COMPETITIVE INTERACTIONS OF HYDROGEN AND ALUMINIUM IONS IN MODIFYING TRACE METAL AVAILABILITY TO AN ESTUARINE CLAM

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PREFACE

The long sweep of human history reveals an escalating trajectory of alterations and transformations of Earth - of the geosphere - biosphere that sustains life. This trajectory has not been uniform, but has been punctuated by spasms in the extent of change. The episodic outbreak of transformations that has characterised latter half of the century has occurred with such incredible magnitude, pace and variety that it has virtually eclipsed all earlier bouts of change: it has been truly global in scope has progressed with unprecedented speed and has wrought through up-heavals in the basic bio-geochemical fabric. Not withstanding all this human induced alterations of earth have raised complex issues that focused global environmental agenda.

Resolution of various issues emanating from the global metamorphosis is however, essential to the sustenance and development of a balanced ecosystem. Global environmental change includes both systematic changes that operate globally through the major systems of the geosphere - biosphere and cumulative changes that represent the global accumulation of localised changes. An understanding of the human dimensions of change requires attention to both types through research that integrates finding from spatial scales ranging from the global to the local. A regional or mesoscale focus represents a particularly promising avenue of approach.

This investigation is aimed at a study on the behaviour of aluminium in the Cochin estuary and the interactive effect of H^+ and Al^{3+} on the bioavailability of Cu, Cd and Hg to an estuarine clam, *Villorita cyprinoides* var *cochinensis* (Hanley). Estimation of the different forms of aluminium and of the variation of these different forms with respect to the physico-chemical parameters of the estuary have been the main focus of attention. The role of H^+ and Al^{3+} on the variation of toxicity of trace metals Cu, Cd, and

Hg to the test specimen, bioaccumulation of these metals and physiological and biochemical changes were systematically analysed vis-a-vis the interactive effect of H^+ and Al^{3+} on the health of this aquatic organism.

The results of this investigation are presented in six chapters. Chapter I provides a broad and general perspective on the aquatic acidification problem and trace metal pollution as well as an appreciation of the spectrum of the health effects produced by Cu, Cd, Hg and Al in biological systems. Chapter II describes the different experimental designs employed in this study and the procedural details of various parameters like LC50, filtration rate, metabolic rate, uptake and elimination of trace metals and various biochemical constituents. Chapter III surveys the behaviour of aluminium in the Cochin estuary and the variation of different Al fractions with physico-chemical parameters like salinity, pH, DOC, POC etc. This includes the descriptions of the speciation scheme employed and the experimental techniques applied for the measurement of various physico-chemical parameters. Chapter IV deals with the toxicity of different trace metals Cu, Cd, and Hg in presence of Al (under different pH) to the test specimen and also discusses the interactive effect of trace metals (at different pH) on the filtration rate and oxygen consumption of the test organism exposed to different experimental conditions. Chapter V focuses on the accumulation and depuration of trace metals by the test organism. Chapter VI reports on the biochemical effects of Cu, Cd and Hg and on the interactive effect of H^+ and Al^{3+} ions on them. The variations in glycogen, lactic acid, lipid, protein and ascorbic acid of the test specimen were measured with a view to using them as indicators of trace metal stress. A summary of the investigation and a bibliography follow Chapter VI.

CONTENTS

CHAPTER I	*INTRODUCTION*	1
CHAPTER II	*MATERIALS AND METHODS*	16
CHAPTER III	*BEHAVIOUR OF ALUMINIUM IN THE COCHIN ESTUARY*	28
CHAPTER IV	*TOXICITY, FILTRATION RATE AND METABOLIC RATE*	49
CHAPTER V	*BIOAVAILABILITY OF TRACE METALS*	85
CHAPTER V1	*BIOCHEMICAL EFFECTS OF TRACE METALS*	118
	SUMMARY	143
	REFERENCES	148

CHAPTER I

INTRODUCTION

The agricultural and industrial revolutions emerged from the need to manage the human environment: the environmental revolution now seeks to manage ecosystems to ensure its sustainable development. Earlier in this century, wanton disposal of wastes and the consequent alteration of ecosystems resulted in inhibiting the ecosystems from extending their innate services. Environmental risk assessment therefore is no longer an optional extra but forms a central and crucial aspects of human life on Mother Earth.

Aqueous acidification and trace metal pollution are serious water quality problems facing many regions of the world today. The problems associated with acidity of precipitation currently occupies a lot of interest (Drablos and Tollan, 1980; Watt Committee, 1984). It is claimed (Evans *et al.*, 1981) that acid rain is the principal cause of the acidification of soils and fresh waters observed in certain parts of the world. Acid deposition consists of delivering acidic substances or their precursors (principally sulfur and nitrogen oxides, acids and salts) from the atmosphere to the earth's surface. These compounds are introduced into the atmosphere in industrialised areas at rates that greatly exceed natural emission rates. Deposition processes include "wet deposition" (or familiarly "acid - rain") i.e., the delivery of material through precipitation, dry deposition (eddy transport followed by absorption, adsorption, adhesion or other processes) and impaction of cloud or fig droplets. In view of the possible large, social, economic, ecological and aesthetic consequences of acid deposition (Stephen, 1989), this phenomenon has become the subject of widespread concern. This concern is reflected in existing and pending legislation and regulations to reduce acid deposition by reducing emissions (McPoland, 1986).

Increased concentration of H^+ and trace metals resulting from acidification can be detrimental to all trophic levels (Haines,

1981) including benthic invertebrates (Kimmel *et al.*, 1985; Allard and Moreau, 1986; 1987). There are, however differences in the results between these studies as to the extent of specific invertebrate parameters altered at acidic sites (e.g., total invertebrate density and biomass, feeding group compositions and abundance). Lack of agreement between these studies may be as a result of several factors, differences in experimental approach viz., acidification of natural or artificial aquatic systems, comparison of existing acidic and non acidic aquatic systems (Kimmel *et al.*, 1985), and differences in monitoring of water chemistry parameters deemed important in altering benthic parameters viz pH only (Kimmel *et al.*, 1985; Allard and Moreau, 1987; Smith *et al.*, 1990). Additionally, species information may vary based on the geographic region studied. Clearly,then there is a need for further process level information of the relation between acidity and benthic invertebrates as well as an understanding of regional variation in these relationships.

At elevated H^+ ion concentrations, several inter-related physiological functions of aquatic organisms are impaired: namely Ca^{2+} and Na^+ regulation, acid-base balance and respiration (Fromm, 1980; Haines, 1981; Havas, 1981; Okland and Okland, 1986). Lowered oxygen consumption, a response often observed in fish exposed to low pH (Havas, 1981) was attributed by Packer (1979) to reduced O_2 carrying capacity of the blood and decreased oxygen transfer at the gill. The same may be applicable to aquatic invertebrates.

The wide spread regional acidification of lakes and streams is a relatively recent phenomenon; but one which is spreading and accelerating (Oden and Ahl, 1970; Grahn *et al.*, 1974; Brackke, 1976). A major concern is that acidified lakes are unable to support a diverse flora and fauna. The decline of fish populations attributed to acid precipitation was first reported in Norway (Leivestad *et al.*, 1976). Similar trends have been observed in Sweden, United States and Canada (Beamish and Harvey, 1972; Dickson, 1975; Schofield, 1976). While attention has focused primarily on fish, studies have also shown that phytoplankton,

zooplankton and benthic invertebrates have also decreased in diversity in acidified waters (Havas and Hutchinson, 1982). There is ample evidence to suggest that pH is a major variable in determining the distribution of species. For example, gastropods are very sensitive to low pH (Okland, 1969) as are blue green algae (Brock, 1973). The alga, *Euglena mutabilis,* in contrast, is only found in waters of extreme acidity (Whitton, 1975).

An important consequence of acidification is the mobilization of Al from the edaphic to the aquatic environment. Elevated levels of aluminium may have serious ramifications for biological communities (Driscoll *et al.*, 1980). Mortality during exposure to acutely toxic concentration of Al appears to result from severe necrosis of the gill epithelium. With the increased transport of Al to aquatic systems, there may be indirect effects upon phosphorus availability through increased inorganic precipitation of Al phosphates (Cronan and Schofield, 1979). Accelerated Al leaching also has implications for soil forming processes, the health of plant communities and clay mineralogy. These are the perturbing factors which prompted initiation of this study on the behaviour of Al in Cochin estuary.

Metals and their compounds are indispensable to the safety and economy of most nations and have been key factors in the liberation of modern civilization from hunger, disease and discomfort. 82% of the 108 known elements are metals and the number of commercial uses continue to grow with the development of modern science and technology. Inevitably, each industrial process generates wastes along with the ever growing list of new metallic compounds which are discharged into the environment. It has been estimated that the toxicity of all the metals being released annually into the environment exceeds the combined toxicity of all the radioactive and organic wastes. Metals are non-degradable and the continuous build up of such toxins in life support systems is of grave concern.

Trace metals are released into the environment from a wide spectrum of natural and anthropogenic sources. The principal anthropogenic sources of trace metals in the atmosphere are smelting of metallic ores, industrial fabrication and commercial applications of metals, as well as burning of fossil fuels. Metal pollution in soils is derived mostly from atmospheric fallout, coal-fly ash and bottom ash, urban refuse, animal wastes, agricultural wastes and food wastes. On the other hand, metals find their way into natural waters mainly through discharge of domestic and industrial (including mine and smelter) waste waters and the dumping of sewage sludge. For most metals the order of magnitude of input is soil > water > air.

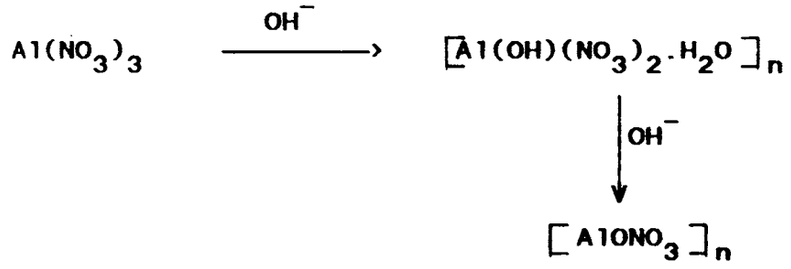
In pre-technological times, the cycling of each trace metal was more or less at a steady state and a tight control was maintained on its distribution in any given ecosystem. The anthropogenic inputs now however, have upset the natural biogeochemical cycles of trace metals in many ecosystems and have consequently resulted in the inevitable build up of such toxins in the human food chain. The increased environmental exposure is reflected in the elevations in the concentration of trace elements in body fluids and organ systems of contemporary populations. Some of the toxic metals specifically Cd and Hg, have no beneficial effects in humans nor are there any known homeostasis mechanism for them. Any long term exposure may therefore, lead to severe disruptions in the normal functioning of the organ systems where the metals get accumulated.

Much attention has been directed recently towards concentration and effects of trace metals in aquatic environments. Although, it is known that many metals are toxic to aquatic organisms, the exact pathway of the action of the metals and the levels at which they become harmful are still being investigated. The hazards of trace metal pollution have served to focus attention onto the presence of metals in aquatic animals. Al, Cu, Cd and Hg were chosen for the present investigation. The selection of these metals was based on their important health effects, on their

chemical nature and on their biochemical features. Although, Cu is the only recognized essential metal while the others are not. But there is some evidence that Cd may be required for animal growth (Schwarz and Spallholz, 1979). The chemistry and health effects of metals studied are discussed below.

1.1. ALUMINIUM

Aluminium exhibits trivalence and amphoteric character; its simple cationic and anionic salts are water-soluble. Aluminium forms binary salts of the type $MAl(SO_4)_2.12H_2O$ or M_2SO_4 $Al_2(SO_4)_3.24H_2O$ (where M is any mono or divalent metal): high atomic weight of the M ion confers great stability to Al salts. Halogen anion complexes such as $[AlF_6]^{3-}$ are stable and more soluble than CaF_2. Aluminium can exist as the central atom in salts such as $[K_9Al(MoO_4)_6].xH_2O$ and in hetero-6-polyacids $H_9[MO_6]$, where M is any trivalent metal such as Al. In dilute aqueous solutions and especially near neutral pH, simple cationic Al salts are hydrolyzed and olated. The olated compounds can be oxolated and the oxolated forms get aggregated into colloidal, water-insoluble, and chemically inactive hydrous oxides.

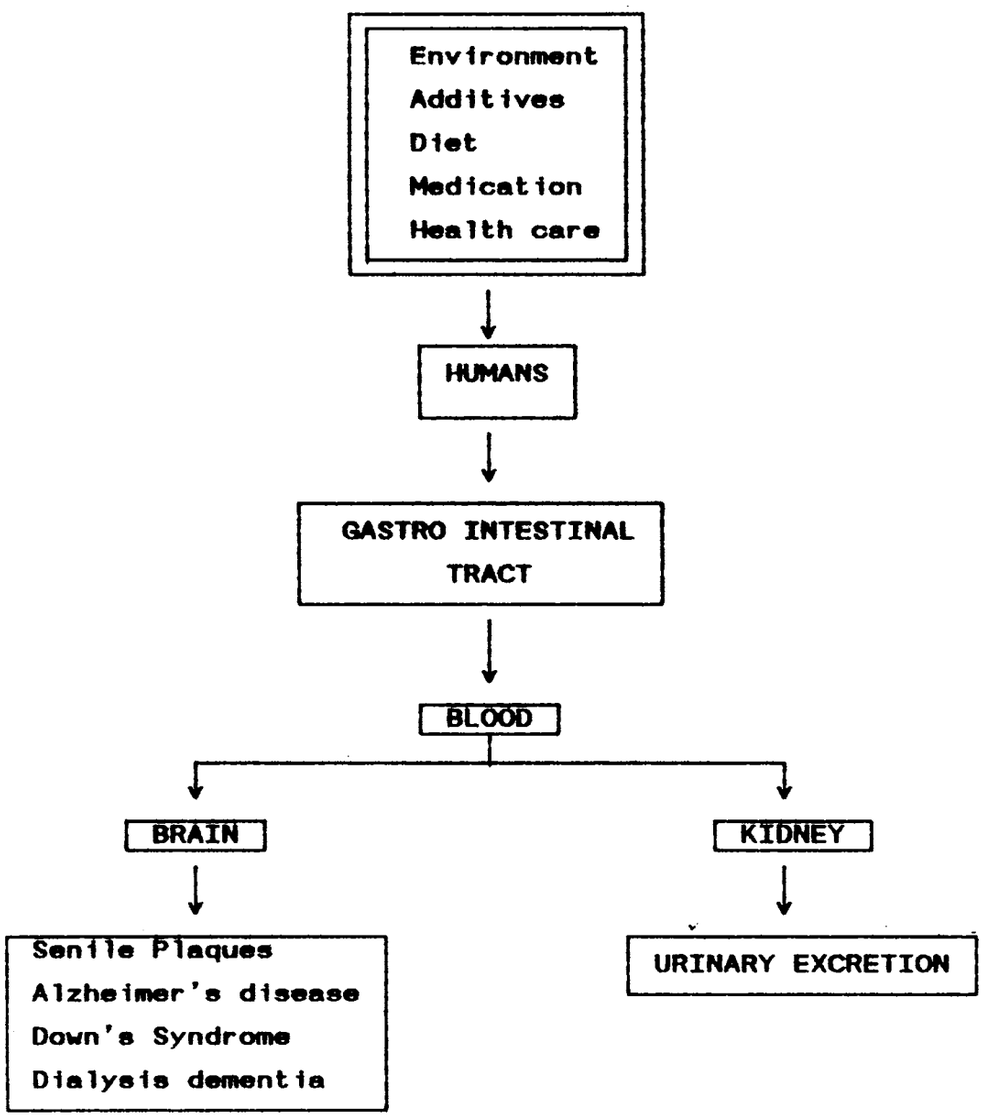


oxolated aggregate

The hydrous oxides can be peptized by formic, acetic, glycolic, and tartaric acids. Continued olation results in aggregate complexes with zero charge and decreased chemical activity. The solubility and chemical reactivity of Al compounds

change in the gastro-intestinal tract, where the pH ranges from 2 to 7.1. Enzymes, co-factors, and phosphates present in the digestive tract and in food also affect the solubility of Al compounds. Aluminium has a high affinity to both free and bound phosphate groups in biological macro molecules, and is capable of forming complex compounds with proteins.

Aluminium pathways in humans



No other metal is more exposed to materials meant for human consumption than Al. During the last decade, there has been an increased interest in the biological importance of Al because of evidence for the systematic toxicity of the element and the development of accurate analytical techniques. Al is used in building and vehicle constructions and in the manufacture of paint, electrical equipment, packaging containers and cooking utensils. It is used therapeutically as an antacid, in drinking water purification and as an antiperspirant. The effects of Al intake are well documented (Shore and Wyatt, 1982; Wisniewski *et al.*, 1982; Wurtman, 1986) and the indications are that it is neuro-toxic. Kidney dialysis with Al rich solutions is known to cause dementia (Alfrey *et al.*, 1976). Rabbits and cats injected with solutions containing aluminium salts develop symptoms of neuro-toxicity and the metal is found to accumulate in the neural tissue (Wisniewski *et al.*, 1982). Accidental implantation of metallic Al in the brain has been reported to result in complications leading to epilepsy (Foncin and El Hachimi, 1986). Recently it was found that senile plaque cores of patients with Alzheimer's type of dementia contain abnormally high levels of Al (Candy *et al.*, 1986).

Involvement of Al was found responsible for the defective formation of egg shells and impairment in wild passerine birds (Nyholm, 1981).

Although, no convincing evidence has been presented, Krishnan *et al.* (1988) have reported that Al is known to be toxic to brain tissue. It has been suggested that the localisation of aluminium at the calcification fronts in bone would have a direct inhibitory effect on the calcification mechanisms. Renal osteomalacic osteodystrophy (aluminium bone disease) was first reported in 1973 and an elevation of the aluminium content in the bone was associated with this disorder (Cameron *et al.*, 1988).

In the past decade tissue storage (human) of as yet unidentified aluminium complexes has been associated with at least two human syndromes occurring in patients with chronic renal failure — dialysis encephalopathy and osteomalacia (Alfrey *et al.*, 1976; Pierides *et al.*, 1981). In domestic animals, high concentrations of dietary aluminium have been accompanied by malabsorption of fluoride, phosphate and magnesium (Allen and Robinson, 1980). The rationale for extending information gained from one population at risk from environmental pollution (such as fresh water fish) to other population that may be similarly exposed has previously been established (Quehee *et al.*, 1979).

Bioaccumulation of aluminium in viscera, gills and other fish parts has been demonstrated in carp which survived a 48h exposure to inorganic aluminium salts alone or in the presence of EDTA or NTA (Muramoto, 1981). There may also be long term effects with respect to bioaccumulation of Al. For example, an unidentified aluminium complex that accumulates in bone marrow tissue of wild passerine birds has been implicated in the reproductive failure of this species, when it feeds on insects at the shorelines of acidified, aluminium contaminated lakes (Nyholm, 1981).

The relevance of continuous exposure experiments in understanding the episodic exposures assumes significance in the context of Al being a major determinant of response by aquatic organisms. This is the importance of the present study.

Aluminium in the environment - Summary of representative values
(Jones and Bennett, 1986)

	Concentration
Atmosphere	
Urban	1000 ng m^{-3}
Rural	200 ng m^{-3}
Lithosphere	
Agricultural soil	70000 µg l^{-1}
Hydrosphere	
Fresh water (dissolved)	50 µg l^{-1}
Ocean (dissolved)	2 µg l^{-1}
Biosphere	
Terrestrial plants	100 µg g^{-1}
Foods	8 µg g^{-1}
Man	
Body burden	60 mg
Serum/plasma	7 µg g^{-1}
Tissues	3 µg g^{-1}
Transfer rates	
Intake	
Ingestion	20 mg day^{-1}
Inhalation - urban	15 µg day^{-1}
- rural	3 µg day^{-1}
Absorption	
Gastrointestinal tract	0.01%
Lungs - retention	0.35%
- absorption	0.03%

1.2. COPPER

The predominant oxidation states of Cu are +1 and +2. Cu (III) is unstable in solution. Cu^{+} forms several kinds of polynuclear complex in which four Cu atoms lie at the vertices of a

tetrahedron. Cuprous ion can also form co-ordination compounds with co-ordination numbers 2 and 3 having linear and planar structures. The d^9 configuration makes Cu^{2+} subject to Jahn teller distortion if placed in an environment of cubic (regular, octahedral or tetrahedral) symmetry and this has a proportional effect on all its stereochemistry. Cu^{2+} forms co-ordination compounds with co-ordination numbers 4, 5 and 6.

Cu is present in a number of metalloenzymes and other proteins. The protein binding capacity of Cu is high, it confers stability and/or maintains the conformation of proteins. Copper metalloenzymes are involved in oxidation - reduction reactions with oxygen as the electron acceptor when copper participates directly in the electron trnsfer. The cupric ion is present in phenolases and other oxidases, and predominates in oxygen carriers and enzymes reacting directly with molecular oxygen. Cuprous ions are oxidised to the stable cupric state by peroxide accumulation in the tissues; however, Cu^{2+} can bind with -SH containing compounds and reversibly react to form Cu^{+} and disulfide. Copper oxidase functions through a cyclic shuttling between the valence states of copper with a continuous uptake of molecular oxygen.

The range between deficiency and toxicity of Cu is wide for mammals, although it is narrow for bacteria and fungi, and Cu is highly toxic to aquatic organisms. Symptoms of chronic copper poisoning in mammals include nausea, vomiting, epigastric pain jaundice etc., and those of acute copper toxicity include sporadic fever, tachycradia, hypotension, oliguria, uremia, coma, cardiovascular collapse and death. Inhalation of dusts and fumes of metallic Cu and its salts causes congestion of nasal mucous membrane. Contact dermatitis and eczemetom dermatitis caused by internal exposure to Cu in the form of intrauterine devices have been reported (Venugopal and Luckey, 1978).

The mechanism of copper toxicity in mammals is complex. It involves increased cellular permeability in erythrocytes with consequent lysis inhibition of glutathione reductase and loss of

intercellular reduced glutathione agglutination and an excessive stimulation of the hexose monophosphate shunt. These lead to oxidative stress in erythrocytes and to accelerated loss of intercellular reduced glutathione. Copper ion induces mitochondrial swelling and inhibits oxygen consumption. The affinity of Cu^{2+} to -SH groups of hemoglobin erythrocyte, and other membranes increases the permeability and lysis of erythrocytes. Copper increases the frangility of lysosomal membranes and releases acid hydrolases causing cell degeneration. Subcutaneous bleeding and anemia in animals are caused by failure of collagen formation in the walls of arterioles in copper deficient animals. A high incidence of cancer among copper smiths and of stomach cancer in people living in regions with a high Cu:Zn ratio in the soil are suggestive of a carcinogenic capacity of Cu (Venugopal and Luckey, 1978).

1.3. CADMIUM

Cadmium, like mercury, is easily vaporisable, and Cd^{2+} is similar to Ca^{2+} in ionic size and charge. Normally, Cd exhibits a maximum valence of 2+, and forms stable cationic salts. However, with its large number of inner electrons, Cd has a tendency to form outer orbital complexes with less electronegative elements, involving covalent bonds. Cadmium forms tetrahedral complexes with a coordination number of 4. It forms halogen complexes such as $[CdX_3]^-$ and $[CdX_6]^{4-}$; Cd is also amphoteric and forms stable salts such as sodium cadmate, $Na_2[Cd(OH)_4]$ in which the hydroxyl groups can be replaced by water and monovalent groups. Cadmium resembles zinc in its electronic configuration and affinity toward organic ligands, but it has greater affinity than zinc to thiol groups and will replace zinc in some metal enzyme complexes. However, Zn is bound more tightly than Cd to oxygen and nitrogen containing ligands. The binding of Cd with S is stronger than that of any essential metals except Cu; among the toxic metals, only Hg and Pb bind more strongly with S ligands than Cd. Cadmium has a high affinity toward hemoglobin and metallothionein, a protein present in mammalian kidney and liver. Cadmium binds to nucleotides *in vitro* and can presumably bind with nucleic acids. It

forms stable chelates with some of the common chelating agents *in vitro* but not *in vivo*. Cadmium could not be removed from tissues with chelating agents which can mobilize zinc from tissues; apparently it is bound very firmly in the tissues.

Inseparable in nature, Cd and Zn are paradoxical pair— one is a threat to life and one is required for it. Cadmium is similar to the heavier essential metals in the ability to bind with various organic complexes. Cd binds more tightly to sulphur donors than Zn, which probably underscores the toxic effects in biological systems. Living organisms have evolved a defense against small amount of Cd contamination through a metal binding proteins (Friberg *et al.*, 1971). However, with rising levels of Cd intake, this defense mechanism can be overtaxed. The presence of this toxic element in vital metal ores, in fossil fuels and phosphate fertilizers, and in manufactured products from which it is virtually unrecoverable portends an increasingly polluted environment which is and will continue to be a potential hazard to all living organism.

Cadmium is an insidious poison. It accumulates in the kidney. Chronic poisoning produces proteinuria and affects the proximal tubules of the kidney causing the formation of kidney stones. As with arsenic, the toxic effects of cadmium have been attributed to its action on sulphhydryl groups of essential enzymes. Japanese workers have described a specific distressing disease, associated with multiple fractures, attributed to Cd. Cd possesses cardiotoxic properties (Carmignani *et al.*, 1983).

Cd has a deleterious effect on most if not all, marine species tested (Newman and MaClean, 1974). Invertebrates are more sensitive to Cd than are finfish (Eisler, 1971).

1.4. MERCURY

Mercury, the heaviest among the transition elements, exhibits valences of +1 and +2 and forms stable cationic salts; the divalent salts are more soluble in water than are the monovalent (mercurous)

salts. The solubility of mercury in water is about 0.02 mg l^{-1} and in body fat 0.6 - 2.7 mg l^{-1}. Hg is volatile at room temperature and some Hg^{2+} salts sublime. Mercury cannot form stable ring complexes because the bond angle of Hg is 180^{o}. Electrons in the inner orbitals of the Hg atoms are involved in coordinate covalent bonds; the more common coordination number is 4 and the geometry of the Hg coordination complexes is tetrahedral. Mercury complexes with primary and secondary amines and forms halogen complexes such as $[Hg\ X_3]^{-1}$ and $[Hg\ X_4]^{2-}$. The affinity of Hg toward reactive groups is: $-SH > -CONH_2 > -NH_2 > -COOH > PO_4^{3-}$. Among the metals, Hg has the greatest affinity towards thiol groups. In biological fluids and tissues, the thiol groups of proteins and other compounds bind the available Hg ions by forming reversible complexes. This binding causes protein agglutination, inhibition of thiol groups containing enzymes, and clumping of erythrocytes.

The metabolism and physiological behaviour of elemental Hg vapour, inorganic Hg salts such as $HgCl_2$ and $Hg(NO_3)_2$, organic alkyl Hg salts such as $CH_3Hg^{+}Cl$ and organic mercurials such as diphenyl mercury and mercurochrome differ greatly according to their chemical structure and ionization.

Mercury has been added steadily to air and water since the beginning of geologic time through the process of weathering of mercury-laiden rock add by volcanic activity (Klein and Goldberg, 1970). Alkyl mercuric compounds have been used in increasing amount in both agriculture and industry and an estimated 4000 to 5000 tons of mercury per year are discharged into various bodies of water (Johnes and Westermark, 1969). Ultimately much of this reaches the estuarine and marine environments. Hg concentration in coastal marine organisms may be several orders of magnitude greater than the surrounding sea water (Klein and Goldberg, 1970). The highly toxic character of corrosive sublimate (mercuric chloride) and other soluble compounds of Hg has been known at least since the middle ages and there are numerous records of death caused by mercury poisoning. Inorganic salts of mercury taken by mouth lead to bleeding of the intestinal tract, kidney injury, suppression of

urine and ultimately death from kidney failure. Since Hg combines with sulphhydryl groups, soluble mercury salts are toxic to all cells. The dangers to health associated with the industrial wastes have been greatly accentuated by selective uptake of mercury from the water by marine organisms and the capacity of marine organisms to convert mercury into the much more toxic and highly volatile dimethyl mercury and into methyl mercury salts. In regard to the second characteristic, biological methylation, mercury appears to be unique.

Al, Cd, and Hg can adversely affect human reproduction (Nriagu, 1988). Metal-induced immuno suppression often occurs at low doses which elicit no evident toxicity in the organism. Al, Cu and Cd and their compounds have been shown to be initiators or promotors of carcinogenic activity in animals (Jennette, 1981; Babich *et al.*, 1985).

1.5. BIOLOGICAL INDICATOR

Bioindicators or sentinel organisms are species employed to quantify the biologically available levels of conservative contaminants in aquatic ecosystems. Selection of the species to be used as bioindicators requires the consideration of several inter related factors (Phillips, 1977; 1980). These include the metabolic regulation of pollutants by bioindicators, pollutant-interactions in bioindicators, the tissue integration capacity as well as degree to which bioindicators can reflect contamination in a sampling area.

The understanding of natural fluctuations of metal concentration in a bioindicator is indispensable for a good assessment of the disturbance due to pollution. Numerous monitoring programs such as the "mussel watch" recommend the use of filter feeder molluscs as indicators of pollution. The genus, *Villorita cyprinoides* var *cochinensis* (Hanley) has been found to offer most of the features of a biological indicator such as non-migrant nature with long life, reasonable size, easy sampling, fairly

abundant distribution, tolerance to brackish waters and ability to concentrate numerous pollutants (Babukutty, 1991; Sujatha, 1992). Hence *Villorita cyprinoides* var *cochinensis* (Hanley) was chosen as the test organism for this investigation.

1.6. OBJECTIVES AND SCOPE OF THE PRESENT STUDY

It is the paucity of information on the interactive effects of H^+ and Al^{3+} ions on the bioavailability of Cu, Cd and Hg which prompted initiation of this investigation. Objectives of this study were four fold:

- to investigate the relationship between various Al fractions and the different physico-chemical parameters like salinity, pH, dissolved oxygen, dissolved organic carbon, particulate organic carbon, and suspended matter in the Cochin estuary

- to assess the interactive effect of H^+ and Al^{3+} on the toxicity of Cu, Cd and Hg to the test organism and to the physiological features such as filtration rate and oxygen consumption of the test organism

- to study the interactive effect of H^+ and Al^{3+} on the bioavailability of Cu, Cd and Hg to the test specimen and

- to obtain a deeper insight into the biochemical changes (glycogen, lactic acid, lipid, protein and ascorbic acid) triggered by sublethal levels of Cu, Cd and Hg occurring at varying pH and Al^{3+} ion concentrations.

This study is a modest attempt at focusing attention on the hitherto unexplored behaviour of Al in the Cochin estuary and on the pH-dependence of Al^{3+} on the toxicity and bioavailability of Cu, Cd and Hg to a common estuarine clam, *Villorita cyprinoides* var *cochinensis* (Hanley).

CHAPTER II

MATERIALS AND METHODS

The present investigation was primarily intended to assess the interactive effects of H^+ and Al^{3+} on the bioavailability of Cu, Cd and Hg to an estuarine clam, *Villorita cyprinoides* var *cochinensis* (Hanley) and also to ascertain the metal induced responses of the biochemical constituents. The details of the materials used and the experimental techniques employed are presented in this Chapter.

2.1. TEST ORGANISM

Villorita cyprinoides var *cochinensis* (Hanley) is a purely brackish water species and it is capable of tolerating a wide range of salinity upto a maximum of 34×10^{-3} (Prashad, 1921; Satyamurti, 1960) and it is a cheap source of protein rich food and a raw material for the manufacture of cement and lime. It is found in parts of the backwaters nearer to the barmouth, wherever the bottom deposit consists of sand and silt. Large specimens were obtained during December to April and young one during August and September when the salinity was comparatively low. The optimum habitat salinity of the clam has been reported to be 13×10^{-3} (Nair and Shynamma, 1975). Specimens of *Villorita cyprinoides* var *cochinensis* (Hanley) were obtained from Kumbalam, a place along the southern part of the Cochin estuary which is considered to be a relatively pollution free zone. In order to avoid differences in respiration, only individuals of approximately the same length (25-30 mm) were used. Specimens from the collection site were maintained in polyethylene tubs of 50 l capacity containing constantly aerated, filtered water of the habitat salinity.

In the laboratory, the animals were cleaned free of sediment and of other detrital matter and then transferred into large perspex tanks of 50 l capacity and were then acclimated to the laboratory conditions (temp. = $30 \pm 1^{o}C$, salinity = 10×10^{-3}, DO >

80% saturation and pH = 7.6) by aeration for 48h. The animals were fed daily with blue green algae *Synechocystis salina* prior to 24h of the commencement of the experiment.

2.2. EXPOSURE MEDIUM

Seawater collected ≈ 8-10 km off Cochin barmouth was filtered through 0.45 μm glass fibre filter paper and stored in the dark for 10 days. After appropriate dilution using deionized water to obtain the required salinity (≈ 10 x 10^{-3}), this was aerated to saturation prior to use. The pH of the solution was adjusted using 2N H_2SO_4 and 2N NaOH. The different pH values of the exposure media employed in this study were 5.5, 6.0, 6.5, 7.6 and 9.0. The pH was maintained throughout this study with a deviation of ± 0.01.

2.3. METAL SOLUTIONS

Stock trace metal solutions of Cu and Cd were prepared from 99.9% pure metals (BDH-Analar grade). The oxide layers on the surface of the metals were cleaned off using 0.1N HNO_3 and the purified metals were then dried using acetone. 1g each of the metals was dissolved in the minimum amount of 1:1 HNO_3. Any excess acid present was carefully evaporated off, the solution was cooled and then diluted to 1000 ml with milli-Q water (low in organics, inorganics, benthic and particulate matter).

1000 ppm stock solutions of mercury and aluminium in milli-Q water were prepared using mercuric chloride and aluminium sulphate (BDH-Analar grade) respectively. The stock solutions were later diluted to the concentrations required for the experiments.

The concentrations of the metals selected for the acute lethal toxicity experiments as determined from the preliminary range-finding tests were given in Table 2.1. The sublethal concentrations of Cu, Cd, Hg and Al used in this study (for investigation on filtration rate, metabolic rate, bioaccumulation

and variation in biochemical parameters) were arrived from the acute mortality results and are given in Table 2.2.

Table 2.1. Concentrations (ppm) of metals used for lethal toxicity studies.

Copper	Cadmium	Mercury	Aluminium
0.50	1.00	0.25	0.30
0.75	2.00	0.50	1.00
1.00	4.00	1.00	
2.00	8.00	2.00	
5.00	10.00	4.00	

Table 2.2. Concentrations (ppm) of trace metals used for sublethal studies.

Copper		Cadmium		Mercury		Aluminium	
Cu-1	Cu-2	Cd-1	Cd-2	Hg-1	Hg-2	Al-1	Al-2
0.05	0.10	0.10	0.50	0.05	0.10	0.30	1.00

2.4. ACUTE LETHAL TOXICITY STUDIES

The short term acute toxicities of trace metals to the test organism were determined at different pH by measuring the 120h-LC50 values using static renewal bioassay technique in accordance with the procedures recommended by Ward and Parrish (1982).

Ten animals of the required size group (25 mm ± 2 mm) were maintained in 5 l of filtered seawater (salinity = 10 $\times 10^{-3}$) at the required pH in specially manufactured dye-free polyethylene troughs of 10 l capacity. The pH of the exposure medium was kept constant throughout the exposure period. Calculated volumes of metal solutions were added individually to maintain the required concentrations of toxicants. A minimum of five test concentrations of the metals was set up in each experiment besides the respective

controls. The test medium was renewed daily with seawater having the similar conditions. The troughs were not aerated or the animals fed during the experiments. The mortality of the animals was noted every 12h (an animal was considered dead when the valve gap was at least 4 mm and when there was no response to gentle prodding) and the 120h LC50 values were calculated from the cumulative percentage mortalities using log probit method (Litchfield and Wilcoxon, 1949). The experiment was repeated thrice and the mean value was calculated for each metal at a particular pH. The different pH used were 5.5, 6.0, 6.5, 7.6 and 9.0. There was no mortality of the test specimen exposed to Al^{3+} upto 10 ppm. Then the influence of Al^{3+} on the LC50 values of trace metals were determined by repeating the above experiments in presence of Al-1 (0.3 ppm) and Al-2 (1.0 ppm) for each metal at different pH. LC50 values were calculated as above.

2.5. SUBLETHAL STUDIES

Sublethal studies involved the exposure of test organism for a period of 120h to sublethal concentrations as represented in Table 2.2 at different pH and the measurement of filtration rate, metabolic rate, accumulation of trace metals, and biochemical parameters.

The clams were acclimatised as mentioned earlier and used in the sublethal studies. Ten clams of the size group 25 ± 2 mm were then carefully transferred into a 10 l pre-cleaned plastic trough containing 5 l of seawater having the required experimental conditions (metal concentrations at different pH). The troughs were neither aerated nor were the animals fed during the experiments. The salinity and other characteristics of the test media were kept constant. The test medium was renewed daily and characteristics retained. The different experimental conditions used in the present investigation are presented as follows (Table 2.3).

Table 2.3. Experimental conditions during sublethal studies.

Exposure medium	Metal concentration		
1. Copper	Cu-1	Cu-1+Al-1	Cu-1+Al-2
	Cu-2	Cu-2+Al-1	Cu-2+Al-2
2. Cadmium	Cd-1	Cd-1+Al-1	Cd-1+Al-2
	Cd-2	Cd-2+Al-1	Cd-2+Al-2
3. Mercury	Hg-1	Hg-1+Al-1	Hg-1+Al-2
	Hg-2	Hg-2+Al-1	Hg-2+Al-2
4. Aluminium	Al-1		
	Al-2		
pH of the test media: 5.5, 6.0, 6.5, 7.6 and 9.0			

The pre-exposed animals were sampled at different intervals for a period of 120h for the determination of the sublethal parameters.

2.5.1. Filtration rate

The filtration rate was measured with the standard suspension of neutral red, 3-amino 7-dimethyl amino 2-methyl phenonzine hydrochloride (Badman, 1975). A concentrated solution of neutral red (AR-BDH) was prepared in deionised water to obtain a concentration of 1 mg l^{-1} of the dye. Three pre-exposed animals were then carefully transferred into a pyrex glass beaker containing 400 ml of filtered seawater (salinity 10 x 10^{-3}) previously aerated to saturation and containing the dye and the pH of the system was maintained at the pH of the pre-exposure medium. Measurements were taken after an initial period of 1h and after making sure that the animals were filtering actively. 10 ml of the test solution was withdrawn at 30 minute-intervals for 3h and the concentration of the dye in each solution was determined by

measuring the optical density at 530 nm for the experiments at pH 5.5, 6.0 and 6.5 and at 435 nm for the experiments at pH 7.6 and 9.0. The absorbances were measured on a Hitachi (150-20) UV-VIS spectrophotometer. The rate of filtration was calculated using the equation,

$$m = \frac{M}{n\ t} \log \frac{C_o}{C_t}$$

where M is the volume of the test solution, n the number of animals used in the experiment, C_o the initial concentration of the dye, C_t the concentration of dye at time t and m the rate of filtration in ml h^{-1} $animal.^{-1}$ The animals were removed from their shells at the termination of the experiment and dried to constant weight by keeping the tissue sample at 70 to 80°C for 48h. Filtration rates were then expressed in ml h^{-1} g^{-1}. The maximum value obtained was taken as the filtration rate. The experiment was repeated five times and the filtration rate was expressed as the mean value ± standard deviation. The concentration range of the dye, the $\lambda_{max.}$ of the dye at different pH, time limit employed and the sampling interval of 30 minutes were decided on the basis of pilot experiments.

The filtration rate of the test animals pre-exposed to the different experimental conditions as given in the Table 2.3 at 0 h, 24h, 48h, 72h, 96h and 120h were determined as mentioned above and the values are presented in the Table.4.3.

2.5.2. Metabolic rate

Three test animals each, pre-exposed to the selected experimental conditions (Table 2.3) for 0h, 24h, 72h, 96h and 120h were kept in conical flasks of 1 l capacity containing 1000 ml of filtered seawater of salinity 10 x 10^{-3} after initial saturation by aeration and kept at the same pH of the pre-exposure medium. The oxygen content of the water in the conical flasks was measured initially. Gas exchange from the atmosphere was prevented by

sealing the flask with liquid paraffin. The duration of the experiment was 3h and the frequency of the sampling was 30 minutes. The oxygen contents of the water sample collected at 0h (O_i) and at 30-minute intervals (O_t) upto 3h were estimated in triplicate by Winkler's method (Grasshoff, 1983). The oxygen consumed by the clams was determined as follows:

Oxygen consumed by the clams = $(O_i - O_t)$

After the experiments, soft tissues of the clams were scooped out, cleaned in distilled water and dried to constant weight at 70-80°C. The metabolic rate was expressed as ml O_2 h^{-1} g^{-1} (dry weight).

2.5.3. Accumulation and depuration studies

Five animals pre-exposed to different experimental conditions (Table 2.3) were sampled after 0h, 24h, 48h, 72h, and 120h in each experiment and their whole soft tissues were scooped out, washed with deionised water and pressed between the folds of the filter paper. These were then dried to constant weight at 70-80°C and used for digestion and estimation of different trace metals. The experiment was repeated in triplicate.

Depuration studies were conducted by transferring the animals after 120h accumulation (under different experimental conditions for 120h) into filtered seawater (salinity = 10 x 10^{-3}, pH = 7.60 ± 0.01, D.O > 80% saturation, temp. = 30 ± 1°C) and maintaining them for a further period of 120h. The whole tissues of the animals were sampled at 24h intervals. As in the accumulation study, the samples were dissected out, washed using deionised water, and pressed between the folds of the filter paper and dried to constant weight at 70-80°C. The dried material was powdered and stored in a desiccator till the analyses of the metals were carried out.

2.5.4. Digestion procedure for Cu, Cd, Hg and Al

A definite amount of the dried and powdered soft tissue (0.1 - 1 g) was digested in a Kjeldahl's flask using concentrated HNO_3 and $HClO_4$ in the ratio 3:1 (v/v) (Martincic *et al.*, 1984). Teflon vessel was used for samples containing Al. After pre-heating, the samples were digested for 8h. The solutions were then cooled and made upto 25 ml using milli-Q water.

For the determination of mercury, a definite weight of the dried and powdered sample was refluxed for 8h with concentrated nitric acid and concentrated sulfuric acid in the ratio 4:1 (v/v) as recommended by BITC (1976) using the Bethge apparatus (Shaw and Panigrahi, 1986). The solution was cooled and made upto 25 ml using 0.6N HNO_3.

2.5.5. Estimation of Cu, Cd, Hg and Al

Cu, Cd and Al were estimated by atomic absorption spectrophotometry using Perkin Elmer 2380 model instrument by directly aspirating the sample into air-acetylene flame for Cu and Cd and N_2O - acetylene flame for Al. Small amount of KCl was added to the sample containing Al before estimation. The Al contents were also cross checked against pyro catechol violet method (Grasshoff, 1983).

Mercury was estimated using an atomic absorption spectrophotometer (Perkin Elmer 2380) having a hydride generation system (MHS-10) as an accessory. The metal solutions were first reduced in alkaline sodium borohydride (3% sodium borohydride solution prepared in 0.1% sodium hydroxide) and aspirated to the AAS for the estimation of Hg.

2.5.6. Biochemical parameters

The biochemical constituents namely glycogen, lactic acid, lipid, protein and ascorbic acid of *Villorita cyprinoides* var

cochinensis (Hanley) exposed to different experimental conditions were determined by the standard procedures.

For the determination of the biochemical parameters, 2 animals were taken from each of the three experimental troughs containing a particular metal concentration. The whole soft tissues were pooled together to compensate for any individual effects, homogenised and dried between the folds of the filter paper. Each of these was later sub-sampled and then analysed for the different biochemical parameters.

a) Glycogen

The glycogen content of the clams was estimated by the method of Pluger, modified by Hassid and Abraham (1957). A known weight of the bivalve soft tissue was dissolved in potassium hydroxide (3 ml of 30%) and digested by placing the tube containing the sample in a boiling water bath for 20-30 minutes. 0.5 ml of saturated sodium sulphate solution was added and the glycogen was precipitated by the addition of 95% alcohol. It was then centrifuged for 20 minutes (5000 rpm). Decant off the mother liquor and the precipitate was redissolved in 2 ml water and reprecipitated with 2.5 ml of 95% ethyl alcohol. Again decant off the supernatant liquid and the purified glycogen was hydrolysed to glucose by refluxing with 6 ml of 0.6N HCl in a water bath for 3h and the sugar was estimated by Heath and Barnes (1970) method. The concentration of sugar thus obtained was converted to glycogen using the conversion factor 0.93. The glycogen thus obtained was expressed as mg glycogen g^{-1} of the soft tissue. The mean value and standard deviation were presented in the Table 6.1 (n = 10).

b) Lactic acid

Lactic acid content in the whole soft tissues of the clam was estimated by the method of Barker (1957) in which the lactic acid was converted to aldehyde by heating with concentrated sulphuric acid and then complexed with p-hydroxy diphenyl reagent. The soft

tissue of the pre-exposed animal was dried between the folds of the filter paper, weighed and homogenised with trichloro acetic acid (10 ml 10%) and purified sea sand. The mixture was centrifuged and the supernatant liquid was treated with copper sulphate solution (1 ml 20%). This solution was then diluted to 10 ml, shaken well with 1 g calcium hydroxide powder and kept aside for 30 minutes and again centrifuged. Duplicate aliquots of the supernatant liquid (1 ml) were pipetted out into clean test tubes and chilled after addition of 1 drop of 4% $CuSO_4$ solution. This was then treated with sulphuric acid (3 ml of 6%) and allowed to hydrolyse by placing in a water bath. After cooling, the solution was treated with 3 drops of 1.5% p-hydroxy diphenyl reagent, kept aside for 30 minutes and the absorbance was measured at 560 nm. Lithium lactate was used for the preparation of standard curve and the lactic acid content in the whole soft tissue was expressed as mg lactic acid g^{-1} of the tissue.

c) Total lipid

The sulpho phosphovanilin method (Barnes and Blackstock, 1973) was used for the estimation of lipid. A definite amount of the soft tissue was extracted thrice with 2:1 chloroform-methanol mixture. The above extract was mixed with 0.2 ml of 0.9N sodium chloride in a test tube and was then capped with non-absorbent cotton and was allowed to equilibrate overnight at 4-5^{o}C. It was then transferred to a separating funnel and the lower layer was drained into a clean test tube. After adding concentrated sulphuric acid (0.5 ml), the solution was warmed for 10 minutes in a boiling water bath, cooled and 5 ml phosphovanilin reagent was added. Shaken well and the optical density was measured at 520 nm. Standard graph was prepared using cholesterol to calculate the lipid concentration and the lipid content thus obtained was expressed as mg lipid g^{-1} of the soft tissue.

d) Protein

The protein content of the soft tissue of the test specimen exposed to different experimental designs was estimated by the modified Folin-Ciocalteu method (CMFRI, 1982). Protein was extracted from approximately 20 mg of the bivalve soft tissue using sodium hydroxide (10 ml of 0.1N) solution and the extract was made upto 25 ml using double distilled water. To 1 ml of the sample, 5 ml alkaline copper reagent was added followed by 5 ml of potassium antimonyl tartrate and mixed well. The optical density was measured at 500 nm after 30 minutes. Bovin serum albumin was used as the standard. Blanks were run for each batch of the experiments. Protein content in the whole soft tissue was expressed as mg protein g^{-1} of the soft tissue.

e) Ascorbic acid

The ascorbic acid content of the soft tissue of the clam was determined by following the procedure described by Roe (1954). The soft tissue of the clam pre-exposed to different experimental conditions (Table 2.3) was weighed accurately and homogenised with 6 ml of 5% TCA. It was then centrifuged and after the extraction 100 mg acid washed norit was added to the extract and shaken well and extracted by centrifugation. To 1.5 ml of the supernatant, 0.5 ml of freshly prepared 2,4-dinitro phenyl hydrazine thiourea reagent (prepared by adding 0.4 g thiourea, 0.05 g $CuSO_4$ $5H_2O$ and 3 g 2,4-dinitro phenyl and brought to a total volume of 100 ml with 9N sulphuric acid) was added and incubated at 37°C for 3h. After the incubation, the tube was transferred to a beaker containing ice water. To each tube, 2.25 ml of ice cold 85% sulphuric acid was added drop by drop to avoid sudden rise in the temperature. The tubes were shaken well. The blank was conducted using 1.5 ml milli-Q water and following all other procedures. After 30 minutes the tubes were brought to the room temperature and the optical density was measured at 520 nm. The pure ascorbic acid was used as the standard and the ascorbic acid content was expressed as mg ascorbic acid g^{-1} of the soft tissue.

Unless otherwise stated extra pure BDH and EMerck chemicals were used in this investigation. Hitachi 150-20 UV-VIS spectrophotometer was used to measure the optical densities of all the samples. Cell to Cell and blank corrections were employed for all sets of readings.

2.8. STATISTICAL ANALYSIS

ANOVA, Regression and Correlation matrix were used for the comparison of the interactive effects due to different metal concentrations at different pH and period of exposure.

CHAPTER III

BEHAVIOUR OF ALUMINIUM IN THE COCHIN ESTUARY

Estuaries must be regarded as dynamically evolving land forms that go through a life cycle from valley creation, followed by the drowning phase and ending up with the progressive back fillings with sediments. Estuaries are ephemeral in respect to geologic time and the origin of most modern estuaries dates back to the rise of the sea level after last ice age. Furthermore, estuaries are generally subjected to large inputs of contaminants due to the fact that industrial areas and cities are generally located adjacent to them. Estuaries are the interface between the continents and the oceans and with regard to the movement of trace metals along the hydrological cycle, the question arises how efficient are the estuaries and the coastal zones in trapping the particulate and dissolved metals. This question is especially important for assessing the inputs of trace metals from natural and anthropogenic sources into the world oceans.

The major elemental composition of the ocean can be better understood with a knowledge of elemental mass balances and estuarine activity. The constancy of the chemical composition of seawater requires a removal of river-introduced dissolved constituents (Krauskopf, 1956; Mackenzie and Garrels, 1966). Compared with the behaviour of metals in the oceans or lakes the estuarine system is more complex and dynamic due to the strong gradients in chemical composition of water, variable suspended matter concentrations and complex hydrodynamic processes.

Removal process in estuaries may therefore be expected to significantly influence the chemical balance between the rivers and oceans. The removal of trace inorganic elements like Al should be more accentuated than that of the major elements like Na, K, Ca, Mg

and Cl, since the concentrations of the former constituents are generally higher in river water than in seawater.

Eventhough considerable work has been done on the hydrography of Cochin estuary (Sankaranarayanan and Qasim, 1969; Sankaranarayanan *et al.*, 1986; Anirudhan and Nambisan, 1990; Nair, 1992; Shibu, 1992) detailed accounts of inter-relationship between various hydrographical parameters and Al are yet in deficiency. The aluminium chemistry of surface waters is of interest for the following reasons:

1. Certain forms of the metal mostly, Al^{3+}, and its monomeric hydrolysis products and freshly precipitated aluminium oxyhydroxides are toxic to fish and biota (Muniz and Leivestad, 1980; Dickson, 1983; Helliwell *et al.*, 1983).

2. The concentration and chemical speciation of Al are sensitive to deposition catchment interactions and therefore provide a means to assess rates and extents of acidification by atmospheric pollutants (Johnson *et al.*, 1981; Reuss and Johnson, 1986).

3. Hydrolysis of Al may control solution pH and may result in the formation of (oxy) hydroxides with adsorptive properties (Driscoll, 1985).

4. Interaction of Al species with dissolved organic matter may influence the latter's solubility and thereby, surface tension of water (Hall *et al.*, 1984).

5. Adsorption of Al species by particles may affect aggregation, sedimentation and deposition of the suspended particles in streams and lakes.

Aluminium is the most abundant metallic element in the earth's crust (8.1 wt %) and it is the most widely used non-ferrous metal because of its important features —— low density, high electrical and thermal conductivity, high reflectivity and corrosion resistance. However, since aluminium is very reactive, the free

metal is not found in nature. As a group III element, Al occurs only in the trivalent state. The aluminium ion is capable of four-fold coordination with oxygen and forms many of the same kinds of compounds as silicon. The activity of Al^{3+} in soil and ground waters is largely controlled by precipitation/dissolution reactions and can be estimated from pH, total Al and activities of F^- and SO_4^{2-} (EPRI, 1984). Gibbsite, $Al(OH)_3$ has a common control on the solubility of aluminium minerals in many geochemical environments at lower pH values and at higher sulphate concentration. Ion exchange process is an important retention mechanism for aluminium in acid to neutral pH regimes (EPRI, 1984). The concentration of aluminium in natural waters depends primarily on pH and the presence of complexing agents. Although, aluminium is an abundant element, it rarely occurs in natural water having concentration greater than a few tenths of a milligram per litre as long as the pH is nearly neutral. The typical concentration in seawater has been reported as 0.01 ng l^{-1} and is probably present as $Al(OH)_4^-$, AlF_2^+ and AlF^{+2}. However, in US surface fresh water the mean concentrations of suspended and dissolved aluminium measured were at 3860 $\mu g\ l^{-1}$ and 74 $\mu g\ l^{-1}$ respectively (Singer, 1974). While the suspended form was observed in 97% of the samples, the dissolved Al was detected in only 31% of the samples. The concentration of Al in river waters can vary significantly with flow rate. Al readily precipitates in natural waters to form particulate or colloidal hydroxides; however, under turbulent conditions of high flow it can be redissolved from suspended minerals, perhaps aided by complex formation (Singer, 1974).

Al is readily soluble at pH <4. High concentrations of Al have been detected in acid mine waters, acid sulphate soil waters, acid geothermal waters and poorly buffered lakes, streams and ground waters that receive large inputs of acid runoff (Odonnel, *et al.*, 1984). In aqueous solution, Al^{3+} does not occur as the free ion, but is surrounded by six molecules of water to form $Al(H_2O)_6^{3+}$. As the pH increases, protons are progressively removed from the coordinated water molecules to give the hydrolysis products shown below.

$$Al^{3+} + H_2O \rightleftharpoons AlOH^{2+} + H^+$$

$$Al^{3+} + 2H_2O \rightleftharpoons Al(OH)_2^+ + 2H^+$$

$$Al^{3+} + 3H_2O \rightleftharpoons Al(OH)_3 + 3H^+$$

$$Al^{3+} + 4H_2O \rightleftharpoons Al(OH)_4^- + 4H^+$$

$$Al^{3+} + 5H_2O \rightleftharpoons Al(OH)_5^{2-} + 5H^+$$

$$2Al^{3+} + 2H_2O \rightleftharpoons Al_2(OH)_2^{4+} + 2H^+$$

The most important forms of dissolved aluminium are Al^{3+}, $Al(OH)_2^+$ and $Al(OH)_4^-$. Each species predominates over a certain pH range:

Al^{3+}	:	<4.0
$Al(OH)_2^+$	:	5-6
$Al(OH)_4^-$	:	>7.0

Aluminium also shows a strong tendency to form polymers and ultimately the neutral mineral, gibbsite is precipitated (Odonnell *et al.*, 1984). The polymerisation of aluminium hydroxide species is affected by the presence of dissolved silica. When enough silica is present, the aluminium is rapidly precipitated as poorly crystallized clay mineral species. Certain anions like SO_4^{2-}, F^- etc., form strong aqueous complexes that can dominate solution speciation at higher ligand concentrations (EPRI, 1984). AlF_2^+ and AlF^{2+} are most likely in natural water containing fluoride ion concentration ranging from a few tenths of mg l^{-1} to a few mg l^{-1} (Hem, 1970). Soluble phosphate complexes of aluminium have been reported and the sulphate complex $AlSO_4^+$ may predominate in acid solution in the presence of elevated sulphate levels. The influence of phosphate on aluminium is not clear. Polymeric aluminium orthophosphates are known (Callis *et al.*, 1954). Again,

the stability and degree of aggregation depend primarily on pH. The most stable compound is probably $Al(PO_4)$, which has a solubility product of 6.3×10^{-19} (Odonnell *et al.*, 1984).

When various ligands compete for Al, the distribution of aluminium species depends mainly on the concentration of the ligands and on pH (OH^- is a ligand as well). For instance, in a solution containing fluoride in excess of aluminium, almost all the aluminium would be associated with fluoride ions below neutral pH. Above neutral pH, hydroxy complexes would dominate under suitable conditions.

3.1. Al SPECIATION

Speciation of an element is the process yielding evidence of the atomic or molecular form of an analyte (IUPAC). It is now well established that speciation measurements are necessary for the understanding of trace metal transport in rivers and estuaries and for the study of toxicity of metals on aquatic organisms. Natural waters provide a favourable environment for speciation studies because of the prevailing variable chemical matrix and the variety of metal forms that may exist there. There have been three major approaches to the elucidation of the speciation of metals in natural waters (Harrison, 1987):

a) Modelling studies based upon the knowledge of stability constants of ion pairs and metal complexes

b) Laboratory studies on simplified systems

c) Analytical studies of real water samples

Because of the complexity of natural waters, the third approach has been adopted in the present study to establish the real nature of the distribution of Al in the Cochin estuary. The modified fractionation scheme including a cation exchange step adopted is a combination of methods used by Driscoll *et al.* (1980)

and Ramamoorthy (1988). Seven different Al fractions were determined:

- Total dissolved aluminium (acid digested) —— TDA
- Total monomeric aluminium —— TMA
- Filterable cation exchangeable Al (Non labile) —— FCA
- Filterable anion exchangeable Al —— FAA
- Particulate aluminium —— PA
- Labile aluminium —— LA
- Colloidal aluminium —— CA

3.2. COCHIN ESTUARY —— LOCATION

The Cochin estuary, the longest along the south west coast of India extends between 09°40' — 10°10' N and 76°15'— 76°30'E. The estuary has a length of about 70 km and the width varies between a few hundred meters to about 6 km. It covers an area of 300 km^2 and falls under the category of a tropical positive estuary (Pritchard, 1952). Since the year 1976, the hydrographical conditions of this estuary have undergone extensive changes by way of (i) inter-basin transfer of water from river Periyar to river Muvattupuzha to facilitate a hydroelectric project (ii) construction of a salt water barrier (Thanneermukkom bund) on the southern part of the estuary and (iii) extensive dredging and harbour operations. Depending upon the degree of mixing between the fresh water and salt water, the estuary may vary from a well-mixed type to a stratified type (Sankaranarayanan *et al.*, 1986).

