STUDIES ON THE INTER-COMPARTMENTAL EXCHANGE OF TRACE METALS IN AN ESTUARINE SYSTEM

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CERTIFICATE

This is to certify that this thesis is a bonafide record of research carried out by Sri. Babukutty Y. under my guidance, in partial fulfilment of the requirements for the degree of ***Philosophiae Doctor*** *of the Cochin University of Science and Technology.*

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DECLARATION

I hereby declare that this thesis entitled "Studies on the Inter-compartmental Exchange of Trace Metals in an Estuarine System" is an authentic record of research carried out by me under the supervision of Dr.Jacob Chacko, Reader, Chemical Oceanography Division, School of Marine Sciences, Cochin University of Science and Technology, and that no part of it has previously formed the basis of the award of any degree, diploma, associateship, fellowship or other similar title or recognition.

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PREFACE

Human life is based on the consumption of resources provided to him by the benevolence of Mother Nature. The unending quest of man, concomitant with the exponential growth of population and economic development has however tilted away the balance between resources and consumption. This imbalance has led to a plethora of environmental problems of diverse nature.

The varied and increasingly complex problems arising out of anthropogenic use of the ocean have now raised man's consciousness regarding the pressing need to protect marine ecosystems and hence to develop sensitive and precise diagnostic tools with a predictive capability for environmental impact assessment. Rivers and estuaries, the most polluted areas of the aqueous environment, deserve special attention in pollution management programmes. A thorough assessment of the relative concentrations of pollutants in the different environmentally significant compartments of an aquatic system is an essential pre-requisite for any systematic hazard evaluation programme.

The present study is an attempt at investigating the inter-compartmental exchange of trace metals (copper, cadmium, zinc, lead and nickel) in the Cochin estuary. The nature and extent of distribution in the different compartments with special reference to the transport from environmental compartments to biological compartments have been dealt with in detail. The suitability of the shells of *Villorita cyprinoides* var. *cochinensis* (Hanely) in pollution monitoring activities has been assessed. A mathematical model (**SAAMPLE - S**hells in the **A**ssessmet of **A**quatic **M**etal **P**ollution **LE**vels) based on kinetic laws that govern the inter- compartmental exchange has been proposed, tested and verified.

The results of the present investigation have been published /are under publication/ have been presented in seminars as detailed hereunder

1. Toxicity of a bipartite metal mixture on *Villorita cyprinoides* var. *cochinensis*.

Y. Babukutty, T.S. Geetha and J. Chacko *J. BioSci.* (under publication).

2. Trace metal enrichment in an estuarine bivalve -- shells vs. soft tissues.

Y. Babukutty and J. Chacko, *Ambio* (under publication)

3. SAAMPLE - A predictive mathematical model for assessment of aquatic metal pollution.

Y. Babukutty, N. Chandramohanakumar and J. Chacko, *Mar. Ecol. Prog. Ser.* (under publication)

4.Combined toxicity of copper and cadmium on *Villorita cyprinoides* var. *cochinensis*, an estuarine clam.

Y. Babukutty, T. S. Geetha and J. Chacko, Presented at the National Seminar on Estuarine Management, Trivandrum, 1987.

5. Carotenoids as an index of heavy metal stress in an estuarine clam.

Jacob Chacko, T.S. Geetha and Y. Babukutty, Presented at theInternational Symposium on the Fate and Effects of Toxic Chemicals in large Rivers and their Estuaries, Quebec, Canada, 1988.

6. Trace metal levels in the different phases of an aquatic ecosystem.

Y. Babukutty and Jacob Chacko. Accepted for presentation at the World Fisheries Congress, Athens, Greece, 1992.

Chapter 1

INTRODUCTION

The life supporting environmental regime, we call ecosystem, is a very closely interwoven fabric of all living things, coupled with the natural processes, that determine the character, quantity and quality of life that can be supported. Life in the ocean, as life on land, is intimately related to its environment. During the past few decades, human influence on marine ecosystems has been quite significant. Until, man understands the complexities of the aquatic environment so as to fully realize the disadvantageous consequences of his actions, he cannot hope to safely exploit the environment to his advantage. The increasing awareness of the magnitude of environmental problems triggered by human intervention has served to focus attention on the urgent need for sensitive and precise diagnostic tools with a predictive capability for environmental impact assessment.

From oceans life expanded into estuaries, rivers and

lakes. An estuary is "an inlet of the sea reaching into a river valley as far as the upper limit of the tidal rise" (Fairbridge, 1980). Thus, estuaries are highly dynamic systems subject to changes occurring over a spectrum of durations ranging from very short periods to geologic time spans. The flood plain soils of estuaries constitute some of the most valuable agricultural land on earth. Regional seas and near shore areas extending to the edge of the continental shelf often constitute the world's richest fishing zones. Large ports have developed on estuaries or immediately upstream on the navigable rivers that flow into them. Extensive industrial developments are located in these ports and along the shores of estuaries and on the rivers flowing into them. Estuaries are often linked with refineries and oil storage depots, steel and paper mills as well as a diversity of chemical industries (Allan, 1990). In short, the banks of rivers and estuaries became the foci of civilization, because of the favourable features such as the profuse vegetation, fertile soil, access to navigational facilities etc. that have catalyzed the flourishing of human habitats in those regions.

Human association with estuaries and its associated rivers has unfortunately led to their contamination by a variety of pollutants. Public concern for this gross pollution has generated a global demand for initiating regulatory measures to control estuarine and near shore pollution. Pollution has come

to assume such gigantic proportions that it has become virtually impossible to plan its total eradication. A scientific management of the hazard alone seems feasible, if man is to reconcile with the dual imperatives of use and conservation of his planet's resources. Statutory pollution management guidelines would have to be based on sound scientific advice emerging out of systematic, quantitative and definitive assessment programmes.

GESAMP (1980) have defined aquatic pollution as "the introduction by man, directly or indirectly of substances or energy resulting in such deleterious effects as harm to living resources, hazards to human health, hindrance to aquatic activities including fishing, impairment of quality for use of water and reduction of amenities". Identification of a substance as "hazardous to the environment" involves the determination of its potential for biological damage and the assessment of the chemical's potential for environmental exposure. The potential biological damage can be evaluated by toxicity tests, both acute and chronic. Acute toxicity tests determine the dose of a particular chemical that will elicit a specific response or measurable end point from a test organism in a relatively short period of time, while chronic toxicity tests investigate the exposure of the organism to a chemical over a prolonged period (van Leeuwen, 1988). Environmental exposure assessment is quantified in terms of its potential

environmental distribution (PED) and potential environmental concentration (PEC), which involve the assessment of environmental fate and transport, identification of exposed populations and environments as well as estimation of expected levels in the environment (Klein *et al.*, 1988).

Of the two hazard evaluation criteria discussed above, toxicity tests have gained wider acceptance in view of their experimental simplicity and have been the subject of several monographs (Philips, 1980; Bayne, 1985; Rand and Petrocelli, 1985). However, its inherent limitations severely restrict the applicability of the laboratory results in predicting the biological impact of the aquatic environment which is distinctly different from that in the laboratory experiments. Environmental exposure assessment, though involving rigorous experimental designs, provides a more realistic assessment of the pollutant's fate in the aquatic environment.

Precise information on the nature of distribution, bioavailability and exchange of trace metals among the different environmental as well as biological compartments of an aquatic ecosystem is essential in evaluating their hazardousness. The present investigation relates to the distribution of trace metals among the different compartments of the Cochin estuary.

The term "trace metal"/"trace element" is used in current literature to designate those elements which occur in small concentrations in natural systems. For all practical purposes, the terms such as "trace metals", "trace inorganics", "heavy metals", "micro elements" and "micro nutrients" are treated as synonymous with the term "trace elements" (Wittmann, 1983). Metals such as Fe, Zn, Cu, Mo, Cr, Co and Mn are essential for life but can be toxic when present at higher levels.

The Cochin estuary has been the subject of several studies, most of which have centered around biological assessment of toxicities of trace metals to estuarine or marine organisms (Lakshmanan, 1982; Sivadasan, 1987; Baby, 1987; Latha Thampuran 1986; Prabhudeva, 1988; Krishnakumar, 1987). Lethal and sublethal effects of trace metal pollutants on biochemical constituents as well as on accumulation and depuration rates have also been documented (Lakshmanan and Nambisan, 1985; 1989; Abraham *et al.*, 1986; Prabhudeva and Menon, 1988; Sathyanathan *et al.*, 1988; Krishnakumar *et. al.*, 1990). Other investigations pertaining to the Cochin estuary relate mainly to studies on nutrients and organic compounds (Sankaranarayanan and Rosamma Stephen, 1978; Murty and Veerayya 1981; Ramani *et al.*, 1981; Venugopal *et. al.*,1982; Paul and Pillai, 1983 a and b; Sankaranarayanan *et. al.*, 1986; Lakshmanan *et al.*, 1987; Shibu *et al.*, 1990, Nair *et. al.*, 1990, Nair *et. al.*, 1991, Ouseph, 1987; 1990).

Scope of the present study

The present study is a marked deviation from conventional toxicity assessments and attempts to initiate investigations in an entirely new perspective - environmental exposure assessment. A pollutant upon release into the aquatic system is carried away from the source by the medium and distributed in due course among the various compartments of the system. A knowledge of the processes that govern the partitioning of the toxicant from the primarily loaded compartment to the adjacent compartments, the factors controlling the equilibria, the sediment-water exchange processes, the inter-compartmental mass transfer rates, etc. define the potential risks of the toxicant to aquatic organisms (van de Meent, 1988).

A scheme of study encompassing all these aspects provides the frame work for the present investigation. Two clams *Villorita cyprinoides* var.*cochinensis* (Hanley) and *Meretrix casta* (Chemnitz) were taken as representative bivalves. Considering the relevance and significance of shells in revealing past environmental history, the bivalves were analysed for trace metal concentrations in soft tissues as well as in shells. Water samples were analysed for trace metal concentrations in both dissolved and particulate phases. Sediment samples were sequentially extracted to separately

analyse the different chemically extractable chemical species present in them. Correlations were struck between various combinations of biological factors and environmental variables. The equilibria existing between the different environmental and biological compartments were evaluated and a mathematical "shell-model" was developed to predict the environmental levels of trace metals from a knowledge of the trace metal concentrations in the shells.

The objectives of the present study were three fold:
(i) to establish the significance of shells in the assessment of aquatic metal pollution levels
(ii) to quantify trace metal bioavailability in terms of the various biological and environmental variables
(iii) to develop a mathematical model for predicting the trace metal concentrations in the various environmental compartments.

In tune with these objectives, the thesis has been divided into six chapters. Chapter 1 gives a general introduction to the subject and highlights the importance of hazard evaluation, risk assessment and management of toxic chemicals and the necessity for evironmental monitoring and pollution abatement activities. Earlier reports on the distribution of trace metals in the aquatic biosphere with special reference to the Cochin estuary are also highlighted.
Chapter 2 describes the location of the study area. Details of

procedures adopted for sampling, processing and analyses in respect of the investigations carried out in Chapters 3, 4 and 5 are also presented here.

Chapter 3 details the studies carried out on the shells of bivalves with a view to highlighting its significance in environmental stress assessment. Spectroscopic data (IR and EPR) have been used to address the question of influence of mineralogy trace metal enrichment.

Chapter 4 deals with the bioavailability of the trace metals, copper, cadmium, zinc, lead, and nickel to the bivalve *V. cyprinoides*.var.*Cochinensis* (Hanley) which has been assessed in terms of the relationships between the biological factors and the environmental variables (sediment-related as well as water-related). Chapter 5 describes the various equilibria existing between the environmental and the biological compartments of the estuarine ecosystem. The transfer of toxicants from the environmental to the biological system is discussed and a mathematical model has been proposed to aid in the evaluation of environmental stress.

Chapter 6 summarizes the salient results of the present investigation.

Chapter 2

MATERIALS and METHODS

A brief description of the Cochin estuarine system and the various methods employed in the present study are given in this chapter.

The study area

The Cochin estuary located along the south west coast of India extends between 9°40'N and 10°12'N and 76°10'E and 76°30'E. It has a length of about 70 km and a width which varies between a few hundred meters to about 6 km and covers an area of about 250 km^2. The Cochin estuarine system is connected to the sea through a permanent opening, the Cochin barmouth, which is about 450 m wide and 10 to 13 m deep. Here the depth is maintained by dredging as this opening is used for navigational activities. This barmouth is also responsiblefor the tidal flux of the Cochin estuary. Though less important, two other openings also exist, which are only seasonal in nature, one at Azhikode (northern region) and the other one at Andhakaranazhi (southern region).

Two major rivers discharge freshwater into this estuarine system; the river Periyar and the river Muvattupuzha joining the northern and the southern parts of this estuary respectively. A multitude of industrial concerns punctuate the banks of the estuary. Saline water intrusion to southern parts of the estuary is regulated by the Thanneermukkam bund, a salt water barrier commissioned in 1975. The depth of the estuary varies moderately around 3m except for the shipping channel which is dredged periodically to a depth of about 13m. The tides are of semi-diurnal type with an average range of about 90cm.

The study area and the location of the sampling sites are depicted in Fig.1. The stations selected were clam habitats located in diverse environmental characteristics. The hydrographical parameters (pH, temperature, salinity, dissolved oxygen and suspended solids) determined by standard methods (Grasshoff, 1983 a and b) are presented in Table 1.pH was determined by using a Philips portable pH meter (model PP - 9046 with a glass electrode).

Sampling procedure

Monthly field collections spread over a period of 15 months (from October 1988 to December 1989) were carried out at all stations except Station 5 and 6 (located at the northern end of

Table 1. Hydrographical parameters of the sampling sites

	STATIONS					
	1	2	3	4	5	6
Temperature °C	29.4 ± 1.2 28.0 - 31.5	29.5 ± 1.3 27.5 - 32.0	29.8 ± 1.3 27.5 - 32.0	30.2 ± 1.3 28.5 - 33.0	30.1 ± 0.7 29.5 - 31.0	31.2 ± 1.1 30.0 - 33.0
Salinity ‰	7.2 ± 6.6 0.0 - 17.6	8.6 ± 7.4 0.2 - 18.9	5.4 ± 4.7 0.0 - 12.8	2.0 ± 1.7 0.0 - 5.3	23.0 ± 11.3 3.8 - 32.0	4.2 ± 4.3 0.0 - 9.9
Dissolved Oxygen $ml\ l^{-1}$	3.2 ± 0.5 2.4 - 4.0	3.8 ± 1.3 1.3 - 6.8	4.9 ± 1.6 3.2 - 8.7	4.9 ± 1.0 3.7 - 7.3	4.2 ± 0.6 3.5 - 5.1	4.8 ± 0.6 4.1 - 5.6
pH	7.0 ± 0.2 6.7 - 7.5	7.0 ± 0.2 6.7 - 7.3	7.0 ± 0.2 6.7 - 7.5	7.0 ± 0.2 6.6 - 7.3	7.3 ± 0.4 6.9 - 7.8	7.0 ± 0.0 6.9 - 7.2
Suspended Solids $mg\ l^{-1}$	23.7 ± 9.0 10.4 - 36.1	24.0 ±14.2 5.2 - 56.4	16.1 ± 7.0 4.2 - 27.4	14.5 ± 8.9 5.0 - 36.4	23.8 ± 12.8 11.3 - 43.1	14.8 ± 7.9 6.1 - 34.3

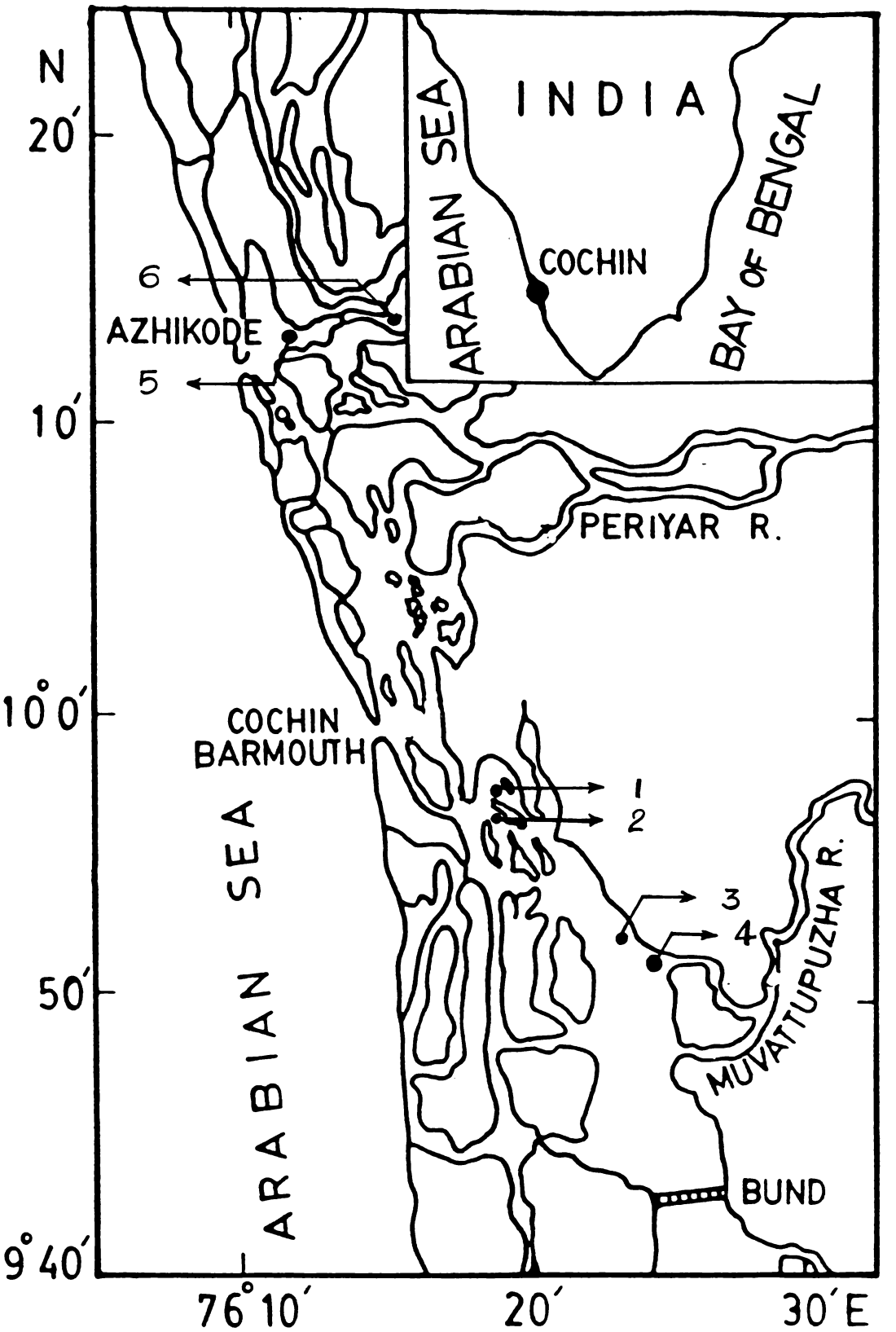


Fig.1 Map of Cochin estuary showing location of stations (1 - 6)

the estuary), where, owing to practical difficulties, only six monthly collections (between November 1988 and September 1989) were carried out. While *Villorita cyprinoides* var. *cochinensis* (Hanley) was sampled from all the stations except Station 5, *Meritrix casta* (Chemnitz) was the species sampled from Station 5. The clams were collected from the beds using a van Veen grab and were washed free of epiphytes and adhering sediments and were transported to the laboratory. Bottom water samples were collected from these stations using a pre-cleaned teflon Hytech water sampler and stored in pre-cleaned, acid-washed polyethylene bottles. A stainless steel, plastic-lined van Veen grab was used to collect sediment samples and aliquots were carefully transferred to polyethylene bags which were stored at -5°C till analyses were performed.

Chemical analyses

All glassware used for the analyses were soaked in 5N nitric acid and thoroughly washed with distilled water before use. All the reagents used were of BDH-AnalaR grade, unless otherwise specified.
Deionised, double-distilled water was used for the chemical analyses.

Water

The water samples were filtered using acid washed, 0.45 μm

Whatman membrane filters. The filtrate was used for the analyses of dissolved metals while the residue was used to estimate the particulate metal concentrations.

The dissolved metal concentration was estimated by the method described by Danielsson *et al.*(1978; 1982). The filtered water samples were subjected to solvent extraction using ammonium- 1-pyrrolidine dithiocarbamate / diethylammonium diethyl dithiocarbamate / chloroform (APDC / DDDC / Chloroform) mixture. The extract was acidified with concentrated nitric acid and the metals (copper, cadmium, zinc, lead, and nickel) were brought into the aqueous phase by equilibration with a definite volume of water and then analysed on an atomic absorption spectrophotometer.

The particulate matter separated above was digested according to the APHA (1985) procedure. The dry residue in the membrane filter was leached with 10 ml of an acid solution ($HClO_4$, HNO_3 and HCl in the ratio 1:1:3) at 90°C for 6 hours. The resultant solution was centrifuged and then made upto 10 ml with 0.1N HCl for analysis on the AAS.

Sediment

Trace metals in the sediment were extracted according to the scheme depicted in the Fig.2. The extractants, the sequence

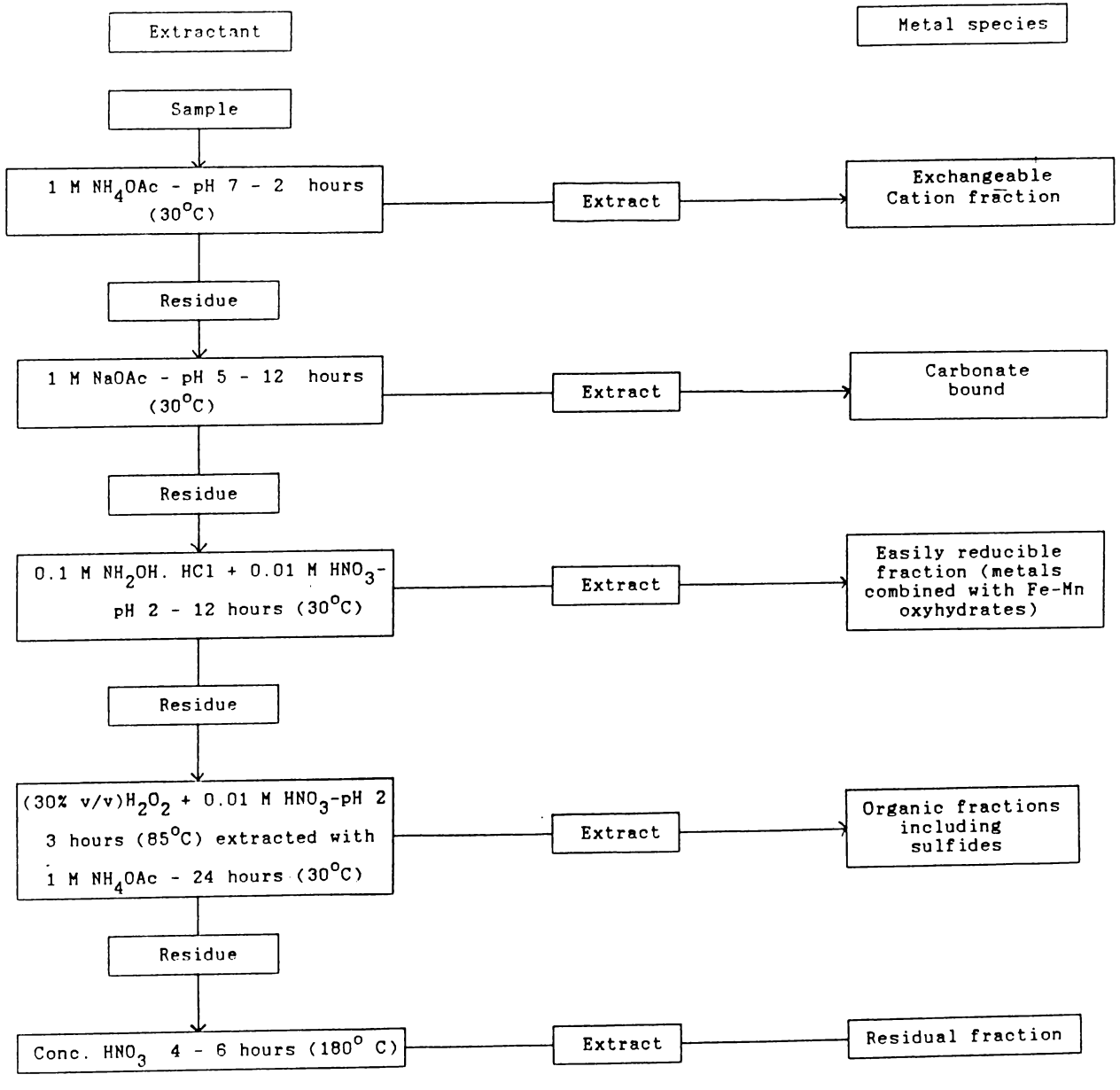


Fig.2 Sequential extraction scheme

and the procedure followed were adapted from methods employed by Tessier *et al.* (1979; 1984) and Nair *et al.* (1991). The different metal species studied are:

Fraction 1 - Exchangeable cation fraction

Fraction 2 - Carbonate bound fraction

Fraction 3 - Easily reducible fraction (metals combined with Fe/Mn oxides)

Fraction 4 - Organically bound fraction

Fraction 5 - Residual fraction

Eight to ten gram aliquots of the wet sediment samples were weighed out into 250ml Erlenmeyer flasks and allowed to equilibrate with 50 ml of the extractant. The phases were separated by centrifugation. The supernatant liquid separated was analysed for trace metals (by AAS), whereas the residue was carefully washed back into the flask with the next extractant of the sequence and the operation repeated. The percentage of moisture in the samples were determined separately and were used to recalculate and express the metal concentrations obtained above on a dry weight basis. The total metal concentrations referred to (in Chapters 4 and 5) are the summation of the metal concentrations in the sequentially extracted fractions.

Bivalves

Sample pre-treatment

The individual clam collected from each of the Stations was washed free of attached epiphytes and adhering sediments and their dimensions noted. The shells were then opened and the soft tissue was separated from the shell and the shell-weight (W_s) recorded. Each shell was heated to 110°C overnight and the final weights were noted (W_{sf}).

Analyses of shells

The analyses of shells were carried out according to two schemes explained below:

(i) For the first part of the studies reported in Chapter 3 (pages 28 to 32) *viz.* attempts to ascertain the relationship between the metal concentration in shells and their respective shell weights, shells were analysed individually. The individual shell was warmed at 60°C for one hour in a 40% solution of 30 v/v hydrogen peroxide (Glaxo) so as to completely dissolve away the periostracum and any other attached organic matter. They were then washed well with distilled water and allowed to remain in 0.05M HCl for 2 - 3hr to strip off any surface adsorbed elements, periostracum residue or any surface contamination. They were then dried to

constant weight at 110°C.

Each of these shells was dissolved in the minimum amount of 0.5 M HCl added drop-wise. Any excess acid present was evaporated off and the residue redissolved and made up to a definite volume (dependent on the weight of the shell) in deionised, double-distilled water for subsequent metal analyses.

(ii) When the results of the above individual shell analyses revealed that the trace metal concentrations of bivalve shells were totally independent of shell-weights, it was decided that, henceforth, all shell analyses be carried out on four composite samples of shells, each sample being a powdered mixture of a minimum of four individual shells of varying weights. The values of metal concentrations reported in the latter half of Chapter 3 (*i.e.* for intercomparison of metal concentrations - Table 10) as well as in Chapters 4 and 5, are the mean of the concentrations of the four composite samples. For metal analyses, 0.5 g of each of these composite samples was dissolved in the minimum amount of 0.5M HCl added drop wise. The excess acid was evaporated off and the residue redissolved and made up to 25ml in deionised, double- distilled water.

Determination of valve thickness

The valve thicknesses of the shells were determined by the

method of Goldberg *et al.* (1978). An aluminum foil of uniform thickness and known weight per unit area was pressed over the shells and cut carefully along the edges of the shells. The aluminium foil was then weighed and the effective shell area calculated. The shell thickness was obtained as the ratio between the weight of the shell and the shell area.

Infrared spectra

Infrared spectra of the shell samples were recorded as KBr pellets on a Perkin-Elmer (model-183) infra-red spectrophotmeter.

Electron paramagnetic resonance spectra

The Q- band EPR spectra of powdered shell samples were recorded at 35.5 GHz, at room temperature (RT) and at liquid nitrogen temperatures (LNT) using a Varian E112 X/Q band EPR Spectrometer. The spectra were calibrated using diphenylpicryl hydrazide as the field marker.

Analysis of soft tissue

The soft tissue of each composite sample (obtained as described on page 19) were dried at ~ 80°C for 24 hrs. (For the investigations in Chapter 3 alone, the soft tissues were grouped according to weights of the bivalve shells for reasons indicated on page 38). The dried tissues were powdered and a

definite weight digested in a Kjeldahl's flask (Martincic *et al.*, 1984). To about 0.1 - 1.0 g of sample, 5 - 10 ml of conc. HNO_3 and 0.5 - 1.0 ml of conc. $HClO_4$ were added. After pre-heating, the samples were digested for about 3 - 6 hrs. The solutions were cooled and made up to a specific volume for analyses on the AAS.

Analysis of trace metals using AAS

A Perkin Elmer Atomic Absorption Spectrophotometer (model 2380) was used for the analyses of the trace metals. The sample solutions were directly aspirated into the flame (Air - Acetylene) and the concentration in the digest was measured. For the analysis of shells, the standards were prepared in approximately 2% solution of $CaCO_3$ (BDH - ARISTAR) dissolved in 0.5M HCl. Blanks were also prepared and read wherever necessary.

Analysis of data

All data were statistically processed wherever necessary. Regression analyses were performed between various biological factors and the environmental variables referred to in Chapter 4. In Chapter 5, Chi-square analysis was done to find out the level of significance between the observed and expected environmental concentrations of the trace metals.

Chapter 3

METAL ENRICHMENTS IN BIVALVE SHELLS

Introduction

The biogeochemical cycling of many elements on this planet has a very strong anthropogenic influence. Rapid industrialization and agricultural professionalization have led to the generation of enormous quantities of untreated waste materials which have not been attended to with the priority they warrant eventhough the treatment and disposal of waste products have been recognised problems of prime concern from a socio-ecological view point.The aquatic environment, which receives most of such waste materials disposed off with scant regard to the environmental safe guards, is consequently getting polluted at an alarmingly increasing rate. The elevated concentrations of such pollutants in the water body makes it unfit for industrial and biological consumption. The deterioration of water quality influences not only the aquatic biotic processes, but the terrestrial life activities also. As the rural/urban agglomerations are often traversed by rivers/ estuaries, the impact of a polluted aquatic environment poses severe health hazards even to human life. Proper assessment and monitoring activities have to be carried out periodically in order to find out the nature and extent of pollution and to devise effective risk management programmes.

Bivalves and other aquatic organisms have been extensively used to quantify chemotoxicity and bioaccumulation of pollutants and the results have been of much significance in establishing methods for environmental hazard assessment (Phillips, 1976; Goldberg *et al.*, 1978; Zaroogian, 1980). Studies on the use of aquatic organisms for mapping pollutant levels in the aquatic system have centered around two major foci: (i) for periodic monitoring purposes and (ii) as biological indicators.

Monitoring studies primarily quantify pollutant levels in the different aquatic organisms. Indicator/sentinel organism studies involve comparison of pollutant levels in a single species distributed on a geographical basis and this has a built-in frame work for environmental hazard assessment. Gastropods and bivalves have widely been used as indicators of aquatic pollutants. By virtue of their sedentary and filter feeding nature, bivalves can reflect even minute changes in the surrounding environment. They have a remarkable ability to accumulate and concentrate substances from the ambient medium to much higher levels in their tissues (Goldberg, 1975; 1980; 1986; Goldberg *et al.*, 1978; Boyden, 1977; Ireland and Wootton, 1977; Phillips, 1980; Marigomez and Ireland, 1989).

Although earlier studies on shells of aquatic organisms had focussed on the influence of environmental parameters like

salinity, temperature etc. on the formation, growth and mineralogy of shells (Pilkey and Hower, 1960; Dodd,1965; Segar *et al.*, 1971; Bertine and Goldberg, 1972; Compere and Bates, 1973; Sturesson, 1976; 1978 and others), most of the recent investigations emphasised the importance of shells in pollution monitoring (Phillips, 1980; Hubbard *et al.*, 1981; Koide *et al.*, 1982; Al-Dabbas *et al.*, 1984; Sturesson, 1984; Szefer, 1986; Szefer and Wenne, 1987; Carrel *et al.*, 1987; Bourgoin, 1988; 1990; Bourgoin and Risk, 1987). Shells have some very pertinent advantages over soft tissues, *viz.* much better ease of handling, negligible rate depuration and a much less variability in the results *vis-a-vis* those obtained from soft tissues. These features make them a convenient and spectacular record of environmental history (Carrel *et al.*, 1987).

The shell is a three layered structure consisting of an outer periostracum, an inner nacreous layer and a middle prismatic layer. While the periostracum is formed of an organic material called conchiolin, the prismatic layer consists of crystals of calcium carbonate separated by thin layers of conchiolin. The inner nacreous layer is a thin array of calcium carbonate crystals. The newly formed shell layers are added onto the nacreous layer.

Depuration of metals trapped inside the inter- crystalline matrix of the shells can occur only through a very slow process

of solid state diffusion. The average life time of the bivalve would be insignificant in relation to the time span required for this diffusion process. Hence the outflow of materials from the shells is almost negligible and consequently the shells are able to integrate metal concentrations over the years and preserve them even after the death of the organisms. Bourgoin and Risk (1987) analyzed lead in recent and fossil shells of *Mya truncata* and observed a five-fold enrichment in the recent samples. This increased higher accumulation in the recent shell sample was indicative of the present day anthropogenic input of lead to the environment. Deep inroads into the history of industrial pollution, of nuclear activities, of weapon tests etc. could, thus, be made by such analyses (Carrel *et al.*, 1987).

The influence of salinity on the strontium, copper and magnesium contents in the shells of *Macoma balthica* was reported by Sturesson and Reyment (1971). Koide *et al.*,(1982) analyzed the trace metal and trans-uranic metal contents in the shells, byssal threads and soft tissues of *M. edulis* and obtained stronger correlations between the different metal levels in the shells than those in the soft tissues. The enhanced concentrations of actinides in the byssal threads and relative behaviour of plutonium and americium in their accumulation were also investigated.

Bourgoin (1990) reported that the relationship between lead concentration in the suspended matter of the aqueous phase and that in the nacreous layer of the shell were very similar to that exhibited between lead concentration in the suspended matter and that in the soft tissue. It was even suggested that shell could therefore replace soft tissues in its function as bioindicators. Szefer and Wenne (1987) reported on the species and the region-dependent variations of uranium and thorium in molluscs of the Gdansk Bay. The concentrations of uranium showed species variation while those of thorium showed both species as well as spatial variations.

Shells find yet another important application in dating techniques. Amino acid racemisation dating was effectively used for estimating the ages more precisely than by conventional radio-carbon dating methods (Masters and Bada, 1978). Normally the amino acid present in the proteins of living organisms consists of only the l-enantiomer. But on death and decay, the l-amino acids slowly undergo racemisation to the corresponding d-amino acids. In fossils, both l- and d-amino acids are present and the d/l amino acid ratio was found to increase with increasing age of the sample till the formation of the racemic mixture. Out of the numerous amino acids, aspartic acid (having half life for racemisation equal to 15,000 years) was mainly used for the dating studies. Since the rate of decay is much slower than that of carbon-14, this

method is useful in dating very old fossils.

Although the reports cited underscored the capability of molluscan shell to function as unerasable records of trace metal pollution, no attempt was hitherto made to evaluate the potential of shells of the organisms of the Cochin estuary. The only available reports pertaining to studies carried out in the Cochin estuary relate to those by Sreevalsan (1985) who analysed the infrared spectra and Ca/Mg levels in the shells of *C. madrasensis*, and by Sivadasan (1987) who estimated the trace metal (copper, mercury and zinc) contents in the exoskeleton of the crustacean *Metapenaeus dobsoni*.

This investigation was designed to be a systematic rigorous attempt at critically evaluating the relevance of shells in the environmental quality assessment of the Cochin estuary with special reference to the bivalve *V. cyprinoides* and the trace metals, lead, manganese, cobalt, copper, cadmium and zinc. Details of the sampling sites, of the cleaning procedures and of the analytical methodologies are presented in Chapter 2.

