1H0,1X,12I10)
2001  FORMAT(1H0,I8,4X,12F12.4)
      END
C     ***************************************************************
C     MATRIX INVERSION
C     ***************************************************************
      SUBROUTINE MATINV(A,N1,B,M1,DETERM,ID)
      DIMENSION A(20,20),B(20,1),INDEX(20,3)
      N=N1
      M=M1
10    DETERM=1.0
15    DO 20 J=1,M
20    INDEX(J,3)=0
30    DO 550 I=1,N
40    AMAX=0.0
45    DO 105 J=1,N
      IF(INDEX(J,3)-1)60,105,60
60    DO 100 K=1,N
      IF(INDEX(K,3)-1)80,100,716
80    IF(AMAX-ABS(A(J,K)))85,100,100
85    IROW=J
90    ICOLUM=K
      AMAX=ABS(A(J,K))
100   CONTINUE
105   CONTINUE
      INDEX(ICOLUM,3)=INDEX(ICOLUM,3)+1
206   INDEX(I,1)=IROW
270   INDEX(I,2)=ICOLUM
130   IF(IROW-ICOLUM)140,310,140
140   DETERM=-DETERM
150   DO 200 L=1,N
160   SWAP=A(IROW,L)
170   A(IROW,L)=A(ICOLUM,L)
200   A(ICOLUM,L)=SWAP
      IF(M)310,310,210
210   DO 250 L=1,M
220   SWAP=B(IROW,L)
230   B(IROW,L)=B(ICOLUM,L)
```

```
250   B(ICOLUM,L)=SWAP
310   PIVOT=A(ICOLUM,ICOLUM)
      DETERM=DETERM*PIVOT
330   A(ICOLUM,ICOLUM)=1.0
340   DO 350 L=1,N
350   A(ICOLUM,L)=A(ICOLUM,L)/PIVOT
355   IF(M)380,380,360
360   DO 370 L=1,M
370   B(ICOLUM,L)=B(ICOLUM,L)/PIVOT
380   DO 550 L1=1,N
390   IF(L1-ICOLUM)400,550,400
400   T=A(L1,ICOLUM)
420   A(L1,ICOLUM)=0.0
430   DO 450 L=1,N
450   A(L1,L)=A(L1,L)-A(ICOLUM,L)*T
455   IF(M)550,550,460
460   DO 500 L=1,M
500   B(L1,L)=B(L1,L)-B(ICOLUM,L)*T
550   CONTINUE
      DO 710 I=1,N
610   L=N+1-I
620   IF(INDEX(L,1)-INDEX(L,2))630,710,630
630   JROW=INDEX(L,1)
640   JCOLUM=INDEX(L,2)
650   DO 705 K=1,N
660   SWAP=A(K,JROW)
670   A(K,JROW)=A(K,JCOLUM)
700   A(K,JCOLUM)=SWAP
705   CONTINUE
710   CONTINUE
      DO 730 K=1,N
      IF(INDEX(K,3)-1)715,720,715
715   ID=2
      GO TO 740
720   CONTINUE
730   CONTINUE
      ID=1
      GO TO 740
716   ID=2
740   RETURN
      END
C       ************************************************************************
      SUBROUTINE STAND(X,N,M,N1,M1)
C       ************************************************************************
      DIMENSION X(N1,M1)
      DO 100 I=1,M
      SX=0.0
      SXX=0.0
      DO 101 J=1,N
```

```
      SX=SX+X(J,I)
      SXX=SXX+X(J,I)**2
101   CONTINUE
      XM=SX/FLOAT(M)
      SD=SQRT((SXX*FLOAT(N)-SX*SX)/FLOAT(N*(N-1)))
      DO 102 J=1,N
      IF(SD.NE.0.0)GO TO 103
      WRITE(2,104)J
104   FORMAT(3X,'SD=0.0 FORJ=',I5)
      GO TO 102
103   CONTINUE
      X(J,I)=(X(J,I)-XM)/SD
102   CONTINUE
100   CONTINUE
      RETURN
      END
C       ***************************************************************
      SUBROUTINE READM(A,N,M,N1,M1)
C       ***************************************************************
      DIMENSION A(N1,M1)
      M=20
      N=4
1     FORMAT(2I10)
      DO 2 I=1,N
      READ(1,*)(A(I,J),J=1,M)
      WRITE(2,*)(A(I,J),J=1,M)
2     CONTINUE
3     FORMAT(15F6.2)
      RETURN
      END
C       ***************************************************************
      SUBROUTINE SUBM(A,B,C,N,M,N1,M1)
C       ***************************************************************
      DIMENSION A(N1,M1),B(N1,M1),C(N1,M1)
      DO 100 I=1,N
      DO 101 J=1,M
      C(I,J)=A(I,J)-B(I,J)
101   CONTINUE
100   CONTINUE
      RETURN
      END
C       ***************************************************************
      SUBROUTINE MMULT(A,B,C,L,N,M,NA,MA,NB,MB,NC,MC)
C       ***************************************************************
      DIMENSION A(NA,MA),B(NB,MB),C(NC,MC)
      OPEN(UNIT=1,FILE='S15.DAT',STATUS='OLD')
      DO 100 I=1,L
      DO 101 J=1,M
      C(I,J)=0.0
      DO 102 K=1,N
```

```
      C(I,J)=C(I,J)+A(I,K)*B(K,J)
102   CONTINUE
101   CONTINUE
100   CONTINUE
      RETURN
      END
```