Because of the existence of various chemical industries on the northern part of the estuary, six Stations (Fig. 3.1) were fixed along the 15 km from the barmouth to the riverine region of the river Periyar. Stations 1 and 2 were located in the fresh water zone of the river Periyar. Station 3 was situated 100 m down-stream of the effluent discharge area of chemical industries and Station 4 was in the middle of this part of the estuary near *Cheranellore*. Station 5 was adjacent to a coconut husk retting

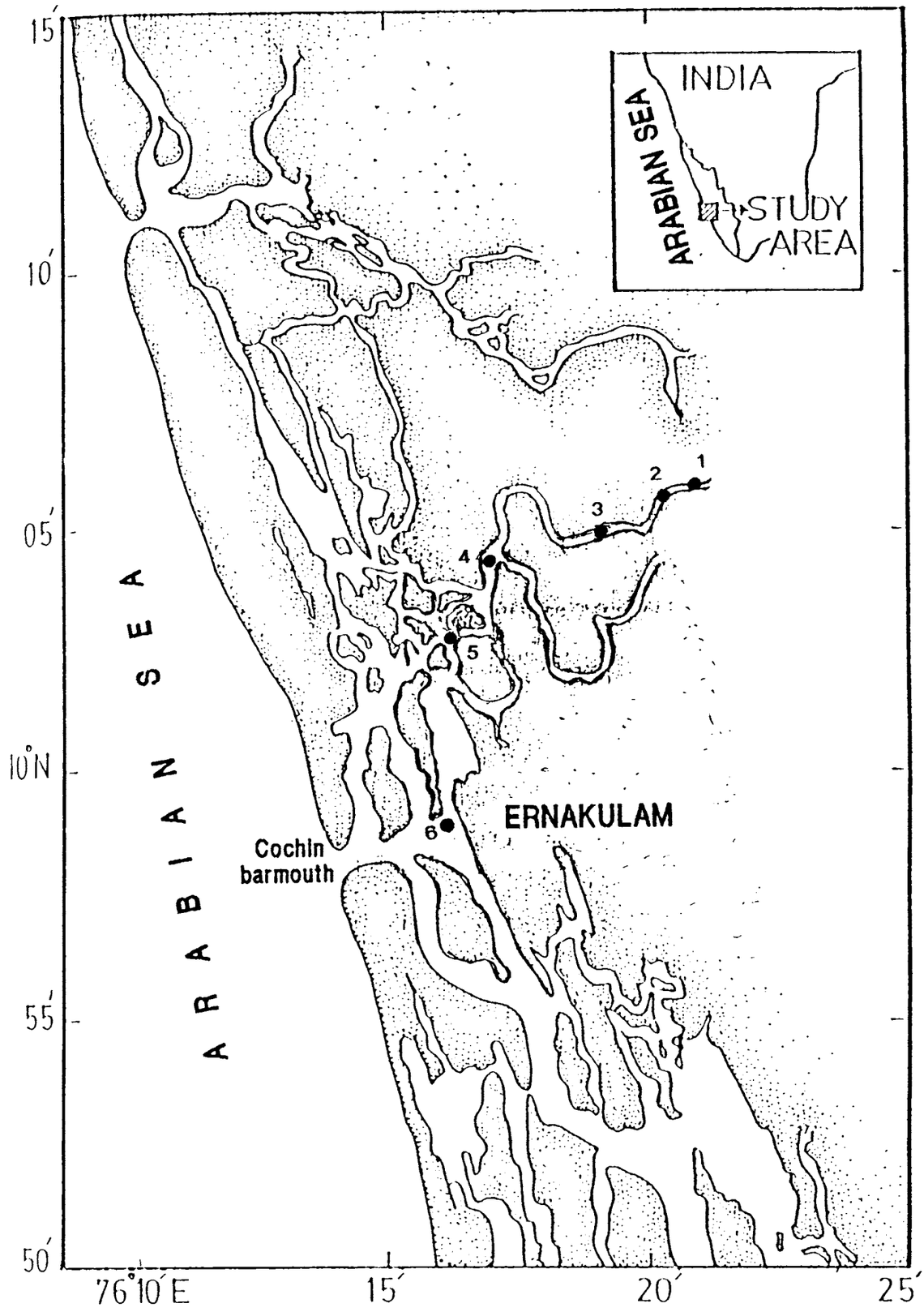


Fig. 3.1. Map of the Cochin estuary showing location of sampling sites.

ground near *Vaduthala* and was about 4 km distant from Station 4 and Station 6 represented the confluence region and was close to the barmouth. Bimonthly samplings were conducted using a fibre glass dinghy to eliminate any metal contamination arising from the vessel. The sampling period extended for 11 months from February 1992 to December 1992 and spanned all the three seasons —— pre-monsoon (February - April), monsoon (June - August) and post-monsoon (October-December). All samplings were timed to coincide with the phase of ebb tide in the estuary.

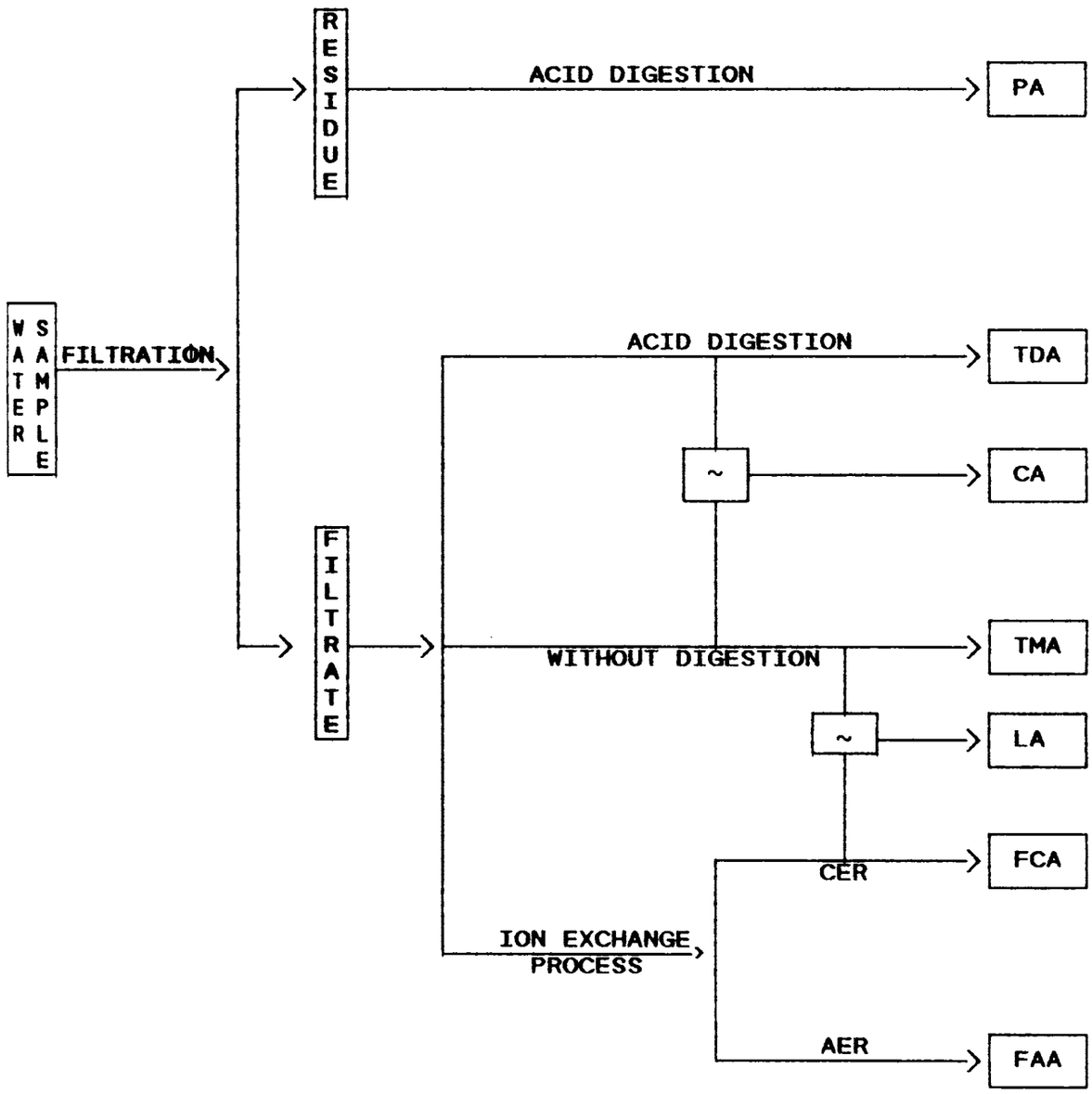
3.3. COLLECTION AND TREATMENT OF SAMPLES

Water samples (10 l) were collected (0.5 m below the surface) using a pre-cleaned, and acid washed teflon water sampler. Samples were transfered to polyethylene bottles which were filled to the top and tightly closed to avoid degasing of CO_2. The samples were filtered immediately after collection, through acid washed, pre-weighed, 0.45 μm Whatmann membrane filters. The filtered samples were stored at $-4^{o}C$. The particulates retained on membrane filters were dried to constant weight at $40^{o}C$.

3.4. EXPERIMENTAL PROCEDURE

The following scheme was adopted for the speciation of aluminium.

Aluminium speciation scheme



1. Total Dissolved Aluminium (TDA): The total dissolved aluminium was determined after acid digestion of the filtered water. 200 ml water sample was digested using a mixture of $HClO_4$, HNO_3 and HCl for about one hour.

2. Particulate Aluminium (PA) : The particulate aluminium fraction was obtained by the strong acid digestion of the particulate matter using a mixture of $HClO_4$, HNO_3 and HCl.

3. Total Monomeric Aluminium (TMA): Filtered samples analysed for aluminium without acid digestion was called total monomeric aluminium.

4. Filterable Cation exchangeable Aluminium (FCA): The fraction retained by the cation exchange column was called filterable cation exchange aluminium.

5. Filterable Anion exchangeable Aluminium (FAA): The fraction retained by the anion exchange column was called filterable anion exchange aluminium.

6. Labile Aluminium (LA): The difference between total monomeric aluminium and filterable cation exchange aluminium was the labile aluminium fraction.

7. Colloidal Aluminium (CA): The difference between the aluminium in the acid digested water sample and the total monomeric aluminium was the colloidal aluminium fraction.

Amberlite IR-120(H) and Amberlite IR-45(OH) were used as the Cation Exchange Resin (CER) and the Anion Exchange Resin (AER) respectively in this study. The resin was swelled in deionized water prior to use and it was taken in the chromatographic column.

The water sample (3 l) was filtered through a 0.45 μm Whatman membrane filter paper to remove the particulate matter if any, the filtrate was used for the determination of the total monomeric aluminium. The supernatant water (filtrate) free from particulate matter was eluted through the CER column and collected in a conical flask. This eluate was free from filterable cation exchange Al. The

CER column was then eluted with 0.1M nitric acid solution and collected in a polypropylene bottle. This was used for the determination of the FCA. The eluate free from FCA was eluted through the AER column and collected in a polypropylene bottle with milli-Q water rinsing. The eluate finally obtained is free from both cationic and anionic Al species. The AER column was eluted with 0.1M NaOH solution and collected in a polypropylene bottle. This was used for the determination of the FAA. The labile aluminium fraction was calculated by subtracting the value of FCA from the total monomeric Al (without acid digestion).

Aluminium analysis was carried out by pyrocatechol violet method (Grasshoff *et al.*, 1983). PCV reacts slowly with aqueous aluminium even in the absence of competing ligands in solution. Short reaction times (4 minutes) have been used by some investigators (Seip *et al.*, 1984) in order to minimise reaction of PCV with polymeric aluminium and adsorbed aluminium, but in general large reaction times (10 minutes) are preferred because the rate of colour development is then much lower and experimental errors are minimised (Goenaga and Williams, 1988). The magnitude of total Al measurement was cross-checked against atomic absorption spectrophotometry (APHA, 1985). Aqueous samples were prepared by addition of H_2O_2 (2%) to prevent carbide formation during the atomisation step and 1% nitric acid was added for analysis using AAS to prevent the loss of Al by sublimation during the ashing step and to minimise adsorption losses during storage of sample before analysis.

3.5. PHYSICO-CHEMICAL PARAMETERS

The physico-chemical parameters likely to influence the concentration and distribution of Al species in the Cochin estuary along with predominant controlling factors are summarised below:

Parameter	Analytical method	Controlling factor
1.Temperature	Hg in glass thermometer (1/10°C)	Climate
2.Particulate matter	Filtration through 0.45 μm filter paper (gravimetric)	Weathering, discharge,tidal action & productivity
3.Salinity	Strickland and Parsons (1977)	River discharge and tidal action
4.Dissolved oxygen	--do--	Productivity & reaeration
5.pH	Using a Philips pH meter	Ionic strength
6.Dissolved organic carbon	APHA (1985)	River input and anthropogenic activity.
7.Particulate organic carbon	El Wakeel and Riley (1957)	Fluvial transport, urban run off and productivity

3.6. RESULTS AND DISCUSSION

The study on the physico-chemical parameters of the Cochin estuary is of great importance in assessing the distribution of Al. The variations in the physico-chemical parameters are shown in Fig. 3.2 for comparison and the absolute values are given in Table 3.1.

The temperature in this estuary varied between 32.5°C during pre-monsoon and 26.2°C during monsoon. The maximum value was

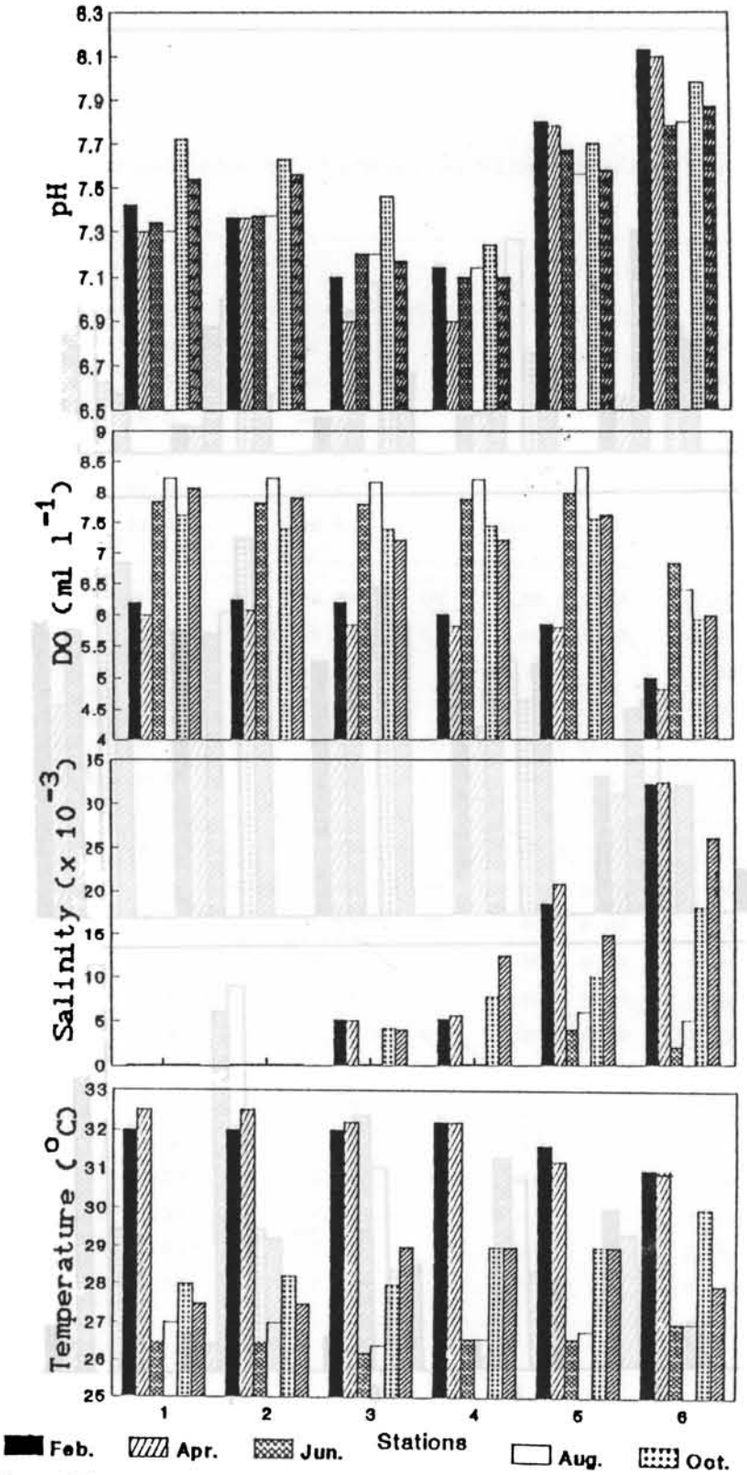


Fig. 3.2. Bimonthly distributions of hydrographical parameters in the Cochin estuary.

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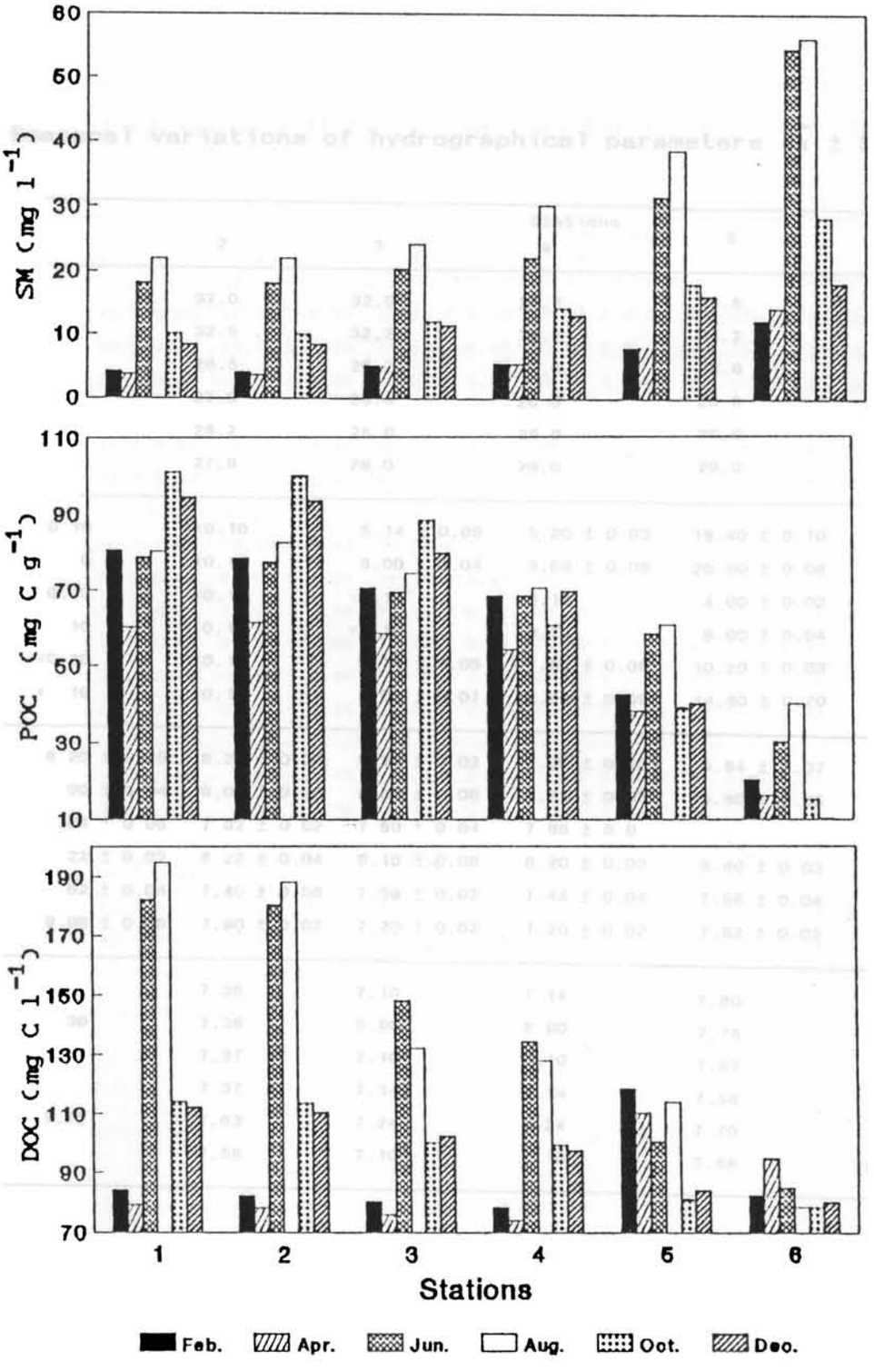
SM (mg l-1)
POC (mg C g-1)
DOC (mg C l-1)
Stations
1
2
3
4
5
6
Feb.
Apr.
Jun.
Aug.
Oct.
Dec.

Fig.3.2

Table 3.1. Seasonal variations of hydrographical parameters ($\bar{x}$ ± SD, n = 6)

Parameter	Months	Stations 1	2	3	4	5	6
Temperature	Feb.	32.2	32.0	32.0	32.2	31.6	31.0
(^{o}C)	Apr.	32.5	32.5	32.2	32.2	31.2	31.0
	Jun.	26.5	26.5	26.2	26.6	26.6	27.0
	Aug.	27.0	27.0	26.4	26.6	26.8	27.0
	Oct.	28.0	28.2	28.0	29.0	29.0	30.0
	Dec.	27.5	27.6	29.0	29.0	29.0	28.0
Salinity	Feb.	<0.10	<0.10	5.14 ± 0.05	5.20 ± 0.03	18.40 ± 0.10	32.20 ± 0.07
($\times 10^{-3}$)	Apr.	<0.10	<0.10	5.00 ± 0.04	5.60 ± 0.08	20.80 ± 0.08	32.40 ± 0.12
	Jun.	<0.10	<0.10	<0.10	<0.10	4.00 ± 0.02	2.00 ± 0.09
	Aug.	<0.10	<0.10	<0.10	<0.10	8.00 ± 0.04	5.00 ± 0.03
	Oct.	<0.10	<0.10	4.12 ± 0.05	7.80 ± 0.08	10.20 ± 0.03	28.00 ± 0.05
	Dec.	<0.10	<0.10	4.00 ± 0.07	12.40 ± 0.09	14.80 ± 0.20	28.00 ± 0.04
Dissolved	Feb.	6.20 ± 0.05	6.24 ± 0.08	6.20 ± 0.02	6.00 ± 0.02	5.84 ± 0.07	4.98 ± 0.03
Oxygen	Apr.	6.00 ± 0.04	6.08 ± 0.04	5.84 ± 0.06	5.82 ± 0.08	5.80 ± 0.05	4.82 ± 0.04
($ml\ l^{-1}$)	Jun.	7.84 ± 0.08	7.82 ± 0.02	7.80 ± 0.04	7.88 ± 0.02	7.98 ± 0.02	6.82 ± 0.04
	Aug.	8.22 ± 0.02	8.22 ± 0.04	8.16 ± 0.08	8.20 ± 0.02	8.40 ± 0.03	6.40 ± 0.04
	Oct.	7.62 ± 0.04	7.40 ± 0.08	7.38 ± 0.02	7.44 ± 0.04	7.56 ± 0.04	5.92 ± 0.07
	Dec.	8.08 ± 0.08	7.90 ± 0.02	7.20 ± 0.02	7.20 ± 0.02	7.62 ± 0.02	5.98 ± 0.05
pH	Feb.	7.42	7.36	7.10	7.14	7.80	8.13
	Apr.	7.30	7.36	6.90	6.90	7.78	8.10
	Jun.	7.34	7.37	7.10	7.10	7.67	7.78
	Aug.	7.30	7.37	7.14	7.14	7.56	7.80
	Oct.	7.72	7.63	7.24	7.24	7.70	7.98
	Dec.	7.54	7.56	7.10	7.10	7.58	7.87

(Contd...)

Table 3.1. Seasonal variations of hydrographical parameters ($\bar{x}$ ± SD, n = 6)

Parameter	Months	Stations 1	2	3	4	5	6
DOC	Feb.	84.12 ± 2.24	82.01 ± 6.24	80.14 ± 3.52	78.12 ± 5.64	118.40 ± 4.52	82.40 ± 4.27
(mg C l^{-1})	Apr.	79.34 ± 4.20	78.12 ± 4.28	76.12 ± 6.48	74.14 ± 8.24	110.22 ± 5.26	94.62 ± 2.24
	Jun.	182.40 ± 6.24	180.40 ± 3.54	148.12 ± 5.48	134.42 ± 3.20	100.10 ± 4.26	84.74 ± 1.84
	Aug.	194.60 ± 3.54	188.22 ± 7.21	132.30 ± 9.80	128.12 ± 5.40	114.12 ± 5.24	78.62 ± 2.14
	Oct.	114.16 ± 6.24	113.42 ± 3.56	100.10 ± 9.42	99.12 ± 2.10	81.12 ± 3.24	78.43 ± 1.34
	Dec.	112.23 ± 3.28	110.12 ± 5.42	102.20 ± 8.40	97.34 ± 5.42	84.12 ± 3.48	80.12 ± 1.24
POC	Feb.	80.20 ± 1.02	78.17 ± 2.44	70.23 ± 0.54	68.20 ± 1.24	42.34 ± 1.24	20.10 ± 0.24
(mg C g^{-1})	Apr.	60.24 ± 0.05	61.24 ± 2.24	58.30 ± 0.64	54.20 ± 1.48	38.20 ± 2.42	18.20 ± 0.24
	Jun.	78.46 ± 0.54	77.14 ± 1.48	69.14 ± 0.47	68.14 ± 2.46	58.12 ± 3.68	30.12 ± 0.54
	Aug.	80.12 ± 0.86	82.12 ± 1.68	74.12 ± 0.14	70.20 ± 0.84	60.40 ± 2.86	40.14 ± 0.63
	Oct.	101.01 ± 5.40	100.14 ± 2.49	88.12 ± 1.42	80.40 ± 1.46	39.10 ± 5.46	15.14 ± 0.28
	Dec.	94.26 ± 6.21	93.14 ± 1.24	79.34 ± 0.84	69.45 ± 0.54	40.12 ± 2.68	10.20 ± 0.14
SM	Feb.	4.12 ± 0.08	4.02 ± 0.12	5.24 ± 0.04	5.48 ± 0.12	7.82 ± 0.04	12.20 ± 0.12
(mg l^{-1})	Apr.	3.80 ± 0.12	3.60 ± 0.06	5.14 ± 0.06	5.40 ± 0.14	8.21 ± 0.05	14.20 ± 0.28
	Jun.	18.22 ± 0.08	18.10 ± 0.06	20.40 ± 0.24	22.12 ± 0.05	31.40 ± 0.14	54.50 ± 1.24
	Aug.	22.04 ± 0.05	22.02 ± 0.14	24.20 ± 0.30	30.20 ± 0.06	38.64 ± 0.30	56.22 ± 1.40
	Oct.	10.20 ± 0.20	10.00 ± 0.20	12.12 ± 0.25	14.26 ± 0.04	18.12 ± 0.42	28.20 ± 1.02
	Dec.	8.40 ± 0.15	8.42 ± 0.03	11.40 ± 0.16	13.12 ± 0.04	16.14 ± 0.24	18.20 ± 0.84

observed in April at Stations 1 and 2, and the minimum was found in June at Station 3. The monthly variations were less significant and found to be the minimum in the estuarine region (Stations 3-6).

The salinity distribution in this estuary is strongly dependent on the seawater intrusion through the Cochin barmouth and the discharge of river water. The estuarine features alternate between a well mixed type during pre-monsoon and a stratified type during monsoon (Qazim and Gopinathan, 1969; Lakshmanan *et al.*, 1987). In the present study, estuarine conditions were observed at Stations 3 - 6 during pre-monsoon and post-monsoon seasons whereas, fresh water conditions prevailed at Stations 1 and 2 during all seasons and at Stations 3 and 4 during monsoon. Very low salinity values were observed at Stations 5 & 6 during monsoon. The maximum salinity value of 32.40×10^{-3} was obtained at Station 6 during pre-monsoon (April).

The dissolved oxygen (DO) values varied between 8.40 mg l^{-1} and 4.82 mg l^{-1} (Fig. 3.2) in this estuary. DO values were low in saline regions compared to the fresh water zone. These were low also during pre-monsoon and found to be the maximum during monsoon. Similar observations have been reported (Nair *et al.*, 1990). Among the different Stations, the maximum value was observed at Station 5 and the minimum value at Station 6.

The pH of the surface waters varied between 8.13 and 6.90, and the minimum pH was found during the pre monsoon season at Stations 3 and 4 whereas, pH values were generally lower during monsoon at all other Stations. The pH values decreased from fresh water zone (Station 1) to the estuarine region (Station 3 and 4) and then increased to the maximum value near the barmouth (Station 6). The lowering of pH values at Stations 3 and 4 may be attributed to the discharge of industrial effluents. While photosynthesis exerts only a mild influence on the pH of this estuary, it is the change in salinity which predominantly regulates the pH (Shibu, 1992).

The dissolved organic carbon (DOC) content was found to be

maximum in fresh water zone (Station 1) in monsoon and post-monsoon whereas, DOC was maximum at Station 6 during April. The DOC varied between 194.60 mg C l^{-1} and 74.14 mg C l^{-1}. In Stations except 5 and 6, the DOC values were found to be enhanced during the monsoon and post-monsoon periods. This behaviour has been attributed to the varying biological productivity as reported earlier by Gopinanthan *et al.* (1984) and Joseph (1989). DOC values decreased from fresh water zone to saline region during the entire period of study.

The particulate organic carbon (POC) decreased from the fresh water zone to the Station 6 during the whole period of study (Fig. 3.2). The maximum POC value was 101.01 mg C g^{-1} observed at Station 1 during October and the minimum value was 10.20 mg C g^{-1} at Station 6 during December.

The distribution of suspended matter (SM) generally showed an increase towards the mixing zone of the estuary (Fig. 3.2). The maximum recorded value was 56.22 mg l^{-1} in August at Station 6 and the minimum value was 3.6 mg l^{-1} in April at Station 2. The constantly high values in the saline region can be explained as due to the tidal influence which inturn affect the estuarine circulation resulting in resuspension of the bottom sediments (Pillai, 1989).

Distributions of different fractions of aluminium in the Cochin estuary are depicted in the Fig. 3.3 and their absolute values are given in Table 3.2. The maximum and the minimum values of the different Al fractions along with Station and period are given in the Table 3.3.

The maximum values of different Al fractions were found in the fresh water zone (Station 1) except FAA, LA and CA whereas the minimum values were observed in the saline region (barmouth, Station 6). The concentrations of different Al fractions except PA, LA and CA, were high during monsoon period and decreased through post-monsoon to pre-monsoon. Particulate Al during monsoon period was minimum at Stations 1 and 2 and high PA values were

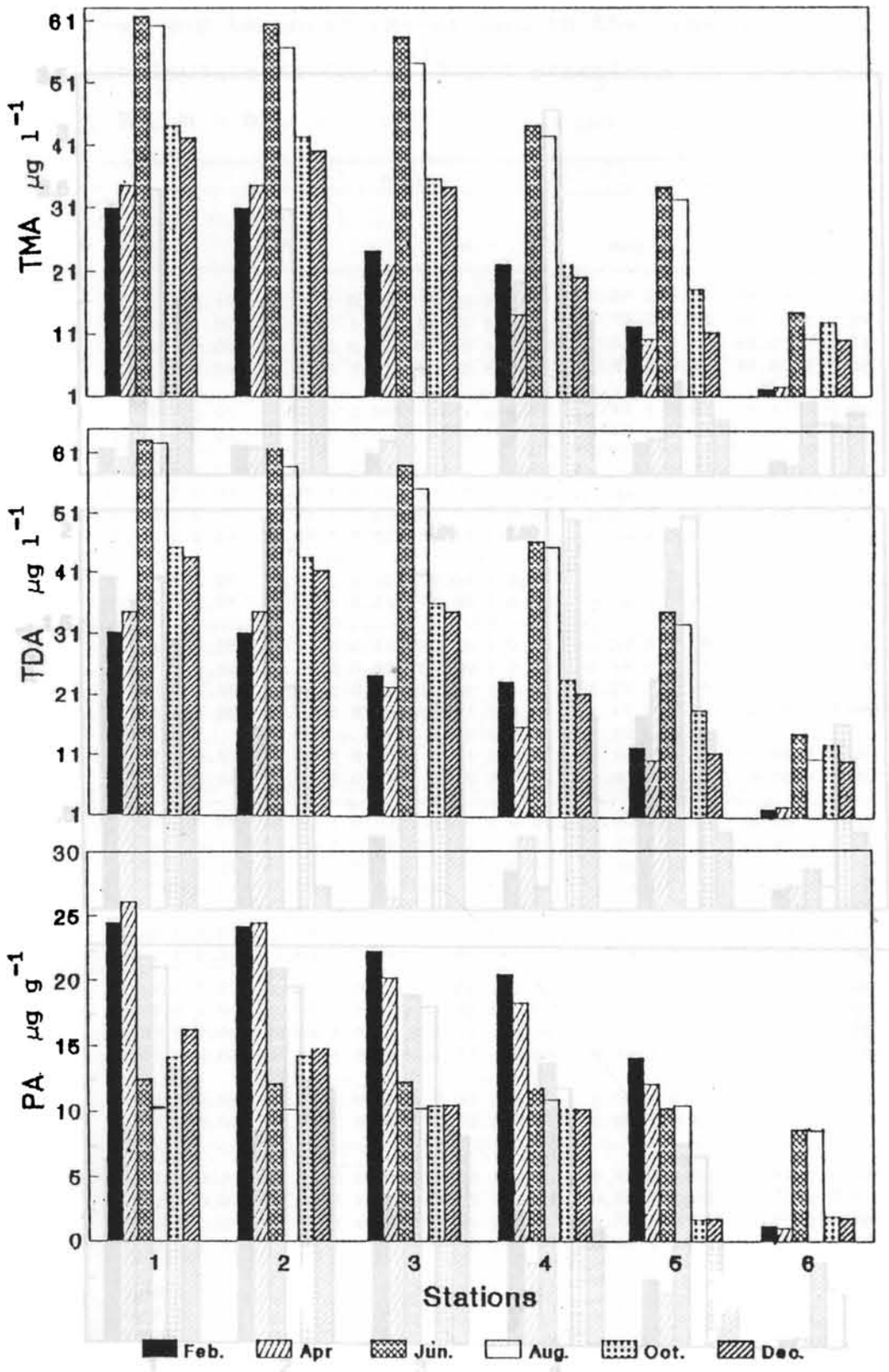

Fig. 3.3. Bimonthly distributions of different fractions of aluminium in the Cochin estuary.

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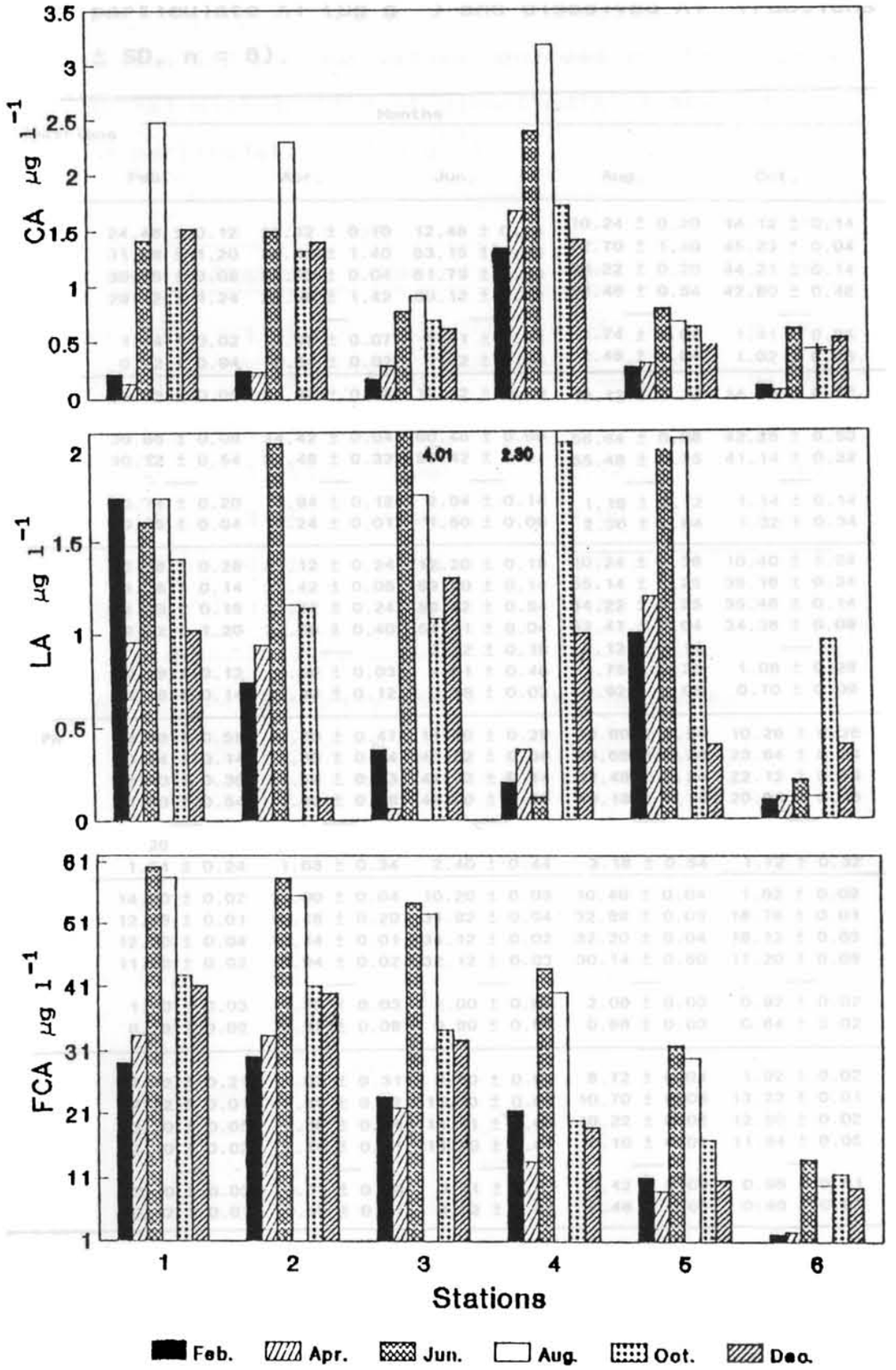
CA µg l⁻¹
LA µg l⁻¹
FCA µg l⁻¹
4.01
2.80
Stations
Feb.
Apr.
Jun.
Aug.
Oct.
Dec.

Fig.3.3

Table 3.2. Spatial and temporal variations in the absolute concentratins of particulate Al ($\mu g\ g^{-1}$) and dissolved Al fractions ($\mu g\ l^{-1}$) ($\bar{x} \pm SD$, n = 6).

Stations	Al-fractions	Months					
		Feb.	Apr.	Jun.	Aug.	Oct.	Dec.
1	PA	24.48 ± 0.12	26.12 ± 0.10	12.48 ± 0.14	10.24 ± 0.20	14.12 ± 0.14	16.24 ± 0.06
	TDA	31.08 ± 1.20	34.56 ± 1.40	63.15 ± 0.08	62.70 ± 1.40	45.23 ± 0.04	43.66 ± 0.50
	TMA	30.86 ± 0.08	34.42 ± 0.04	61.73 ± 0.10	60.22 ± 0.20	44.21 ± 0.14	42.14 ± 0.12
	FCA	29.12 ± 1.24	33.48 ± 1.42	60.12 ± 1.32	58.48 ± 0.54	42.80 ± 0.48	41.12 ± 0.24
	FAA	—	—	—	—	—	—
	LA	1.74 ± 0.02	0.98 ± 0.07	1.61 ± 0.04	1.74 ± 0.02	1.41 ± 0.05	1.02 ± 0.10
	CA	0.22 ± 0.04	0.14 ± 0.02	1.42 ± 0.04	2.48 ± 0.84	1.02 ± 0.08	1.52 ± 0.42
2	PA	24.12 ± 0.05	24.48 ± 0.08	12.12 ± 0.20	10.12 ± 0.14	14.20 ± 0.12	14.84 ± 0.14
	TDA	31.11 ± 0.01	34.66 ± 0.03	61.96 ± 0.04	58.94 ± 0.01	43.60 ± 0.06	41.52 ± 0.31
	TMA	30.86 ± 0.08	34.42 ± 0.04	60.46 ± 0.09	56.64 ± 0.06	42.28 ± 0.50	40.12 ± 0.04
	FCA	30.12 ± 0.54	33.48 ± 0.32	58.42 ± 0.21	55.48 ± 0.25	41.14 ± 0.32	40.00 ± 0.21
	FAA	—	—	—	—	—	—
	LA	0.74 ± 0.20	0.94 ± 0.12	2.04 ± 0.14	1.16 ± 0.12	1.14 ± 0.14	0.12 ± 0.04
	CA	0.25 ± 0.04	0.24 ± 0.01	1.50 ± 0.06	2.30 ± 0.04	1.32 ± 0.34	1.40 ± 0.21
3	PA	22.18 ± 0.28	20.12 ± 0.24	12.20 ± 0.18	10.24 ± 0.36	10.40 ± 1.24	10.42 ± 0.34
	TDA	24.38 ± 0.14	22.42 ± 0.05	59.20 ± 0.14	55.14 ± 0.25	36.16 ± 0.34	34.76 ± 0.12
	TMA	24.20 ± 0.18	22.12 ± 0.24	58.42 ± 0.54	54.22 ± 0.25	35.46 ± 0.14	34.14 ± 0.15
	FCA	23.82 ± 1.20	22.06 ± 0.40	54.41 ± 0.04	52.47 ± 0.04	34.38 ± 0.08	32.84 ± 0.02
	FAA	—	—	3.12 ± 0.18	2.12 ± 0.14	—	—
	LA	0.38 ± 0.12	0.06 ± 0.03	4.01 ± 0.48	1.75 ± 0.25	1.08 ± 0.28	1.30 ± 0.13
	CA	0.18 ± 0.14	0.30 ± 0.12	0.78 ± 0.02	0.92 ± 0.08	0.70 ± 0.09	0.62 ± 0.08
4	PA	20.36 ± 0.58	18.20 ± 0.47	11.80 ± 0.28	10.80 ± 0.54	10.26 ± 0.36	10.12 ± 0.24
	TDA	23.34 ± 0.14	15.86 ± 0.24	46.52 ± 0.36	45.66 ± 0.24	23.84 ± 0.24	21.54 ± 0.04
	TMA	22.00 ± 0.36	14.18 ± 0.13	44.12 ± 0.14	42.48 ± 0.24	22.12 ± 0.36	20.12 ± 0.24
	FCA	21.80 ± 0.54	13.80 ± 0.68	44.00 ± 0.56	40.18 ± 0.74	20.08 ± 0.46	19.12 ± 0.54
	FAA	—	—	—	—	—	—
	LA	0.20 ± 0.05	0.38 ± 0.18	0.12 ± 0.02	2.30 ± 0.12	2.04 ± 0.18	1.00 ± 0.14
	CA	1.34 ± 0.24	1.68 ± 0.34	2.40 ± 0.44	3.18 ± 0.54	1.72 ± 0.32	1.42 ± 0.24
5	PA	14.00 ± 0.02	12.00 ± 0.04	10.20 ± 0.03	10.40 ± 0.04	1.62 ± 0.02	1.73 ± 0.01
	TDA	12.48 ± 0.01	10.46 ± 0.20	34.92 ± 0.04	32.88 ± 0.03	18.76 ± 0.01	11.68 ± 0.05
	TMA	12.20 ± 0.04	10.14 ± 0.01	34.12 ± 0.02	32.20 ± 0.04	18.12 ± 0.03	11.20 ± 0.01
	FCA	11.20 ± 0.02	8.94 ± 0.02	32.12 ± 0.03	30.14 ± 0.50	17.20 ± 0.08	10.80 ± 0.01
	FAA	—	—	—	—	—	—
	LA	1.00 ± 0.03	1.20 ± 0.03	2.00 ± 0.02	2.06 ± 0.03	0.92 ± 0.02	0.40 ± 0.01
	CA	0.28 ± 0.02	0.32 ± 0.08	0.80 ± 0.03	0.68 ± 0.03	0.64 ± 0.02	0.48 ± 0.02
6	PA	1.12 ± 0.21	1.02 ± 0.31	8.60 ± 0.04	8.72 ± 0.04	1.92 ± 0.02	1.78 ± 0.04
	TDA	2.32 ± 0.01	2.68 ± 0.02	15.03 ± 0.05	10.70 ± 0.06	13.22 ± 0.01	10.54 ± 0.02
	TMA	2.20 ± 0.05	2.60 ± 0.04	14.41 ± 0.04	10.22 ± 0.01	12.80 ± 0.02	10.00 ± 0.03
	FCA	2.10 ± 0.02	2.48 ± 0.01	14.20 ± 0.07	10.10 ± 0.01	11.84 ± 0.05	9.60 ± 0.04
	FAA	—	—	—	—	—	—
	LA	0.10 ± 0.03	0.12 ± 0.08	0.21 ± 0.03	0.12 ± 0.08	0.96 ± 0.01	0.40 ± 0.02
	CA	0.12 ± 0.01	0.08 ± 0.01	0.62 ± 0.04	0.48 ± 0.03	0.40 ± 0.03	0.54 ± 0.02

Table 3.3. Maximum and minimum values observed in the temporal and spatial distribution of dissolved Al fractions ($\mu g\ l^{-1}$) and particulate Al ($\mu g\ g^{-1}$).

Fraction	Maximum			Minimum		
	Conc.	Station	Period	Conc.	Station	Period
TDA	63.15	1 (R)	Jun.	2.32	6 (M)	Feb.
TMA	61.73	1 (R)	Jun.	2.20	6 (M)	Feb.
FCA	60.12	1 (R)	Jun.	2.10	6 (M)	Feb.
FAA	3.12	3 (E)	Jun.	2.12	3 (E)	Aug.
LA	4.01	3 (E)	Jun.	0.06	3 (E)	Apr.
CA	3.18	4 (E)	Aug.	0.08	6 (M)	Apr.
PA	26.12	1 (R)	Apr.	1.02	6 (M)	Apr.

R — Riverine, E — Estuarine and M — Marine zones.

observed during pre-monsoon while, minimum values were found in all other Stations during post-monsoon. The FAA fraction (Table 3.2) was obtained only at Station 3 and the values varied between 3.12 $\mu g\ l^{-1}$ and 2.12 $\mu g\ l^{-1}$. The magnitudes of Al fractions in this estuary were found to depend on various physico-chemical parameters like pH, salinity, DO, DOC, POC and SM.

The percentage Al fractions —— TMA, FCA, LA and CA with respect to the TDA are given in the Table 3.4 . The maximum percentage of TMA i.e., 99.59 was observed at Station 1 and the minimum, 89.40 was found at Station 4 during pre-monsoon. The percentage of FCA varied between 98.39 at Station 3 during pre-monsoon and 84.23 at Station 4 during post-monsoon. LA and CA possessed the highest contribution during pre-monsoon at Stations 5 and 4 and the values were 11.47 and 10.59 respectively. The minimum LA and CA values were observed at Stations 4 and 1. The minimum values were 0.26 and 0.41 during monsoon and pre-monsoon respectively.

Correlation of Al fractions to physico-chemical parameters.

The inter-relationships between different Al fractions and various physico-chemical parameters in the Cochin estuary are depicted in the Fig. 3.4 to Fig 3.9. All aluminium fractions were negatively correlated to salinity and pH in this estuary (r values varied between -0.360 and -0.767 for salinity, -0.217 and -0.579 for pH, n = 36). The different Al fractions were positively correlated to DOC and POC whereas very small negative correlation existed between PA and DOC (r = 0.106). Inter-relationship was found between PA and SM only and that was negative (r = -0.493). All Al fractions except PA were positively correlated to DO and negatively correlated to temperature. The positive correlation was found between PA and temperature (r = 0.465).

But the variations of these different Al fractions were dependent on seasons and the correlations varied with sampling periods. The correlation coefficients for the hydrographical

Table 3.4. Spatial and temporal variations in the percentage of dissolved Al fractions in the Cochin estuary.

Stations	Al-Fraction	Months					
		Feb.	Apr.	Jun.	Aug.	Oct.	Dec.
1	TMA	99.29	99.59	99.33	99.29	97.74	96.52
	FCA	93.69	96.87	95.20	92.60	94.62	94.18
	LA	5.60	2.78	2.55	2.76	3.12	2.34
	CA	0.71	0.41	2.25	3.93	2.26	3.48
2	TMA	99.23	99.31	97.58	96.10	96.97	96.63
	FCA	96.85	96.60	94.29	94.12	94.36	96.34
	LA	2.38	2.71	3.29	1.97	2.61	0.29
	CA	0.80	0.69	2.42	3.90	3.03	3.37
3	TMA	99.26	98.66	98.68	98.33	98.06	98.22
	FCA	97.70	98.39	91.91	95.15	95.08	94.48
	LA	1.56	0.27	6.77	3.17	2.99	3.74
	CA	0.74	1.34	1.32	1.67	1.94	1.78
4	TMA	94.25	89.40	94.84	93.04	92.79	93.40
	FCA	93.40	87.02	94.58	88.00	84.23	88.77
	LA	0.85	2.40	0.26	5.04	8.56	4.64
	CA	5.74	10.59	5.16	6.96	7.21	6.59
5	TMA	97.76	96.94	97.71	97.93	96.59	95.89
	FCA	89.74	85.47	91.98	91.67	91.68	92.47
	LA	8.01	11.47	5.73	6.27	4.90	3.42
	CA	2.24	3.06	2.29	2.07	3.41	4.10
6	TMA	94.82	97.01	95.87	95.51	96.82	94.88
	FCA	90.52	92.54	94.48	94.39	89.56	91.08
	LA	4.31	4.48	1.40	1.12	7.26	3.80
	CA	5.17	2.99	4.13	4.49	3.18	5.12

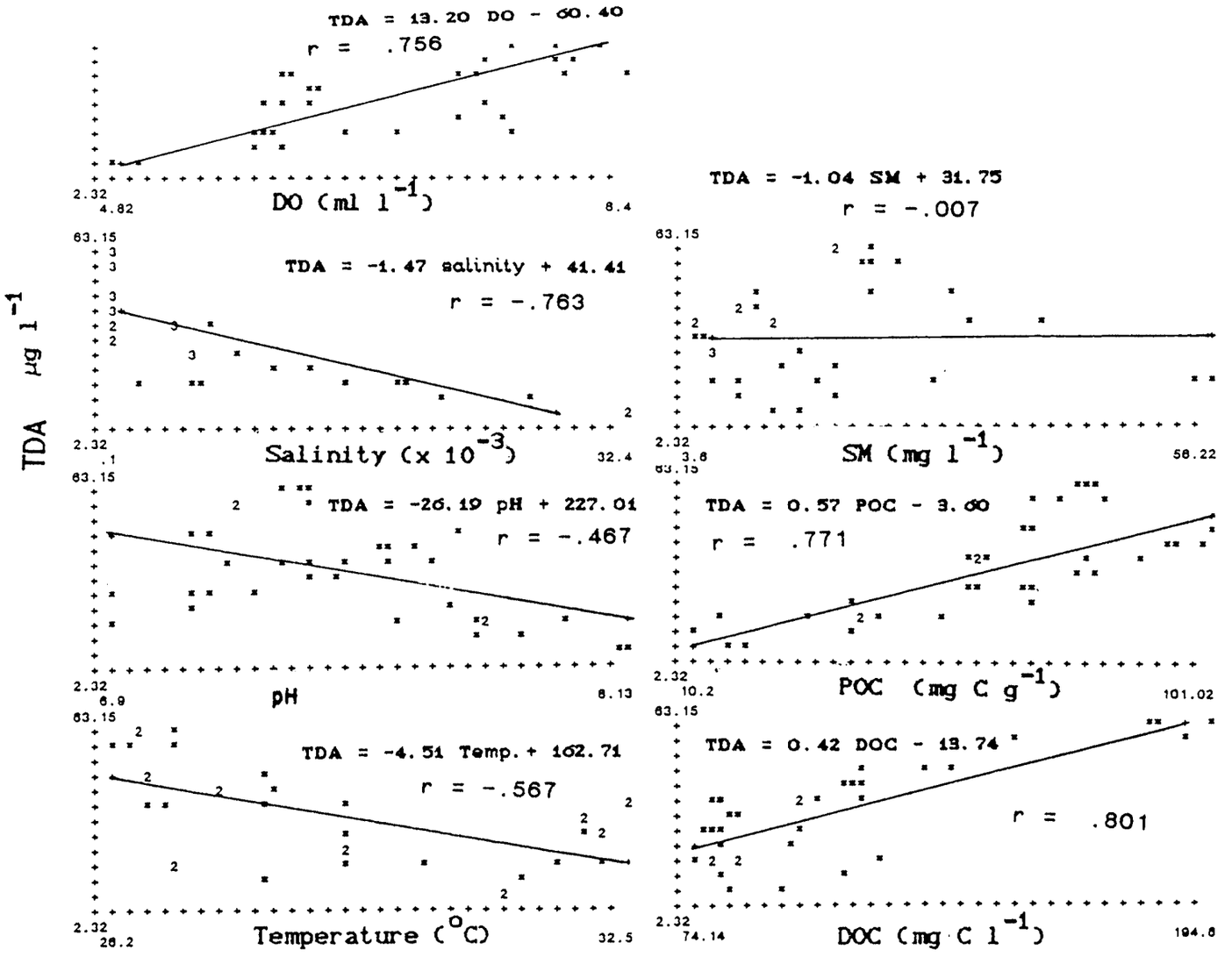

Fig. 3.4. Correlation of TDA vs different hydrographical parameters.

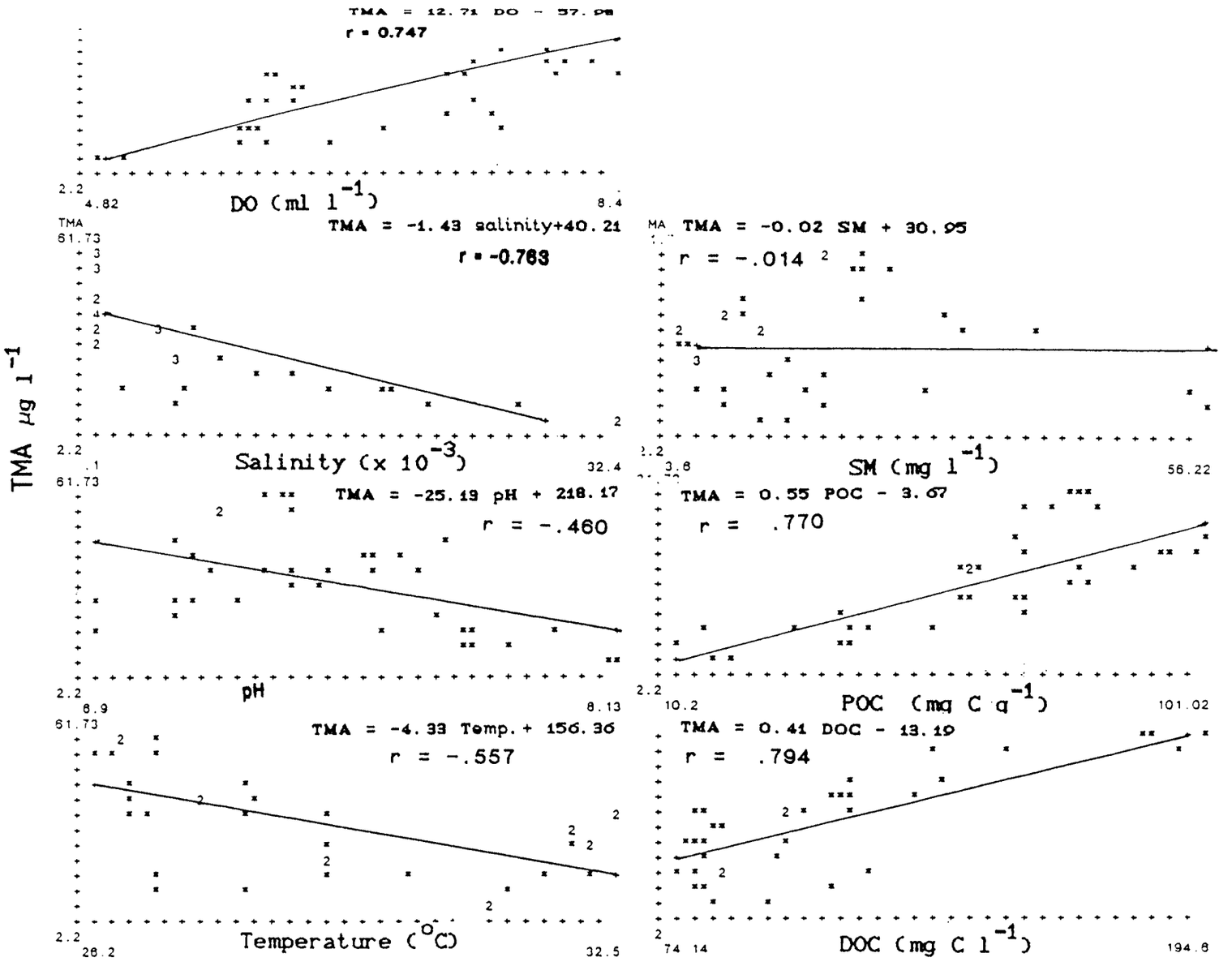

Fig. 3.5. Correlation of TMA vs different hydrographical parameters.

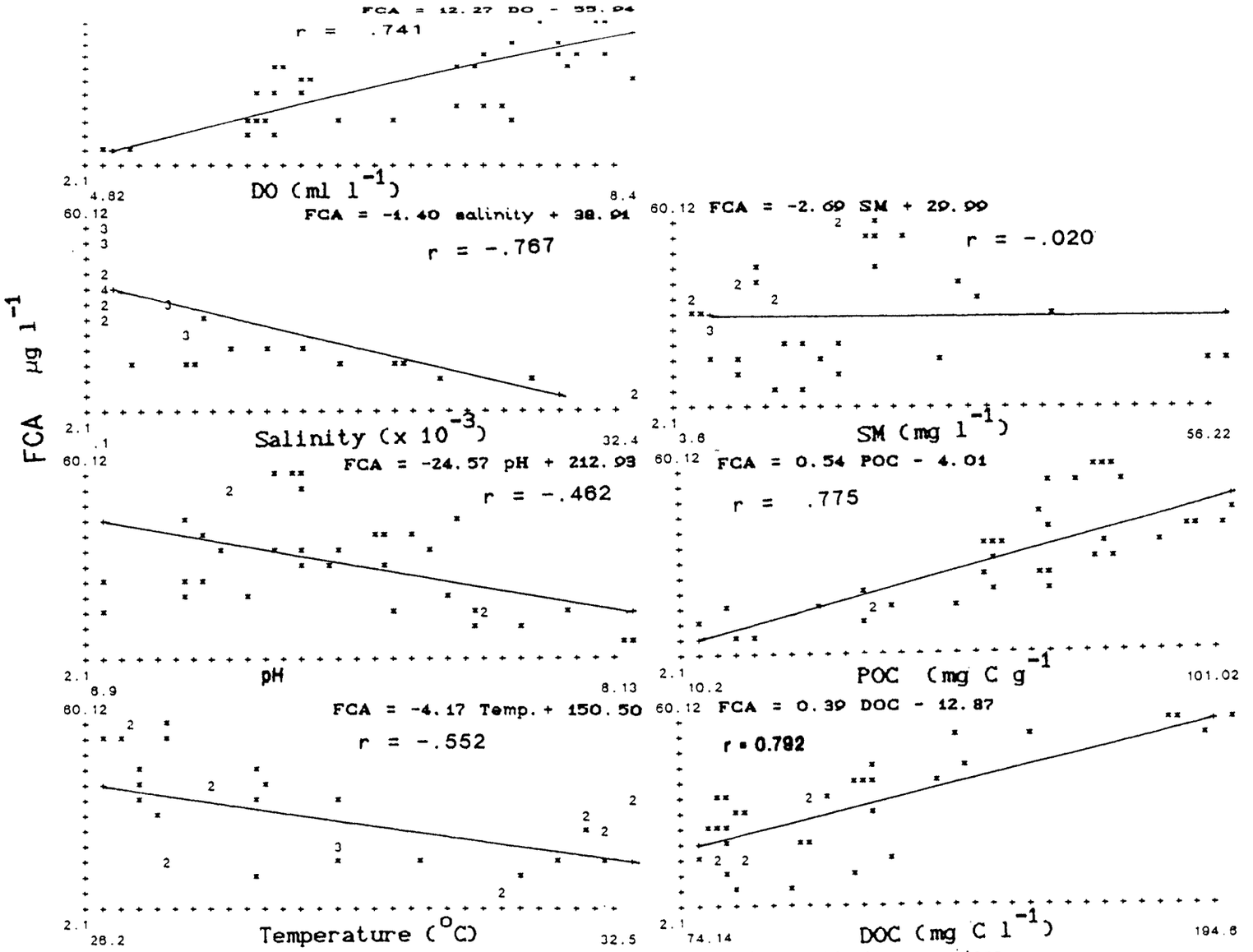

Fig. 3.6. Correlation of FCA vs different hydrographical parameters.