Results

(i) Variations of metal concetration in shells

Shell weights (W_s and W_{sf}), valve thicknesses(V_t) and concentrations of cadmium, zinc, copper, lead, manganese and cobalt in the shells of the *V. cyprinoides* from the Stations 1 to 4 are given in Tables 2-5 respectively. Figures 3-6 depict the relationships between metal concentrations and shell weights as well as that between metal concentrations and valve thicknesses (except Fig. 5 corresponding to bivalves from Station 3; since all the bivalves sampled from the Station where small in size and had weights less than 2g, their valve thicknesses were not ascertained). The nature of the plots convincingly proclaimed that metal concentrations (excepting zinc which shows an anomalous behaviour, especially in Station 2 and 3; this anomalous behaviour observed in the case of zinc could be the effect of a wide spread of the values centered around a small concentration level e.g. 3.79 $\mu g\ g^{-1}$ at Station 2) in the individual bivalves did not vary to any appreciable degree as a function of valve weights or valve thicknesses (and consequently of bivalve ages). A similar result was obtained in investigations conducted elsewhere on gastropod shells sampled from various locations on the globe (P. Foster, personal communication). Hence it was decided that individual sampling of bivalves was not warranted in studies aimed at analysing the

Table 2. Metal concentrations of *V. cyprinoides* shells (Station 1)

Weight of shell W_s (g)	Dry wt. of shell, W_{sf} (g)	Valve thickness V_t (mg/cm²)	Cd	Zn	Cu	Pb	Mn	Co
			μg g^{-1} dry wt					
14.597	14.557	403.03	3.83	5.18	8.04	36.55	26.35	42.07
16.494	16.459	507.42	4.38	4.63	7.64	47.71	28.32	45.76
19.453	19.413	508.48	3.60	3.33	6.53	35.42	21.95	36.06
20.108	20.059	535.70	4.07	3.22	6.87	39.58	23.50	41.00
21.667	21.623	559.03	3.21	3.64	6.32	36.48	21.66	36.39
22.519	22.478	567.97	2.96	4.14	6.07	35.04	18.00	36.30
22.833	22.783	529.05	3.60	5.06	8.41	42.05	27.74	41.67
23.768	23.712	560.49	3.09	4.06	6.74	35.92	20.95	33.60
26.885	26.829	570.86	3.44	4.92	7.04	37.12	26.51	38.20
27.825	27.752	572.78	3.78	6.43	7.06	44.59	24.43	36.35
		Mean	3.59 ±0.44	4.46 ±0.98	7.07 ±0.75	39.04 ± 4.35	23.94 ±3.33	38.74 ±3.72

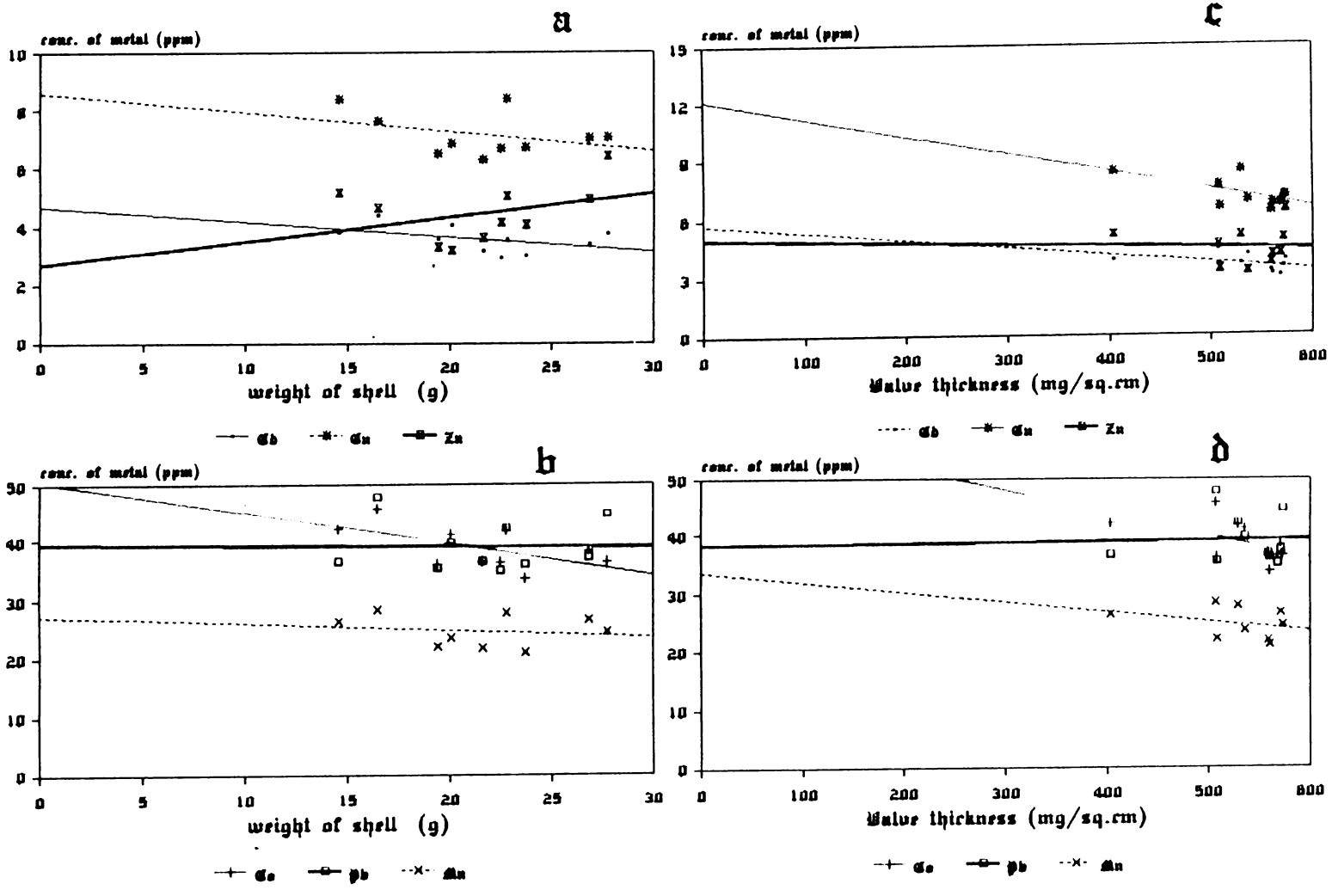


Fig.3 Variation of metal conc. with shell weights (a & b) and valve thicknesses (c & d) of *V. cyprinoides* (Station 1)

Table 3. Metal concentration of V. cyprinoides shells (Station 2)

Weight of shell W_s (g)	Dry wt. of shell, W_{sf} (g)	Valve thickness V_t (mg/cm²)	Cd	Zn	Cu	Pb	Mn	Co
			μg g^{-1} dry wt					
1.723	1.717	217.99	3.42	5.86	6.20	31.00	26.29	39.26
2.519	2.510	256.68	2.80	6.99	7.61	38.07	23.29	51.11
2.695	2.689	284.07	2.84	6.53	6.36	43.72	16.86	50.33
3.924	3.913	300.29	2.93	3.88	6.40	39.96	18.08	39.86
4.483	4.476	327.95	3.08	3.53	7.45	32.63	21.75	36.22
4.875	4.861	325.46	2.40	3.61	7.42	41.45	21.28	42.69
5.329	5.314	336.30	2.63	3.30	6.38	33.89	16.91	39.00
6.992	6.977	364.54	3.55	4.83	6.75	36.82	18.22	44.15
7.220	7.198	345.36	3.58	2.79	6.49	47.20	16.82	47.54
7.784	7.590	348.27	3.02	3.24	6.59	43.94	25.62	39.52
8.449	8.421	386.96	4.17	4.86	8.08	40.37	23.55	42.12
12.846	12.811	446.12	3.42	2.49	6.05	40.31	14.25	36.74
13.144	13.091	446.59	3.48	2.93	6.47	35.58	18.52	37.23
13.249	13.220	414.17	3.85	2.38	6.36	30.21	16.91	39.35
17.254	17.206	519.10	3.39	3.27	6.15	36.91	19.38	35.41
18.367	18.328	501.63	3.02	2.39	6.93	34.64	18.36	38.21
21.534	21.493	472.22	3.24	3.67	6.38	36.79	22.88	35.29
22.928	22.881	536.40	3.36	2.67	7.03	37.48	25.83	39.09
26.730	26.661	614.49	3.70	2.72	6.34	37.49	18.41	36.34
		Mean	3.26 ±0.43	3.79 ± 1.39	6.71 ±0.56	37.81 ± 4.44	20.17 ± 3.55	38.76 ± 9.67

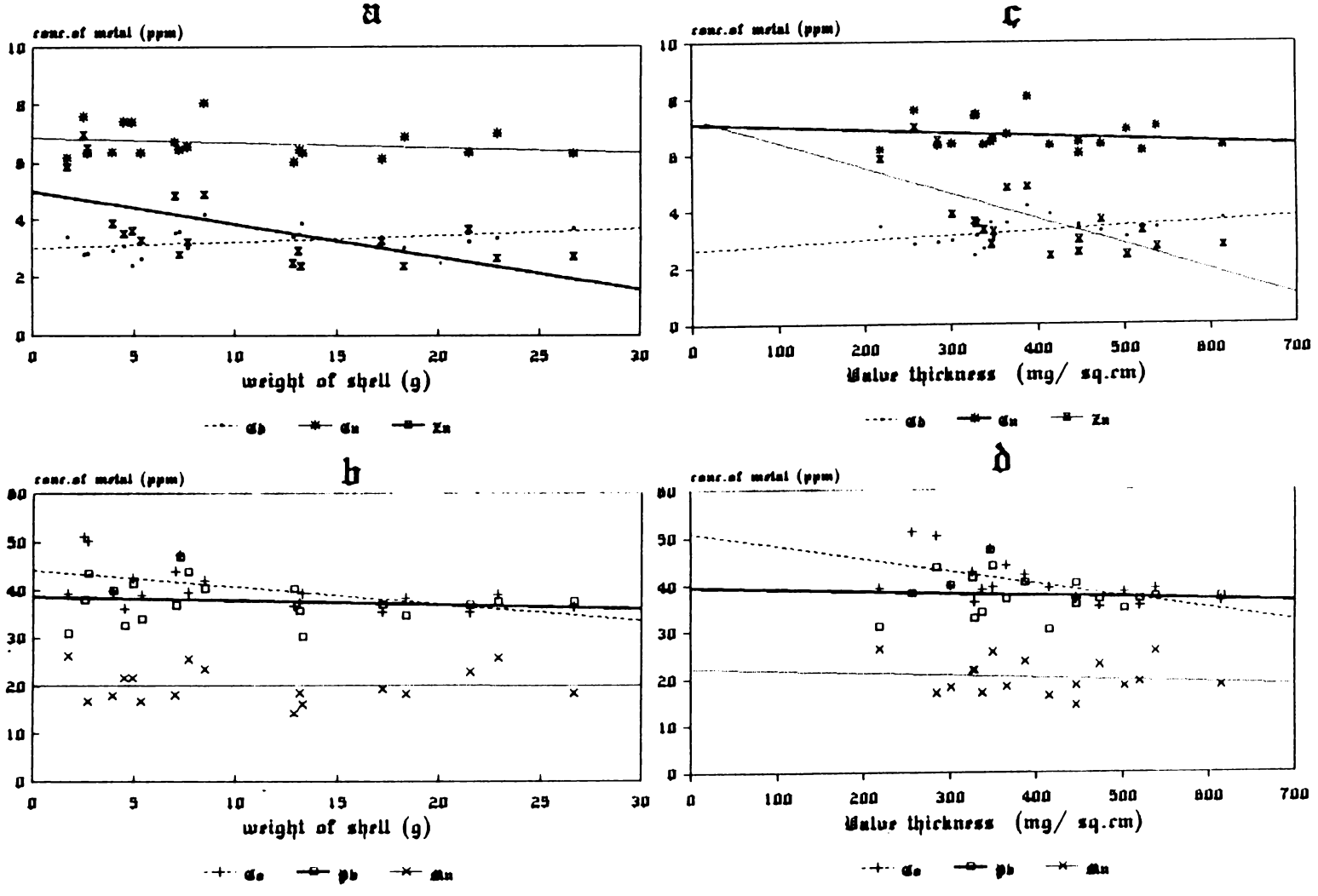


Fig.4 Variation of metal conc. with shell weights (a & b) and valve thicknesses (c & d) of *V. cyprinoides* (Station 2)

Table 4. Metal concentrations of V. cyprinoides shells (Station 3)

Weight of shell W_s (g)	Dry wt. of shell, W_{sf} (g)	Valve thickness V_t (mg/cm²)	Cd	Zn	Cu	Pb	Mn	Co
			μg g^{-1} dry wt					
	0.823		3.91	6.35	9.04	45.20	38.34	48.31
	0.978		3.58	5.12	7.58	43.36	36.78	46.79
	1.079		3.24	4.44	6.77	29.39	33.24	39.47
	1.638		3.02	4.43	7.08	41.88	43.02	42.64
	1.687		3.27	4.31	6.87	40.62	29.15	43.15
	1.837		2.21	3.17	7.48	40.20	35.38	39.74
		Mean	3.21 ±0.57	4.64 ± 1.05	7.47 ± 0.83	40.11 ± 5.56	35.99 ± 4.69	43.35 ± 3.60

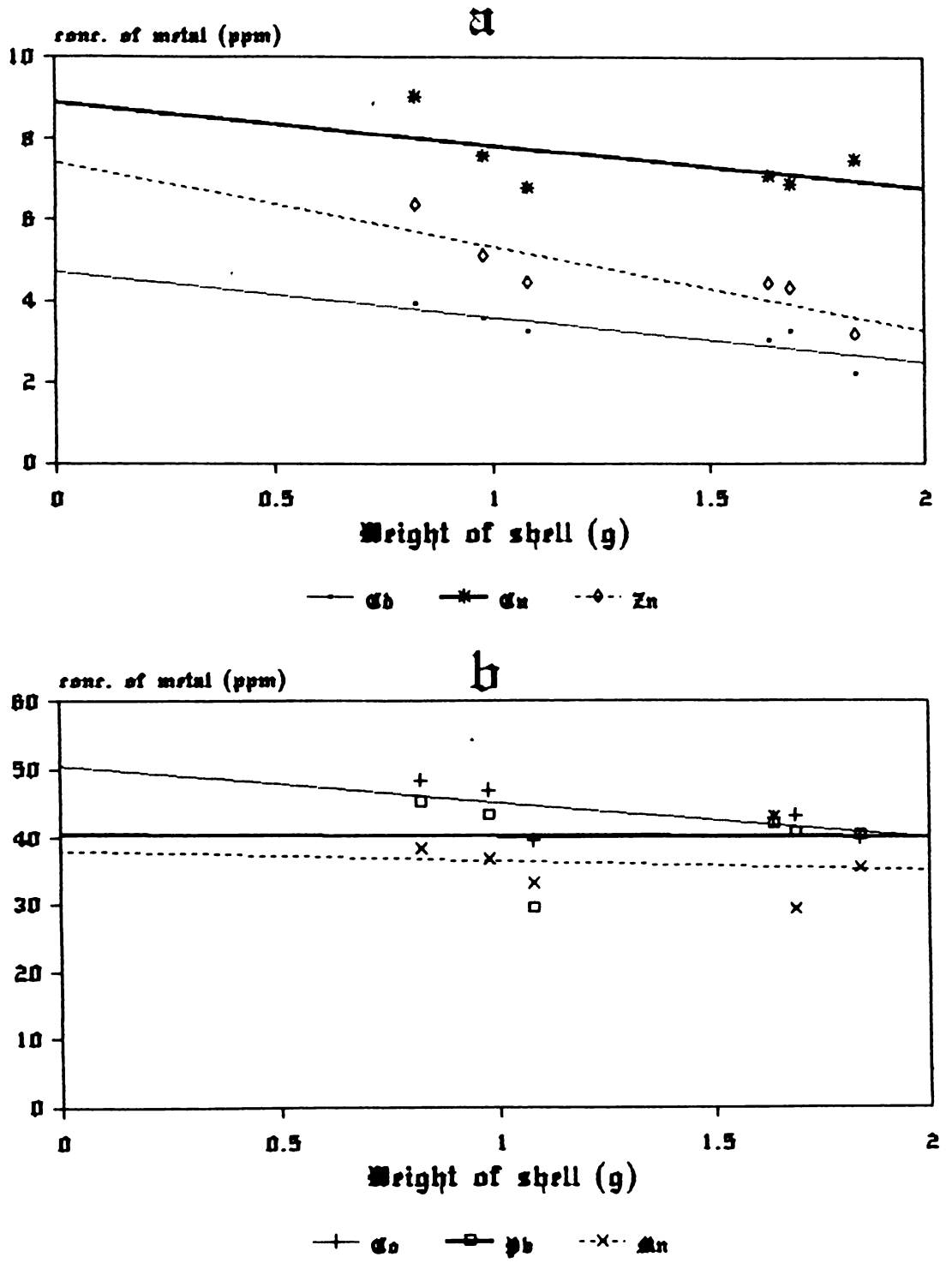


Fig.5 Variation of metal conc. with shell weights (a & b) and valve thicknesses (c & d) of *B. cyprinoides* (Station 3)

Table 5. Metal concentrations (in ppm dry wt) of *V. cyprinoides* shells (Station 4)

Weight of shell W_s (g)	Dry wt. of shell, W_{sf} (g)	Valve thickness V_t (mg/cm²)	Cd	Zn	Cu	Pb	Mn	Co
			μg g^{-1} dry wt					
	1.504		4.01	4.50	7.64	34.70	29.43	43.93
	1.594		3.42	4.64	7.21	32.75	29.16	41.45
	2.259		4.07	3.00	8.31	46.20	29.19	43.39
4.202	4.190	368.30	3.78	4.21	8.14	40.71	26.18	42.47
4.324	4.313	387.13	4.55	4.40	8.76	38.93	26.82	46.38
5.090	5.077	406.61	3.96	4.33	6.77	38.09	21.54	43.83
5.171	5.157	375.22	3.85	3.73	8.23	37.03	22.69	44.99
7.553	7.537	401.74	4.36	2.79	6.23	31.13	27.60	42.35
8.352	8.331	463.18	3.62	3.45	6.03	35.64	24.41	37.86
8.236	8.220	495.89	3.53	3.12	7.17	35.89	23.92	38.36
8.648	8.631	422.49	3.22	2.88	6.34	31.70	23.79	39.29
10.610	10.588	474.07	2.42	2.44	6.38	36.46	20.32	43.64
12.038	11.993	560.54	2.96	2.60	5.73	34.03	21.27	35.05
12.229	12.207	522.09	3.25	2.93	5.90	37.92	23.56	35.97
19.120	19.081	574.53	3.77	2.92	7.13	35.65	26.93	39.45
20.184	20.142	607.56	3.46	2.99	7.34	39.31	21.12	40.72
24.579	24.539	696.08	3.20	3.90	6.46	38.76	24.66	42.13
		Mean	3.61 ± 0.52	3.46 ±0.73	7.05 ±0.93	36.76 ± 3.63	24.86 ±2.98	41.25 ± 3.17

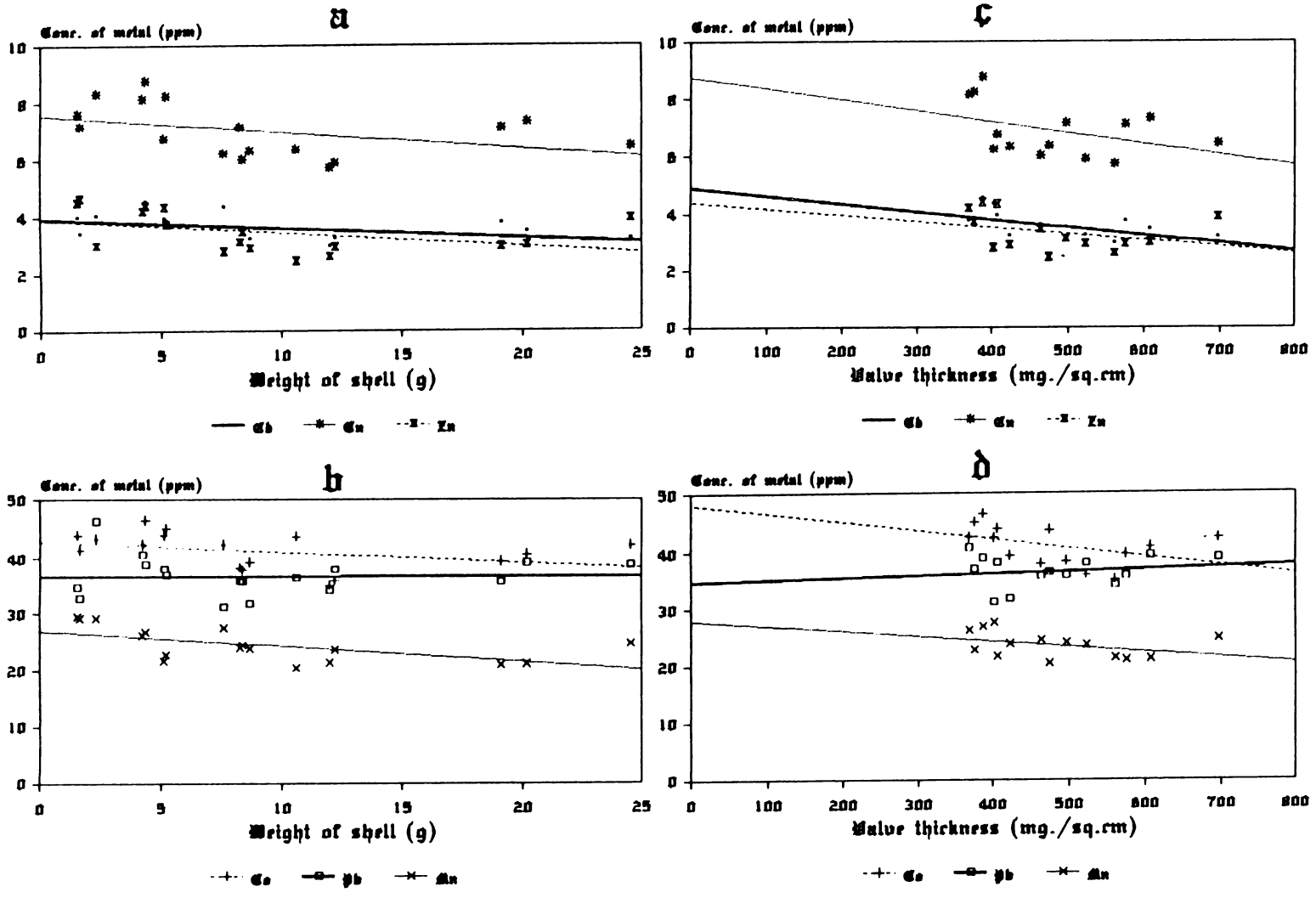


Fig.6 Variation of metal conc. with shell weights (a & b) and valve thicknesses (c & d) of *M. cyprinoides* (Station 4)

metal content of the shells. However to provide for any statistical variability, the available individual organisms were pooled in to fourconvenient weight groups (*vide infra*) for assessing the inter relationship between metal levels in the shells and in the soft tissues as described in the following section (p 38). But in all subsequent analyses (for intercomparison of metalconcentrations, Table 10) reported in this Chapter as well as for studies reported in Chapters 4 and 5 the shells of all organisms collected from a particular location were pooled in to four composite samples, each sample consisting of a minimum of four shells of varying weights.

At all the four Stations studied, cadmium was found to be the least abundant in the shell followed by zinc, with values varying from 3.21 $\mu g\ g^{-1}$ to 3.61 $\mu g\ g^{-1}$ and from 3.46 μgg^{-1} to 4.64 μgg^{-1} respectively. Cobalt was the most abundant metal in the shells obtained from Stations 2, 3, and 4, while lead was the most abundant in those from Station 1. Manganese concentrations ranged between 20.17 $\mu g\ g^{-1}$ and 35.99 μgg^{-1} among the different stations. The concentrations of copper did not show any appreciable spatial variation and generally exhibited values intermediate between those of manganese and zinc.

Table 6. Metal concentrations in shells and soft tissues of different weight groups of V. cyprinoides ($\mu g\ g^{-1}$ dry wt.)

		Station 1						Station 2					
		Upto 5g GP I	5 - 12g GP II	12 - 20g GP III		>20g GP IV		GP I		GP II		GP III	GP IV
Cd	Shell	-	-	3.97 ±	0.33	3.35 ±	0.31	3.12 ± 0.64		3.39 ± 0.58		3.43 ± 0.29	3.43 ± 0.23
	S.tissue	-	-	6.01 ±	0.83	6.41 ±	1.03	5.95 ± 0.43		7.44 ± 0.86		5.76 ± 0.43	4.86 ± 0.35
Zn	Shell	-	-	4.09 ±	0.96	4.71 ±	1.00	5.32 ± 1.56		3.80 ± 0.97		2.69 ± 0.39	3.02 ± 0.56
	S.tissue	-	-	85.71 ±	10.31	89.64 ±	10.12	95.27 ± 10.38		104.21 ± 12.30		87.37 ± 9.50	81.52 ± 9.73
Cu	Shell	-	-	7.27 ±	0.69	6.94 ±	0.82	6.83 ± 0.63		6.86 ± 0.69		6.39 ± 0.34	6.58 ± 0.35
	S.tissue	-	-	13.89 ±	1.30	14.07 ±	0.93	9.22 ± 1.14		10.99 ± 1.18		11.30 ± 1.32	10.16 ± 1.07
Pb	Shell	-	-	39.82 ±	5.50	38.53 ±	3.85	27.25 ± 4.81		40.44 ± 5.36		35.53 ± 3.66	37.25 ± 0.40
	S.tissue	-	-	3.94 ±	0.42	4.12 ±	0.54	2.32 ± 0.18		2.18 ± 0.17		2.01 ± 0.25	2.83 ± 0.15
Mn	Shell	-	-	25.03 ±	3.67	23.22 ±	3.67	20.74 ± 3.45		19.61 ± 4.87		17.34 ± 2.08	22.37 ± 3.75
	S.tissue	-	-	8.64 ±	0.53	9.44 ±	1.08	10.65 ± 0.94		12.18 ± 1.43		9.00 ± 1.17	11.08 ± 1.23
Co	Shell	-	-	41.23 ±	4.00	37.09 ±	2.68	43.97 ± 5.39		42.46 ± 3.51		37.39 ± 1.49	36.91 ± 1.96
	S.tissue	-	-	4.52 ±	0.83	5.13 ±	0.43	6.18 ± 0.53		5.78 ± 0.68		5.98 ± 0.38	6.61 ± 0.84

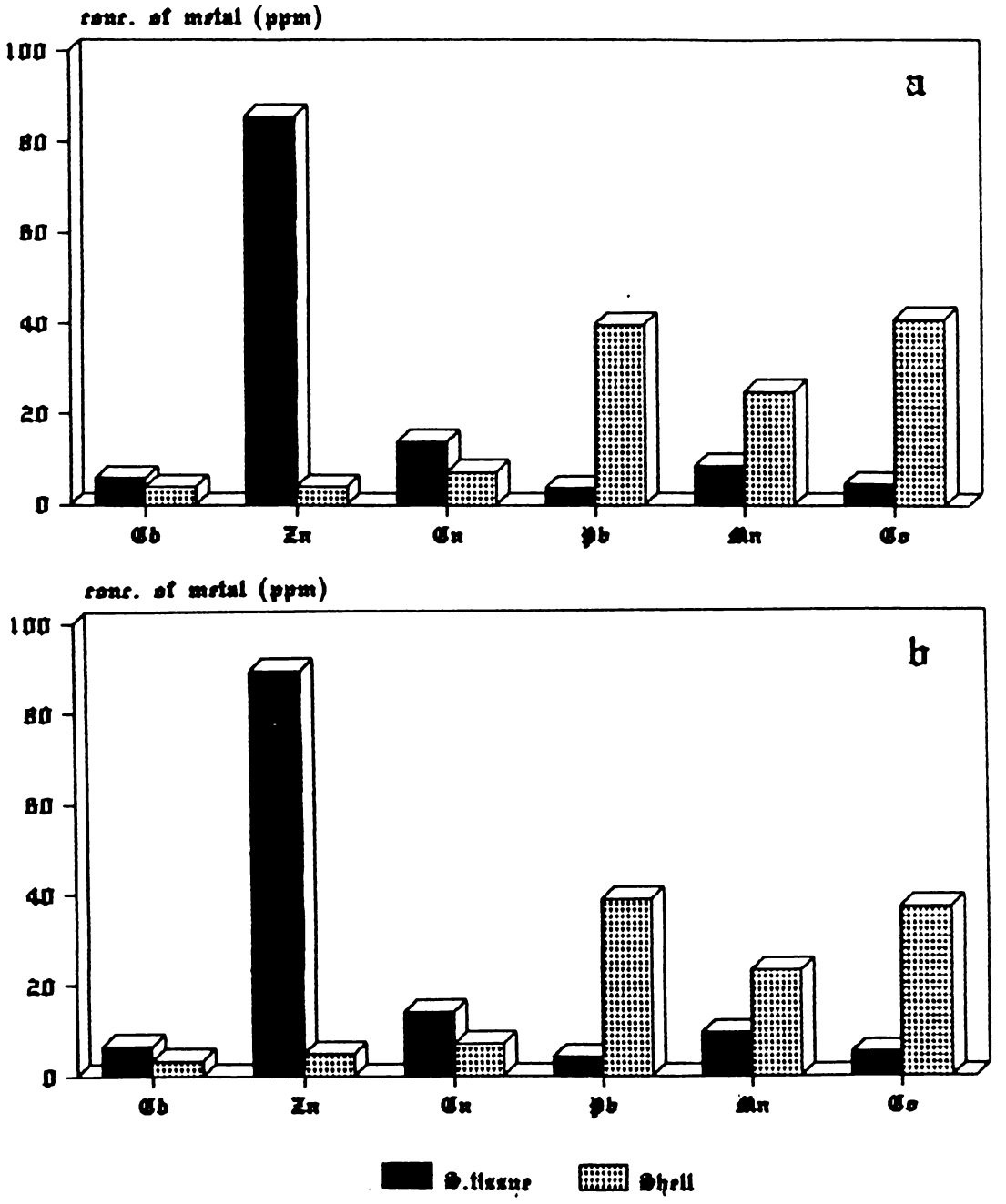


Fig.7 Metal concentrations in shells and tissues of *V. cyprinoides* (Station 1)
Wt. Group a) 12–20g b) 20g

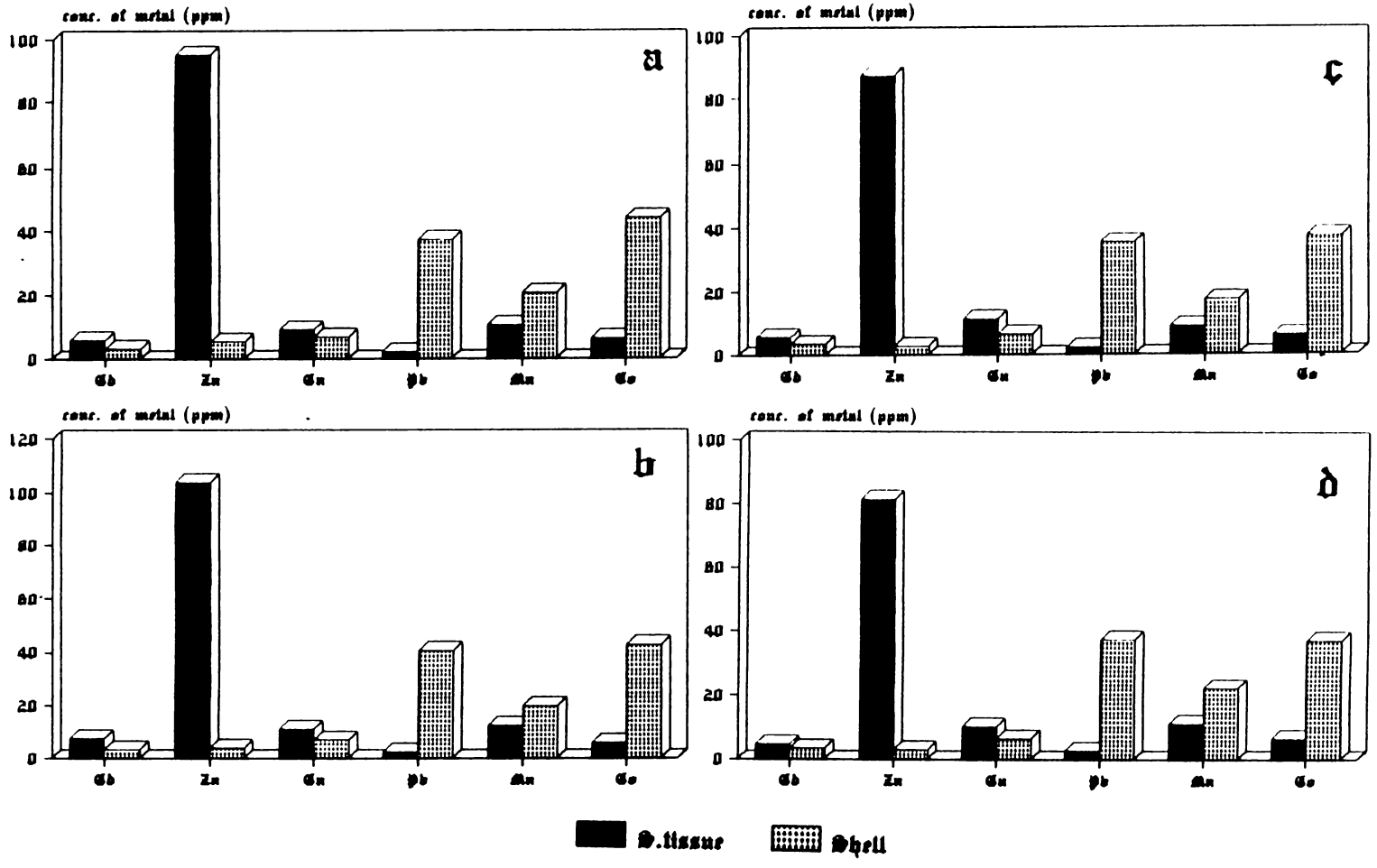


Fig.8 Metal concentrations in shells and tissues of
V. cyprinoides (Station 2)
Wt. Group a) 5 b) 6-12g c) 12-20g d) 20g

Table 7. Metal concentrations in shells and soft tissues of different weight groups of *V. cyprinoides* (µg g^{-1} dry wt)

		Station 3				Station 4			
		Upto 5g GP I	5 - 12g GP II	12 - 20g GP III	> 20g GP IV	GP I	GP II	GP III	GP IV
Cd	Shell	3.21 ± 0.57	-	-	-	3.96 ± 0.41	3.56 ± 0.61	3.32 ± 0.41	3.33 ± 0.18
	S.tissue	6.21 ± 0.54	-	-	-	9.26 ± 1.13	10.71 ± 1.32	8.99 ± 1.04	9.94 ± 1.07
Zn	Shell	4.63 ± 1.05	-	-	-	4.15 ± 0.66	3.24 ± 0.64	2.81 ± 0.18	3.45 ± 0.64
	S.tissue	129.87 ± 15.30	-	-	-	108.86 ± 13.30	97.21 ± 10.81	88.47 ± 10.30	101.25 ± 12.34
Cu	Shell	7.47 ± 0.83	-	-	-	8.01 ± 0.60	6.73 ± 0.75	6.25 ± 0.76	6.90 ± 0.62
	S.tissue	14.68 ± 1.43	-	-	-	11.21 ± 0.89	16.93 ± 2.01	17.18 ± 1.98	16.02 ± 0.93
Pb	Shell	40.12 ± 5.56	-	-	-	38.66 ± 5.28	35.13 ± 2.66	35.86 ± 1.95	39.04 ± 0.38
	S.tissue	6.28 ± 0.40	-	-	-	5.21 ± 0.43	4.93 ± 0.81	5.13 ± 0.43	5.43 ± 0.73
Mn	Shell	35.98 ± 4.69	-	-	-	28.16 ± 1.53	23.46 ± 2.32	23.92 ± 2.84	22.89 ± 2.50
	S.tissue	6.73 ± 0.47	-	-	-	7.92 ± 0.87	5.41 ± 0.47	8.03 ± 0.76	11.12 ± 1.18
Co	Shell	43.35 ± 3.60	-	-	-	43.52 ± 1.85	41.47 ± 2.91	36.87 ± 2.32	41.43 ± 0.99
	S.tissue	4.53 ± 0.63	-	-	-	4.66 ± 0.35	4.53 ± 0.51	4.83 ± 0.55	4.78 ± 0.58

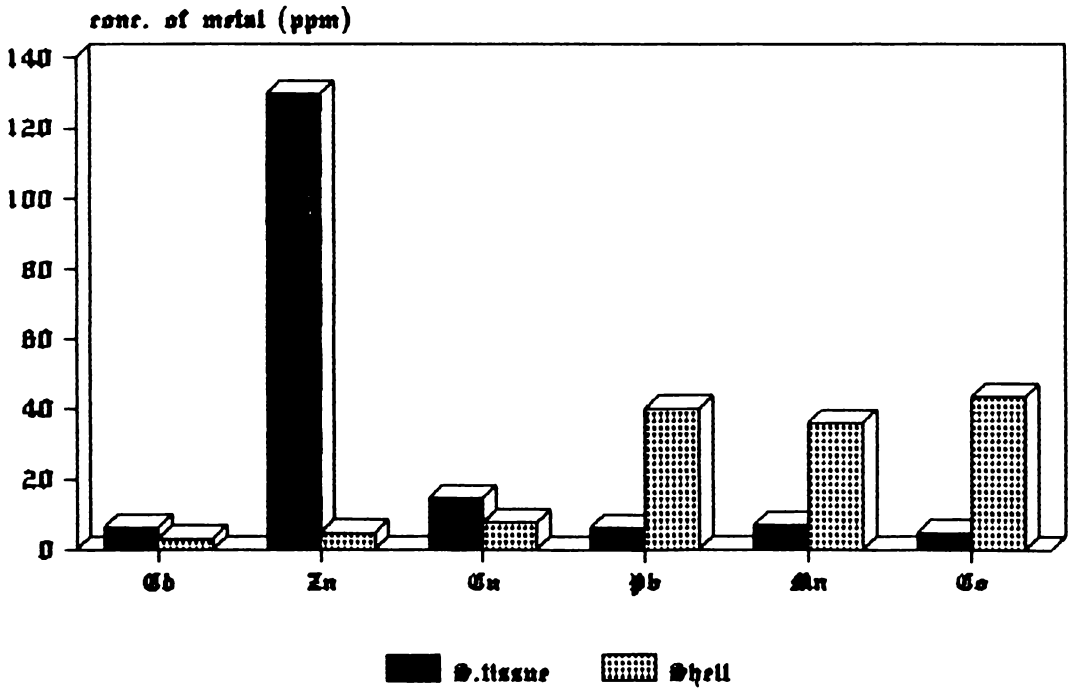


Fig.9 Metal concentrations in shells and tissues of V. cyprinoides (Station 3) Wt. Group a) 5