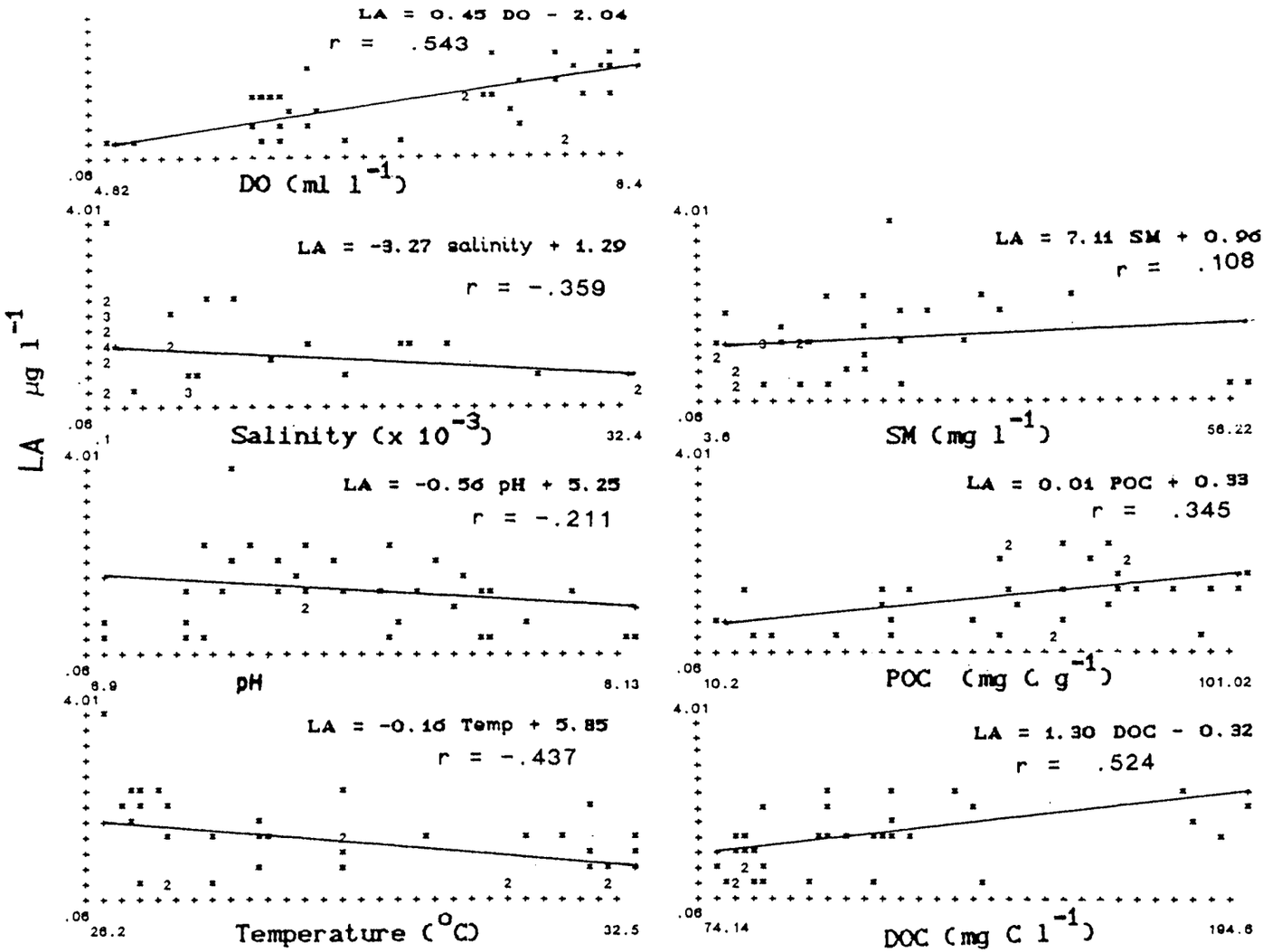

Fig. 3.7. Correlation of LA vs different hydrographical parameters.

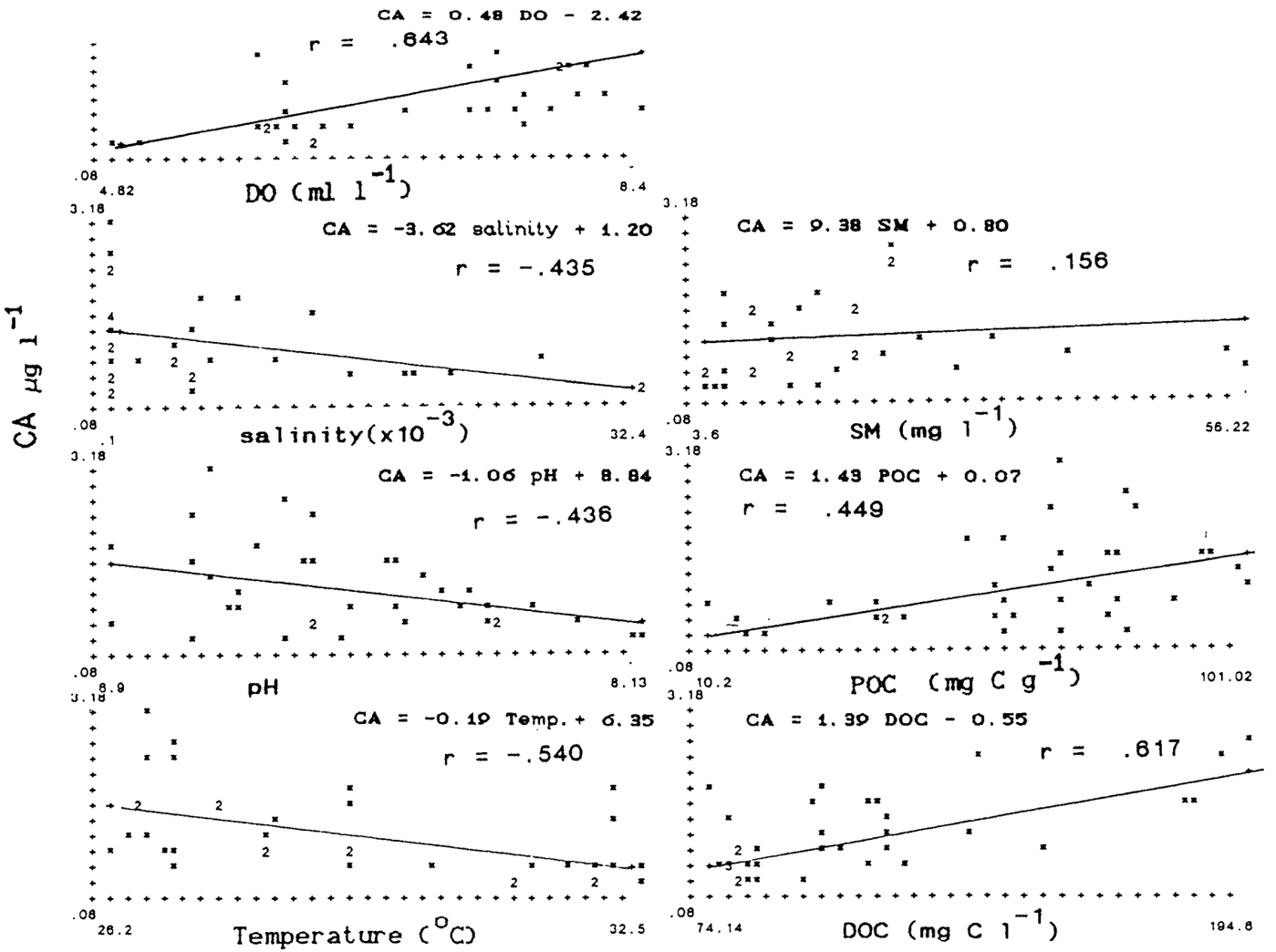

Fig. 3.8. Correlation of CA vs different hydrographical parameters.

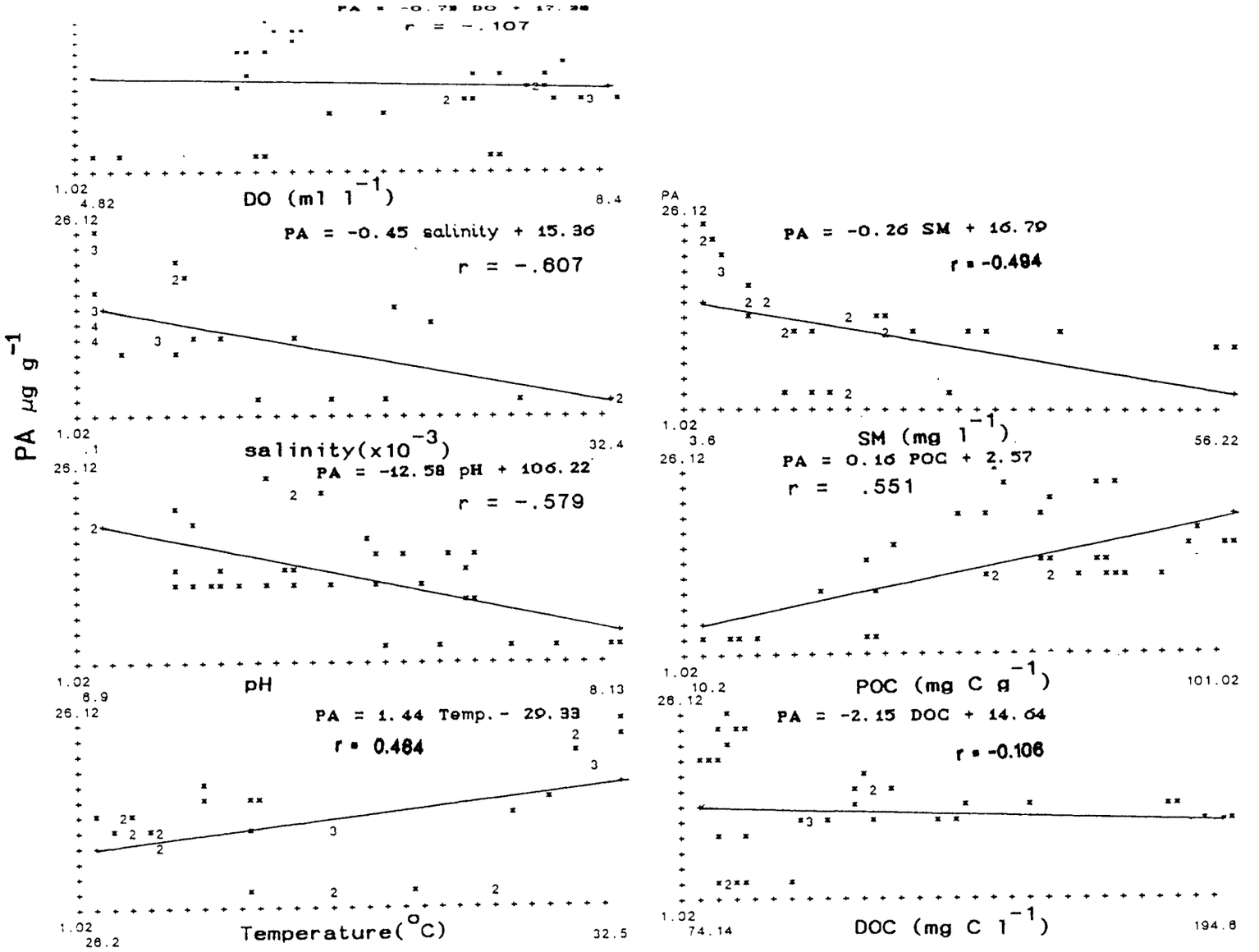

Fig. 3.9. Correlation of PA vs different hydrographical parameters.

parameters to different Al fractions during different months are given in the Table 3.5, and various Al fractions were correlated to the physico-chemical parameters as given below:

1. Dissolved oxygen and temperature: All aluminium fractions were positively correlated with the amount of dissolved oxygen during all seasons while this relationship was small in the case of colloidal Al. There was no well defined relationship between the temperature and Al in this estuary. PA, TDA, TMA and FCA were positively correlated to temperature during pre-monsoon whereas these fractions were negatively correlated to temperature, CA and LA were not well correlated except during certain seasons.

2. pH: The Al fractions decreased with increase in pH during monsoon and pre-monsoon periods. But during post-monsoon, there was not well defined relationship between the pH and the amount of various Al fractions except LA and CA.

3. Salinity: The PA, TDA, TMA and FCA were found to be negatively correlated to salinity during all seasons (r values varied between -0.881 and -0.992, n = 36) except during monsoon (August) where the variation is slightly depressed (r= -0.486). The labile and colloidal Al fractions were generally not well correlated to salinity.

4. DOC: The PA, TDA, TMA and FCA fractions decreased with increase in DOC during pre-monsoon (r values varied between -0.212 and -0.844 whereas these fractions were positively correlated to DOC during monsoon and post-monsoon (r values varied between 0.900 and 0.989) except during monsoon in the case of particulate metal where r value is equal to 0.480, or in other words the magnitude of these fractions were increased with increase in the DOC. The other fractions were not well correlated to DOC ($r < 0.387$), whereas CA was positively correlated to DOC during post-monsoon.

5. POC: All aluminium fractions, except labile and colloidal Al fractions were found to be elevated with increase in the POC

Table 3.5. Correlation coefficients for the hydrographical parameters to different Al fractions (n = 36).

	Aluminium Fractions	PERIOD					
		Feb.	Apr.	Jun.	Aug.	Oct.	Dec.
DO	PA	0.987	0.928	0.764	0.938	0.557	0.682
	TDA	0.933	0.810	0.723	0.787	0.605	0.688
	TMA	0.932	0.795	0.726	0.787	0.594	0.684
	FCA	0.930	0.785	0.723	0.768	0.585	0.688
	LA	0.483	0.555	0.443	0.881	0.336	0.060
	CA	0.168	0.191	0.446	0.473	0.518	0.577
TEMPERATURE	PA	0.945	0.948	-0.840	-0.489	-0.814	-0.496
	TDA	0.898	0.914	-0.828	-0.189	-0.923	-0.526
	TMA	0.882	0.897	-0.857	-0.198	-0.924	-0.521
	FCA	0.891	0.905	-0.828	-0.179	-0.923	-0.539
	LA	0.276	0.125	-0.839	-0.559	-0.107	0.411
	CA	0.504	0.216	-0.122	0.033	-0.320	-0.499
pH	PA	-0.875	-0.741	-0.864	-0.832	-0.459	-0.390
	TDA	-0.826	-0.545	-0.758	-0.828	-0.232	-0.288
	TMA	-0.812	-0.515	-0.745	-0.816	-0.208	-0.285
	FCA	-0.831	-0.530	-0.758	-0.802	-0.185	-0.288
	LA	-0.826	0.192	-0.250	-0.756	-0.736	-0.714
	CA	-0.459	-0.558	-0.711	-0.706	-0.752	-0.294
SALINITY	PA	-0.990	-0.983	-0.729	-0.486	-0.905	-0.900
	TDA	-0.992	-0.913	-0.691	-0.842	-0.960	-0.942
	TMA	-0.987	-0.895	-0.681	-0.829	-0.954	-0.942
	FCA	-0.989	-0.899	-0.702	-0.833	-0.949	-0.941
	LA	-0.449	-0.238	-0.125	-0.334	-0.239	-0.278
	CA	-0.250	-0.233	-0.546	-0.743	-0.511	-0.622
DOC	PA	-0.212	-0.644	0.910	0.480	0.989	0.989
	TDA	-0.352	-0.617	0.949	0.905	0.954	0.980
	TMA	-0.344	-0.596	0.946	0.900	0.944	0.975
	FCA	-0.373	-0.619	0.959	0.907	0.934	0.974
	LA	0.333	0.387	0.371	0.317	0.394	0.306
	CA	-0.226	-0.366	0.443	0.644	0.615	0.783
POC	PA	0.984	0.979	0.969	0.729	0.941	0.946
	TDA	0.994	0.882	0.966	0.990	0.982	0.929
	TMA	0.991	0.864	0.961	0.987	0.977	0.925
	FCA	0.993	0.867	0.968	0.985	0.972	0.921
	LA	0.453	0.264	0.444	0.579	0.232	0.351
	CA	0.224	0.233	0.535	0.662	0.473	0.741
SM	PA	-0.998	-0.984	-0.975	-0.744	-0.872	-0.974
	TDA	-0.981	-0.884	-0.958	-0.994	-0.885	-0.978
	TMA	-0.998	-0.867	-0.952	-0.993	-0.875	-0.974
	FCA	-0.977	-0.865	-0.956	-0.990	-0.866	-0.975
	LA	-0.489	-0.376	-0.487	-0.611	-0.357	-0.239
	CA	-0.225	-0.229	-0.540	-0.621	-0.600	-0.761

content during all seasons in this estuary (r values varied between 0.729 and 0.993). The labile and colloidal Al fractions were not correlated to POC except CA during Aug. and Dec.

6. Suspended matter: Except the labile and colloidal Al fractions, all the other four Al fractions got depleted well with increase in the suspended matter (r values varied between -0.866 and -0.998) during all seasons. The labile fraction was negatively correlated to SM during monsoon (r= -0.611), and the colloidal fraction was negatively correlated to SM during monsoon and post-monsoon (r >0.540).

The mobility and redistribution of heavy metals in aqueous environmental systems are related to the chemical/physical state of the element which is highly dependent on the hydrochemical conditions of the system. The concentrations of metals like Al in the aquatic environment are, however, rarely controlled by the formation of well defined sparingly soluble stoichiometric compounds of the element itself. Mechanisms controlling the transport and solubility of Al under environmental conditions are rather related to sorption phenomenon in the presence of solid phases as well as the precipitation/co-precipitation of sparingly soluble major components which can act as particulate solid adsorbents or carriers of the dissolved trace constituents. Thus, both transport and the redistribution of metals at trace levels in environmental aqueous system would largely be determined by the presence or formation of particulate matter and the interaction with the dissolved metals.

The values of dissolved Al concentrations in river waters cover an extremely wide range of concentration. The dissolved aluminium concentrations in some of the aquatic systems are given as follows:

Aquatic system	Al $\mu g\ l^{-1}$	Reference
1. Amazon river	20-60	Gibbs (1972)
2. Anglia river	4-98	Hydes and Liss (1977)
3. Californian streams	1-10	Jones *et al.* (1974)
4. Esk and Duddon Cumbria	5-940	Bull and Hall (1986)
5. Fresh water	2-100	Florence (1982)
6. Galveston Bay	2-80	Benoit *et al.* (1994)
7. Lake Orta	90	Mosello *et al.* (1989)
8. Ocean and coastal water	0.3-10	Hydes and Liss (1977)
9 Open ocean	0.5	Florence (1982)
10. Rhine estuary	60	Duinker and Nolting (1977)
11. Rivers of North America	12-2250	Durum and Haffty (1963)
12. Cochin estuary	2.2- 61	Present study

The minimum Al value observed in this study was at a Station very close to barmouth. The wide range of Al content reflects the sensitivity of solubility controlling reactions to pH although, stabilization of dissolved and micro colloidal Al, by complexation with natural organic material may be an additional factor (Beck *et al.*, 1974; Perdue *et al.*, 1976; Crerar *et al.*, 1981). Low dissolved Al concentration of 2.2 $\mu g\ l^{-1}$ at Station 6 and a short oceanic residence time of around 100 years reflect the inherent instability of Al in solution with respect to inorganic precipitation processes (Moore and Millwarg, 1984) although biological uptake is possibly the most significant removal process (Mackenzie *et al.*, 1978; Stoffyn, 1979).

The influence of estuaries in the geochemical cycling of Al is not well established. The distribution of Al concentration observed in the estuarine environments reveals the existence of an Al removal process. Varying degrees of removal of dissolved Al from surface waters within estuarine low salinity regions have been observed (Hosokawa *et al.*, 1970; Hydes and Liss, 1977; Crerar *et*

al., 1981). So increase in the seaward salinity and physical dilution were responsible for the lowering of Al concentration towards barmouth. For Hydes and Liss (1977), the Al adsorbed on the fine clay fraction could be trapped by clay coagulation during the mixing of fresh and shelf waters. They have also shown that there is a progressive decrease from estuarine zone to the open sea. Thus it is concluded that the dissolved Al concentration is controlled by the dispersion of comparatively Al rich estuarine waters. It must be pointed out that Hydes and Liss (1977) have considered short time mixing process in which the oceanic environment is regarded as a chemically inert reservoir towards Al. However, the very low average residence time of Al in the oceanic system (Brewer, 1979) indicates that there must exist in this system phenomena able to remove rapidly dissolved Al. It is suggested that the biological productivity played a prominent role in controlling the concentration of Al (Moore, 1981). The dissolved Al distribution in coastal waters appears to be controlled predominantly by a combination of biologically related removal process and input from both atmospheric and fluvial sources and therefore exhibit seasonal and geographical variability (Moran and Moore, 1988).

Distribution of total dissolved Al showed a regular pattern; high concentrations were recorded at the fresh water region which gradually decreased seaward, varying between 63.15 $\mu g\ l^{-1}$ at Station 1 during monsoon and 2.32 $\mu g\ l^{-1}$ observed at barmouth region during pre-monsoon. During pre-monsoon and post-monsoon , identically similar conditions were recorded for Al. The marked correlation values between salinity and total dissolved Al and the marked departure from the dilution line (Fig. 3.10) suggest that Al behaves non-conservatively in this estuary.

The main mechanism controlling the estuarine circulation during monsoon period is the discharge of river water giving rise to stratification with the onset of monsoon, except the barmouth, the entire estuary attains nearly fresh water conditions. This is also the period when the estuaries being flushed out, attributable

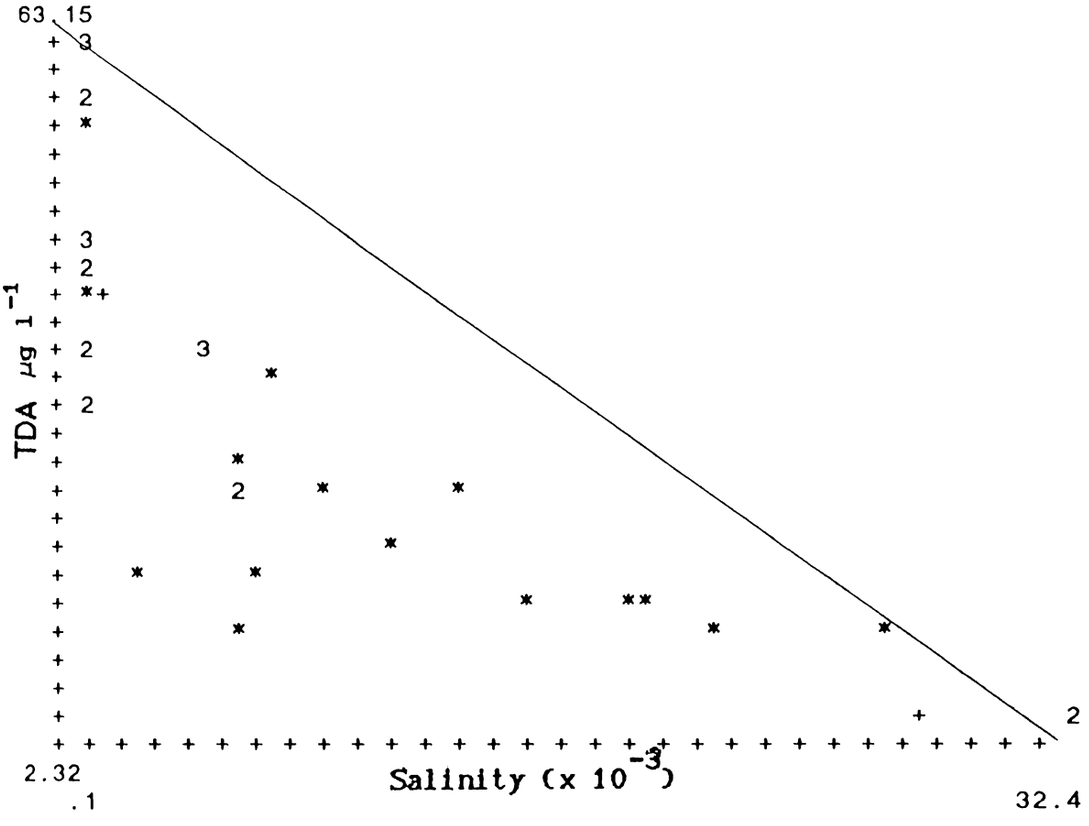

Fig. 3.10. Dilution line -- Salinity vs TDA.

as a characteristic phenomenon in a tropical estuary. Post-monsoon season is essentially a time of stabilization of the relative forces acting in the estuary namely the tidal currents versus fresh water influx. The features of transition period from the monsoon fresh water conditions to the pre-monsoon condition are characteristics of this estuary.

Dissolved Al in the surface water was found to be very high (Hydes, 1983). The high concentration of Al observed during monsoon in the fresh water region of the Cochin estuary may be attributed to the acidification of soil system on the catchment area of river Periyar. Acidification of soil system may cause a transfer of Al into aqueous solution (Dickson, 1980; Seip *et al.*, 1989) where Al may be present in different physico-chemical forms varying for instance in size and charge properties (Lydersen *et al.*, 1987). In acid soils with low base saturation, significant amount of Al^{3+} may remain in solution. Mobile strong acid anions such as SO_4^{2-} and NO_3^- readily transport H^+ and Al^{3+} out of the soil. While degassing will reduce bicarbonate concentration, these strong acid anions remain unaffected. The recent increasing trends in the dissolved Al contents of rivers and lakes have been associated with acidification processes as reported earlier (Reuss *et al.*, 1987).

The few studies that have been carried out on the estuarine behaviour of Al have showed removal of dissolved fractions. The percentage removal was 26 to 30 in Cauvery river estuary (Hydes and Liss, 1977) and more than 50 in the Chikugogawa estuary (Hosokawa *et al.*, 1970). In both cases most of the removal occurred at a salinity of 5×10^{-3}. During mixing of filtered river water and seawater, 20% of the dissolved Al was removed (Sholkovitz, 1976). In this estuary, $\approx$ 93% Al was removed at the Station 6 during pre-monsoon compared to the freshwater zone. Removal of Al as well as other trace metals has been reported in the low saline region of Tamar estuary (Morris, 1986).

A decrease in Al concentration with increasing pH was observed in the present study. A similar trend was observed in lake Orta,

Italy (Mosello *et al.*, 1989). Ionic strength as opposed to pH is the cause of Al removal (Crerar *et al.*, 1981, Hunter and Liss, 1982).

The relationship between DOM and dissolved Al is complex. While Sholkovitz (1976) and May *et al.* (1979) have reported on river water rich in DOM and dissolved Al, truly linear relationship was proposed by Perdue *et al.* (1976). The influence of iron and chloride ions to compete with Al for the available sites in DOM was indicated by Perdue *et al.* (1976). The seasonal dependence of Al content and DOC observed in the Cochin estuary can be attributed to the adsorption of Al onto DOM and desorption of Al by Fe and chloride ions.

Although, for many trace metals the particulate phase is a major carrier in river systems (Gibbs, 1973), in Cochin estuary, this is not so with respect to Al. It is observed that particulate matter and its associated Al were removed with respect to salinity in this estuary. Therefore, Cochin estuary acts as a sink for Al, including dissolved species.

F^- is usually bound by an excess of Al and transported in complexed form through the water cycle (Ares, 1990). The presence of F^- content in this estuary (Mohanachandran, 1995) may be responsible for the anion exchangeable Al, which was present only in Stations 3 and 4 during monsoon.

The presence of cation exchangeable Al (FCA) was found to be > 84% at all Stations during all seasons and it is one of the prominent characteristics of Al in this estuary. The absence of FAA may be due to the removal from dissolved state by the formation of precipitates.

The concentration of Al in the fresh water zone was high but was very low nearer to sea. So the removal of dissolved Al in this estuary therefore depended on the role of salinity, pH, physical dilution and the biological activity. The sorptive behaviour of

suspended matter is crucial in this estuary and negative relationship between dissolved organic carbon and dissolved Al was observed during certain seasons. The existence of higher level of Al in the surface water of the river Periyar during the monsoon season indicated the presence of acidification processes in the soil system adjacent to the river. The contribution of this estuary to the Al level in the Arabian ocean is very small and the Cochin estuary acts as a sink for Al, a metal which is toxic to the aquatic organism and human beings.

CHAPTER IV

TOXICITY, FILTRATION RATE AND METABOLIC RATE

4.1. TOXICITY.

Environmental perturbations have traditionally been monitored by quantifying changes in population density and community structure and function relative to an expected or controlled value. Sensitive changes at the population or community level of organisation occur only after death or after partial impairment of individual organisms and are irreversible. The goal of toxicologists, therefore is to predict significant population effects.

The acute toxicity test has become the "work horse" in monitoring the interactive effects of toxicants as they serve to establish an initial benchmark or a relative toxic potential. They are ecologically relevant because they can be compared to or calibrated with observation in the real world. They are repeatable, rapid, easily interpretable, cost-effective and legally defensible (Sprague, 1970). Acute toxicity tests follow basically four types of test designs: static, static-renewal, continuous or intermittent flow in-situ. The static-renewal method was employed in the present study.

The dose-response toxicity curve relates the responding proportion of populations to dose exposure concentrations, death being taken as the end point by permitting the evaluation of the asymptotic (incipient lethal level) LC50. The extrapolation of toxicity curves has been used to assess the effect of chronic exposure (which are relatively difficult to be experimentally determined) from acute toxicity data (Stephan, 1982).

The acute toxicity test determines the highest concentration of a toxicant that would lethally affect a particular percentage (e.g, 50%) of a limited number of organisms. The acute toxicity has been expressed as the incipient LC50 (= lethal threshold concentration) which is the level of the toxicant lethal to 50% of individuals exposed for periods sufficiently long so that continued exposure does not entail any further lethality.

Organisms respond to their environment at different hierarchial levels, ranging from the community level to the cellular and genetic levels. These responses are often modelled as a function of spatial and temporal variation of environmental variables; either for the purpose of describing and understanding the processes which are operating or for the purpose of environmental monitoring and management.

Bivalve molluscs are one group of organisms which have established their identity as biological indicators (Phillips, 1980). They are ubiquitous, sedentary and responsive to their environment at both the micro and macro geographical scales and at all levels of biological organization. *Villorita cyprinoides* var *cochinensis* (Hanley) satisfies all the criteria specified for such organisms and are consequently being increasingly used in biological monitoring investigations (Babukutty, 1991).

The results of the present attempts to evaluate the interactive effects of H^{+} and Al^{3+} ions on the acute toxicities of the trace metals Cu, Cd, and Hg to *Villorita cyprinoides* var *cochinensis* (Hanley) are presented in this Chapter.

MATERIALS AND METHODS

Experimental techniques and methodologies employed are described in Chapter II. The test organisms were acclimated to conditions (salinity = 10×10^{-3}, pH = 7.60 ± 0.02, D.O = 7.7 ± 0.3 ml l^{-1} and temperature = 29 ± 1°C) very close to natural habitat. pH 7.6 without Al was chosen as the standard for comparison of the

interactive effects of H^+ and Al^{3+}. No mortality was observed in the absence of trace metals Cu, Cd, and Hg at all pH employed. The interactive effects of Al^{3+} on their toxicities of Cu, Cd and Hg to the test organisms were much less during the first 3 days of exposure and was found to be predominant and measurable on the fifth day. Therefore, 120h LC50 values were measured in the present study.

RESULTS AND DISCUSSION

The effects of H^+ and of Al^{3+} on the acute toxicities of Cu, Cd, and Hg expressed in terms of their 120h LC50 are presented in Table 4.1 and Fig. 4.1. Table 4.2 affords a comparison between the percentage variations of the different LC50 values and the values corresponding to pH 7.6 and 0 mg l^{-1} Al^{3+}. No mortality was recorded in the absence of Cu, Cd and Hg concentrations. Enhanced mucus production was a rather conspicuous effect of H^+ and Al^{3+} stress.

Toxicity of copper

In the absence of Al, Cu was found to be much less toxic at the two extremes of H^+ concentration tested; it was most toxic at pH 6. The response of the organisms to Al^{3+} in the presence of Cu varied with pH and the concentration of Al^{3+}. Toxic effect of Cu was increased by the presence of Al^{3+} ions at all pH employed. This was evidenced by the lowering of LC50 values ranging from 0.470 mg l^{-1} Cu at pH 6.0 to 1.134 mg l^{-1} Cu at pH 5.5. These results demonstrated that although H^+ mitigated Cu toxicity, the presence of Al^{3+} ions enhanced the toxic action of Cu and the interaction increased with the concentration of Al^{3+}. In presence of 300 μg l^{-1} Al^{3+}, the percentage deviation of LC50 value compared to pH 7.6 and 0 mg l^{-1} Al^{3+} was in between 6.19 at pH 6.5 and 38.00 at pH 5.5 whereas, the reduction in LC50 values was observed at pH 7.6 and 6.0 (1.03% and 33.99% respectively). The high concentration of Al^{3+} (1 ppm Al) diminished the LC50 values compared to that of control in all pH employed except 5.5 where the value was 1.134 mg

Table 4.1. Interactive effects of pH and aluminium ions on the 120h LC50 values* (mg l^{-1}) during the exposure of *V.cyprinoides* to Cu, Cd and Hg.

Metal	pH				
Medium	5.5	6.0	6.5	7.6	9.0
Cu	1.214 (1.032-1.292)	0.852 (0.743-0.882)	0.932 (0.823-0.952)	0.871 (0.655-1.089)	1.302 (1.180-1.426)
Cu+Al-1	1.202 (1.141-1.334)	0.575 (0.564-0.612)	0.925 (0.892-0.993)	0.862 (0.841-0.962)	0.943 (0.912-0.994)
Cu+Al-2	1.134 (1.023-1.324)	0.470 (0.432-0.512)	0.803 (0.782-0.821)	0.851 (0.841-0.871)	0.824 (0.803-0.853)
Cd	3.632 (3.312-3.820)	3.732 (3.524-3.932)	3.524 (3.233-3.718)	3.144 (2.992-3.314)	4.016 3.923-4.215)
Cd+Al-1	3.726 (3.641-3.912)	3.548 (3.401-3.741)	3.412 (3.208-3.498)	2.987 (2.716-3.084)	3.943 (3.612-4.213)
Cd+Al-2	3.892 (3.413-3.981)	3.432 (3.312-3.628)	3.314 (3.102-3.513)	2.834 (2.452-3.021)	3.841 (3.642-4.026)
Hg	0.612 (0.597-0.623)	0.666 (0.642-0.681)	0.754 (0.714-0.774)	0.789 (0.751-0.801)	0.819 (0.762-0.792)
Hg+Al-1	0.593 (0.572-0.604)	0.651 (0.632-0.672)	0.743 (0.731-0.764)	0.776 (0.751-0.761)	0.801 (0.781-0.821)
Hg+Al-2	0.542 (0.522-0.563)	0.642 (0.621-0.654)	0.733 (0.715-0.741)	0.763 (0.741-0.751)	0.789 (0.751-0.811)

*(95% confidence limits).

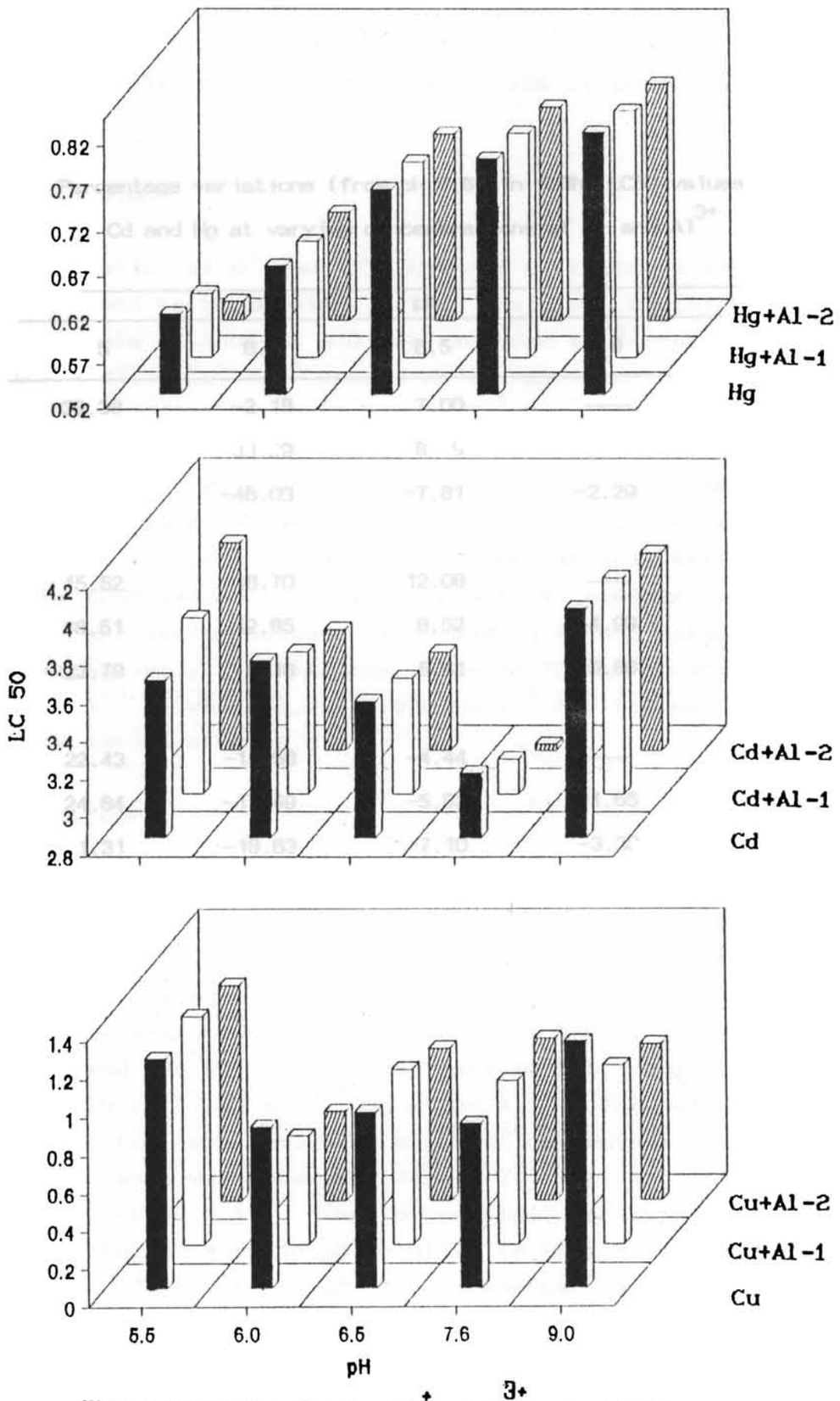

Fig. 4.1. Interactive effects of H^{+} and Al^{3+} on the LC 50 values (mg l^{-1}) of Cu, Cd and Hg to V. cyprinoides.

Table 4.2. Percentage variations (from pH 7.6) in 120h LC50 values of Cu, Cd and Hg at varying concentrations of H^{+} and Al^{3+} ions.

Metal	pH				
medium	5.5	6.0	6.5	7.6	9.0
Cu	39.38	-2.18	7.00	——	49.48
Cu+Al-1	38.00	-33.99	6.19	-1.03	8.26
Cu+Al-2	30.19	-46.03	-7.81	-2.29	-5.39
Cd	15.52	18.70	12.08	——	27.71
Cd+Al-1	18.51	12.85	8.52	-4.99	25.41
Cd+Al-2	23.79	9.16	5.41	-9.86	22.17
Hg	-22.43	-15.58	-4.44	——	3.80
Hg+Al-1	-24.84	-17.49	-5.83	-1.65	1.52
Hg+Al-2	-31.31	-18.63	-7.10	-3.30	——

l^{-1} Cu. The toxicity range was between -2.29% at pH 7.6 and 46.03% at pH 6.0.

Toxicity of cadmium

The interaction of H^+ and Al^{3+} towards Cd toxicity to the test organism was found to be complex. The toxicity diminished with decreasing pH upto a value of 6.0 but recorded an increase at 5.5. However, the addition of Al^{3+} caused an increase in the toxicity. The LC50 values varied between 2.834 mg l^{-1} Cd at pH 7.6 and 4.016 mg l^{-1} at pH 9.0. The percentage variation in LC50 values of Cd with increase in pH, relative to that at pH 7.6 was in the range 12.08 to 27.71. In presence of added aluminium concentrations (300 μg l^{-1} and 1 mg l^{-1}) also, the percentage variation in corresponding LC50 values followed an almost a similar trend for increasing pH and varied between -4.99 and 25.41 and between -9.86 and 23.79 respectively. The maximum value 23.79 was observed at pH 5.5 with 1 mg l^{-1} Al whereas, with 300 μg l^{-1} Al, the maximum value (25.41) was observed at pH 9.0.

Toxicity of mercury

Increase in the H^+ concentration was found to diminish the LC50 values. This established the synergistic action of H^+ on Hg toxicity to the test organisms. In the absence of Al, LC50 values varied between 0.819 mg l^{-1} Hg and 0.612 mg l^{-1} Hg at different pH. At low Al^{3+}concentration, the acute toxicity increased with decreasing pH and the values recorded were in the range 0.801 mg l^{-1} Hg at pH 9.0 to 0.593 mg l^{-1} Hg at pH 5.5. The variation in toxicity was further enhanced with high Al^{3+}concentration at all pH values and the range was between 0.789 mg l^{-1} Hg at pH 9.0 and 0.542 mg l^{-1} Hg at pH 5.5. The percentage variations in LC50 values of Hg with increase in pH relative to that at pH 7.6 were, in the range -22.43 to 3.80. In presence of added Al^{3+} concentration (300 μg l^{-1} and 1 mg l^{-1}) also, the percentage variation in corresponding LC50 values followed a similar trend for

increasing pH and varied between -24.84 and 1.52, and between -31.31 and 0 respectively.

The present study clearly established the role of H^+ and Al^{3+} ions on the trace metal toxicity to *Villorita cyprinoides* var *cochinensis* (Hanley). Trace metal toxicity was attenuated by increase in hydrogen ion concentration whereas, the toxicity wasenhanced by the presence of Al^{3+} ions. Synergistic action of H^+ was observed at pH 6.0 and pH 5.5 in the case of Cu^{2+} and Cd^{2+} respectively. In the case of Hg, the toxicity increased with increase in H^+ (decreasing pH). The lethal response to total dissolved Cu was a complex one over the H^+/Al^{3+} combinations tested. Increasing H^+ ion content has a variable effect; there was an initial decrease, then an increase and subsequently a further decrease in Cu toxicity.

The studies on the response of aquatic organisms to low pH and or elevated metal concentrations span the regimes of biology, liminology and toxicology. The response elicited when a metal species (electrophile) interacts with a biological system is dependent upon a complex set of inter-relationships involving physical, chemical, biological and pharmacological factors. To a first approximation, physico-chemical factors associated with the intrinsic nature of both the metal system and the type of biological ligands present are primary determinants (in a broad sense) as to whether the interaction will be beneficial (eg as in the case of essential trace element Cu as therapeutic agent), detrimental (toxic agent) or innocuous to the organism. In order to understand the molecular basis of such a response, one needs to characterise the metal biological system interactions in terms of the nature of the reacting species and the types of products (or lessions) formed. While the characterisation of species in a simple chemical system is a relatively straight forward task, the characterization of metal species in a biological medium is an exceedingly complex one, if not an intractable problem. Thus, one has to rely on basic chemical, biological informations and fundamental concepts obtained from simplified model systems.

In terrestrial animals, the toxicity of a particular metal is mainly related to its dose. If a metal is not absorbed then it is not toxic, irrespective of its reactivity in aqueous solution (Mc Donald *et al.*, 1989). In aquatic animals, the external surfaces are generally much more structurally and physiologically delicate than comparable liquid-exposed surfaces in terrestrial animals. Thus, a particular metal could be toxic to an aquatic animal because of its surface activity as well as whatever internal effects it might have. Metals which are most likely to be internally toxic only are those that are readily adsorbed and have little, if any, surface activity. Most prominent metals in this category are Hg, Pb, As and Sn. In aqueous medium, these are typically found as lipid soluble organometallic complexes that are readily permeable to biological membranes. Most other metal contaminants of aquatic environments tend to occur as water soluble cations and are potentially active at the exposed surface of the test organism. Indeed the surface activity of some metals and probably of H^+ (Mc Donald, 1983) may be all that is needed to explain their respective toxicants.

In order to understand the surface interactions of metals and hydrogen ions, it is necessary to consider: the structural and physiological targets of surface active metals, the extrinsic factors governing the concentration and chemical form of metals at the surface and the differences in their chemistry. The main structural target of surface active metals is likely to be the gills and the main physiological target is likely to be the ion regulation (Oura, 1972; Oura *et al.*, 1972, Cunningham and Tripp, 1975a; Engel and Fowler, 1979a). The gill surface probably has a substantial net negative charge because of a variety of anionic ligands and as a result is likely to have a relatively high affinity for the cationic forms of metals. The surface anions of gills have not been characterized to any extent but (Oschman, 1978) are likely to consist of the following: sialic acid residues of mucus and membrane glycoproteins, carboxyl groups of membrane glycolipids and various polyelectrolytes of intercellular cements,

hyaluronic acid, heparin and chondrotin sulphate with carboxyl phosphate and sulphate groups. Although, there is probably a variety of anions on the surface, their unique feature is the predominance of oxygen as a donor ligand. This becomes important when considering the binding preference of metals. The specific biochemical action of metals is likely to fall into one or more of three categories (Ochiai, 1977). Competitive or non competitive blockade of functional groups on proteins (e.g., blockade of ion channels or carriers), displacement of essential metal ions from proteins (e.g., displacement of Ca^{2+} from surface ligands) and conformational change in proteins (e.g., oxidation of SH groups to -S-S-).

The aquo-ion activity (M^{Z+}) has often proven to be the best predictor of metal availability towards aquatic biota (Nelson *et al.*, 1981; Pagenkopf, 1983; Sunda and Huntsman, 1983). Simple models involving the surface complexation of metals at the biological interface has been developed to explain these results. Consider the interaction between the metal ion having the charge +Z (M^{Z+}) and the cellular ligand (L) (Pagenkopf, 1983).

$$M^{Z+} + L \leftrightarrow M^{Z+}\text{-}L \qquad (1)$$

In such free ion toxicity models, the surface complex is represented by M^{Z+}– L.

Then the biological response is proportional to the concentration of surface complexes

i.e., biological response $\propto$ $[M^{Z+}\ L]$

substituting for $[M^{Z+}\text{-}L]$ from (1)

$$\text{biological response} = k\ [M^{Z+}]\ [L]$$

By analogy, one might hypothesize that the toxic response of aquatic biota to dissolved aluminium would be best predicted on the basis of concentration of the $[Al(H_2O)_6]^{3+}$ (Cameron *et al.*, 1986; Kinraide and Parker, 1989). Since Cu^{2+}, Cd^{2+}, Hg^{2+}, Al^{3+} and H^{+} undoubtedly react with biological ligands in fundamentally different ways, the differences in their toxicities might be related to one or more of their properties such as charge, ionic radius, ligand binding preference etc.

Classification of Cu, Cd, Hg and Al ions according to their affinity for sulphur and other ligands (Lithner, 1989).

	Class A	Borderline ions	Class B
Metal ions	Al^{3+}	Cu^{2+} and Cd^{2+}	Cu^{+} and Hg^{2+}
Ligand Ions	O^{2-}, PO_4^{3-}, RCO–, RCOH–, $ROPO_3^{2-}$	‹ —— varying —— ›	–S–H, –S–S, –S–R, $-NH_2$

Biesinger and Christensen (1972) correlated the chronic toxicity of metals towards *Daphnia magna* in soft water with the metal's electronegativity and the ability of metals to form complexes together with the insolubility of metallic sulphides. The affinity for sulphur is also used as the basis for a classification system where the metal ions are divided into class A ions, class B ions and borderline ions (Ahrland *et al.*, 1958; Nieboer and Richardson, 1980). Class A ions have the greatest affinity for oxygen, whereas class B ions (Cu^{+}, Hg^{2+}) have the greatest affinity for sulphur. Among the borderline metal ions, the affinity for oxygen decreases whereas, the affinity for sulphur increases in the order Cd ‹ Cu. Properties of class B ions are described by the covalent index or X^2R, where X is the

electronegativity of the metal ion and R is the ionic radius and the properties of class A ions are described by an ionic index or Z^2/R, where Z is the ionic charge. Class B properties and thus the affinity for sulphur will increase with increasing electronegativity, as also demonstrated by Biesinger and Christensen (1972). Based on the above considerations the trace metals employed in this study, can be arranged in the order of decreasing toxicity as follows:

$$Hg^{2+} > Cu^{2+} > Cd^{2+} > Al^{3+}$$

The same trend was observed in the exposure of the organism to different metal concentrations.

The toxic actions of Cu, Cd, Hg and Al at different pH were attributed to their bioavailability to the clam and the different mode of actions. The difference in the bioavailability of metals at different pH may be due to the influence of pH on metal speciation (i.e., complexation, precipitation, adsorption etc.) (Hahne and Kroontje, 1975; Florence, 1977; O'shea and Mancy, 1978) and the competitive interactions of H^+ with different species of metals. The physiological changes in the clam due to the interactive effect of H^+ also play a crucial role in the modification of the toxicities of trace metals. The lethal actions of Cu, Cd, Hg and Al depend on variety of factors such as those indicated below.

Copper is highly toxic to most freshwater and marine invertebrates. LC50's are generally less than 0.5 mg l^{-1}, though they may range from 0.008 mg l^{-1} to > 225.0 mg l^{-1} under certain conditions (Moore and Ramamoorthy, 1984).

Copper toxicity in the marine environment and its severe effects on marine organisms are well documented in several reviews (Shuster and Pringle, 1969; Bryan, 1971). Despite the fact that copper is known to be an essential trace element for biological processes, extremely low concentrations of Cu have been shown to be

lethal to several marine species. pH was found to be important in determining the toxicity of copper (Meador, 1991). pH is especially important because ionic copper concentration decreases about one order of magnitude for every 0.5 increase in pH above 6 (Stumm and Morgan, 1981). Ionic copper is thus highly correlated with toxicity (Andrew *et al.*, 1977; Borgmann, 1983).

Some copper hydroxyl species have also been correlated with toxicity (Cowan *et al.*, 1984) and there seems to be general agreement that the carbonate species (e.g., $CuHCO_3^+$, $CuCO_3$ and $Cu(CO_3)_2^{2-}$ are much less toxic than other copper complexes (Chakaoumakos *et al.*, 1979). Additional complexation of copper by variable organic carbon causes a decrease of both free copper and copper hydroxides (Stumm and Morgan, 1981).

Increasing toxicity of Cu^{2+} exposed to pH has been discussed by several authors (Borgmann, 1983; Campbell and Stokes, 1985) although detailed studies on the relationship between pH and ionic copper effects on invertebrates are rare. The increase in the toxicity response from ionic copper under the influence of increasing pH can be explained as due to competition. As hydrogen ions (protons) decreased, there is a reduction in competition with the ionic form of copper at the cell surface/ receptor (Pagenkopf, 1983, Cusimano *et al.*, 1986; Crist *et al.*, 1990). It is assumed that as hydrogen ions increase, these are able to displace copper from the cell surface. Hence the toxicity potential of ionic copper is different not because of its change in its abundance, but is rather due to a transformation at the surface which would lead to a shift in the number of available receptors (physiological bioavailability).

The effect of pH on Cu toxicity may be due to the ability of copper to compete directly with H^+ to inhibit sodium uptake (Lauren and Mc Donald, 1985). The lethal response to total dissolved copper was a complex one over the different Al/H^+ combinations tested. Increasing H^+ contents had a variable effect; there was a

decrease, then an increase and subsequently a decrease in copper toxicity.

There was also a striking effect of pH at high Al concentration with Cu being especially toxic at pH 7.6, when hydroxyl copper complexes are known to accumulate between the gill filaments (Lloyd and Herbert, 1962). Excretion of carbon dioxide at the gill surface lowered pH in the intermediate area which led to the ionization of hydroxides and liberated large amounts of cupric ion which are taken into the gill as postulated for Zn (Mount, 1966). Whether sufficient CO_2 is generated to change pH at the gill surface has not actually been documented. Cu^{2+} was clearly more toxic at pH 6.0 than at pH 5.5. The reverse might have been expected, since water of pH 5.5 would seem to impose a stress on the organism that might predictably add to the stress of copper (NAS/NAF, 1974). At pH 6.0 copper toxicity was enhanced in the present study. Similar reports were made by Andrew *et al.* (1977) and Nriagu (1979).

As far as cadmium is concerned, hydrated Cd ions are generally considered to be the major toxic species (Peterson *et al.*, 1984) while metal organic complexes are less toxic (Giesy *et al.*, 1977). Although, the dominant Cd species present during the present exposures was not determined, knowledge of the inorganic chemistry of Cd allowed a reasonable estimation of the predominant inorganic species. At the lower pH of 5.5, the free Cd^{2+} will be the dominant form. Increasing the pH values above 7.0 will result in inorganic complexation with carbonate and in the formation of $CdCO_3$. This was likely the case during the exposure to pH 7.6.

A decrease in toxicity of Cd to crustacean zoo-plankton community with increasing H^+ was observed by Lawrence and Holoka (1987). Decrease in pH would be expected to ameliorate Cd^{2+} toxicity due to H^+ competition for cellular binding sites (Peterson *et al.*, 1984; Campbell and Stokes, 1985). Casterline and Yip (1975) exposed oysters (*Crassostrea virginica*) to 0.1 ppm Cd for 8 days and found that 87.5% of the Cd in the tissue homogenates was

associated primarily with proteins. Noel-Lambot (1976) proposed the existence of a low molecular weight Cd binding protein in the cytosol fractions of *Mytilus edulis* and suggested that this Cd-binding protein might play a protective role by limiting the amount of free Cd^{2+} ions in the tissue. Toxicity of Cd to estuarine invertebrates varied by a factor of at least 5000; crustaceans appear to be most sensitive followed by molluscs and polychaetes (Moore and Ramamoorthy, 1984).

Although on the one hand, the 96h LC50 for *M.edulis* was 1.55 mg l^{-1} Cd (Amiard-Triquet *et al.*, 1986), on the other hand natural population of the fresh water mussel *Dreissena polymorpha* was affected at concentrations >0.1-5 $\mu g\ l^{-1}$ Cd (Van urk and Marquenie, 1989).

Cadmium toxicity to clams and mussels can be explained as follows; the process of concentration to high levels is rapid without any direct toxic effects. Detoxification by metal binding proteins will require energy and resources which are drawn from the energy and nitrogen (protein) pool of the animal. A delayed effect will occur in which the population size diminishes due to the constant drain of energy and protein causing energy exhaustion (Van urk and Marquenie, 1989). So the clam is subjected to rapid accumulation and slow elimination with the overall effect of a steady increase in the Cd concentration and a consequent declining population size (Engel and Fowler, 1979b). One effect directly or indirectly caused by the accumulated cadmium is a change in the activity of the clams. The clams were found to be less active with respect to opening closure patterns of their valves (vide Chapter 4.2.) which was also reported by Balogh and Salanki (1984) and Herwig (1989).

Dissolved mercury taken up from sea water by bivalve molluscs is accumulated initially by the gills, then redistributed to other organs (Cunningham and Tripp, 1975b; Roesijadi and Hall, 1981). 96h LC50 for *Perna viridis* Linnaeus was found to be 0.35 ppm Hg (Lakshmanan and Nambisan, 1979). At present, there is

substantial proof demonstrating the impact of pH on the Hg content in fish (Brouzes *et al.*, 1977; Hakanson, 1980). The Hg toxicity decreased with increase in pH. Verma *et al.* (1985) also found that the toxicity of mercuric chloride decreased with change in pH from 5.8 to 7.8. Marking and Hogan (1967) performed experiments at pH 8.4 to 8.8 and 7.2 to 7.6 on fish of the family of bay luscidae and recorded that at all exposure periods, the chemical was more toxic to rainbow trout and other test fish at lower pH values as compared to higher pH levels. Mostly, inorganic chemicals (pollutants) are weak acids or bases and exist in molecular or ionic forms when dissolved in water. Since the dissociation of chemicals depends on the pH medium, the toxicity of chemical also depends on pH. The increase in the bioavilable form of mercury and its nature may be responsible for the toxicity of mercury at low pH. Acute toxicity of mercuric chloride to marine and fresh water invertebrates depends on the species, the developmental stage and the environmental conditions (Moore and Ramamoorthy, 1984).

The importance of Al in acidic lakes has only recently been recognized by aquatic scientists although geochemists, agronomists and plant ecologists had been long aware of the complex chemistry of Al and of its toxicity to plants.

The depletion of fish population in parts of North America and Scandinavia has been attributed in part to elevated Al concentration (Grahn, 1980; Schofield and Trojnar, 1980). Laboratory experiments have confirmed that the concentration of Al found in many of these lakes are toxic to fishes (Baker, 1982; Van Coillie *et al.*, 1983). The toxicity of Al to invertebrates has been the subject of several laboratory studies which have indicated impaired osmoregulation (Witters *et al.*, 1984; Havas and Likens, 1985) and/or precipitation of aluminium hydroxide on gas exchange surface as the reasons for the observed toxicity (Herman and Anderson, 1986; Mc Cahon *et al.*, 1987). Although organic complexation of Al is known to moderate its toxicity to fish (Baker and Schofield, 1982), the evidence for a similar effect on

invertebrates is sparse (Burton and Allan, 1986; Petersen *et al.*, 1986).

The complex nature of Al speciation, which is controlled by pH, renders the precise interpretation of the contribution of Al toxicity difficult. Mortality was dependent on the pH and Al concentration (Dietrich and Schlatter, 1989).

$Al(H_2O)_6^{3+}$ is stable only in acidic solution so that at pH values more basic than 3, hydrolysis occurs with the appearance of $[Al(H_2O)_5(OH)]^{2+}$, $[Al(H_2O)_4(OH)_2]^{+}$. The formation of $Al(OH)_3$ dictated the limiting solubility of Al at about pH 6.5 and at more basic pH values $Al(OH)_4^-$, the aluminate ion is formed. At pH 7.4, the major species is considered to be $Al(OH)_4^-$. These species interacts with Al preferring hard ligands (e.g., oxygen). Al^{3+} achieved the toxicity predicted on the basis of its large Z^2/r value. This is probably because of its ability to block external class A site in aquatic organisms (Ochiai, 1977). The results obtained in this study suggested the toxic effects of Al on the acid sensitive *Villorita cyprinoides* var *cochinensis* (Hanley). Similar toxic effects were observed by Havas and Likens (1985) who exposed *Daphnia magna* clones to acid and Al in laboratory bioassay. They found that Al interfered with ion exchange by affecting the Na^+, K^+ ATP pump, such that the Na^+ was not effectively transported into the animals. Interestingly Havas and Likens (1985) found the effects of Al on net Na^+ flux to be most pronounced at pH 6.5 and 5.0. It is in this range that most zooplankton species are eliminated during lake acidification (NRCC, 1981).

At pH values lower than 5.4, and Al concentrations higher than 100-200 $\mu g\ l^{-1}$, two major mechanisms have been reported to death of the trout. One being electrolyte loss possibly being induced by an interaction of aluminium with enzymes and epithelial junctions and the second being cytotoxicity of labile Al to the gill epithelia leading to proliferations and fusions of the secondary lamellae and finally resulting in the impairment of gas exchange. At high pH levels these two effects which seemed to be induced mainly by

polymeric Al got decreased in intensity whereas mucification become higher. The predominance of one of these mechanisms may depend on the susceptibility of the fish strain, the pH and the aluminium concentration.