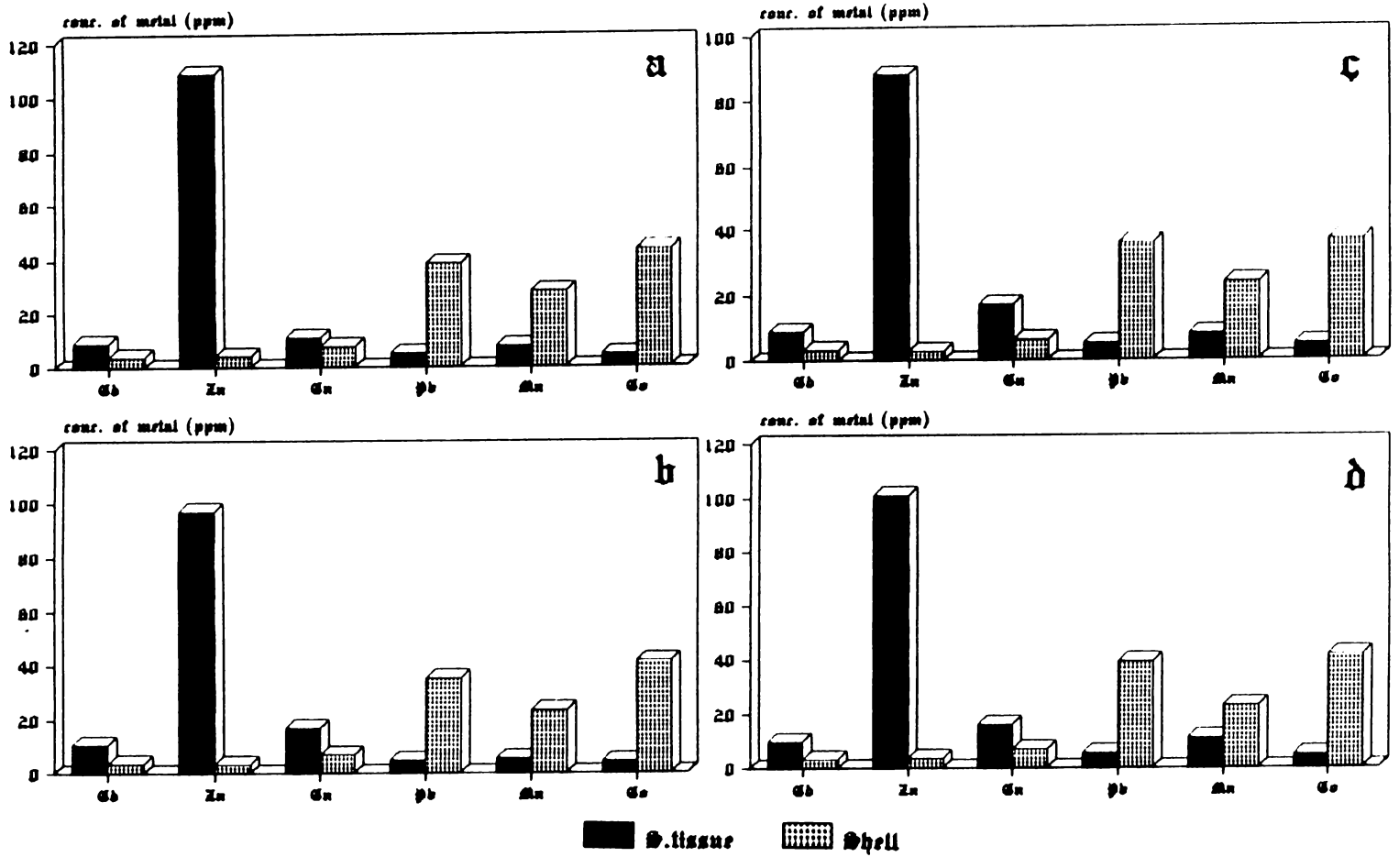


Fig.10 Metal concentrations in shells and tissues of
B. cyprinoides (Station 4)
Wt. Group a) 5 b) 6-12g c) 12-20g d) 20g

Table 8. Ratio of metal concentrations in shells and soft tissues of V. cyprinoides

	Station 1				Station 2			
	GP I	GP II	GP III	GP IV	GP I	GP II	GP III	GP IV
Cd	-	-	0.66 (0.59 ± 0.09)	0.523	0.524	0.456 (0.57 ± 0.1)	0.595	0.705
Zn	-	-	0.048 (0.050 ± 0.0031)	0.052	0.056	0.036 (0.04 ± 0.01)	0.031	0.037
Cu	-	-	0.523 (0.508 ± 0.02)	0.493	0.74	0.62 (0.643 ± 0.07)	0.56	0.65
Pb	-	-	10.11 (9.73 ± 0.53)	9.35	16.06	18.55 (16.36 ± 2.36)	17.67	13.16
Mn	-	-	2.89 (2.68 ± 0.30)	2.46	1.95	1.61 (1.87 ± 0.17)	1.92	2.01
Co	-	-	9.12 (8.18 ± 1.33)	7.23	7.11	7.34 (6.64 ± 0.70)	6.25	5.84

Table 9. Ratio of metal concentrations in shells and soft tissues of V. cyprinoides

	Station 3				Station 4			
	GP I	GP II	GP III	GP IV	GP I	GP II	GP III	GP IV
Cd	0.52	-	-	-	0.43	0.33 (0.37 ± 0.04)	0.37	0.34
Zn	0.036	-	-	-	0.038	0.033 (0.034 ± 0.002)	0.032	0.034
Cu	0.51	-	-	-	0.71	0.40 (0.48 ± 0.131)	0.36	0.43
Pb	6.38	-	-	-	7.21	7.13 (7.13 ± 0.08)	6.99	7.18
Mn	5.35	-	-	-	3.56	4.33 (3.23 ± 0.90)	2.97	2.06
Co	9.57	-	-	-	9.33	9.15 (8.69 ± 0.77)	7.62	8.67

(ii) Shell-soft tissue metal interrelationships

The available individual organisms from each station were pooled according to their shell weights into four convenient weight groups *viz.* upto 5 g (GP I), 6-12 g (GP II), 12-20 g (GP III) and more than 20 g (GP IV). Groups I and II were absent at Station 1 while only group I was present at Station 3. Stations 2 and 4 had all the four groups of organisms. Metal concentrations in the different weight groups of *V. cyprinoides* obtained from various Stations are presented in Tables 6 and 7. The shell metal concentrations given (Tables 6 and 7) are mean values of the respective metal concentrations of all individual shells falling into one of the above weight groups. Tissue-metal concentrations given are mean value obtained for four replicate analyses carried out in each weight group. The relative abundances of metal concentrations in the shells and soft tissues in the different weight groups of *V. cyprinoides* obtained from the various stations are pictorially represented in the Fig. 7-10. Data given are the mean values of the individual analysis of each weight range.

The ratio between metal concentrations in the shells and that in the soft tissues (denoted as $\phi = M_{sh}/M_{st}$) in each weight group and the mean value for the different weight groups of bivalves sampled from each of the Stations are given in

Tables 8 and 9. For manganese and cobalt the highest ϕ values, 5.35 and 9.57 respectively were observed for shells sampled from Station 3 whereas the lowest values were for those taken from Station 2 (1.87 and 6.64 respectively). In contrast lead recorded maximum and minimum (16.36 and 6.38 respectively) for shells obtained from Stations 2 and 3. In the case of cadmium ϕ had the highest value (0.59) for shells sampled from at Station 1, and the lowest value (0.37) for those obtained from Station 4. Zinc concentrations also showed a similar pattern with the highest and lowest ratios being recorded for the shells taken from Stations 1 and 4 respectively. For copper the ratio was a maximum for shells sampled from Station 2 and a minimum those from Station 4. These three metals exhibited higher ratios in lower weight ranges and lower ratios in higher weight ranges. Out of the metals studied manganese, cobalt and lead were seen to be preferentially accumulated in the shells whereas cadmium, zinc and copper were seen accumulated more in the soft tissues.

(iii) Infrared spectral analysis

Analysis of the infrared spectrum of powdered *V. cyprinoides* shell showed strong absorptions around 710 cm^{-1} (doublet) and around 1070 cm^{-1}, characteristic of an aragonite lattice structure. These results were compared with spectra of

other bivalve shells (of *P. viridis, M. casta, M. senhousia* and *S. scripta*). All the shells studied herein showed the similar spectral bands indicating an aragonite mineralogy.

(iv) Strontium/Magnesium Ratio

Table 11 and 11a gives the concentrations of strontium and magnesium and their ratios for the different bivalves studied. Very low values of ratio were observed for *M. senhousia* and *M. casta,* while it was the highest for *V. cyprinoides.*

(v) EPR Spectra

Q band EPR spectra were recorded at RT/LNT. Although isotropic spectra with signals characteristics of Mn(II) (d^5) ions were obtained for powdered shells of *V. cyprinoides* (a typical spectrum is reproduced in Fig.11), no EPR signals corresponding Co(II) systems (d^7) ions could observed.

Discussion

The one question that has often been addressed in studies involving shells of aquatic organisms is whether or not shells are better accumulators of metals than soft tissues. In the absence of any definite answer, there has been a general disinterest to indulge in detailed investigations on the shells. Much of the hesitation to use shells stems from an

Table 10. Metal concentration in the shells of different bivalve species

	Cu	Cd	Zn	Pb	Mn	Co
			$\mu g\ g^{-1}$			
P. viridis	5.69	3.92	3.67	40.95	9.10	19.88
M. senhousia	6.25	4.86	5.53	38.78	35.65	19.26
S. scripta	5.77	4.14	2.72	41.67	8.01	19.29

Table 11. Sr, Mg and Sr/Mg levels in the shells of different bivalve species

	Sr	Mg	Sr/Mg
		μg g^{-1}	
P. viridis	1046.80	477.87	2.19
S. scripta	1144.02	513.53	2.23
M. senhonsia	995.73	484.82	2.05
M. casta	1152.98	559.14	2.06

Table 11a. Sr, Mg and Sr/Mg levels in the shells of *V. cyprinoides* from Stations 1 to 4

Stations	Sr	Mg	Sr/Mg
		μg g^{-1}	
1	1208.27	496.71	2.43
2	1349.13	516.89	2.62
3	1421.78	517.27	2.77
4	1380.79	495.05	2.80

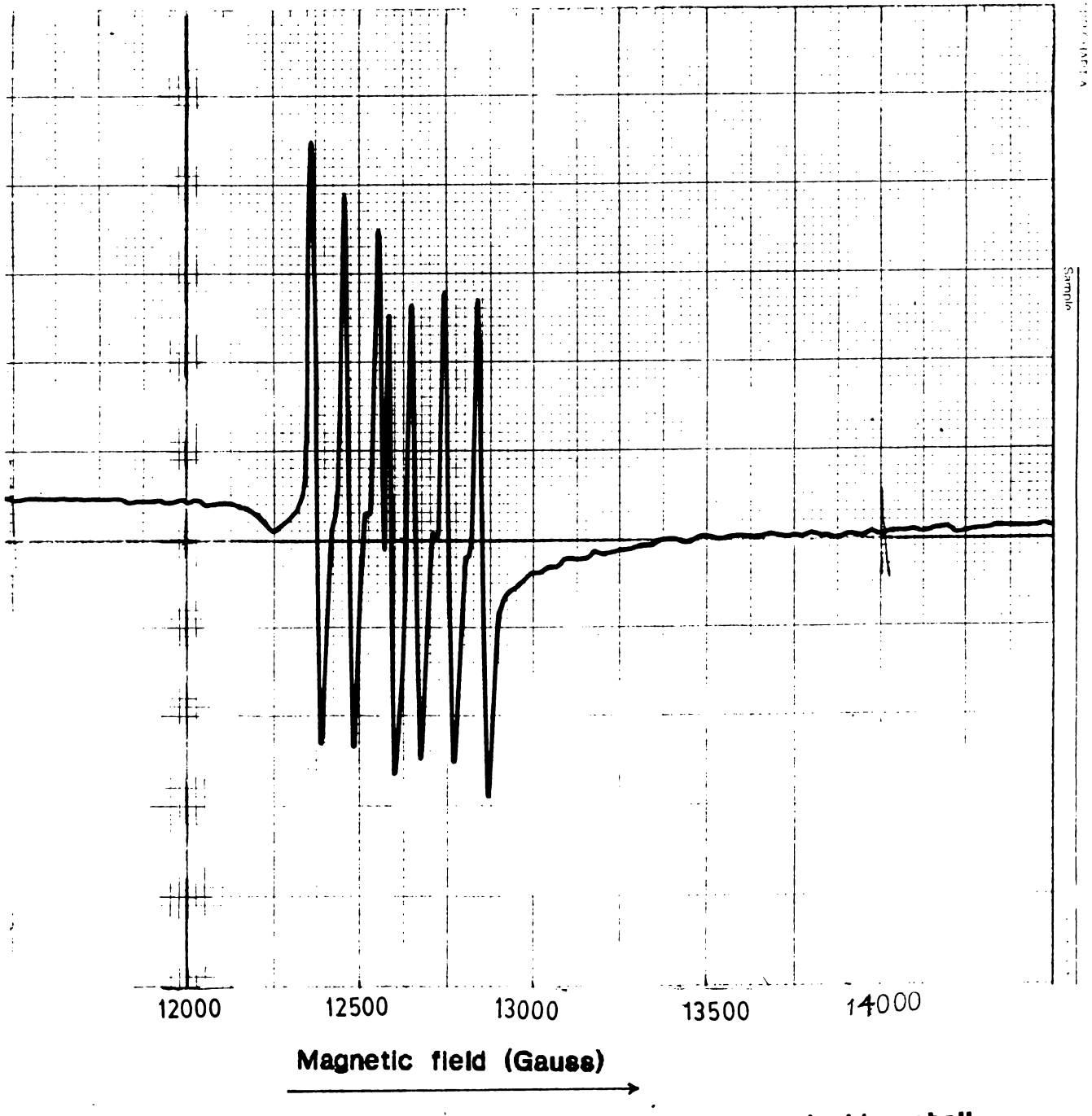


Fig.11 Q-band EPR spectra of powdered *V. cyprinoides* shell

unjustified notion that shells are vulnerable to severe variabilities in their metal content. The apparent variabilities in shell compositions can be traced to poor and non-uniform shell cleaning and treatment procedures before digestion, especially since the periostracum has sometimes been included as an integral part of the shell matrix. Rosenberg (1980) has summarized the short comings and pitfalls in the earlier studies on molluscan shell chemistry. In an effort to avoid contamination due to adsorbed metals and to estimate only metals which are incorporated into the shell matrix a cleaning procedure (P.Foster, Personal communication) was adopted *(vide* Chapter 2), which entirely removed the periostracum and thus eliminated the variability associated with the determination of adsorbed metals.

The incorporation of metals into shells normally occur *via.* two different processes .

(a) A passive enrichment, probably composed of several processes, mainly adsorptive in character and taking place on surfaces exposed to the aquatic medium i.e., the periostracum and the unprotected carbonates (Sturesson, 1976).

(b) An active accumulation of trace metals triggered by metabolic functions and/or mineralogic considerations which ultimately results in the integration of metals into the shell matrix. This occurs in conjuction with shell formation which

can be considered to happen in two major phases: (1) cellular processes of ion transport, protein synthesis and secretion and (2) a series of physico-chemical processes in which crystals of $CaCO_3$ are nucleated, oriented and grown in intimate association with a secreted organic matrix (Wilbur and Saleuddin, 1983). Trace metals are either bonded directly with the various structural components of the shell like conchiolin, organometallic pigments etc., or alternatively, may be incorporated within the inorganic lattice (Fox, 1966; Sturesson, 1976). In the present study, since the periostracum as well as organic and surface adsorbed materials were completely removed, the metal concentrations observed in these shells could have resulted only through physiological or mineralogical processes. It has been suggested (Phillips, 1980) that wherever adsorption of metals were nonexistent, the integration of metals into bivalve shells could only occur *via* a biological process during their synthesis.

The biogenic carbonates in the shells occur mainly as calcites and aragonites. The outer mantle epithelium is responsible for shell secretion. Shell formation occurs within the extrapallial space into which the mantle epithelium secretes the extrapallial fluid. It may contain contaminants like trace metals along with the components for biomineralization like Ca^{2+}, HCO_3^-, organic molecules etc.

During the formation of shells, any trace metal actively incorporated within the shell matrix, is assimilated by the organism (Wilbur and Saleuddin,1983). The incorporation of different kinds of trace elements into the shell during its formation is influenced by environmental as well as physiological processes like concentration of elements in ambient water, its seasonal and environmental variations, its salinity, mineralogy of the shell etc. (Pilkey and Goodell, 1963; Dodd, 1965; Pilkey and Harris, 1966; Frazier, 1976; Buchardt and Prosi, 1978, and others.). Trace or minor elements are generally incorporated into the shell either as substituents for calcium or as constituents of separate mineral phases or organic compounds (Carriker *et al.*, 1980).

The biogenic carbonates, calcites and aragonites, are easily identified on the basis of their ν_1 and ν_3 stretching vibrations in their infrared spectra (Chester and Elderfield, 1967). Calcites exhibit only a single peak at ~ 710cm^{-1} and do not show the ν_1 peak (~ 1070 cm^{-1}). The infrared spectral bands observed (in *V. cyprinoides* and other bivalve shells) ~710 cm^{-1} (ν_3 doublet) and ~1070cm^{-1} (ν_1) are clearly diagnostic of orthorhombic aragonite mineralogy. Carbonates of metals having ionic radii larger than Ca^{2+} are known to give rise to aragonitic mineralogy while those with ionic radii less than Ca^{2+} generally yield calcitic crystal lattices. Therefore

aragonites could normally be expected to take up metals larger than Ca^{2+} while inclusion of metals smaller than Ca^{2+} could occur in the case of calcites. The nature of the adsorption processes, the complexation capacity of shell proteins,the ionic radii of the metals, genetic variations of the organisms involved, etc. independently or jointly govern the uptake of metals into the shells (Carrel *et al.*, 1987; Chester and Elderfield, 1967; Segar *et al.*, 1971; Bertine and Goldberg, 1972; Sturesson, 1978; Carriker *et al.*, 1980; Al-Dabbas *et al.*, 1984).

As reported earlier lead, manganese and cobalt were found to be preferentially enriched in the shells while copper, cadmium and zinc were observed to be accumulated in the soft tissues. Some of causative factors of the preferential enrichment of the metals (lead, manganese and cobalt) are discussed below.

The accumulation of lead can take place in two ways i.e., either by adsorption on the outer surface or by incorporation into the shell matrix. Ferrell *et al.*, (1973) and Sturesson, (1976), have explained the pathway of the enrichment of lead due to the surface adsorption. Since the cleaning procedure employed in the present study ensures stripping of any surface adsorbed metals, the concentration of lead observed herein

could have resulted only through a gradual incorporation from soft tissues during growth.The presence of lead in *V. cyprinoides* and other bivalve shells considered here is fully explainable from mineralogical considerations as well. Pb^{2+} ions can be expected to substitute some of the Ca^{2+} ion in the 9-coordinate aragonite lattice because lead carbonate (cerrusite) and aragonite are isostructural (Chester and Elderfield, 1967).

Manganese enrichment in the shells is believed to take place by chemical as well as environmentally significant processes. Frazier (1975) studied the dynamics of manganese transport in *C. virginica* and reported that about 20 μg of manganese were deposited in their shells each day during shell growth. Carriker, *et al.* (1980) and Phillips (1980) have also reported enhanced enrichment of manganese in shells. Manganese enrichment is believed to be facilitated either by the complexation of manganese with the organic matter of the shell matrix or by incorporation into the lattice structure of the carbonates and the oxides (Goldberg, 1957; Fox, 1966; Horiguchi and Tsujii, 1967; Bourget, 1974). According to Bryan and Uysal (1978) the incorporation of manganese into shells occurs largely during shell deposition by the mantle. The incorporation of manganese into the lattice structure could take place by two routes. One is the usual substitution in the

calcite. This is quite possible in view of the identical crystal structure of calcite and rhodochrosite (manganese carbonate). The second pathway of manganese incorporation is by a geologically improbable inclusion of Mn^{2+} into the aragonite lattice by biomineralization occurring during shell formation (Rosenberg, 1980).

Blanchard and Chasteen (1976) reported the occurrence of manganese in the calcitic shell of *M. edulis*. But White *et al.* (1977) reported the presence of Mn^{2+} in the aragonitic lattice of *M. arenaria*. EPR is a sensitive method for detecting systems with unpaired electrons and hence have been used to detect the presence of these in molluscan shells. "g" values provide considerable information about the stereochemistry and symmetry of the metal ion within the crystal lattice. The results of the room temperature EPR spectra of powdered *V. cyprinoides* shell samples used in the present study, yielded g_{iso} and A_{iso} values of about 2.011 and 100G respectively, which are in excellent agreement with results reported earlier by White *et al.*(1977). These Mn^{2+} signals indicate an unusual isomorphous substitution of Ca^{2+} in the aragonite lattice.

Reports on the enrichment of cobalt in the shells and/or soft tissues of aquatic organisms are scanty in literature. Szefer (1986) and Szefer and Szefer (1985) reported on the

values of cobalt in the soft tissues and shells of organisms like *M. edulis, C. glaucum, M. arenaria, M. balthica* etc., the levels of cobalt in the shells being considerably less than that in the soft tissues. In marked contrast, the results of the present analyses of the shells of *V. cyprinoides* show increased accumulation of cobalt in the shells than in the soft tissues. Cobalt, though an essential element, is not accumulated in the soft tissues to any appreciable levels. The increased enrichment of cobalt in the shells, therefore leads to the conclusion that biological transport of excess amount of cobalt into the shell structure is an active process prevailing in the body. The Co^{2+} (d^7) ion has a 4 $T_{1g}(F)$ ground state with extensive spin orbit coupling, and EPR measurements are possible only at low temperatures. Because of spin relaxation problems, only a single peak is expected with a g_{iso} value of 4.33 (Drago, 1977). However, the absence of any EPRsignal (at room temperature or even at liquid nitrogen temperatures) in the shells of *V. cyprinoides* clearly shows that the cobalt present in the shells (confirmed by atomic absorption analyses) do not exist as Co^{2+} (d^7) ion but exists as the low spin, diamagnetic Co^{3+} ion. The superior stability of Co^{3+} complexes of N-ligands (like amines, proteins etc.) over those of Co^{2+} is well established (Huheey, 1978). Thus the presence of Co in the *V. cyprinoides* shells attests the fact that mineralogical incorporation is not the principal

enrichment process and that, other factors like genetic/ physiological processes do exert a decisive influence in regulating metal uptake by shells.

Copper, cadmium and zinc were observed to be preferentially accumulated in the soft tissues. Lattice incorporation of these metals is totally unexpected in view of the incompatibility of their effective ionic radii *vis-a-vis* that of Ca^{2+}. Cu^{2+} ions, due to its significant physiological function have a distinct affinity for the soft tissues than for the shells. Zinc is an essential metal in the biological realm because it is an integral part of a number of metallo-enzymes. The concentration of Zn^{2+} can regulate many metabolic processes through initiation and/or regulation of the activity of these enzymes (Leland and Kuwabura, 1985). Eisler (1981) observed that accumulation of zinc is mediated by many factors including interaction effects with salts of calcium, cobalt, iron, cadmium and various organic substances. Metallothionein is believed to play an important role in the enhanced enrichment of metals like copper, cadmium, mercury, zinc, cobalt etc. Metallothioneins are low molecular weight cytosolic proteins rich in cysteine. They are induced by several factors including exposure to metals such as Cd, Hg, Cu, Zn, Au, Ag etc. Metallothioneins have a high affinity for these metals and

it is generally accepted that the binding of these metals to metallothionein constitute a metal detoxifcation (Klerks and Bartholomew, 1991). Soft tissue concentrations of cadmium were comparatively lower than those of other metals in the soft tissues. These results are in agreement with studies on other clams and gastropods reported by Segar *et al.* (1971), Frazier (1976), Lande (1977) etc. Although cadmium has no known biological function in molluscan tissues (Eisler, 1981), it is regarded as a toxic element because of its ability to substitute Zn^{2+} (Graham,1972). Cadmium can therefore be expected to mimic Zn^{2+} in its behaviour and hence to be concentrated more in the soft tissues than in the shells. The observed slight accumulation in the shells might have resulted by the substitution of Ca^{2+} ions by Cd^{2+} ions in view of their comparable ionic radii.

Strontium and magnesium are two other important elements found strongly associated with shells of bivalves. Even though the concentrations of these elements are much higher than that of the other elements studied, the influence of environmental variables on the enrichment of these elements are not completely well understood. Rosenberg (1980) has listed the detailed, but unsuccessful attempts of earlier workers to correlate strontium/magnesium levels in the shells and environmental factors such as salinity, temperature etc.

The concentrations of strontium were generally higher compared to that of magnesium and variations in *V. cyprinoides* were not very significant. The concentrations ranged between 1208.2 $\mu g\ g^{-1}$ to 1421.78 μgg^{-1} . The lower values were obtained from the more saline stations whereas the higher values were observed in less saline environments. The concentration of strontium in the estaurine bivalve *V. cyprinoides* was also notably greater than that in the other bivalves found in the region, *viz.P. viridis* (1046.8 $\mu g\ g^{-1}$) *S. scripta* (1144.02 μgg^{-1}) *M. senhousia* (995.73 $\mu g\ g^{-1}$) and *M. casta* (1157.98 μgg^{-1}) which had more saline habitat. The Sr/Mg ratio was also greater in *V. cyprinoides* than in the other bivalves mentioned above.

Stronium enrichment in the shell is determined by several environmental factors and variables such as illumination, nutrition, water circulation, mussel growth rate, temperature, salinity, Sr/Ca ratio in the environment etc. (Nelson, 1961; Muller, 1968, 1978; Gunatilaka, 1975; Crisp, 1975; Lorens and Bender, 1977).

The mineralogy of the biogenic carbonates also critically influences the enrichment of strontium. Sr^{2+} has larger ionic radius than that of Ca^{2+} and is known for its preference to be

included in the more open, octahedral, crystal structure of aragonite (Rosenberg, 1980; Lorens, 1981). The distribution coefficient of strontium was reported to be ten times more for aragonites than for calcites (Kinsman, 1969; Kitano *et al.*,1971). In view of the aragonitic structure, clearly evidenced by the infrared spectra of the shells analysed herein the relative increase in concentration of strontium and the high values of the Sr/Mg ratio can be fully justified. The Sr/Mg ratio in *V. cyprinoides* was also seen to be affected by the environmental characteristics of the habitats.

The concentration of magnesium does not show any significant variation. The values ranged between 495.05 $\mu g\ g^{-1}$ and 517.27 $\mu g\ g^{-1}$ for *V. cyprinoides* sampled from the different Stations. The level of magnesium is generally lower in molluscs than in other invertebrates (Lowenstam, 1963; Dodd, 1967; Wyckoff, 1972). Even though magnesium is three times more abundant than calcium in the sea water, bivalves have developed a highly enhanced capability for discriminating against magnesium. (Lowenstam, 1963). Milliman (1974) opined, that the higher solubility of $MgCO_3$ than $CaCO_3$ could be a reason for such an unusual behaviour. Eisma *et al.*, (1976) compared *C. glaucum, M. balthica, M. arenaria* and *M. edulis* sampled from different areas of salinity and found only weak trends between the magnesium concentrations annd salinity. Moreover, even for

a single species, the trend was inconclusive. Similar results were obtained in the present study also as the magnesium concentrations in the shells of *V. cyprinoides* obtained from the different stations as well as that in the different bivalves did not convey any definite trend. The infrared spectra of all the shells analysed in the present investigations indicated a predominant aragonitic lattice which could be responsible for a low concentration of magnesium.

The present study highlights the use of shells as bio-indicators. Besides their use in dating techniques, chemical ontogeny etc. are indeed quite promising. However more exhaustive studies employing advanced analytical techniques are needed to fully exploit the potential of shells to be used as store house of environmental records.

Chapter 4

TRACE METALS IN THE COCHIN ESTUARY - DISTRIBUTION AND BIOAVAILABILITY

Introduction

The exponential expansion of human activities, industrialization and exploitation of the available resources has resulted in an undesirable enrichment of nutrients and trace metals in the aquatic environment. Municipal, industrial, urban, agricultural and atmospheric wastes pose a serious threat, to the integrity and the conservation of the global aquatic ecosystem. It has, therefore, become necessary to mobilise a concern for environmental and ecological sustainability at the global level.

A pollutant on being discharged into the aquatic environment, is subjected to a series of complex physical, chemical and biological processes, which ultimately result in the partitioning of the pollutant into the different phases of the aquatic system. As a result of various physico-chemical changes and reactions in the aquatic environment, a major fraction of the metals introduced into the system will be associated with the bottom sediment. Hence it has become necessary to quantify the pollutant distribution profile in the different segments of the aquatic system (Tessier and Campbell, 1987). Mechanisms of transport and fate of metals in estuaries have become the focus of attention in attempts to elucidate the route of metal uptake in biological tissues (Connell *et*

al.,1991). An adequate knowledge of the processes that preside over the distribution and concentration of the metal in sediment, biota and overlying water is essential for any meaningful assessment of water quality.

Studies relating to the distribution of trace metals in oneorthe other segments of the aquatic systems are numerous. Förstner (1983 a), Danielsson (1980), Moore (1981), Duinker and Nolting (1982), Paul and Pillai (1983), Sathyanarayana *et al.*, (1985) Campbell *et al.* (1988), Windom *et al.* (1989), Forstner *et al.* (1990), Shibu *et al.* (1990), Turner and Millward (1990) and others studied the distribution of trace metals in the water column. Several authors have reported on the distribution of trace metals in the sediments (Forstner,1983 b; Moore, 1980; Cosma *et al.*,1982; Howell, 1985; Morris and Kwain, 1988; Nair *et al.*, 1990; Salomons and Eagle, 1990). Trace metal levels in biota have been studied extensively (Bertine and Goldberg, 1972, Sturesson 1976; Boyden, 1977; Ireland and Wootton, 1977; Davenport and Manley, 1978; Goldberg *et al.*, 1978; Theede *et al.*,1979; Kumaraguru *et al.*, 1980; Strong and Luoma, 1981; Lakshmanan and Nambisan, 1983; Prosi, 1983;1983; Al-Dabbas *et al*, 1984; Borchardt *et al.*, 1985; Brix and Lingby, 1985; Szefer and Szefer, 1985; Szefer, 1986; Marcovecchio, 1988; Phillips and Rainbow, 1988; Sivadasan and Nambisan,1988; Mariogomez and Ireland, 1989). Luoma and Phillips (1988) studied the distribution, variability and impacts of trace elements in

water and biota of the San Francisco Bay. However investigations in which all the three phases of the aquatic environment were considered are relatively few. Mahajan *et al.* (1987) studied the anthropogenic influence on the enrichment of trace metals in the sediments and biota in the estuarine region of Bombay island. Patel and Chandy (1988) also reported on studies relating to trace metal levels of sediments and clams of Bombay coast. Ajmal *et al.*, (1987) had reported on the relative abundance of heavy metals in water, sediment, fish and plants. Schirmer (1990) determined metal concentrations in water (dissolved and particulate), sediment and biota (algae and crustacea) and attempted to relate the environmental factors with biological tissue concentrations.

"Ecotoxicology is concerned with the toxic effects of chemical and physical agents on living organisms especially on populations and communities, within defined ecosystems; it includes, the transfer pathways of those agents and their interactions with their environment" (Butler, 1978). Such studies attempt at assessing the structure and function of an ecosystem, in the hope of being able to stumble upon minute, yet pertinent, fluctuations in both biotic and abiotic factors (Boudou and Ribeyre, 1989). An understanding of the distribution of toxic substances in an aquatic system is therefore central to any ecotoxicological investigation. The essentiality or toxicity of a chemical is decided by the extent

of biological requirement necessary for the metabolic processes being carried out in the organism into which the chemical is bioaccumulated.

It may be appropriate to explain some of the terms frequently used in such studies.

Bioavailable:- The fraction of the total chemical in the surrounding environment, which is available for uptake by the organisms (Spacie and Hamelink, 1985), is referred to as the `bioavailable' fraction.

Bioaccumulation:- It refers to the ability of the organism to accumulate a chemical from its environment by any means (Isensee *et al.*, 1973). It can occur only if the rate of uptake of a substance in an organism is more than elimination.

Bioconcentration:- It is the accumulation of chemical residue in organisms by transportation of the chemical through it either by gills or other membranes or both.

Biomagnification : It is the process whereby pollutants are passed from one trophic level to another and exhibit increasing concentrations in organisms related to their trophic status (Connell and Miller, 1984).

The static state of bioconcentration is attained by the dynamic process of bioaccumulation which is accelerated by the bioavailability of a substance in the environment. Bioavailability, which enhances bioaccumulation, is influenced

by the species of the metal bioaccumulated (and not by the total metal concentration) and the nature of the bioaccumulating organism and is a property dependent on the physicochemical, geochemical and physiological processes that determine the fate of a chemical in the environment. Bioavailability and hence bioaccumulation are thus influenced by characteristics of the interface, reactivity of the metal form, presence of other metal ions that enhance or inhibit metal uptake, variations in temperature, physiological state of the organism, etc. (Luoma, 1983). As the uptake of a chemical is effected from the surrounding water, the suspended material and the sediment, the bioavailability of the chemical from all these compartments will have to be considered for appreciating the process in its totality.

Bioaccumulation of metals occurs mainly by diffusion, transport and adsorption. Diffusion can occur across any barrier that is semipermeable to the chemical and across which a concentration gradient exists, (e.g., gills, lining of the mouth gastrointestinal tract etc.). While the lipid bilayer of simple biological membranes permit rapid diffusion of non-polar molecules, proteinaceous pores present in the membrane allow the passage of essential polar molecules. This semipermeable character may be altered by salinity and/or physiology of the organism (Spacie and Hamelink, 1985). The transport mechanism operates through carrier molecules and includes: (i) transport

via. carriers specific for nutritionally essential cations (e.g. Ca, Mo, Zn etc.) (ii) nonspecific complexation of metal forms with carrier molecules, which could result in either "accidental" transport across the interface or immobilization at the external interface (iii) transport of metals complexed with essential nutrients (e.g. amino acids or proteins) on carriers specific for the nutrient and (iv) transport of nutritionally essential metal complexes (Luoma, 1983). Adsorption can be effected by binding the chemical to the surface by covalent, electrostatic or molecular forces. It is important as the initial step in the accumulation process and is mainly operative in micro organisms which have extremely high surface to volume ratio (Spacie and Hamelink, 1985).

Although several attempts have been made to unravel the complexities and ascertain the mechanisms of bioaccumulation, very little is yet known of the behaviour of the metals (or of any other substances) in the aquatic environment to be sure of the factors that regulate bioavailability. Most of the studies were performed in simulated conditions and reported on the enrichment of trace metals in the tissues resulting from an applied concentration (Denton and Burden-Jones, 1981; Ahsanullah *et al.*,1981; Davenport and Redpath, 1984; Amiard-Triquet *et al.*, 1986; Wolmarans and Van aardt, 1986; Sivadasan, 1987; Neimann and Mitz, 1988; Lakshmanan and Nambisan, 1989; Marigomez and Ireland, 1989; Krishnakumar *et*

al., 1990). Zamuda and Sunda (1982) have, however studied on the bioavailability of dissolved copper to the oyster *Crassostrea virginica*.

The influence of the physico-chemical characteristics of the aqueous phase (such as salinity, hardness of water, pH etc.) on bioaccumulation. were probed into by Sivadasan (1987), Wright and Zamuda (1987) Sprague (1985) and others. Sivadasan (1987) and Wright and Zamuda (1987) observed an inversely proportional relationship between salinity and accumulation of trace metals. Variations in salinity determine the nature of metal species and thus regulate the bioavailability of the metal. Dilution of water which resulted in low salinity facilitated the formation of an increased proportion of free metal ions which were more readily bioavailable than any other metal species. Hardness of water has also been reported to have an inverse effect on trace metal accumulation (Miller and Mackay, 1980). The enhanced concentration of calcium and magnesium ions probably inhibits the smaller ions from forming complexes with organic molecules. Also, the higher levels of calcium in the tissues make the cell membranes in the gills less permeable, thwarting any attempts of metals to enter the tissues (Sprague, 1985). The influence of pH on trace metal accumulation and toxicity is related to the maintenance of ionic species concentration in the aqueous medium.

From the aqueous phase, metals are accumulated into the tissues both, from the dissolved and from the particulate forms. Even though ionic species are the preferred form for uptake by the organisms, their concentrations in the water medium is very low as they get complexed with ligands and get adsorbed onto the surface of suspended particles. Metal uptake from solution is enhanced by diffusion (both active and passive), transport (active and facilitated transport), adsorption to the binding sites, concentration of free metal ions in the medium etc. (Luoma, 1983; Spacie and Hamelink, 1985). On the other hand, environmental processes like adsorption to suspended solids, to sediments, to humic acids and to macromolecules, formation of colloidal suspensions, chelation, complexation etc. reduce the amount of ionic species in solution and consequently decrease its bioavailability from water (Spacie and Hamelink, 1985). The particulate matter ingested by the organisms are solubilized by the acidic juices in the gut, thereby rendering the metals contained therein bioavailable (Waldichuk, 1985).

The characteristics of the sediment phase also play a dominant role in determining the bioavailability of the metal. Detritus feeding organisms, being directly exposed to the sediment bound metals, have been widely used in studying the bioavailability from sediments (Luoma, 1983). The attributes of the sediment that influence metal bioavailability include

the nature of donor ligands and competing cations, the redox potential, the ionic strength, the adsorptive power etc. (Luoma, 1983; Campbell and Tessier, 1989).

Since sediments represent the phase with maximum trace metal concentration in the aqueous system, several workers have attempted to elucidate and predict the availability of metals from the sediment phase both by conventional and sequential extraction techniques (Luoma and Jenne, 1976; Bryan and Hummerstone, 1978; Luoma and Bryan, 1979; Luoma ,1983; 1989; Tessier and Campbell, 1984; 1987; Campbell and Tessier,1989; Gunn *et al.*, 1989).

Distribution of trace metals in the waters of Cochin estuary has been studied by several authors. Sankaranarayanan and Rosamma Stephen (1978) reported on the particulate Fe, Mn, Cu and Zn in Cochin backwaters. Paul and Pillai (1983 a & b) have studied the distribution, speciation and biological transfer of trace metals in the Periyar river. Trace metal levels in the various aquatic organisms of this region have been studied and reported by various authors (Sankaranarayanan *et al.*, 1978; Lakshmanan, 1982; Krishnakumar, 1987; Sivadasan and Nambisan,1988). Metal levels in the sediments of Vembanad lake have been studied by Murty and Veerayya (1981), Venugopal *et al.*, (1982), Ouseph (1987, 1990), Nair *et al.*,(1990) and others. Nair *et al.*, (1991) have carried out chemical partitioning studies on sediments of Cochin estuary. Ouseph

(1990) has reported on the distribution of mercury in the sediment, the particulate and the dissolved phases of the estuarine environment.