Growth of *Anacystis nidulans*, a typically rod shaped unicellular cyanobacterium was inhibited by low pH and Al^{3+} ions (Lee *et al.*, 1991). Al was most toxic at about pH 5.0 - 5.5 (Baker and Schofield, 1982). Zooplankton abundance and species richness declined in both acid and H^{+}+ Al^{3+} exposure (Havens and Heath, 1989). Al monomers are considered to be the most toxic form of Al to fishes (Baker, 1982), however Al precipitates and polymers are thought to coagulate at or adsorb onto the gill and body surfaces and to cause damage (Kane and Rabeni, 1987). Total dissolved monomerical Al and total dissolved Al for varying Al and pH levels are different. Concentrations of total monomeric Al decreased from pH 5.5 to 6.5 and then increased to 9.0 (Palmer *et al.*, 1989). In acidic water the surficial uptake of Al on or within the gills of salmonids is considered to be a sensitive indicator of toxicity correlated with decrease in plasma electrolyte causing death (Neville, 1985; Ramamoorthy, 1988). Increase in the Al concentration was responsible for the dramatic accumulation of Al and hence the mortality of Atlantic salmon (*Salmo salar*) (Peterson *et al.*, 1989).

Many authors (Leivestad *et al.*, 1976; Grahn, 1980; Muniz and Leivestad, 1980; Baker, 1982; Dickson, 1983) found that fish damaged by exposures to waters supersaturated with respect to $Al(OH)_3$ had gills clogged with mucous and showed symptoms of asphyxiation. This mucification and subsequent asphyxiation was attributed to the physical irritation of the gill epithelia by precipitation of polymeric $Al(OH)_3$ onto the gill surface. Havas (1986) found marked binding of Al to chloride cells in *Daphnia* and *Holopedian sp.* Grahn (1980) as well as Muniz and Leivestad (1980) observed that Al bound to the gills of fish resulted in mucus clogging, with decreased plasma pO_2 and electrolyte levels. Toxicity is associated with respiratory stress and problems with

salt regulation (Muniz and Leivestad, 1980). For fish, it is generally agreed that Al^{3+} and hydroxide complexes are the most toxic and organic complexes are non toxic (Driscoll *et al.*, 1980; Schofield and Trojnar, 1980; Neville, 1985) with fluoride complexation being of intermediate toxicity. In contrast, Clark and Lazerte (1985) found no difference in toxicity between Al^{3+} and AlF to amphibians and there was no marked difference in the toxicity of Al to juvenile, Atlantic salmon in the presence of F^- (Wilkinson, *et al.*, 1990). Both Al uptake and mortality rose with increasing pH and indicated that $Al(OH)_x$ may bind to/ precipitate on biological membranes more easily than Al^{3+}, thus potentially increasing its toxicity. Thus effects of Al were focused on ion regulating and respiratory structures in both the zooplankton and aquatic vertebrates. The observed toxicities of invertebrates would also be based on similar effects.

Mercury has been reported to be more toxic than copper to many species of aquatic organisms e.g., *Gammarus Duebeni* (Moulder, 1980), *Metapenaeus dobsoni* (Sivadasan, 1987), *Crassostrea virginica* and *Gigas* (MacInnes and Calabrese, 1978), *Meretrix casta* (Eknath, 1978; Mathew and Menon, 1983). Toxicity to the test organism was in the decreasing order Hg > Cu > Cd which is similar to the trend observed by Umadevi and Rao (1989), when fiddler crabs were exposed to these metals. The toxic responses of *Daphnia magna* are strongly correlated to EC50 values of *C.subglobosa* for most of the metal ions. The order of toxicity was as follows: Hg > Cu > Cd > Al (Banerji and Singh, 1991). Among Hg, Cu and Zn tested with marine invertebrates Hg seemed to be more toxic (Sivadasan *et al.*, 1986).

Results from 120h LC50 tests established that Hg has the highest toxic effect on the test organism followed by copper and cadmium. These findings are in agreement with those reported by others. Eisler and Hennekey (1977) reported the acute toxicity of Cd, Cr, Hg, Ni and Zn to six estuarine macro faunal species. In general, the order of toxicity of metals tested was:

Hg >> Cd >> Zn > Cr > Ni.

Ahsanullah (1982) studied the acute toxicity of Cr and Hg to the amphipod *Allorchestes compressa* and suggested that Hg exerted the most toxic effect followed by Cr. Papathanassiou (1983) studied the effects of Cd and Hg ions upon the longevity of *Palaemon serratus* and suggested that Hg was more toxic than Cd. Delramo *et al.* (1987) established that Hg was more toxic than Cd to the freshwater cray fish *Procambarus clarkii* (Girard).

On the basis of this study the following conclusions were drawn which should be useful in predicting the trace metal interactions in low pH environments.

1. Reduction of pH modified the toxic effects of Cu and Cd in a largely protective fashion whereas in the case of Hg, the toxicity increased.

2. Alumunium was found to augment the toxicity of trace metals by joint interaction.

The possibility of joint toxicity of hydrogen ion, Al and trace metals in acidified water should not therefore be overlooked. Studies done in near neutral pH have clearly shown that trace metal mixtures interact to produce acute lethality to the clam — *Villorita cyprinoides* var *cochinensis* (Geetha, 1992). Therefore, attempts to describe or to predict the impact of acidification should consider the entire trace metal environment of acidified water and should not be confined to the effects of H^+ and Al alone.

4.2. FILTRATION RATE

Sublethal studies carried out at concentrations of pollutants which are debilitating but not directly lethal are important in the environmental management. The sublethal concentration of toxicants exerts stress on animals. Stress is a measurable alteration of a physiological (behavioural, biological or cytological) steady state which is induced by an environmental change and which renders

individuals (or the population) more vulnerable to further environmental change (Bayne, 1976). The change in the concentration of hydrogen ions and trace metals also will exert stress on the test specimen. Lamellibranch molluscs (both fresh water and marine), show shell valve movements, which may be of great use to investigators in estimating the physiological condition of the animal (Bernard and Noakes., 1990). Changes in physiology and or behaviour of aquatic organisms could be used as rapid and sensitive indicators of toxic stress (Janssen *et al.*, 1993). Filtration rate was suggested as a reliable sublethal toxicity index (Abel, 1976) for filter feeding bivalves. The flow of water through bivalve can be measured as filtration rate (the volume of water containing equivalent number of particles retained per unit time) or as pumping rate or ventilation rate (the volume of water passing through the animal per unit time). These two parameters are almost identical in most circumstances as their is virtually 100% retention of particles >2 μm in diameter (Vahl, 1972).

The bivalve molluscs pump water through the palial cavity by means of ciliated gills. The bivalve pumping system comprises of inhalant and exhalant areas of relatively small size, leading to a chamber separated by a large area of gills bearing numerous ciliated slits or pores between gill filaments which act as a series of pumps. The gill can be considered to consist of a large number of pumps in parallel so that pressure is equivalent to a single pump but the total flow is proportional to the total number of pumps (Fox, 1974). The general pattern of bivalve pumping is the drawing in of the inhalant stream at low velocity, its passage between the gill filaments, and its ejection at higher velocity through the exhalant aperture or siphon. Published differences between inhalant and exhalant pressure have varied from less than 100 Pa (100 Pa = 1.0197 mm H_2O) for bivalves with short siphons (Foster-Smith, 1976) to as much as 230 Pa measured in the exhalant siphons (Jones and Allen, 1986).

The rate of filtration of *Perna viridis* as an index of sublethal toxicity to metal pollutants has been studied by Eknath

(1978), Murthy (1982) and Mathew and Menon (1984). Mathew (1990) studied the sublethal effect of Cu, Cd and Hg on the filtration rate of *Perna indica* and *Donax incarnatus* (Gmelin). In view of the paucity of information on the filtration rate of euryhaline clam, *Villorita cyprinoides* var *cochinensis* (Hanley), an attempt is made in the present investigation to study the interactive effects of trace metals Cu, Cd and Hg with H^+ and Al on the filtration rate of the test specimen. The specific objective of the present study was to estimate the role of Al^{3+} in changing the filtration rate of the test organism exposed to Cu, Cd and Hg at different pH.

MATERIALS AND METHODS

The conditions of exposure medium and the procedural pattern employed were discussed in Chapter II.

RESULTS AND DISCUSSIONS

The filtration rates of *Villorita cyprinoides var cochinensis (Hanley)* exposed to different metal concentrations at different pH and periods of exposure are given in Table 4.3. The percentage variations in the filtration rate of the test specimen exposed to different experimental conditions from the control value at 0h are depicted in the Fig. 4.2 to Fig. 4.5 for comparison.

The pH was maintained constant with a maximum variation of ± 0.02 throughout the period of exposure and no mortality was observed in different experimental designs throughout the exposure period. Temperature, hardness and other factors except the concentration of H^+, Cu, Cd, Hg and Al were kept constant throughout the experiment. The filtration rate of the test organism at pH 7.6 and 0h was 691 ml h^{-1} g^{-1} (dry weight) and considered as the control value because the test organisms used for the different experimental conditions were acclimatised at pH 7.6.

Filtration rate of the test organism significantly decreased ($P<0.01$) initially with change in H^+ content compared to pH 7.6 and

Table 4.3. Filtration rate (ml h^{-1} g^{-1} dry wt) of *V.cyprinoides* exposed to varying pH and metal concentrations.

(Control value = 691 ± 0.4)

pH/metal Conc.	Exposure period (h)				
	24	48	72	96	120
pH 5.5/	434 ± 1.2	470 ± 1.5	455 ± 2.8	512 ± 2.4	580 ± 1.4
Al-1	418 ± 1.4	409 ± 2.5	430 ± 1.6	450 ± 1.4	440 ± 0.8
Al-2	404 ± 2.3	402 ± 1.2	390 ± 0.6	404 ± 0.4	408 ± 1.4
Cu-1	410 ± 1.6	408 ± 0.7	422 ± 1.5	448 ± 2.2	497 ± 3.4
Cu-1+Al-1	384 ± 0.6	380 ± 1.2	410 ± 1.4	424 ± 3.2	476 ± 1.3
Cu-1+Al-2	340 ± 2.0	324 ± 3.2	366 ± 0.5	386 ± 1.8	420 ± 2.1
Cu-2	388 ± 1.4	371 ± 0.6	391 ± 2.1	410 ± 2.4	485 ± 4.2
Cu-2+Al-1	360 ± 2.1	374 ± 3.2	398 ± 1.8	441 ± 1.6	510 ± 2.6
Cu-2+Al-2	355 ± 3.7	370 ± 2.5	418 ± 3.4	412 ± 1.2	401 ± 3.2
Cd-1	498 ± 2.2	484 ± 5.2	530 ± 2.3	548 ± 4.5	560 ± 1.4
Cd-1+Al-1	485 ± 1.6	471 ± 4.6	512 ± 3.2	539 ± 2.4	548 ± 2.6
Cd-1+Al-2	462 ± 4.2	468 ± 1.2	524 ± 2.6	570 ± 2.4	564 ± 1.7
Cd-2	422 ± 2.1	448 ± 1.3	468 ± 2.4	494 ± 3.4	534 ± 1.2
Cd-2+Al-1	418 ± 1.8	404 ± 1.2	445 ± 2.6	512 ± 1.8	545 ± 1.4
Cd-2+Al-2	390 ± 0.9	398 ± 1.4	426 ± 5.6	478 ± 2.7	541 ± 2.4
Hg-1	345 ± 0.8	334 ± 2.8	370 ± 3.6	394 ± 1.5	410 ± 1.6
Hg-1+Al-1	352 ± 1.5	348 ± 2.6	384 ± 2.5	412 ± 0.8	443 ± 1.4
Hg-1+Al-2	368 ± 1.5	345 ± 1.8	395 ± 1.9	404 ± 1.4	416 ± 1.9
Hg-2	312 ± 1.7	305 ± 1.7	371 ± 1.2	420 ± 0.7	441 ± 1.3
Hg-2+Al-1	304 ± 1.2	312 ± 0.6	386 ± 1.4	440 ± 1.6	465 ± 1.2
Hg-2+Al-2	334 ± 0.8	307 ± 2.9	395 ± 1.7	460 ± 1.8	490 ± 1.8
pH 6.0/	545 ± 1.2	576 ± 2.3	620 ± 3.2	645 ± 2.2	748 ± 1.8
Al-1	510 ± 3.4	504 ± 2.6	518 ± 1.0	540 ± 4.2	551 ± 2.3
Al-2	460 ± 1.2	480 ± 3.4	490 ± 1.4	498 ± 2.3	515 ± 1.4
Cu-1	505 ± 2.2	520 ± 1.3	542 ± 1.7	551 ± 1.5	620 ± 4.5
Cu-1+Al-1	475 ± 1.8	465 ± 1.4	489 ± 0.8	510 ± 2.1	585 ± 2.0
Cu-1+Al-2	430 ± 2.1	450 ± 2.3	474 ± 1.2	504 ± 1.8	570 ± 3.2
Cu-2	480 ± 0.6	495 ± 0.4	519 ± 0.5	520 ± 0.4	530 ± 0.3
Cu-2+Al-1	460 ± 0.2	454 ± 1.1	496 ± 1.2	510 ± 0.8	524 ± 1.3
Cu-2+Al-2	402 ± 0.6	420 ± 1.3	450 ± 0.8	490 ± 2.5	502 ± 1.2
Cd-1	497 ± 2.1	489 ± 1.7	499 ± 1.7	512 ± 1.5	540 ± 2.3
Cd-1+Al-1	484 ± 2.4	498 ± 1.8	518 ± 2.6	612 ± 2.4	624 ± 1.4
Cd-1+Al-2	468 ± 1.8	454 ± 2.4	442 ± 3.2	470 ± 1.7	512 ± 4.2
Cd-2	412 ± 2.6	428 ± 1.6	418 ± 1.3	490 ± 1.4	520 ± 3.7
Cd-2+Al-1	405 ± 2.4	404 ± 5.2	428 ± 4.1	472 ± 4.2	538 ± 1.6
Cd-2+Al-2	411 ± 3.2	420 ± 3.1	464 ± 1.8	512 ± 1.1	524 ± 1.2
Hg-1	420 ± 1.4	404 ± 1.6	387 ± 2.1	424 ± 1.2	458 ± 1.3
Hg-1+Al-1	408 ± 0.6	387 ± 1.0	397 ± 2.0	418 ± 1.2	470 ± 0.8
Hg-1+Al-2	372 ± 2.8	345 ± 0.8	367 ± 1.9	398 ± 1.6	447 ± 3.6
Hg-2	358 ± 6.1	340 ± 1.3	380 ± 0.8	425 ± 1.3	470 ± 2.8
Hg-2+Al-1	321 ± 2.4	318 ± 0.8	365 ± 1.6	410 ± 1.4	445 ± 3.4
Hg-2+Al-2	304 ± 1.6	302 ± 1.3	348 ± 0.5	445 ± 2.3	458 ± 0.6

(Contd...)

Table 4.3. (Contd...)

pH 6.5/	652 ± 0.7	665 ± 1.4	678 ± 1.2	691 ± 1.4	698 ± 1.0
Al-1	631 ± 3.2	620 ± 2.4	624 ± 1.8	646 ± 1.4	648 ± 0.8
Al-2	584 ± 1.4	581 ± 2.1	604 ± 0.6	608 ± 0.8	610 ± 1.0
Cu-1	623 ± 1.3	610 ± 1.4	580 ± 2.1	620 ± 1.4	612 ± 0.8
Cu-1+Al-1	612 ± 0.8	598 ± 2.4	575 ± 1.6	632 ± 1.2	640 ± 0.5
Cu-1+Al-2	568 ± 0.2	547 ± 1.2	564 ± 0.5	578 ± 0.3	604 ± 0.4
Cu-2	591 ± 1.2	572 ± 0.4	560 ± 0.4	580 ± 0.4	590 ± 2.1
Cu-2+Al-1	570 ± 1.4	558 ± 2.6	549 ± 0.5	561 ± 1.4	568 ± 0.8
Cu-2+Al-2	542 ± 0.8	518 ± 0.4	539 ± 1.3	548 ± 1.6	552 ± 1.0
Cd-1	598 ± 2.8	580 ± 1.8	594 ± 1.6	612 ± 2.4	620 ± 2.1
Cd-1+Al-1	580 ± 2.6	560 ± 2.5	572 ± 1.3	594 ± 0.5	610 ± 1.2
Cd-1+Al-2	548 ± 1.4	538 ± 5.4	568 ± 1.4	584 ± 1.4	595 ± 1.3
Cd-2	512 ± 1.7	510 ± 1.2	541 ± 0.8	562 ± 1.2	564 ± 0.5
Cd-2+Al-1	488 ± 0.9	494 ± 0.7	512 ± 3.2	540 ± 4.2	584 ± 1.8
Cd-2+Al-2	474 ± 3.6	488 ± 1.1	494 ± 1.4	512 ± 3.2	568 ± 1.6
Hg-1	480 ± 1.6	448 ± 1.2	474 ± 0.8	497 ± 1.3	554 ± 2.3
Hg-1+Al-1	462 ± 2.4	431 ± 0.6	485 ± 3.6	510 ± 2.5	570 ± 1.5
Hg-1+Al-2	412 ± 1.8	405 ± 1.5	471 ± 2.7	488 ± 2.1	542 ± 0.6
Hg-2	428 ± 3.4	414 ± 1.4	480 ± 2.4	495 ± 1.5	516 ± 0.4
Hg-2+Al-1	404 ± 1.8	394 ± 1.2	412 ± 2.6	460 ± 1.6	485 ± 5.2
Hg-2+Al-2	378 ± 1.6	340 ± 1.3	360 ± 1.7	334 ± 0.8	358 ± 4.1
pH 7.6/	710 ± 1.4	702 ± 0.6	685 ± 2.1	695 ± 1.0	701 ± 1.2
Al-1	645 ± 0.8	620 ± 0.4	603 ± 1.4	628 ± 0.8	670 ± 1.4
Al-2	618 ± 0.6	590 ± 0.6	570 ± 1.4	585 ± 0.6	604 ± 1.0
Cu-1	680 ± 1.6	640 ± 1.0	675 ± 2.4	705 ± 3.2	720 ± 1.6
Cu-1+Al-1	665 ± 1.4	621 ± 1.2	603 ± 1.6	652 ± 2.4	742 ± 4.6
Cu-1+Al-2	640 ± 2.0	601 ± 1.3	587 ± 1.6	611 ± 0.9	640 ± 1.2
Cu-2	650 ± 1.6	630 ± 1.2	622 ± 0.7	645 ± 2.3	650 ± 0.4
Cu-2+Al-1	610 ± 1.3	580 ± 1.5	547 ± 3.6	562 ± 1.7	578 ± 1.5
Cu-2+Al-2	580 ± 0.4	591 ± 1.3	525 ± 4.2	670 ± 3.8	730 ± 2.7
Cd-1	678 ± 1.1	674 ± 1.4	665 ± 0.7	689 ± 2.4	694 ± 1.2
Cd-1+Al-1	641 ± 1.2	638 ± 1.2	646 ± 3.4	658 ± 0.9	674 ± 1.7
Cd-1+Al-2	634 ± 0.8	624 ± 0.5	612 ± 4.1	664 ± 2.8	645 ± 2.3
Cd-2	594 ± 1.7	598 ± 1.4	584 ± 2.5	570 ± 3.1	592 ± 1.7
Cd-2+Al-1	540 ± 2.3	548 ± 1.3	552 ± 1.6	574 ± 1.7	589 ± 0.6
Cd-2+Al-2	536 ± 1.6	510 ± 0.9	508 ± 3.8	565 ± 1.6	614 ± 1.4
Hg-1	551 ± 1.2	540 ± 2.3	548 ± 1.5	570 ± 1.7	598 ± 1.6
Hg-1+Al-1	542 ± 1.6	521 ± 1.4	562 ± 1.3	592 ± 0.9	612 ± 0.9
Hg-1+Al-2	501 ± 2.1	518 ± 1.3	571 ± 1.6	602 ± 2.3	628 ± 1.2
Hg-2	532 ± 1.8	510 ± 1.4	490 ± 1.8	518 ± 2.6	534 ± 1.8
Hg-2+Al-1	504 ± 1.3	487 ± 1.2	498 ± 2.3	534 ± 1.7	562 ± 1.4
Hg-2+Al-2	491 ± 1.7	465 ± 3.4	510 ± 0.8	548 ± 2.9	574 ± 1.3

(Contd...)

Table 4.3. (Contd...)

pH 9.0/	565 ± 0.7	514 ± 1.3	625 ± 1.4	653 ± 2.1	660 ± 1.4
Al-1	498 ± 3.4	470 ± 2.8	484 ± 1.6	478 ± 1.5	462 ± 0.8
Al-2	420 ± 1.3	410 ± 1.7	395 ± 1.1	384 ± 0.9	391 ± 2.3
Cu-1	512 ± 2.8	508 ± 2.9	520 ± 3.1	537 ± 0.7	536 ± 1.0
Cu-1+Al-1	460 ± 1.3	470 ± 2.6	464 ± 2.6	410 ± 1.9	400 ± 1.7
Cu-1+Al-2	380 ± 1.5	387 ± 2.4	412 ± 0.5	426 ± 0.4	405 ± 1.3
Cu-2	480 ± 5.1	471 ± 4.6	498 ± 2.1	510 ± 1.4	526 ± 0.8
Cu-2+Al-1	420 ± 1.4	417 ± 1.2	404 ± 1.4	412 ± 0.3	390 ± 5.1
Cu-2+Al-2	318 ± 1.0	380 ± 0.5	410 ± 2.3	418 ± 1.8	440 ± 3.7
Cd-1	590 ± 2.3	580 ± 1.7	610 ± 0.7	618 ± 1.2	602 ± 1.2
Cd-1+Al-1	562 ± 1.4	571 ± 0.8	588 ± 1.7	589 ± 2.6	598 ± 0.7
Cd-1+Al-2	510 ± 1.2	518 ± 1.6	541 ± 1.4	538 ± 1.8	520 ± 2.6
Cd-2	565 ± 0.7	540 ± 1.2	548 ± 0.8	580 ± 1.6	578 ± 1.7
Cd-2+Al-1	579 ± 2.5	548 ± 0.9	560 ± 1.6	535 ± 1.8	530 ± 1.6
Cd-2+Al-2	514 ± 1.6	511 ± 3.5	502 ± 1.7	498 ± 1.7	474 ± 3.4
Hg-1	541 ± 0.8	536 ± 1.2	534 ± 0.9	560 ± 1.2	565 ± 1.4
Hg-1+Al-1	510 ± 0.7	498 ± 1.3	502 ± 1.3	540 ± 1.6	551 ± 1.5
Hg-1+Al-2	438 ± 1.3	434 ± 1.6	426 ± 1.2	471 ± 1.3	498 ± 1.6
Hg-2	505 ± 1.2	494 ± 0.9	485 ± 1.8	510 ± 2.1	512 ± 1.3
Hg-2+Al-1	540 ± 1.3	512 ± 0.8	508 ± 0.8	542 ± 3.2	548 ± 0.8
Hg-2+Al-2	460 ± 1.4	454 ± 1.1	498 ± 0.7	514 ± 1.2	540 ± 0.7

(Value = $\bar{x}$ ± SD, n = 6)

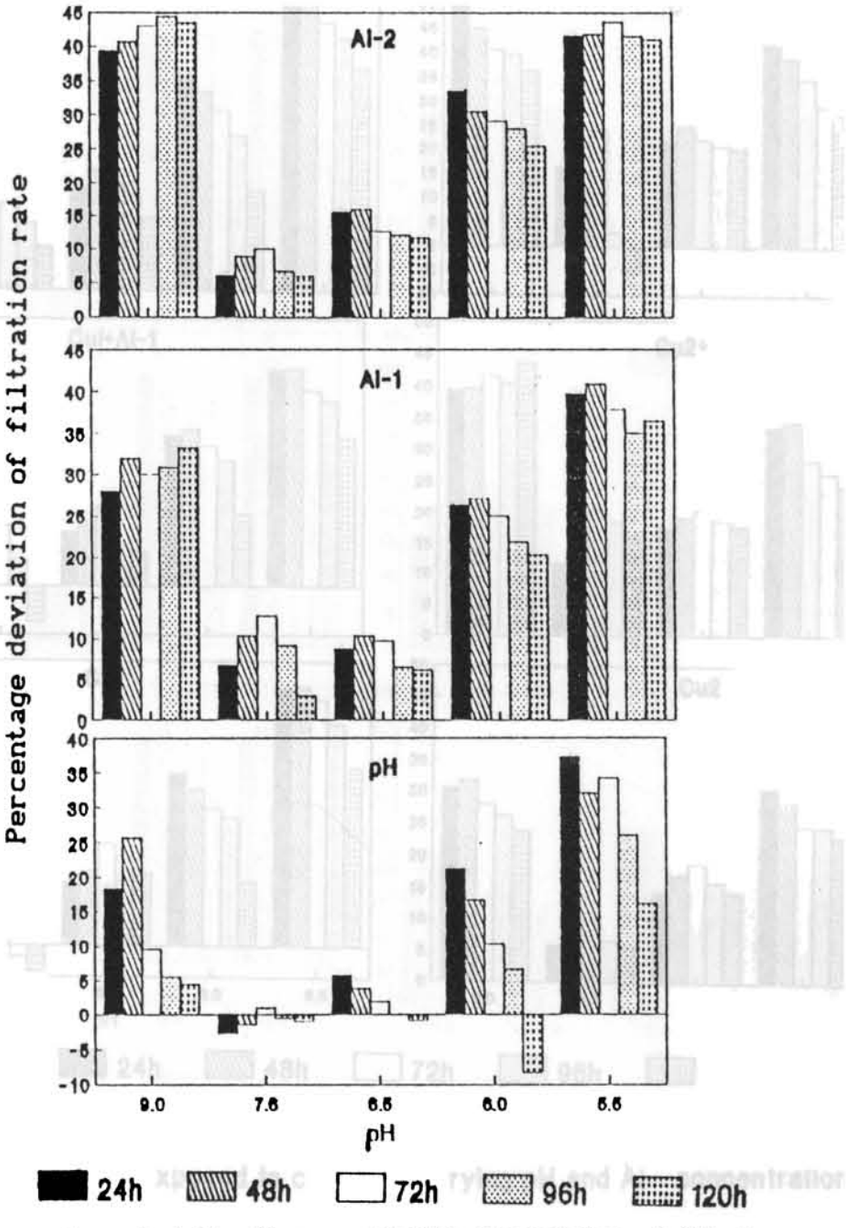

Fig.4.2. Percentage deviation (from control) in suppression of filtration rate of V. cyprinoides exposed to varying pH and Al^{3+} concentrations.

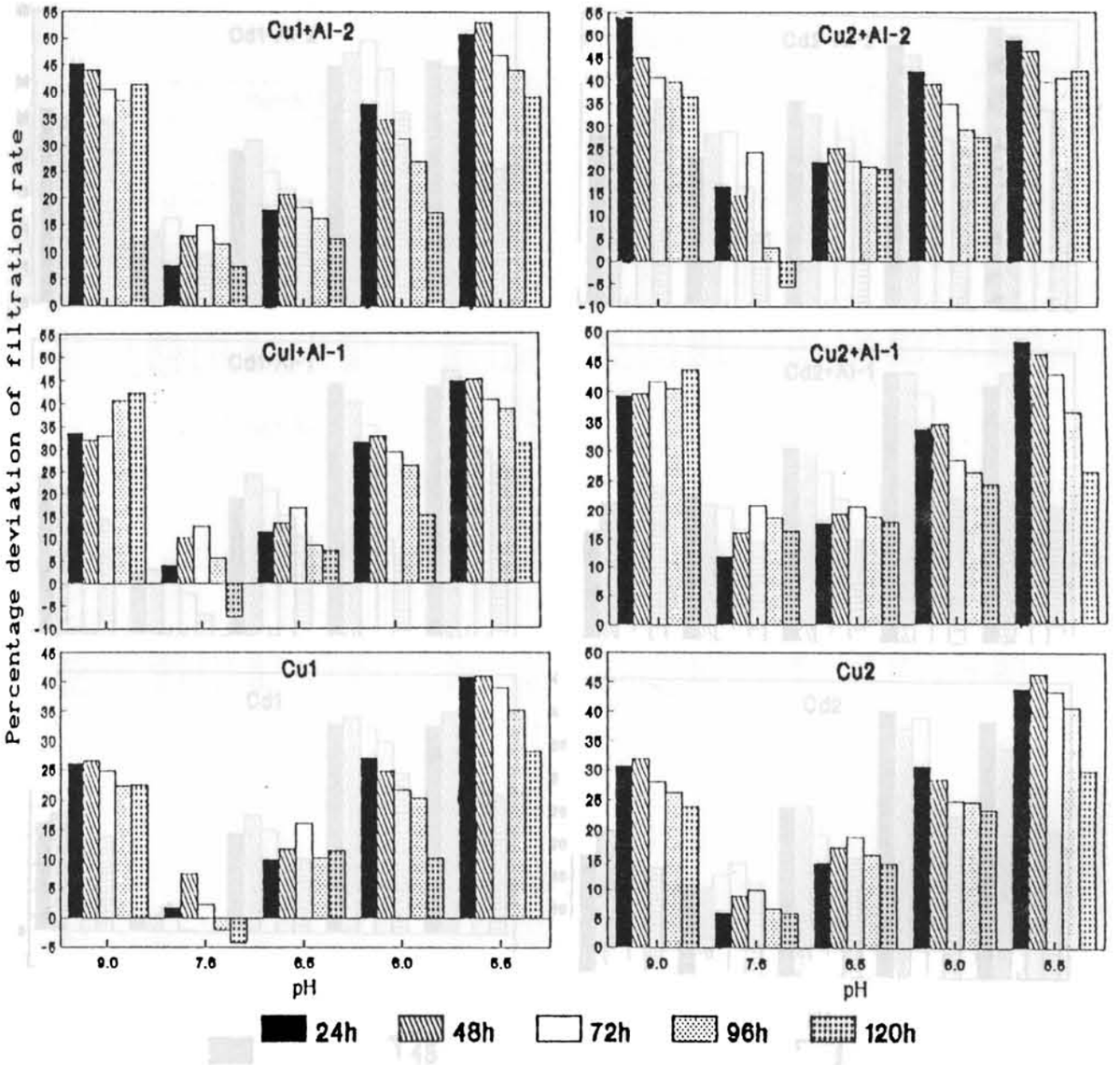

Fig. 4.3. Percentage deviation (from control) in suppression of filtration rate of V. cyprinoides exposed to copper at varying pH and Al^{3+} concentrations.

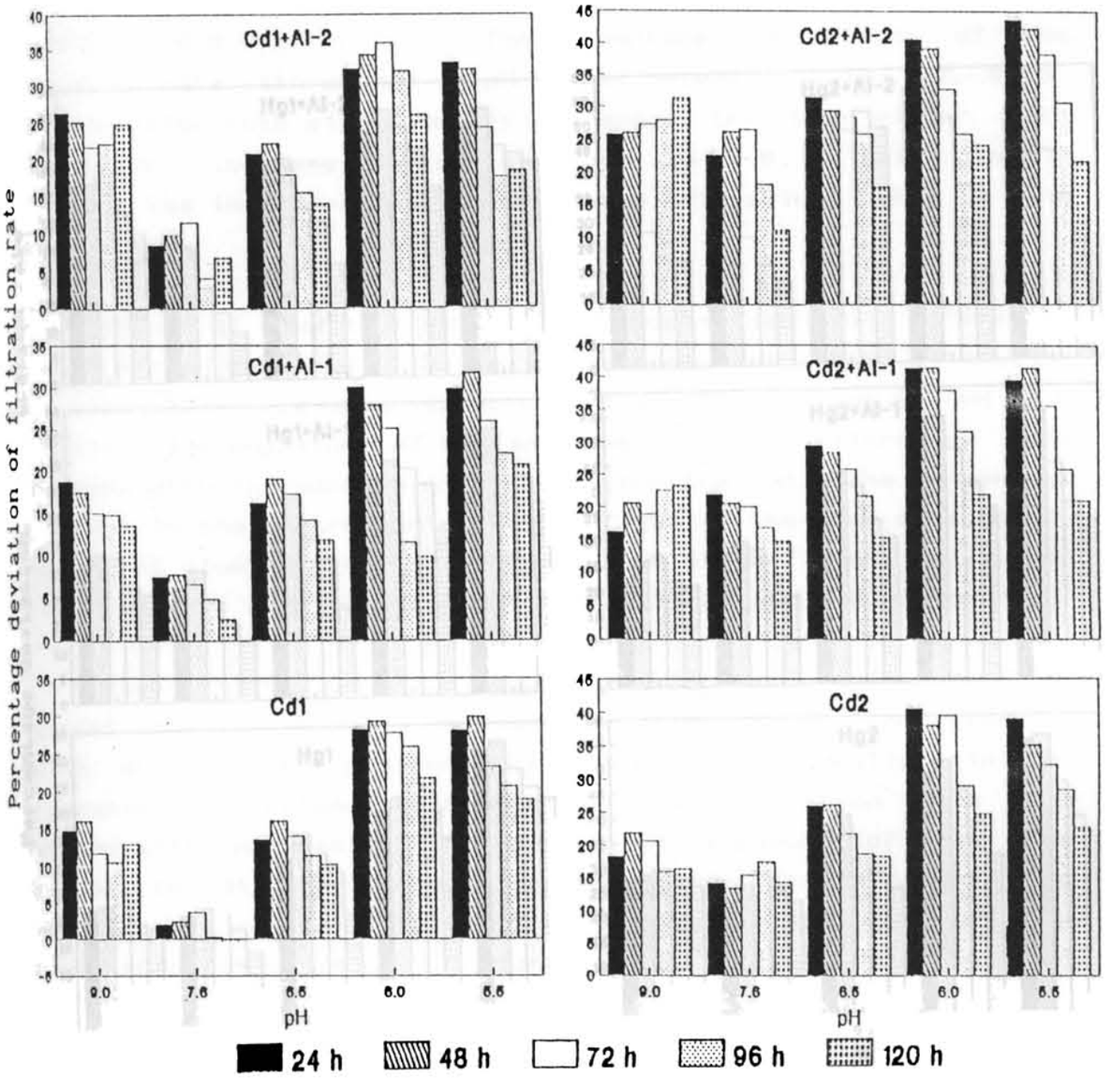

Fig. 4.4. Percentage deviation (from control) in suppression of filtration rate of V. cyprinoides exposed to cadmium at varying pH and Al^{3+} concentrations.

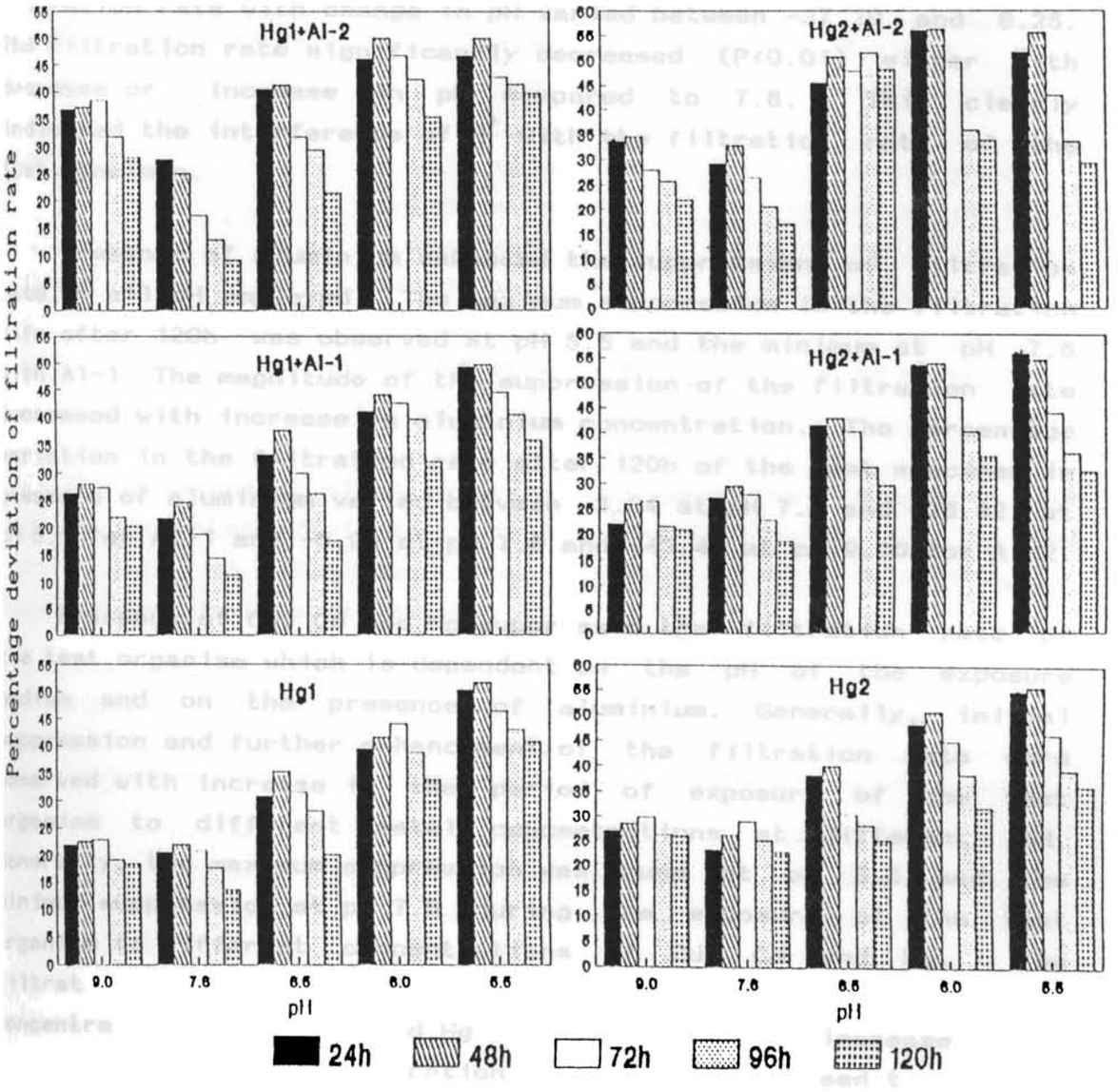

Fig.4.5. Percentage deviation (from control) in suppression of filtration rate of V. cyprinoides exposed to mercury at varying pH and Al^{3+} concentrations.

then gradually increased to a higher value at 120h at pH 6.0, 6.5 and 7.6 whereas, lower values compared to the control were observed at pH 5.5 and 9.0 after 120h. The percentage variation of the filtration rate with change in pH varied between -37.19 and 8.25. The filtration rate significantly decreased ($P<0.01$) either with decrease or increase in pH compared to 7.6. This clearly indicated the interference of H^+ with the filtration rate of the test specimen.

Presence of aluminium enhanced the suppression of filtration rate at all pH employed. The maximum suppression in the filtration rate after 120h was observed at pH 5.5 and the minimum at pH 7.6 with Al-1. The magnitude of the suppression of the filtration rate increased with increase in aluminium concentration. The percentage variation in the filtration rate after 120h of the test specimen in presence of aluminium varied between -3.04 at pH 7.6 and -36.32 at pH 5.5 for Al-1 and -5.93 at pH 7.6 and -43.42 at pH 9.00 for Al-2.

Presence of Cu, Cd and Hg suppressed the filtration rate of the test organism which is dependent on the pH of the exposure medium and on the presence of aluminium. Generally, initial suppression and further enhancement of the filtration rate were observed with increase in the period of exposure of the test organism to different metal concentrations at different pH. Generally, the maximum suppression was found at pH 5.5 and the minimum suppression at pH 7.6 during the exposure of the test organism to different concentrations of Cu, Cd and Hg. The filtration rates of the test organism exposed to different concentrations of Cu, Cd and Hg were increased with increase in pH upto 7.6 and again the filtration rates were suppressed to a lower value at pH 9.0. Increase in the concentration of Cu, Cd and Hg and the presence of aluminium further decreased the filtration rate at all pH values and the interference of aluminium was found to be increased with increase in concentration except in the case of the test organism exposed to Hg-1 at pH 7.6, Hg-2 at different pH except 6.5 and 9.0, Cu-1 at pH 9.0, Cu-2 at pH 7.6 and 9.0 and Cd-1 at pH 5.5 and 7.6.

The percentage deviation in the filtration rate (from the control value), of the test specimen exposed to different metal concentrations varied after 120h as follows:

Metal concentration	Minimum value	Maximum value
Cu-1	4.20 at pH 7.6	- 28.08 at pH 5.5
Cu-2	-5.93 at pH 7.6	-29.81 at pH 5.5
Cd-1	0.43 at pH 7.6	-21.85 at pH 6.0
Cd-2	-14.33 at pH 7.6	-24.75 at pH 6.0
Hg-1	-13.46 at pH 7.6	-40.67 at pH 5.5
Hg-2	-22.72 at pH 7.6	-36.18 at pH 5.5

The presence of aluminium also suppressed the filtration rate and the percentage deviation from the control value due to the interference of aluminium after 120h varied in different experimental designs as follows:

Aluminium conc.	Metal conc.	Minimum value	Maximum value
Al-1	Cu-1	7.38 at pH 7.6	-42.11 at pH 9.0
	Cu-2	-16.35 at pH 7.6	-43.56 at pH 9.0
	Cd-1	-2.46 at pH 7.6	-20.69 at pH 5.5
	Cd-2	-14.76 at pH 7.6	-23.30 at pH 9.0
	Hg-1	-11.43 at pH 7.6	-35.89 at pH 5.5
	Hg-2	-18.67 at pH 7.6	-35.60 at pH 6.0
Al-2	Cu-1	-7.38 at pH 7.6	-41.39 at pH 9.0
	Cu-2	5.64 at pH 7.6	-41.97 at pH 5.5
	Cd-1	-6.66 at pH 7.6	-25.90 at pH 6.0
	Cd-2	-11.14 at pH 7.6	-31.40 at pH 9.0
	Hg-1	-9.12 at pH 7.6	-39.80 at pH 5.5
	Hg-2	-16.93 at pH 7.6	-48.19 at pH 6.5

Minimal interference of aluminium in filtration rate of the test organism exposed to trace metals was found to occur at pH 7.6 and the maximum suppression in the filtration rate occured was different. It depends on the nature of the metal and on the concentration of aluminium as shown above.

The magnitude of the suppression in the filtration rate varied between different trace metals employed in the order Hg> Cu > Cd. The presence of aluminium altered this order to Cu > Hg > Cd for low metal concentrations and Hg > Cu > Cd for high metal concentrations. ANOVA results proved the significant interaction of Cu-1, Cd-1, Hg and Al at all pH ($P<0.01$) on the filtration rate during the period of exposure except at pH 9.0 for Cu and Cd where the changes were not significant during the period of exposure.

These observations clearly indicated the interactions of H^+, Al^{3+} and trace metals on the filtration rate of the test organism. The filtering rates of bivalves are known to be influenced by environmental parameters such as salinity, temperature, dissolved oxygen, pH and suspended matter (Badman, 1974; 1975; Foster-Smith, 1975; Walting and Walting, 1982). Competitive effects of eight metals on the filtration rates of *Perna perna* have been measured (Walting and Walting, 1982) and the range of toxicity was similar to the results obtained in the present study.

In the undisturbed animals, the valve movements are initiated by intrinsic periodic stimuli originating in the various ganglia (Barnes, 1955). Changes from the normal pattern of movements are seen in the presence of altered chemical or other conditions of the medium. A depression in the filtration rates of bivalves has been shown to take place following exposure to a variety of metal ions (Abel, 1976; Walting and Walting, 1982; Howell *et al.*, 1984; Grace and Gainey, 1987; Micallef and Tyler, 1990). The behavioural response of *Mytilus edulis* to increasing copper concentration has been reported as a three stage process of shell valve movement—shell valve adduction followed by a period of testing and then

complete closure (Manley and Davenport, 1979). The shell valve adduction and closure responses of *Mytilus* sp. to increasing dissolved metal concentrations have been described previously (Davenport, 1977; Manley and Davenport, 1979; Redpath and Davenport, 1988).

There have been few studies on the effects of trace metals and patterns of pumping activity in bivalves. Filtration in *Perna perna*, *Chormytilus meridionalis*, *Crassostrea margaritacea* and *Crassostrea gigas* is reduced 50% by Zn, Cu, and Cd in the ranges 750 - 2000 $\mu g\ l^{-1}$, 60 - 160 $\mu g\ l^{-1}$ and 0.6 - 28 $ng\ l^{-1}$ respectively (Walting and Walting, 1982).

Reduction of filtration rates and disturbed ciliary activity were observed in response to uptake of dissolved chromium by *Myaarenaria* L and uptake of dissolved and particulate chromium by *Mytilus edulis* L (Capuzzo and Sasner, 1977). According to Badman (1975), the clams perform less pumping of water during the period of intense valve activity under hypoxic conditions than when oxygen is available to them. Thus, the postulation that the animals undergo the intense activity in order to supply more oxygen to their tissues cannot hold. But during this activity, accumulated metabolic wastes are released from the tissues and expelled from the mantle cavity. The observed enhancement in the filtration rate with increase in the period of exposure may be due to the above fact.

The water current through the mantle cavity is generated by the lateral cilia of the gills (Silvester and Sleigh, 1984). Brown and Newell (1972) found that both Zn and Cu inhibited the ciliary activity. Howell *et al.* (1984) suggested that the action of copper is an inhibition of the branchial nerve which inturn controls ciliary action and not a direct effect on the cilia or shell valve closure. Numerous studies have demonstrated that bivalves exhibit daily and seasonal cycle of physiological and behavioural activity (Higgins, 1980; Doherty *et al.*, 1987). Exposure of bivalves to toxicants (e.g., metals and halogens) produced alteration in

behaviour (e.g. cessation of siphoning and valve closure) that resulted in isolation of the soft tissues from the toxic effects of the pollutant. (Davenport, 1977; Akberali and Black, 1980). Consequently these responses may interrupt the accumulation of metals. Manley and Davenport (1979) reported that the threshold of detection (interruption of valve movements) for Cu by the representatives of the marine bivalves generally ranged from 0.02 to 0.14 ng l^{-1}. Akberali and Black (1980) reported that specimen of *Scrobicularia plana* interacted with their environment in the presence of Cu at concentrations 0.01 and 0.05 mg l^{-1}. A reduction in activity was observed at 0.1 mg l^{-1} while, exposure to Cu at a concentration of 0.05 mg l^{-1} resulted in rapid (less than one minute) retraction of the siphon and closure of the valve. Rodgers *et al.* (1980) reported an inverse relationship between Cu and visually assessed filtering activity by Asiatic clams. They observed that >50% of the test specimens exposed to Cu at a concentration of 0.01 mg l^{-1} was filtering after 17h of exposure. No specimen was active in solutions of 0.05 mg l^{-1}. Kraemer (1983) noted an atypical clustering of cilia on the highly innervated epithelial surface of the anal siphon of *Corbiculidae flumnea* and suggested that these cilia tufts may be the sensory organelles responsible for the high degree of sensitivity of the anal siphon. Akberali *et al.* (1982) studied the influence of external calcium on Cu induced contraction of the isolated siphon of the estuarine bivalve *S.plana*. They found that siphonal contraction in the presence of Cu was stronger dependent on the presence of calcium in the breathing medium.

The suppression in the filtration rate of the test specimen with change in H^+ concentration may be attributed to the avoidance behaviour of the clam (Crenshan, 1972). Measurements of oxygen tension in the extra pallial fluid of *Mercenaria* sp. (Crenshan and Neff, 1969; Crenshan, 1972) revealed that the clam become completely anaerobic within 25 minutes of shell closure. Valve closure in bivalves such as *S.plana*, *M.edulis* and *M.mercenaria* during short term stress conditions or natural quiescence, thus resulted in cessation of pumping and the introduction of largely

anaerobic respirations. This contributed to the fall of pH in the mantle cavity due to the accumulation of acid metabolites. The resumption of activity is characterised by a rapid series of valve adductions whose probable function, at least in *S.plana* and possibly in other bivalves, is to hyper ventilate the mantle cavity and probably to accelerate the removal of metabolites.

The hydrodynamics of bivalve pumping depends on mucus production. Mucus may reduce friction drag (Daniel, 1981) as demonstrated for fish in turbulent flow (Rosen and Cornford, 1971). This may facilitated the filtration with increase in the period of exposure as observed in the present study.

Doherty *et al.* (1987) demonstrated that the valve closure response of the Asiatic clam, *Corbicula fluminea* is a phenomenon dependent on the exposure concentration of dissolved Cd and Zn. Exposure of Asiatic clam to Cd resulted in valve closure at a rate approximately three times faster than which occurred on exposure to Zn. The decrease in the filtration rate of the test specimen exposed to Cd was also due to the valve closure for the avoidance of the pollutant. Suppression in physiological activity decreased with the increase in exposure period as observed in this study agrees with that of Radhakrishniah (1988).

The result for the effect of mercury on the filtration rate showed that mercury rapidly decreases the filtration rate. Other studies also showed a rapid decrease in the filtration rate of bivalves following exposure to inorganic mercury (Micallef and Tyler, 1990). Walting and Walting (1982) reported a 10 min EC50 of 0.04 mg l^{-1} and 0.025 mg l^{-1} Hg for the filtration rates of *M.edulis* and *Perna perna* respectively, Dorn (1976) observed a reduction of about 90-100% in the filtration rate of mussels exposed to methyl mercury (0.4-2.4 ng Hg l^{-1}) for 48h. Filtration rate of bivalve *Villorita cyprinoides var cochinensis* (Hanley) decreased exponentially with increasing metal concentration (Abraham *et al.*, 1986). The order of metals which decrease the filtration rate in the present study was in good agreement with the

results based on EC50, the concentration which reduced the rate of filtration by 50% (Abraham *et al.*, 1986) and similar trends were observed by Abel and Papathanassiou (1986).

Effects of H^+ and Al^{3+} on the ventilation of brook trout *(Salvelinus fontinalis)* and rainbow trout *(Salmo gairdneri)* were studied by Walker *et al.* (1988) and reported mucous clogging of the gills and reduction of oxygen extraction from the water. Two fold increase in the ventilation volume when brook trout (*Salvelinus fontinalis*) exposed to acid was documented (Walker *et al.*, 1988) and also suggested that Al interfered with the mechanism controlling the ventilator response. No study was undertaken for the interaction of H^+, Al and trace metals on the filtration rates of invertebrates. The moderate and extensive production of mucus and the bioavailability of metal and its interaction with the body at different pH were responsible for the reduction in the filtration rate of the test organism exposed to Cu, Cd, Hg and Al at different pH. The possibility of the effects of body size, physiological status, experimental techniques, salinity, temperature and other parameters of water quality except pH has to be ruled out in this study because those factors were kept constant throughout the experimental design. The minimum suppression in the filtration rate was observed at pH 7.6, the pH at which the animals were acclimatized. This may be due to the minimum disturbance by H^+. The maximum suppression was observed at pH 5.5 in the case of animals exposed to Cu, Cd and Hg. This may be due to the increased H^+ concentration in the exposure medium compared to that of control, which may cause the avoidance behaviour. The changes in the pH value at which the maximum suppression when the organism is exposed to Cu, Cd and Hg in presence of aluminium may be due to the contribution of aluminium. The hydroxide form of Al^{3+} which is the predominant species at higher pH may interfere with gills thereby the filtration rate is suppressed initially and further increased to a higher value for the removal of metabolic wastes due to the stress as stated earlier.

The amount of water that passes over the gills of lamellibranches is of considerable interest in the study of respiration or excretory activities of these animals. It is also of value in studying the effects of water pollution on commercially important bivalves. The present study clearly indicated the reduction in the filtering activity of the test organism due to the presence of H^+, Al, Cu, Cd and Hg. The variations observed can be explained based on the production of mucus during the exposure to different metals at different pH, bioavailability of trace metals, interaction of H^+ and trace metals on the physiological action of the test species and variation of valve closure due to the stress.

4.3. METABOLIC RATE

The changes in behavioural and physiological activity of aquatic organisms serve as useful indicators of sublethal effects. Rate of oxygen uptake is a meaningful index to assess the toxicity and stress in animals as it indicates the energy expenditure required to meet the demands of an environmental alteration (Thurberg *et al.*, 1974). The environmental factors which affect metabolic rate have been classified into two— controlling factors and limiting factors. The controlling factors consist of several parameters relating to maximal and minimal metabolic rates which include salinity and temperature while, the limiting factors are those which interfere into the metabolic processes of the organism and include the availability of oxygen and substrate supply. Newell and Northcroft (1967) distinguished two extreme rates of oxygen consumption, the lowest or "standard" rate being the oxygen during minimal activity and the highest or "active" rate being the requirement during maximal activity.

Several authors have reported altered oxygen consumption due to exposure of aquatic organisms to sublethal levels of trace metals (De Coursey and Vernberg, 1972; Byczkowski and Sorenson, 1984; Baby and Menon, 1986; Sivaramakrishna, 1991; Geetha, 1992; Sujatha, 1992). In bivalves, feeding and respiration occur as a result of a water current drawn into the mantle cavity by

specialized cilia found on each gill filament, so that the physiological processes of filtration and respiration are intimately connected. In bivalves, these physiological processes have been shown to be influenced by natural and man made environmental changes. However, rarely have any of the studies dealt with the interactive effects of H^+ and Al^{3+} on the metabolic rates of *Villorita cyprinoides* var *cochinensis* (Hanley) exposed to Cu, Cd and Hg. The specific objective of this section was to study the interactive effects of H^+ and Al^{3+} on the variation of the metabolic rate of the test organism exposed to sublethal concentrations of Cu, Cd and Hg.

MATERIALS AND METHODS

Experimental conditions and method employed for the determination of oxygen consumption are given in Chapter II. Salinity, temperature and hardness of the exposure medium were kept constant throughout the study. 5.5, 6.0, 6.5, 7.6 and 9.0 were the different pH employed (± 0.01).

RESULTS AND DISCUSSION

The metabolic rates measured at 24h intervals for a period of 120h are presented in the Table 4.4. The percentage variations in metabolic rate from the control value for the different experimental conditions (Fig. 4.6 to Fig. 4.9) indicate the trend in the variation of metabolic rates under the influence of H^+, Al^{3+}, Cu^{2+}, Cd^{2+} and Hg^{2+}. The metabolic rate of the test organism at pH 7.6 i.e., the pH at which the test organisms were acclimatized, and at 0h was 2.132 ml O_2 h^{-1} g^{-1} (dry weight). Throughout the exposure period no mortality of organisms was registered in the different experimental designs. The metabolic rates of the test organism exposed to different pH were suddenly decreased at 24h and then gradually increased to a value lower than the control value. The rate of oxygen consumption was significantly diminished ($P<0.01$) with alteration in the H^+ contents compared to pH 7.6 and the range was between 2.045 ml O_2 h^{-1} g^{-1} dry weight at

ble 4.4. Metabolic rate (ml O_2 h^{-1} g^{-1} dry wt) of *V. cyprinoides* exposed to varying pH and metal concentrations.

(Control value = 2.132±0.003)

Exposure period (h)					
metal conc.	24	48	72	96	120
pH 5.5/	1.248±0.002	1.316±0.005	1.298±0.003	1.402±0.006	1.581±0.002
-1	1.121±0.004	1.284±0.003	1.212±0.010	1.312±0.006	1.340±0.008
-2	0.986±0.005	0.981±0.004	1.106±0.002	1.112±0.005	1.121±0.005
u-1	0.942±0.002	0.940±0.007	0.952±0.005	0.962±0.004	1.023±0.003
u-1+Al-1	0.811±0.002	0.808±0.002	0.941±0.007	0.951±0.002	0.986±0.008
u-1+Al-2	0.681±0.003	0.672±0.004	0.733±0.002	0.801±0.007	0.941±0.004
u-2	0.812±0.007	0.810±0.005	0.921±0.007	1.102±0.010	0.982±0.004
u-2+Al-1	0.688±0.001	0.687±0.002	0.732±0.008	0.898±0.007	0.986±0.003
u-2+Al-2	0.558±0.004	0.568±0.001	0.702±0.004	0.713±0.001	0.712±0.003
d-1	1.213±0.002	1.112±0.001	1.561±0.002	1.578±0.001	1.712±0.002
d-1+Al-1	1.192±0.004	1.082±0.004	1.513±0.007	1.521±0.002	1.692±0.004
d-1+Al-2	0.972±0.003	1.001±0.003	1.521±0.011	1.601±0.004	1.618±0.001
d-2	1.986±0.005	2.012±0.001	1.994±0.017	1.681±0.003	1.612±0.008
d-2+Al-1	1.892±0.001	1.872±0.003	1.861±0.008	1.621±0.002	1.542±0.009
d-2+Al-2	1.713±0.003	1.722±0.002	1.812±0.019	1.912±0.004	1.412±0.023
g-1	0.571±0.002	0.560±0.002	0.592±0.003	0.602±0.001	0.613±0.001
g-1+Al-1	0.558±0.004	0.501±0.001	0.589±0.002	0.612±0.003	0.686±0.012
g-1+Al-2	0.412±0.003	0.401±0.001	0.512±0.012	0.586±0.004	0.618±0.014
g-2	0.372±0.001	0.361±0.002	0.392±0.008	0.621±0.014	0.672±0.006
g-2+Al-1	0.314±0.003	0.301±0.003	0.386±0.006	0.671±0.025	0.686±0.002
g-2+Al-2	0.269±0.002	0.252±0.001	0.392±0.014	0.712±0.036	0.722±0.003
pH 8.0/	1.712±0.002	1.723±0.002	1.735±0.001	1.782±0.012	1.988±0.004
l-1	1.681±0.006	1.691±0.004	1.701±0.005	1.712±0.010	1.734±0.002
l-2	1.641±0.001	1.652±0.006	1.671±0.004	1.801±0.010	1.812±0.008
u-1	1.013±0.002	1.123±0.004	1.312±0.002	1.323±0.002	1.403±0.004
u-1+Al-1	0.989±0.004	0.992±0.002	1.212±0.003	1.318±0.001	1.401±0.002
u-1+Al-2	0.871±0.003	0.982±0.003	1.192±0.002	1.302±0.012	1.392±0.001
u-2	0.987±0.001	0.992±0.001	1.012±0.001	1.014±0.001	1.213±0.003
u-2+Al-1	0.962±0.002	0.951±0.002	1.008±0.003	1.128±0.014	1.208±0.001
u-2+Al-2	0.868±0.003	0.902±0.003	0.958±0.002	1.102±0.009	1.201±0.008
d-1	1.413±0.002	1.402±0.002	1.421±0.001	1.513±0.003	1.612±0.005
d-1+Al-1	1.381±0.001	1.398±0.003	1.523±0.015	1.620±0.001	1.713±0.001
d-1+Al-2	1.361±0.002	1.351±0.002	1.362±0.003	1.401±0.002	1.512±0.003
d-2	1.123±0.004	1.213±0.001	1.198±0.003	1.480±0.004	1.523±0.001
d-2+Al-1	1.002±0.003	1.012±0.008	1.201±0.008	1.408±0.012	1.501±0.005
d-2+Al-2	0.986±0.004	0.992±0.002	1.398±0.001	1.512±0.018	1.486±0.001
g-1	0.695±0.002	0.612±0.002	0.598±0.002	0.712±0.001	0.726±0.002
g-1+Al-1	0.681±0.001	0.597±0.003	0.612±0.001	0.692±0.002	0.748±0.001
g-1+Al-2	0.586±0.008	0.532±0.008	0.571±0.014	0.598±0.004	0.698±0.012
g-2	0.483±0.007	0.441±0.011	0.512±0.011	0.543±0.001	0.562±0.013
g-2+Al-1	0.412±0.001	0.408±0.001	0.486±0.021	0.512±0.002	0.531±0.002
g-2+Al-2	0.386±0.001	0.381±0.001	0.398±0.001	0.528±0.003	0.541±0.004

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:H 6.5/	1.783±0.007	1.792±0.009	1.813±0.008	1.901±0.011	1.922±0.006
il-1	1.802±0.002	1.787±0.002	1.831±0.004	1.892±0.002	1.901±0.005
il-2	1.798±0.006	1.792±0.003	1.821±0.005	1.832±0.003	1.844±0.003
ju-1	1.412±0.002	1.398±0.002	1.513±0.012	1.561±0.002	1.602±0.004
ju-1+Al-1	1.212±0.003	1.201±0.001	1.193±0.010	1.432±0.024	1.586±0.002
ju-1+Al-2	1.193±0.007	1.012±0.003	1.212±0.008	1.234±0.014	1.497±0.012
ju-2	1.234±0.004	1.215±0.002	0.986±0.014	1.524±0.030	1.544±0.008
ju-2+Al-1	1.013±0.005	0.998±0.003	0.987±0.006	1.232±0.001	1.432±0.007
ju-2+Al-2	1.002±0.003	1.001±0.001	1.132±0.004	1.192±0.008	1.386±0.020
jd-1	1.712±0.002	1.698±0.001	1.701±0.003	1.812±0.005	1.876±0.001
jd-1+Al-1	1.697±0.008	1.641±0.004	1.681±0.002	1.712±0.002	1.801±0.008
jd-1+Al-2	1.405±0.004	1.398±0.002	1.455±0.005	1.621±0.003	1.714±0.007
jd-2	1.512±0.006	1.523±0.003	1.541±0.002	1.642±0.001	1.812±0.012
jd-2+Al-1	1.481±0.001	1.492±0.001	1.514±0.003	1.572±0.002	1.728±0.020
jd-2+Al-2	1.211±0.003	1.312±0.002	1.322±0.001	1.520±0.005	1.601±0.009
jg-1	0.786±0.008	0.763±0.002	0.771±0.002	0.822±0.002	0.981±0.001
jg-1+Al-1	0.698±0.007	0.671±0.003	0.692±0.003	0.723±0.001	0.943±0.001
jg-1+Al-2	0.612±0.003	0.601±0.004	0.712±0.001	0.801±0.003	0.902±0.013
jg-2	0.653±0.010	0.641±0.001	0.687±0.006	0.713±0.012	0.786±0.014
jg-2+Al-1	0.602±0.002	0.598±0.003	0.621±0.010	0.686±0.010	0.732±0.001
jg-2+Al-2	0.586±0.014	0.541±0.009	0.568±0.012	0.532±0.009	0.561±0.008
:H 7.6/	2.045±0.005	2.011±0.007	1.989±0.004	2.023±0.002	2.089±0.004
il-1	1.812±0.007	1.801±0.005	1.781±0.004	1.812±0.011	1.863±0.009
il-2	1.706±0.008	1.686±0.012	1.613±0.004	1.602±0.007	1.721±0.010
ju-1	1.212±0.004	1.241±0.004	1.563±0.005	1.621±0.002	1.643±0.008
ju-1+Al-1	1.198±0.002	1.083±0.009	0.986±0.008	1.134±0.003	1.542±0.007
ju-1+Al-2	1.162±0.007	0.984±0.014	0.956±0.006	1.012±0.004	1.213±0.012
ju-2	1.013±0.003	1.423±0.012	1.401±0.002	1.571±0.008	1.602±0.013
ju-2+Al-1	0.821±0.004	0.796±0.013	0.782±0.008	0.798±0.004	0.812±0.007
ju-2+Al-2	0.789±0.005	0.732±0.007	0.741±0.005	0.724±0.008	0.801±0.014
jd-1	1.413±0.002	1.403±0.002	1.400±0.002	1.512±0.005	1.684±0.003
jd-1+Al-1	1.381±0.003	1.372±0.001	1.402±0.004	1.508±0.002	1.612±0.008
jd-1+Al-2	1.351±0.004	1.324±0.006	1.310±0.008	1.581±0.003	1.521±0.007
jd-2	1.213±0.002	1.324±0.007	1.512±0.002	1.584±0.005	1.613±0.001
jd-2+Al-1	1.191±0.004	1.213±0.023	1.192±0.005	1.081±0.010	1.292±0.002
jd-2+Al-2	0.986±0.005	0.912±0.009	0.910±0.007	1.072±0.008	1.223±0.003
jg-1	1.121±0.002	1.078±0.003	1.102±0.001	1.213±0.002	1.314±0.002
jg-1+Al-1	1.023±0.001	0.998±0.001	1.201±0.001	1.325±0.001	1.413±0.001
jg-1+Al-2	0.992±0.004	1.021±0.002	1.212±0.006	1.398±0.001	1.424±0.003
jg-2	0.987±0.005	1.112±0.010	1.134±0.004	1.342±0.002	1.425±0.001
jg-2+Al-1	0.798±0.001	0.776±0.002	0.797±0.002	0.976±0.003	1.213±0.002
jg-2+Al-2	0.562±0.002	0.486±0.015	0.812±0.004	0.923±0.005	1.112±0.003

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H 8.0/	1.621±0.009	1.513±0.006	1.712±0.015	1.791±0.008	1.812±0.017
1-1	1.121±0.008	1.008±0.004	1.112±0.004	1.009±0.006	0.992±0.007
1-2	0.892±0.007	0.872±0.008	0.862±0.009	0.853±0.006	0.862±0.004
2-1	1.412±0.002	1.401±0.003	1.421±0.002	1.512±0.003	1.511±0.001
2-1+A1-1	1.008±0.003	1.107±0.008	1.102±0.007	1.001±0.007	0.986±0.003
2-1+A1-2	0.712±0.007	0.886±0.002	0.912±0.003	1.022±0.004	1.002±0.002
2-2	1.113±0.006	1.092±0.001	1.124±0.007	1.215±0.012	1.312±0.001
2-2+A1-1	0.801±0.004	0.800±0.001	0.792±0.004	0.798±0.002	0.912±0.002
2-2+A1-2	0.602±0.004	0.713±0.002	0.798±0.001	0.812±0.001	1.013±0.001
3-1	1.623±0.003	1.602±0.003	1.712±0.020	1.728±0.002	1.692±0.002
3-1+A1-1	1.510±0.002	1.528±0.002	1.586±0.006	1.612±0.008	1.686±0.003
3-1+A1-2	1.442±0.001	1.501±0.010	1.546±0.009	1.526±0.001	1.510±0.001
3-2	1.432±0.008	1.411±0.005	1.426±0.005	1.510±0.030	1.486±0.002
3-2+A1-1	1.414±0.004	1.401±0.001	1.398±0.003	1.296±0.006	1.213±0.001
3-2+A1-2	1.214±0.001	1.201±0.002	1.198±0.001	1.002±0.001	0.986±0.002
4-1	0.986±0.001	0.971±0.003	0.972±0.003	0.998±0.001	1.121±0.001
4-1+A1-1	0.891±0.002	0.912±0.001	0.898±0.001	0.974±0.002	0.985±0.001
4-1+A1-2	0.831±0.001	0.820±0.001	0.802±0.005	0.912±0.010	0.934±0.010
4-2	0.632±0.008	0.598±0.004	0.586±0.001	0.886±0.024	0.898±0.001
4-2+A1-1	0.452±0.004	0.448±0.001	0.686±0.001	0.792±0.011	0.816±0.010
4-2+A1-2	0.248±0.007	0.241±0.005	0.456±0.025	0.892±0.036	0.962±0.007

alue = $\bar{x}$ ± SD, n = 6)

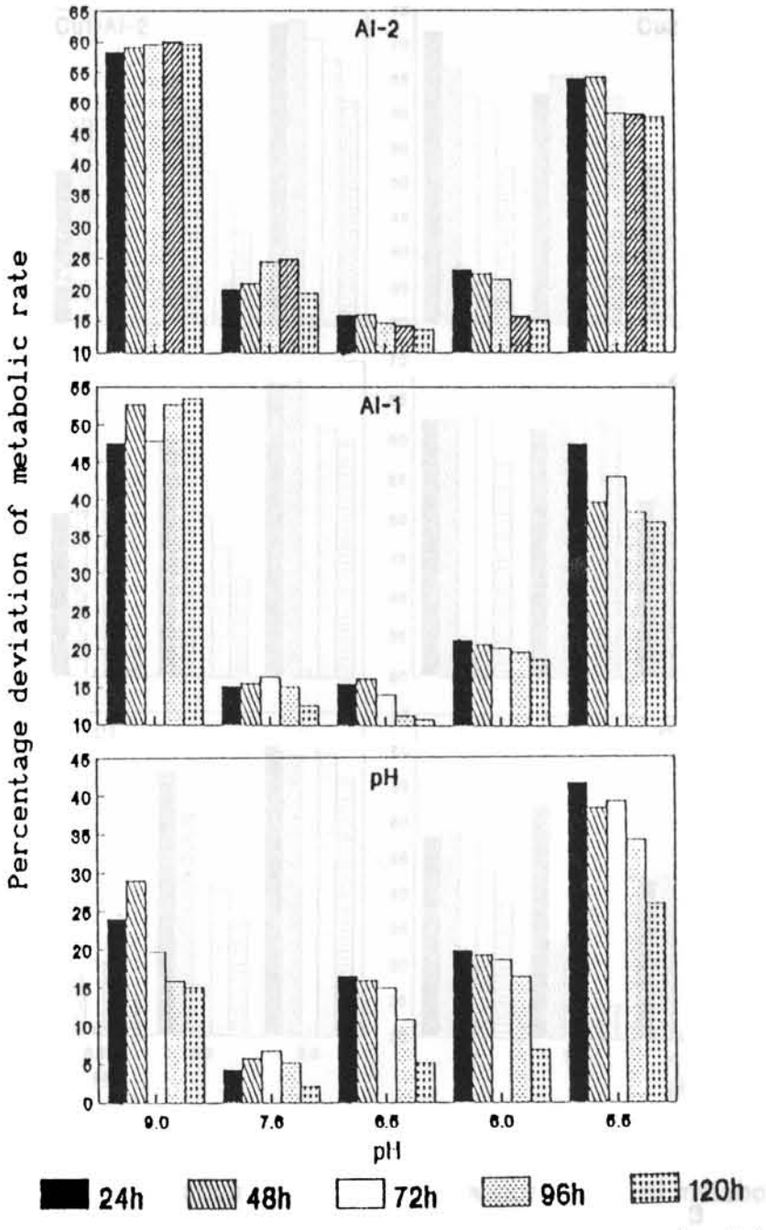


Fig. 4.6. Percentage deviation (from control) in suppression of metabolic rate of $V.^{3+}$ cyprinoides exposed to varying pH and Al concentrations.

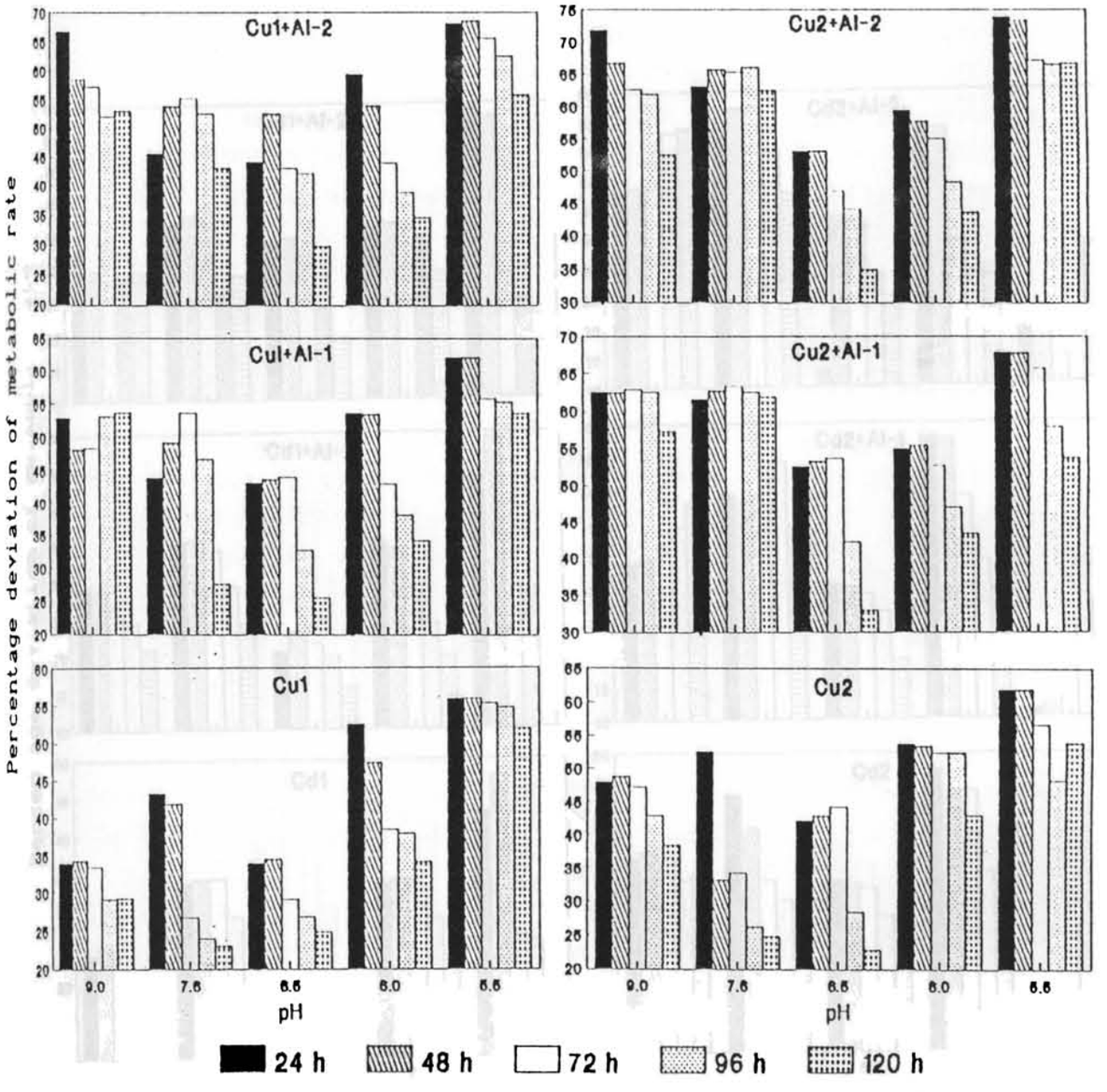


Fig.4.7. Percentage deviation (from control) in suppression of metabolic rate of *V. cyprinoides* exposed to copper at varying pH and Al^{3+} concentrations.

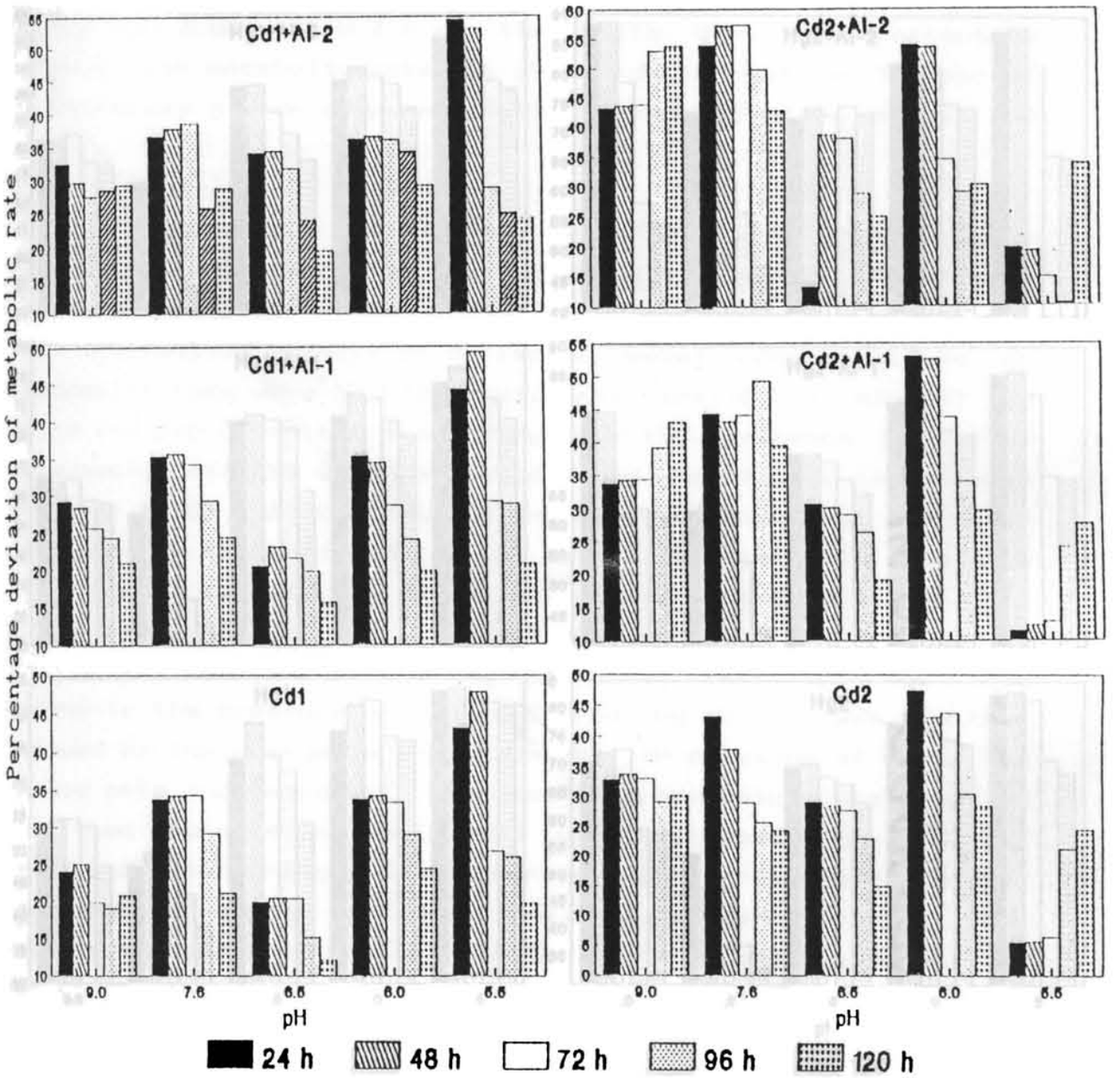


Fig. 4.8. Percentage deviation (from control) in suppression of metabolic rate of V. cyprinoides exposed to cadmium at varying pH and Al^{3+} concentrations.

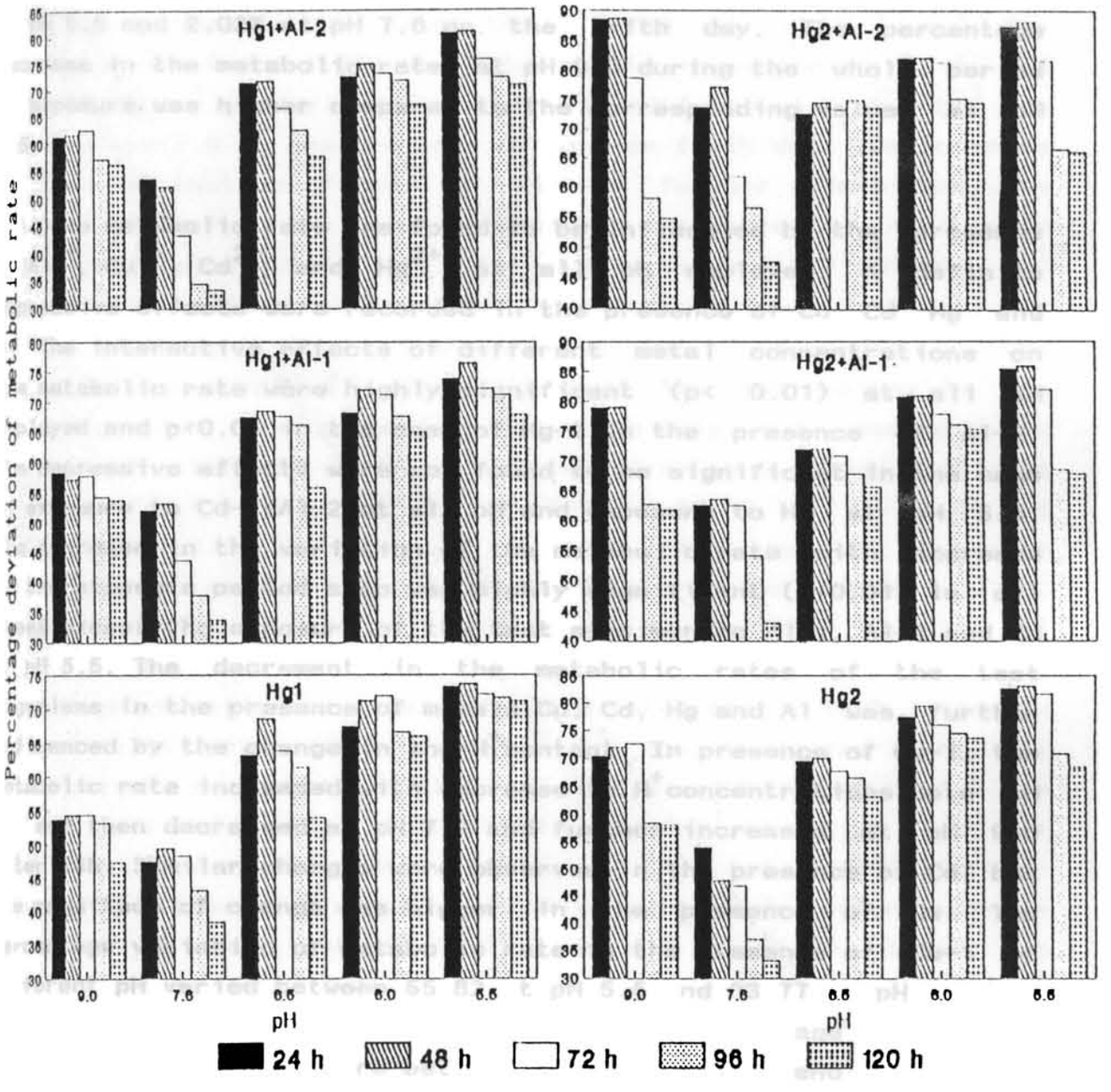


Fig. 4.9. Percentage deviation (from control) in suppression of metabolic rate of $V.^{3+}$ cyprinoides exposed to mercury at varying pH and Al concentrations.

pH 7.6 and 1.248 ml O_2 h^{-1} g^{-1} dry weight at pH 5.5 after 24h. The percentage variations in the metabolic rate decreased with increase in pH upto 7.6 and then increased at pH 9.0. The same trend was observed throughout the exposure period and varied between 25.84% at pH 5.5 and 2.02% at pH 7.6 on the fifth day. The percentage decrease in the metabolic rates at pH 9.0 during the whole period of exposure was higher compared to the corresponding values at pH 6.5.

The metabolic rate was found to be influenced by the presence of Al^{3+}, Cu^{2+}, Cd^{2+} and Hg^{2+} at all pH employed. A definite depressive effects were recorded in the presence of Cu, Cd, Hg and Al. The interactive effects of different metal concentrations on the metabolic rate were highly significant ($p< 0.01$) at all pH employed and $p<0.05$ in the case of Hg-2 in the presence of Al-2. The depressive effects were not found to be significant in the case of exposure to Cd-1+Al-2 at all pH and exposure to Hg at pH 5.5. The increase in the variation of the metabolic rate with increase in the exposure period also was highly significant ($p<0.01$) in all cases except the exposure of the test specimen to Al-1, Al-2 and Cd at pH 5.5. The decrement in the metabolic rates of the test organisms in the presence of metals Cu, Cd, Hg and Al was further influenced by the change in the H^+content. In presence of Cu-1, the metabolic rate increased with decrease in H^+concentrations upto pH 6.5 and then decreased at pH 7.6 and further increased at pH 9.0 after 24h. Similar changes were observed in the presence of Cd, but the magnitude of change was higher in the presence of Cu. The percentage variation of metabolic rate in the presence of Cu-1 at different pH varied between 55.82 at pH 5.5 and 33.77 at pH 6.5 and 9.0 after 24h. In the presence of Cd-1 the percentage deviations from the control value were between 43.11 at pH 5.5 and 19.70 at pH 6.5 after 24h. Presence of mercury also diminished the metabolic rate. The highest value 0.987 ml O_2 h^{-1} g^{-1} dry weight was observed at pH 7.6 and the minimum 0.372 ml O_2 h^{-1} g^{-1} at pH 5.5 after 24h in the case of Hg-2.

The metabolic rate increased with time in all cases and the magnitude varied between 1.023 ml O_2 h^{-1} g^{-1} dry weight at pH 5.5 and 1.511 ml O_2 h^{-1} g^{-1} dry weight at pH 9.0 in the case of test organisms exposed to Cu-1 after 120h. 1.612 ml O_2 h^{-1} g^{-1} dry weight at pH 6.0 and 1.878 at pH 6.5 in the presence of Cd-1 and 0.813 ml O_2 h^{-1} g^{-1} dry weight at pH 5.5 and 1.314 ml O_2 h^{-1} g^{-1} dry weight at pH 7.6 in the presence of Hg-1 on fifth day. The increase in the concentration of Cu, Cd and Hg further diminished the metabolic rates of the test organisms. Presence of Al-1 also diminished the metabolic rate. The maximum value 1.812 ml O_2 h^{-1} g^{-1} dry weight was observed at pH 7.6 and the minimum 1.121 ml O_2 h^{-1} g^{-1} dry weight at pH 5.5 and 9.0 at 24h. Increase in the aluminium concentration further augmented the depression in the metabolic rate. The percentage deviation (Fig. 4.6) compared to the control after 24h varied between 47.42 at pH 5.5 and 9.0 and 15.01 at pH 7.6 in the presence of Al-1 and 58.16 at pH 9.0 and 15.67 at pH 6.5 in the presence of Al-2. Presence of aluminium diminished the metabolic rates of the test organism exposed to Cu, Cd and Hg. This interference increased with the increase in aluminium concentration. In presence of low Al concentration, the percentage deviations in the metabolic rates of the test organism exposed to Cu after 120h varied in the following range at different pH, i.e., between 25.61 at pH 6.5 and 53.75 at pH 5.5 and 9.0 for Cu-1 and between 32.83 at pH 6.5 and 61.91 at pH 7.6 for Cu-2. Increase in the concentration of Al i.e., Al-2 further diminished the metabolic rate and the percentage deviations were in the following range, between 29.78 at pH 6.5 and 55.86 at pH 5.5 for Cu-1 and between 34.99 at pH 6.5 and 66.66 at pH 5.5 for Cu-2.

The percentage deviation in the metabolic rate (from the control value) of the test specimen exposed to different metal concentrations varied after 120h as given below:

Metal conc.	Minimum value	Maximum value
Cu-1	22.94 at pH 7.6	52.02 at pH 5.5
Cu-2	22.89 at pH 6.5	53.94 at pH 5.5
Cd-1	12.01 at pH 6.5	24.39 at pH 6.0
Cd-2	15.01 at pH 6.5	30.30 at pH 9.0
Hg-1	38.37 at pH 7.6	71.25 at pH 5.5
Hg-2	33.16 at pH 7.6	73.64 at pH 6.0

The presence of aluminium also suppressed the metabolic rate and the percentage deviation due to the interaction of Al^{3+} after 120h varied in different experimental designs as given below:

Al^{3+} conc.	Metal conc.	Minimum value	Maximum value
Al-1	Cu-1	25.61 at pH 6.5	53.75 at pH 5.5 & 9.0
	Cu-2	32.83 at pH 6.5	61.91 at pH 7.6
	Cd-1	15.53 at pH 6.5	24.39 at pH 7.6
	Cd-2	18.95 at pH 6.5	43.11 at pH 9.0
	Hg-1	33.72 at pH 7.6	67.82 at pH 5.5
	Hg-2	43.11 at pH 7.6	75.09 at pH 6.0
Al-2	Cu-1	29.78 at pH 6.5	55.86 at pH 5.5
	Cu-2	34.99 at pH 6.5	66.66 at pH 5.5
	Cd-1	19.61 at pH 6.5	29.17 at pH 9.0
	Cd-2	24.91 at pH 6.5	53.75 at pH 9.0
	Hg-1	33.21 at pH 7.6	71.01 at pH 5.5
	Hg-2	47.84 at pH 7.6	74.62 at pH 6.0

Similar interactive effect was observed in the presence of Cd and Hg with change in magnitude. The percentage deviation in the metabolic rate of the test organism exposed to Al^{3+} in presence of cadmium varied between 15.53 at pH 6.5 and 24.39 at pH 7.6 for Cd-1

and between 18.95 at pH 6.5 and 43.11 at pH 9.0 for Cd-2 after 120h. Increase in the concentration of Al decreased the metabolic rate and the percentage deviation varied between 19.61 at pH 6.5 and 29.17 at pH 9.0 for Cd-1 and between 24.91 at pH 6.5 and 53.75 at pH 9.0 for Cd-2 after 120h.

The addition of Al also diminished the metabolic rate in presence of mercury. The percentage deviation after 120h varied between 33.72 at pH 7.6 and 67.82 at pH 5.5 for Hg-1 and between 43.11 at pH 7.6 and 75.09 at pH 6.0 for Hg-2. Increase in the concentration of Al further diminished the metabolic rate. The percentage deviation in the metabolic rate after 120h varied between 33.21 at pH 7.6 and 71.01 at pH 5.5 for Hg-1 and between 47.84 at pH 7.6 and 74.62 at pH 6.0 for Hg-2. 120h exposure to Hg-2+Al-2 was found to lead a slight enhancement in the metabolic rate. Similar discrepancies were observed in the case of exposure to Cd-1 at pH 6.0 and to Cd-2 at pH 9.0 (after 120h).

The cumulative effect of Cu^{2+}, Cd^{2+}, Hg^{2+}, Al^{3+} and H^{+} on the oxygen consumption after 120h was found to be in the following order:

$Hg^{2+} > Cu^{2+} > Cd^{2+} > Al^{3+} > H^{+}$ —— at pH 6.0, 6.5 & 7.6,

$Al^{3+} > Hg^{2+} > Cu^{2+} > Cd^{2+} > H^{+}$ —— at pH 9.0,

and $Hg^{2+} > Cu^{2+} > Al^{3+} > H^{+} > Cd^{2+}$ —— at pH 5.5.

Heavy metals can either depress or elevate the rate of oxygen uptake in marine bivalves. Mathew and Menon (1983) have reported that sublethal concentrations of Cu and Hg function as respiratory depressants. As mentioned earlier, the oxygen consumption of the aquatic invertebrates may be affected by a variety of environmental stimuli. The manner in which these organisms respond to these factors ultimately delineates its suitability to a given environment. The immediate response of mussels to inorganic ions was to secrete copious amount of mucus. The effect of heavy metals

on oxygen consumption in both species of fiddler crabs *Uca annulipes Latreille* and *Uca triangularis* (Milne Edwards) was in the order of Hg > Cd > Cu (Umadevi and Rao, 1989). But in the present study this was in the order Hg > Cu > Cd as mentioned above. Among the three metals Hg, Cd and Zn, cadmium was found to be the least toxic and mercury the most (Baby and Menon, 1986). Scott and Major (1972) found that metabolic rates were reduced in *Mytilus edulis* by about 12% on exposure to 300 ppb Cu. Brown and Newell (1972) concluded that the reduction in oxygen consumption of *Mytilus edulis* in the presence of 500 ppb Cu was due to the suspension of ciliary activity rather than direct inhibition of respiratory activity. Shapiro (1964) suggested that the respiratory activity depression in *Mytilus gallo provincialis* (L) could either be due to valve closure or direct impairment of metabolic activity.

Mercuric chloride decreased metabolic activity in fiddiler *Uca pugilator* (De Coursey and Vernberg, 1972) and in *Mytilus edulis* (Micallef and Tyler, 1990). In *Congeria leucophaeata*, the metabolic rate increased with increased sublethal concentration of mercury following a 48h exposure (Dorn, 1974). On the other hand, short term experiments with marine molluscs showed that oxygen consumption was depressed in the presence of mercury (Saliba and Vella, 1977).

Sivaramakrishna *et al.* (1991) observed the depression in oxygen consumption of mussel, *Lamellidens marginalis*, exposed to mercury. In the crab *Uca*, when larvae or adults were exposed to mercury pollution, loco-motor activity, respiration and osmoregulation were depressed (Vernberg *et al.*, 1974; Depledge, 1984). Thurberg *et al.* (1974) recorded a depression in oxygen consumption during exposure of 3 species of crab to Cd. The results obtained in this study indicated initial suppression in oxygen consumption with a gradual recovery in the specimen exposed to sublethal concentrations of trace metals. The similar trend was observed by Radhakrishnaiah, (1988) in the rates of oxygen

consumption of *Lamellidens marginalis* after exposure to sublethal concentrations of Cd.

The change in oxygen consumption could be brought about by one or a combination of three mechanisms: a decrease in the ventilation volume over the gills, a decrease in the permeability of the gill cells or a reduction of blood flow through the gills.

Singh and Singh (1979) proposed that the suppression in oxygen consumption was probably due to the reduced efficiency of gills besides the irreversible interaction of metal ions with cellular metabolic enzymes. Fortification of gills epithelium, high metal storing capacity and synthesis of metal binding proteins are some of the possible reasons for the metabolic recovery (Engel and Fowler, 1979a). The initial decrease in the oxygen consumption could have been the result of a decrease in loco-motor activity and in the ventilating system.

Respiratory problems (mucus clogging, increased ventilation, coughing, hypoxia) have been observed in laboratory exposure of fish to a wide pH range (Neville, 1985; Dalziel *et al.*, 1986). These effects appear to be associated with supersaturation of Al (Rosseland and Skogheim, 1984) and may therefore, be caused by precipitation on the gill surface. Aluminium accumulates on the respiratory structure of invertebrates (Havas, 1986). The observed decrease in the metabolic rates during the exposure of the test specimen to both low and high Al concentrations and its interactive effects in the presence of trace metals Cu, Cd and Hg at different pH may be explained based on the above facts.

The results from the short term laboratory experiments have also shown that exposure of fragments of mussel gills to Cu and Cd can result in the separation of gill filaments although the cilia remains active (Sunila, 1981). Ultrastructural investigations by Sunila and Lindstrom (1985) have shown that the ciliary connection forming the inter-filamentary functions of the mussel gill break and the ciliary disks slide away upon exposure to Cu and Cd.

Howell *et al.* (1984) pointed in the direction of neuronal mechanisms of inhibitions and /or separation of gill filaments. This is a view shared by Grace and Gainey (1987) as well. The effects of trace metals on metabolic rates of crabs depend to a considerable degree on the organism's reaction mechanisms, the entrance speed of substance to an organism and the competition which may occur for an active place of several enzymes (Ramirez *et al.*, 1989). Alterations occurring in the mechanisms governing oxygen transfer, which may be the result of a reduction in the efficiency of oxygen transfer over the gill, or of vascular constriction in the gill capillaries resulting inturn, in an inhibited ability for gas exchange. Thus, one of the causes of the organism's death together with intoxication may be due to anoxia produced by trace metals.

Low concentrations of Cd caused an increase in ATP and total adenylates in clam tissues (Giesy *et al.*, 1983) and this could indicate concomitant increase in respiration rates during exposure which was recorded in the study.

The mechanisms implied in the changes observed were assumed to be due to some damage at membrane and into mitochondria levels as suggested by Thurberg *et al.* (1977) and Byczkowski and Sorenson (1984). Thus, the presence of significant decrease in gill tissue oxygen uptake rate and respiration activity may indicate difference in toxicity due to species sensitivity and environmental parameters.

According to Shapiro (1964), the reduction in oxygen consumption resulted either from the valve closure or from a direct metabolic effect. The rate of ciliary activity is correlated with the rate of oxygen consumption. Coleman (1973) proposed the capillary activity but decreased effective irrigation of the mantle cavity during valve closure. During valve closure, due to the decreased ciliary activity, the effective irrigation of the mantle cavity decreases whereas the effective uptake of oxygen is retained due to decreased diffusion distances in water in the closed

condition. Hence, the shell closing mechanisms exhibited by most of the bivalves may also help them in lowering the metabolic rate by a reduced oxygen uptake. Studies conducted by Engel and Fowler (1979a) demonstrated that accumulation of Cu and Cd caused significant physiological and cellular changes in the gill tissue of the oyster and indicated that Cu was able to enter tissues such as gills and alter cell morphology as well as oxygen consumption rates. The cellular swelling and mitochondrial damage observed by electron microscopy suggested that the observed increase in gill tissue respiration may be related to increased cellular or mitochondrial membrane permeability.

Based on the above observations the difference in the rate of mucification, loco-motor activity, filtration rate, osmoregulation, accumulation of Cu, Cd, Hg and Al at the gill surface, chemistry of metal speciation at different pH and cellular morphology were responsible for the variations in the metabolic rates observed in this investigation.

Further studies are needed to clarify this phenomena in the case of the test specimen and its impact on control of respiration including possible effects of cellular ion transport.

CHAPTER V

BIOAVAILABILITY OF TRACE METALS

The effects and possible interactions of trace metals within the aquatic environment have become the focus of considerable research during the past few decades. A better assessment of the effects can be made only if the distribution and accumulation in biological communities are known. Trace metals like Cu, Cd, and Hg accumulate in different parts of the food web. The biological consequence of this is a direct toxic effect on the organisms involved with injury to their physiological system and the resultant large scale mortality. In addition, primary production and trophic relationships are disturbed by comparatively low concentrations of these metals. The biotic cycle gets disturbed and destabilizes the aquatic ecosystem. Bioaccumulation studies are important to define the maximum permissible concentration of these metals in the hydrosphere. Bioaccumulation is distinct from biomagnification in that the latter requires transfer of contaminant between trophic levels via ingestion whereas, bioaccumulation requires independent direct uptake at each trophic levels. Bioconcentration is thus process of accumulation of water-borne chemicals by fish and other aquatic animals through non-dietary routes (Robards and Worsfold, 1991).

The extent of trace metal accumulation which is the net effect of two compelling processes, uptake and depuration, is related to the level of environmental contamination. The ever-increasing influx of heavy metals to the aquatic environment has heightened concern over possible detrimental impacts on shell fish resources. Consequent efforts to monitor these impacts have substantially increased the body of literature dealing with the accumulation of metals in bivalves (Langston, 1988; Chan, 1989; Lakshmanan and Nambisan, 1989; Coimbra and Carraca, 1990; Luoma *et al.*, 1990;

Szefer and Szefer, 1990; Babukutty, 1991; Geetha, 1992; Falandysz and Kowalewska, 1993).

By using organisms as indicators of heavy metal pollution, biological availability of metals can be monitored over extended periods of time. Bivalve suspension feeders, especially *Villorita cyprinoides var cochinensis* (Hanley), are particularly suitable as bioindicator organism. Previous research indicated the existence of a metal concentration factor for *Villorita cyprinoides var cochinensis* (Hanley) between metal content of the organisms and that of the surrounding water (Babukutty, 1991; Geetha, 1992).

The specific objectives of the present investigations were to evaluate the interactive effects of H^+ and Al^{3+} on the bioavailability of Cu, Cd and Hg to *Villorita cyprinoides* var *cochinensis* for a period of 120h.

5.1. MATERIALS AND METHODS

The different pH employed were 5.5, 6.0, 6.5, 7.6 and 9.0. The metal concentrations in the exposure medium and their combination with Al^{3+} are given in the Table 2.2. Other experimental procedures relating to the static exposure system (Table 2.3), the digestion of soft tissues and quantitative analyses of different metals have been detailed in Chapter II.

Regression analyses were carried out for the study of variation in the accumulation of metals after 24h, 48h, 72h, 96h and 120h. ANOVA was used for predicting the effect of pH and different metal concentrations in the bioavailability of metals to the test organism.

5.2. RESULTS AND DISCUSSION

The quantities of Cu, Cd, Hg and Al accumulated in the soft tissues of the clam under different conditions and different periods of exposure are presented in Tables 5.1 to 5.4. The

Table 5.1. Tissue copper concentrations (μg g^{-1} dry weight) in *V. cyprinoides* exposed to copper and its combinations with aluminium at varying pH (accumulation phase).

(Control value = 4.28±0.08)

Exposure conditions	Exposure period (h) 24	48	72	96	120
pH 5.5/Cu-1	11.17±0.12	16.50±1.20	20.15±1.02	21.51±0.90	23.30±0.80
Cu-1+Al-1	14.06±1.02	17.12±0.21	18.23±0.14	20.12±0.14	24.71±0.28
Cu-1+Al-2	17.11±0.23	19.02±0.14	22.13±1.21	24.71±0.25	27.83±0.36
Cu-2	15.32±0.24	19.09±0.08	21.18±0.14	27.94±0.36	30.05±0.14
Cu-2+Al-1	18.98±0.54	20.26±0.21	24.41±0.18	25.24±1.10	31.66±0.15
Cu-2+Al-2	20.07±0.21	22.14±0.12	26.32±0.25	29.96±1.05	34.12±0.28
pH 6.0/Cu-1	15.05±0.12	19.01±1.08	21.85±0.08	24.00±0.24	25.38±0.24
Cu-1+Al-1	16.71±0.25	18.32±0.35	22.46±0.14	26.12±0.12	32.02±0.18
Cu-1+Al-2	19.12±0.24	20.14±0.18	24.32±0.24	28.34±0.18	33.24±0.08
Cu-2	19.13±0.18	22.08±0.64	23.15±0.18	30.42±0.26	30.93±0.30
Cu-2+Al-1	20.97±1.06	24.28±0.36	29.21±0.09	36.12±0.08	40.24±0.10
Cu-2+Al-2	22.12±0.98	25.98±0.87	31.82±0.14	38.23±0.05	42.22±0.08
pH 6.5/Cu-1	15.62±0.10	19.77±0.12	23.40±0.05	29.76±0.10	31.79±0.24
Cu-1+Al-1	17.52±0.12	20.30±0.11	24.75±0.09	33.50±0.08	35.12±0.14
Cu-1+Al-2	20.84±0.24	23.95±0.14	28.50±0.10	36.18±0.24	39.12±0.20
Cu-2	20.84±0.28	23.95±0.32	32.01±0.04	41.12±0.32	45.15±0.34
Cu-2+Al-1	21.96±0.35	26.66±0.08	34.18±0.12	40.18±0.01	41.12±0.12
Cu-2+Al-2	22.75±0.17	27.68±0.07	38.54±0.03	42.19±0.08	46.26±0.08
pH 7.6/Cu-1	17.76±0.14	22.23±0.10	28.42±0.10	30.63±0.05	33.12±0.05
Cu-1+Al-1	21.54±0.08	24.61±0.08	28.69±0.08	34.12±0.24	39.32±0.01
Cu-1+Al-2	28.10±0.10	32.12±0.14	39.36±0.31	46.17±1.02	47.18±0.36
Cu-2	22.05±0.24	27.26±0.21	37.12±0.14	44.11±0.28	48.24±0.45
Cu-2+Al-1	24.56±0.22	29.11±0.20	39.32±1.02	45.35±0.36	53.13±0.57
Cu-2+Al-2	26.72±0.09	29.91±0.14	44.14±1.24	46.12±0.34	59.12±0.32
pH 9.0/Cu-1	19.87±0.12	22.12±0.14	29.12±0.20	32.34±0.24	37.06±0.14
Cu-1+Al-1	23.62±0.24	24.61±0.15	26.23±0.24	28.14±0.12	31.30±0.11
Cu-1+Al-2	29.19±1.30	30.12±0.54	32.18±0.30	34.15±0.54	35.09±0.12
Cu-2	24.94±0.65	28.34±0.80	34.14±0.18	39.36±0.24	48.83±0.08
Cu-2+Al-1	26.50±0.78	28.12±0.41	31.12±0.08	31.26±0.17	34.77±0.01
Cu-2+Al-2	27.21±0.54	26.52±0.37	32.23±0.01	32.48±0.28	34.90±0.07

(Value = $\bar{x}$ ± SD, n = 6)

Table 5.2. Tissue cadmium concentrations ($\mu g\ g^{-1}$ dry weight) in *V. cyprinoides* exposed to cadmium and its combinations with aluminium at varying pH (accumulation phase).

(Control value = 4.12±0.32)

Exposure conditions	Exposure period (h)				
	24	48	72	96	120
pH 5.5/Cd-1	11.12±0.25	14.12±0.12	20.12±0.12	26.42±0.12	32.12±0.14
Cd-1+Al-1	11.23±1.10	15.12±0.10	21.12±0.20	29.43±0.20	33.48±0.11
Cd-1+Al-2	13.12±0.84	17.34±0.08	24.32±0.14	31.23±0.08	38.14±0.10
Cd-2	15.24±0.20	18.19±0.24	24.64±0.24	44.15±0.21	57.03±0.20
Cd-2+Al-1	16.72±0.42	19.12±0.32	25.16±0.30	45.16±0.14	58.12±0.08
Cd-2+Al-2	18.12±0.32	19.23±0.10	28.48±0.57	49.12±0.12	60.11±0.03
pH 6.0/Cd-1	8.42±0.05	16.22±0.05	20.42±0.12	26.12±0.12	30.04±0.03
Cd-1+Al-1	9.12±0.04	18.13±0.10	24.12±0.04	28.13±0.05	32.14±0.12
Cd-1+Al-2	12.13±0.03	21.12±0.21	28.12±0.06	31.12±0.03	36.12±0.04
Cd-2	14.12±0.10	15.12±0.30	21.12±0.08	34.14±0.01	48.24±0.01
Cd-2+Al-1	15.12±0.24	18.32±0.14	24.28±0.25	35.14±0.10	49.94±0.12
Cd-2+Al-2	16.27±0.14	20.12±0.20	26.12±0.14	37.12±0.12	51.12±0.17
pH 6.5/Cd-1	10.13±0.04	11.23±0.14	20.42±0.10	28.12±0.04	36.14±0.14
Cd-1+Al-1	11.24±0.12	14.12±0.11	24.12±0.04	30.12±0.02	38.12±0.02
Cd-1+Al-2	12.12±0.03	15.16±0.10	22.13±0.08	34.12±1.02	39.28±0.03
Cd-2	16.23±0.04	18.42±0.03	28.42±0.13	40.12±0.35	52.12±0.12
Cd-2+Al-1	18.12±0.10	19.12±0.08	26.43±0.01	42.12±0.65	53.14±0.07
Cd-2+Al-2	19.24±0.20	21.12±0.07	28.12±0.12	44.13±0.47	58.12±0.18
pH 7.6/Cd-1	12.12±0.05	17.12±0.02	24.12±0.08	28.12±0.21	40.12±0.02
Cd-1+Al-1	14.32±0.03	18.34±0.04	26.42±0.07	29.12±0.12	42.24±0.05
Cd-1+Al-2	17.24±0.01	19.12±0.03	27.18±0.21	32.84±0.14	44.62±0.12
Cd-2	18.12±0.08	28.32±0.10	43.03±0.36	45.58±0.31	61.10±0.21
Cd-2+Al-1	19.23±0.06	29.12±0.12	46.12±0.45	49.12±0.12	64.20±0.15
Cd-2+Al-2	28.12±0.07	35.88±0.14	48.12±0.25	56.75±0.14	69.92±0.47
pH 9.0/Cd-1	10.13±0.04	12.18±0.03	24.16±0.05	26.12±0.06	28.12±0.06
Cd-1+Al-1	10.12±0.03	13.18±0.14	24.16±0.05	28.12±0.02	30.12±0.05
Cd-1+Al-2	12.13±0.12	14.56±0.20	26.17±0.16	31.23±0.25	34.18±0.14
Cd-2	16.12±0.14	18.12±0.21	31.12±0.34	36.12±0.24	41.23±0.12
Cd-2+Al-1	17.28±0.28	19.34±0.14	30.23±0.25	38.24±0.18	44.24±0.24
Cd-2+Al-2	19.34±0.32	24.12±0.10	32.12±0.14	40.52±0.17	49.42±0.34

(Value = $\bar{x}$ ± SD, n = 6)

able 5.3. Tissue mercury concentrations ($\mu g\ g^{-1}$ dry weight) in *V. cyprinoides* exposed to mercury and its combinations with aluminium at varying pH (accumulation phase).

(Control value = not detectable)

Exposure conditions	Exposure period (h)				
	24	48	72	96	120
pH 5.5/Hg-1	18.12±0.04	20.12±0.26	44.56±0.21	78.12±0.24	101.10±0.02
Hg-1+Al-1	17.10±0.14	20.01±0.12	42.44±0.14	64.23±1.02	85.18±0.14
Hg-1+Al-2	16.19±0.25	19.12±0.54	38.44±0.35	59.98±2.04	87.34±0.47
Hg-2	27.43±0.28	29.12±0.24	64.48±0.68	99.46±2.18	142.10±1.25
Hg-2+Al-1	26.12±0.38	28.12±0.34	58.46±0.47	82.12±1.82	112.54±1.47
Hg-2+Al-2	24.34±0.64	26.19±0.28	54.12±0.62	80.24±1.57	101.24±1.24
pH 6.0/Hg-1	17.18±0.36	28.21±0.28	49.24±0.12	64.36±1.04	104.27±0.14
Hg-1+Al-1	16.23±1.25	24.12±0.24	46.42±0.20	58.42±0.54	84.24±1.24
Hg-1+Al-2	14.12±1.05	18.42±0.14	40.14±0.31	54.14±0.65	80.12±0.57
Hg-2	26.24±0.65	31.12±0.31	62.36±0.14	92.94±0.12	136.04±0.58
Hg-2+Al-1	24.12±0.25	28.24±0.04	49.54±0.14	74.58±0.14	91.65±0.28
Hg-2+Al-2	22.13±0.14	24.16±0.08	44.21±0.15	70.12±0.47	87.77±0.60
pH 6.5/Hg-1	18.12±0.25	21.23±0.24	40.23±0.24	54.32±0.26	88.63±0.21
Hg-1+Al-1	17.18±1.25	20.23±0.35	38.46±0.64	52.54±0.02	56.23±0.14
Hg-1+Al-2	14.23±0.32	19.23±0.10	24.52±0.36	25.10±0.05	29.12±0.12
Hg-2	28.12±0.56	28.86±0.12	50.12±0.25	84.84±0.31	126.26±0.14
Hg-2+Al-1	23.12±0.54	24.12±0.14	42.24±0.14	64.12±0.01	80.08±0.12
Hg-2+Al-2	18.24±0.34	22.13±0.15	23.12±0.36	25.18±0.20	34.12±0.08
pH 7.6/Hg-1	19.03±0.21	22.12±0.14	38.12±0.12	61.46±0.21	81.55±0.12
Hg-1+Al-1	17.62±0.04	20.23±0.08	36.24±0.05	42.56±0.02	58.58±0.10
Hg-1+Al-2	15.61±0.08	19.12±0.03	34.23±0.04	31.18±0.10	30.82±0.58
Hg-2	29.10±0.06	32.46±0.07	54.23±0.05	78.42±0.12	110.25±0.36
Hg-2+Al-1	24.12±0.24	26.24±0.01	50.23±0.04	56.12±0.14	86.40±0.04
Hg-2+Al-2	19.18±0.04	20.24±0.02	24.34±0.09	26.24±0.21	35.05±0.12
pH 9.0/Hg-1	18.12±0.10	20.21±0.10	42.84±0.21	60.18±0.12	74.13±0.21
Hg-1+Al-1	17.24±0.05	19.21±0.15	38.42±0.18	58.12±0.32	70.12±0.18
Hg-1+Al-2	18.13±0.12	21.12±0.21	44.13±0.17	62.14±0.04	83.36±0.17
Hg-2	26.20±0.05	28.12±0.17	51.21±0.25	74.28±0.01	100.69±0.34
Hg-2+Al-1	24.10±0.32	24.23±0.10	48.12±1.20	68.12±5.40	83.36±0.08
Hg-2+Al-2	25.14±0.17	25.98±0.08	52.14±3.22	75.16±2.67	109.57±0.06

(Value = $\bar{x}$ ± SD, n = 6)

Table 5.4. Tissue aluminium concentrations ($\mu g\ g^{-1}$ dry weight) in *V. cyprinoides* exposed to aluminium and its combinations with Cu, Cd and Hg at varying pH (accumulation phase).

(Control value = 34.60±0.97)

Exposure conditions	Exposure period (h)				
	24	48	72	96	120
pH 5.5/Al-1/	34.86±1.02	36.12±0.35	42.23±0.25	50.01±1.32	57.80±0.24
+Cu-1	35.12±1.24	38.01±0.25	44.16±0.12	51.12±1.25	56.12±0.28
Cu-2	34.96±1.25	37.01±0.28	42.01±0.54	50.12±2.40	54.02±0.10
Cd-1	35.01±0.98	38.12±0.14	44.06±0.25	52.01±0.25	58.12±0.12
Cd-2	34.18±0.45	36.12±0.03	40.16±0.36	48.12±0.65	57.01±0.34
Hg-1	35.10±0.68	37.22±0.25	42.22±0.45	48.14±0.25	54.12±0.02
Hg-2	34.58±0.47	38.12±0.68	44.16±0.25	47.12±0.18	52.02±0.14
Al-2/	38.14±0.36	40.12±0.47	52.12±0.36	64.07±0.37	76.96±0.01
+Cu-1	36.02±0.58	38.01±0.36	50.01±0.18	60.12±0.64	74.12±0.54
Cu-2	40.23±0.20	41.18±0.24	51.18±1.12	62.08±0.98	72.03±0.12
Cd-1	41.12±0.35	44.48±0.54	50.08±1.25	60.12±0.54	69.12±0.03
Cd-2	39.11±0.25	40.22±0.25	52.14±1.54	64.01±0.65	74.12±0.21
Hg-1	35.12±0.62	36.20±0.36	50.12±0.58	60.21±0.21	70.12±0.14
Hg-2	36.12±0.24	38.12±0.25	48.22±0.26	55.14±0.10	68.16±0.15
pH 6.0/Al-1/	36.12±0.24	38.12±0.24	58.12±0.14	68.12±0.12	73.50±0.12
+Cu-1	37.02±0.14	40.13±0.36	59.21±0.02	69.01±0.08	72.10±0.05
Cu-2	35.12±0.18	37.12±0.10	60.12±0.17	64.12±0.15	71.98±0.08
Cd-1	34.16±0.26	38.10±0.08	58.19±0.14	62.09±1.20	69.12±2.40
Cd-2	35.72±0.15	34.10±0.02	54.12±0.16	60.12±0.35	68.12±0.36
Hg-1	36.12±0.34	37.12±0.01	48.12±0.13	58.42±2.56	66.20±0.24
Hg-2	34.78±0.18	36.10±0.47	46.20±0.03	56.20±0.21	64.12±1.10
Al-2/	42.13±0.10	44.12±0.01	69.12±0.01	98.01±0.02	150.64±1.40
+Cu-1	40.12±0.09	48.12±0.24	74.21±1.40	102.12±0.05	140.12±0.02
Cu-2	38.10±0.14	46.20±0.01	72.12±2.64	101.02±0.14	141.12±0.01
Cd-1	39.20±0.11	44.12±0.02	74.12±3.84	104.86±0.13	142.12±0.14
Cd-2	41.12±0.12	45.00±0.24	76.12±1.24	106.12±0.02	144.26±0.12
Hg-1	38.16±1.20	42.46±0.18	74.00±0.26	101.20±0.01	138.12±0.03
Hg-2	37.12±0.24	40.12±0.28	68.12±1.38	98.12±0.01	126.21±1.25
pH 6.5/Al-1/	38.66±0.04	42.43±0.03	51.98±0.12	61.23±0.03	70.43±0.14
+Cu-1	38.34±0.21	41.56±0.14	52.32±0.10	60.12±0.01	69.21±0.25
Cu-2	37.15±0.05	40.22±1.24	53.12±0.08	62.14±1.24	69.12±1.60
Cd-1	38.42±0.15	42.13±1.54	54.25±0.07	61.23±2.34	68.27±3.50
Cd-2	35.10±0.10	41.22±2.02	53.16±1.02	60.12±0.24	67.18±0.01
Hg-1	34.10±0.14	39.12±1.54	50.20±0.03	56.12±0.35	62.12±0.02
Hg-2	36.21±0.04	38.20±0.58	51.22±0.06	54.12±0.36	64.12±0.04

(Contd...)

Table 5.4. (Contd...)

Al-2/	35.12±0.06	39.12±0.12	74.86±0.01	98.12±0.28	126.32±1.12
+Cu-1	36.17±0.35	42.24±0.35	72.36±0.02	99.21±0.36	125.10±0.05
Cu-2	44.16±0.10	48.42±0.14	74.86±0.03	104.26±0.34	126.10±0.14
Cd-1	40.12±0.05	46.24±0.17	68.16±0.06	90.84±0.24	120.24±0.31
Cd-2	38.12±0.21	44.12±0.18	65.22±1.20	92.12±0.21	118.12±0.02
Hg-1	36.22±0.05	42.24±0.24	64.46±0.65	98.21±0.01	116.22±0.01
Hg-2	37.12±0.06	44.12±0.24	65.16±0.42	102.12±0.02	121.12±0.05
pH 7.6/Al-1/	38.64±0.02	42.45±0.11	48.86±0.05	59.42±0.06	68.64±0.10
+Cu-1	39.12±0.35	41.23±0.10	49.22±0.24	58.24±0.10	67.12±0.06
Cu-2	37.12±1.20	40.23±0.24	49.12±0.36	58.12±0.09	66.36±0.26
Cd-1	34.76±0.32	38.12±0.32	46.23±0.14	56.24±0.12	64.32±0.35
Cd-2	35.16±0.02	39.46±0.01	47.26±0.65	59.41±0.08	67.65±0.27
Hg-1	36.02±0.15	37.60±0.14	45.21±0.24	54.81±0.02	64.32±0.81
Hg-2	34.96±0.14	36.86±1.26	42.84±0.28	53.94±0.14	62.12±0.11
Al-2/	44.32±0.18	48.42±1.54	62.94±0.10	84.16±1.20	102.22±0.28
+Cu-1	42.12±0.17	48.12±1.34	58.12±0.09	71.20±3.20	88.05±0.26
Cu-2	40.12±0.16	46.21±1.24	54.20±0.05	80.12±3.62	93.08±0.57
Cd-1	39.28±0.14	47.12±0.25	55.20±0.36	81.22±1.78	94.22±0.28
Cd-2	41.32±0.12	49.20±1.20	57.12±0.12	82.14±1.65	96.17±0.18
Hg-1	40.23±0.09	47.86±1.56	54.12±0.01	70.21±1.40	88.12±0.15
Hg-2	34.81±0.34	37.12±1.25	42.10±0.02	48.12±0.02	50.83±0.37
pH 9.0/Al-1/	36.12±0.14	48.12±0.26	62.13±0.12	73.18±0.14	84.67±0.26
+Cu-1	34.01±0.10	49.21±0.12	64.24±0.26	75.12±0.12	88.12±1.12
Cu-2	38.42±0.04	48.12±0.36	62.14±0.35	74.01±0.14	86.05±1.23
Cd-1	36.01±0.36	39.12±0.26	60.68±0.14	72.12±0.09	87.12±0.25
Cd-2	35.12±1.50	38.14±0.54	64.52±0.18	70.01±0.25	81.02±1.47
Hg-1	34.78±2.10	42.12±0.15	62.12±0.16	72.11±0.18	79.12±2.10
Hg-2	34.56±0.25	44.16±0.27	60.21±0.48	70.12±0.28	80.10±2.34
Al-2/	42.12±0.35	58.16±0.28	72.48±0.26	112.12±0.34	156.03±0.68
+Cu-1	41.12±0.24	71.12±0.29	84.26±0.35	118.01±0.12	154.12±1.57
Cu-2	44.22±0.14	74.21±0.45	85.12±0.10	112.21±0.11	148.12±1.47
Cd-1	40.12±0.54	64.16±0.46	92.12±2.17	121.12±0.10	155.16±1.64
Cd-2	42.12±0.21	62.12±0.12	90.01±1.12	118.12±0.23	152.02±1.28
Hg-1	38.16±0.26	42.18±0.10	84.16±0.26	102.16±0.10	132.12±1.36
Hg-2	36.12±0.28	50.12±0.14	78.16±0.01	104.12±0.25	128.24±1.47

(Value = $\bar{x}$ ± SD, n = 6)

corresponding values during depuration are given the Table 5.5 to 5.8. The bioconcentration factors (BCF) of different metals used were calculated and are depicted in the Fig. 5.1 and 5.2 for comparison.