Although various speciation/fractionation schemes have been developed for assessing the metal partitioning in the aqueous phase no attempts have been made to compare or relate their results to the metal concentration present in the biota. Any rigorous assessment of metal bioavailability in an aquatic environment would, obviously,need to consider the contributions from all the constituent phases.

Results and Discussion

In the present study metal concentrations in the different phases - sediment, particulate matter of the aqueous phase as well as soft tissues of organisms - have been separately identified and are normalized with respect to the dissolved metal concentrations. The results of trace metal concentrations in the sediment, water (dissolved and particulate) and bivalves (*V. cyprinoides* var. *cochinensis* from stations except 5, and *M. casta* from station 5) are analysed and the trace metals are given in this chapter. Metals under consideration are copper, cadmium, zinc, Nickel and lead.

The relationship between metal levels in the bivalves (both shell and tissues) were determined along with the metal

concentrations in water and sediment. Since dissolved metal concentration is known to play an important role in regulating trace metal concentrations in the other phases, the ratio of metal concentrations in the respective compartments to that with Correlations were attempted between biological factors, soft tissue metal levels, BCF, log BCF, BCR, log BCR and BAF on the one hand and environmental factors such as dissolved metal concentrations, particulate metal concentration, MPR, GAF, besides metal concentration in different fractions (*viz.* exchangeable, carbonate bound, Fe/Mn oxide bound, organically bound and residual) obtained by the sequential extraction of the sediment (*vide* Chapter-2) as well as total sediment-metal concentration on the other hand. The trends obtained between these two sets of factors are discussed below for each of the metals investigated.

The six sampling sites (Chapter 2) selected were clam habitats which represented a reasonably good spatial variation within the estuary. The present investigation was based on extensive, monthly, field collections spread over a period of fifteen months from October 1988 to December, 1989. However, owing to practical difficulties only six monthly collections (between November 1988 and September 1989) were carried out at Stations 5 and 6 located at the northern end of the estuary. While *V. cyprinoides* was sampled from all the Stations except Station5, *M. casta*, was the only species available at Station5

For analysis of the metal distribution profile, calculations of annual mean metal concentrations were limited to 12 sets of monthly data (December 1988 to Novemeber 1989) pertaining to Stations 1 to 4 and to the 6 sets of montly data pertaining to Stations 5 and 6. The annual mean concentrations (a.m.c.) of the trace metals (copper, cadmium, zinc, lead and nickel) in the various environmental (sediment, dissolved and particulate phases of the aquatic environment) as well as biological (soft tissues and shells) compartments at different Stations are tabulated metal wise. The highest and lowest concentrations observed at each station, along with their respective standard deviations would give an idea about the temporal as well as the spatial fluctuations in the concentrations of trace metals.

V. cyprinoides was chosen for the bioavailability studies in view of its wide distribution and easy availability within the Cochin estuary. All available monthly sets of data (15 sets pertaining to stations 1 to 4 and 6 sets pertaining to station 6; *M. casta* sampled from Station 5 was not considered) were used in the regression analysis for evaluating the bioavailability of the trace metals.

Although metal concentrations in both shells and soft tissues of *V. cyprinoides* were taken in to account in the

regression analyses, significant relationships became obvious only between metal concentrations, in the soft tissue (and allied ratios- *vide infra*) on the one hand and that in the water/ sediment (and related ratios - *vide infra*) on the other hand. (The role of shells, however lay in their distinct ability to be used as a convenient index for evaluating the metal concentrations present in the other segments of the aqatic system. This aspect has been dealt with separately in chapter 5).

Correlations were found out between the different environmental variables and the biological factors. The degree of correlation existing between the various environmental variables and biological factors was taken as the index of bioavailability.

The various environmental variables and bilogical factors considered in the regression analysis are explained below.

Environmental variables

Metal concentration in the sediment phase, denoted as X

Metal concentration in dissolved phase, denoted as X1

Metal concentration in particulate phase, denoted as X2

Exchangeable fraction of metal concentration in the sediment, denoted as X5

Percentage of exchangeable fraction to the total metal concentration in the sediment, denoted as X6

Ratios related with Envrionmental Variables

Metal partitioning ratio (MPR) was defined as the ratio between metal concentration in the particulate phase to that in the dissolved phase and denoted as X3

X3 = X2/X1

Geoaccumulation factor (GAF) was defined as the ratio between metal concentration in the sediment to that in the dissolved phase and denoted as X4

X4 = X/X1

Biological variables

Metal concentration in the soft tissue, denoted as Y1

Ratios related with Biological variable

Bio concentration factor (BCF), was defined as the ratio between metal concentration in the soft tisse to that in the ambient water, and was denoted as Y2

Y2 = Y1 (X1 + X2)

Bioconcentration ratio (BCR), was defined as the ratio between metal concentration in the soft tissue to that in dissolved phase of water, and was denoted as Y3

Y3 =Y1/X1

Bioaccumulation factor (BAF), was defined as the ratio between metal concetration in the soft tissues to that in sediment and was denoted as Y4

Y4 = Y1/X.

Copper

The distribution profile of copper in the Cochin estuary is summarized as follows. Table 12 gives the annual mean,standard deviation and the lowest and the highest concentrations of copper in the different compartments observed during a period of 12 months. The monthly variations are depicted in Figs. 12-14

Distribution in Water

Dissolved

The highest value of dissolved copper (9.42 $\mu g\ l^{-1}$) was recorded at station 4 and the lowest (1.49 $\mu g\ l^{-1}$) at Station 2. Station 4 also recorded the maximum a.m.c. (annual mean concentration) (4.36 $\mu g\ l^{-1}$); the minimum a.m.c. being observed at Station 2 (3.34 $\mu g\ l^{-1}$). In the southern side of the estuary, the maximum a.m.c. was observed at Station 4 where the Moovattupuzha river debounches into the estuary. But on the northern side the a.m.c. was higher at Station 5 (estuarine), than at the Station 6 (riverine) *viz.* Manjali.

Particulate

The highest and lowest values of particulate copper for the whole period of study were 7.97 $\mu g\ l^{-1}$ and 0.75 $\mu g l^{-1}$

Table 12. Distribution of copper in the Cochin estuary

COMPARTMENTS	STATIONS					
	1	2	3	4	5	6
Soft tissue $\mu g\ g^{-1}$	17.52 ± 6.93 (9.29 - 28.46)	18.73 ± 5.90 (11.28 -28.28)	22.52 ± 8.05 (10.14 - 33.43)	26.97 ± 8.09 (14.53 - 42.74)	25.11 ± 4.14 (18.42 - 30.98)	17.01 ± 3.65 (11.39-22.54)
Shell $\mu g\ g^{-1}$	5.70 ± 0.84 (4.53 - 7.49)	6.22 ± 0.92 (5.18 - 8.32)	5.48 ± 0.60 (4.05 - 7.47)	5.60 ± 0.95 (4.27 - 8.0)	4.93 ± 0.31 (4.57 - 5.28)	5.13 ± 0.46 (4.60- 6.05)
Sediment $\mu g\ g^{-1}$	5.65 ± 1.13 (3.41 - 7.73)	5.66 ± 1.31 (3.15 - 7.56)	6.72 ± 0.67 (5.68 - 7.99)	7.72 ± 2.40 (4.37 - 11.48)	6.32 ± 0.73 (5.02 - 7.52)	6.06 ± 0.63 (5.26- 7.15)
Dissolved $\mu g\ l^{-1}$	3.90 ± 1.64 (1.93 - 7.24)	3.34 ± 1.39 (1.49 - 7.42)	3.50 ± 1.13 (1.75 - 5.15)	4.36 ± 2.07 (1.90 - 9.42)	4.33 ± 0.77 (2.78 - 5.04)	4.02 ± 1.03 (2.70- 5.49)
Particulate $\mu g\ l^{-1}$	2.80 ± 1.48 (0.96 - 5.94)	2.22 ± 1.63 (0.77 - 6.56)	2.44 ± 1.06 (1.20 - 4.78)	3.78 ± 1.78 (1.31 - 7.97)	2.65 ± 0.90 (1.13 - 3.56)	1.61 ± 0.48 (0.75- 2.13)

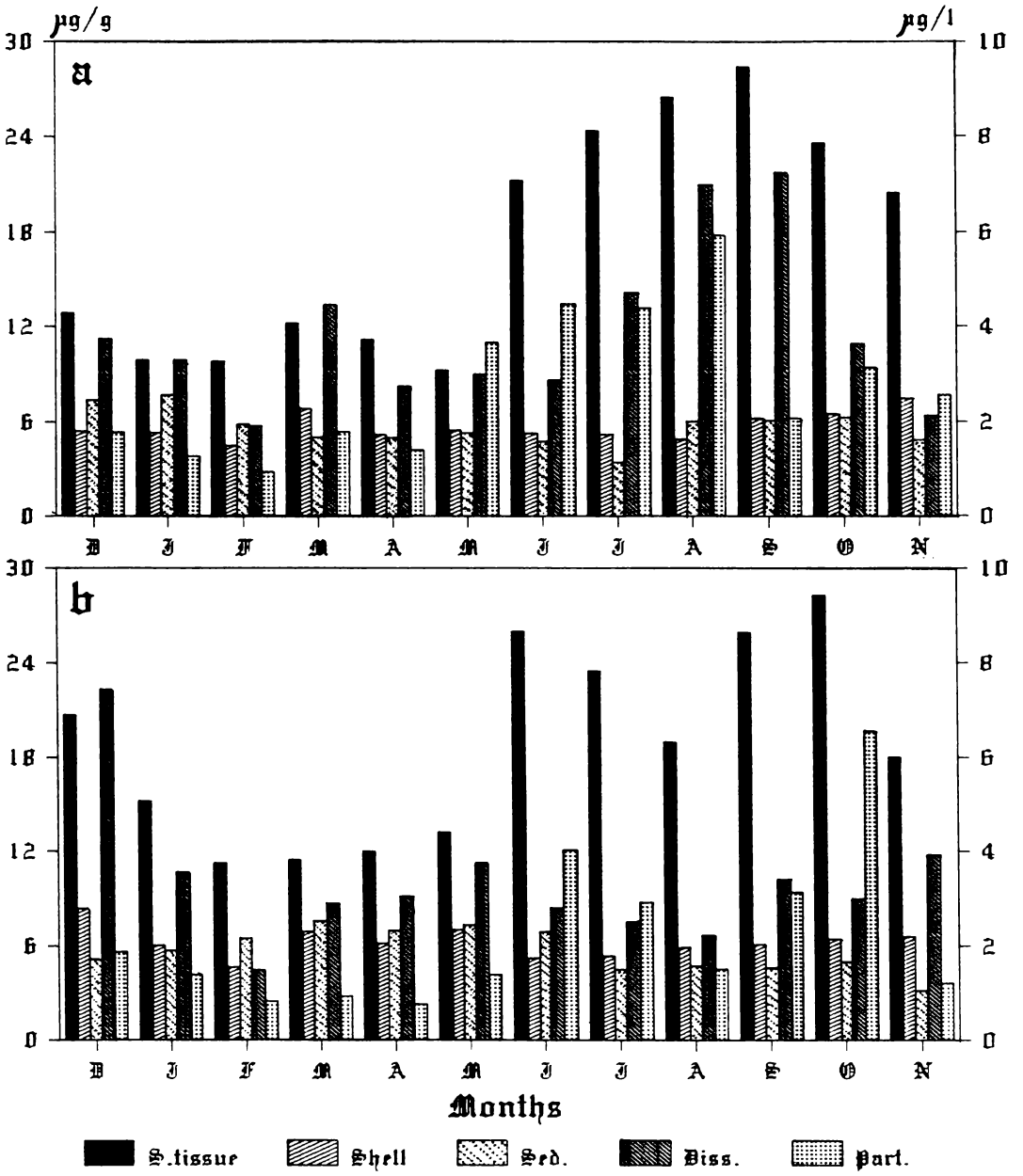


Fig.12 Trace metal partitioning in Cochin estuary (copper)
a) Station 1 b) Station 2

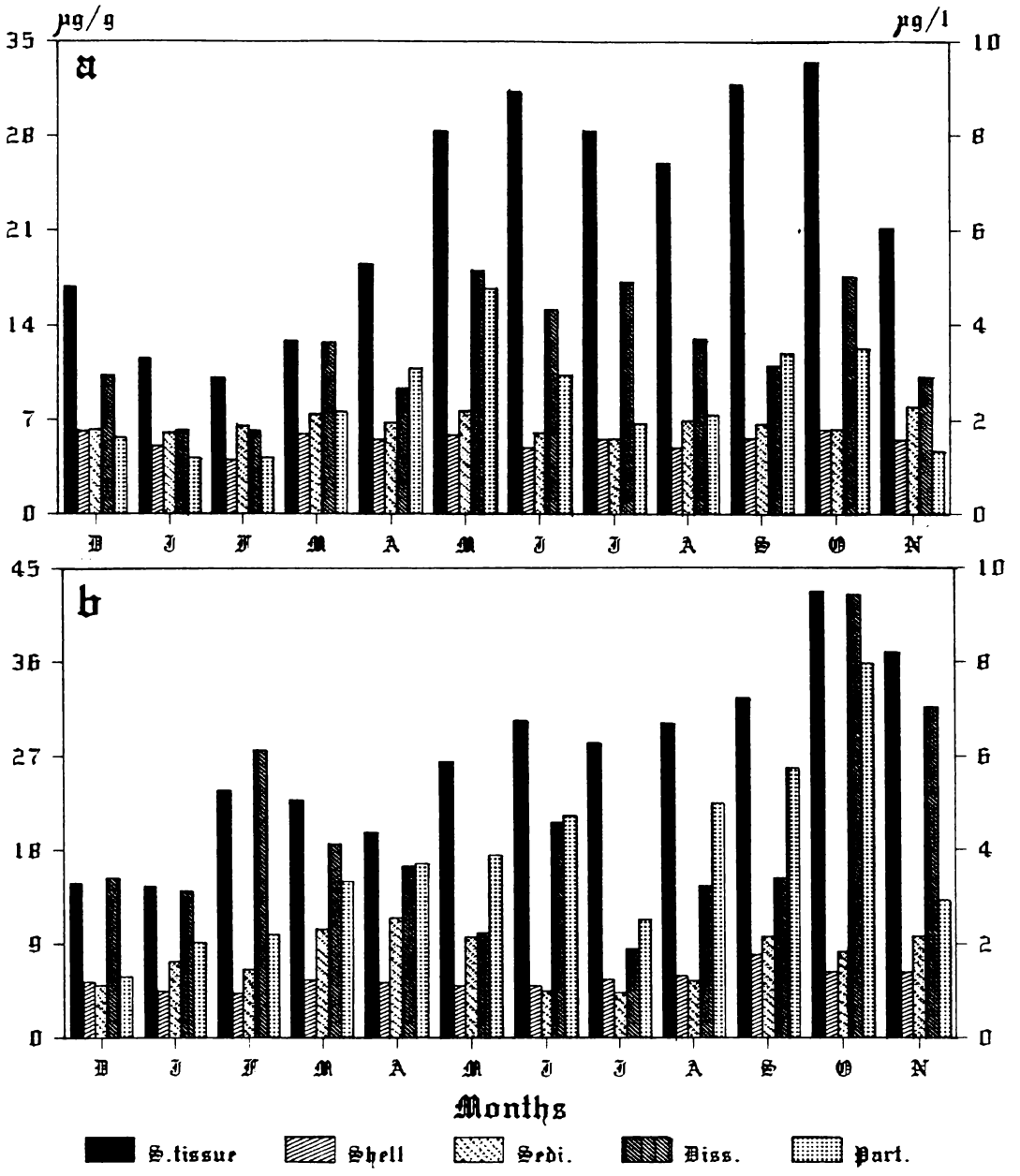


Fig. 13 Trace metal partitioning in Cochin estuary (copper)
a) Station 3 b) Station 4

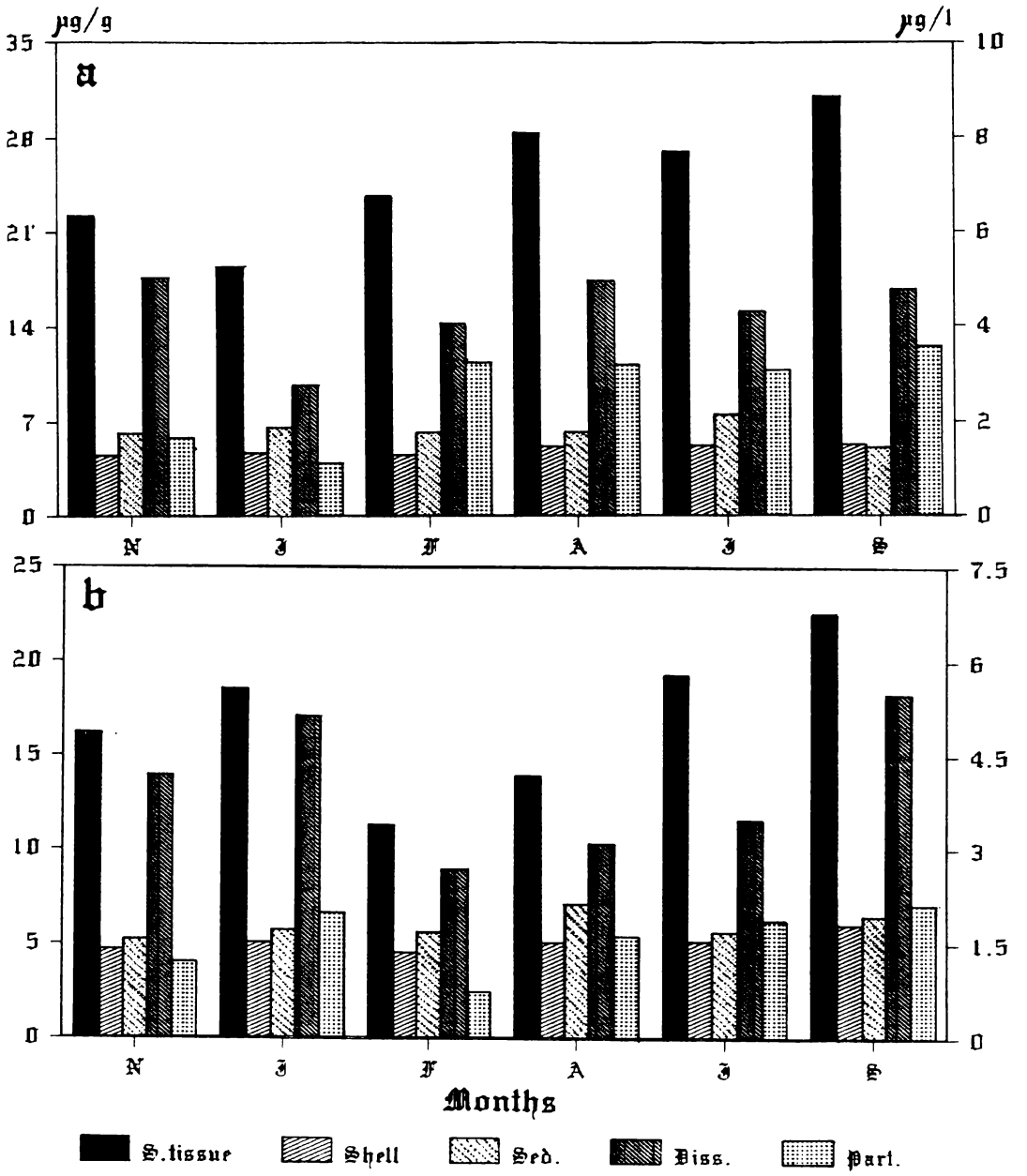


Fig. 14 Trace metal partitioning in Cochin estuary (copper)
a) Station 5 b) Station 6

respectively recorded at Stations 4 and 6. The a.m.c. varied between 3.78 μgl^{-1} and 1.61 μgl^{-1} at Stations 4 and 6 respectively, both of them are riverine in nature. The a.m.c. at the other estuarine Stations (1,2,3, and 5) varied between 2.22 μgl^{-1} to 2.80 $\mu g\ l^{-1}$.

Distribution in Sediment

The highest concentration was observed at Station 4 (11.4 μgg^{-1}) and lowest at station 2 (3.14 $\mu g\ g^{-1}$). The sediment a.m.c. showed comparatively smaller variation ranging between 5.65 $\mu g\ g^{-1}$ at Station 1 and 7.72 μgg^{-1} at Station 4, both Stations located in the southern region of the estuary. In the northern region, maximum a.m.c. of estuarine Station was 6.32 $\mu g\ g^{-1}$ and that of riverine station was 6.06 $\mu g\ g^{-1}$.

Distribution in Bivalves

Shells and soft tissues were analysed separately to ascertain their metal contents. Even though the concentrations in the shells were comparatively lower than that in the soft tissues, they assume relevance as they form the major body weight of the organisms (see also chapter 5).

Shells

Copper concentrations in shells did not show any large

variation with changes in environmental factors or with seasons. The highest concentration of copper for *V. cyprinoides* was recorded at Station 2 (8.32 $\mu g\ g^{-1}$) and the lowest value was 4.05 $\mu g\ g^{-1}$ at Station 3. For *M. casta* collectedfrom the estuarine Station in the northern region, the lowest and the highest values were 4.57 $\mu g\ g^{-1}$ and 5.28 $\mu g g^{-1}$ with an a.m.c. of 4.93 $\mu g\ g^{-1}$. While the maximum a.m.c. for *V. cyprinoides* was 6.22 $\mu g\ ^{-1}g$ recorded at station 2, the minimum a.m.c. was observed at Station 6 (5.13 $\mu g\ g^{-1}$). The Stations 2 and 3 which showed the highest and the lowest concentrations of copper in *V. cyprinoides* were adjacent ones in the southern region.

Soft tissues

Soft tissue concentrations of metals are often very sensitive in reflecting changes in the ambient environment and hence important in assessing the environmental quality. Copper concentrations observed in the soft tissues, exhibited wide variations with respect to the sampling sites. Generally, high concentrations of copper were observed at all Stations during the monsoon months (June to September) and in October 1989, which incidentally, had all the monsoonal characteristics.

The highest concentration of copper (42.74 $\mu g\ g^{-1}$)was observed at Station 4 and the lowest concentration (9.29 $\mu g g^{-1}$)

at Station 1. The a.m.c. of *V. cyprinoides* varied between 17.01 $\mu g\ g^{-1}$ and 26.97 μgg^{-1} and were observed at the riverine stations 6 and 4 respectively. For *M. casta*, the a.m.c. obtained was 25.11 $\mu g\ g^{-1}$ with the lowest value of 18.42 $\mu g\ g^{-1}$ and the highest concentration of 30.98 $\mu g\ g^{-1}$. In the southern region of the estuary,the a.m.c. decreased gradually from riverine to estuarine Stations. Seasonal variations in the metal concentrations within the Stations were prominent except at the northern riverine Station, probably due to its unpolluted environment.

Bioavailability

The different correlation coefficient between the various biological factors and environmental variables are given in Table 13. The soft tissue concentration (Y1) was seen to be positively well correlated with the dissolved metal concentration (X1), the particulate metal concentration (X2) and their ratio, MPR (X3) ($p<0.001$, in all the cases). The BCF (Y2) and log BCF were negatively correlated with X1 ($p<0.001$) while no significant correlations were obtained with any other environmental variables..The BCR (Y3) and log BCR were correlated, negatively with X1 and positively with X3 ($p<0.001$), BAF (Y4) reflected good relationship ($p<0.001$) with particulate metal concentration (X2).

Table 13. Correlation coefficients between Environmental variables and Biological factors (Copper)

	S.tissue Y1	BCF Y2	log BCF log Y2	BCR Y3	log BCR log Y3	BAF Y4
Dissol. X1	0.539**	-0.42**	-0.398**	-0.462**	-0.461**	0.347*
Part. X2	0.724**	-0.129	-0.123	0.329*	0.342*	0.543**
MPR X3	0.381**	0.192	0.172	0.732**	0.709**	0.342*
GAF X4	-0.383**	0.351*	0.343*	0.405**	0.412**	-0.576**
Exch. X5	0.326*	0.006	0.020	0.185	0.171	0.233
Exch % X6	0.224	0.115	0.101	0.245	0.207	0.636**

n = 66 * $p < 0.01$ ** $p < 0.001$

The strongest correlation observed between the biological factors (the dependent variables) and environmental variables pertaining to the aqueous phase, was that relating Y3 to X3, represented by the equations,

$$Y3 = 4083\ X3 + 2098.67 \quad (r = 0.73,\ p<0.001)$$

$$\log Y3 = 0.274\ X3 + 3.54 \quad (r = 0.71,\ p<0.001)$$

and Figs. 15 a and b.

The bioavailability of metals from the sediment was characterised on the basis of correlation between the biological factors (Y1 to Y4) and environmental factors pertaining to the sediment (X4, X5 and X6). BCR (Y3) was found to be well correlated ($p<0.001$) with GAF (X4); (Y4) showed strong negative correlation with GAF and good positive correlation with percentage of exchangeable fraction (X6) of the sediment ($p<0.001$ in all the cases). The other biological factors (Y1 and Y2) showed only less significant ($p<0.01$) relationship with the sediment parameters. The significant regression lines were defined by the following equations and depicted in Figs. 16 a and b.

$$Y3 = 1.15\ X4 + 3746.25 \quad (r = 0.41,\ p<0.001)$$

$$Y4 = 1.07\ X6 + 1.043 \quad (r = 0.64,\ p<0.001)$$

Copper is an essential trace element necessary for many metabolic processes. It is a transition metal with three oxidation states, Cu^{0},Cu^{I}, Cu^{II}. Despite being an essential

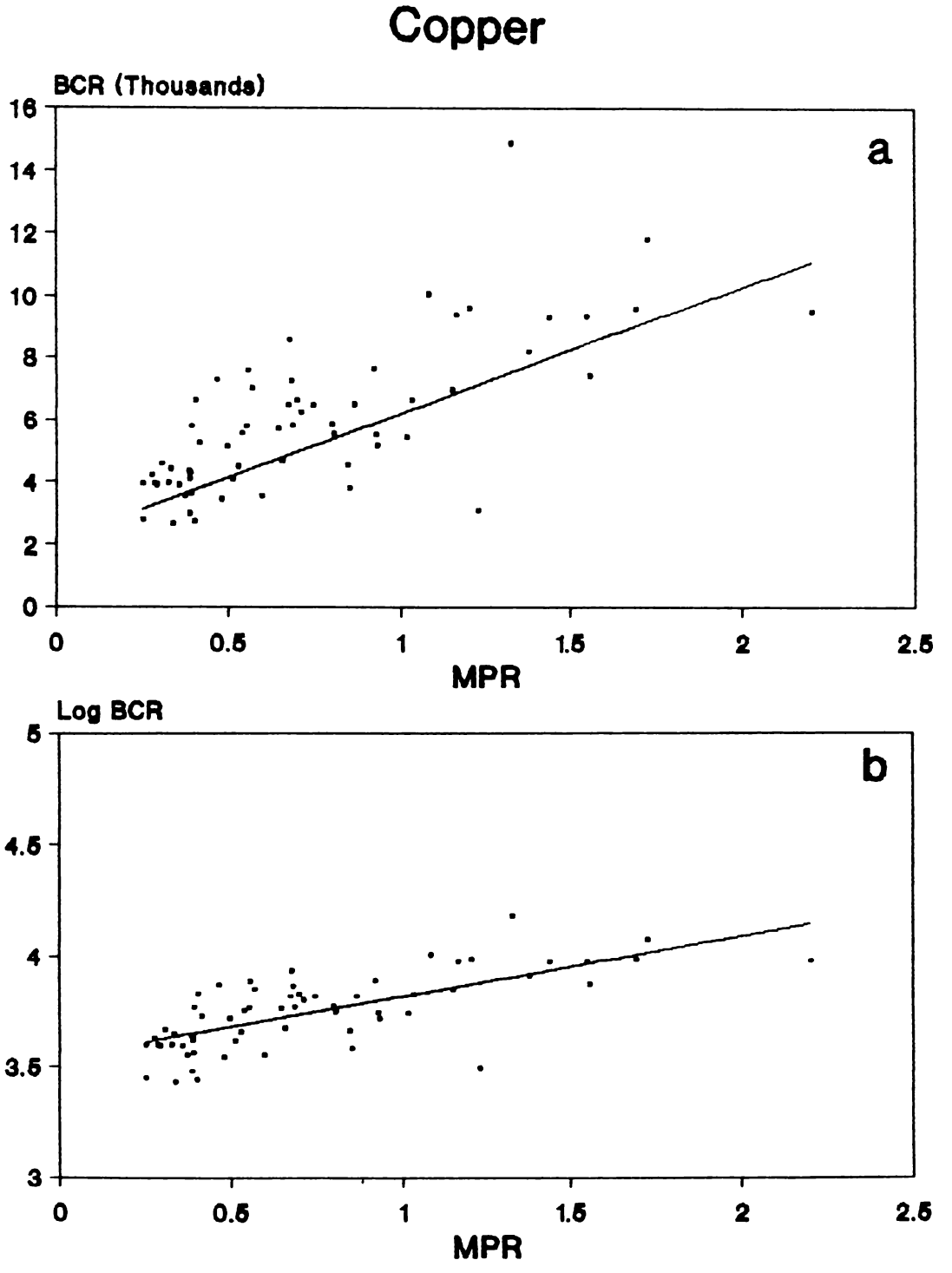


Fig.15 Regression analysis (copper)
a) MPR vs BCR b) MPR vs Log BCR

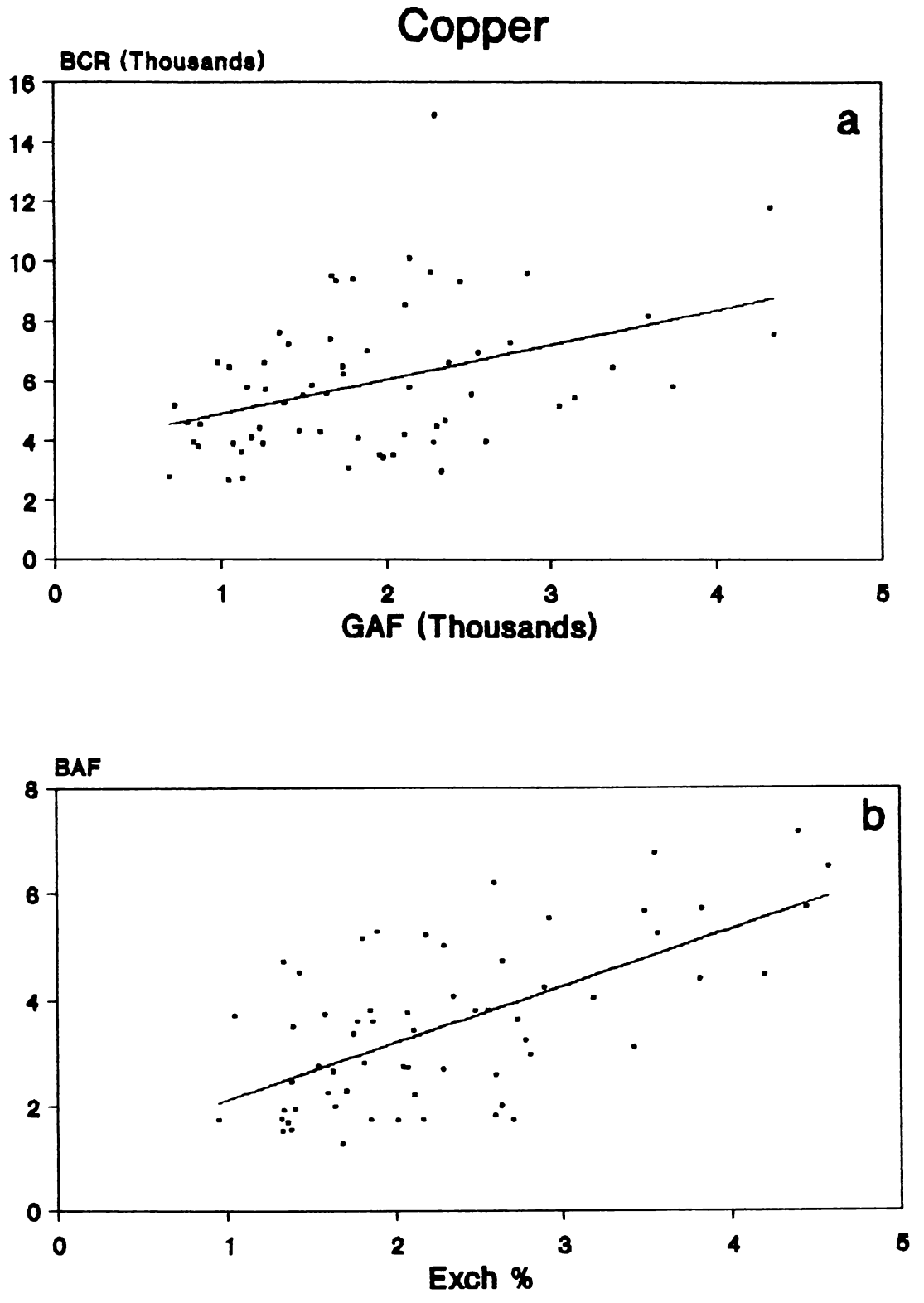


Fig.16 Regression analysis (copper)
a) GAF vs BCR b) Exch.% vs BAF

element copper could prove to be toxic at elevated concentrations. For a better understanding of the toxicity threshold of copper, a deeper insight into the mechanisms of copper uptake from the environment is essential (Flemming and Trevors, 1989).

In the present study environmental factors like copper concentrations in water and sediment phases have been determined and compared with the metal concentration in the clam *Villorita cyprinoides* var. *cochinensis*. The results of the study showed that copper concentration in the soft tissues of the clam correlated well with the dissolved, the particulate copper concentrations as well as with the MPR (in all cases $p<0.001$).The BCF, however showed only a negative relationship with the dissolved copper (and no relationship with the particulate copper) and hence it cannot be considered as a factor for predicting copper bioavailability from water. But BCR showed a good relationship with environmental factors like particulate concentrations and MPR ($p<0.01$ and $p<0.001$ respectively), whereas with dissolved metal concentrations, it showed only a negative relationship. Out of all the biological and environmental factors considered, BCR showed a maximum correlation with MPR ($r= 0.732$, $p<0.001$); soft tissue concentration exhibited a fairly good correlation with particulate metal ($r = 0.724$ $p<0.001$) and with dissolved metal ($r = 0.537$, $p<0.001$) levels in water. Among the five metals

studied, only in the case of copper there was a positive correlation between soft tissue concentration and dissolved copper concentration.

Although accumulation of copper had been studied by several authors (Phillips, 1976; Ahsanullah *et al.*, 1981; Amiard - Triquet *et al.*, 1986; Sivadasan, 1987; Lakshmanan and Nambisan, 1989; Marigomez and Ireland, 1989), no attempt has been made to comment on the bioavailability of copper to the organisms. Zamuda and Sunda (1982) and Wright and Zamuda (1987) carried out studies on the bioavailability of copper by simulating different cupric ion activities and changes in salinity and reported that the free cupric ion was more available to the organism and that the bioaccumulation was significantly influenced by changes in salinity.

Copper is more readily bioaccumulated when present as the free cupric ion. Although the dissolved phase of water may contain an appreciable concentration of the free ions, considerably larger proportion of the labile ions exists in the particulate phase being adsorbed onto them. On ingestion by the organism, these adsorbed metal ions are solubilized by the acidic nature of the gastric juice and thus rendered bioavailable. This explains the significant correlation observed between metal concentrations in the soft tissues and in the aqueous particulate form.

The increased correlation observed between BCR and MPR may be due to the effect of normalization with respect to an environmental factor (dissolved metal concentration), which governs the bioaccumulative processes. The soft tissue concentrations of metals have comparatively higher values in monsoon periods than during other seasons. The weak-acid exchangeable fraction whichis greater during the monsoon period outweighs any other environmental factor.

Sediment bioavailability was studied in which the sediment was chemically partitioned by a sequential extraction scheme. Correlations were better for the relatively easily extractable metals than for the more tightly bound fractions or for the total trace metal concentration indicating that the availability of particular metalis inversely related to itsbinding strength to the various substrates in the sediment. Correlations, if any, were struck between the various sediment fractions and the biological factors. Soft tissue concentrations were seen to be correlated ($p<0.001$) to the exchangeable metal concentrations (X5), BCR to GAF (X4) ($p<0.001$) and BAF (Y4) to the percentage exchangeable fraction X6) ($p<0.001$).

The strong relationship between metal concentrations in the soft tissues and that in the exchangeable fraction in the sediment was a reflection of the weakly bound labile ions attached to the substrate, being released by the nature of the

extractant and conditions of the extraction process. Gunn *et al.*, (1989) have reported a good correlation between copper concentration in tubificid worms and that in the exchangeable fraction in the sediment. Tessier *et al.*, (1984) partitioned the surficial sediments sampled from the habitat of the organism, *Elliptio complanata,* by a sequential extraction scheme similar to the one employed in this study and compared the soft tissue concentrations of copper, lead and zinc to their respective concentrations in the sediment fractions. Their results were quantified in terms of the ratio of the total metal concentrations present in the exchangeable, the carbonate bound and the Fe/Mn oxide bound fractions considered together to the concentration of iron in Fe/Mn oxide bound fraction. The copper concentration in the soft tissue of the organism was found to be well correlated with this ratio.

Salinity and run off have been suggested to be relevant in regulating metal bioavailability (Cossa and Rondeau, 1985; Nugegonda and Rainbow, 1989). However, Cain and Luoma (1990) reported that neither of these factors could be clearly related to fluctuations in copper and silver content of *M. balthica* in San Francisco Bay. Thus, metal bioavailability is a complex phenomenon influenced by a wide range of geochemical, hydrological and biological features..

From the present investigations it is seen that the best correlation available for metal bioavailability from aqueous

phase was that between BCR and MPR. In the sediment phase, BAF and percentage of metal concentration in the exchangeable sediment fraction revealed a close inter-relationship.

Cadmium

Concentrations of cadmium in the different compartments of the aqueous environment in the study area are given below. Table 14 gives the annual mean concentration, standard deviation and the range of values recorded at each station. Figs. 17 - 19 depict the monthly variations.