Mode of trace metal entry into bivalves

The interaction between bivalve and their environment may occur at the exposed body surface and at the digestive and respiratory epithelia. The endogenously produced epithelial mucus layers may function as binding sites for metals. Mucus sheet may act as massive unstirred layers that completely dominate the effective permeabilities of the underlying epithelia or they may be collecting systems that are subsequently modified once they enter the micro environment of the digestive system. The mucus sheets of the oyster accumulates Al^{3+}, Cu^{2+} and Hg^{2+} which are then ingested via this route (Korringa, 1952).

Endocytosis that is the uptake of material from surface of a cell by an infolding of the plasma membrane to form a vesicle has been described in many molluscan epithelia, e.g., association of Hg with lysosomes in *Mytilus edulis* (Lowe and Moore, 1979) and *Mercenaria mercenaria* (Fowler *et al.*, 1975) have been reported.

There is extensive evidence that Cu in *Biomaphalaria glabrata* (Cheng and Sullivan, 1974) and in *Protothaca staminea* (Roesijadi, 1980) and in *M edulis* (George and Coombs, 1977) and Hg in *M mercenaria* (Fowler *et al.*, 1975) are taken up by bivalves at a rate approximately proportional to the concentration in the water.

The complex nature of chemistry of ion species in aquatic systems will influence this uptake. It is possible that mucus binding may provide a ligand source for increasing metal ion uptake, although other workers have suggested intracellular sources of metallothioneins, copper chelation or low molecular weight compounds as alternative mediating molecules (Roesijadi, 1980). In the case of Cd, absorption is facilitated by diffusion of $CdCl_2$

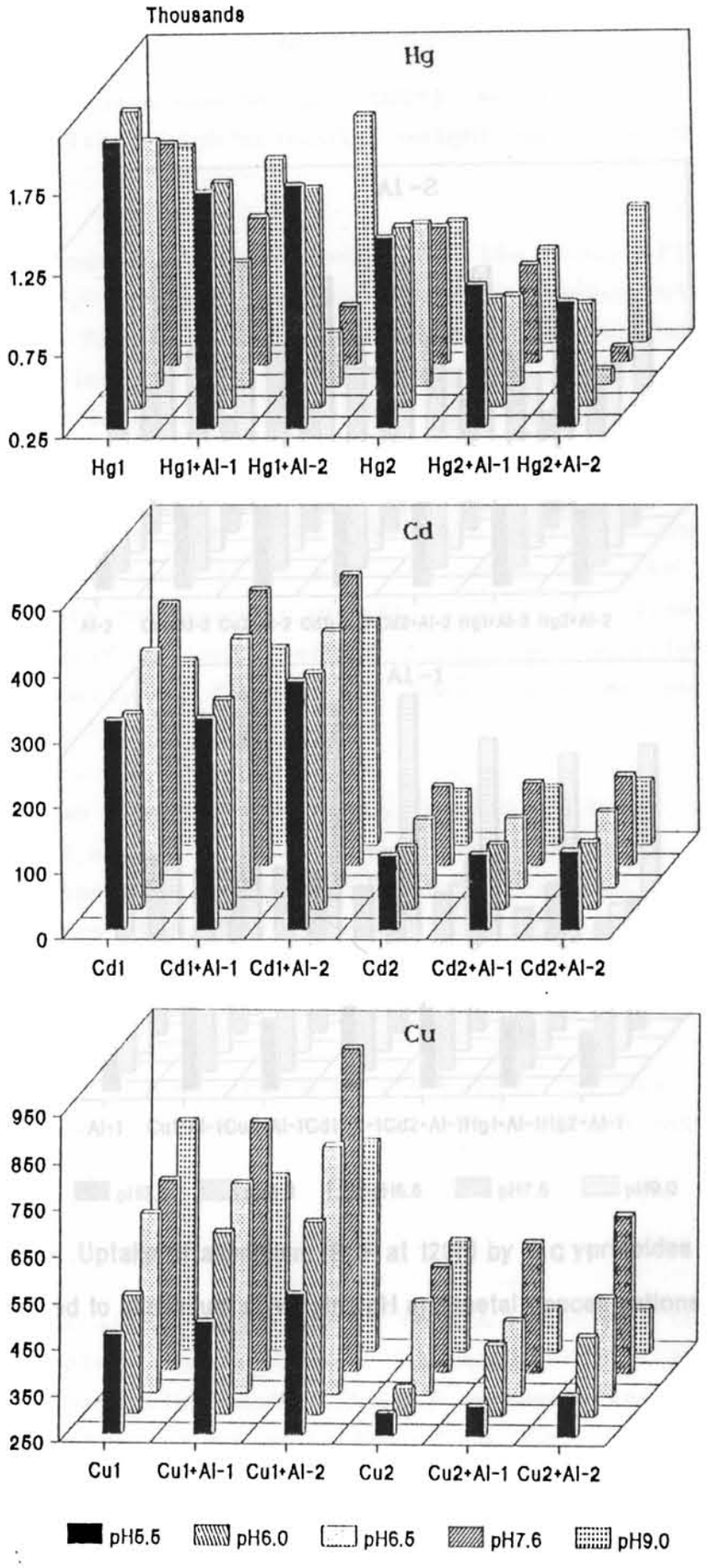


Fig. 5.1. Uptake of metals (BCF at 120h) by V.cyprinoides exposed to metals at varying pH and Al^{3+} concentrations.

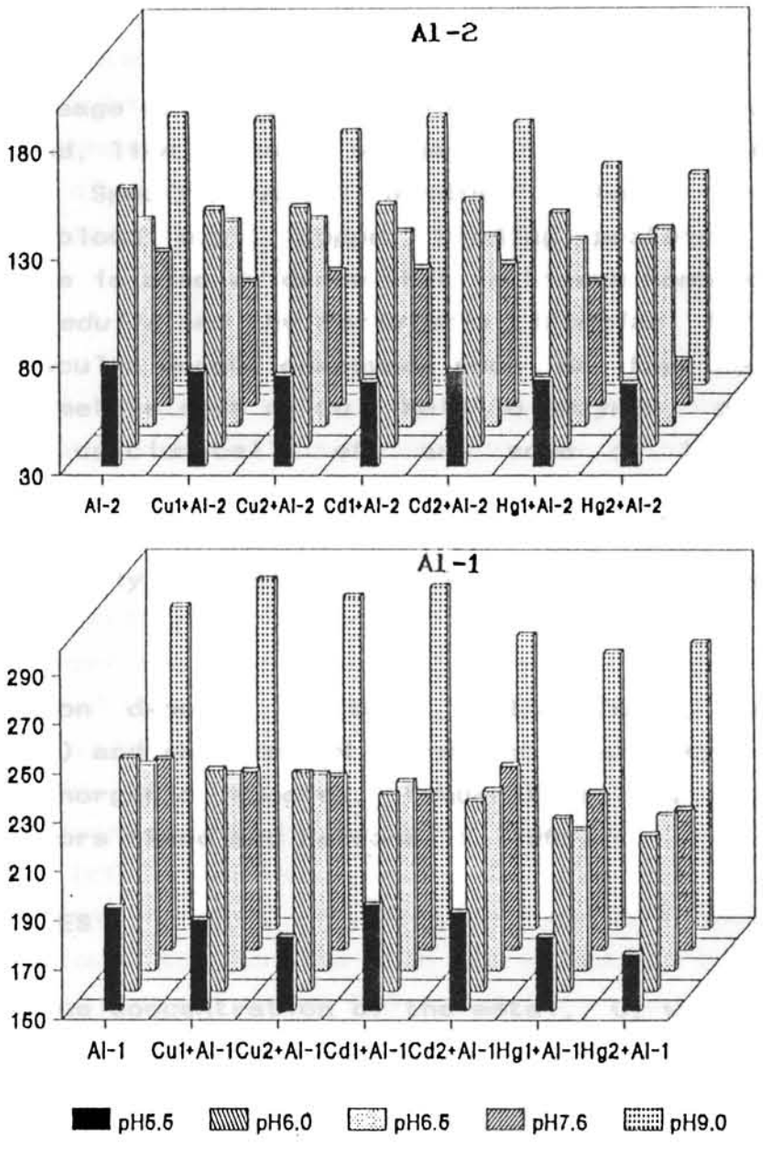


Fig. 5.2. Uptake of aluminium (BCF at 120h) by V. cyprinoides exposed to aluminium at varying pH and metal concentrations.

across the gills (Carpene and George, 1981) or by some type of complex formation with a high molecular weight compound (George *et al.*, 1978).

After the passage of metal ion into the body fluid, the amebocytes in blood, like mucus on epidermis is responsible for the metal metabolism. Specific metal binding proteins have been reported in the blood e.g., copper binding protein in *Sepia officianalis*. There is also evidence that in tissue homogenates of the *Oyster Ostrea edulis* and the *Periwinkle Littorina littorea*, a number of low molecular weight compounds such as taurine, lysine and hemarine bind metals such as Cu. Metallo enzymes, pore cells connective tissue, calcium cells etc are some of the cellular storage systems which accommodate metals in bivalves. Bivalves also have cellular detoxification systems like the metallothioneins which bind specifically Cu, Cd and Hg ions (Simkiss and Mason, 1983).

Bioaccumulation depends on both biotic (size, age and nutritional system) and abiotic (pH, hardness, salinity, presence of organic and inorganic species, temperature and metal-metal interactions) factors (Rand and Petrocelli, 1985).

ACCUMULATION STUDIES

The soft tissue concentration of the metal, C_t was taken as the difference between the concentration at time t (C) and that at zero time (C_o).

$$C_t = C - C_o$$

The bioconcentration factor (BCF) is the ratio of the amount of metal accumulated in the test organism to the metal concentration in the environment. The BCF values after 120h were used for the comparison of accumulation of Cu, Cd, Hg and Al at different experimental conditions.

Accumulation of Cu, Cd, Hg and Al significantly increased ($P < 0.01$) with the period of exposure at all pH employed. The r values were found to be greater than 0.92 (n = 5) in all cases except Hg-1+Al-2 at pH 7.6 where the value was 0.81. Al^{3+} as well as Cu^{2+}, Cd^{2+} and Hg^{2+} in presence of Al^{3+} make significant contributions (with some discrepancies discussed under each section) ($P < 0.01$) to the concentration of metals at all pH employed.

Copper

Lowering of pH inhibited the uptake of Cu by the test organism (Table 5.1). The copper uptake was reduced by the interaction of H^+ in such a way to produce the change in BCF (Fig. 5.1) after 120h from 741.2 at pH 9.0 to 466.0 at pH 5.5 for Cu1 and 488.3 at pH 9.0 to 300.5 at pH 5.5 for Cu2. Eventhough, BCF values decreased with increase in concentration of copper, the enhancement in the accumulation of copper with increase in the concentration of copper in the exposure medium was observed. The presence of Al^{3+} diminished the BCF values at pH 9.0 in the case of the test specimen exposed to Cu-1 and Cu-2 and the magnitude of the depressive effect increased with increase in the aluminium ion concentration. The BCF values were reduced to 626 and 701.8 for Cu-1 and 347.7 and 349 for Cu-2. The accumulation of copper in the test specimen was found to increase with the presence of Al^{3+} at all other pH employed except at pH 6.5 and 9.0 for Cu-2 and pH 9.0 for Cu-1 and at pH 9.0 for Cu-1. The increase in the H^+ concentration suppressed the accumulation of copper in the animal. The BCF values varied between 786.4 at pH 7.6 and 494.2 at pH 5.5 for Cu-1+Al-1, 943.6 at pH 7.6 and 556.6 at pH 5.5 for Cu1+Al-2, 531.3 at pH 7.6 and 316.6 at pH 5.5 for Cu-2+Al-1 and 591.2 at pH 7.6 and 341.2 at pH 5.5 for Cu2+Al-2. The maximum and minimum BCF values for the uptake of Cu at different pH and varying metal combinations are given below:

Exposure medium	BCF		
	Maximum	Minimum	
Cu-1	741.2 at pH 9.0	466.0	at pH 5.5
Cu-2	488.3 at pH 9.0	300.5	
Cu-1+Al-1	786.4 at pH 7.6	494.2	
Cu-1+Al-2	943.6 at pH 7.6	556.6	
Cu-2+Al-1	531.3 at pH 7.6	316.6	
Cu-2+Al-2	591.2 at pH 7.6	341.2	

Cadmium

Cd accumulation was also influenced by pH and Al^{3+}. Cd uptake decreased upon increase in H^{+}, but the trend was found to be complex. The highest rate of Cd accumulation was observed at pH 7.6 (Table 5.2) and the minimum at pH 9.0. A small enhancement in the bioavailability of Cd was observed at pH 5.5 and a sudden decrease in the rate of absorption of Cd at pH 9.0. The presence of low and high Al^{3+} concentrations enhanced the accumulation of Cd at all pH studied. The maximum and minimum BCF (Fig. 5.1) values under different experimental conditions for Cd exposure at different pH are given hereunder:

Exposure medium	BCF			
	Maximum		Minimum	
Cd-1	401.2	at pH 7.6	281.2	at pH 9.0
Cd-2	122.2		82.5	
Cd-1+Al-1	422.4		301.2	
Cd-1+Al-2	446.2		341.8	
Cd-2+Al-1	128.4		88.5	
Cd-2+Al-2	139.8		98.9	

As in the case of Cu, the amount of Cd accumulated increased with increase in the concentration of Cd exposed but BCF values were found to be decreased. So there was no proportionate increase in the concentration of Cd in the organism with increase in the concentration of Cd in the exposure medium.

Mercury

The accumulation of mercury in the clam was very complex. The decrease in the H^+ content lowered the quantity of Hg accumulated in the animal i.e.,acidification favoured the accumulation of mercury (Table 5.3). The BCF values (Fig. 5.1) varied between 1482.6 at pH 9.0 and 2022.0 at pH 5.5 for Hg-1. Although, increase in the concentration of mercury in the exposure medium led to an increase in the amount of mercury accumulated by the organism, BCF values were found to be lowered. The BCF values varied between 1006.9 at pH 9.0 and 1421.0 at pH 5.5 for Hg-2 after 120h. The addition of Al^{3+} was found to have an inhibitory effect on the mercury uptake by the clam, the effect being more predominant at pH 6.5. At pH 9.0, however, higher concentrations of Al were found to bring about an enhancement in the rate of mercury accumulation. Generally, BCF values were found to undergo a lowering under the influence of added Al stress except at pH 5.5 for Hg1+Al-2 and at pH 9.0 for Hg-1+Al-2 and Hg-2+Al-2 where a slight enhancement were observed.

The maximum and minimum BCF values of mercury in the test specimen at different experimental conditions are given below:

Exposure medium	BCF	
	Maximum	Minimum
Hg-1	2085.4 at pH 6.0	1482.6 at pH 9.0
Hg-2	1421.0 at pH 5.5	1006.9 at pH 9.0
Hg-1+Al-1	1703.6 at pH 5.5	1024.6 at pH 6.5
Hg-1+Al-2	1746.8 at pH 5.5	582.4 at pH 6.5
Hg-2+Al-1	1125.4 at pH 5.5	800.8 at pH 6.5
Hg-2+Al-2	1012.4 at pH 5.5	341.2 at pH 6.5

From the above it is amply clear that pH and Al^{3+} have profound influences on the accumulation of mercury.

Aluminium

The uptake of Al was also influenced by the pH (Table 5.4) but the effects of the interaction of other metals were very little. The accumulation of Al was minimum at pH 5.5 and maximum at pH 9.0. The depressive effect of H^+ in the accumulation of Al was observed at pH 5.5 while, an enhancement was observed at pH 6.0 and 9.0. The amount of aluminium accumulated increased with increase in the concentration of Al^{3+} but BCF values for Al-2 were always lower than that for Al-1. While the presence of Cu-1, Cu-2 and Cd-1 at pH 9.0 enhanced the uptake of Al, the accumulation of Al was either reduced or only marginally affected by Cu, Cd and Hg at all pH.

The changes in BCF (Fig. 5.2) values of Al with increase in the concentration of Al were as follows:

	BCF	
Al conc.	Maximum	Minimum
Al-1	282.2 at pH 9.0	192.7 at pH 5.5
Al-2	156.0 at pH 9.0	77.0 at pH 5.5

Comparison of the accumulation of Cu, Cd, Hg and Al

By comparing the BCF values of Cu, Cd, Hg and Al, the order of accumulation of different metals in the soft tissue of the test specimen at all pH employed was as follows:

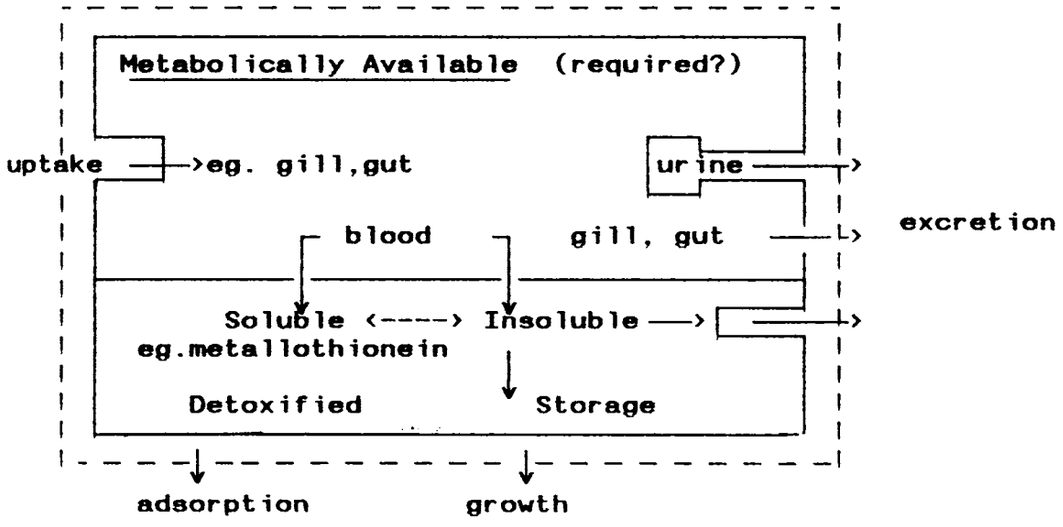
Hg > Cu > Cd > Al

and this is in very good agreement with the LC50 values obtained during the toxicity studies.

The major factor affecting the uptake of metals by organisms is the biological availability of the metals. Aquatic organisms are literally bathed in a solution of metals and may therefore take up

metals directly in the dissolved form. Such metals may be adsorbed passively on the body surface and may be available for physical exchange with metals in the medium or may be taken up across permeable surfaces into the body, a transfer potentially under metabolic control. Although ion pumps are available to move certain metal ions (particularly of group A) across membrane against concentration gradients, it is usually not necessary to invoke the presence of active transfer mechanisms (Coombs and George, 1978). In spite of usually high body concentrations of trace metals relative to the external medium, there is often a concentration gradient across a permeable surface promoting passive entry of metal ions. Absorbed metals may be bound by internal proteins thereby rendering them unavailable for passive re-export in the setting up of any equilibrium (Carpene and George, 1981) and therefore may be removed from the vicinity of the site of uptake by body fluids.

Schematic representation of the processes controlling the levels of accumulated trace metals (Rainbow, 1985) in aquatic organisms is shown below.



Net metal content in the organism must be a resultant of the process of uptake and loss, whether or not under metabolic control,

although body metal concentrations will also be affected by changes in body weight due to growth, gamete production, storage or depletion of energy reserves etc. Metals are taken into the body at sites such as epithelia of respiratory surfaces and alimentary tract,but a certain fraction of body metal may be adsorbed passively on to the body surface. This exchangeable adsorbed metal, beyond metabolic control, may represent a significant proportion of the total body metal (Jennings and Rainbow, 1979).

With the progress that has been made in aquatic toxicology over the past 10 - 15 years, there is wide recognition that a particular metal in water can exist in more than one form, and that different forms have different availabilities to living organisms. It is now axiomatic that a value for total metal in a given water sample is rarely adequate to predict even in general term the potential toxicity. In fact, there is steadily increasing body of evidence, derived from laboratory experiments with a variety of aquatic organisms (e.g., algae, bacteria, micro crustacea, fish, bivalves) demonstrating that the biological response elicited by a dissolved metal is usually a function of the free metal ion concentration, $M^{z+}(H_2O)_n$, which inturn is determined not only by the total dissolved metal concentration but also by the concentration and nature of the ligands present (Borgmann, 1983).

Complexes with organic and inorganic ligands tend to dissociate as pH decreases, resulting in increased free ion concentrations. This trend has led to the general acceptance of the idea that metals are more likely to be toxic to biota in acidic than in neutral waters. This is indirectly supported by the generally low species richness, low productivity, and small standing crop of biota in acidic waters.

In the present study, metal bioavailability and toxicity over the pH range 5.5 - 9.0 were tested and the emphasis was directed at assessing the possible changes in metal fluxes occasioned by the change in pH as well as by variation in biological susceptibility.

THEORETICAL CONSIDERATIONS

Influence of pH on metal speciation in solution

Even at constant dissolved metal concentration, changes in metal speciation can be anticipated as a result of a change in pH e.g., a shift in the hydrolysis equilibrium to the left, favouring the aquo-ion,

$$M^{z+}(H_2O)_n \xrightarrow{K1} M(H_2O)_{n-1}(OH)^{(z-1)+} + H^+ \qquad (1)$$

a shift in complexation equilibria (competition between M^{z+} and H^+ for the same ligands)

$$M^{z+} + nL \xrightarrow{K2} ML_n^{z+} \qquad (2)$$

$$H^+ + L \xrightarrow{K3} HL^+ \qquad (3)$$

where M is metal cation of charge +z (shown without its hydration sphere for simplicity) and L is ligand (shown uncharged for simplicity).

The degree to which the speciation of a particular metal is affected by a decrease in pH will depend on the acid-base properties of L and on the stability of the various ML species.

Influence of pH on metal - surface interactions at the cell medium interface

To elicit a biological response from a target organism and/or to get accumulate with in this organism, a given metal must obviously interact with/traverse a cell membrane. This interaction of the metal with the cell surface, normally involving either M^{z+} or ML_n^{z+} as the reactive species, can be represented in the form of the formation of M—X—Cell surface complexes, where X—Cell = a

cellular ligand and the biological response is proportional to (M^{z+}—X—Cell) (Nelson *et al.*, 1981; Sunda and Huntsman, 1983).

$$M^{z+} + X\text{——}Cell \xrightarrow{K4} M^{z+}\text{——}X\text{——}Cell \qquad (4)$$

$$[M^{z+}\text{——}X\text{——}Cell] = K4\,[X\text{——}Cell]\,[M^{z+}] \qquad (5)$$

$$ML_n^{z+} + X\text{——}Cell \xrightarrow{K5} M^{z+}\text{——}X\text{——}Cell + n\,L \qquad (6)$$

In the latter case (equation 5), if equilibrium conditions are assumed, the concentration of cell bound metal can be expressed as

$$[M^{z+}\text{——}X\text{——}Cell] = \frac{K_5[X\text{——}Cell][ML_n^{z+}]}{[L]^n} \qquad (7)$$

rearranging equation (2) one obtains,

$$\frac{[ML_n^{z+}]}{[L]^n} = K_2[M^{z+}] \qquad (8)$$

substitution of equation (8) into (7) then yields,

$$[M^{z+}\text{——}X\text{——}Cell] = K_5K_2\,[X\text{——}Cell]\,[M^{z+}] \qquad (9)$$

Which shows the same dependence on M^{z+} as equation (5). Equations 4 and 5 are the simplest representation of the interaction of the metal with the cell surface. The mechanisms leading to a biological effect might then involve the reaction of M^{z+} and a metabolically active site on the cell surface resulting in a direct biological response (Pagenkopf, 1983) alternatively, binding at the cell surface site might simply precede transport into the cell. The reaction with the metabolically active centre occurs intra-cellularly (Sunda and Huntsman, 1983).

Functional groups potentially present at cell surfaces, conceivably involved in metal binding, include the following; alcohol, carboxylic acid, amine, amide, hydroxamic acid, phosphate ester, thiol and imidazole (Crist *et al.*, 1981; Nelson *et al.*, 1981). This binding may involve individual functional groups or more likely a concerted action between different functional groups, e.g., chelation. Clearly, there exists a potential for binding cations other than M^{Z+} and these sites notably the hydrogen ion itself, H^{+}. Depending upon the pK values of the functional groups involved, changes of pH might thus affect the binding degree of protonation.

$$H^{+} + X\text{——Cell} \quad \langle \xrightleftharpoons{K6} \rangle \quad H^{+}\text{——}X\text{——Cell} \qquad (10)$$

In such a case, the proton would play a protective role analogous to the well - documented protection afforded by the hardness cations, Ca^{2+} and Mg^{2+} (Borgmann, 1983; Pagenkopf, 1983) and thereby, reducing the availability of the binding sites for toxic metals. An increase in the H^{+} concentration might thus be expected to influence the distribution of M^{Z+} among the various cellular ligands, X—Cell present at the cell surface, and in some cases, to a decrease in the overall concentration of surface bound metal.

Several additional points merit consideration. First, the reasoning developed to this point strictly applies to situations where equilibrium exists between the metal present in solution and the metal bound at the cell surface. If the reaction of M^{Z+} at the cell surface (equation (4)) is not reversible then the biological response will be determined not only by thermodynamic equilibrium considerations but rather by the kinetics of the reaction of M^{Z+} with the cellular ligand:

$$M^{Z+} + X\text{——Cell} \longrightarrow M^{Z+}\text{——}X\text{——Cell} \qquad (11)$$

An increased H^{+} concentration would decrease the proportion of unprotonated binding sites, X—Cell and reduce the rate of

formation of the biologically relevant M^{z+}——X—Cell surface complex. Thus even under conditions of kinetic control, decrease in pH could retard the biological response, secondly, changes in pH might also induce allosteric conformational changes at the metal binding sites and thus alter their affinities (pK_4) for the toxic metal of interest. Finally of importance for those metals moving passively across the membrane, changes in pH may affect the membrane potential. In order to regulate intra-cellular pH and maintain a negative potential across the membrane, protons are pumped out by the cell (Raven, 1980). As the pH is lowered, there will be an increasing binding tendency for H^+ to enter the cell, and the proton pumps will respond to maintain the internal charge differential. Eventually, depolarisation of the membrane may occur, related to the decreased pH, with consequent effects on the uptake of metal ions.

Prior to approximately 1970, the effects of pH changes on aquatic organisms, in the presence or absence of trace metals, were generally interpreted in terms of the physiological response of the organism with little or no consideration of pH induced chemical changes in the external medium. In recent years, however, the research pendulum has swung in the opposite direction and the effects of pH changes now tend to be interpreted in terms of speciation changes in the external medium. A change in pH may reasonably be expected to affect both metal speciation in solution and biological sensitivity at the cell surface level. These two responses to acidification are antagonistic and have the potential to cancel each other, the observed overall response at the organism level to a pH change from 9.0 to 5.5, at constant total metal concentration may well be positive, negative or null.

The present study focused initially on these metals that exist predominantly in cationic form and can be considered "very toxic and relatively accessible" in an environmental context. The Al was additionaly included because of its known implication in the response of watersheds to acid depositions (Cronan and Schofield, 1979; Johnson *et al.*, 1981).

Copper

The accumulation of Cu in molluscs depends on different well-documented factors such as salinity, age and size, presence of chelators, inherent species differences, interaction with other metals , seasonal variation, sex and temperature (Eisler, 1981; Babukutty, 1991).

A number of studies also have demonstrated the importance of free metal ion activity in controlling biological availability and toxicity of free metals. The potential mechanisms that would explain the dependence of accumulation rate on cupric ion activity are (1) copper transport directly across the external cell membrane as the free cupric ion i.e., $(Cu(H_2O)_6)^{2+}$ or as inorganic complex species whose concentration co-varies with that of cupric ion.(e.g., $CuCO_3$, $CuOH^+$, $CuCl^+$ or (2) uptake mediation by binding of copper to ligand sites at cell surfaces, because biological membranes (i.e., lipid bilayer membranes) are relatively impermeable to charged or highly polar species (e.g., $CuCO_3$) (Finkelstein and Cass, 1968).

Invertebrates, inhabiting polluted freshwater generally carry residues of 5 - 200 mg kg^{-1} dry weight of Cu in soft tissues. Residues in marine invertebrates are often much higher than those reported for fresh waters. This reflected the presence of bioavailable copper which is then sequestered in tissues. As in fresh water, copper may not be accumulated to higher trophic levels. Some of the highest residues for marine invertebrates (Moore and Ramamoorthy, 1984) have been reported for the Bristol channel and Severn Estuary (U.K.). Stenner and Nickless (1974) reported maximum levels of 1750 mg kg^{-1} Cu (dry weight) for the soft tissues of the mollusca *Nucella lapillus* whereas the oyster *Crassostrea gigas* contained upto 6480 mg kg^{-1} (Boyden and Romeril, 1974). On the other hand, crustaceans and molluscs from many other parts of Europe and North America be on levels of <60 mg Kg^{-1}).

The linear relationships between the period of exposure and the amount of copper accumulated in the present study were in good agreement with the Phase 1 of the study conducted by Shuster and Pringle (1969). During Phase 1 the rate of accumulation increased rapidly however, during succeeding Phase 2 and 3, the rate of uptake progressively decreased.

Cadmium

The accumulation of cadmium depended on size, moisture content, seasonal changes, presence of other metals, chelating agents, chemical form of water, metabolic changes (Eisler, 1981). The reaction of Cd with biologically active sites of enzymes like carbolic, phenoxyl, sulfhydryl, disulphide, phosphate etc., inhibits ATPase, phosphatase, carbohydrase, peptidase and aldolase and Cd uptake (Mc Leese and Ray, 1984; Skwarzec *et al.*, 1984; Diaz-Mayans *et al.*, 1986; Holwerda *et al.*, 1989).

The Cd accumulation with age in organs of Japanese scallop is due to metal binding by cytoplasmic proteins (Evtushenko *et al.*, 1990). Cd uptake by bivalves was also reported by Borchardt, (1983) Amiard *et al.* (1986) and Coleman *et al.* (1986).

Cd may be weakly bounded to phospho-protein particles. However, due to the chemical make up of these particles , the possibility for class A metals (Nieboer and Richardson, 1980) to associate with phospho-protein particles is to a greater extent than either class B (Hg) or border line (Cu, Cd) metals which are strongly attracted to sulphur residue (Robinson and Ryan, 1988).

Large variations in Cd accumulation have been reported in different specimen of molluscs (Greig, 1979). The importance of Cd speciation in the accumulation of Cd in oyster was demonstrated by Zamuda and Sunda (1982).

The experiments with Cd suggested that the concentration of this metal in the soft tissues was a function of metal

concentrations in the water. Similar results have obtained for a number of crustaceans and other invertebrates (Ahsanullah, 1981). Cd uptake patterns have previously been studied in *Mytilus edulis* (Riisgard *et al.*, 1987). In the present investigation, the Cd uptake was found to be linear with time. Linear Cd accumulation with time, as found in the present work has been previously demonstrated in *Mytilus edulis* (George *et al.*, 1978; Kohler and Riisgard, 1982; Poulsen *et al.*, 1982) and in oyster *Crassostrea virginica* (Shuster and Pringle, 1969).

In bivalves, Cd accumulation during chronic exposure is usually linear with time, the accumulation rate is proportional to the exposure concentration and saturation is seldom observed (Phillips, 1981; Poulsen *et al.*, 1982; Ritz *et al.*, 1982).

George and Coombs (1977) found that when *Mytilus edulis* was exposed to ionic cadmium, there is an initial lag period before accumulation occured, perhaps, because the Cd must be complexed before uptake occurred. Viarengo *et al.* (1981) presented evidence for the occurrence in molluscs of metal binding ligands which are induced by exposure to pollutants. The accumulation of Cd results from intra-cellular complexation with available ligands and is controlled by lysosomal activity. After initial cadmium binding to available ligands further stable Cd binding to metallothioneins like protein occurs. (Viarengo *et al.*, 1987). Cd accumulation and storage in bivalves are mediated primarily by metal-binding proteins. Although, the metellothioneins of bivalves and other invertebrates are similar to those of vertebrates, they do possess some unique properties (difference in amino acid composition and cadmium binding capacity) and are therefore often referred to as metallothionein like proteins (Engel and Brouwer, 1984). The relative amount of Cd bound to the various types of proteins (high molecular weight, very low molecular weight proteins or metallothioneins) varies between species and with concentration and duration of exposure (Engel and Brouwer, 1984; George and Viarengo, 1985).

The finding that Cd accumulation rate may vary with the manner in which a pollutant dose is administered showed that, even in the absence of other complicating factors the relationship between metal levels in mussel tissues and average metal levels in the surrounding water is not necessarily a simple one (Coleman *et al.*, 1988). This was proved to be correct in this study.

Reduction in the pumping activity of the clam (Chapter 4.2) exposed to different experimental conditions was also responsible for the reduction in Cd accumulation. The same argument was put forward by Zarooigan (1980) for the reduction in Cd accumulation in oyster

George and Coombs (1977) reported that chemical form of Cd affected the Cd uptake in *Mytilus edulis*, and that the complexed or chelated Cd produced in doubling the final concentration in all tissues. Metabolic activity is strongly implicated in metal uptake (Cunningham and Tripp, 1975b; Boyden, 1977). So the variation in the metabolic rate with change in the experimental conditions will also affect the accumulation of Cd in the soft tissues of the clam.

Mercury

The accumulation of mercury in molluscs is modified by several factors: chemical form (Jenner *et al.*, 1991), water temperature, salinity, tissue specificity (Eisler, 1981) and environmental factors (Wren *et al.*, 1991). Biota can accumulate Hg either directly from the water or food, and the relative importance of these two factors seems to be species and site specific.

Mercuric chloride has been reported to change the structural properties and permeability of trout mucus (Varanasi *et al.*, 1975). Changes in the rate of production and structural properties of the mucus which covers the gill epithelium may thus account for the increased efficiency of Hg uptake observed in the present investigation. Mason *et al.* (1976) suggested two distinct phases of metal uptake in Oysters, C.*virginica* exposed to $HgCl_2$ — a short

term logarithmic phase followed by a long term linear phase. They hypothesised that the logarithmic phase prevailed until a heavy metal threshold concentration was attained. Below this threshold value, accumulation was reversible, above the threshold a second irreversible linear phase was initiated. In the present observations, no such threshold concentration was attained, but irreversible linear phase was observed during the whole period of exposure.

Hg induces the biosyntheis of metallothioneins in animal tissue. Metallothioneins are often considered to be the most important specific cellular metal ligand and may also be related to the reduction of cytotoxic effects produced by metal accumulation (Roesijadi, 1981). Many metals having similar electronic characteristics may react with the same classes of biological compounds and are detoxified by the cells by similar mechanisms. However, due to the atomic differences existing among the different metals (atomic number, electronegativity etc.) they also show differences in their interactions with the cellular components (Viarengo *et al.*, 1985). Radoux and Bouquegneau (1979) demonstrated that $HgCl_2$ intoxification induced increased mucus production by the gills of *Servanus cabrilla*, and established that cations can be complexed by mucus secretions. This may limit the rate of entry of the pollutant which was not observed in the present study.

Windom and Smith (1972) suggested that speciation of the metal is an important consideration in metals uptake by *Crassostrea virginica*. When organic mercury was substituted for mercuric chloride, Cunningham and Tripp (1975b) observed four fold increase in Hg accumulation by C.*virginica*.

The rapid transformation of Hg to a complexed form during uptake will facilitate Hg bioconcentration by removing the metal from the electrochemical gradient across the environmental interface of the animal (Fletcher, 1970). Slow exchange of the complexed form will further potentiate steady state concentrations by necessitating high concentration of bound Hg before flux out of

such a compartment will equal flux into the compartment. The more rapid the flux into the bound compartment, the higher the steady state concentration.

Oyster, *Crassostrea virginica* accumulated Hg 1400 times and 2800 times above the environmental concentration of 100 and 10 ppb mercury respectively. In the present investigation, Hg accumulated 1482 times and 1006 times above the concentration in the exposure medium containing 50 ppb and 100 ppb mercury respectively.

Accumulation of Hg by teleosts occurred almost entirely across the gills (Olson *et al.*, 1973) and is directly proportional to methyl mercury concentration (Rodgers and Beamish, 1981). Hence, the change in the metabolic rate during the exposure to Hg also will influence the uptake of Hg.

Aluminium

Little is known about the effects of aluminium on aquatic invertebrates. Al in molluscs was found to be in the range 32-174 mg kg^{-1} dry wt (Culkin and Riley, 1958) and in *Mytilus californianus* 6.0 to 4.4 mg Al kg^{-1} dry wt (Goldberg *et al.*, 1978).

Al intake by trout, *Salvelius namay chush* aged 1-12 years was 200 to 180 ppb fresh weight (Chowtong *et al.*, 1974). Al uptake by bivalves was documented (Reynold, 1979; Eisler, 1989). In fish, most of this Al seems to be associated with gills rather than internal organs. The same may be true in bivalves.

Effect of H^+ on the bioavailability of Cu, Cd, Hg and Al

Much information about the influence of pH changes on metal adsorption at abiotic surfaces exists in the scientific literature e.g., metal oxides and oxyhydroxides (Stumm and Morgan, 1981), humic materials (Kerndorff and Schnitzer, 1980). In contrast, relatively few data exists regarding the pH dependence of metal adsorption at biological surfaces.

The increase in H^+ concentration may reduce uptake and toxicity of metal ions (Borgmann, 1983; Campbell and Stokes, 1985). H^+ may compete for a limited number of active binding sites on the outer gill membranes, thereby reducing the uptake of metals. Low pH may also have a negative physiological effect (e.g., reduced respiration rates) on the organism resulting in reduced uptake of metals. pH induced effects could be expected in the diffuse double layer present at the cell wall surface. A variation in the H^+ concentration in the bulk solution would lead to a change in the surface potential which inturn might affect the partitioning of the trace metal of interest between the surface and the bulk solutions. Similar trend in surface bound metal as a function of pH could be anticipated on the basis of competition between H^+ and M^{Z+} for specific binding sites at the cell surface. Metal transport into the cell might also be expected to be sensitive to pH changes. The transmembrane flux will be a function of the permeability of the cell membrane and the living force for movement into the cell: for passive metal transport. This latter component is determined by the concentration gradient from outside to inside the cell membrane, and by the transmembrane potential (Clarkson, 1974). Since the metal concentration gradient is determined by the inter facial rather than the bulk aqueous concentration, any pH induced changes in the partitioning of the metal between the bulk solution and the surface layer close to the membrane will affect metal transport. A pH effect might also be observed if metal transport involved initial binding at specific transport sites at the membrane surface (Sunda and Huntsman, 1983); specific competition between H^+ and M^{Z+} for such binding sites would decrease the equilibrium concentration (M^{Z+}—L— membrane), and lower the metal fluxes into the clam would result. Finally, change in pH may also affect the transmembrane potential (Raven, 1980) and hence, influence this component of the driving force for metal movement into the cell.

It is clear that acidification may thus be expected to affect both metal speciation in solution and sensitivity of the organism to metals (metal accumulation and toxicological response).

The electrical charge of the gill becomes more positive at higher H^+ concentrations (Mc Williams and Potts, 1978) consequently uptake of cations should decrease. pH has little effect on gill permeability or charge (Mc Williams and Potts, 1978).

The biological availability of copper to aquatic organisms is believed to be controlled by the free metal ion concentration in the water. This relation seems to hold for such different organisms as bacteria, algae, molluscs, crustaceans and fishes (Rai *et al.*, 1981; Babich and Stotzky, 1983; Borgmann, 1983; Campbell and Stokes, 1985). The chemical speciations of copper in salt solution are highly pH dependent. At low pH, the cupric ion is the dominant inorganic species, but with increasingly alkaline conditions its concentration declines quickly and carbonate and hydroxide species become the dominant inorganic forms (Zuehlke and Kester, 1983).

The first step in the absorption of copper from a solution by an organism is probably the binding to some kind of carrier and the cell solution interface to form complex that transports the metal across the cell membrane. Metal species with the highest free energy e.g., the free metal ion should react most readily with the carrier. At this point, pH may influence the uptake process in two antagonistic ways, firstly, by altering the chemical speciation of copper in the solution and secondly, by the protonation of binding sites which may be present at the cell surface (Da Silva and Williams, 1976; Williams, 1981). Carriers, however are not necessarily the only molecular instruments available for the translocation of copper across a solution cell interface. Passive diffusion of polar metal species through hydrophilic pores may provide an alternative for uptake. Metal species which are lipid soluble or of reduced polarity (i.e., non-electrolytes, lipid soluble organic complexes) may cross lipid bilayers by passive diffusion through the hydrophobic core of the membrane (Simkiss,

1983; Ahsanullah and Florence, 1984). Additionally, changes in pH can alter the physiological condition of an organism and thus, indirectly affect the uptake process (Knutzen, 1981). From all this, it can be anticipated that pH must have profound and complex effects on the biological availability of copper to aquatic organisms.

At pH below 8, Cu speciation is dominated by $CuCO_3$, with a decrease in pH, Cu^{2+} becomes progressively more important. At first glance, the results obtained concerning the effect of pH on the biological availability of copper to the test specimen was inconsistent with the widespread view that the biological availability of copper to aquatic biota is closely related to the cupric ion concentration in the medium. Indeed, over the pH range 5.5 to 9.0, 80 fold decrease in cupric ion concentration observed and $\approx$2 fold increase in copper accumulation observed. This could either mean that one or more of the inorganic complexes (i.e., $CuCO_3$, $Cu(CO_3)_2^{2-}$, $Cu(OH)^+$ and $Cu(OH)_2$) which prevail in alkaline media are biologically available and for that the decrease in uptake of copper with increasing hydrogen ion concentration was the result of protonation of binding sites.

Functional groups, potentially present at cell membrane surfaces and thus, conceivably involved in copper binding include carboxylic acid, amine, amide phosphate, thiol and imidoxole. This binding may involve one (monodentate) or more (multidentate) functional groups. The pH influenced the interaction between the copper and the binding site. This will also influence the Cu uptake.

Mucus is a strong chelator of copper particularly, near neutral pH however, cupric ion binding capacity decreased with decreasing pH. This is likely due to a decrease in total negative charge on the mucus molecules as a result of increased H^+ concentrations in the water (Miller and Mackay, 1982).

The experiments on the influences of pH on the aqueous uptake of Cd by the test organisms revealed significant effects. The bioavailability of aqueous Cd is dependent principally on the chemical species. The pH has marked effect on the speciation. In the present investigation, uptake of Cd was significantly reduced in alkaline conditions. The free Cd^{2+} ion usually is thought to be the most readily bioavailable species. Although, enhanced free Cd^{2+} ion concentration may be expected at decreasing pH values (O'Shea and Mancy, 1978), the proportionate amount of Cd^{2+} was not found accumulated. Since, most of the Cd at pH 7.6 was already present in readily available form of Cd^{2+}, a shift to pH 5.5 was not likely to have any appreciable effect on the bioavailability. Graney *et al.* (1984) however, observed a reduced accumulation of Cd at pH 5.0 in experiments with clam, *Corbicula fluminea* and attributed this effect to interference by filtration rates caused by the stress of low pH.

Influence of pH on mercury bioaccumulation was reported as early as 1975 (Hakanson, 1980). Bioaccumulation of mercury was found to be inversely dependent on the pH. A lowering of pH has been associated with increase in the dissociation of $HgCl_2$ (Moore and Ramamoorthy, 1984). A consequent increase in the bioaccumulation would be expected as has been observed in the present investigation. Wren and Maccrimmon (1983) also have reported higher Hg levels at lower pH in their studies on *Lepomis gibbosus*.

Bioaccumulation of Al has been found to be pH dependent with maximum accumulation occuring at pH 9.0 and minimum at pH 5.5 which could be due to the predominance of $Al(OH)_3$ in the bioaccumulative process. Increased bioaccumulation of Al with increasing pH was observed by Havas (1985) in the case of *D.magna*.

Metal accumulation in molluscs was found at two main sites, the hepatopancreas and the kidney. These organs are also the sites of large accumulation of phosphate, pyrophosphate and oxalate (Howard *et al.*, 1981; Overnell, 1981) salts together with

metallothionein type protein with large thiol content (Roesijadi, 1981). The first of these deposits will clearly trap class A metals (Al^{3+}), while the latter will accumulate other groups of metals (Cu^{2+}, Cd^{2+} and Hg^{2+}).

A xenobiotic metal entering into an organism may be retained in the body for one of the following two reasons: it may either parasitize the metabolic pathway of an essential element, disrupt it and produce toxic effects or it may be trapped by the same detoxifying sink. The extent to which an organism modified its metabolism to compensate for a pollutant metal will depend on the type of metal ions. Metal BCF's were strongly related to electrochemical properties of the elements. It appears that the oxidation potential may be a particularly good index of tissue incorporation. A consideration of metal chemistry in relation to the affinity of different elements for biological tissues might assist in interpreting the results. Based on the charge to radius ratio, oxidation state and ionisation potential, the metals could be arranged in the following increasing order of accumulation:

$$Al < Cd < Cu < Hg.$$

The results of the present investigation conform to and ratify this sequence.

Martin (1986) showed that some of the metal ions that form the strongest bonds undergo rapid ligand exchange whereas, other metals (for e.g., Al^{3+}) with low ionisation potentials have exchange rates which are around eight orders of magnitude slower than those of Cu^{2+}. The low BCF's observed for Al were consistent with slow ligand exchange rates.

The variations in the accumulation of Cu, Cd and Hg at different pH may be attributed to the difference in the speciation, the competitive interaction of H^{+}, the nature of metals and the nature and quality of mucus production and the physiological

changes like filtration rate and metabolic rate as observed in the Chapter IV.

The interactive effect of Al which increased the accumulation of Cu and Cd in the test specimen may be due to the reduced competitive effect of H^+ and the changes brought about by the Al^{3+} in the metabolic rate and filtration rate. In the case of Hg accumulation, Al^{3+} acts as an inhibitor. This may be due to the electrical nature of changes in the absorption sites and the variation in the physiological charges which are not suitable for Hg accumulation.

The interactive effect of Cu, Cd and Hg on the accumulation of Al was found to be negligibly small reflecting the mechanisms of Al accumulation which inturn is depended on the nature of the ligands and the nature of the bonds. This is clearly established by the fact that Al belongs to class A metals which prefer ligands like PO_4^{3-}, RCO^-, $RCOH^-$, $ROPO_3^{2-}$, whereas Cu^+ and Hg^{2+} being class B prefer —SH, —S—S, —SR and —NH_2. The preference of Cu^{2+} and Cd^{2+} which are border line varied between the above two.

The variation in the accumulation of Cu, Cd, Hg and Al in the test specimen is in very good agreement with their variation in toxicities i.e., Hg > Cu > Cd > Al which have explained in Chapter IV.

DEPURATION STUDIES

The rate of metal loss can be defined in terms of the biological half life of a particular metal. It is affected by the total body burden and can be calculated over a definite time interval (depuration period). The biological half life ($B_{1/2}$) of a metal is defined as the time required for half the accumulated tissue metal to be eliminated as a result of biological process and can be determined by different methods. In the present investigation, the tissue concentration in the test specimens which are pre-exposed to the different experimental conditions and

undergoing depuration at pH 7.6 were analysed at 24h interval for a period of 120h. The values are given in the Tables 5.5 to 5.8. The tissue concentration of the metal in the specimen is equal to the difference between the concentration at a definite time interval and that at in the control. The quantity of metals excreted was found to be directly proportional to time. ($r > 0.96$ in 95% cases and $r > 0.90$ in all other cases, $n = 6$) or in other words the depuration was found to be a linear one. $B_{1/2}$ values are depicted in the Figs. 5.3 and 5.4.

The equation $Y = a + bx$ was used for each experiment using regression analysis for the calculation of $B_{1/2}$ values. (y = number of days, x = metal concentration at time t). The biological half life was obtained as the value of Y when x was 50% of zero hour value.

The rate of elimination of metals Cu, Cd and Hg from the animal body was found to be significantly influenced by the presence of Al ($p<0.01$) whereas the influence of other metals on the depuration of Al was negligibly small. The loss of Cu, Cd, Hg and Al from the test specimen was found to be proportional to time and this was found to be significant ($p<.01$) in almost all of the different experimental conditions employed. Discrepancies were noticed in the effect of Al on the depuration of Cu-2+Al-1 and Cu-2+Al-2. where the effects were not significant.

The elimination of metals was a maximum in the case of animals pre-exposed to alkaline medium. Hence, $B_{1/2}$ values were found to be a minimum in alkaline medium and a maximum in acid medium. Discrepancies were noticed in the elimination of Cd and Hg during Hg-2 and Cd-2 exposures.

Copper:

The depuration of copper increased with decrease in H^+ ion concentration to which the animals were pre-exposed. The increase in the concentration of copper in the animal body increased the

able 5.5. Tissue copper concentrations ($\mu g\ g^{-1}$ dry wt) in *V. cyprinoides* pre-exposed to copper and its combinations with aluminium at varying pH (depuration phase).

Pre-exposure conditions	Exposure period (h) 24	48	72	96	120
pH 5.5/Cu-1	22.18±0.05	20.24±0.04	16.40±0.04	12.52±0.12	8.12±0.03
Cu-1+Al-1	22.38±0.03	20.48±0.21	17.52±0.12	14.13±0.32	9.12±0.01
Cu-1+Al-2	26.42±0.41	23.12±0.30	15.12±0.10	16.23±0.10	11.23±0.08
Cu-2	29.54±0.21	26.42±0.02	20.14±0.25	17.19±0.21	10.12±0.20
Cu-2+Al-1	30.12±0.25	28.12±0.08	26.12±0.32	18.23±0.14	11.23±0.10
Cu-2+Al-2	31.23±0.09	29.13±0.01	28.12±0.60	20.84±0.32	12.12±0.24
pH 6.0/Cu-1	24.50±0.12	22.62±0.10	19.54±0.10	16.52±0.04	15.65±0.20
Cu-1+Al-1	30.12±0.20	26.12±0.02	22.12±0.08	18.38±0.02	17.12±0.08
Cu-1+Al-2	29.98±0.31	26.82±0.14	23.14±0.06	20.12±1.02	19.12±0.54
Cu-2	29.71±0.20	26.13±0.14	25.12±0.04	24.27±0.03	16.17±0.20
Cu-2+Al-1	36.12±0.15	29.34±0.20	27.32±0.20	25.12±0.15	18.29±0.10
Cu-2+Al-2	38.10±0.12	31.12±0.14	29.14±0.07	26.28±0.01	20.82±0.14
pH 6.5/Cu-1	30.41±0.05	25.21±0.04	21.12±0.04	19.54±0.05	9.46±0.12
Cu-1+Al-1	31.55±0.08	30.12±0.32	24.85±0.03	20.73±0.14	12.12±0.08
Cu-1+Al-2	32.12±0.10	30.18±0.01	26.12±0.54	23.12±0.28	14.38±0.02
Cu-2	40.12±0.14	34.38±0.10	26.12±0.24	24.12±0.60	14.18±0.10
Cu-2+Al-1	38.12±0.36	35.12±0.07	24.03±0.36	18.12±0.01	16.19±0.35
Cu-2+Al-2	40.16±0.54	32.12±0.04	21.98±0.07	20.72±0.20	20.30±0.20
pH 7.6/Cu-1	28.42±0.05	27.51±0.14	18.51±0.05	14.32±0.02	10.28±0.05
Cu-1+Al-1	34.12±0.02	29.46±0.12	24.21±0.04	18.52±0.03	14.32±0.07
Cu-1+Al-2	37.40±0.10	29.81±0.25	25.43±0.20	22.12±0.12	15.12±0.12
Cu-2	44.12±0.28	36.38±0.14	28.12±0.18	26.45±0.08	17.12±0.10
Cu-2+Al-1	44.86±0.30	37.12±0.08	24.13±0.04	12.44±0.24	12.12±0.20
Cu-2+Al-2	45.16±0.41	33.27±0.04	23.98±0.20	22.69±0.17	18.72±0.06
pH 9.0/Cu-1	30.16±0.12	24.52±0.01	20.12±0.03	17.18±0.10	12.12±0.14
Cu-1+Al-1	29.12±0.08	25.16±0.05	24.12±0.04	20.18±0.08	15.23±0.03
Cu-1+Al-2	32.12±0.01	26.23±0.06	27.18±0.10	24.12±0.14	17.12±0.24
Cu-2	44.12±0.01	38.16±0.10	30.23±0.52	26.42±0.11	20.43±0.14
Cu-2+Al-1	31.12±0.05	26.12±1.02	24.12±0.26	24.08±0.21	22.18±0.37
Cu-2+Al-2	32.34±0.03	28.19±1.14	26.17±0.80	22.48±0.17	22.28±0.09

(Value = $\bar{x}$ ± SD, n = 6)

Table 5.6. Tissue cadmium concentrations ($\mu g\ g^{-1}$ dry wt) in *V. cyprinoides* pre-exposed to cadmium and its combinations with aluminium at varying pH (depuration phase).

Pre-exposure conditions	Exposure period (h)				
	24	48	72	96	120
pH 5.5/Cd-1	31.18±0.02	30.24±0.02	28.26±0.06	26.82±0.02	26.01±0.12
Cd-1+Al-1	32.18±0.04	31.12±0.01	30.02±0.10	28.21±0.11	24.01±0.14
Cd-1+Al-2	35.12±0.10	30.18±0.24	28.12±0.21	28.98±0.25	28.02±1.10
Cd-2	54.12±0.25	52.13±0.01	48.12±0.05	44.16±0.03	44.02±0.68
Cd-2+Al-1	52.13±0.02	50.12±0.12	46.23±0.04	42.12±0.25	40.02±0.48
Cd-2+Al-2	58.12±0.07	55.16±0.32	53.12±0.05	50.12±0.18	48.23±0.54
pH 6.0/Cd-1	29.08±0.05	28.14±0.02	26.12±0.14	25.14±0.05	24.12±0.05
Cd-1+Al-1	31.12±0.28	29.12±0.18	26.14±0.12	24.12±0.04	22.13±0.35
Cd-1+Al-2	30.13±0.14	28.46±0.06	26.54±0.25	24.28±0.11	26.12±0.04
Cd-2	46.24±0.28	44.26±0.04	40.12±0.14	38.24±0.21	38.02±0.06
Cd-2+Al-1	47.18±0.08	45.27±0.15	44.32±0.31	41.23±0.07	40.12±0.07
Cd-2+Al-2	50.14±0.09	48.17±0.28	46.12±0.12	40.24±0.09	35.26±0.10
pH 6.5/Cd-1	35.12±0.02	32.13±0.07	33.18±0.03	31.12±0.02	31.23±0.05
Cd-1+Al-1	36.23±0.10	34.18±0.01	32.12±0.01	32.28±0.02	31.46±0.08
Cd-1+Al-2	38.42±0.14	34.24±0.12	33.22±0.08	32.12±0.01	32.01±0.10
Cd-2	50.12±0.09	48.12±0.01	47.18±0.10	46.28±0.03	42.46±0.01
Cd-2+Al-1	51.12±0.07	50.48±0.06	48.23±0.05	46.72±0.10	45.23±0.01
Cd-2+Al-2	56.12±0.02	54.21±0.04	50.25±0.04	48.43±0.12	41.56±0.12
pH 7.6/Cd-1	39.12±0.03	38.12±0.03	36.45±0.02	34.12±0.20	34.10±0.02
Cd-1+Al-1	41.12±0.01	39.12±0.14	38.46±0.01	35.23±0.14	32.12±0.31
Cd-1+Al-2	42.12±0.10	36.23±0.24	35.24±0.14	33.48±1.58	30.23±0.05
Cd-2	60.12±0.12	58.16±0.27	55.23±0.54	52.12±0.20	48.12±0.08
Cd-2+Al-1	62.13±0.14	56.24±0.14	53.12±0.17	48.42±0.25	42.26±0.10
Cd-2+Al-2	64.12±0.14	61.12±0.02	51.23±0.29	44.62±0.12	38.14±0.11
pH 9.0/Cd-1	26.23±0.05	24.12±0.02	21.34±0.12	21.02±0.03	20.08±0.05
Cd-1+Al-1	28.12±0.04	28.20±0.01	20.12±0.02	19.21±0.05	19.21±0.07
Cd-1+Al-2	32.12±0.04	30.46±0.18	28.12±0.09	22.12±0.11	20.18±0.10
Cd-2	40.12±0.08	38.23±0.14	34.52±0.27	33.56±0.42	32.09±0.21
Cd-2+Al-1	41.23±0.07	37.41±0.20	35.42±1.10	34.41±0.12	30.12±0.04
Cd-2+Al-2	46.28±0.14	44.32±0.26	40.12±0.02	36.12±0.11	34.13±0.01

(Value = x ± SD, n = 6)

Table 5.7. Tissue mercury concentrations ($\mu g\ g^{-1}$ dry wt) in *V. cyprinoides* pre-exposed to mercury and its combinations with aluminium at varying pH (depuration phase).