Distribution in Water

Dissolved

The highest concentration of cadmium observed was 2.15 $\mu g\ l^{-1}$ at Station 5 and the lowest was 0.28 $\mu g\ l^{-1}$ at Stations 3 and 4. The maximum a.m.c. was observed at Station 5 (1.04 $\mu g\ l^{-1}$) and the minimum at Station 3 (0.45 $\mu g\ l^{-1}$), both stations representing estuarine characters. Stations 4 and 6, the riverine stations in the southern and northern regions respectively, had a.m.c. 0.46 $\mu g\ l^{-1}$ and 0.53 $\mu g\ l^{-1}$.

Table 14. Distribution of cadmium in the Cochin estuary

COMPARTMENTS	STATIONS					
	1	2	3	4	5	6
S.tissue ug g^{-1}	9.10 ± 3.90 (3.54 - 16.73)	5.67 ± 1.84 (3.31 - 9.75)	7.27 ± 3.47 (3.16 - 15.15)	8.60 ± 3.48 (4.08 - 16.06)	5.29 ± 3.00 (3.16 - 11.87)	3.09 ± 0.65 (2.14 - 4.16)
Shell ug g^{-1}	4.03 ± 0.34 (3.34 - 4.68)	4.24 ± 0.52 (3.38 - 5.72)	4.33 ± 0.73 (3.43 - 6.57)	4.41 ± 0.75 (3.49 - 6.52)	3.91 ± 0.30 (3.53 - 4.34)	4.12 ± 0.45 (3.03 - 5.96)
Sediment ug g^{-1}	0.73 ± 0.16 (0.54 - 1.11)	0.76 ± 0.31 (0.50 - 1.53)	0.66 ± 0.10 (0.5 - 0.89)	0.58 ± 0.06 (0.48 - 0.71)	0.69 ± 0.09 (0.6 - 0.87)	0.69 ± 0.05 (0.61 - 0.78)
Dissolved · ug l^{-1}	0.60 ± 0.18 (0.35 - 1.68)	0.71 ± 0.20 (0.4 - 1.02)	0.45 ± 0.15 (0.28 - 0.86)	0.46 ± 0.12 (0.28 - 0.72)	1.04 ± 0.60 (0.5 - 2.15)	0.53 ± 0.12 (0.35 - 0.74)
Particulate ug l^{-1}	0.57 ± 0.40 (0.21 - 1.74)	0.59 ± 0.19 (0.29 - 1.03)	0.69 ± 0.38 (0.24 - 1.72)	0.41 ± 0.15 (0.23 - 0.86)	0.40 ± 0.18 (0.21 - 0.68)	0.43 ± 0.20 (0.21 - 0.83)

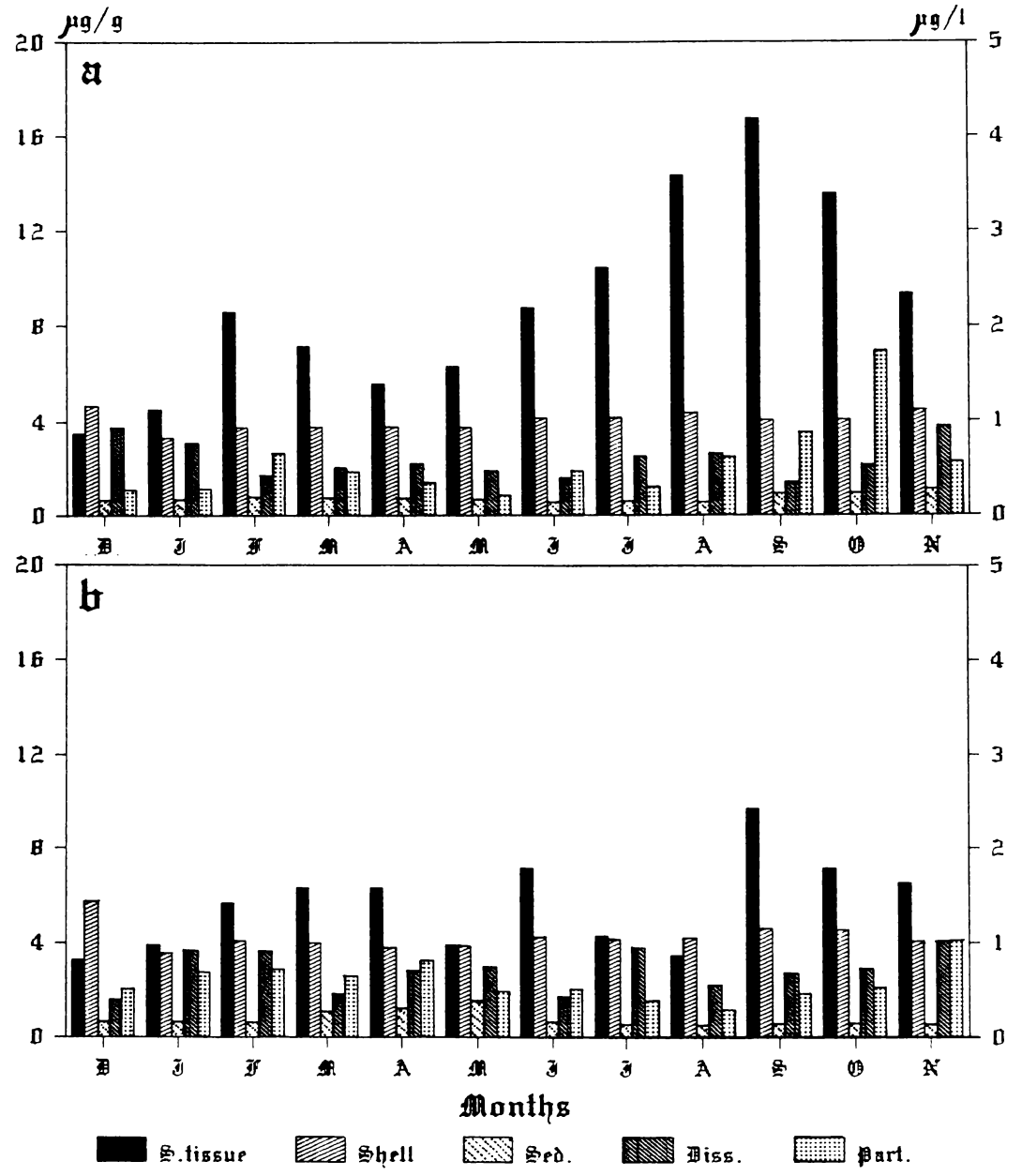


Fig.17 Trace metal partitioning in Cochin estuary (cadmium)
a) Station 1 b) Station 2

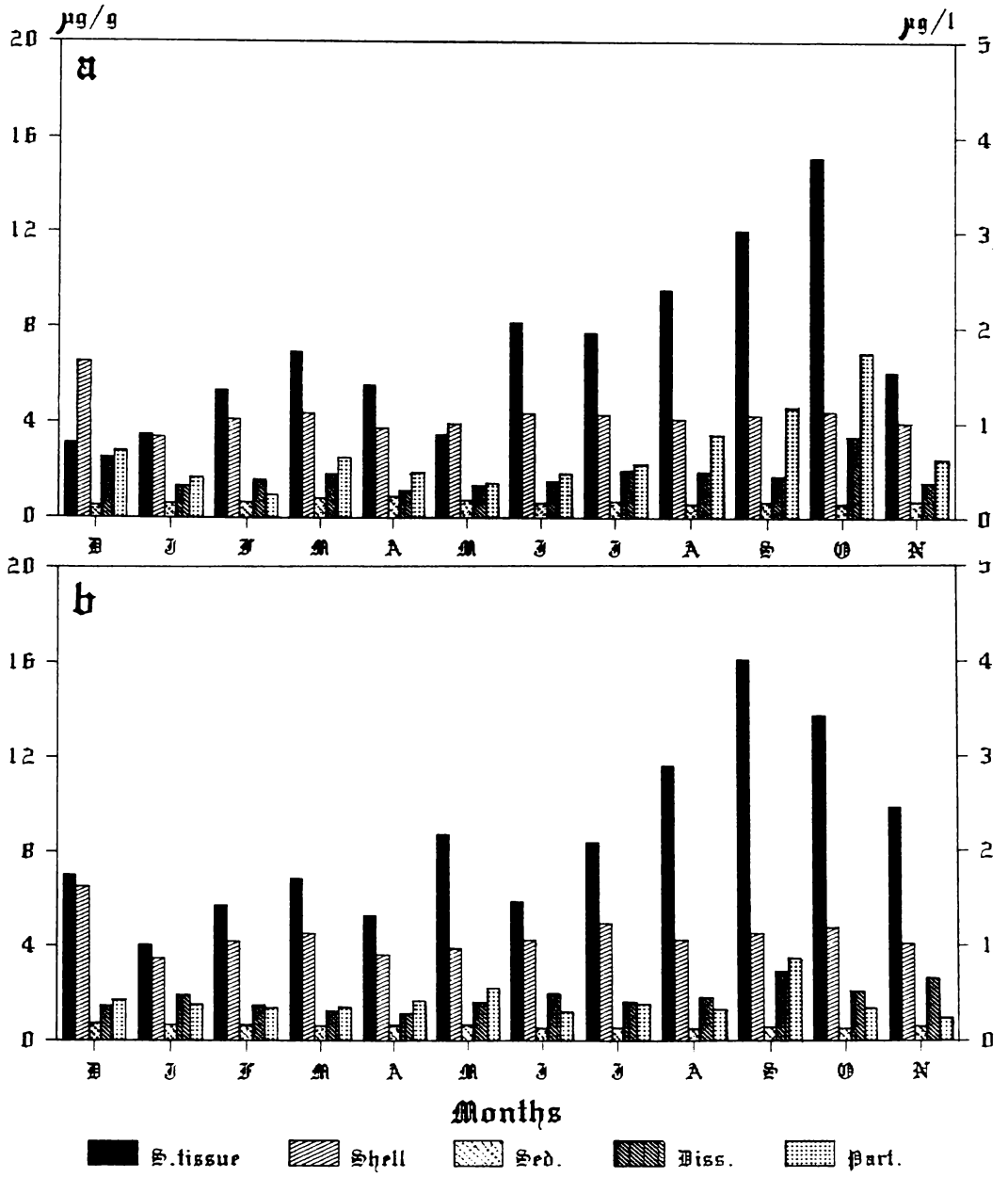


Fig.18 Trace metal partitioning in Cochin estuary (cadmium)
a) Station 3 b) Station 4

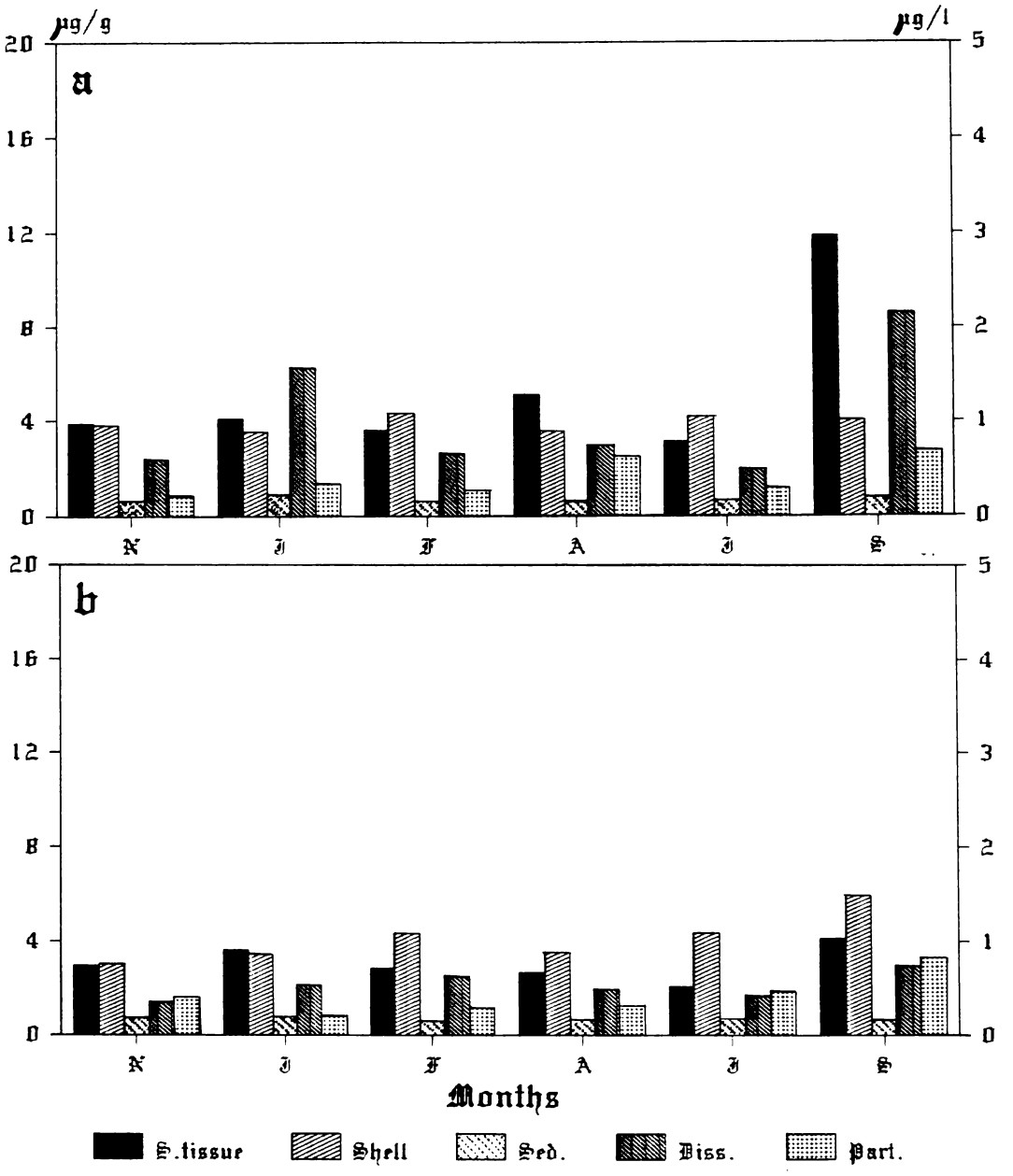


Fig.19 Trace metal partitioning in Cochin estuary (cadmium)
a) Station 5 b) Station 6

Particulate

Like dissolved cadmium, concentrations of particulate cadmium was also low in concentration. The highest concentration of 1.74 $\mu g\ l^{-1}$ was recorded at Station 1 and the lowest concentration of 0.21 was observed at three stations, *viz*. Stations 1, 5 and 6 .The a.m.c. ranged between a minimum of 0.40 $\mu g\ l^{-1}$ at Station 5 and maximum of 0.69 $\mu g\ l^{-1}$ at Station 3, being just reverse of the trend in dissolved cadmium values. Not much variation was seen either among the stations or within the stations.

Distribution in Sediment

The concentration of cadmium in the sediment did not vary appreciably. The highest concentrations (1.53 $\mu g\ g^{-1}$ and the lowest concentration of 0.48 $\mu g\ g^{-1}$ were recorded at station 2 and 4 respectively. The a.m.c. values ranged between 0.58 $\mu g/g$ and 0.73 $\mu g/g$ (station 1 and station 4 respectively). Out of all the stations, maximum variation was also observed at station 2.

Distribution in Bivalves

Cadmium is sequestered in the shells and soft tissues of *V. cypriniodes* and *M.casta*. Different mechanisms and environmental factors involved in sequestration are responsible for the differences in cadmium content of these two compartments.

Shells

Concentrations of cadmium in the shells of *V.cyprinoides* remained more or less the same at all Stations. The highest concetration (6.57 $\mu g\ g^{-1}$) was observed at Station 3 and the lowest (3.03 $\mu g\ g^{-1}$) at Station 6. These two Stations are far apart on the southern and northern regions of the estuary. The a.m.c. in *V. cyprinoides* shells varied between 4.03 $\mu g\ g^{-1}$ and 4.41 $\mu g\ g^{-1}$ observed at Stations 1 and 4 respectively, whereas that in *M. casta* the a.m.c. was 3.91 $\mu g\ g^{-1}$. Station 6, which is riverine in the northern region, had a.m.c. 4.12 $\mu g\ g^{-1}$, with the values ranging between 3.03 $\mu g\ g^{-1}$ and 5.96 $\mu g\ g^{-1}$. Except on one occasion, concentrations of cadmium in the shells were generally greater than that in the soft tissues at this Station. In the southern region, there is a gradual decrease in a.m.c. corresponding to a change from riverine to estuarine character

Soft tissues

Cadmium concentrations in the soft tissues showed more fluctuations with respect to environmental parameters. The highest concentration of cadmium for *V. cyprinoides* was seen at Station 1 (16.73 $\mu g\ g^{-1}$) and the lowest (2.14 $\mu g\ g^{-1}$) at station 6. The maximum and minimum a.m.c. were 9.10 $\mu g\ g^{-1}$ (at Station 1) and 3.09 $\mu g\ g^{-1}$ (at Station 6). *M. casta* recorded an a.m.c. of 5.29 $\mu g\ g^{-1}$ with values ranging between 3.16 μg

g^{-1} and 11.87 $\mu g\ g^{-1}$. The seasonal trends exhibited by both the bivalves, *V. cyprinoides* and *M. casta* were similar in nature. There were considerable differences in the a.m.c. values obtained from the riverine Stations of the southern and northern regions. The former had concentration of 8.60 $\mu g\ g^{-1}$ while, the latter had a value of 3.09 $\mu g\ g^{-1}$.

Bioavailability

The important correlation coefficients obtained between the different biological factors and environmental variables are given in Table 15. The environmental factors such as dissolved (X1) and particulate (X2) metal concentrations and the MPR (X3) were seen to be correlated with the biological factors. The soft tissue concentration (Y1) of cadmium was significantly correlated with the particulate metal concentration ($r = 0.49$ ($p<0.001$) and to the MPR ($r = 0.45$, $p<0.001$). The BCF and log BCF were strongly and negatively correlated ($p<0.001$) with the dissolved metal concentration. The strongest relationship was observed between the BCR and the MPR ($r = 0.65$, $p<0.001$) and consequently BCR and dissolved metal concentration exhibited a strong negative correlation. No significant relationship was observed between any of the other biological factors and environmental variables. The equations of the significant regression lines are given below and are also represented in Figs. 20 a and b, and 21 a.

Table 15. Correlation coefficients between Environmental variables and Biological factors (Cadmium)

	S.tissue Y1	BCF Y2	log BCF log Y2	BCR Y3	log BCR log Y3	BAF Y4
Dissol. X1	0.058	-0.412**	-0.489**	-0.464**	-0.576**	0.018
Part. X2	0.492**	-0.128	-0.121	0.242	0.183	0.351*
MPR X3	0.454**	0.192	0.225	0.646**	0.608**	0.278
GAF X4	-0.049	0.240	0.325*	0.459**	0.489**	-0.232
Exch. X5	-0.230	-0.141	-0.098	-0.103	-0.086	-0.330*
Exch % X6	-0.194	0.006	0.025	-0.006		-0.072

n = 66 * $p < 0.01$ ** $p < 0.001$

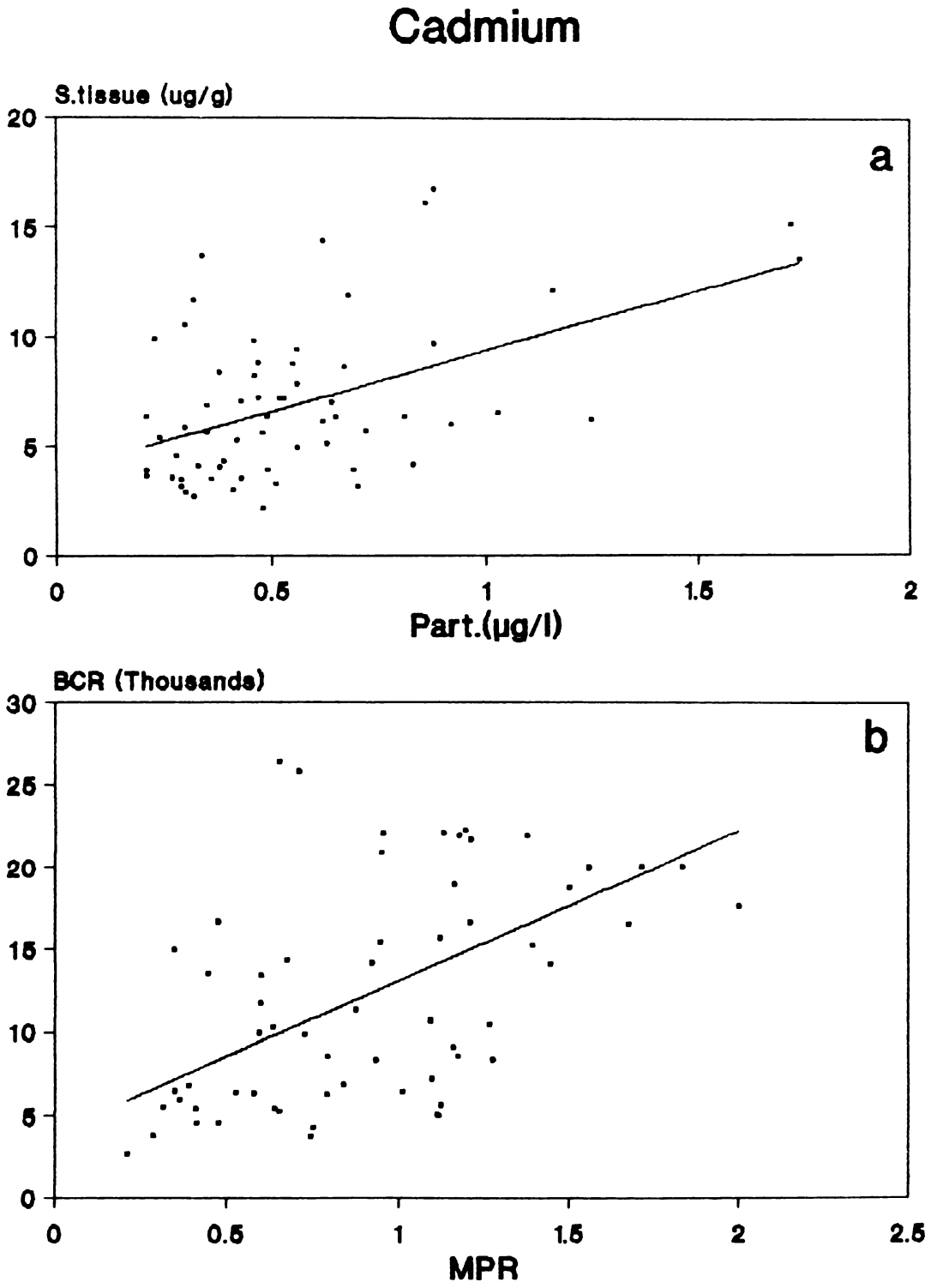


Fig.20 Regression analysis (cadmium)
a) Part. vs S.tissue b) MPR vs BCR

$$Y1 = 5.5\ X2 + 3.85 \quad (r = 0.49,\ p<0.001)$$

$$Y3 = 9135.54\ X3 + 3941.49 \quad (r = 0.65\ \ p<0.001)$$

$$\text{Log}\ Y3 = 0.288\ X3 + 3.747 \quad (r = 0.60,\ p<0.001)$$

The BCR was seen to maintain a significant relationship with GAF values as represented by the equation and Fig. 21b.

$$Y3 = 6.63\ X4 + 4431.56 \quad (r = 0.46,\ p<0.001)$$

However, BAF showed only negative relationships with GAF ($p<0.05$) and with the exchangeable metal fraction, X5, ($p<0.001$). Weak correlations were observed between BAF and percentage distribution of carbonate bound fraction as well as with organically bound sediment fraction. Soft tissue metal levels were also seen to be negatively correlated with the exchangeable metal; however BCF exhibited only a poor relationship with GAF ($P<0.05$).

Cadmium is a highly toxic non-essential metal. Its chemical similarity to zinc, enables it to mimic the essential element zinc in its metabolic functions. Although its concentrations in the aqueous environment, both in water and in sediment are low, several fold enrichment is observed in the bivalves. Eventhough several studies have been carried out on the accumulation of cadmium by the organisms (Denton and Burden Jones, 1981; Ahsanullah *et al.*, 1981; Amiard-Triquet *et al* ., 1986; Amiard *et al.*, 1987; Chan, 1988; Giles, 1988; Marigomez and Ireland, 1989), only scanty attempts were made to

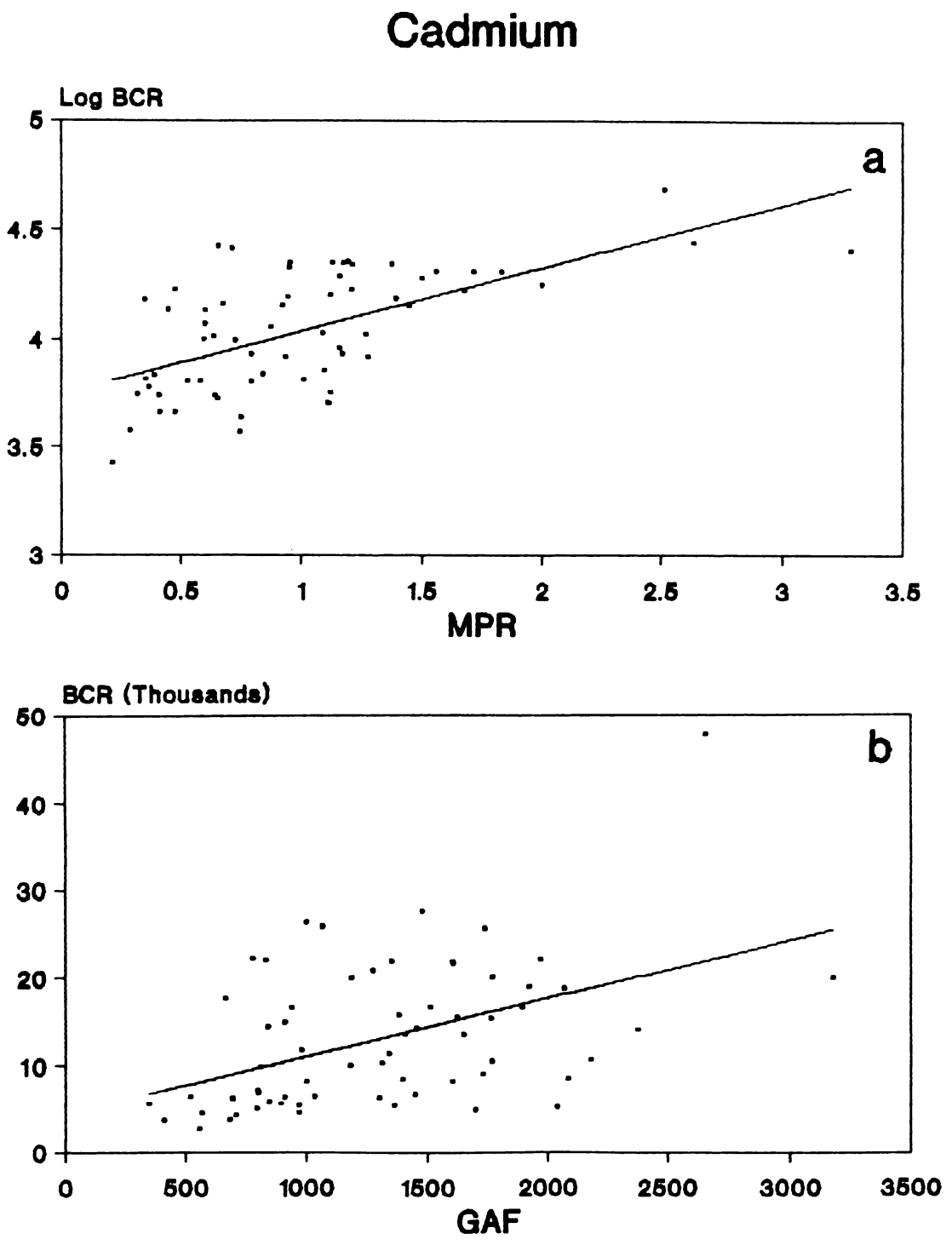


Fig.21 Regression analysis (cadmium)
a) MPR vs Log BCR b) GAF vs BCR

assess the metal bioavailability. The present attempt to quantify bioaccumulation indicated that BCR and MPR values exposed a strong correlation. This result is indicative of the increased labile ion concentration in the particulate phase (as compared to the dissolved phase) which predominantly influences the metal bioavailability. Lacerda *et al.*(1987) also reported the availability of particulate bound metals to the organisms.The absence of any significant relationship with metal concentration in the soft tissues and that in the aqueous phase, in studies carried out by Gunn *et al.*, (1989), lends credence to the above observation.

During the monsoon period cadmium concentrations are seen to be comparatively higher within the soft tissues. Cadmium is known to form fairly stable chloro-complexes. When the salinity is appreciable, cadmium ions will form chloro-complexes (Stumm and Brauner,1975) and hence the free cadmium ion (the aqueous ion) concentrations are very much reduced and result in low bioavailable levels of cadmium. Ionic species have become recognized as the prefered form of metal uptake by biological organisms (Zamuda ad Sunda 1982). Conversely, during the monsoon period, much higher levels of `free' cadmium ions are available for uptake by the organism. In the present study, during the monsoon period (June to October,1989) salinity was found to be less than 1%.. Ingestion of complexes of cadmium with low molecular weight molecules present in the

aqueous phase is yet another route of metal uptake by organism (Luoma, 1983).

Several authors have attempted to study the availability ofcadmium from the sediment (Luoma and Jenne, 1976; Bryan and Hummerstone,1978; Ward and Young, 1984; Gunn *et al.*, 1989). While Luoma and Jenne (1976) extracted the available metal fractions from different sediment samples using different chemical extractants, sequential extractions of the same sediment sample with different chemical extractants were carried out by Gunn *et al.*, (1989) who found that the exchangeable fraction of the sediment correlated well with the metal content of tubificid worms.

In the present study it was seen that none of the sequentially extracted sediment fractions correlated significantly with any of the biological factors. However, a positive relationship ($p<0.05$) was found between BAF and percentage distribution of concentration of organically bound fraction of cadmium. Luoma and Jenne (1976) obtained a high correlation ($p<0.05$) between cadmium concentration in the soft tissues of *M. balthica* and that in ethyl alcohol extract of the sediment. The ethyl alcohol has obviously extracted organically bound cadmium and this observation therefore support the present investigation.

Studies on cadmium speciation in the aqueous/sediment

phases held in conjunction with bioavailability are onlv scantly reported in literature. The low distribution levels o cadmium in the environment could be one of the reasons for this lack of emphasis. Results of the present study clearly point to MPR as the environmental factor and BCR as the biological factor that could be a predictive tool in the assessment o aqueous bioavailability. GAF and BCR respectively could be used for quantifying sediment bioavailability. Luoma (1989) has corroborated the use of such enrichment factors in bioavailability prediction programmes, as they can reflect the enhanced or reduced vulnerability of biota to metal contamination by exposing the differences in the bioavailability.

Zinc

Intercompartmental partitionig of zinc in the Cochin estuary are given below. Table 16 illustrates the annual mean concetration, standard deviation and the range of values in each compartment. Figures 22 - 24 depict the monthly variations for one year. Correlation coefficients between environmental variables and biological factors are given in Table 17.

Table 16. Distribution of Zinc in the Cochin estuary

COMPARTMENTS	STATIONS					
	1	2	3	4	5	6
Soft tissue $\mu g\ g^{-1}$	174.92 ± 84.57 (56.75 - 324.5)	142.69 ±45.44 (71.63 - 231.4)	152.80 ± 47.37 (74.83 - 243.2)	181.06 ± 50.92 (86.56 - 284.1)	101.69 ± 26.17 (59.48 - 140.51)	158.51 ±60.56 (97.51-243.92)
Shell $\mu g\ g^{-1}$	3.48 ± 0.64 (2.67 - 4.53)	3.77 ± 0.75 (2.75 - 5.00)	3.16 ± 0.44 (2.54 - 3.96)	3.35 ± 0.62 (2.68 - 4.64)	2.87 ± 0.18 (2.66 - 3.18)	3.49 ± 0.68 (2.58 - 4.68)
Sediment $\mu g\ g^{-1}$	25.97 ± 5.72 (14.21 - 33.42)	21.34 ± 4.91 (18.18 - 31.76)	26.25 ± 2.02 (23.29 - 29.52)	28.42 ± 10.05 (16.69 - 46.68)	21.15 ± 2.45 (17.73 - 25.65)	19.08 ± 2.92 (14.94 - 24.16)
Dissolved $\mu g\ l^{-1}$	80.17 ± 62.06 (10.4 - 214.00)	28.49 ± 7.68 (18.15 - 43.43)	13.84 ± 4.65 (7.89 - 21.93)	16.23 ± 5.91 (9.05 - 25.13)	45.67 ± 14.27 (26.4 - 67.74)	20.02 ± 4.09 (13.38 - 26.48)
Particulate $\mu g\ l^{-1}$	37.51 ± 21.83 (14.33 - 79.32)	18.88 ±10.78 (6.44 - 44.56)	12.29 ± 6.95 (4.71 - 29.19)	13.86 ± 2.73 (9.34 - 18.38)	26.81 ± 7.76 (12.13 - 36.35)	13.42 ± 2.38 (9.96 - 17.20)

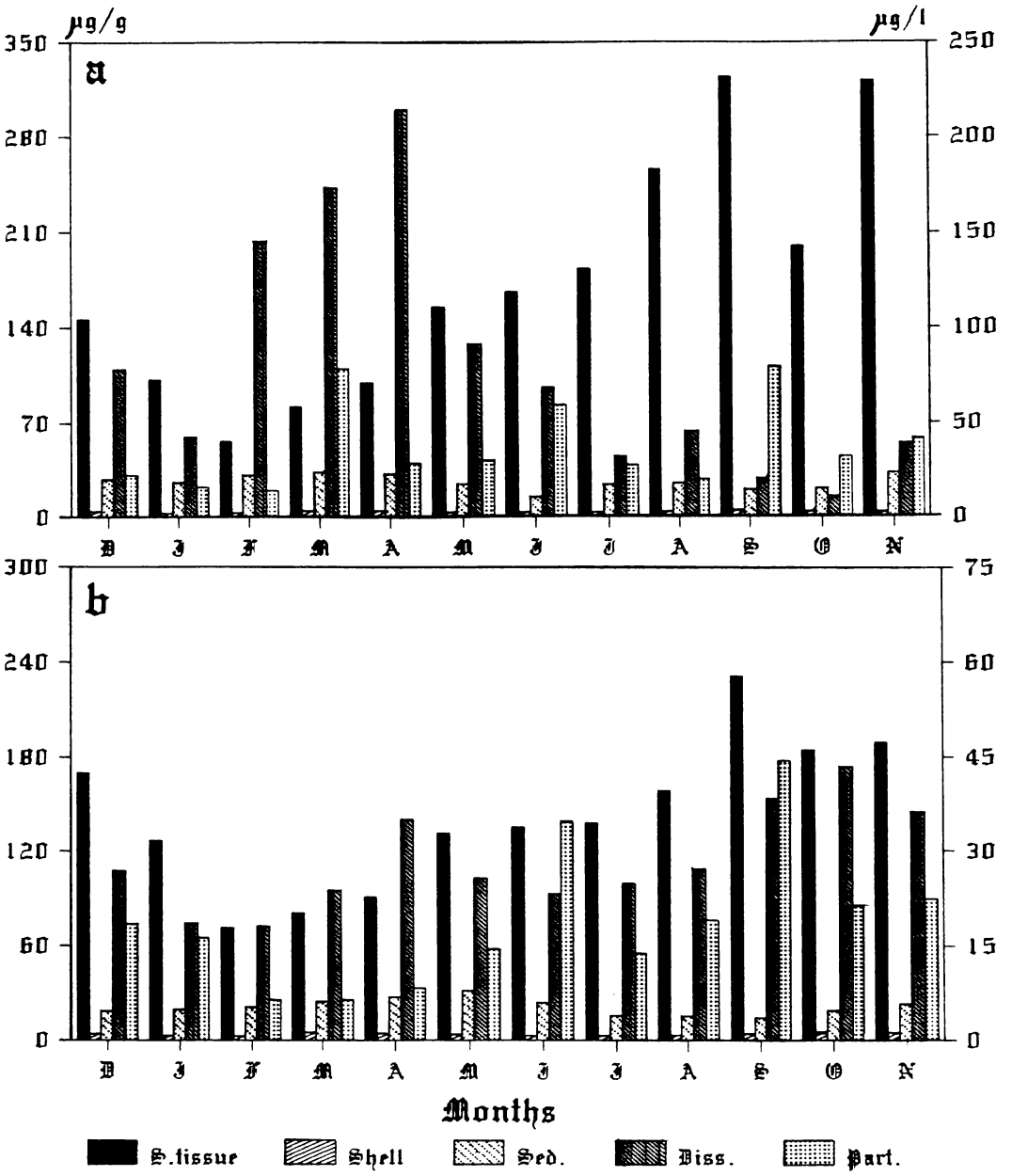


Fig.22 Trace metal partitioning in Cochin estuary (zinc)
a) Station 1 b) Station 2

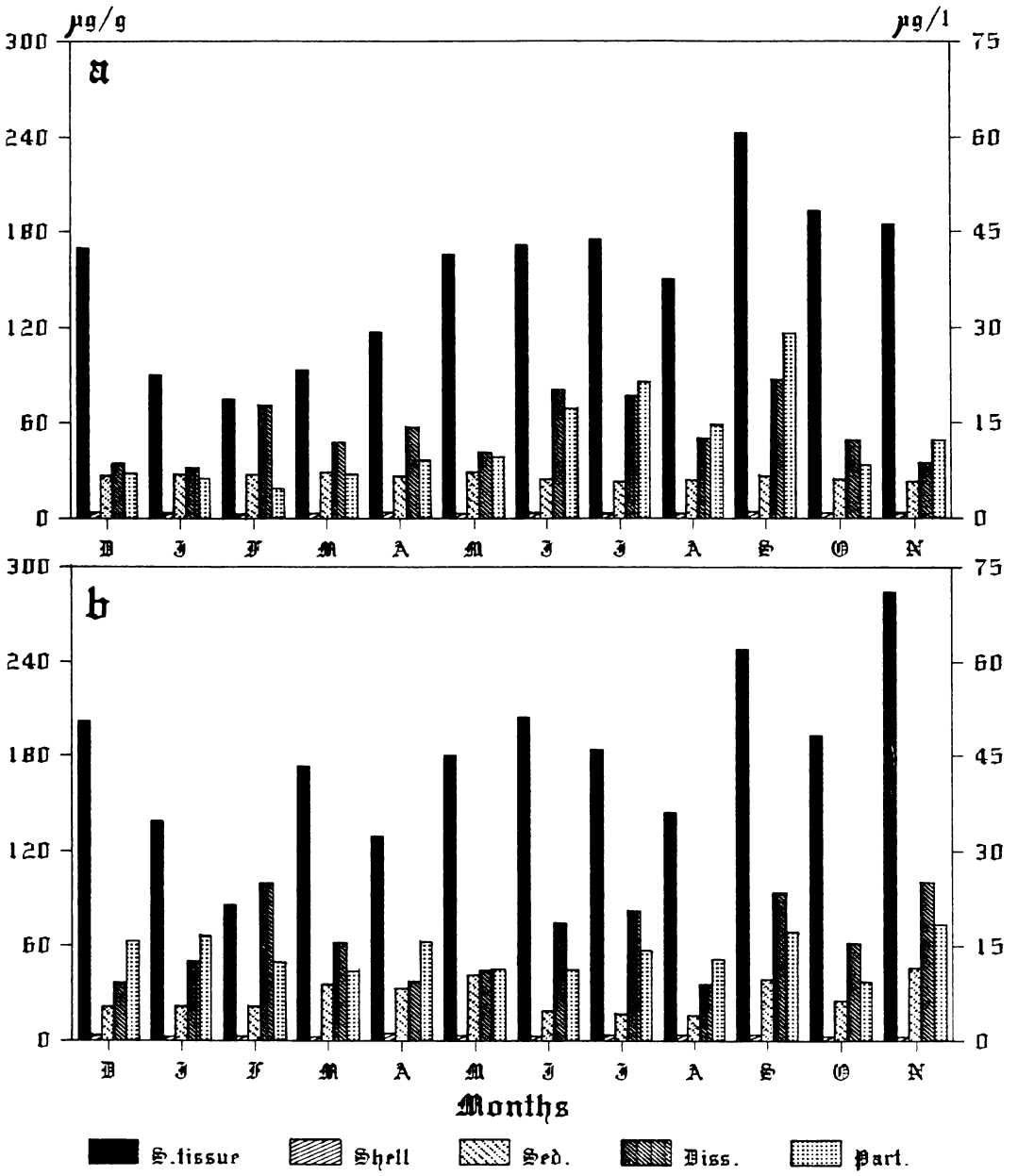


Fig.23 Trace metal partitioning in Cochin estuary (zinc)
a) Station 3 b) Station 4

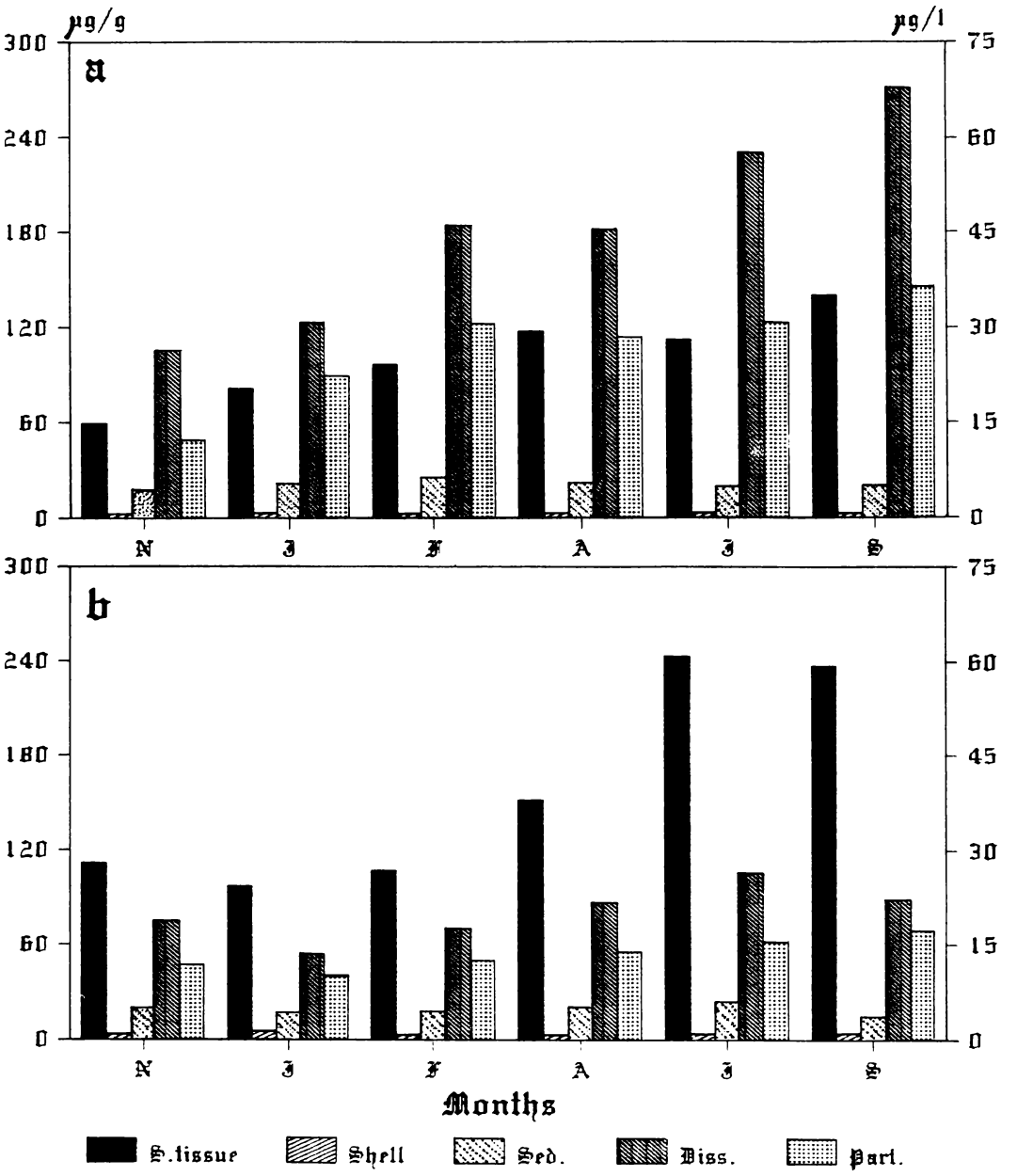


Fig.24 Trace metal partitioning in Cochin estuary (zinc)
a) Station 5 b) Station 6

Distribution in Water

Dissolved

The concentration of dissolved zinc varied widely in the different Stations in the estuary. The highest value of 214 $\mu g\ l^{-1}$ was reported in Station 1 and lowest value of 7.89 $\mu g\ l^{-1}$ at Station 3. The maximum and minimum a.m.c. were seen at Station 1 (80.17 $\mu g\ l^{-1}$) and Station 3 (13.84 $\mu g\ l^{-1}$) respectively. Station 5, the estuarine one in the northern region had a comparatively higher concentration, with an a.m.c. of 45.67 $\mu g\ l^{-1}$, and with the values ranging from 26.4 $\mu g\ l^{-1}$ to 67.74 $\mu g\ l^{-1}$. The riverine Station of northern side showed an a.m.c. of 20.02 $\mu g\ l^{-1}$ with concentrations varying between 13.38 $\mu g\ l^{-1}$ and 26.48 $\mu g\ l^{-1}$. Station 4, the riverine one in the southern region had only an a.m.c. of 16.23 $\mu g\ l^{-1}$ and the values ranged between 9.05 $\mu g\ l^{-1}$ and 25.13 $\mu g\ l^{-1}$.

Particulate

The values of particulate zinc from all over the estuary showed a trend similar to that of dissolved fraction. The highest and lowest values of 79.32 $\mu g\ l^{-1}$ and 4.71 $\mu g\ l^{-1}$ were seen at Stations 1 and 3 respectively. Station 1 showed maximum a.m.c. of (37.51 $\mu g\ l^{-1}$) varying between 14.3 $\mu g\ l^{-1}$ and 79.32 $\mu g\ l^{-1}$.Minimum a.m.c. (12.29 $\mu g\ l^{-1}$) was obtained at Station 3. Station 5, which is an estuarine one in the

northern region showed fairly high values of a.m.c. (26.81 $\mu g\ l^{-1}$) varying from 12.13 $\mu g\ l^{-1}$ to 36.35 $\mu g\ l^{-1}$ and the riverine Station of the same region had only 13.42 $\mu g\ l^{-1}$ as a.m.c.. Fairly high variations were obtained in the values and they do influence the distribution of the metal in the biological system.

Distribution in Sediment

The concentrations of zinc in the sediments were fairly high compared to copper and cadmium concentrations. The highest value of 46.68 $\mu g\ g^{-1}$ was observed in Station 4 and the lowest value of 14.21 $\mu g\ g^{-1}$ was present at Station 1. Seasonal variations did exist within the Stations. Station 4 and 6, the two riverine Stations in the southern and northern arms of the estuary respectively recorded the maximum value (28.42 $\mu g\ g^{-1}$) and the minimum (19.08 $\mu g\ g^{-1}$) a.m.c.s respectively. All other estuarine Stations also showed fairly high concentrations having a.m.c. between 21.15 $\mu g\ g^{-1}$ to 25.97 $\mu g\ g^{-1}$. Stations 3, 5 and 6 showed values without much significant variation.

Distribution in Bivalves

The concentrations of zinc in the bivalves were fairly high in the soft tissues. The shells had the least values among all the metals studied. Unlike copper and cadmium, zinc is a very

essential trace element and hence its distribution studies are also important.

Shells

Compared to the concentrations of zinc in the soft tissues, concentrations in the shells were insignificant. But on account of the contribution of shells in the total body weight of the organism, the metals in the shells also need to be considered. The highest concentration (5.0 $\mu g\ g^{-1}$) among all the stations for the whole period of study was recorded in Station 2 for *V. cyprinoides* . The a.m.c. of shells of *V. cyprinoides* varied from 3.16 $\mu g\ g^{-1}$ at Station 3 to 3.77 $\mu g\ g^{-1}$ at Station 2. *M. casta* collected from Station 5 also presented a comparable value of 2.87 $\mu g\ g^{-1}$ as a.m.c.

Soft tissues

The highest and lowest recorded values for *V. cyprinoides* were 324.50 $\mu g\ g^{-1}$ and 56.75 $\mu g\ g^{-1}$ obtained at the same station, (Station 1). The a.m.c. varied from 142.69 $\mu g\ g^{-1}$ to 181.06 $\mu g\ g^{-1}$ as represented in Stations 2 and 4 respectively. In *M. casta* collected from Station 5, the a.m.c. was 101.69 $\mu g\ g^{-1}$, with the seasonal values varying between 59.48 $\mu g\ g^{-1}$ and 140.51 $\mu g\ g^{-1}$. The concentration of zinc in *V. cyprinoides* obtained from the Station 6 in the northern region was more than that in *M. casta* from Station 5.

Bioavailability

Soft tissue concentrations of *V. cyprinoides* correlated well ($p<0.01$) with particulate metal concentration and much better with MPR ($p<0.001$). But it was weakly and negatively correlated ($p<0.05$) with dissolved metal concentration in water. The BCF and log BCF are significantly and negatively correlated ($p<0.001$) with dissolved and particulate metal concentration. But MPR showed a positive but feeble relationship ($0.02>p<0.05$) with BCF and a significant one ($p<0.01$) with log BCF. The BCR is negatively ($p<0.001$) correlated with dissolved metal concentration and positively with the MPR ($P<0.001$). The BAF values exhibited a weak negative relationship with dissolved metal concentration but fairly significant positive correlation ($P<0.001$) with particulate metal concentration and the MPR. Although most of the relationships are well correlated, the strongest relationship is between the BCR and the MPR (Fig.25 a and b). The equations of significant regression lines are

$Y1 = 49.32\ X3 + 114.737$ $(r = 0.49;\ p < 0.001)$

$Y3 = 5543.24\ X3 + 3490.42$ $(r = 0.62;\ p < 0.001)$

The GAF generally showed better relationship with biological factors than other sediment fractions. Soft tissue concentrations showed only a weak relationship ($p<0.05$) with exchangeable metal concentration, whereas with the other sediment parameters it did not have any worthy relationship.

Table 17. Correlation coefficients between Environmental variables and Biological factors (Zinc)

	S.tissue Y1	BCF Y2	log BCF log Y2	BCR Y3	log BCR log Y3	BAF Y4
Dissol. X1	-0.223	-0.608**	-0.850**	-0.569**	-0.841**	-0.204
Part. X2	0.325*	-0.458**	-0.499**	-0.192	-0.310*	0.382**
MPR X3	0.490**	0.240	0.310*	0.673**	0.543**	0.484**
GAF X4	-0.007	0.450**	0.440**	0.444**	0.444**	-0.300
Exch. X5	0.239	0.015	0.002	0.053	0.038	0.006
Exch% X6	0.142	-0.140	-0.056	-0.53	0.005	0.470**

n = 66 * $p < 0.01$ ** $p < 0.001$

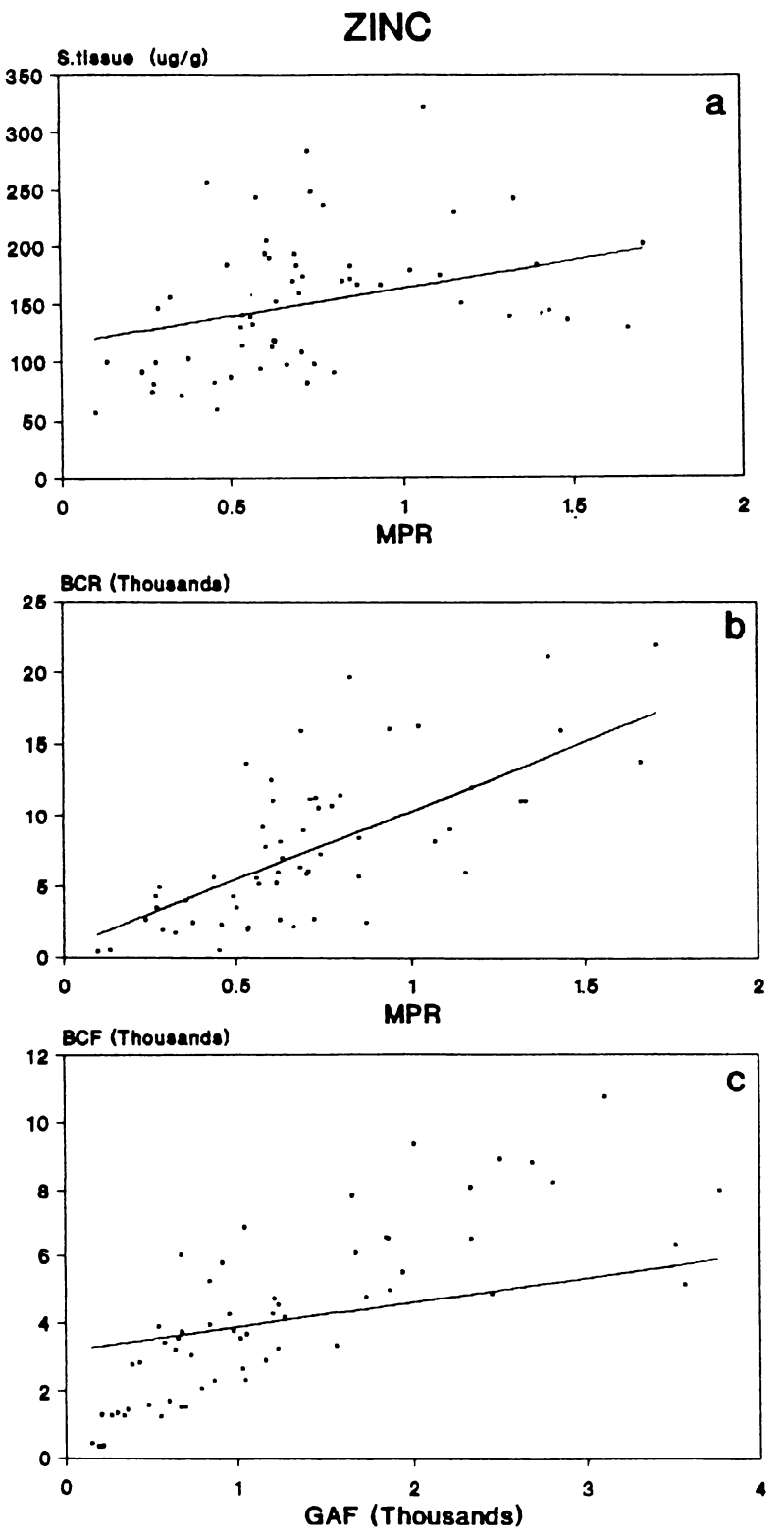


Fig.25 Regression analysis (zinc)
a) MPR vs S.tissue b) MPR vs BCR c) GAF vs BCF

The BCF and log BCF were strongly related with GAF values ($p<0.001$); The BCR and its log value showed good relationship with the GAF ($p<0.001$). The BAF showed a negative relationship ($p<0.001$) with GAF and a highly significant ($p<0.001$) relationship with the percentage distribution of exchangeable metal concentrations.The significant regression lines are given below along with the plot of lines (Figs 25 c; 26 a and b).

$Y2 = 0.734\ X4 + 3176.37$ ($r = 0.45$ $p<0.001$)

$Y3 = 1.621\ X4 + 5725.45$ ($r = 0.44$ $p<0.001$)

$Y4 = 7.135\ X6 + 2.44$ ($r = 0.47$ $P<0.001$).

Zinc is an essential trace element in living systems for normal cell differentiation and growth. It forms an integral part of a number of metallo-enzymes and a co-factor for regulating the activity of zinc specific enzymes (Leland and Kuwabara, 1985). It acts also as a structural component in many enzymes taking part in the energy metabolism. Deficiency of zinc can result in severe growth depression, skin lesions and sexual immaturity (Kiekens, 1990). But prolonged exposure to sublethal concentrations of zinc could cause extensive edema and necrosis of liver tissues (Leland and Kuwabara, 1985). Thus studies on the distribution and bioavailability of zinc is important as the environmental levels can play a great role in determining the concentrations in the living organisms. The various aspects of zinc bioaccumulation were investigated

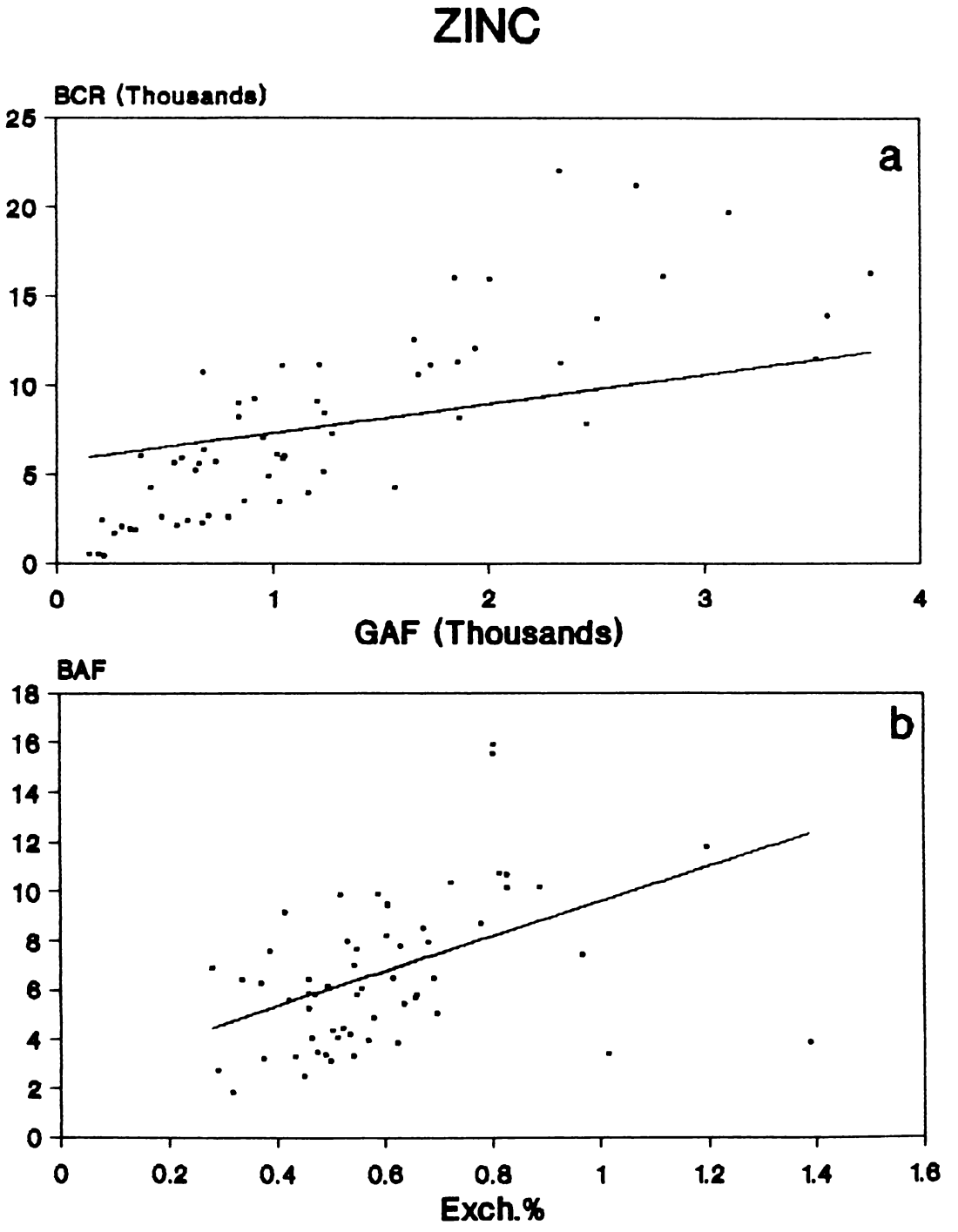


Fig.26 Regression analysis (zinc)
a) GAF vs BCR b) Exch.% vs BCF

by several authors (Styron *et al.*, 1976; Zingde *et al.* 1976; Ahsanullah *et al.*, 1981; Amiard-Triquet *et al.*, 1986; Amiard *et al.*, 1987; Memmert, 1987; Sivadasan, 1987; Chan, 1988; Neiman and Mitz, 1988; Kelly and Whitton, 1989; Lakshmanan and Nambisan, 1989). Though accumulation studies are well documented, bioavailability studies and speciation studies have not received any appreciable attention. Although the studies conducted in the laboratory under simulated conditions are useful, the environmental effects can truly be gauged, only if such studies are translated to the field conditions The observed highly significant correlation between MPR and soft tissue metal concentration as well as the absence of any positive correlation between dissolved zinc levels and any biological factors, suggests that the role of dissolved zinc is in regulating the uptake and not in actually getting accumulated.

The results of the sediment bioavailability studies indicate that zinc concentrations in the soft tissue bear, a weak relationship ($P<0.05$) with that in the exchangeable fraction. The best correlation obtained was between BAF and percentage distribution of zinc in the exchangeable fraction. Luoma and Bryan (1979) reported that the partitioning in sediments will vary with changes in the relative abundance of substrates which bind zinc. Since the bioavailability of zinc

to the organism varies with the nature of substrates, the change in the relative abundance of the substrate concentration should correlate with the changes in the biological availability of the metal to the organisms, whose burden is controlled by the sediments. The biological availability of the sediment bound zinc was, thus, defined as the concentration in the organism relative to that in the sediment (BAF). Laundrum (1989) also used the BAF concept to estimate bioavailability. In the present study also, the strongest correlation was obtained between BAF and the percentage distribution of exchangeable metal concentration. The protective influence of iron, can be a factor that reduce, the availability of zinc as well. This was evidenced from the studies of Tessier *et al.*, (1984) which showed a more significant correlation between zinc concentration in tissues of *Elliptio complanata* and the sum of exchangeable, carbonate bound and Fe/Mn-oxide bound fractions normalized to the iron in the Fe/Mn-oxide fraction. But a more conclusive and more predictive result was obtained by using the ratio of organically bound zinc to that with the iron in the Fe/Mn-oxide bound fraction. Out of the various chemically extractable fractions, exchangeable fraction (extracted with NH_4OAc) had correlation ($p<0.05$) with the soft tissue zinc concentration. A similar result was also reported by Luoma and Jenne (1976), using different extractants from different types of sediments. It was observed that NH_4OAc is the best

extractant for bioavailable zinc. In another report by Luoma and Bryan (1979), the BAF values of zinc with the ratio of iron extracted from the sediment by hydroxyl ammonium hydrochloride to manganese extracted with NH_4OAc was found to have good correlation. These factors corresponds to the control of substrate availability of zinc and protective action of iron and manganese on the uptake of zinc.

From the present study, it is found that BCR and MPR, (the biological factor and environmental variable) could be used as a predictive tool for assessing zinc bioavailability from the aqueous phase. For sediment bioavailability, BAF and percentage distribution of exchangeable fraction, correspondingly could be used as the predictive tools.

As the shells have been subjected to a rigorous cleaning process (*vide* Chapter - 2) which removes all the adsorbed metals, the metal concentrations observed in the shells have resulted through a process of biomineralization and/or biological transport. Zinc being an essential element will be transported from the soft tissue over to the shell only if a sufficiently excess gets accumulated in the soft tissues. Zinc concentrations in the shells were seen to decrease with increase in shell weight, probably a consequence of the decreased transport rate of zinc in older organisms.

Lead

The annual mean concentrations of lead observed during a period of one year in the selected six stations in Cochin estuary with standard deviations and the lowest and highest values at each station are given in Table 18. The monthly variations are given in Figs. 27 - 29.

Distribution in Water

Dissolved

The concentrations of dissolved lead in the environment varied widely depending on the various environmental conditions. Variations were visible both among the Stations and within the Stations. The highest concentration obtained was 16.8 $\mu g\ l^{-1}$ (Station 5) and lowest value was 1.2 $\mu g\ l^{-1}$ (Station 2).The maximum a.m.c. of 11.23 $\mu g\ l^{-1}$ was recorded in the estuarine Station of northern region and the minimum at Station 2 (2.10 $\mu g\ l^{-1}$) which was also estuarine in nature. The riverine Station in the northern region (Station 6) had an a.m.c. of 3.94 $\mu g l^{-1}$ while riverine Station in the southern side had 5.51$\mu g l^{-1}$. High variations were observed at Station 5 and the values ranged from 5.14 $\mu g\ l^{-1}$ to 16.8 $\mu g l^{-1}$.

Table 18. Distribution of Lead in the Cochin estuary

COMPARTMENTS	STATIONS					
	1	2	3	4	5	6
Soft tissue $\mu g\ g^{-1}$	7.58 ± 3.10 (3.38 - 11.78)	7.91 ± 2.57 (4.38 - 12.35)	10.11 ± 2.67 (6.84 - 15.46)	9.13 ± 3.02 (5.21 - 16.66)	9.21 ± 2.41 (5.67 - 13.23)	6.03 ± 1.93 (4.34 - 9.38)
Shell $\mu g\ g^{-1}$	40.60 ± 3.63 (34.58 - 46.21)	42.55 ± 3.92 (36.08 - 49.58)	42.35 ± 4.77 (34.67 - 51.57)	43.02 ± 5.53 (34.00 - 51.83)	41.32 ± 7.26 (33.54 - 54.35)	43.37 ± 5.45 (35.83 - 53.13)
Sediment $\mu g\ g^{-1}$	10.25 ± 3.10 (6.37 - 15.26)	10.38 ± 4.62 (4.73 - 20.25)	8.54 ± 1.45 (6.49 - 11.53)	7.64 ± 1.07 (6.20 - 9.09)	10.66 ± 1.97 (8.39 - 14.64)	9.17 ± 1.06 (7.79 - 10.72)
Dissolved $\mu g\ l^{-1}$	3.55 ± 2.23 (1.58 - 9.14)	2.10 ± 0.88 (1.20 - 4.53)	3.97 ± 1.70 (1.75 - 8.00)	5.51 ± 4.13 (2.50 - 14.00)	11.23 ± 4.35 (5.14 - 16.80)	3.94 ± 1.62 (2.40 - 7.14)
Particulate $\mu g\ l^{-1}$	3.28 ± 1.06 (1.58 - 5.33)	4.09 ± 2.76 (1.75 - 9.71)	1.35 ± 0.66 (0.60 - 2.93)	2.92 ± 1.30 (1.40 - 6.13)	3.21 ± 1.10 (1.60 - 4.83)	1.82 ± 0.30 (1.47 - 2.27)

Particulate

Concentrations of particulate lead did not vary much like that of dissolved species. The highest concentration of 9.71 $\mu g\ l^{-1}$ was obtained in monsoon period at Station 2 and the lowest concentration of 0.6 $\mu g\ l^{-1}$ was obtained in pre-monsoon period at Station 3. The maximum and minimum a.m.c.were 4.09 $\mu g\ l^{-1}$ and 1.35 $\mu g\ l^{-1}$ observed at Stations 2 and 3 respectively. Except for Station 3, estuarine stations were having higher concentrations than the riverine stations. Variations were maximum at Station 2 where the a.m.c. of 4.09 $\mu g\ l^{-1}$ varied between 1.75 $\mu g\ l^{-1}$ to 9.71 $\mu g l^{-1}$.

Distribution in Sediment

The concentrations of lead among the Stations were fairly steady. The highest and lowest concentrations (20.25 $\mu g\ g^{-1}$ and 4.73 $\mu g\ g^{-1}$ respectivey) were obtained at Station 2. The maximum and minimum values of a.m.c. were 10.66 $\mu g\ g^{-1}$ (Station 5) and 7.64 $\mu g\ g^{-1}$(Station 4). It was noted that the estuarine Stations had more lead concentrations in the sediment than the riverine Stations. Variability within the Station was also maximum at Station 2, followed by Station 1 with the values ranging between 6.37 $\mu g\ g^{-1}$ and 15.26 $\mu g g^{-1}$. All other Stations irrespective of being estuarine or riverine, did not show much variations seasonally within them.

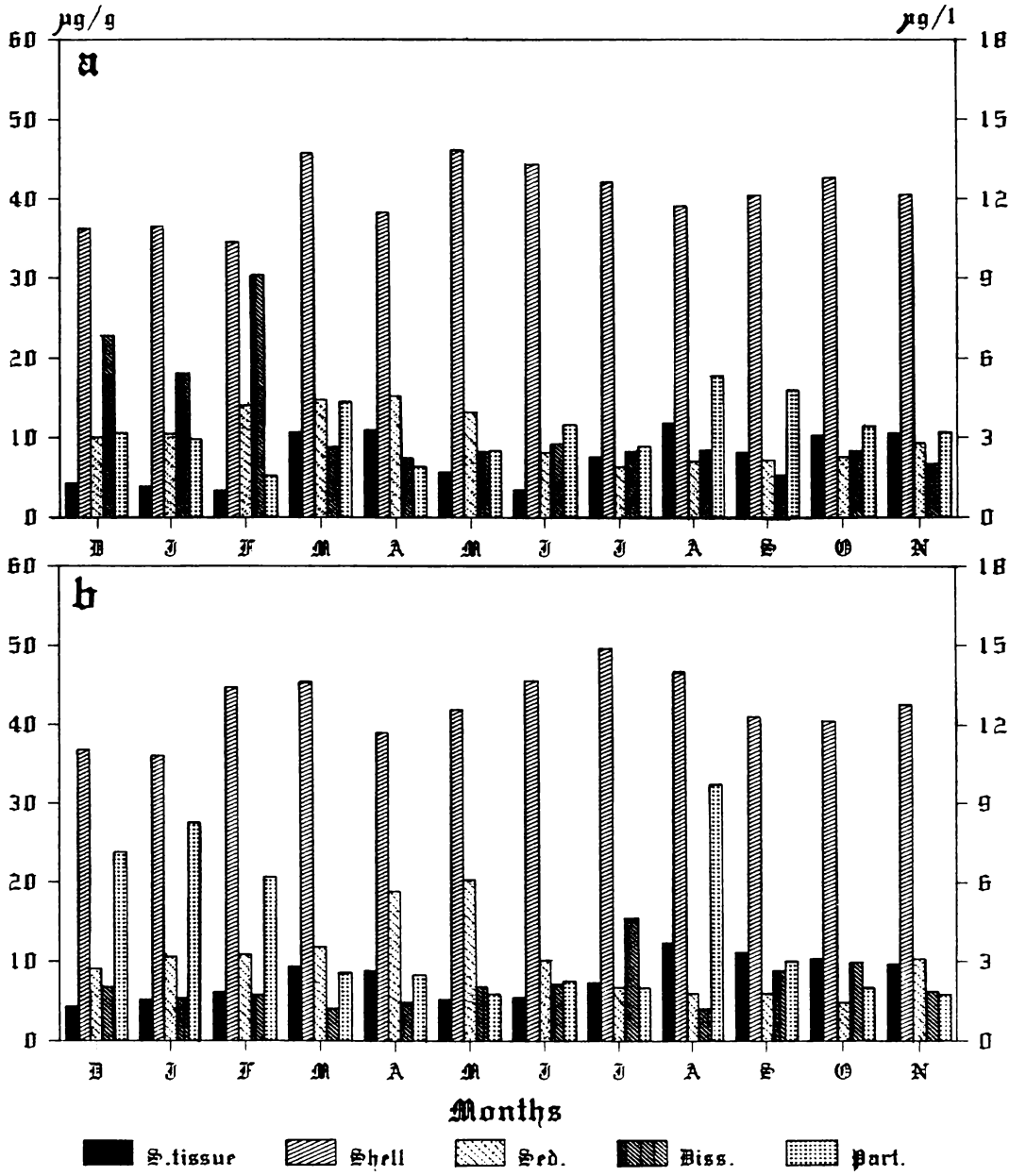


Fig.27 Trace metal partitioning in Cochin estuary (lead)
a) Station 1 b) Station 2

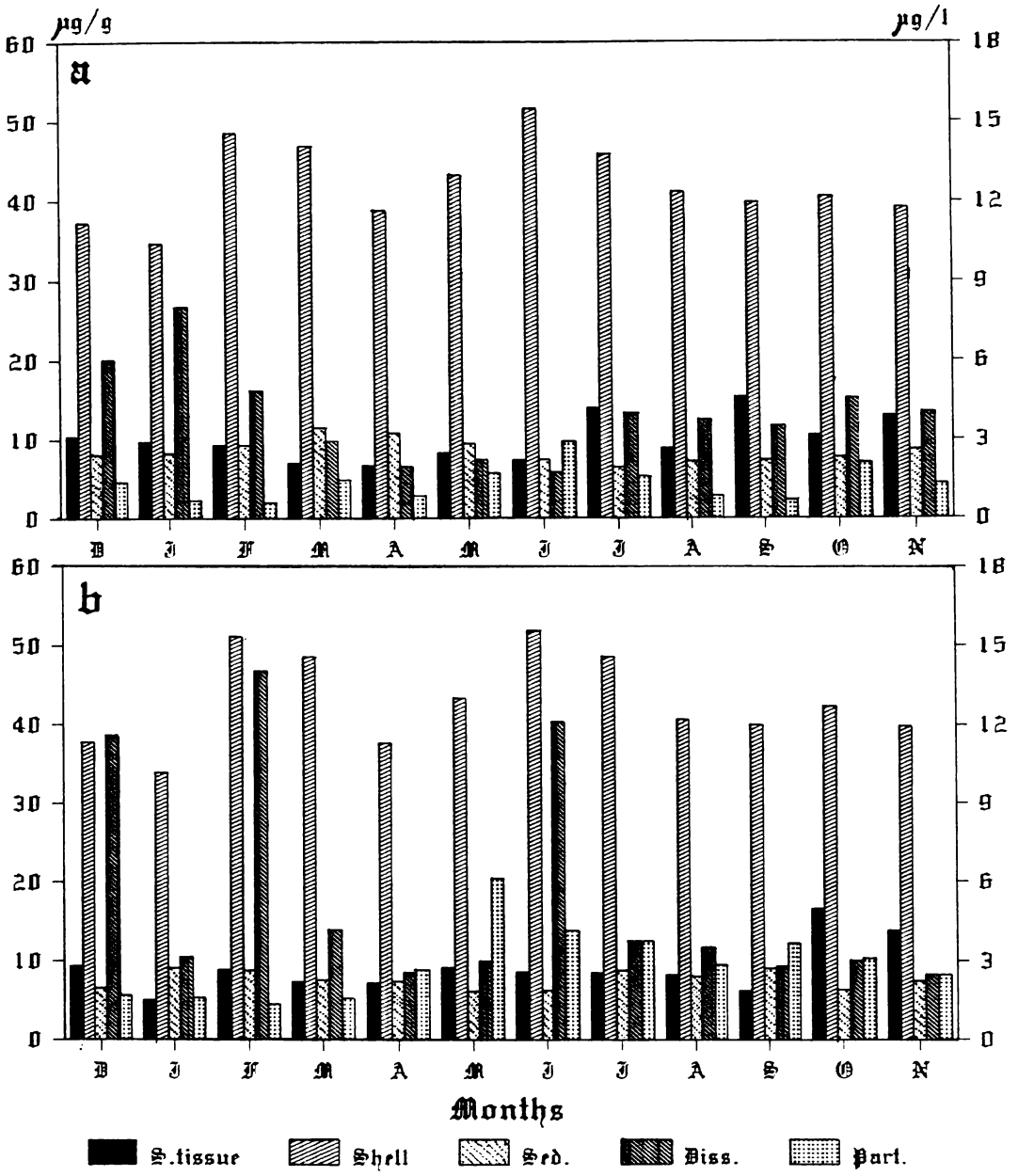


Fig.28 Trace metal partitioning in Cochin estuary (lead)
a) Station 3 b) Station 4

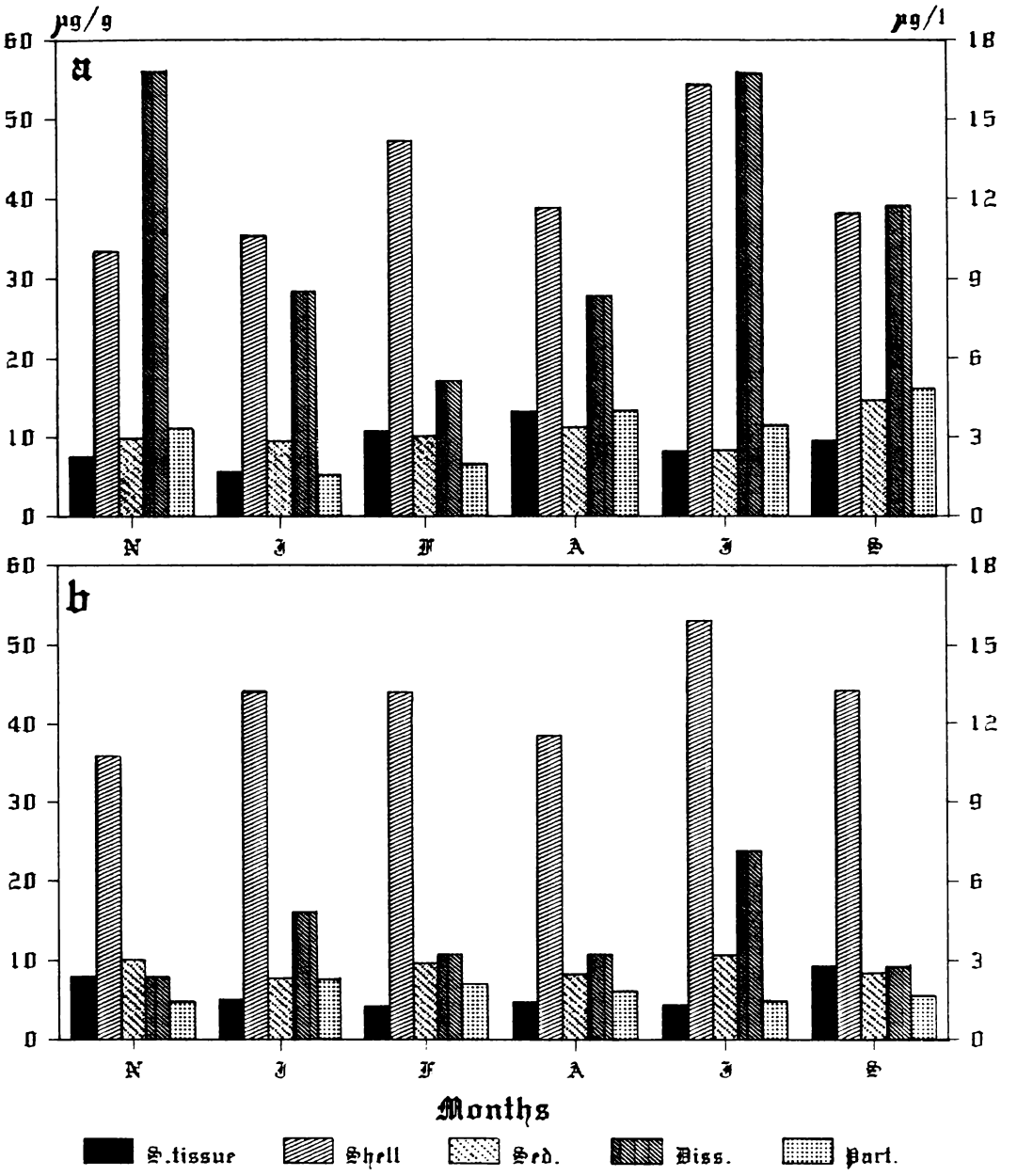


Fig.29 Trace metal partitioning in Cochin estuary (lead)
a) Station 5 b) Station 6

Distribution in Bivalves

Lead is a non essential trace element to the living organisms. But accumulation of lead into the living system occurs quite often as evidenced by the presence of lead in soft tissues and shells of organisms. It has been pointed out that if the diet did not contain the necessary requirements of calcium, lead storage occurs within the tissues.