Pre-exposure conditions	Exposure period (h) 24	48	72	96	120
pH 5.5/Hg-1	100.02±0.01	94.56±0.02	84.12±0.04	80.14±0.06	79.12±0.07
Hg-1+Al-1	84.12±0.05	82.13±0.03	78.42±0.08	72.18±0.02	60.18±0.31
Hg-1+Al-2	86.12±0.02	84.13±0.54	76.12±0.04	70.12±0.31	62.12±0.11
Hg-2	140.12±1.25	136.14±0.15	120.12±1.10	105.16±0.25	92.32±0.10
Hg-2+Al-1	110.43±2.68	108.12±0.36	98.12±2.36	87.86±2.65	84.12±0.40
Hg-2+Al-2	98.12±3.64	94.13±0.28	90.14±1.36	83.12±0.08	81.14±0.08
pH 8.0/Hg-1	100.27±0.21	94.56±0.06	88.01±0.03	85.14±0.05	82.12±0.05
Hg-1+Al-1	82.12±0.18	80.18±0.04	74.13±1.20	68.12±0.14	60.12±0.03
Hg-1+Al-2	79.23±0.06	74.12±0.08	70.12±0.36	64.23±0.32	58.19±0.14
Hg-2	128.12±0.35	120.41±0.11	104.12±0.24	98.11±0.01	92.48±0.10
Hg-2+Al-1	89.21±0.14	87.41±0.02	80.42±0.12	78.12±0.10	70.21±0.11
Hg-2+Al-2	86.11±0.01	84.21±0.14	81.12±0.25	75.16±0.11	68.42±0.08
pH 6.5/Hg-1	84.12±0.04	81.34±0.05	80.48±0.06	75.16±0.06	71.17±0.02
Hg-1+Al-1	54.23±0.05	52.14±0.08	48.16±0.12	44.23±0.10	43.01±0.05
Hg-1+Al-2	28.01±0.06	26.48±0.14	27.12±0.34	25.16±0.10	24.18±0.03
Hg-2	120.41±0.03	114.50±0.10	98.12±0.01	85.16±0.05	83.12±0.04
Hg-2+Al-1	78.12±0.54	76.14±0.01	64.12±0.02	62.13±0.14	61.12±0.10
Hg-2+Al-2	33.44±0.10	32.20±0.02	30.12±0.17	28.20±0.35	26.07±0.11
pH 7.6/Hg-1	78.12±0.05	76.12±0.03	68.12±0.06	66.42±0.02	65.12±0.06
Hg-1+Al-1	56.12±0.08	54.13±0.01	52.12±0.02	44.14±0.11	43.45±0.21
Hg-1+Al-2	29.12±0.01	28.04±0.14	26.42±0.11	25.12±0.17	24.56±0.32
Hg-2	108.12±0.02	104.13±0.02	92.12±0.25	87.12±0.14	85.47±0.11
Hg-2+Al-1	84.20±0.03	82.18±0.36	79.24±0.36	75.12±0.24	70.12±0.05
Hg-2+Al-2	34.13±0.11	33.12±0.01	32.04±0.57	30.08±0.22	30.01±0.04
pH 9.0/Hg-1	72.13±0.05	68.42±0.02	59.32±0.04	50.23±0.12	54.02±0.02
Hg-1+Al-1	68.12±1.20	66.24±0.11	60.12±0.01	57.12±1.26	55.01±0.03
Hg-1+Al-2	82.28±1.11	80.12±0.65	75.22±0.04	70.40±0.36	64.23±0.12
Hg-2	98.12±2.00	96.12±0.25	84.12±0.14	78.13±0.25	77.12±0.15
Hg-2+Al-1	81.12±0.58	79.41±0.02	65.12±0.36	58.12±0.14	54.13±0.14
Hg-2+Al-2	104.17±0.65	98.12±0.01	78.12±1.25	54.12±1.65	50.12±0.17

(Value = $\bar{x}$ ± SD, n = 6)

ble 5.8. Tissue aluminium concentrations ($\mu g\ g^{-1}$ dry wt) in *V. cyprinoides* pre-exposed to aluminium and its combinations with Cu, Cd and Hg at varying pH (depuration phase).

re-exposure	Exposure period (h)				
nditions	24	48	72	96	120
H 5.5/Al-1/	53.21±0.12	50.12±0.15	44.68±0.14	39.12±0.10	38.16±0.25
+Cu-1	55.16±0.14	51.12±1.24	42.18±0.25	38.06±1.10	36.22±0.14
Cu-2	52.13±0.25	48.12±0.36	40.22±0.31	37.12±1.02	34.88±0.15
Cd-1	56.12±1.26	49.24±0.12	45.12±0.14	39.14±3.60	35.01±0.11
Cd-2	54.01±0.19	52.12±0.05	44.18±0.02	38.12±3.12	36.21±0.01
Hg-1	53.12±1.42	50.21±0.02	40.18±0.25	39.12±2.14	37.18±1.54
Hg-2	50.04±0.39	48.12±0.36	44.15±0.14	41.12±2.30	36.09±0.25
Al-2/	74.12±1.24	72.13±0.14	61.23±0.11	50.12±1.58	40.12±1.36
+Cu-1	72.01±1.56	68.14±0.25	57.99±0.09	46.12±1.87	41.20±0.58
Cu-2	70.01±0.32	66.94±0.01	58.12±0.14	42.16±1.65	38.86±0.14
Cd-1	64.12±1.21	60.21±0.14	50.18±0.08	41.24±0.89	39.12±0.14
Cd-2	72.31±1.11	70.12±0.11	58.42±0.06	50.12±0.24	37.18±0.24
Hg-1	64.16±1.02	62.38±0.45	54.12±0.12	48.12±0.25	36.06±0.28
Hg-2	65.36±0.25	60.41±0.25	55.12±0.14	42.38±1.62	39.12±0.14
H 6.0/Al-1/	71.23±0.12	68.12±0.12	58.12±0.25	49.89±0.25	39.12±0.36
+Cu-1	70.12±0.25	64.66±0.25	56.12±1.26	48.12±0.14	39.14±0.58
Cu-2	68.12±0.14	67.12±0.14	54.12±0.36	40.18±0.36	36.46±0.17
Cd-1	67.22±0.11	65.13±0.25	52.18±1.58	48.12±0.14	37.81±0.25
Cd-2	64.66±0.25	60.12±0.26	50.18±2.62	41.19±0.03	36.12±0.11
Hg-1	64.12±0.36	60.22±0.24	51.21±0.35	44.12±0.25	40.01±0.58
Hg-2	62.13±0.24	58.12±0.24	50.01±0.48	45.21±0.15	39.12±0.14
Al-2/	148.12±0.36	145.13±0.21	110.12±0.17	68.12±0.01	44.16±0.25
+Cu-1	137.13±1.11	118.12±0.18	90.21±0.19	64.15±0.01	45.12±0.14
Cu-2	140.12±0.58	125.16±0.36	88.12±0.25	51.12±0.25	41.14±0.36
Cd-1	138.04±0.25	130.62±0.24	101.21±0.01	64.84±0.26	39.21±0.11
Cd-2	128.12±0.23	101.21±0.23	71.12±0.02	50.18±0.36	48.12±0.18
Hg-1	130.12±0.15	110.21±0.14	81.12±0.21	51.24±0.25	39.86±0.25
Hg-2	118.02±0.69	108.12±0.18	74.12±0.15	48.12±0.14	40.12±0.26
H 6.5/Al-1/	68.12±1.23	64.21±0.14	58.01±0.12	48.12±0.11	39.42±0.23
+Cu-1	66.18±0.58	62.12±1.65	54.98±0.36	45.14±0.25	38.92±2.36
Cu-2	64.12±1.26	60.13±0.25	51.92±0.24	42.48±0.14	35.71±1.87
Cd-1	65.12±2.31	64.12±0.48	52.31±1.21	43.31±0.16	36.12±2.56
Cd-2	66.18±0.25	65.16±0.59	54.88±2.10	40.88±0.13	34.68±1.47
Hg-1	60.01±0.14	58.16±0.65	50.12±3.65	44.12±1.20	41.12±0.25
Hg-2	62.21±0.58	61.12±0.47	51.10±1.25	41.12±0.58	39.02±3.68

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Al-2/	124.12±0.47	118.12±0.36	88.18±0.98	58.12±0.47	41.18±0.25
+Cu-1	118.12±0.26	108.12±1.25	72.16±0.14	49.12±0.36	42.01±1.25
Cu-2	120.13±0.19	112.88±0.58	82.12±0.58	54.18±0.14	38.12±0.36
Cd-1	118.12±0.68	101.12±0.69	80.18±0.35	56.21±0.21	36.76±4.56
Cd-2	108.01±1.25	104.01±0.23	80.01±0.36	51.12±0.37	34.14±1.25
Hg-1	110.12±2.50	102.18±0.58	79.12±0.14	49.12±0.19	36.12±0.36
Hg-2	118.01±1.36	106.12±0.01	76.12±0.25	52.14±1.35	34.86±0.69
pH 7.6/Al-1/	60.12±0.21	58.12±0.25	48.21±0.23	40.12±0.14	38.12±0.21
+Cu-1	65.16±0.32	57.12±0.36	44.21±1.20	41.12±1.10	39.01±0.23
Cu-2	60.01±1.23	59.12±0.14	45.12±0.58	40.12±0.03	37.86±0.14
Cd-1	62.12±0.56	54.01±0.26	46.12±0.48	39.12±0.65	36.04±0.14
Cd-2	65.19±0.14	52.12±1.20	44.18±0.27	39.01±0.14	36.12±0.12
Hg-1	62.10±0.25	54.12±1.10	48.12±0.38	42.01±0.12	37.03±0.25
Hg-2	60.01±0.56	54.17±0.28	46.12±0.15	40.12±0.02	36.12±0.14
Al-2/	98.14±2.31	92.12±1.24	74.18±2.16	56.12±0.15	37.98±1.20
+Cu-1	86.12±1.25	76.21±0.39	65.12±3.65	54.16±0.14	36.12±2.50
Cu-2	90.04±0.09	88.42±1.29	70.12±0.26	52.14±1.10	38.12±1.54
Cd-1	91.12±0.06	79.12±0.23	62.13±1.35	53.12±0.35	48.12±2.31
Cd-2	94.01±0.12	84.22±0.32	64.16±0.39	41.12±0.14	36.28±1.12
Hg-1	87.01±0.25	81.12±0.25	70.21±1.24	50.12±0.25	40.12±0.09
Hg-2	48.08±0.56	46.16±0.02	41.12±2.30	40.12±0.14	39.22±0.12
pH 9.0/Al-1/	82.12±0.12	80.18±0.12	60.24±0.24	40.12±0.11	36.12±0.14
+Cu-1	80.18±0.21	76.14±0.12	66.12±0.12	50.28±1.20	37.18±0.26
Cu-2	84.16±0.36	80.23±0.25	58.52±0.14	42.13±1.57	38.12±1.00
Cd-1	85.12±1.20	84.18±0.02	55.16±0.18	37.18±2.60	34.98±1.25
Cd-2	78.01±0.25	74.12±0.03	50.12±0.09	38.46±3.24	36.24±0.38
Hg-1	74.01±1.36	70.28±0.24	54.26±0.03	38.11±2.58	37.98±0.17
Hg-2	78.12±0.14	76.12±0.03	56.12±0.02	42.38±3.41	38.22±0.28
Al-2/	150.12±1.54	138.12±0.02	98.12±0.25	54.12±1.20	35.12±0.03
+Cu-1	138.16±0.26	128.92±0.25	89.12±0.40	50.43±2.10	34.98±0.05
Cu-2	140.21±1.45	137.12±0.02	97.40±0.14	48.12±2.30	38.42±0.04
Cd-1	150.12±0.26	121.12±0.03	89.28±0.12	51.12±1.25	37.18±0.38
Cd-2	132.13±0.12	101.20±0.14	75.16±0.25	42.32±0.26	39.14±0.28
Hg-1	118.18±1.56	100.12±0.18	74.12±0.25	40.12±0.36	36.32±0.17
Hg-2	122.16±0.25	112.18±0.29	90.12±0.13	51.28±0.45	38.14±0.28

(Value = $\bar{x}$ ± SD, n = 6)

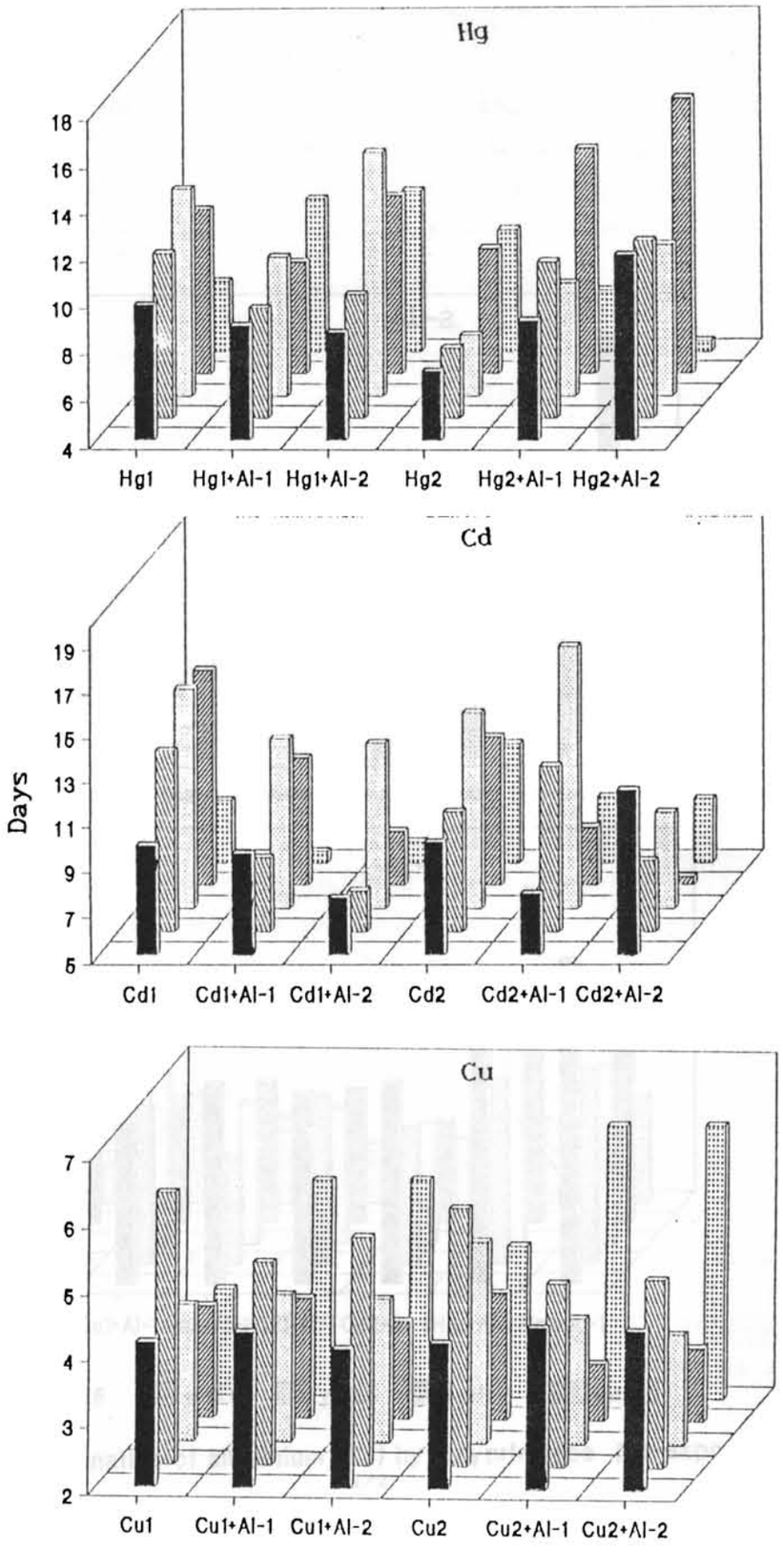

Fig. 5.3. Elimination of metals ($B_{1/2}$) by V.cyprinoides pre-exposed to metals at varying pH and Al concentrations.

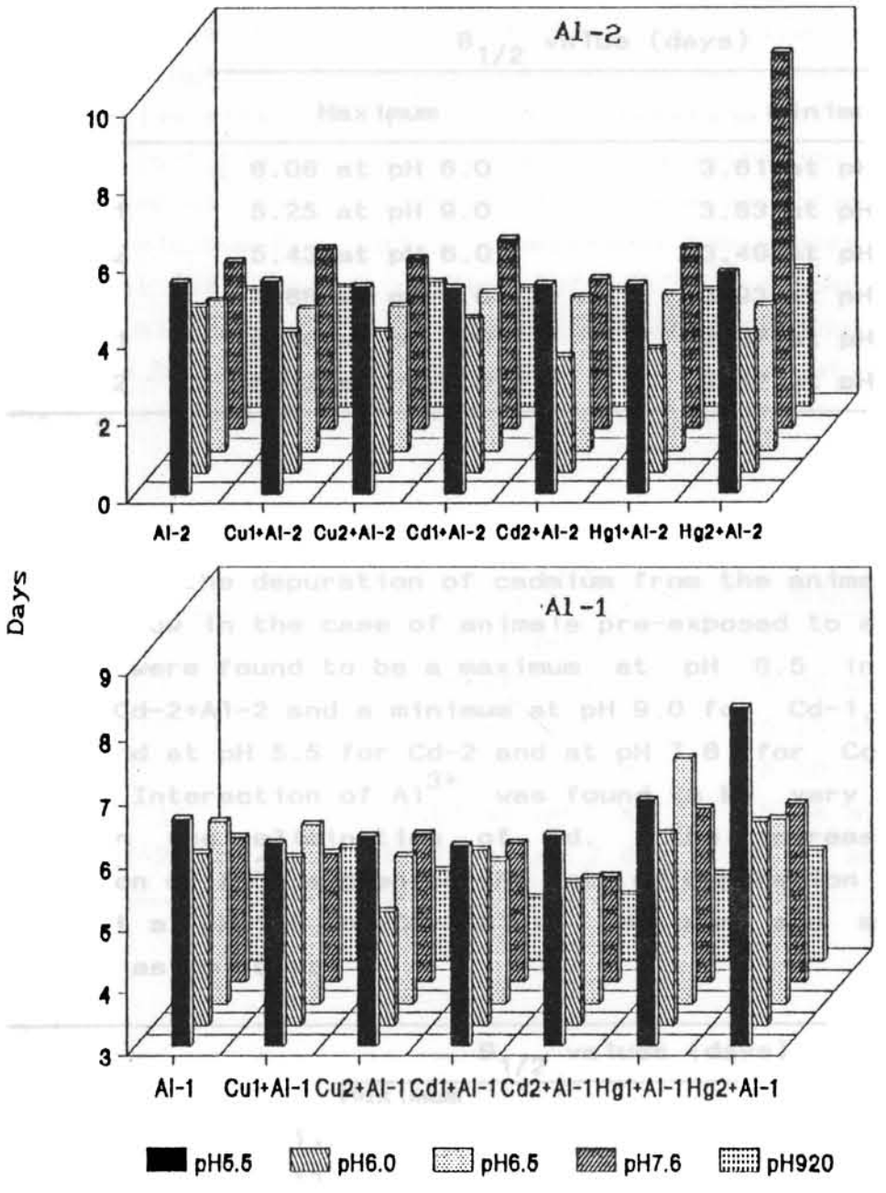

Fig. 5.4. Elimination of aluminium ($B_{1/2}$) by V.cyprinoides pre-exposed to aluminium at varying pH and metal concentrations.

loss of copper and thereby, lowered the $B_{1/2}$ value. The interaction of Al^{3+} decreased the rate of depuration in all cases except during Cu-1+Al-1 at pH 6.0 and Cu-2 at pH 6.0, 6.5 and 7.6. The maximum and minimum $B_{1/2}$ values were as follows:

Exposure Medium	$B_{1/2}$ value (days)	
	Maximum	Minimum
Cu-1	6.08 at pH 6.0	3.61 at pH 9.0
Cu-1+Al-1	5.25 at pH 9.0	3.83 at pH 7.6
Cu-1+Al-2	5.43 at pH 6.0	3.49 at pH 7.6
Cu-2	5.88 at pH 6.0	3.93 at pH 7.6
Cu-2+Al-1	6.10 at pH 9.0	2.87 at pH 7.6
Cu-2+Al-2	6.12 at pH 9.0	3.12 at pH 7.6

Cadmium

Generally the depuration of cadmium from the animal body was found to be low in the case of animals pre-exposed to acid medium. $B_{1/2}$ values were found to be a maximum at pH 6.5 in all cases except for Cd-2+Al-2 and a minimum at pH 9.0 for Cd-1, Cd-1+Al-1, Cd-1+Al-2 and at pH 5.5 for Cd-2 and at pH 7.6 for Cd-2+Al-1 and Cd-2+Al-2. Interaction of Al^{3+} was found to be very significant ($p<0.01$) in the elimination of Cd. The increase in the concentration of Al^{3+} augmented the rate of depuration of Cd in all cases except at pH 5.5 for Cd-2+Al-2. Maximum and minimum $B_{1/2}$ values were as follows:

Exposure Medium	$B_{1/2}$ values (days)	
	Maximum	Minimum
Cd1	14.84 at pH 6.5	7.80 at pH 9.0
Cd1 + Al-1	12.58 at pH 6.5	5.51 at pH 9.0
Cd1 + Al-2	12.40 at pH 6.5	5.95 at pH 9.0
Cd2	13.77 at pH 6.5	9.92 at pH 5.5
Cd2 + Al-1	16.77 at pH 6.5	7.61 at pH 7.6
Cd2 + Al-2	12.28 at pH 5.5	5.58 at pH 7.6

Mercury

The depuration of mercury from the animal body was not well defined. The rate of depuration was found to be a maximum in the case of animals pre-exposed to alkaline medium during Hg-1, Hg-2+Al-1 and Hg-2+Al-2 whereas the reverse was true for others. The interactive effect of Al^{3+} on the depuration of mercury were significant ($p<0.01$). The low Al^{3+} concentration reduced the maximum $B_{1/2}$ value for Hg-1 while the reverse was true for Hg-2. In the case of minimum $B_{1/2}$ value, a decrement was observed with Hg-2 and increment with Hg-1 in presence of low Al^{3+} concentration. The presence of high aluminium concentration enhanced the magnitude of variation in $B_{1/2}$ values in the case of Hg-2 except at pH 9.0. The maximum and minimum $B_{1/2}$ values were as follows:

Exposure Medium	$B_{1/2}$ values (days)	
	Maximum	Minimum
Hg-1	12.88 at pH 6.5	7.02 at pH 9.0
Hg-1+Al-1	10.55 at pH 6.5	8.68 at pH 5.5
Hg-1+Al-2	14.43 at pH 6.5	8.56 at pH 5.5
Hg-2	9.36 at pH 7.6	6.59 at pH 6.5
Hg-2+Al-1	13.64 at pH 7.6	6.64 at pH 9.0
Hg-2+Al-2	15.70 at pH 7.6	4.47 at pH 9.0

Aluminium

The elimination of accumulated aluminium was found to be influenced by the interactive effects of other metals i.e., Cu, Cd and Hg. But the effects were very small. The rate of elimination of Al was found to be very high in the case of animals pre-exposed to alkaline medium. Another important observation was that the amount of aluminium eliminated increased with the increase in the amount of Al^{3+} accumulated. The maximum and minimum $B_{1/2}$ values were as follows:

Exposure Medium		$B_{1/2}$ values (days) Maximum	Minimum
Al-1		6.65	4.33
Al-2		5.51	3.08
Cu-1	+Al-1	6.27	4.82
Cu-2	+Al-1	6.38	4.45
Cd-1	+Al-1	6.24	4.03
Cd-2	+Al-1	6.40	4.08
Hg-1	+Al-1	6.95	4.40
Hg-2	+Al-1	8.38	4.78
Cu-1	+Al-2	5.56	3.13
Cu-2	+Al-2	5.39	3.26
Cd-1	+Al-2	5.35	3.11
Cd-2	+Al-2	5.45	2.99 at pH 6.0 (3.05 at pH 9.0)
Hg-1	+Al-2	5.46	3.04 at pH 9.0
Hg-2	+Al-2	5.77	3.58 at pH 6.0 (3.60 at pH 9.0)

(Maximum: at pH 5.5; Minimum: at pH 9.0)

The minimum $B_{1/2}$ values were obtained in the case of test specimens pre-exposed to pH 9.0 in all cases except during the exposures to Cd-2+Al-2 and Hg-2+Al-2 where slight enhancement was observed at pH 6 0.

Comparison of metals based on depuration

The order of elimination of metals investigated in this study was found to be dependent on the pH at which the clams were pre-exposed and decreased in the following order:

Cu > Al > Cd > Hg at pH 5.5, Al> Cu> Hg> Cd at pH 6.0 and Cu> Al> Hg> Cd at pH 6.5, 7.6 & 9.0	for low metal concentrations.
Cu> Al> Hg> Cd at pH 5.5, Al> Cu> Hg> Cd at pH 6.0, 6.5 & 9.0 and Cu> Al> Hg> Cd at pH 7.6	for high metal concentrations.

Trace metal depuration may be related to the deposition or binding characteristics of each metal.In the present investigation Cu, Cd, Hg and Al were eliminated by *Villorita cyprinoides var cochinensis* (Hanley). These metals may be found in amebocytes as described by George *et al.* (1978). These mobile amebocytes may then transport the trace metals to the epithelia where mucus elimination of the metals can occur. Cunningham and Tripp (1975b) have recognised the following categories of metal release: I) increase in $B_{1/2}$ with increased body burden of trace metal II) decrease in $B_{1/2}$ with increase in body burden of trace metal. and III) stable $B_{1/2}$ when an equilibrium is maintained by a proportionate increase in the rate of trace metal loss as its body burden increases

In the present investigation, metals can be grouped generally into the first or the second category and was found to be dependent on the conditions during accumulation phase. Cd belonged to the group I whereas Cu and Al belonged to group II. Hg belonged to group I during low body burden and changed to group II with increase in the amount of Hg accumulated. Discrepancies were noticed in certain exposures and that may be due to the interactive effects of H^+ and metals during accumulation phase and changes in the mechanism of accumulation.

Although the uptake of the soluble forms of trace metals like Cu, Cd and Hg seems to be mainly a passive process, the elimination of these metals from animal cells seems to be mediated principally by an active mechanism involving their accumulation in lysosomes with the subsequent excretion of the metal rich residual bodies (George and Viarengo, 1985). The same may be true in the present study.

After 21 days, the mean Cu concentration in depuration had fallen from 37.0 to 6.9 $\mu g\ g^{-1}$ (Dixon and Sprague, 1981). The copper elimination by *Noemacheilus barbatulus* showed a similar trend. The rate of loss was rapid in all tissues except liver, where significant levels were retained for more than two weeks (Solbe and Cooper, 1976).

Metallothioneine, induced by the accumulated Cd, bound the metal which will not be available for excretion. The increase in $B_{1/2}$ value may be due to the binding nature of accumulated Cd in metallothioneine (Vierango *et al.*, 1987). Galtsoff (1964) reported that gills serve as an excretory organ. Cd could therefore, be eliminated through the gills. If the gills are involved in excretion of Cd, the change in either the metabolic rate or the filtration rate may influence the depuration of Cd.

Scholz (1980) showed that during exposure of *Mytilus edulis* to a concentration of 100 μg Cd l^{-1}, no equilibrium is reached within 20 days. After transferring the mussels to unspiked running seawater, the elimination was found to be an exponential process with a half life between 14 and 29 days. In contrast, Viarengo *et al.* (1985) showed a minimum elimination of Cd for *Mytilus edulis* whereas Borchardt (1983) found a half life for Cd in *Mytilus edulis* between 96 and 190 days with prolonged duration under conditions of decreasing food availability presumably as a result of metabolic processes.

The accumulation of Hg in organisms was a more rapid process than depuration. Total self purification was not achieved within a

6 months depuration period (Cunningham and Tripp, 1973). Cunnigham and Tripp (1975b) also monitored Hg loss in *Crassostrea virginica* which was characterised by a decrease in $B_{1/2}$ as internal concentration of Hg increased. This pattern of loss could have occurred if Hg were associated primarily with wandering amebocytes and mucus which could be rapidly eliminated from the body and not associated with major tissues.

The depuration of Cu, Cd and Hg at different pH and in presence of Al^{3+} can be explained on the basis of variations in the metabolic processes under these conditions. The elimination of different metals Cu, Cd, Hg and Al may be attributed to self cleaning processes.

In general, the variation in the accumulation and depuration of Cu, Cd, Hg and Al at different pH and the interactive effect of Al^{3+} are complex processes defined by such variables as interaction of H^{+} for binding site, metal chemistry, the enhanced and varied mucus secretion, the variation in the speciation of metals, several detoxification mechanisms, the alteration in the metabolic processes such as metabolic rate, and filtration rate and other biochemical changes. The observed changes in the bioavailability of Cu, Cd and Hg to the test organism with pH and Al were undoubtedly the result of several superimposed effects. It is however clear that unless the pH of the medium is stated and the assumption is made that the complexation invariably decreases availability, the data on the metal ion concentration alone do not provide on the indication of the biological availability of the metals.

CHAPTER VI

BIOCHEMICAL EFFECTS OF TRACE METALS

Biochemical responses to trace metals have been identified as indices for environmental monitoring purposes. Sensitivity to sublethal stress, ease of measurement and specificity of the responses are salient features of such a response. The indices of sublethal pollutant stress which appears to offer the most promise for use in field monitoring programmes are measurements of the physiological or biochemical parameters that are related to growth and reproduction, as the survival of a population is inevitably depended upon these processes.

There are many levels of organizations which can be affected by contaminants and processes at each of these levels can be used to monitor or predict the effects of toxic materials on aquatic organisms (Cairns, 1986). Toxic materials exist as atom or molecules and each exerts their toxic effects by interacting with biological systems at the cellular and sub-cellular levels of organization (Giesy *et al.*, 1988). Therefore, one would expect that biochemical and physiological measures of toxicant induced stress would be useful as sensitive specific predictors of effects at the level of response of whole animals to the effects of toxicants. Furthermore, because changes must occur at the biochemical, cellular and tissue levels of organisation before effects will be observed at the organism level, it has been suggested that such measures may prove to be useful acute measures of more chronic effects at higher levels of organization (McKee and Knowles, 1986).

For this reason, biochemical measures of toxic effects, such as energy content, protein content, RNA/DNA ratio or concentration of specific enzymes or substrates have been suggested as potential short-term functional measures, which can be used to predict the

toxic effects of chronic exposures to xenobiotics on the survival and fecundity of organisms (Giesy *et al.*, 1988).

Most of the information available on the effects of toxic materials on aquatic organisms suggested that responses of whole organisms provide the most useful information for setting standards to protect aquatic organisms (Sprague, 1976; Mount, 1988). The present study also verified the use of biochemical and physiological measures for monitoring adverse effects in aquatic systems and for elucidating modes of toxic action. The utility of measuring biochemical and physiological parameters, which are often classified as 'clinical measures', is in determining the mode of toxic action of chemical under laboratory conditions, determining the probable causes to lethal or sublethal effects under field conditions or monitoring from the combined adverse effects of long term exposure to mixtures of toxicants. Also, because surface waters are seldom acutely toxic to aquatic organisms, clinical measures have been useful in monitoring for effects before they reach population or community levels (Versteeg *et al.*, 1988).

There are multitude of energetic responses which organisms exhibit in response to toxicant induced stress, and in many ways, these responses can be directly related to the general adaptation syndrome (GAS) (Selye, 1976). The implications of GAS and its implication for measuring the degree of stress under which an organism exists are reviewed elsewhere (Giesy *et al.*, 1988). In very simple terms, the theory of the GAS states that there are a number of quantifiable, general responses of organisms under stress and that organisms can resist the stressful effects of exposure to toxicants for a finite period of time. The basis for the use of energetics is that energy is required to resist the effects of toxicants and to maintain homeostasis. Eventually, the organism expends all of its energy reserves and it passes into exhaustion and ultimately dies. This energy can be used for movement, to transport ions or biomolecules, to replace damaged or biotransformed structural or functional proteins and to metabolize toxicants.

The increased energy demand associated with stress may reduce energy stores such as glycogen and lipid and /or alter their distribution among tissues. Reduction or redistribution of energy stores is generally considered to be a non-specific indicator which is responsive to any kind of stressor, natural or man made and can be applied as a biochemical indicator of stress in both field monitoring and laboratory hazard assessment schemes. The most successful approach involves assessment of the entire energy budget for an organism. Both physical and chemical stressors cause changes in the concentrations of energy storage and transport of biomolecules in fish and invertebrates. For example, blood sugar concentration of freshwater fish does not vary with age but can be affected by nutrition. Anaerobiosis or oxygen stress can result in the mobilisation of glycogen reserves (Wilps and Zebe, 1976). Therefore, when choosing an appropriate organism for use in bioassays through measurement of energetic responses, background informations on its physiology and biochemistry are essential. In utilizing these biochemicals to assess the effects of stressors, it must be remembered that many factors including species, sex, season, temperature and hormonal status can affect the basal concentration of energy substrates (Lehtinen *et al.*, 1984).

The objectives of the present study were to determine whether the biochemical parameters such as glycogen, lactic acid, lipid, protein and ascorbic acid were altered in the test specimen during sublethal pollutants stress (interactive effect of H^+, Al^{3+}, Cu, Cd and Hg).

6.1. MATERIALS AND METHODS

The experimental techniques used for the determination of glycogen, lactic acid, lipid, protein and ascorbic acid, and the experimental designs employed in the present study were detailed in Chapter II.

6.2. RESULTS AND DISCUSSION

Bivalves maintained at 0h and pH 7.6 were considered as control and the variations in the magnitude of the biochemical parameters compared to those of the control were measured at 24h, 72h and 120h after the exposure of the test specimen to different experimental conditions. The percentage variations of the biochemical parameters at 120h were used for the inter comparison of the interactive effects of H^+, Al^{3+}, Cu, Cd and Hg. ANOVA was carried out for the prediction of the effect of different experimental conditions on the variation in biochemical effects.

Glycogen

Glycogen is a branched polymer of glucose residues and represents the readily mobilizable storage form of glucose for most organisms. The glycogen contents of the clam at 0h, 24h, 72h, and 120h under different experimental conditions are given in the Table 6.1 and percentage variations of glycogen content after 120h from the control value are given in the Fig. 6.1.

The glycogen content of the clam at 0h and pH 7.6 (2.98 ± 0.04 mg g^{-1} wet weight) was taken as the control value. Significant depletion ($P<0.01$) in glycogen content was observed with different metal concentrations at different pH. Glycogen content was found to be significantly decreased ($P<0.01$) with increase in the period of exposure in all experimental conditions.

The decrease in the glycogen content increased with decrease in pH or, conversely acidification was found to enhance the rate of depletion in all cases. The minimum depression was at pH 9.0 (2.11 mg g^{-1} after 120h). and the maximum depletion was observed at pH 5.5 (1.42 mg g^{-1} after 120h). Although the presence of Cu, Cd, Hg and Al enhanced the depletion in the glycogen contents, the contribution due to Al was found to be very little. The maximum and minimum percentage variations in the glycogen content (Fig. 6.1) after 120h in different experimental conditions were as follows:

Table 6.1. Metal induced variations in the glycogen conten (mg g^{-1} wet wt.) of *V. cyprinoides* at varying pH.

(Control value = 2.98±0.04)

pH	Metal conc.	Exposure period (h)		
		24	72	120
5.5	H^+	1.98±0.02	1.87±0.01	1.42±0.04
	Al-1	1.78±0.01	1.64±0.02	1.40±0.05
	Al-2	1.62±0.02	1.52±0.04	1.38±0.05
	Cu-1	2.72±0.04	2.02±0.02	1.96±0.04
	Cu-1+Al-1	2.69±0.06	1.98±0.01	1.34±0.04
	Cu-1+Al-2	2.32±0.07	1.84±0.07	1.22±0.02
	Cu-2	2.64±0.10	1.72±0.04	1.62±0.02
	Cu-2+Al-1	2.48±0.03	1.70±0.06	1.22±0.01
	Cu-2+Al-2	2.32±0.04	1.58±0.06	1.08±0.02
	Cd-1	1.96±0.01	1.69±0.01	1.38±0.05
	Cd-1+Al-1	1.88±0.02	1.87±0.02	1.32±0.08
	Cd-1+Al-2	1.72±0.01	1.68±0.02	1.24±0.01
	Cd-2	2.08±0.01	1.54±0.03	1.12±0.02
	Cd-2+Al-1	1.97±0.03	1.38±0.01	1.22±0.02
	Cd-2+Al-2	1.96±0.01	1.24±0.02	1.20±0.01
	Hg-1	1.89±0.04	1.78±0.02	1.36±0.02
	Hg-1+Al-1	1.88±0.02	1.58±0.02	1.22±0.01
	Hg-1+Al-2	1.86±0.02	1.40±0.01	1.18±0.01
	Hg-2	1.87±0.03	1.74±0.03	1.38±0.08
	Hg-2+Al-1	1.86±0.01	1.78±0.02	1.20±0.07
	Hg-2+Al-2	1.78±0.06	1.60±0.01	0.98±0.04
6.0	H^+	2.02±0.01	1.92±0.02	1.84±0.03
	Al-1	1.98±0.01	1.88±0.02	1.62±0.06
	Al-2	1.79±0.01	1.64±0.03	1.54±0.07
	Cu-1	2.84±0.01	2.23±0.02	1.98±0.03
	Cu-1+Al-1	2.78±0.01	2.62±0.01	1.58±0.01
	Cu-1+Al-2	2.68±0.03	1.80±0.01	1.38±0.01
	Cu-2	2.76±0.02	1.94±0.01	1.78±0.02
	Cu-2+Al-1	2.62±0.02	1.76±0.01	1.50±0.02
	Cu-2+Al-2	2.41±0.01	1.62±0.01	1.21±0.04
	Cd-1	2.20±0.02	2.46±0.02	2.10±0.02
	Cd-1+Al-1	2.12±0.01	1.88±0.03	1.66±0.02
	Cd-1+Al-2	1.90±0.07	1.72±0.04	1.40±0.02
	Cd-2	2.58±0.02	1.58±0.03	1.38±0.02
	Cd-2+Al-1	2.24±0.03	1.42±0.02	1.24±0.04
	Cd-2+Al-2	2.12±0.02	1.38±0.01	1.12±0.03

(Contd...

Table 6.1. (Contd...)

	Hg-1	2.01±0.02	1.88±0.04	1.58±0.04
	Hg-1+Al-1	1.98±0.04	1.86±0.05	1.50±0.05
	Hg-1+Al-2	1.90±0.01	1.72±0.06	1.40±0.03
	Hg-2	1.92±0.02	1.76±0.06	1.42±0.07
	Hg-2+Al-1	1.90±0.01	1.82±0.04	1.48±0.04
	Hg-2+Al-2	1.86±0.01	1.68±0.03	1.32±0.02
6.5	H^+	2.48±0.01	2.17±0.01	2.01±0.02
	Al-1	2.32±0.02	1.98±0.02	1.76±0.04
	Al-2	1.81±0.02	1.72±0.02	1.62±0.04
	Cu-1	2.90±0.01	2.38±0.01	2.00±0.01
	Cu-1+Al-1	2.81±0.01	1.84±0.01	1.64±0.02
	Cu-1+Al-2	2.78±0.01	1.76±0.01	1.52±0.01
	Cu-2	2.88±0.01	2.12±0.01	1.84±0.01
	Cu-2+Al-1	2.74±0.01	1.86±0.01	1.53±0.01
	Cu-2+Al-2	2.66±0.01	1.78±0.01	1.39±0.01
	Cd-1	2.38±0.04	2.58±0.04	2.32±0.03
	Cd-1+Al-1	2.28±0.03	1.91±0.03	1.72±0.01
	Cd-1+Al-2	1.98±0.01	1.76±0.01	1.54±0.01
	Cd-2	2.62±0.02	1.68±0.01	1.41±0.01
	Cd-2+Al-1	2.42±0.01	1.58±0.01	1.38±0.02
	Cd-2+Al-2	2.31±0.03	1.42±0.03	1.28±0.03
	Hg-1	2.38±0.04	1.98±0.01	1.62±0.01
	Hg-1+Al-1	2.26±0.01	1.86±0.01	1.58±0.01
	Hg-1+Al-2	2.12±0.01	1.72±0.01	1.42±0.01
	Hg-2	2.38±0.01	1.79±0.01	1.38±0.02
	Hg-2+Al-1	2.12±0.01	1.72±0.01	1.22±0.02
	Hg-2+Al-2	2.01±0.02	1.68±0.01	1.01±0.02
7.6	H^+	2.87±0.01	2.67±0.02	1.92±0.03
	Al-1	2.67±0.04	2.02±0.05	1.81±0.04
	Al-2	2.18±0.02	1.98±0.03	1.78±0.02
	Cu-1	2.99±0.01	2.42±0.07	2.14±0.02
	Cu-1+Al-1	2.92±0.01	1.98±0.04	1.78±0.03
	Cu-1+Al-2	2.89±0.01	1.92±0.03	1.62±0.01
	Cu-2	3.12±0.01	2.21±0.04	1.98±0.01
	Cu-2+Al-1	3.01±0.02	1.88±0.05	1.62±0.01
	Cu-2+Al-2	2.78±0.02	1.86±0.01	1.42±0.04

(Contd...

Table 6.1. (Contd...)

	Cd-1	2.81±0.04	2.61±0.02	2.42±0.06
	Cd-1+Al-1	2.30±0.03	2.22±0.01	1.78±0.07
	Cd-1+Al-2	2.06±0.04	1.86±0.01	1.64±0.01
	Cd-2	2.78±0.03	2.54±0.03	2.32±0.02
	Cd-2+Al-1	2.60±0.03	1.82±0.04	1.78±0.03
	Cd-2+Al-2	2.48±0.01	1.80±0.05	1.70±0.01
	Hg-1	2.67±0.02	2.02±0.01	1.76±0.04
	Hg-1+Al-1	2.58±0.02	1.98±0.02	1.70±0.05
	Hg-1+Al-2	2.52±0.04	1.86±0.04	1.62±0.06
	Hg-2	2.46±0.03	2.00±0.03	1.59±0.08
	Hg-2+Al-1	2.40±0.02	1.87±0.09	1.68±0.08
	Hg-2+Al-2	2.32±0.01	1.76±0.01	1.58±0.01
9.0	H^+	2.89±0.02	2.62±0.01	2.11±0.04
	Al-1	2.67±0.01	2.41±0.03	1.98±0.02
	Al-2	2.01±0.01	1.89±0.02	1.78±0.02
	Cu-1	2.87±0.02	2.58±0.01	2.00±0.01
	Cu-1+Al-1	2.56±0.01	2.18±0.01	1.72±0.01
	Cu-1+Al-2	2.48±0.05	2.01±0.02	1.69±0.01
	Cu-2	2.79±0.06	2.42±0.02	1.81±0.04
	Cu-2+Al-1	2.43±0.06	1.98±0.07	1.62±0.05
	Cu-2+Al-2	2.31±0.05	1.82±0.01	1.51±0.04
	Cd-1	2.78±0.03	2.58±0.01	2.31±0.04
	Cd-1+Al-1	2.28±0.03	2.12±0.02	1.76±0.05
	Cd-1+Al-2	1.92±0.02	1.76±0.01	1.58±0.06
	Cd-2	2.66±0.04	2.32±0.01	2.02±0.02
	Cd-2+Al-1	2.48±0.05	1.62±0.01	1.68±0.03
	Cd-2+Al-2	2.21±0.04	1.58±0.06	1.52±0.04
	Hg-1	2.52±0.01	1.98±0.01	1.88±0.01
	Hg-1+Al-1	2.48±0.01	1.86±0.01	1.62±0.01
	Hg-1+Al-2	2.21±0.02	1.78±0.04	1.50±0.02
	Hg-2	2.26±0.01	1.92±0.05	1.48±0.01
	Hg-2+Al-1	2.10±0.01	1.86±0.08	1.32±0.08
	Hg-2+Al-2	2.08±0.01	1.74±0.01	1.30±0.09

(Value = $\bar{x}$ ± SD, n = 9)

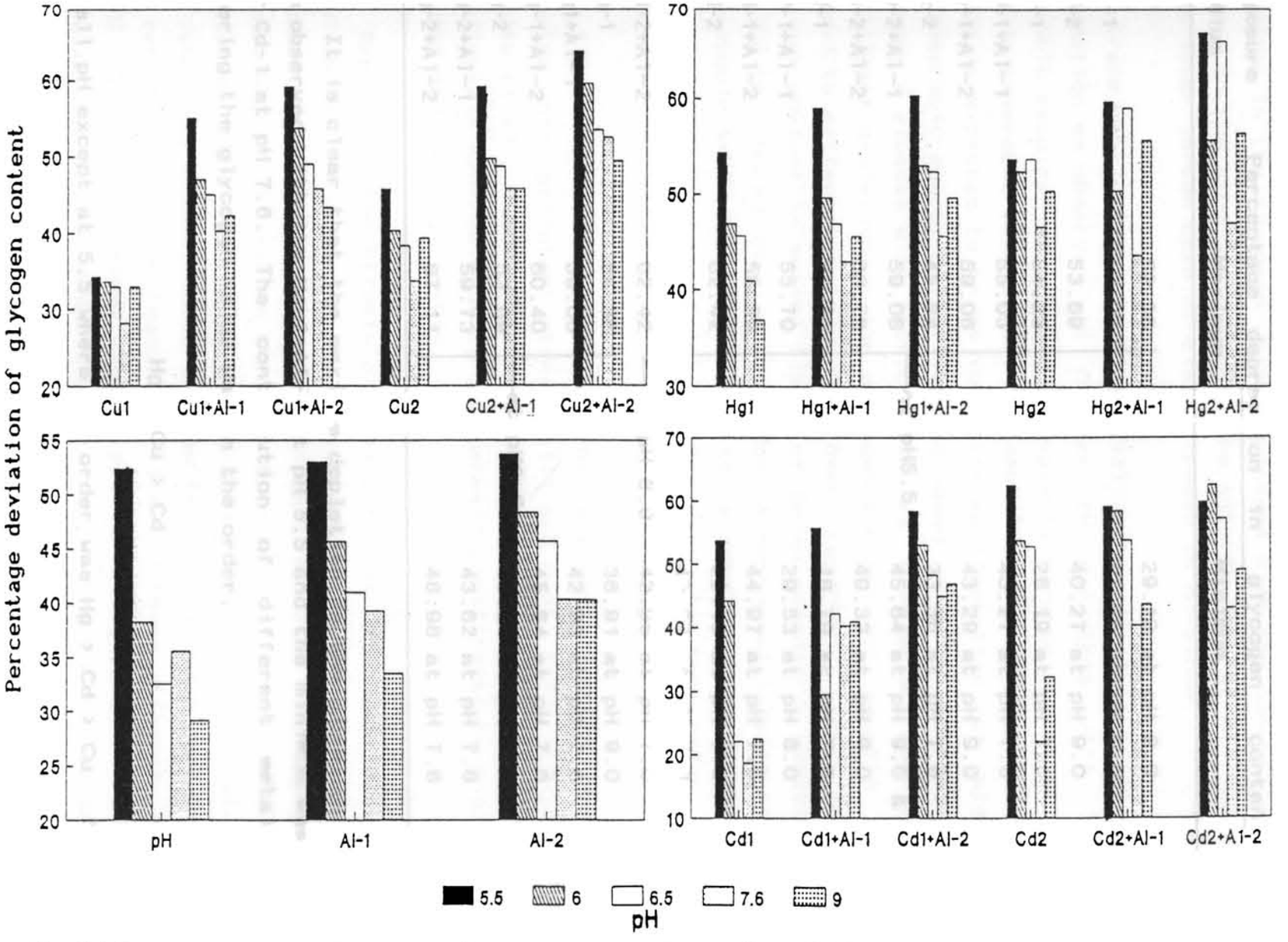

Fig. 6.1. Percetage deviation (from control) of glycogen content of V.cyprinoides exposed to Cu, Cd, Hg and Al at varying pH.

Exposure Medium	Percentage depression in glycogen content		
	Maximum		Minimum
H^+	52.35		29.19 at pH 9.0
Al-1	53.02		33.56 at pH 9.0
Al-2	53.69		40.27 at pH 9.0
Cu-1	34.23		28.19 at pH 7.6
Cu-1+Al-1	55.03		40.27 at pH 7.6
Cu-1+Al-2	59.06		43.29 at pH 9.0
Cu-2	45.64		33.56 at pH 7.6
Cu-2+Al-1	59.06	—at pH5.5	45.64 at pH 9.0 & 7.6
Cu-2+Al-2	63.76		49.33 at pH 9.0
Cd-1	53.69		18.79 at pH 7.6
Cd-1+Al-1	55.70		29.53 at pH 6.0
Cd-1+Al-2	58.39		44.97 at pH 7.6
Cd-2	62.42		22.15 at pH 7.6
Cd-2+Al-1	59.06		40.27 at pH 7.6
Cd-2+Al-2	62.42	— at pH 6.0	42.95 at pH 7.6
Hg-1	54.36		36.91 at pH 9.0
Hg1+Al-1	59.06		42.93 at pH 7.6
Hg-1+Al-2	60.40		45.64 at pH 7.6
Hg-2	53.69	—at pH5.5	46.64 at pH 7.6
Hg-2+Al-1	59.73		43.62 at pH 7.6
Hg-2+Al-2	67.11		46.98 at pH 7.6

It is clear that the maximum depletion in the glycogen content was observed with the Hg-2+Al-2 at pH 5.5 and the minimum was found for Cd-1 at pH 7.6. The contribution of different metals in lowering the glycogen value was in the order,

Hg > Cu > Cd

at all pH except at 5.5 where the order was Hg > Cd > Cu for low metal concentrations and Cd> Hg > Cu at pH 5.5 and 6.0 and Hg > Cd > Cu at pH 6.5 and Hg > Cu> Cd at pH 7.6 and 9.0 for high metal

concentrations. The presence of Al^{3+} further enhanced the depletion in the glycogen which increased with the amount of Al^{3+} present in the exposure medium. The interference of Al^{3+} was found to be lower in the case of clams exposed to H^{+} alone.

Trace metal stress in clams will cause depletion in oxygen consumption as observed in Chapter IV and this will lead to anaerobic respiration. Since glycogen is a ready source of energy even in anaerobic conditions, the depletion of glycogen from the tissue is expected to be an immediate manifestation of hypoxemia. The anaerobic energy metabolism was based on the degradation of glycogen / glucose with lactic acid as the major end product. Significant depletion in the glycogen content of aquatic animals exposed to different metals has been reported. The decreased glycogen content in mussel tissue of *Salmo gairdneri* exposed to Cd was due to reduced insulin secretion. A decreased insulin secretion is probable as Cd has been found to accumulate in pancreas and cause necrotic lesions of the insulin producing beta cells in the islets of lanterns (Larsson and Haux, 1982

Significant depletion in the glycogen content has been reported under pollutant stress (Gill and Pant, 1981; Cyriac, 1990; Sujatha *et al.*, 1995). This was attributed either to decreased vitellogenin synthesis or to glyogenesis facilitated by high blood glucose and cortisol levels.

Reports on the effects of toxicants on glycogen content of bivalves are relatively few. Decrease in glycogen content in presence of Cu and Hg was observed in *Villorita cyprinoides var cochinensis* (Sathyanathan *et al.*, 1988; Geetha, 1992). In *Mytilus edulis*, glycogen was recognized as an important source of energy (de Zwaan and Zandee, 1972) and low food levels with gametogenises was reported to be the reason for its decrease (Zandee *et al.*, 1980). The present study clearly proved that glycogen content of the clam got reduced under sublethal stress of Cu, Cd, Hg and Al at different pH.

Very little is known about inter-conversion of glucose and glycogen in marine bivalves. Synthesis of glucose was found to be inhibited under anaerobic conditions and glycogen was used as the main energy resource during stress. The variation in the glycogen content under different experimental conditions may be attributed to the bioavailability of the different metals. Increased oxygen demand on exposure to Cu at low pH resulted in increased consumption of energy and in a consequent depletion of tissue glycogen content. Hence variation in the oxygen consumption under different experimental conditions as described in Chapter IV would also be responsible for the variation in glycogen content.

The higher glycogen levels found in the clam at certain conditions may be an adaptation to acidification whereby, gluconeogenesis maintains osmotic pressure by increasing plasma glucose to compensate for the electrolyte loss. Similar increase in liver glycogen was observed in rainbow trout exposed to pH 4.2 and 4.8 for 14 days (Lee *et al.*, 1983).

Lactic acid

The lactic acid content in the *Villorita cyprinoides* var *cochinensis* at 0h and pH 7.6 was 0.0278 ± 0.0002 mg g^{-1}(wet weight). This was considered to be the control value for comparison. The lactic acid contents of the test specimen exposed to various concentrations of Cu, Cd, Hg and Al at different pH and the interactive effects of Al^{3+} and H^{+} at 24h, 72h and 120h. are given in the Table 6.2. The percentage variations after 120h compared with the control are depicted in the Fig. 6.2.

The lactic acid content was found to be significantly elevated ($P<0.01$) at all experimental conditions. The percentage variation after 120h increased with decrease in pH. The maximum value of 0.073 ± 0.0002 mg g^{-1} was found at pH 5.5 and the minimum value of 0.048± 0.0004 mg g^{-1} at pH 9.0. The presence of trace metals further augmented this enhancement in the lactic acid content. The

Table 6.2. Metal induced variations in the lactic acid content ($\times 10^{-3}$ mg g^{-1} wet wt.) of *V. cyprinoides* at varying pH.

(Control value = 27.8±0.02)

pH	Metal conc.	Exposure period (h)		
		24	72	120
5.5	H^+	61±0.3	72±0.5	73±0.2
	Al-1	68±0.6	69±0.4	78±0.2
	Al-2	74±0.7	78±0.3	84±0.4
	Cu-1	74±0.2	84±0.1	92±0.7
	Cu-1+Al-1	78±0.6	91±0.3	112±0.1
	Cu-1+Al-2	84±0.7	98±0.4	128±0.3
	Cu-2	81±0.4	90±0.1	102±0.4
	Cu-2+Al-1	82±0.2	94±0.2	112±0.2
	Cu-2+Al-2	90±0.4	98±0.1	128±0.5
	Cd-1	62±0.2	76±0.1	98±0.2
	Cd-1+Al-1	68±0.3	84±0.2	104±0.4
	Cd-1+Al-2	80±0.2	88±0.1	112±0.3
	Cd-2	64±0.1	87±0.4	99±0.1
	Cd-2+Al-1	72±0.4	94±0.3	108±0.1
	Cd-2+Al-2	84±0.3	98±0.2	112±0.2
	Hg-1	54±0.4	62±0.1	80±0.2
	Hg-1+Al-1	64±0.2	74±0.2	91±0.3
	Hg-1+Al-2	65±0.4	68±0.3	94±0.1
	Hg-2	62±0.5	74±0.1	88±0.2
	Hg-2+Al-1	61±0.6	78±0.4	94±0.4
	Hg-2+Al-2	68±0.7	76±0.7	101±0.3
6.0	H^+	52±0.4	62±0.1	68±0.7
	Al-1	62±0.6	68±0.4	74±0.1
	Al-2	70±0.3	72±0.2	81±0.3
	Cu-1	58±0.2	68±0.4	79±0.4
	Cu-1+Al-1	65±0.3	84±0.2	94±0.3
	Cu-1+Al-2	78±0.1	98±0.3	108±0.2
	Cu-2	73±0.7	82±0.1	99±0.1
	Cu-2+Al-1	78±0.1	94±0.1	106±0.1
	Cu-2+Al-2	88±0.2	99±0.2	120±0.2
	Cd-1	58±0.6	74±0.3	84±0.2
	Cd-1+Al-1	62±0.7	76±0.2	92±0.1
	Cd-1+Al-2	84±0.8	90±0.7	98±0.1
	Cd-2	64±0.2	84±0.2	99±0.7
	Cd-2+Al-1	65±0.1	87±0.4	104±0.6
	Cd-2+Al-2	70±0.1	88±0.1	108±0.5

(Contd...)

Table 6.2. (Contd...)

	Hg-1	48±0.1	64±0.4	74±0.3
	Hg-1+Al-1	54±0.1	68±0.2	81±0.2
	Hg-1+Al-2	56±0.2	69±0.7	84±0.4
	Hg-2	54±0.1	71±0.5	80±0.1
	Hg-2+Al-1	55±0.3	74±0.1	81±0.1
	Hg-2+Al-2	58±0.7	80±0.2	92±0.3
6.5	H^+	41±0.7	52±0.1	58±0.3
	Al-1	52±0.7	61±0.2	68±0.4
	Al-2	58±0.8	64±0.1	74±0.3
	Cu-1	44±0.4	58±0.2	65±0.1
	Cu-1+Al-1	58±0.2	72±0.3	81±0.1
	Cu-1+Al-2	64±0.1	81±0.1	92±0.2
	Cu-2	59±0.3	84±0.1	94±0.3
	Cu-2+Al-1	62±0.4	89±0.2	102±0.2
	Cu-2+Al-2	74±0.2	92±0.4	114±0.1
	Cd-1	48±0.3	64±0.4	72±0.1
	Cd-1+Al-1	54±0.2	68±0.5	84±0.2
	Cd-1+Al-2	62±0.1	74±0.1	94±0.3
	Cd-2	58±0.2	70±0.1	81±0.4
	Cd-2+Al-1	64±0.4	76±0.3	95±0.1
	Cd-2+Al-2	72±0.3	84±0.4	104±0.1
	Hg-1	45±0.1	54±0.2	62±0.2
	Hg-1+Al-1	52±0.2	60±0.3	72±0.3
	Hg-1+Al-2	54±0.3	58±0.1	78±0.4
	Hg-2	50±0.4	60±0.7	68±0.2
	Hg-2+Al-1	54±0.5	62±0.2	77±0.3
	Hg-2+Al-2	57±0.6	63±0.8	88±0.1
7.6	H^+	30±0.1	46±0.4	52±0.2
	Al-1	34±0.2	49±0.4	68±0.3
	Al-2	38±0.1	52±0.2	70±0.1
	Cu-1	31±0.2	48±0.2	54±0.1
	Cu-1+Al-1	34±0.1	54±0.3	69±0.3
	Cu-1+Al-2	41±0.2	58±0.6	74±0.4
	Cu-2	36±0.1	59±0.5	78±0.2
	Cu-2+Al-1	40±0.3	58±0.2	82±0.7
	Cu-2+Al-2	46±0.2	62±0.1	84±0.8

(Contd...)

Table 6.2. (Contd...)

	Cd-1	34±0.2	48±0.5	64±0.3
	Cd-1+Al-1	42±0.1	58±0.2	79±0.4
	Cd-1+Al-2	44±0.3	59±0.3	84±0.2
	Cd-2	42±0.2	57±0.1	78±0.2
	Cd-2+Al-1	54±0.4	68±0.4	88±0.5
	Cd-2+Al-2	60±0.2	79±0.7	98±0.1
	Hg-1	39±0.2	50±0.3	68±0.1
	Hg-1+Al-1	48±0.3	58±0.2	72±0.2
	Hg-1+Al-2	54±0.1	62±0.1	76±0.3
	Hg-2	40±0.1	52±0.2	70±0.4
	Hg-2+Al-1	49±0.4	60±0.7	78±0.5
	Hg-2+Al-2	54±0.5	64±0.8	88±0.6
9.0	H^+	29±0.4	34±0.3	48±0.4
	Al-1	38±0.2	42±0.5	56±0.3
	Al-2	44±0.4	54±0.4	59±0.2
	Cu-1	42±0.2	60±0.4	71±0.2
	Cu-1+Al-1	50±0.3	72±0.2	84±0.3
	Cu-1+Al-2	52±0.1	82±0.1	94±0.1
	Cu-2	51±0.2	67±0.7	78±0.4
	Cu-2+Al-1	64±0.7	76±0.9	92±0.5
	Cu-2+Al-2	68±0.5	84±0.8	102±0.6
	Cd-1	32±0.3	40±0.1	58±0.1
	Cd-1+Al-1	40±0.1	50±0.2	59±0.7
	Cd-1+Al-2	44±0.2	58±0.4	72±0.3
	Cd-2	38±0.1	44±0.3	64±0.2
	Cd-2+Al-1	44±0.4	54±0.2	60±0.1
	Cd-2+Al-2	48±0.2	60±0.1	68±0.1
	Hg-1	41±0.2	42±0.7	52±0.5
	Hg-1+Al-1	50±0.2	62±0.2	73±0.3
	Hg-1+Al-2	58±0.3	68±0.1	80±0.4
	Hg-2	51±0.4	64±0.3	84±0.2
	Hg-2+Al-1	54±0.2	68±0.4	85±0.1
	Hg-2+Al-2	55±0.1	78±0.2	89±0.3

(Value = $\bar{x}$ ± SD, n = 9)

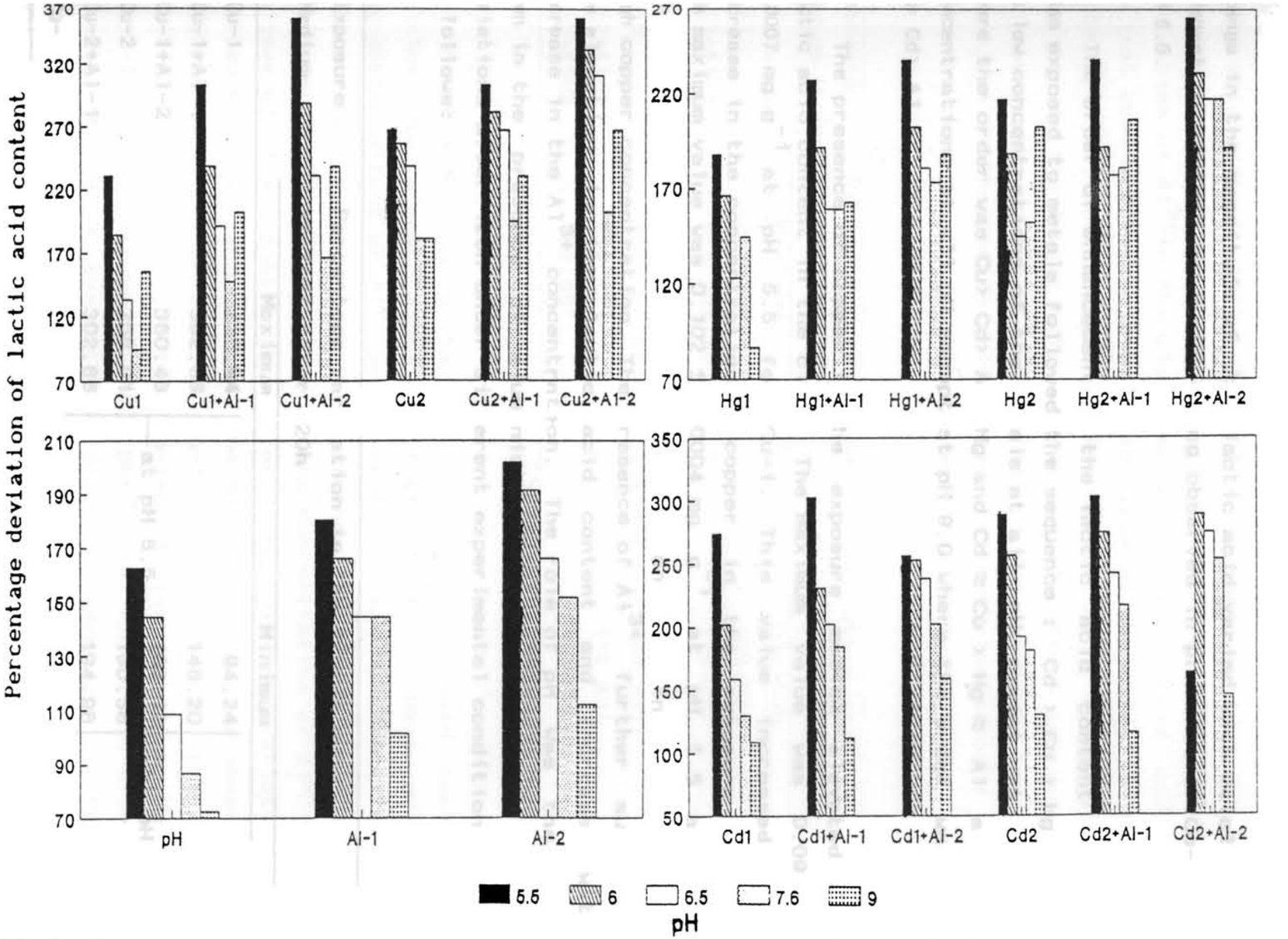

Fig. 6.2. Percentage deviation (from control) of lactic acid content of V.cyprinoides exposed to Cu, Cd, Hg and Al at varying pH.

change in the magnitude of the lactic acid varied with metals with highest percentage variation being observed in presence of Cu-2 at pH 5.5.