Shells

Out of the trace metals studied, lead was having maximum concentration in the shells. The highest concentration of 53.13 $\mu g\ g^{-1}$ was obtained at Station 6 and the lowest concentration of 34.00 $\mu g\ g^{-1}$ at Station 4, in the case of *V.cyprinoides*. Generally higher concentrations were observed in monsoon months. The maximum and minimum a.m.c. recorded were 43.37 $\mu g\ g^{-1}$ at Station 6 and 40.6 $\mu g g^{-1}$ at Station 1 respectively. Shells of *M. casta* from station 5 also presented a comparable value with that of *V. cyprinoides* obtained from all other Stations. The a.m.c. in *M. casta* was 41.32 $\mu g\ g^{-1}$ varying between 33.5 $\mu g\ g^{-1}$ and 54.35 $\mu g g^{-1}$.

Soft tissues

Concentrations of lead in the soft tissues were low compared to the concentration in the shells. The highest concentration of 16.66 $\mu g\ g^{-1}$ was observed at Station 4 and the

lowest concentration of 3.38 $\mu g\ g^{-1}$, at Station 1 in the case of *V. cyprinoides*. These variations occurring within the Stations seasonally indicate the influence of environmental factors on the accumulation/depuration and distribution of the metal within the body. Slight, but conspicuous variations were observed in all the Stations studied, of which the maximum was in Station 1. The maximum and minimum a.m.c. of *V. cyprinoides* were obtained at Stations 3 and 6 respectively (10.11 $\mu g\ g^{-1}$ and 6.03 $\mu g\ g^{-1}$ being the respective values). Both *V. cyprinoides* and *M. casta* were having comparable concentration values. *M. casta* was having a.m.c. of 9.21 $\mu g\ g^{-1}$ varying between 5.67 $\mu g\ g^{-1}$ and 13.23 $\mu g\ g^{-1}$.

Bioavailability

Correlation coefficients been the various environmental variables and biological factors are presented in Table 19. The BCR was found to be significantly correlated both with particulate metal concentration and MPR ($p<0.001$ in both cases). The BCF and BCR (as well as their log values) were strongly correlated, negatively to the dissolved metal concentration, X1. BCF also showed a less significant negative correlation with the particulate metal concentration. Soft tissue concentrations as well as the BAF did not exhibit any significant relationship with either of the environmental factors, X1, X2 or X3. The equations for important lines of significant regression are as follows and are represented

Table 19. Correlation coefficients between Environmental variables and Biological factors (Lead)

	S.tissue Y1	BCF Y2	log BCF log Y2	BCR Y3	log BCR log Y3	BAF Y4
Dissol. X1	-0.030	-0.503**	-0.60**	-0.647**	-0.821**	-0.050
Part. X2	-0.026	-0.342*	-0.324*	0.406**	0.293	0.023
MPR X3	0.008	-0.123	-0.092	0.640**	0.515**	0.049
GAF X4	-0.098	0.303*	0.323*	0.631**	0.621**	-0.314*
Exch. X5	0.244	0.062	0.001	-0.101	-0.136	-0.420
Exch% X6	0.037	0.148	0.107	0.096	-0.090	0.198

n = 66 * $p < 0.01$ ** $p < 0.001$

pictorially in Figs. 30 a, b and 31 a.

$Y3 = 379.26\ X2 + 1724.38 \quad (r = 0.41 \quad p<0.001)$

$Y3 = 772.97\ X3 + 1928.78 \quad (r = 0.64 \quad p<0.001)$

$\log Y3 = 0.107\ X3 + 3.2145 \quad (r = 0.52 \quad p<0.001)$

Despite the presence of appreciable levels of lead in the sediment, there did not seem to exist any significant relationship between the biological factors and lead concentrations in the sediment fractions, *albeit,* the GAF portrayed a strong correlation with BCR ($p<0.001$), defined by the equation,

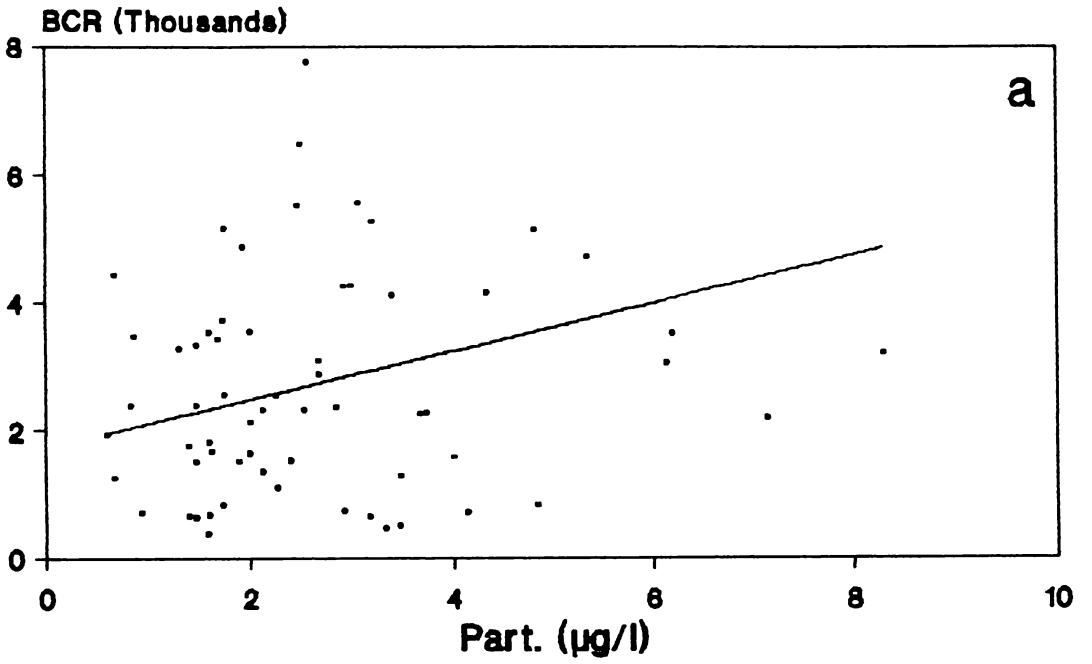
$Y3 = 0.497\ X4 + 1243.8 \quad (r = 0.63 \quad p<0.001)$,

illustrated in Fig. 31 b. Soft tissue metal concentration showed a relationship ($p<0.05$) with the metal concentration in the exchangeable fraction of the sediment.

Lead is reported to have been used by man as early as 7000 B.C. Aquatic pollution of lead can be mostly traced to its use in gasoline, in smelting, in refining, in recycling etc. It is a non essential trace element whose presence even at low concentrations in the biological systems is extremely harmful. Toxicity of lead is mainly due to its chemical nature, which makes it difficult to be removed, once it enters the system.

The accumulation and distribution of lead in the aquatic environment have been widely studied (Denton and Burdon-Jones, 1981; Lakshmanan, 1982; Ajmal *et al.*, 1987; Lacerda *et al.*

Lead



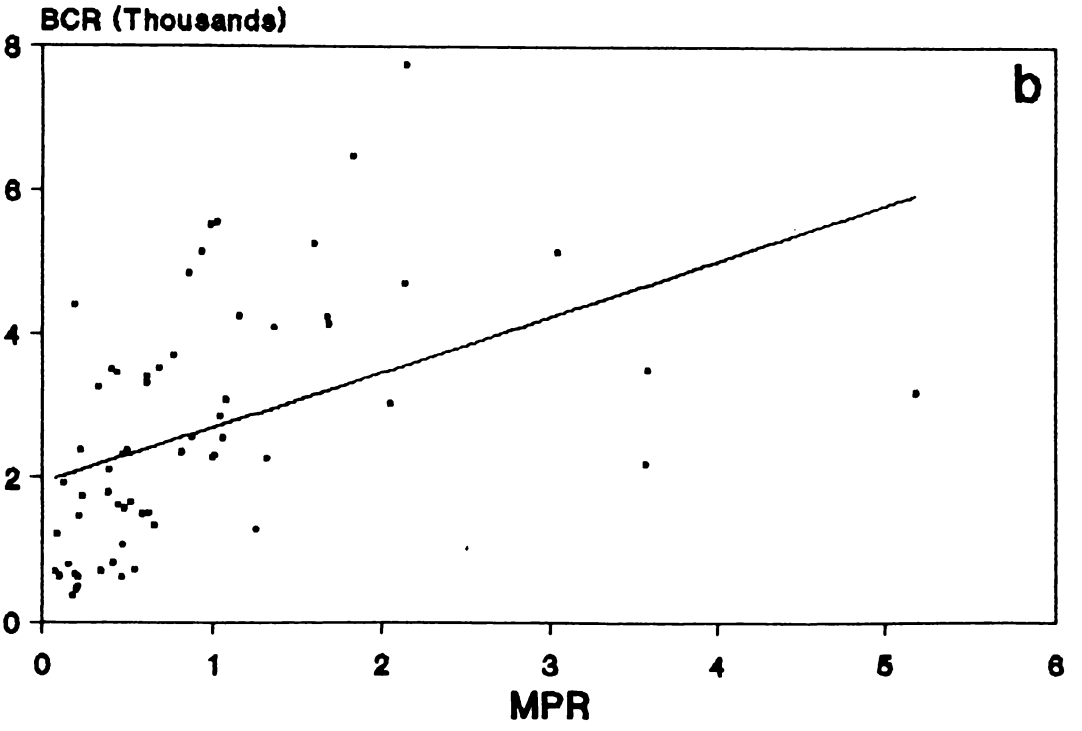


Fig.30 Regression analysis (lead)
a) Part. vs BCR b) MPR vs BCR

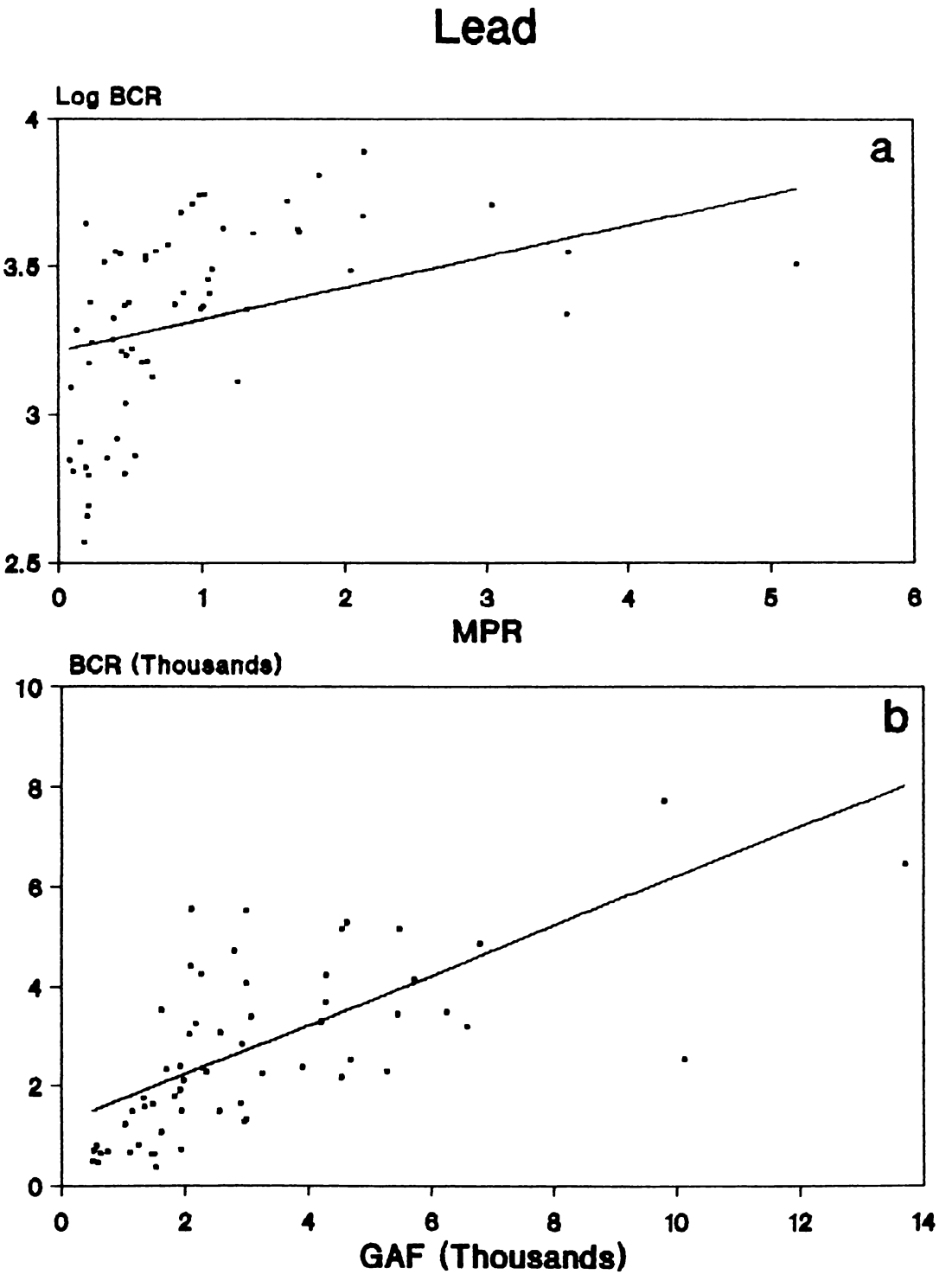


Fig.31 Regression analysis (lead)
a) MPR vs Log BCR b) GAF vs BCR

1988; Malm *et al.*, 1988; Chan, 1988; Kelly and Whitton, 1989; Lakshmanan and Nambisan, 1989). From the present study it is seen that the absolute values of metal concentration in soft tissues do not have any relationship with the environmental variables. Laboratory studies spread over a period of 6 days, carried out by Lakshmanan and Nambisan (1989) indicated that lead was accumulated in the soft tissues in a much higher level than other metals like copper, mercury, zinc etc. The results of the present investigation revealed that the lead concentration in the soft tissues was much less than that of other metals; between soft tissue and shell, lead was seen to concentrate to a less extent in the soft tissues. This observed higher concentration of lead in the shells could possibly be the result of a biological transport mechanism that governs the partitioning of the lead between the shell and the soft tissues.

Amiard *et al.*(1987) studied the accumulation of lead and found that the concentration of lead in the organism and in the environment can be defined by the power equation $Y = ax^b$, where x is the concentration in the organism and Y is the environmental concentration. The relationship between lead in the sediment and lead in the soft tissues of organisms has been studied fairly extensively (Bryan and Hummerstone, 1973; 1978; Luoma and Bryan, 1978; Tessier *et al.*, 1984; Ward and Young, 1984; Gunn *et al.*,1989; Prosi, 1989). Luoma and Bryan (1978) have reported a significant correlation between lead in the

soft tissue of *S. plana* and ratio of lead to iron (1N HCl extractable fraction) in the sediment. Tessier *et al.*, (1984) on the other hand observed a similar significant relation between lead in the soft tissues and the ratio of the concentration of lead in the sum of the mobile fraction to that of iron in the Fe/Mn-oxide bound fraction.(i.e. Pb soft tissue Vs Pb (exch + carbo + Fe/Mn-oxide) /Fe (Fe/Mn-oxide). The above observations emphasize the role of iron in the bioavailability of lead . Iron could be thought to play a protective role in reducing the uptake of lead both inside the digestive system (by complexing with the binding sites) and outside the external medium (by inducing sites for lead ions to get adsorbed on particles)

Gunn *et al.*, (1989) obtained a good relationship between soft tissue concentrations of lead in tubificid worms and that in the exchangeable fraction of the sediment. The present study also has revealed the existence of a similar correlation ($p<0.05$) between lead concentration in soft tissues and that in the exchangeable fraction of sediments. The interstitial water in sediments is known to play an important role in regulating the accumulation of metals in the top layers of sediments (Prosi, 1989). Heavy metals are found to be concentrated to much higher levels in interstitial water than in the overlying water. As a result, sediment dwelling fauna (like bivalves) are subjected to a much higher exposure to the pore water than epifauna, and hence to possibilities of higher metal

accumulation by the organisms. The extraction of the exchangeable fraction of lead from the sediment includes metals in the interstitial water as well. Therefore the observed correlation between lead concentration in the soft tissue and that in the exchangeable fraction of the sediment is only a natural consequence of the benthic nature of the organism.

Lead present in the particulate form in the aqueous phase is more bioavailable to the organisms than that in the dissolved form. The existence of strong positive correlations between BCR and MPR, BCR and particulate lead concentration and strong negative correlation between BCR and dissolved lead concentration is illustrative of the above fact. Luoma and Bryan (1978) compared the concentration of lead in the organism *S. plana* and sea weed *Fucus vesiculosus* from the same location and found no significant correlation between the lead concentrations in the soft tissues of the organism and that in the sea weed. Since the metal concentration in the sea weed is taken as an indicative of the dissolved metal concentration (Bryan and Hummerstone, 1973), the above result suggests that soft tissue concentrations does not bear any positive relationship with the dissolved metal concentration.

Since lead is precipitated as $PbCl_2$ at the normal pH of sea water, the concentration of dissolved lead in sea water is low (Byrne and, Miller, 1984). The precipitation leads to an enhancement of the lead concentration in bottom sediments as

well as in particulates. The higher adsorption rate of lead over that of other metals like cadmium also facilitates a quicker adsorption of lead onto the particulate phase. Lead is rendered more bioavailable from the particulate phase by the mechanisms discussed earlier.

Bioaccumulation of lead is influenced by the blend of several phenomena like capacity of the organism to store lead, the pore water concentration, the availability of binding sites, partioning of lead in the aqueous phase etc. BCR and MPR appear to be the biological and environmental parameters best suited to predict the bioavailabilty from the aqueous phase. From the sediment phase,the BCR and GAF are seen to be the most useful, in bioavailability assessments.

Nickel

Data on the annual mean concentration, standard deviation and the ranges of nickel determined in the different compartments of the system is furnished in Table 20. The trends in monthly variations are depicted in Figs. 32-34.

Distribution in Water

Dissolved

The highest concentration of 5.27 $\mu g\ l^{-1}$ and lowest value of 1.08 $\mu g\ l^{-1}$ were observed at Stations 1 and 5 respectively. The maximum and minimum a.m.c. recorded were 2.67 $\mu g\ l^{-1}$ and 1.66 $\mu g\ l^{-1}$ at Stations 1 and 5 respectively. Both were estuarine stations, but situated on the southern and northern arms of the estuary.

Particulate

Nickel in the particulate form at the different Stations were more abundant than dissolved nickel. The highest (10.66 $\mu g\ l^{-1}$) and lowest (2.41 $\mu g\ l^{-1}$) concentrations were observed at Station 1. The maximum and minimum a.m.c. were recorded at Stations 2 and 6 (6.45 $\mu g\ l^{-1}$ and 3.54 $\mu g\ l^{-1}$). The variability was more between Stations 5 and 6 in the northern region than between the Stations 1 to 4 in the southern region. At the same

Table 20. Distribution of Nickel in the Cochin estuary

COMPARTMENTS	STATIONS					
	1	2	3	4	5	6
Soft tissue ug g^{-1}	5.65 ± 1.70 (4.13 - 10.01)	5.65 ± 2.25 (2.75 - 9.64)	5.59 ± 2.15 (2.72 - 9.75)	7.84 ± 1.86 (4.39 - 10.85)	8.31 ± 1.55 (6.49 - 10.77)	4.71 ± 1.42 (2.81 - 7.08)
Shell ug g^{-1}	22.80 ± 2.38 (18.72 - 26.23)	23.53 ± 1.71 (21.36 - 26.78)	23.18 ± 1.92 (20.40 - 26.77)	22.62 ± 2.08 (19.68 - 26.66)	21.84 ± 1.99 (18.83 - 24.43)	22.42 ± 2.21 (19.98 - 26.17)
Sediment ug g^{-1}	14.76 ± 1.64 (12.53 - 18.33)	16.05 ± 7.43 (8.51 - 34.58)	19.56 ± 2.06 (15.51 - 22.83)	18.42 ± 5.47 (11.68 - 28.92)	17.36 ± 2.40 (13.90 - 20.49)	15.11 ± 1.97 (11.15 - 17.32)
Dissolved ug l^{-1}	2.67 ± 0.89 (1.98 - 5.27)	1.99 ± 0.51 (1.35 - 3.00)	2.14 ± 0.61 (1.26 - 3.55)	2.24 ± 0.88 (1.23 - 3.85)	1.66 ± 0.29 (1.08 - 2.00)	2.13 ± 0.62 (1.48 - 2.98)
Particulate ug l^{-1}	6.04 ± 2.01 (2.41 - 10.66)	6.45 ± 1.94 (2.79 - 9.16)	6.34 ± 1.13 (4.75 - 7.81)	5.68 ± 1.68 (2.85 - 8.49)	5.07 ± 1.71 (3.87 - 8.85)	3.54 ± 0.46 (2.79 - 4.13)

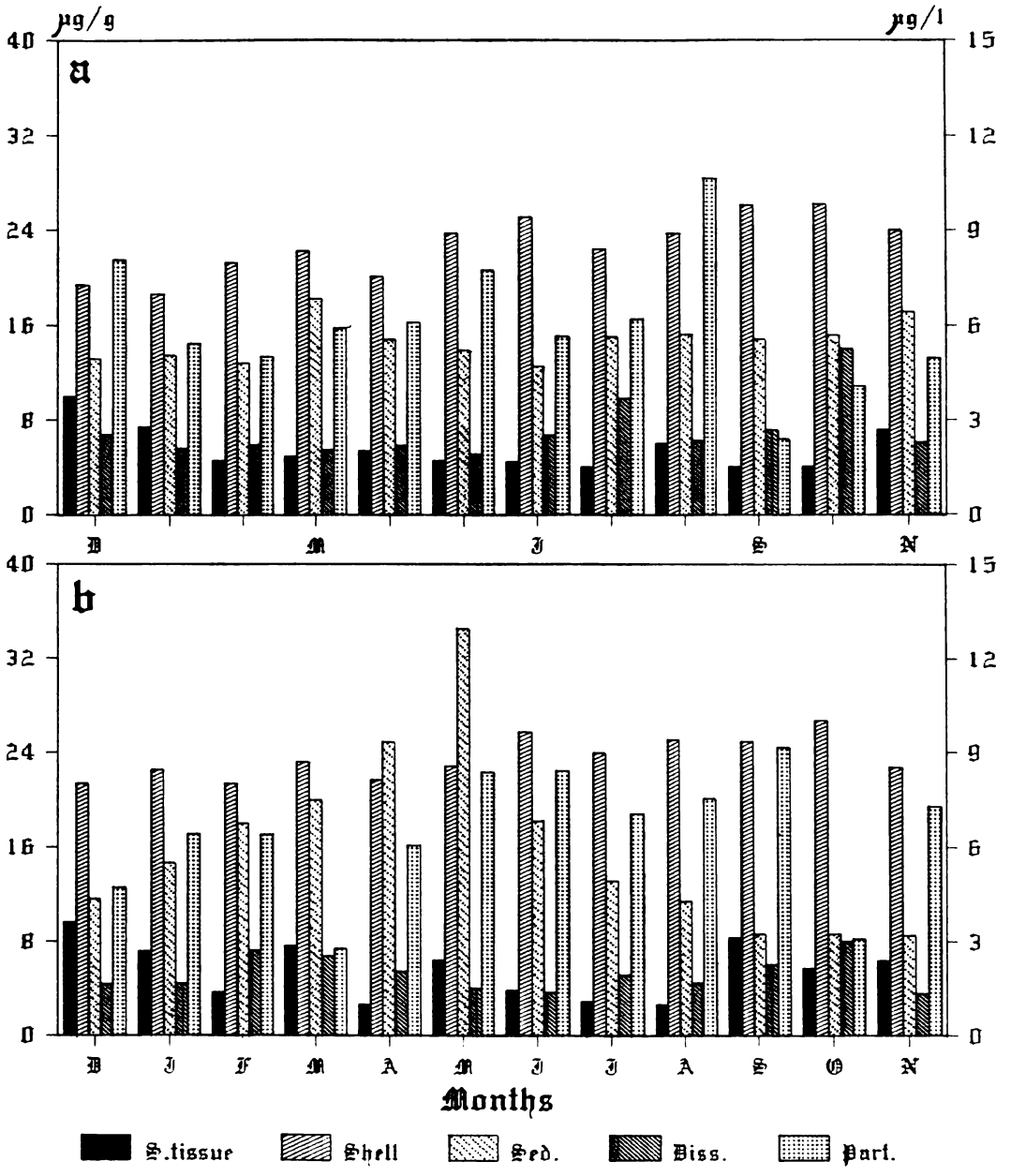


Fig.32 Trace metal partitioning in Cochin estuary (nickel)
a) Station 1 b) Station 2

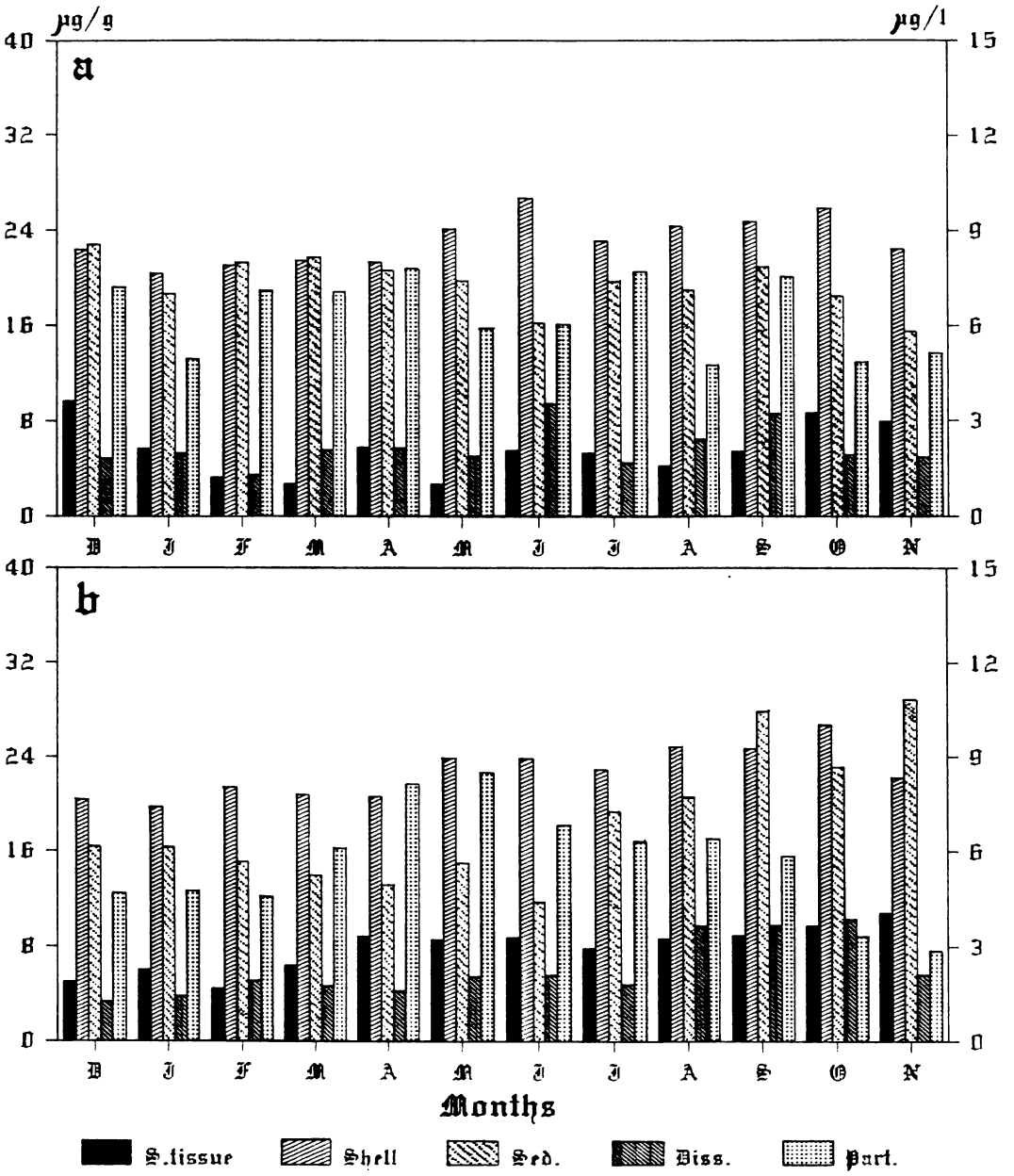


Fig.33 Trace metal partitioning in Cochin estuary (nickel)
a) Station 3 b) Station 4

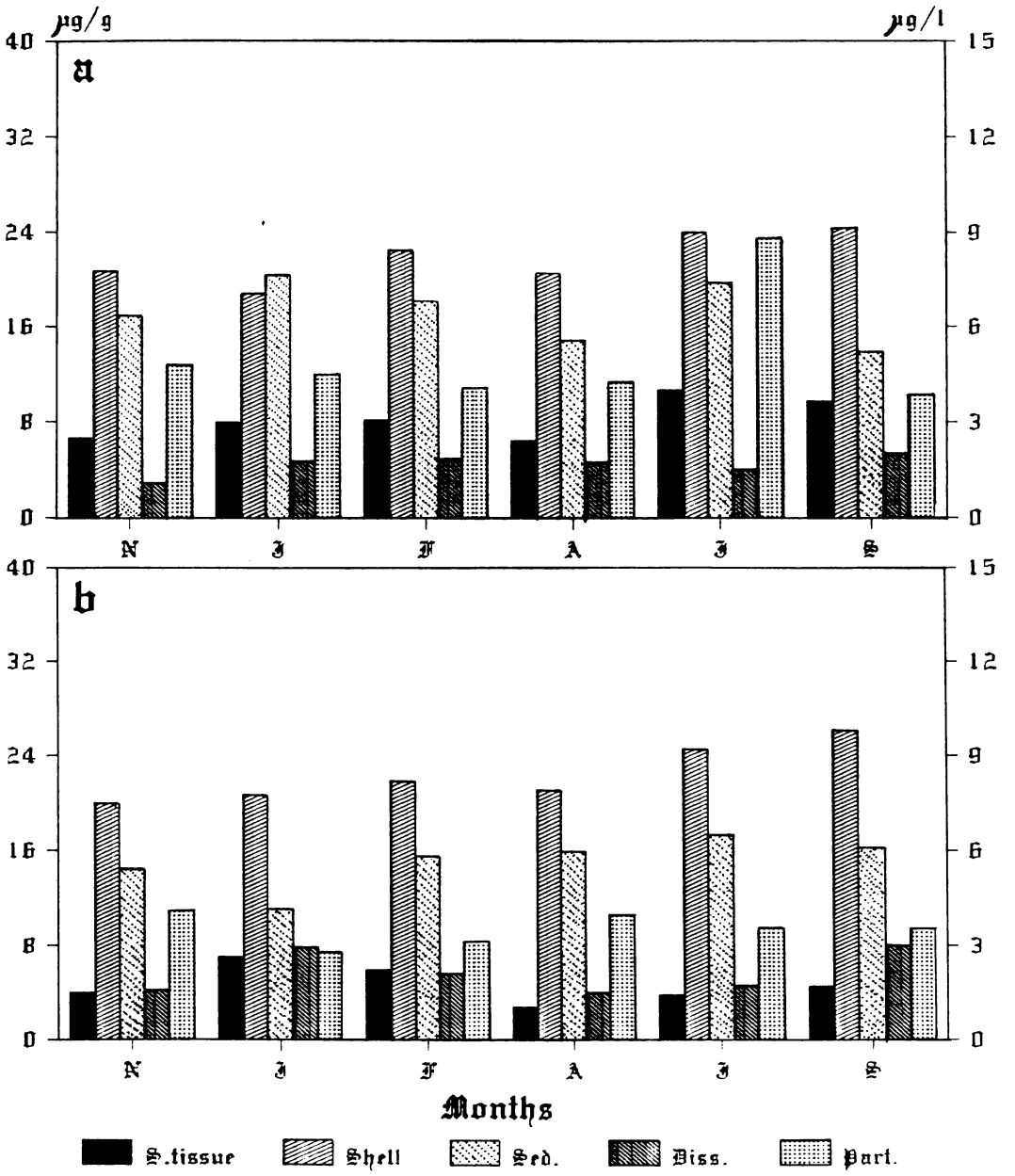


Fig.34 Trace metal partitioning in Cochin estuary (nickel)
a) Station 5 b) Station 6

Stations, the particulate nickel concentration was higher than that of dissolved nickel.

Distribution in Sediment

Nickel concentrations in the sediment were higher than those of copper and cadmium, but less than that of zinc. The highest and lowest overall concentrations were observed at Station 2 (34.58 $\mu g\ g^{-1}$ and 8.51 $\mu g\ g^{-1}$). It varies from 14.76 $\mu g\ g^{-1}$ at Station 1 to 19.56 $\mu g\ g^{-1}$ at Station 3, both being Stations in the southern region. In the northern region, the estuarine Station 5 showed a higher concentration (17.36 $\mu g\ g^{-1}$) than the riverine Station 6 (15.11 $\mu g\ g^{-1}$). Of these Stations, Station 2 and 4 exhibited appreciable seasonal variations. At Station 4 also appreciable variation was present seasonally ranging from 11.68 $\mu g\ g^{-1}$ to 28.92 $\mu g\ g^{-1}$. At other Stations, monthly variations were not conspicous.

Distribution in Bivalves

Nickel is an essential element required only in trace quantities for the living organisms. But the levels observed in the soft tissues were less than that observed in the shells. It may be assumed that the metal present in excess of that required for the normal metabolic functioning of the body may be transported to the nonliving shell material, thereby causing an increase in nickel content in the shells.

Shells

Nickel concentrations in *M. casta* from (Station 5) and *V. cyprinoides* (from all other Stations) reflected comparable levels. The highest and lowest concentrations of nickel in the shells of *V. cyprinoides* were recorded at Stations 2 and 1 and the respective values were 26.78 $\mu g\ g^{-1}$ and 18.72 $\mu g\ g^{-1}$. The maximum and minimum a.m.c. of 23.53 $\mu g\ g^{-1}$ and 22.42 $\mu g\ g^{-1}$ were obtained for *V. cyprinoides* at Stations 2 and 6 respectively. *M. casta* from Station 5 had an a.m.c. of 21.64 $\mu g\ g^{-1}$, comparable to that of *V. cyprinoides*.

Soft tissues

The highest concentration was recorded at Station 4 (10.85 $\mu g\ g^{-1}$ and lowest value was at Station 3 (2.72 $\mu g\ g^{-1}$). Eventhough concentrations were low, within the stations, small variations did exist. Maximum a.m.c. of 7.84 $\mu g\ g^{-1}$ at Station 4 and minimum of 4.71 $\mu g\ g^{-1}$ were recorded for *V. cyprinoides*. For *M. casta*, the a.m.c. was 8.31 $\mu g\ g^{-1}$. The Stations which showed maximum and minimum a.m.c. were riverine Stations on the southern and northern regions respectively. The other three estuarine Stations in the southern region showed fairly identical values (5.65 $\mu g\ g^{-1}$, 5.65 $\mu g\ g^{-1}$ and 5.59 $\mu g\ g^{-1}$ for Stations 1, 2, and 3 respectivey.

Bioavailability

The different biological factors were compared with environmental variables. The important correlation coeffiecients obtained between the different biological factors and environmental variables are given in Table 21.BCR was found to be signifcantly correlated with MPR ($p<0.001$) though it was not correlated to any significant degree with the nickel concentration in the particulate matter of the aqueous phase. As expected, BCR was negatively correlated ($p<0.001$) with the dissolved metal concentrations. These correlations are exemplified by the equations given below and the lines in Figs. 35 a and b.

$Y3 = -102\ X1 + 532.74$ ($r = -0.54$ $p<0.001$)

$Y3 = 47.93\ X3 + 171.9$ ($r = 0.45$ $p<0.001$)

Values of BCR correlated significantly with those of GAF ($p<0.001$), while those of BAF were negatively correlated with GAF ($p<0.001$). BAF was correlated, though less significantly ($p<0.05$) to the various sediment fractions. Thus, eventhough nickel concentrations were comparatively high in the sediment, these did not seem to be of much importance in biological uptake. The most important regression line is represented by

$Y3 = 0.016\ X4 + 171.71$ ($r = 0.40$ $p<0.001$)

Being a transition metal, nickel occurs in a number of oxidation states, of which Ni^{II} is the most common due to its stability over a wide range of pH. Introduction of nickel by

Table 21. Correlation coefficients between Environmental variables and Biological factors (Nickel)

	S.tissue Y1	BCF Y2	log BCF log Y2	BCR Y3	log BCR log Y3	BAF Y4
Dissol. X1	0.019	-0.079	-0.081	-0.541**	-0.597**	-0.035
Part. X2	0.048	-0.489**	-0.475**	0.154	0.142	0.100
MPR X3	0.007	-0.303*	-0.303*	0.453**	0.440**	0.070
GAF X4	-0.013	0.068	0.023	0.403	0.400	-0.399
Exch. X5	-0.310*	-0.273	-0.312*	-0.251	-0.228	-0.295
Exch% X6	-0.220	-0.210	-0.199	-0.186	-0.159	0.270

n = 66 *p < 0.01 **p < 0.001

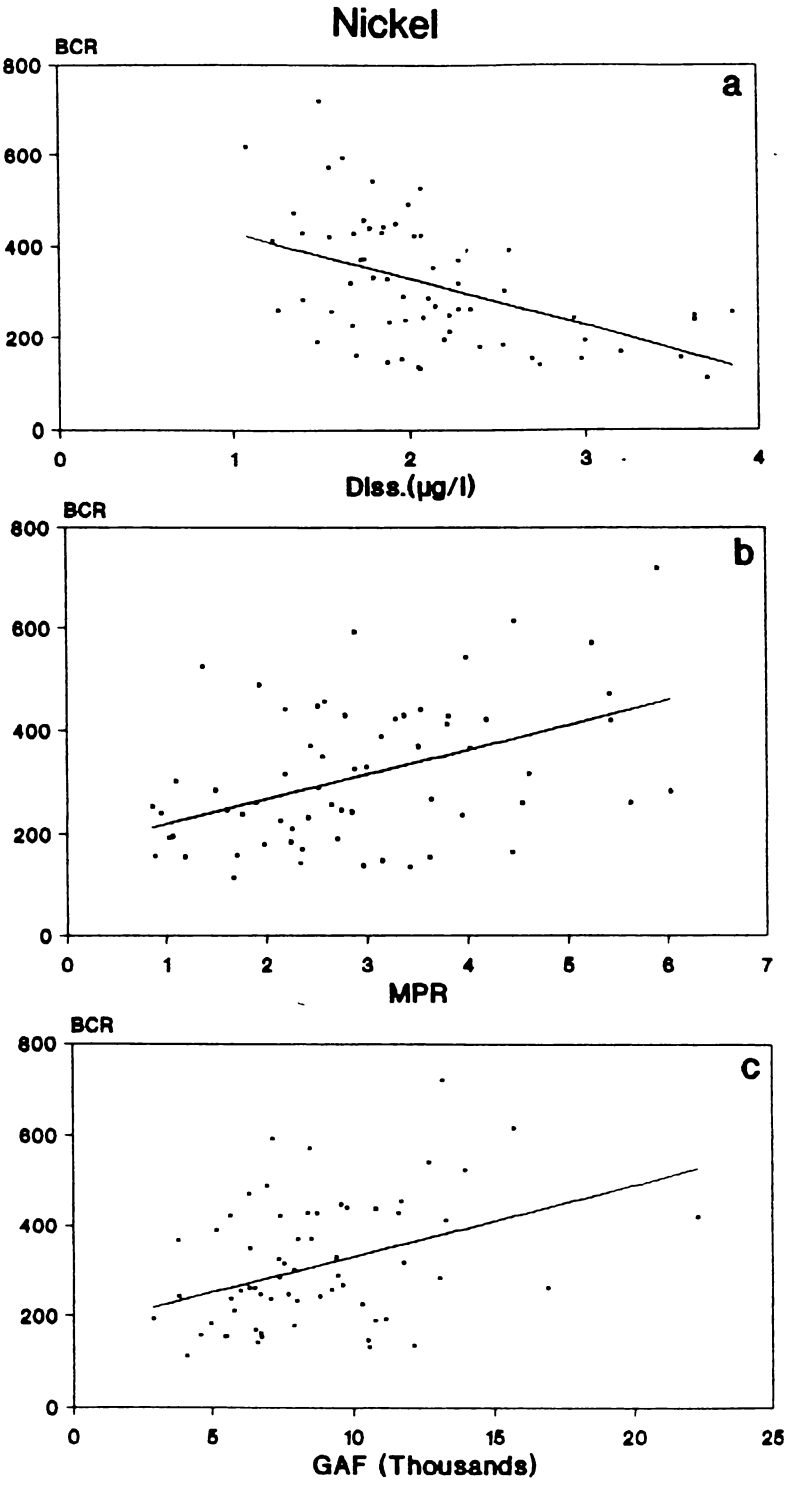


Fig.35 Regression analysis (nickel)
a) Diss. vs BCR b) MPR vs BCR c) GAF vs BCR

anthropogenic source includes industrial activities such as mining, steel plant operations, petrochemical industry etc. Fossil fuel combustion is also known to have introduced sizeable amount of nickel into the atmosphere, which is believed to have ended up in the aquatic and terrestrial biospheres (Nriagu, 1980). The toxic action of nickel is due to its ability to replace essential metals in the metallo-enzymes resulting in the disruption of metabolic pathways (McGroth and Smith, 1990).

Among the metals studied nickel was less abundant than zinc in most of the phases. It showed an unusual behaviour in that there was a significant correlation ($p<0.001$) between nickel concentration in the dissolved phase and that in the shell. Not much interest has been evinced in the studies on nickel in the aquatic environment, probably because it is not a very toxic element. Some of the available literature on the toxicity, bioaccumulation and distribution of nickel were the studies carried out by Eisler and Henneky (1977), Bryan and Hummerstone (1978), Petukhov *et al.* (1982), Pellenberg (1984), Bargagli *et al.* (1985), Campbell *et al.* (1988), Crowder *et al.* (1989), Sadiq (1989)

The main source of nickel input into the aquatic system is from crude oil and its products. Bargagli *et al.* (1985), analysed the nickel concentration in sediments, molluscs and reed leaves and pointed out that considerable variation occurred in molluscs sampled from different stations and in

reed leaves taken from land-sites close to industrial units. Tjalve *et al.* (1988) studied bioaccumulation of nickel by the fish *(Salmo trutta)* and reported a considerable increase in the uptake of nickel caused by external concentrations. Crowder *et al.* (1989) also reported the presence of nickel in the sediment and biota.

Although nickel was present at comparatively high concentration in the aquatic environment, the accumulation of nickel to higher levels in the shells as against that in the soft tissues signified the existence of a biological transport mechanism which regulated the transfer of nickel between the soft tissue and the shell. The observed concentration indicated that nickel was preferentially ingested by the organism from the particulate matter of the aqueous phase. A considerable portion of the nickel ions were reported to exist in sea water as complexes with chloride and sulphate ions, leading to depletion of free nickel ions.

Studies aimed at assessing the sediment bioavailability of nickel have been rare. The observed strong correlation between BCR and GAF may be due to the effect of normalization of soft tissue concentrations and sediment nickel concentration by the dissolved nickel concentration.

From the above studies it was clear that for studying bioavailability from water, MPR and BCR respectively could be used as the environmental variable and biological factor.

Similarly in sediment bioavailability, BCR and GAF may be used for quantifying the metal uptake.

The studies reported herein were aimed at assessing the distribution of copper, cadmium, zinc, lead and nickel in the aquatic environment and at identifying biological or environmental parameters that could be used to predict bioavailability of the metal from both the aqueous as well as the sediment phases. BCR, an index of the distribution of the metal between the soft tissue and the dissolved phase, has emerged as a biological factor, that can be predicted from a knowledge of either MPR (ratio between particulate metal concentrations to dissolved metal concentration in the aqueous phase) or GAF (Ratio between metal concentration in the sediment and that in dissolved phase). The results also suggest that between the absolute values of the different fractions and their corresponding percentages, the latter is to be preferred for a stronger correlation.

The relative abundance of the metals studied in the different phases considered are given below.

Shells	Pb>Ni>Cu>Cd>Zn.
Soft tissues	Zn>Cu>Pb>Cd>Ni.
Sediment	Zn>Ni>Pb>Cu>Cd
Dissolved phase	Zn>Pb>Cu>Ni>Cd
Particulate	Zn>Ni>Pb=Cu>Cd.

In short, the specific processes controlling variations of

metals in organisms are difficult to be conclusively demonstrated in nature because of the complex interaction of uncontrolled and unknown variables. However, there is sufficient understanding from experimental studies, about the processes affecting metal dynamics in organisms and to initiate the application of such studies against field collected data (Cain and Luoma, 1990).

Chapter 5

SAAMPLE - A PREDICTIVE MATHEMATICAL MODEL

Introduction

A thorough understanding of the fate of pollutants discharged into the aquatic system is imperative for any rigorous assessment of their effects on the biological organisms. According to Reuber *et al.*, (1987) the fate and effects of chemicals in the environment are largely determined by the rate at which they migrate between environmental compartments. This inter-compartmental exchange is presided upon by the interplay of a variety of biogeochemical forces that operate within the aquatic biosphere. Mathematical models were conceived to contain and quantify such complex interactive forces (Mackay and Patterson, 1988).

Several models - from relatively simple ones to complex ones - were proposed for assessing/interpreting the effects of pollutants on the different segments of the aquatic system. Mathematical models, being the derivations of the implications of understanding, they can be used to summarize factual information. Bioaccumulation models as well as environmental fate models are useful in interpreting the fate and effects of a pollutant in an environmental set up (Burns and Baughman, 1985).

Environmental modeling (Halfon, 1989) is broadly based on (i) the dynamic approach, in which the changes of concentration with time are considered to be of momentous consequence and (ii) the equilibrium approach, in which the main assumption is that enough time has elapsed for the pollutant loadings in the environment to have reached an equilibrium. The equilibrium models derive their significance from their ability to indicate the compartment that would be the main recepient of the pollutant at equilibrium.

Mackay and Paterson (1981) introduced "fugacity" as an equilibrium criterion and developed a series of evaluative models to characterize the behaviour of chemicals in the biosphere (including air, water and solid phases). Fugacity "can be regarded as the escaping tendency of a chemical from a phase. It has units of pressure and can be related to concentration " (Mackay and Paterson, 1981). Application of the fugacity concept to assess pollutant accumulation in a different environmental sectors led to the evolution of adaptive refinements of the "fugacity model" (Mackay, 1989; Mackay and Paterson, 1988; 1991; Mackay *et al.*, 1983; Rueber *et al.*, 1987; Paterson and Mackay, 1985).

As fugacity is calculated from vapour phase concentrations, it cannot be applied to nonvolatile chemicals like metals or ionic species in the aquatic environment. For metals and other

ions with zero or negligible vapour pressure, Mackay and Diamond (1989), defined a new equilibrium criterion, "aquivalent concentration", for calculating the activities from an aqueous phase base. The "aquivalent concentration" was the equilibrium criterion of "equivalent aqueous concentration". Other models such as EXAMS (Burns *et al.,* 1981), TOXFATE (Halfon 1986) were useful in analysing exposure as well as fate of a pollutant in the environment.

Although both environmental and biological effects need to be quantified in any rigorous scheme aimed at developing a comprenhensive frame work for describing trace metal effects in estuarine systems, no reports were available on such a wholesome approach. The models cited above have, all aimed at conceptualizing the nature of distribution of the pollutant in the environment without making adequate provisions for evaluating the equally relevant bilogical effects stimulated by the pollutant. On the other hand, models specifically designed for the appraisal of bioaccumulation and its effcts (Morairty, 1975; Tuey, 1980; Thomann, 1981; 1989) have been totally oblivious to the environmental distribution effects.

This investigation was therefore an attempt to fill this void by directing systematic efforts to evolve a comprehensive model that would conceptually include the influences of both the segments of the aquatic ecosystem (*viz.* the environmental

segment and the biological segment) within its armpit.

The role of bivalves as ideal/sentinel organisms was established with the introduction of "International Mussel Watch" (Goldberg, 1975; Goldberg *et al.*, 1978). By virtue of the ability of their soft tissues to accumulate trace metals from the aquatic environment, bivalves could convincingly function as indicators of "pollution hot spots".

Development of the Model

The 'Shell - Model' proposed herein has been built-up on the premise that since metal concentrations in bivalve shells represented an unerasable record (unlike tissue - levels, which are intensely stress - dependent) of environmental stress, they should be the epicentre of any rigorous mathematical approach aimed at developing a model that could be used for aquatic pollution assessment. The aquatic environment was considered as a five compartment dynamic equilibrium system defined by three environmental compartments (constituting the environmental system) and two biological compartments (constituting the biological system). The rate of transfer of a metal from one compartment to another of this system would, consequently, be governed by a kinetic law. The environmental system (comprising of the sediment, the particulate and the dissolved phases) is capable of independent existence, even in the absence of the biological system (comprising of the soft tissue and the shell) as represented by the Fig. 36.

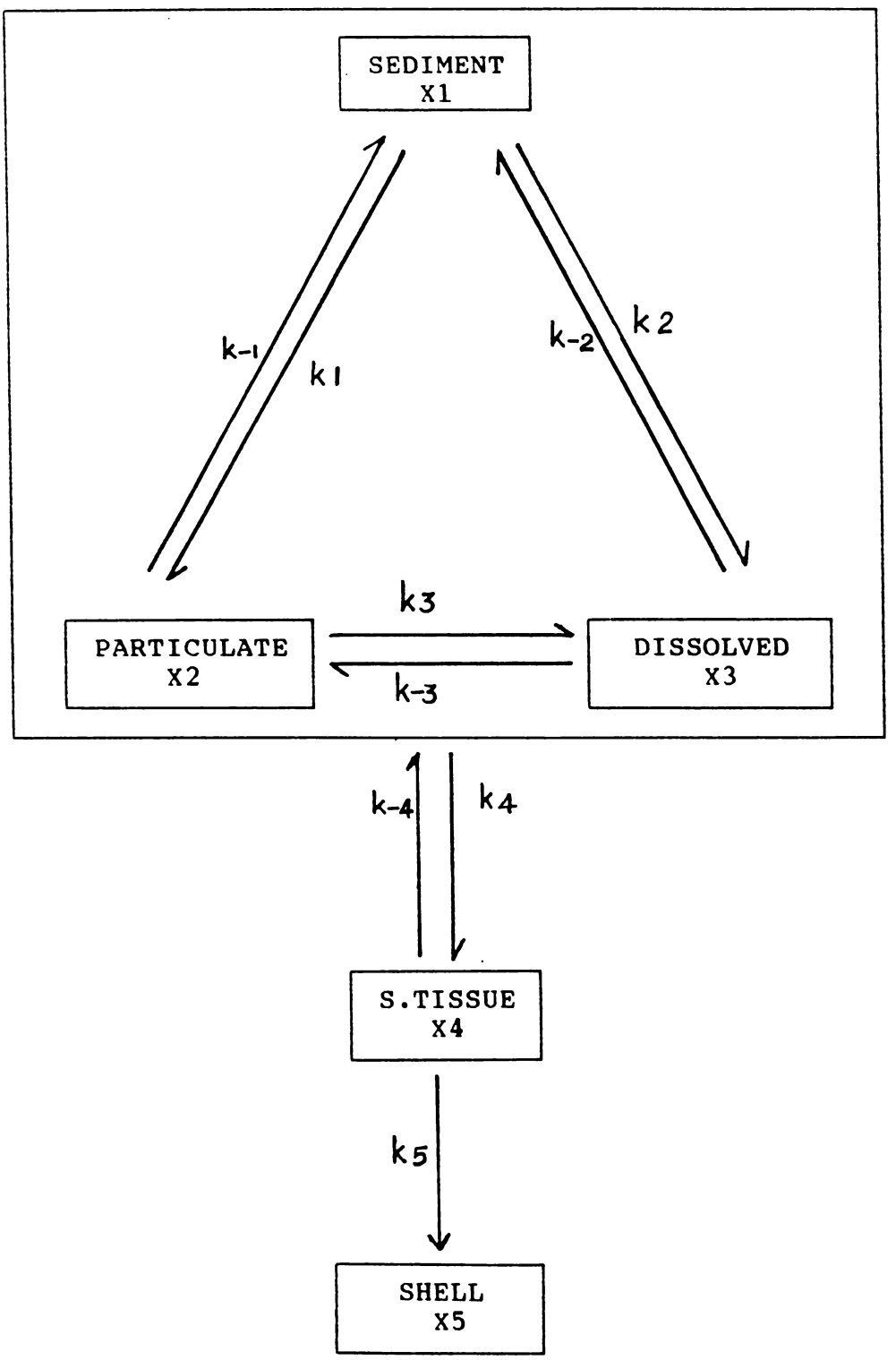


Fig.36 Trace metal partitioning in an estuarine system

TERMS

X1 = Concentration of trace metal in the sediment

X2 = Concentration of trace metal in the particulate phase

X3 = Concentration of trace metal in the dissolved phase

X4 = Concentration of trace metal in the soft tissue

X5 = Concentration of trace metal in the shell

k_1 & k_{-1} = Rate coefficient of transfer between sediment and particulate

k_2 & k_{-2} = Rate coefficient of transfer between sediment and dissolved phase

k_3 & k_{-3} = Rate coefficient of transfer between particulate and dissolved phase

k_4 & k_{-4} = Rate coefficient of transfer between environmental system to the soft tissues.

k_5 = Rate coefficient of transfer from soft tissue to shell.

K1 = The equilibrium coefficient of transfer between sediment and particulate.

K2 = The equilibrium coefficient of transfer between sediment and dissolved

K3 = The equilibrium coefficient of transfer between particulate and dissolved

K4 = The equilibrium coefficient of transfer between enviornment to soft tissue.

K5 = The proportionality constant between soft tissue an shell.

γ = The BIOPINDEX (The biological partitioning index)

A = The ENVOPINDEX (The environmental partitioning index)

The Environmental System

The three inter-dependant compartments (the sediment, the dissolved and the particulate phases) together define the environmental system which would be at equilibrium even in the absence of any one of the compartments identified above.

The rate of transfer of a metal from the sediment

$$-\frac{dX1}{dt} = k_1 X2 - \underline{k}_1 X1 + k_2 X3 - \underline{k}_2 X1 \quad \ldots\ldots\ldots\ldots\ldots\ldots(1)$$

However, at any time,

$$\frac{X3}{X2} = \frac{k_3}{\underline{k}_3} = K3 \quad \ldots\ldots\ldots\ldots\ldots\ldots(2)$$

Substituting (2) in (1) for X2

$$\frac{-dX1}{dt} = \frac{k_1 X3}{K3} - \underline{k}_1 X1 + k_2 X3 - \underline{k}_2 X1$$

$$= X3 \left[\frac{k_1}{K3} + k_2 \right] - X1 (\underline{k}_1 + \underline{k}_2) \quad \ldots\ldots\ldots\ldots\ldots\ldots(3)$$

When the system is at equilibrium, $\frac{dX1}{dt} = 0$.

$$\text{i.e., } X3 \left[\frac{k_1}{K3} + k_2 \right] = X1 (\underline{k}_1 + \underline{k}_2) \quad \ldots\ldots\ldots\ldots\ldots\ldots(4)$$

Hence

$$\frac{X3}{X1} = \frac{(\underline{k}_1 + \underline{k}_2)}{\left(\frac{k_1}{K3} + k_2\right)} = K3 \left[\frac{\underline{k}_1 + \underline{k}_2}{k_1 + K3\ k_2}\right] = K3\ K1 = K2 \quad .....(5)$$

$$\text{Where} \quad K1 = \frac{\underline{k}_1 + \underline{k}_2}{k_1 + K3\ k_2} \quad .....(6)$$

Substituting for X3 in terms of X2

$$\frac{-dX1}{dt} = k_1\ X2 - \underline{k}_1\ X1 + K3\ k_2\ X2 - \underline{k}_2\ X1$$

when the system is at equilibrium, $\frac{-dX1}{dt} = 0$.

$$X2(k_1 + K3\ k_2) = X1\ (\underline{k}_1 + \underline{k}_2) \quad .....(7)$$

$$\frac{X2}{X1} = \left[\frac{\underline{k}_1 + \underline{k}_2}{k_1 + K3\ k_2}\right] = K1, \text{ from equation (6)}$$

$$\frac{X2}{X1} = K1 \quad .....(8)$$

Environment - Biota Equilibrium

For a detritus feeding organism, the concentration of the metal available for uptake by the organism would have contributions from the sediment as well as from the particulate and dissolved phases of the environment. The net concentration, thus available from the above different segments of the environment is defined as X, where

$$X \propto \Sigma\ X_i$$

i.e. $X = \gamma\ (X1 + X2 + X3)$ .....(9)

where γ is defined as the BIOPINDEX (the Biological Partitioning Index) which is a function of the bioaccumulation characteristic of the metal concerned.

Then the rate of accumulation

$$\frac{dX4}{dt} = k_4 X - \underline{k}_4 X4 - k_5 X4 \quad \text{.....(10).}$$

When the system is at equilibrium, $\frac{dX4}{dt} = 0$

$$\text{i.e. } \frac{X}{X4} = \frac{\underline{k}_4 + \underline{k}_5}{k_4} = K4 \quad \text{.....(11)}$$

$$\text{i.e. } \frac{\gamma\ (X1 + X2 + X3)}{X4} = K4 \quad \text{.....(12)}$$

Substituting for X2 and X3 in terms of X1 from equations (8) and (5) respectively,

$$X1 + X2 + X3 = X1 + K1X1 + K2X1$$
$$= X1(\ 1 + K1 + K2)$$
$$= X1\ A \quad \text{.....(13)}$$
$$\text{where } A = (\ 1 + K1 + K2\ ) \quad \text{.....(14)}$$

A is defined as the ENVOPINDEX (the Environmental Partitioning Index), which is characteristic of the environmental being considered.

Similarly, substituting for X1 and X3 in terms of X2 from

(8) and (2) respectively,

$$X1 + X2 + X3 = \frac{X2}{K1} + X2 + K3\ X2$$

$$= \frac{X2}{K1} \ (\ 1 + K1 + K1\ K3)$$

$$= \frac{X2}{K1} \ (\ 1 + K1 + K2)$$

$$= \frac{X2}{K1}\ A \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (15)$$

Again, substituting for X1 and X2 in terms of X3 from (5) and (2) respectively,

$$X1 + X2 + X3 = \frac{X3}{K2} + \frac{X3}{K3} + X3$$

$$= X3\ (\frac{1}{K2} + \frac{1}{K3} + 1)$$

$$= \frac{X3}{K2} \ (\ 1 + K1 + K2)$$

$$= \frac{X3}{K2}\ A \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (16)$$

Substituting (13) in (9)

$$X = \gamma\ X1\ A \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (17)$$

Substituting (15) in (9)

$$X = \gamma\ \frac{X2}{K1}\ A \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (18)$$

Substituting (16) in (9)

$$X = \gamma\ \frac{X3}{K2}\ A \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (19)$$

From equations (12) and (17)

$$\frac{X}{X4} = \frac{\gamma\ X1\ A}{X4} = K4 \quad \text{.....(20)}$$

From equations (12) and (18)

$$\frac{X}{X4} = \frac{\gamma\ X2\ A}{K1\ X4} = K4 \quad \text{.....(21)}$$

From equations (12) and (19)

$$\frac{X}{X4} = \frac{\gamma\ X3\ A}{K2\ X4} = K4 \quad \text{.....(22)}$$

or

$$\left.\begin{aligned} X1 &= \frac{K4\ X4}{\gamma\ A} \\ X2 &= \frac{K1\ K4\ X4}{\gamma\ A} \\ X3 &= \frac{K2\ K4\ X4}{\gamma\ A} \end{aligned}\right\} \quad \text{.....(23)}$$

Biological transport is the governing factor in the enrichment of trace metals into the shells. It has been shown (*vide* Chapter 3; Szefer, 1986), that the shell metal concentration and the soft tissue metal concentration maintain a definite ratio between them.

Hence $\frac{X4}{X5} = K5$, a constant. .....(24)

Substituting X4 from (24) in (23)

$$\left.\begin{aligned} X1 &= \frac{K4\ K5\ X5}{\gamma\ A} \\ X2 &= \frac{K1\ K4\ K5\ X5}{\gamma\ A} \\ X3 &= \frac{K2\ K4\ K5\ X5}{\gamma\ A} \end{aligned}\right\} \quad \ldots\ldots\ldots\ldots(25)$$

Results and Discussion

The model explained above was tested by fitting experimental data (X1, X2, and X3). The values of X1, X2, and X3, the concentrations observed at an estuarine and a riverine station were substituted in the relevant equations to obtain the values of the constants K1, K2, K3, $K4/\gamma A$, $K1K4/\gamma A$, $K2K4/\gamma A$ and K5 (Tables 22 & 23). The model was then tested by using these values of the constants along with those of X5, the metal concentrations in the shell, to calculate the metal (Cadmium, copper, nickel and lead) concentrations X1, X2, and X3 that ought to be present in the environmental compartments. These results (Tables 24 & 25) amply testify to the applicability of this model in both riverine and estuarine stations.

Experimentally determined cadmium concentrations, both riverine and estuarine stations were, in excellent agreement with the respective calculated values (>95% significance in all

Table 22. Computed kinetic constants (Estuarine station)

Metal	K1 (X2/X1)	K2 (X3/X1)	K3 (X3/X2)	K4/γA (X1/X4)	K1K4/γA (X2/X4)	K2K4/γA (X3/X4)	K5 (X4/X5)
Copper	0.000417	0.000663	2.213	0.3543	0.000108	0.000205	3.016
Cadmium	0.000901	0.001084	1.261	0.1471	0.000114	0.000137	1.382
Lead	0.000544	0.000252	0.7508	1.5127	0.000667	0.000285	0.1884
Nickel	0.000473	0.000149	0.3763	3.3992	0.001370	0.000413	0.2441

Table 23. Computed kinetic constants (Riverine station)

Metal	K1 (X2/X1)	K2 (X3/X1)	K3 (X3/X2)	K4/γA (X1/X4)	K1K4/γA (X2/X4)	K2K4/γA (X3/X4)	K5 (X4/X5)
Copper	0.000526	0.000603	1.355	0.3132	0.000137	0.000166	4.7984
Cadmium	0.000704	0.000795	1.288	0.0831	0.000054	0.0006	1.9534
Lead	0.000385	0.000757	2.714	0.9248	0.000327	0.000645	0.2196
Nickel	0.000345	0.000122	0.4372	2.4554	0.000775	0.000289	0.3395

Table 24. Observed and expected metal concentrations based on SAAMPLE (Estuarine Stations)

Metal	No.	X1 (Sediment)			X2 (Particulate)			X3 (Dissolved)		
		Observed $\mu g\ g^{-1}$	Expected $\mu g\ g^{-1}$	% Fitness	Observed $\mu g\ l^{-1}$	Expected $\mu g\ l^{-1}$	% Fitness	Observed $\mu g\ l^{-1}$	Expected $\mu g\ l^{-1}$	% Fitness
Copper	29	**6.01**	**6.36**		**2.00**	**1.94**		**3.26**	**3.68**	
		4.49 - 7.99	4.33 - 8.89	> 95%	0.77 - 4.03	1.32 - 2.44	> 95%	1.49 - 4.49	2.50 - 4.63	> 95%
Cadmium	30	**0.73**	**0.81**		**0.55**	**0.63**		**0.64**	**0.76**	
		0.50 - 1.53	0.68 - 0.95	> 95%	0.24 - 1.25	0.52 - 0.74	> 95%	0.28 - 1.68	0.63 - 0.88	> 95%
Nickel	28	**16.87**	**19.02**		**6.1**	**7.6**		**2.30**	**2.31**	
		11.66 - 21.76	16.93 - 22.22	> 80%	4.06 - 10.66	6.26 - 8.95	> 90%	1.40 - 3.70	1.88 - 2.69	> 95%
Lead	21	**10.04**	**11.91**		**3.46**	**5.25**	Not	**2.41**	**2.25**	
		6.37 - 15.26	10.41 - 13.36	> 80%	0.86 - 8.29	4.53 - 5.75	significant	1.20 - 3.85	1.93 - 2.51	> 95%

Table 25. Observed and expected metal concentrations based on SAAMPLE (Reverine Stations)

Metal	No.	X1 (Sediment)			X2 (Particulate)			X3 (Dissolved)		
		Observed $\mu g\ g^{-1}$	Expected $\mu g\ g^{-1}$	% Fitness	Observed $\mu g\ g^{-1}$	Expected $\mu g\ l^{-1}$	% Fitness	Observed $\mu g\ l^{-1}$	Expected $\mu g\ l^{-1}$	% Fitness
Copper	14	**7.32** 5.26 - 11.48	**8.22** 6.41 - 12.02	70%	**3.09** 1.88 - 5.76	**3.59** 2.80 - 5.26	> 95%	**4.12** 1.90 - 7.05	**4.35** 3.40 - 6.37	> 80%
Cadmium	14	**0.62** 0.48 - 0.78	**0.64** 0.49 - 0.80	95%	**0.36** 0.21 - 0.55	**0.41** 0.31 - 0.52	95%	**0.45** 0.28 - 0.66	**0.46** 0.36 - 0.57	95%
Nickel	14	**17.39** 13.12 - 27.87	**18.75** 16.40 - 22.23	80%	**5.35** 3.15 - 8.49	**5.91** 5.17 - 6.88	80%	**2.22** 1.40 - 3.63	**2.20** 1.93 - 2.62	95%
Lead	14	**8.44** 6.20 - 10.72	**8.52** 6.90 - 10.78	95%	**2.47** 1.47 - 6.13	**3.01** 2.44 - 3.81	80%	**3.72** 2.40 - 7.14	**5.94** 4.81 - 7.52	<50%

cases.) Copper showed very good (>95% significance) agreement between experimental and calculated values in the estuarine environment. In the riverine stations metal concentrations in the sediment and dissolved phases reflected a significance of 70-80% whereas that in the particulate phase compared much better with the corresponding calculated values (95% significance). Dissolved nickel concentrations agreed well with the predicted values in both the environments. The particulate nickel concentrations showed 90% and >80% significance respectively in the estuarine and riverine locations. Lead concentrations in sediment and the dissolved phase of the estuarine stations significantly tallied with the predicted values (>80% and >95% respectively); however, there was no agreement between lead concentrations in the particulate phases. In marked contrast, while lead concentrations in the sediment and particulate phases of riverine stations compared well (>95% and >80% respectively) with the predicted values, the concentrations of lead in the dissolved phase did not show any significant agreement.

Mathematical modelling was conceptually designed to predict the fate and effects of chemicals in the environment. Models transform a collection of disparate quantities (such as the partitioning, the reaction, and the transport data) into an overall behavioural profile that can be mentally assimilated. In a marked deviation from the use of environmental models as

mathematical tools for summarizing experimental data, we have attempted to develop a model characterized by its unique ability to predict past environmental history. Since metals incorporated in to the shells of organisms, are not lost even on death or decay of the organism, the shells provide a spectacular, one time, non erasable record of the past environmental conditions.

The Mussel Watch (Goldberg *et al.*, 1978) was an environmental monitoring programme launched with the goal of determining the degree of pollution in coastal marine waters. Tissues of mussels (preferably of those that remained attached to objects such as rocks, pilings etc.) were sampled and analysed to ascertain the level of pollutants, which would be a function of concentration in waters. Some bivalves like *M. edulis, P. viridis, V. cyprinoides* etc. which are rather widely distributed in the coastal waters have provided a common denominator for the study. Tissue analysis of bivalves have distinguished between the degrees of pollution in some of the several hundreds of locations sampled around the globe. The realistic capability of the Mussel Watch has, thus been to identify zones of high pollutant concentration - pollutant "hot spots".

Although the Mussel Watch enabled a comparison of the severity of pollution at various locations, it does notenvisage

a methodology for translating tissue levels into environmental levels and, therefore, does not afford a means of assessment of the actual environmental levels (of the pollutants) present at those sites.

The shell model fills up this vacuum and, for the first time provides a model that affords a translation between tissue levels and environmental levels. This novel predictive, mathematical tool, SAAMPLE, enables evaluation of metal levels in the different segments of the environment from a knowledge of the metal level in the bivalve shell, is based on two indices - BIOPINDEX and ENVOPINDEX - that reflect the nature of the metal partitioning in the biological and environmental segments respectively. These were initially evaluated for each metal from the values of metal concentrations present in the different compartments of an estuarine and a riverine station and were then used in SAAMPLE to compute the metal concentrations present in the different compartments at the other estuarine and riverine stations. The excellent agreement observed between experimental and computed values have amply proven the worth of the model in the assessment of aquatic trace metal pollution.

The "shell model" developed in this investigation is thus unique in that it enables evaluation of the metal levels that were once (during the life time of the organism) present in the

three constituent environmental compartments from knowledge of metal levels in the shells. Since the model is primarily based on metal concentration in the shell, it has a novel distinction of being able to reveal their respective environmental histories. The successful testing of the model in both estuarine and riverine environments confers on it applicability for use in fresh water and marine systems alike.

Chapter 6

SUMMARY

The concentrations of pollutants in different environmental compartments are related to each other through the action of intercompartment mass transfer processes. Such mass transfer processes decide the fate of pollutants, both organic and inorganic. Any environmental exposure analysis would be meaningful and realistic only if it encapsulates the contr i bution from the different compartments.The present investigation involves such a comprehensive approach, in which the water, the sediment and the biota (represented by bivalves) of the Cochin estuary have been collected and analysed in a single study. The salient result obtained from the study are summarized in the following paragraphs.

The shells of bivalves are important not only as the protective covering to the organism but also as accumulator of trace metal pollutants. Nevertheless the potential of the shells to unravel the complexities of the surrounding environment has not yet acquired proper momentum.

The inherent variability associated with the trace metal concentrations in the periostracum has been the major deterrent to progress in the systematic studies on shells. The cleaning procedure used in the present study was designed to ensure the total removal of the periostracum so that a rigorous estimate of the amount of trace metals incorporated into (the matrix of) the shells by biological transport could be made. Certain metals like lead, manganese and cobalt were observed to be preferentially accumulated in the shells than in the soft tissues. This was only to be expected from mineralogical considerations as well as from the standpoint of bio-mineralization processes. The infrared spectra analysis of the shells revealed the aragonitic nature of the shells of the different species of bivalves available in the Cochin estuary. Electron paramagnetic resonance spectra pointed to a geologically improbable substitution of Ca^{2+} ions by Mn^{2+} ions as the main pathway for incorporation of Mn^{2+} ions in the shells.

The distribution of the trace metals copper, cadmium, zinc, lead and nickel in the three environmental phases (sediment, dissolved and particulate) of the aqueous system and the two biological compartments (soft tissues and shells) was investigated in detail. The bioavailability of trace metals to

the bivalve *Villorita cyprinoides* var. *cochinensis* (Hanley) was evaluated by regression analysis of the various environmental variables and the biological factors. Enrichment factors, which reflected the enhanced or reduced vulnerability of the biota to metal concentrations, were made use of in the evaluation process.

Two new parameters Bio concentration Ratio (BCR) and Metal partitioning Ratio (MPR) were defined and used to predict metal bio availability. Out of the different chemically extractable sequential fractions of the sediment - metal levels, the exchangeable fraction was found to be more bio available than metal concentrations in other fractions considered separately or together.

The study has also enabled the development of a mathematical model, christened as **SAAMPLE** (Shellsin the Assessment of Aquatic Metal Pollution LEvels), for evaluating the concentrations of trace metal pollutants present in the different compartments of aqueous environment from a knowledge of the trace metal concentrations in the (relatively easy to handle) bivalve shells. The model was tested for estuarine and riverine locations and the agreement between calculated and observed values have been excellent.

The present study has, thus, been an integrated attempt at comprehending the complexities of metal partitioning among the different environmental as well as biological compartments of the aquatic system. Based on the studies on the transfer of pollutants from the environmental compartments to the biological compartments and on the dynamics of metals exchange between two biological compartments, the shells have emerged out as an excellent tool for evaluating environmental pollution levels - both past and present.

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