The order of enhancement in the lactic acid content of the clam exposed to metals followed the sequence : Cd > Cu > Hg ≈ Al at low concentrations of the metals at all pH except at pH 9.0 where the order was Cu> Cd> Al> Hg and Cd ≈ Cu > Hg ≈ Al at high concentrations at all pH except at pH 9.0 where the order was Hg> Cu> Cd> Al.

The presence of copper in the exposure medium elevated the lactic acid content in the clam. The maximum value was 0.092 ± 0.0007 mg g^{-1} at pH 5.5 for Cu-1. This value increased with increase in the concentration of copper in the exposure medium. The maximum value was 0.102 ± 0.0004 mg g^{-1} at pH 5.5 and the minimum value was 0.078 mg g^{-1} at pH 7.6 and 9.0 in the case of high copper concentration. The presence of Al^{3+} further augmented the elevation in the lactic acid content and increased with increase in the Al^{3+} concentration. The role of pH was the same even in the presence of aluminium. The maximum and minimum variations after 120h under different experimental conditions were as follows:

Exposure Medium	Percentage varitation in the lactic acid content after 120h			
	Maximum		Minimum	
Cu-1	230.94	at pH 5.5	94.24	at pH 7.6
Cu-1+Al-1	302.88		148.20	
Cu-1+Al-2	360.43		166.19	
Cu-2	266.91		180.58	
Cu-2+Al-1	302.88		194.96	
Cu-2+Al-2	360.43		202.16	

The presence of Cd in the exposure medium also increased the lactic acid content of the clam. In this case, maximum values were observed at pH 5.5 and the minimum values were observed at pH 9.0. Interaction of Al^{3+} in the elevation of the lactic acid content in the test specimen was same as in the case of the animal exposed to copper and Al^{3+}. Here also, the presence of Al^{3+} augmented the elevation in the lactic acid content but the effect increased with increase in the concentration of Al^{3+}. The maximum and minimum variations in the lactic acid contents of the clam exposed to Cd at different experimental conditions were as follows:

Exposure Medium	Percentage variation in the lactic acid content after 120h			
	Maximum		Minimum	
Cd-1	252.52		108.63	
Cd-1+Al-1	274.10	at pH 5.5	112.23	
Cd-1+Al-2	302.88		158.99	at pH 9.0
Cd-2	256.12	at pH 5.5 & 6.0	130.22	
Cd-2+Al-1	288.49	at pH 5.5	115.83	
Cd-2+Al-2	302.88		144.60	

The effect of Hg in the elevation of lactic acid content in the clam was almost equal to that of Al^{3+}. Here also, the interactive effect of Al^{3+} was observed. The presence of Al^{3+} increased the lactic acid content of the clam exposed to Hg and the effect increased with increase in the concentration of Al^{3+}. As in the case of clam exposed to copper, the maximum variation was observed at pH 5.5 and whereas, the minimum variations observed at different pH i.e., pH 9.0 for Hg-1 and Hg-2+Al-2, pH 7.6 for Hg-1+Al-2, pH 7.6 and 6.5 for Hg-1+Al-1 and 6.5 for Hg-2 and Hg2+Al-1. The maximum and minimum percentage variations after 120h in the lactic acid content of the clam exposed to Hg under different experimental conditions were:

Exposure Medium	Percentage variation in the lactic acid content after 120h		
	Maximum		Minimum
Hg-1	187.77	at pH 5.5	87.05 – at pH 9.0
Hg-1+Al-1	227.34		158.99 – at pH 6.5
Hg-1+Al-2	238.13		173.38 – at pH 7.6
Hg-2	216.55		144.60 – at pH 6.5
Hg-2+Al-1	238.13		176.98 – at pH 6.5
Hg-2+Al-2	263.31		191.01 – at pH 9.0

Lactic acid is the final product of anaerobic carbohydrate metabolism and is the primary source of energy in the white muscle of fishes (Pickering *et al.*, 1982). Therefore, short term stressors may cause a rapid depletion of blood glucose and glycogen and an increase in lactic acid in bivalves also. Few studies have investigated on toxicant induced effects on lactate concentration in fish blood. Exposure to 2.6 mg K_2CrO_4 l^{-1} for 120 days caused an increase in lactic acid concentration in plasma and muscle of the teleost fish *Channa punctatus*, while, the lactic acid concentration in liver tissue was less than untreated fish (Sastry and Sunitha, 1983). Similarly, invivo exposure of the fresh water fish *C. fasciatus* to 80% of the 96h LC50 for Ni caused a significant increase in the lactic acid content of the blood (Chaudhry, 1984). An increased tissue lactate : pyruvate ratio in fish exposed to naphthalene indicated a greater aneorobic energy production (Dange and Masurekar, 1982), which is believed to be caused by reduced tissue oxygen due to gill damage (McLeay and Brown, 1974). The concentration of lactic acid in soft tissues of the clam has been studied to only a few trace metals. Accumulation of lactic acid in the bivalve *V. cyprionoides* var *cochinensis* and *Perna viridis* at the whole tissue level was reported (Lakshmanan and Nambisan, 1985 and Sathynathan *et al.*, 1988). Katticaran and Salih (1992) studied the effect of copper on the lactic acid content in the adductor muscle and digestive gland of *Sunetta scripta* and reported an

elevation in the lactic acid content after 24h of exposure of the clam. Suresh (1988) reported the formation of lactic acid during the initial days in the haemolymph lactic acid content of *V.cyprinoides* var *cochinensis* and *S.scripta* exposed to Cu, and Geetha (1992) also observed the accumulation of lactic acid content in the clam exposed to different trace metals.

Valve movement, production of excess mucus etc., by bivalves could result in a shift of aerobic to anaerobic metabolism; however, the major end products reported include succinate / alanine (de Zwaan and Zandee, 1972) and lactate / alanine during anaerobic metabolism (Zurburg and Ebberink, 1981).

In the present investigation also, large accumulation of lactic acid was observed. This may be attributed to the anaerobic metabolism induced by the stress due to H^+ and trace metals. The decrease in the filtration rate and oxygen consumption under different experimental conditions and the bioaccumulation of metals were responsible for the shift to aneorobic metabolism which inturn triggered an increase in the lactic acid content. Hence, elevation in lactic acid content may be considered to be an indicator of stress in the clam.

Lipid

Lipid contents of the clam exposed to Cu, Cd, Hg and Al at different pH were measured at 24h, 72h and 120h and the results are presented in the Table 6.3. The lipid content at 0h and at pH 7.6, 0.926 ± 0.008 mg g^{-1} wet weight was considered as the control value for purposes of comparison. The percentage deviations from the control value after 120h are depicted in the Fig. 6.3.

Total lipid contents of the clam exposed to trace metals at different pH were significantly diminished ($p < 0.01$) at all experimental conditions. But the decrement was small compared to

Table 6.3. Metal induced variations in the lipid content ($x10^{-3}$ mg g^{-1} wet wt.) of *V. cyprinoides* at varying pH. (Control value = 926±8)

pH	Metal conc.	Exposure period (h)		
		24	72	120
5.5	H^+	902±2	908±2	900±1
	Al-1	910±7	906±3	898±4
	Al-2	908±2	904±8	897±5
	Cu-1	868±1	858±2	842±1
	Cu-1+Al-1	860±6	849±5	840±3
	Cu-1+Al-2	854±7	850±8	830±9
	Cu-2	848±2	840±3	832±4
	Cu-2+Al-1	841±1	832±5	828±6
	Cu-2+Al-2	838±3	829±7	816±4
	Cd-1	878±4	870±7	848±1
	Cd-1+Al-1	870±3	866±8	844±2
	Cd-1+Al-2	868±2	858±4	840±3
	Cd-2	864±1	860±3	851±4
	Cd-2+Al-1	858±3	854±2	842±2
	Cd-2+Al-2	844±4	840±1	831±3
	Hg-1	868±3	848±3	831±1
	Hg-1+Al-1	860±4	839±2	830±3
	Hg-1+Al-2	854±5	831±3	824±8
	Hg-2	831±2	820±4	810±7
	Hg-2+Al-1	828±2	812±2	800±4
	Hg-2+Al-2	818±5	800±5	798±3
6.0	H^+	919±4	910±5	908±2
	Al-1	914±4	908±2	900±5
	Al-2	912±3	907±4	898±2
	Cu-1	878±3	862±1	858±9
	Cu-1+Al-1	870±2	861±8	850±2
	Cu-1+Al-2	868±3	852±4	844±3
	Cu-2	862±4	841±3	838±3
	Cu-2+Al-1	848±2	834±1	832±4
	Cu-2+Al-2	840±5	830±2	828±1
	Cd-1	900±2	886±2	878±3
	Cd-1+Al-1	892±8	884±3	870±4
	Cd-1+Al-2	890±4	880±4	866±2
	Cd-2	878±3	864±8	858±8
	Cd-2+Al-1	870±2	858±6	844±4
	Cd-2+Al-2	864±1	851±7	840±2

(Contd...)

Table 6.3. (Contd...)

	Hg-1	870±1	868±3	840±8
	Hg-1+Al-1	868±4	860±2	851±4
	Hg-1+Al-2	861±2	848±3	840±1
	Hg-2	840±3	831±4	818±1
	Hg-2+Al-1	828±4	808±2	806±2
	Hg-2+Al-2	818±5	804±3	802±1
6.5	H^+	918±3	902±2	910±4
	Al-1	916±3	912±4	905±2
	Al-2	912±1	908±4	900±3
	Cu-1	900±6	888±5	870±2
	Cu-1+Al-1	894±7	880±4	868±3
	Cu-1+Al-2	892±2	876±4	860±4
	Cu-2	877±9	860±3	848±1
	Cu-2+Al-1	874±8	861±2	840±3
	Cu-2+Al-2	870±1	868±1	838±7
	Cd-1	908±4	900±1	890±2
	Cd-1+Al-1	901±2	896±3	870±3
	Cd-1+Al-2	900±7	880±4	881±1
	Cd-2	900±8	878±2	862±4
	Cd-2+Al-1	897±9	876±3	858±2
	Cd-2+Al-2	890±8	872±1	854±1
	Hg-1	878±3	870±5	848±3
	Hg-1+Al-1	871±4	868±2	840±1
	Hg-1+Al-2	870±6	860±2	838±8
	Hg-2	848±3	832±4	812±2
	Hg-2+Al-1	840±4	830±2	824±2
	Hg-2+Al-2	838±5	829±6	820±7
7.6	H^+	920±3	918±4	920±2
	Al-1	918±1	914±3	910±2
	Al-2	916±2	912±4	909±5
	Cu-1	908±4	892±3	876±2
	Cu-1+Al-1	906±5	890±4	874±5
	Cu-1+Al-2	904±6	888±1	870±6
	Cu-2	900±1	876±3	868±7
	Cu-2+Al-1	896±2	868±2	853±8
	Cu-2+Al-2	890±1	860±4	850±9

(Contd...)

Table 6.3. (Contd...)

	Cd-1	912±2	910±2	908±3
	Cd-1+Al-1	909±4	908±3	906±1
	Cd-1+Al-2	907±2	908±2	905±1
	Cd-2	908±1	900±4	898±4
	Cd-2+Al-1	906±4	900±5	894±2
	Cd-2+Al-2	904±5	898±6	890±2
	Hg-1	898±8	890±1	870±3
	Hg-1+Al-1	892±2	879±2	861±1
	Hg-1+Al-2	878±3	868±3	860±1
	Hg-2	878±4	864±4	840±2
	Hg-2+Al-1	870±5	862±2	834±1
	Hg-2+Al-2	870±1	860±1	830±4
9.0	H^+	917±4	912±3	909±1
	Al-1	908±1	909±2	900±2
	Al-2	900±2	898±3	902±3
	Cu-1	900±2	898±2	870±9
	Cu-1+Al-1	889±1	890±1	868±8
	Cu-1+Al-2	880±3	878±3	860±4
	Cu-2	870±4	860±4	842±2
	Cu-2+Al-1	858±5	841±8	838±5
	Cu-2+Al-2	850±6	838±7	830±3
	Cd-1	908±2	906±2	908±3
	Cd-1+Al-1	900±4	898±5	872±1
	Cd-1+Al-2	898±7	878±2	870±1
	Cd-2	890±4	879±1	872±4
	Cd-2+Al-1	876±3	870±8	864±2
	Cd-2+Al-2	870±2	868±2	852±2
	Hg-1	880±4	872±5	848±3
	Hg-1+Al-1	878±3	870±2	840±4
	Hg-1+Al-2	870±2	864±3	838±2
	Hg-2	848±1	840±2	821±1
	Hg-2+Al-1	840±3	838±7	836±8
	Hg-2+Al-2	834±4	828±2	810±9

(Value = $\bar{x}$ ± SD. n = 9)

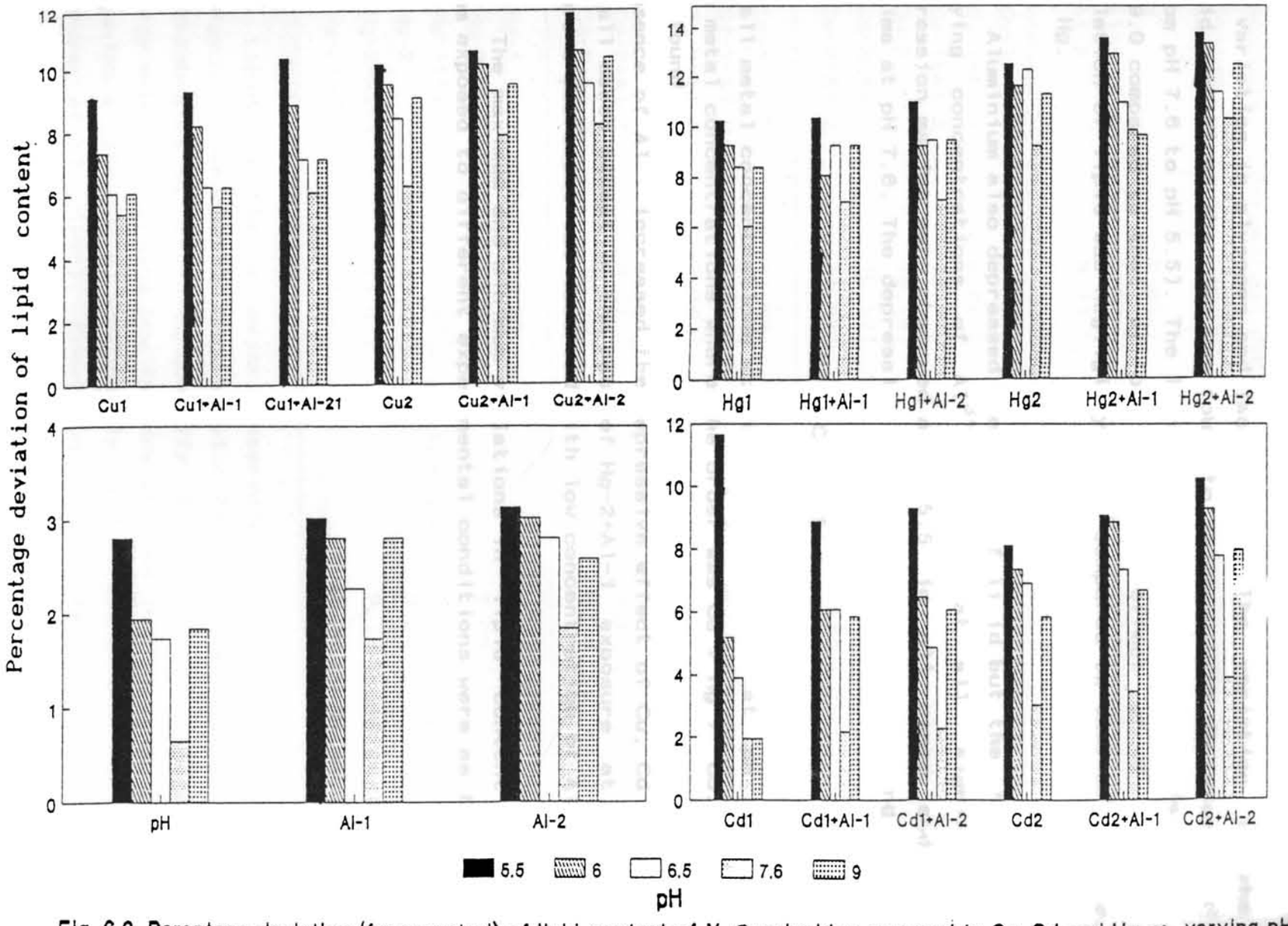

Fig. 6.3. Percntage deviation (from control) of lipid content of V. Cyprinoides exposed to Cu, Cd and Hg at varying pH

the variation in glycogen and lactic acid. The variation in the lipid content of the clam was found to increase with decrease in pH (from pH 7.6 to pH 5.5). The lipid content was also suppressed at pH 9.0 compared to that at pH 7.6. The effect of H^+ on the depletion of lipid was negligibly small compared to that of Cu, Cd and Hg.

Aluminium also depressed the level of lipid but the effect of varying concentrations of Al^{3+} was not at all significant. Depression maxima were observed at pH 5.5 in all cases and the minima at pH 7.6. The depressive effect was in the following order:

$$Hg > Cu > Cd$$

at all metal concentrations at different pH except at pH 5.5 for low metal concentrations where the order was Cd > Hg > Cu. This was found to be in agreement with the toxicity of these metals. Presence of Al^{3+} increased the depressive effect of Cu, Cd and Hg in all cases except in the case of Hg-2+Al-1 exposure at pH 9.0 where a decrement was observed with low concentration of Al.

The maximum and minimum variations in lipid content of the clam exposed to different experimental conditions were as follows:

Exposure Medium	Percentage depletion in lipid content after 120h			
	Maximum		Minimum	
H^+	2.81	at pH 5.5	0.65	at pH 7.6
Al-1	3.02		1.73	
Al-2	3.13		1.84	
Cu-1	9.07	at pH 5.5	5.40	at pH 7.6
Cu-1+Al-1	9.29		5.62	
Cu-1+Al-2	10.37		6.05	
Cu-2	10.15		6.26	
Cu-2+Al-1	10.58		7.88	
Cu-2+Al-2	11.88		8.21	
Cd-1	11.66	at pH 5.5	1.94	at pH 7.6
Cd-1+Al-1	8.86		2.16	
Cd-1+Al-2	9.29		2.27	
Cd-2	8.10		3.02	
Cd-2+Al-1	9.07		3.46	
Cd-2+Al-2	10.26		3.89	
Hg-1	10.26	at pH 5.5	6.05	at pH 7.6
Hg-1+Al-1	10.37		7.02	
Hg-1+Al-2	11.02		7.13	
Hg-2	12.53		9.29	
Hg-2+Al-1	13.61		9.72	at pH 9.0
Hg-2+Al-2	13.82		10.37	at pH 7.6

Lipids can be a major reservoir of biochemically stored energy. Measurement of total lipid content in aquatic invertebrates is needed to quantify this reservoir of potential energy and to determine how factors such as interspecies variation, organism's life cycle, food supply and stress affect lipid content (Gardner *et al.*, 1985). Knowledge of lipid levels in invertebrates is also needed to understand the transfer and partitioning of lipid soluble contaminants in aquatic systems (Kenaga and Goring, 1980).

The importance of lipids in energy metabolism of adult bivalves has already been reported for some species (e.g., *Tapes sp*) (Beninger, 1984), *Pecten jacobeus*, *Petinopelken Yessoensis* and *Macoma balthica* (Wenne and Jurewicz, 1987).

An increase in lipid content during gametogenesis upto a maximum before spawning and a decrease down to a minimum after spawning are typical for those species such as *Mytilus edulis* in which lipids are stored mainly in the gonads (Zandee *et al.*, 1980).

Impairment of lipid metabolism with exposure to lypophilic contaminant has been noted by several investigators (Kato *et al.*, 1982; Capuzzo *et al.*, 1984).

Empirical data on the effect of lipophilic contaminants suggested that linkages clearly exist between 1) energetic, developmental and reproductive abnormalities 2) the physiological and molecular processes involved in uptake 3) the toxicity and /or transformation of lipophilic contaminants (Leavitt *et al.*, 1990).

The composition of lipid fraction of molluscs may be affected by external factors such as fluctuation in the environmental conditions or by internal factors such as sexual maturation. No significant differences were observed in lipid content of gill microsomes between control and experimental bivalve during Cd accumulation (Phelomin and Belcheva, 1991). Cd exerted a direct inhibiting effect in intermediate transfer of lipid in *Mizuhopecten yessoensis* (Chelomin, 1990).

Alterations in the lipid and associated fatty acid composition of organisms exposed to a variety of stressors have been studied extensively. Reasons for observed changes are not always apparent and a variety of mechanisms exists by which the lipid composition of an organism can be affected by toxicant induced stress.

Lipid provides an essentially and readily available energy source for a large number of aquatic invertebrates (Voogt, 1983). Their importance as a primary /secondary energy source varied with species and with seasons. The observed variation in the lipid content of the clam can be explained based on the shift in mobilization and utilization of different energy substrates. The energy pool which may be mobilised for maintaining haemostatis during toxicant induced stress may vary depending on the bioavailability of metals and physiological changes like variation in the filtration rate and metabolic rates.

The greater energy demand caused by the stress of toxicant exposure can result in a mobilization of lipid energy reserves and in a consequent decline of the total lipid content. The depletion in lipid content observed in the present study was similar to the depletion in lipid content noticed in amphipods exposed to fuel oil (Lee *et al.*, 1981), in American lobster exposed to petroleum hydrocarbon (Capuzzo *et al.*, 1984), in *Crrhinus mrigala* exposed to carbamate insecticide carbaryl (Rao *et al.*, 1984) and chlorinated hydrocarbons (Gluth and Hanke, 1984). Alteration in the cholesterol level may be an indirect result of toxicant effects on enzymes and metabolism system. O'Hara *et al.* (1985) have shown that polynuclear aromatic hydrocarbons can interfere with the cyto chrome P-450 enzyme system, such that cholesterol metabolism is impaired. A similar interference of Cu in the enzyme mechanism may be responsible for the depressive effect observed in the lipid content of the clam exposed to Cu.

The lipid content of the estuarine clam under investigation was typical of their environments. Although changes in the whole body lipid content may be of only peripheral relevance in field investigation which are subject to the interplay of a host of extraneous factors, it is significantly relevant in controlled exposure studies.

Protein

Soft tissue protein contents in the clam exposed to Cu, Cd, Hg and Al at different pH under different experimental conditions were measured at 0h, 24h, 72h and 120h. The values are given in the Table 6.4. The tissue protein content of the clam at 0h and at pH 7.6 was 3.72 ± 0.02 mg g^{-1} wet weight. This was used as the control for comparison. The percentage variations in the protein levels after 120h compared to the control as depicted in the Fig. 6.4 were used for inter comparison.

The protein contents were found to be significantly ($p < 0.01$) lowered under all conditions. The maximum protein value (after 120h) 3.60 ± 0.019 mg g^{-1} wet weight was observed at pH 7.6 and pH 9.0 in absence of trace metals and the minimum value 2.80 ± 0.031 mg g^{-1} was recorded during the exposure of the clam to Cu-2+Al-2 at pH 5.5 after 120h.

The protein content was found to be decreased with increase in H^+ content. The minimum variation was observed at pH 9.0 i.e., 3.23% and the maximum at pH 5.5 i.e., 11.59%. The presence of Al^{3+} further lowered the protein content of the animal and the variation was enhanced with increase in the concentration of Al^{3+}. The interactive effect of Al^{3+} with H^+ was found to be very little.

The presence of Cu, Cd and Hg also diminished the protein content of the bivalve. The minimum variation was observed at pH 7.6 in all cases. In the case of Cu, the maximum variation was observed at pH 5.5 under different experimental conditions. The percentage depletion of protein content varied between 9.14 at pH 7.6 during Cu-1 exposure and 24.73 at 5.5 during Cu-2+Al-2 exposure.

In the presence of Cd, the maximum variations were found at pH 6.0 during the exposures to Cd-2, Cd-2+Al-1 and Cd-2+Al-2 and in all other cases the maximum depletion in the protein content was

Table 6.4. Metal induced variations in the protein content (mg g^{-1} wet wt.) of *V. cyprinoides* at varying pH. (Control value = 3.72±0.02)

pH	Metal conc.	Exposure period (h)		
		24	72	120
5.5	H^+	3.58±0.09	3.42±0.03	3.29±0.10
	Al-1	3.50±0.04	3.41±0.02	3.28±0.07
	Al-2	3.48±0.03	3.40±0.01	3.20±0.02
	Cu-1	3.40±0.02	3.31±0.01	3.01±0.02
	Cu-1+Al-1	3.38±0.07	3.30±0.01	3.00±0.04
	Cu-1+Al-2	3.36±0.04	3.28±0.01	2.98±0.03
	Cu-2	3.18±0.11	3.02±0.02	2.90±0.02
	Cu-2+Al-1	3.11±0.09	3.00±0.01	2.86±0.01
	Cu-2+Al-2	3.08±0.02	2.98±0.01	2.80±0.01
	Cd-1	3.44±0.02	3.44±0.02	3.28±0.02
	Cd-1+Al-1	3.40±0.04	3.36±0.01	3.20±0.01
	Cd-1+Al-2	3.35±0.02	3.30±0.03	3.18±0.03
	Cd-2	3.30±0.03	3.28±0.01	3.19±0.04
	Cd-2+Al-1	3.25±0.02	3.20±0.04	3.10±0.02
	Cd-2+Al-2	3.21±0.04	3.19±0.02	3.04±0.01
	Hg-1	3.18±0.02	3.10±0.05	3.00±0.02
	Hg-1+Al-1	3.10±0.03	3.06±0.05	2.98±0.05
	Hg-1+Al-2	3.08±0.01	3.00±0.04	2.96±0.06
	Hg-2	3.10±0.08	3.00±0.07	2.94±0.02
	Hg-2+Al-1	3.08±0.04	2.94±0.02	2.90±0.02
	Hg-2+Al-2	3.00±0.02	2.92±0.03	2.86±0.01
6.0	H^+	3.60±0.02	3.50±0.03	3.40±0.04
	Al-1	3.61±0.03	3.46±0.02	3.41±0.04
	Al-2	3.55±0.04	3.51±0.03	3.36±0.05
	Cu-1	3.48±0.01	3.40±0.02	3.10±0.01
	Cu-1+Al-1	3.46±0.03	3.40±0.01	3.08±0.02
	Cu-1+Al-2	3.42±0.04	3.38±0.02	3.00±0.03
	Cu-2	3.30±0.02	3.18±0.05	3.02±0.02
	Cu-2+Al-1	3.28±0.01	3.16±0.07	3.01±0.04
	Cu-2+Al-2	3.20±0.01	3.08±0.01	3.00±0.03
	Cd-1	3.50±0.05	3.48±0.09	3.30±0.07
	Cd-1+Al-1	3.48±0.07	3.44±0.02	3.21±0.02
	Cd-1+Al-2	3.44±0.02	3.42±0.01	3.18±0.08
	Cd-2	3.28±0.03	3.20±0.04	3.16±0.02
	Cd-2+Al-1	3.18±0.08	3.08±0.03	3.04±0.03
	Cd-2+Al-2	3.16±0.02	3.02±0.05	3.01±0.04

(Contd...)

Table 6.4. (Contd...)

	Hg-1	3.21±0.03	3.18±0.05	3.10±0.04
	Hg-1+Al-1	3.18±0.02	3.09±0.05	3.07±0.03
	Hg-1+Al-2	3.1[illegible]±0.01	3.04±0.04	2.98±0.02
	Hg-2	3.14±0.01	3.08±0.07	3.00±0.[illegible]4
	Hg-2+Al-1	3.10±0.03	3.06±0.02	2.98±0.05
	Hg-2+Al-2	3.08±0.04	3.00±0.03	2.84±0.06
6.5	H^+	3.68±0.05	3.51±0.01	3.48±0.02
	Al-1	3.60±0.04	3.47±0.03	3.40±0.09
	Al-2	3.55±0.02	3.49±0.08	3.38±0.07
	Cu-1	3.50±0.06	3.42±0.04	3.28±0.03
	Cu-1+Al-1	3.46±0.02	3.40±0.02	3.24±0.04
	Cu-1+Al-2	3.41±0.01	3.36±0.03	3.18±0.05
	Cu-2	3.38±0.07	3.30±0.08	3.09±0.01
	Cu-2+Al-1	3.30±0.08	3.28±0.04	3.08±0.01
	Cu-2+Al-2	3.28±0.01	3.20±0.06	3.06±0.02
	Cd-1	3.58±0.06	3.52±0.08	3.38±0.08
	Cd-1+Al-1	3.50±0.07	3.48±0.05	3.30±0.06
	Cd-1+Al-2	3.48±0.02	3.45±0.06	3.28±0.01
	Cd-2	3.41±0.03	3.40±0.07	3.21±0.03
	Cd-2+Al-1	3.40±0.01	3.38±0.04	3.10±0.04
	Cd-2+Al-2	3.38±0.02	3.26±0.05	3.09±0.02
	Hg-1	3.38±0.01	3.30±0.01	3.21±0.02
	Hg-1+Al-1	3.34±0.03	3.28±0.04	3.18±0.02
	Hg-1+Al-2	3.30±0.01	3.18±0.05	3.09±0.06
	Hg-2	3.30±0.02	3.26±0.07	3.00±0.08
	Hg-2+Al-1	3.18±0.03	3.10±0.04	2.98±0.02
	Hg-2+Al-2	3.10±0.04	3.02±0.05	2.94±0.02
7.6	H^+	3.70±0.01	3.68±0.03	3.60±0.04
	Al-1	3.68±0.01	3.64±0.03	3.58±0.02
	Al-2	3.68±0.03	3.62±0.03	3.57±0.02
	Cu-1	3.58±0.01	3.50±0.01	3.38±0.02
	Cu-1+Al-1	3.50±0.02	3.48±0.05	3.30±0.03
	Cu-1+Al-2	3.48±0.03	3.40±0.02	3.26±0.01
	Cu-2	3.50±0.03	3.38±0.01	3.21±0.02
	Cu-2+Al-1	3.48±0.04	3.30±0.07	3.18±0.01
	Cu-2+Al-2	3.40±0.05	3.28±0.01	3.12±0.04

(Contd...)

Table 6.4. (Contd...)

	Cd-1	3.62±0.03	3.60±0.04	3.48±0.05
	Cd-1+Al-1	3.60±0.02	3.56±0.03	3.44±0.04
	Cd-1+Al-2	3.55±0.08	3.51±0.03	3.40±0.03
	Cd-2	3.58±0.09	3.50±0.07	3.48±0.02
	Cd-2+Al-1	3.56±0.06	3.48±0.08	3.40±0.01
	Cd-2+Al-2	3.50±0.07	3.42±0.02	3.36±0.08
	Hg-1	3.50±0.03	3.42±0.03	3.28±0.02
	Hg-1+Al-1	3.44±0.04	3.40±0.04	3.20±0.03
	Hg-1+Al-2	3.42±0.02	3.34±0.02	3.18±0.03
	Hg-2	3.38±0.03	3.30±0.03	3.24±0.01
	Hg-2+Al-1	3.36±0.05	3.27±0.05	3.20±0.09
	Hg-2+Al-2	3.30±0.08	3.18±0.08	3.15±0.07
9.0	H^+	3.71±0.04	3.64±0.03	3.60±0.02
	Al-1	3.66±0.01	3.63±0.02	3.56±0.01
	Al-2	3.60±0.03	3.58±0.04	3.54±0.02
	Cu-1	3.51±0.05	3.40±0.02	3.26±0.03
	Cu-1+Al-1	3.50±0.01	3.38±0.01	3.20±0.04
	Cu-1+Al-2	3.46±0.01	3.30±0.01	3.10±0.05
	Cu-2	3.48±0.02	3.32±0.02	3.20±0.02
	Cu-2+Al-1	3.46±0.04	3.30±0.03	3.16±0.01
	Cu-2+Al-2	3.40±0.07	3.28±0.04	3.10±0.01
	Cd-1	3.60±0.07	3.48±0.03	3.40±0.03
	Cd-1+Al-1	3.55±0.02	3.40±0.04	3.38±0.04
	Cd-1+Al-2	3.51±0.02	3.34±0.05	3.30±0.05
	Cd-2	3.54±0.01	3.40±0.01	3.32±0.02
	Cd-2+Al-1	3.50±0.04	3.36±0.02	3.28±0.01
	Cd-2+Al-2	3.48±0.03	3.30±0.03	3.21±0.08
	Hg-1	3.48±0.01	3.40±0.04	3.21±0.04
	Hg-1+Al-1	3.40±0.03	3.36±0.02	3.18±0.05
	Hg-1+Al-2	3.34±0.06	3.30±0.07	3.16±0.08
	Hg-2	3.30±0.02	3.28±0.08	3.20±0.03
	Hg-2+Al-1	3.28±0.03	3.26±0.04	3.15±0.07
	Hg-2+Al-2	3.24±0.07	3.20±0.05	3.07±0.08

(Value = $\bar{x}$ ± SD, n = 9)

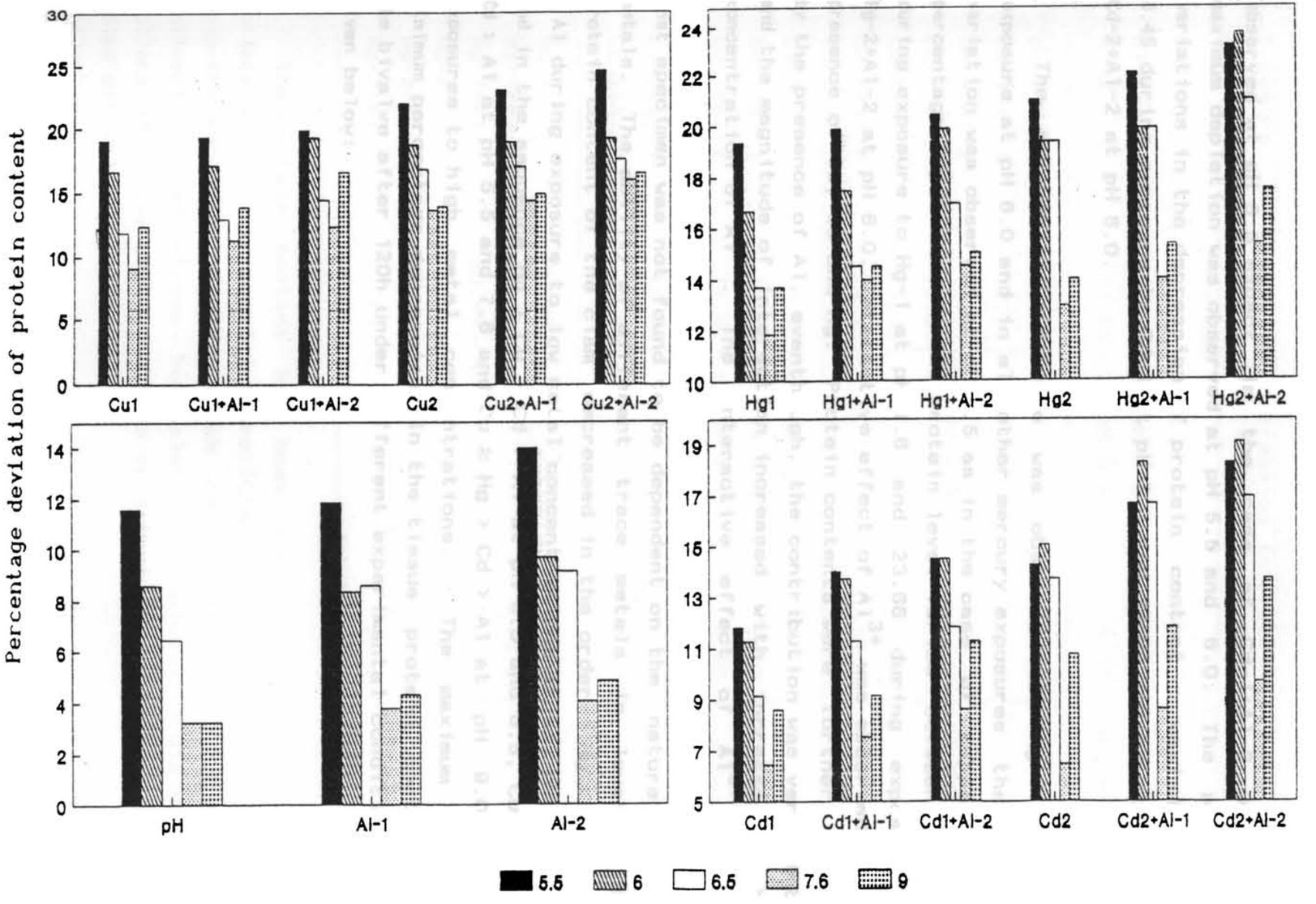

Fig. 6.4. Percentage deviation (from control) of protein content of V.cyprinoides exposed to Cu, Cd, Hg and Al at varying pH.

observed at pH 5.5 expect in the case of Cd-1+Al-2 where the maximum depletion was observed at pH 5.5 and 6.0. The percentage variations in the depression of protein content varied between 6.45 during exposure to Cd-1 at pH 7.6 and 19.09 during exposure to Cd-2+Al-2 at pH 6.0.

The maximum interference was observed during Hg-2+Al-2 exposure at pH 6.0 and in all other mercury exposures the maximum variation was observed at pH 5.5 as in the case of copper. The percentage depression in the protein level varied between 11.83 during exposure to Hg-1 at pH 7.6 and 23.66 during exposure to Hg-2+Al-2 at pH 6.0. Interactive effect of Al^{3+} was observed in the presence of Cu, Cd and Hg. Protein contents were further lowered by the presence of Al, eventhough, the contribution was very little and the magnitude of interaction increased with increase in the concentration of Al^{3+}. The interactive effect of Al^{3+} in the presence of trace metals in lowering the protein content of the test specimen was not found to be dependent on the nature of the metals. The ability of different trace metals in lowering the protein content of the clam decreased in the order Hg > Cu > Cd > Al during exposure to low metal concentrations at all pH employed and in the sequence Hg > Cu > Cd > Al at pH 6.0 and 6.5, Cu > Hg > Cd > Al at pH 5.5 and 7.6 and Cu $\approx$ Hg > Cd > Al at pH 9.0 during exposures to high metal concentrations. The maximum and the minimum percentage depressions in the tissue protein contents of the bivalve after 120h under different experimental conditions are given below:

Exposure Medium	Percentage depression in protein content after 120h	
	Maximum	Minimum
Al-1	11.83 – at pH 5.5	3.76 – at pH 7.6
Al-2	13.98 – at pH 5.5	4.03 – at pH 7.6
Cu-1	19.09	9.14
Cu-1+Al-1	19.35	11.29
Cu-1+Al-2	19.89 – at pH 5.5	12.36 – at pH 7.6
Cu-2	22.04	13.70
Cu-2+Al-1	23.12	14.52
Cu-2+Al-2	24.73	16.13
Cd-1	11.83 – at pH 5.5	6.45
Cd-1+Al-1	13.98 – at pH 5.5	7.53
Cd-1+Al-2	14.52 – at pH 5.5 & 6.0	8.60 – at pH 7.6
Cd-2	15.05 – at pH 6.0	6.45
Cd-2+Al-1	18.28	8.60
Cd-2+Al-2	19.09	9.67
Hg-1	19.35	11.83
Hg-1+Al-1	19.89	13.97
Hg-1+Al-2	20.43 – at pH 5.5	14.52 – at pH 7.6
Hg-2	20.97	12.90
Hg-2+Al-1	22.04	13.98
Hg-2+Al-2	23.66 – at pH 6.0	15.32

The protein content has been shown to be influenced by variety of environmental factors (Claybrook, 1983). It is not only the structural protein which can potentially change during toxicant-induced stress but also the soluble protein. The rationale for changes in soluble or structural proteins are often quite different.

The majority of the changes in structural protein which have been reported can be directly related to the oxidation of aminoacids for energy. During starvation of invertebrates both heamolimph and structural proteins are utilised as energy sources (Claybrook, 1983) and have been mobilised under stressful conditions (Bayne, 1973). Glycogen and/or lipids are generally utilised first; however, depending upon the season, reproductive status of the organism and length of stressful conditions proteins can also become an important energy source (Heath and Barnes, 1970). The significant depletion observed in the present investigation can be attributed to the utilisation of energy to compact the stress under different experimental conditions.

Depletion in protein content by the stressors was previously reported *C.carpio* to temperature (Gluth and Hanke, 1984). During toxicant induced stress, protein increased in oysters exposed to naphthalene (Riley and Mix, 1981) and in freshwater crabs exposed to sumithion (Bhagyalakshmi *et al.*, 1983). Increased protenolysis to meet the energy demand of stress was assumed to cause the decline in structural proteins. Since protein content is generally less variable than either lipids or glycogen, separation of its toxic effect from background 'noise may be easier, although the secondary nature of the protein metabolism for most organisms makes the sensitivity of the measurement questionable. Under laboratory conditions, however, McKee and Knowles (1985) have found protein to be a sensitive indicator for *Daphnia magna* growth and felt that total protein content measured during acute exposures is an effective predictor of chronic survival, growth and reproductive potential.

Tissue protein content has been suggested as an indicator of toxicant induced stress. Total serum protein (TSP) of fish exposed to the least copper concentration had returned to normal, while that of *Salvelinus fontinalis* exposed to 67.5 μg Cu l^{-1} was significantly less than that of controls (Mackim *et al.*, 1970). When brook trout alevins were exposed to methyl mercury, Cd^{2+} and Pb^{2+}, small concentration of methyl mercury caused a decrease in TSP

while a similar concentration of Cd caused an increase in TSP. Higher concentration of Cd had no effect on TSP. Accumulation of Cu and Cd during the reproductive cycle of *Mytilus edulis* was related to the protein synthesis (Coimbra and Carraca, 1990). Protein associated with hemolymph can have abide variety of function such as enzymatic, transport or hormonal and can therefore change for a multitude of reasons. Osmotic stress caused by changes in salinity or toxicant exposure can result in changes in hemolymph protein concentrations (Boone and Schoffeniels, 1979). Oxygen stress can induce the production of transport pigments such as hemoglobin in certain species of crustaceans. However, a decrease in hemoglobin concentration has also been reported for Daphnia exposed to naphthalene (Crider *et al.*, 1982).

Low pH has been shown to result in high protein level in rainbow trout, *Salmo gairdneri* (Brown *et al.*, 1984) possibly due to the nonavailability of the nutrient for normal growth. In the present study, maximum depletion was observed at the low pH of 5.5. This clearly indicated the utility of protein as an energy source even at low pH.

Enzymes, aspartate aminotransterase and glucose 6-phosphate dehydrogenase are important in protein and carbohydrate metabolism respectively (Page *et al.*, 1984). Interference of copper and cadmium will affect the specific activity of the above enzymes and hence the depletion in the protein contents during exposures of bivalves to Cu and Cd may also be due to their interference with the enzymes. The negligibly small magnitude of variation in protein content observed in the case of exposure of the animal to Al^{3+} may be due to the absence of such interference with the enzyme.

Viarengo *et al.* (1982) developed a sublethal stress indicator based on protein synthesis in gill and digestive gland tissue of the marine mussel, *Mytilus galloprovincialis*. Under controlled laboratory conditions, they were able to correlate copper body burden with reduced protein synthesis.

In the present study also the significant depletion in protein content of the clam was found to be related to the accumulation of metals and their toxicity. The order of toxicity of trace metals employed was in good agreement with the order of ability to reduce protein content at low metal concentrations. The changes observed under other conditions may be attributed to the altered biochemical mechanisms.

The observed results suggest that the response of protein to toxicants is not very complex and that the direction of response is dependent upon the duration of exposure as well as concentration of toxicant. Therefore protein appears to be a viable biochemical indicator for controlled exposure studies.

Ascorbic acid

Ascorbic acid is a ubiquitous compound that has been implicated in a wide variety of biological functions. Although, studies involving ascorbic acid have been fairly exhaustive, the role of ascorbic acid in invertebrates remains relatively unexplored. The majority of studies concerning ascorbic acid requirements in aquatic invertebrates has been conducted with penaeid shrimp (Carr and Neff, 1982). In several species of penaeid shrimp, starvation and ascorbic acid deficient dieting have been observed to result in a disease of the connective tissue known as 'black death' which is characterised by lesions composed of masses of hemocytes.

The ascorbic acid contents in the soft tissues of the test specimen exposed to different trace metal concentrations at different pH after 24h, 72h and 120h are given in the Table 6.5. The ascorbic acid content of the clam at pH 7.6 and 0h was 72.18 $\pm$ 1.12 $\mu g\ g^{-1}$ wet weight and this was considered to be the control value for inter comparison. The percentage variations after 120h from the control are depicted in the Fig. 6.5 for comparison. ANOVA was used for predicting the significant change. The ascorbic acid

Table 6.5. Metal induced variations in the ascorbic acid content ($\mu g\ g^{-1}$ wet wt.) of *V. cyprinoides* at varying pH.

(Control value = 72.18±1.12)

pH	Metal conc.	Exposure period (h)		
		24	72	120
5.5	H^+	66.78±0.02	66.12±0.01	64.12±0.02
	Al-1	65.44±0.02	64.12±0.01	64.74±0.03
	Al-2	65.12±0.03	64.92±0.01	64.86±0.07
	Cu-1	67.12±0.14	65.18±0.02	66.17±0.01
	Cu-1+Al-1	67.08±0.02	66.14±0.01	65.98±0.02
	Cu-1+Al-2	68.12±0.03	66.02±0.03	65.14±0.01
	Cu-2	67.14±0.04	66.76±0.02	65.15±0.01
	Cu-2+Al-1	67.02±0.05	65.14±0.01	66.15±0.03
	Cu-2+Al-2	67.02±0.02	66.12±0.04	66.04±0.07
	Cd-1	68.14±0.02	68.02±0.04	66.92±0.01
	Cd-1+Al-1	67.12±0.04	67.04±0.03	66.85±0.07
	Cd-1+Al-2	68.02±0.03	64.03±0.05	62.01±0.02
	Cd-2	67.14±0.02	67.02±0.06	67.02±0.03
	Cd-2+Al-1	64.15±0.04	64.12±0.01	64.08±0.04
	Cd-2+Al-2	64.28±0.02	64.20±0.01	64.12±0.02
	Hg-1	67.42±0.03	67.18±0.03	67.02±0.02
	Hg-1+Al-1	66.12±0.04	66.14±0.01	65.92±0.04
	Hg-1+Al-2	67.18±0.05	66.12±0.01	69.14±0.03
	Hg-2	68.12±0.01	68.14±0.02	68.92±0.02
	Hg-2+Al-1	68.02±0.01	67.98±0.03	67.14±0.01
	Hg-2+Al-2	68.92±0.02	68.48±0.04	68.03±0.03
6.0	H^+	67.12±0.02	66.12±0.01	66.04±0.02
	Al-1	67.02±0.04	66.94±0.03	65.76±0.02
	Al-2	66.98±0.02	66.78±0.07	66.45±0.05
	Cu-1	66.46±0.01	66.02±0.02	64.15±0.03
	Cu-1+Al-1	67.12±0.07	67.04±0.03	66.94±0.04
	Cu-1+Al-2	66.92±0.03	66.90±0.04	66.92±0.05
	Cu-2	65.90±0.04	65.92±0.02	65.90±0.02
	Cu-2+Al-1	64.23±0.02	65.10±0.06	65.08±0.01
	Cu-2+Al-2	64.12±0.03	64.62±0.07	64.48±0.01
	Cd-1	67.98±0.01	68.14±0.02	68.02±0.01
	Cd-1+Al-1	66.12±0.02	66.04±0.03	65.92±0.01
	Cd-1+Al-2	66.08±0.01	60.03±0.04	65.89±0.02
	Cd-2	67.95±0.01	66.86±0.07	66.86±0.03
	Cd-2+Al-1	67.94±0.03	67.87±0.05	65.42±0.07
	Cd-2+Al-2	66.23±0.04	65.94±0.06	65.31±0.01

(Contd...)

Table 6.5. (Contd...)

	Hg-1	68.04±0.01	68.12±0.07	68.04±0.05
	Hg-1+Al-1	68.12±0.02	68.04±0.08	67.94±0.03
	Hg-1+Al-2	67.92±0.01	67.48±0.02	66.98±0.02
	Hg-2	67.86±0.03	67.56±0.03	67.12±0.01
	Hg-2+Al-1	67.92±0.01	67.46±0.09	67.04±0.04
	Hg-2+Al-2	67.12±0.04	67.05±0.04	67.02±0.02
6.5	H^+	69.12±0.03	67.14±0.03	68.92±0.02
	Al-1	69.07±0.02	67.08±0.01	67.02±0.04
	Al-2	68.95±0.03	68.94±0.02	68.42±0.02
	Cu-1	68.04±0.02	66.12±0.05	64.14±0.02
	Cu-1+Al-1	67.98±0.04	66.94±0.02	66.78±0.01
	Cu-1+Al-2	68.02±0.02	68.02±0.02	67.92±0.02
	Cu-2	67.18±0.01	66.92±0.01	66.98±0.01
	Cu-2+Al-1	66.98±0.01	66.48±0.04	66.40±0.04
	Cu-2+Al-2	67.12±0.07	66.24±0.03	66.09±0.01
	Cd-1	68.12±0.03	67.12±0.04	64.13±0.04
	Cd-1+Al-1	67.18±0.04	68.12±0.03	67.02±0.07
	Cd-1+Al-2	67.08±0.02	67.04±0.02	66.92±0.08
	Cd-2	67.14±0.03	66.94±0.01	66.86±0.09
	Cd-2+Al-1	64.12±0.01	65.13±0.01	65.14±0.02
	Cd-2+Al-2	65.18±0.02	66.12±0.02	66.04±0.03
	Hg-1	69.14±0.07	68.12±0.04	68.03±0.01
	Hg-1+Al-1	68.92±0.02	68.88±0.05	68.02±0.04
	Hg-1+Al-2	68.89±0.03	68.72±0.02	68.63±0.02
	Hg-2	68.42±0.01	68.42±0.03	68.01±0.03
	Hg-2+Al-1	68.08±0.01	68.21±0.01	68.12±0.04
	Hg-2+Al-2	68.14±0.08	67.92±0.01	67.13±0.03
7.6	H^+	70.08±0.02	69.16±0.02	69.02±0.03
	Al-1	69.14±0.03	69.02±0.01	68.94±0.03
	Al-2	68.93±0.02	68.14±0.02	67.86±0.04
	Cu-1	69.18±0.02	68.92±0.03	68.46±0.04
	Cu-1+Al-1	68.12±0.05	68.02±0.04	67.48±0.05
	Cu-1+Al-2	68.04±0.06	68.04±0.03	68.10±0.06
	Cu-2	66.48±0 07	66.22±0.02	66.07±0.02
	Cu-2+Al-1	66.24±0.04	66.20±0.01	65.92±0.04
	Cu-2+Al-2	66.12±0.08	65.12±).04	64.18±0.01

(Contd...)

Table 6.5. (Contd...)

	Cd-1	69.12±0.04	68.84±0.05	69.04±0.04
	Cd-1+Al-1	68.95±0.02	68.12±0.06	68.07±0.03
	Cd-1+Al-2	67.98±0.09	67.18±0.07	67.02±0.01
	Cd-2	68.94±0.03	67.99±0.08	66.92±0.01
	Cd-2+Al-1	65.98±0.07	66.94±0.07	64.94±0.02
	Cd-2+Al-2	67.12±0.02	67.02±0.05	67.01±0.02
	Hg-1	68.98±0.01	67.94±0.03	67.95±0.05
	Hg-1+Al-1	68.92±0.02	68.88±0.04	68.76±0.06
	Hg-1+Al-2	68.87±0.07	68.73±0.08	68.69±0.02
	Hg-2	67.12±0.01	68.12±0.01	64.13±0.02
	Hg-2+Al-1	67.18±0.03	67.12±0.04	67.08±0.02
	Hg-2+Al-2	67.12±0.02	67.08±0.05	67.04±0.01
9.0	H^+	69.86±0.03	68.12±0.01	64.13±0.01
	Al-1	68.12±0.02	68.04±0.03	67.92±0.03
	Al-2	66.14±0.04	66.12±0.07	66.05±0.02
	Cu-1	68.12±0.02	67.14±0.02	67.02±0.02
	Cu-1+Al-1	66.08±0.04	66.05±0.03	66.02±0.03
	Cu-1+Al-2	65.02±0.01	64.02±0.04	64.04±0.05
	Cu-2	65.08±0.02	65.02±0.05	64.92±0.04
	Cu-2+Al-1	66.02±0.01	65.92±0.06	65.12±0.06
	Cu-2+Al-2	66.12±0.03	64.32±0.05	65.18±0.07
	Cd-1	69.43±0.01	68.52±0.04	68.39±0.04
	Cd-1+Al-1	68.92±0.02	68.86±0.05	68.74±0.03
	Cd-1+Al-2	68.89±0.01	68.78±0.02	68.68±0.02
	Cd-2	68.87±0.03	68.76±0.07	68.92±0.01
	Cd-2+Al-1	68.84±0.04	67.94±0.03	67.43±0.04
	Cd-2+Al-2	68.82±0.05	68.42±0.06	68.12±0.05
	Hg-1	68.86±0.06	68.48±0.02	67.12±0.08
	Hg-1+Al-1	68.92±0.07	67.12±0.01	66.47±0.02
	Hg-1+Al-2	68.43±0.02	67.08±0.03	66.05±0.03
	Hg-2	68.32±0.03	68.08±0.05	68.04±0.04
	Hg-2+Al-1	68.12±0.08	68.03±0.06	67.94±0.01
	Hg-2+Al-2	67.98±0.04	67.97±0.07	66.45±0.02

(Value = $\bar{x}$ ± SD, n = 9)

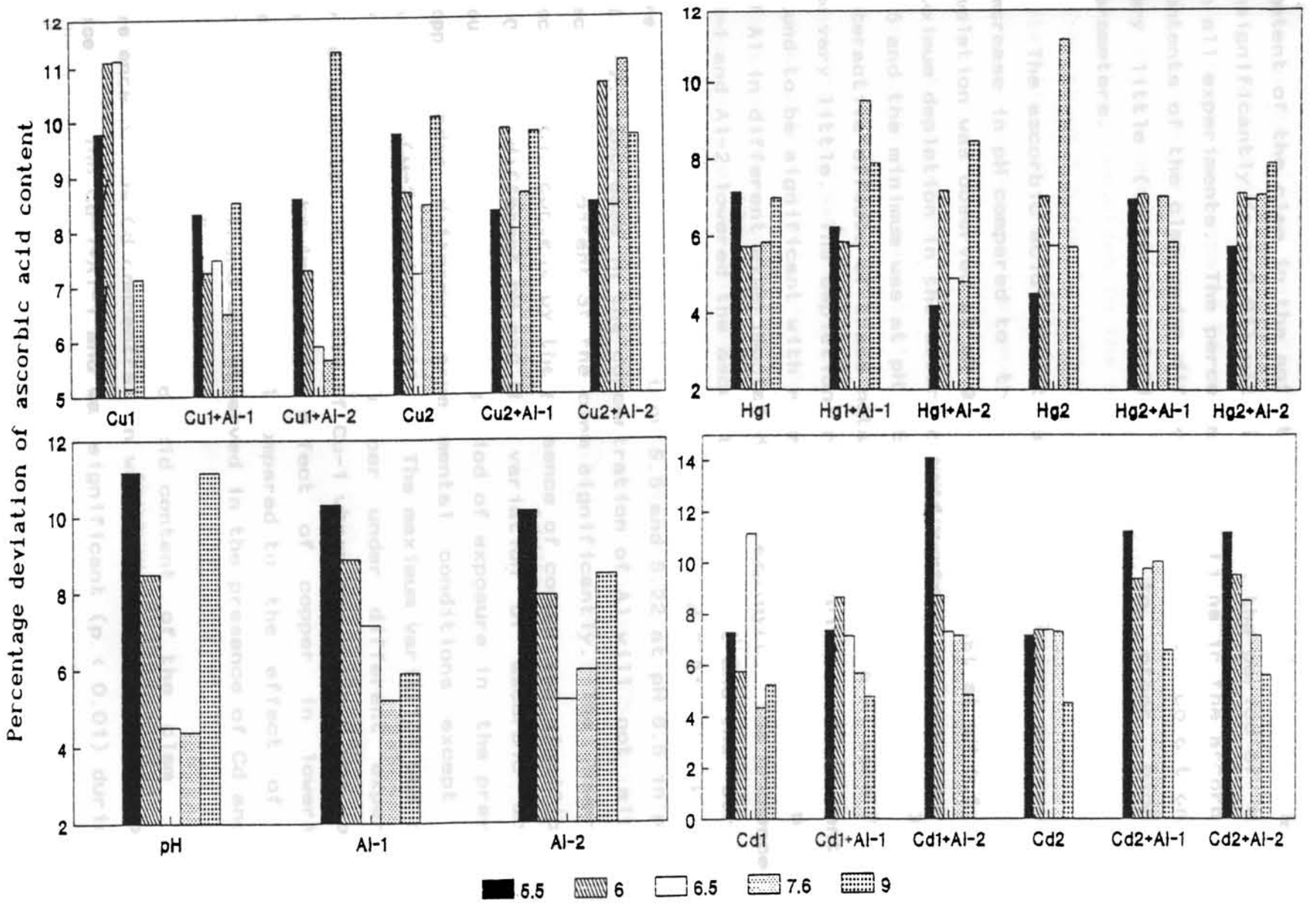

Fig. 6.5. Percentage deviation (from control) of ascorbic acid content of V.cyprinoides exposed to Cu, Cd, Hg and Al at varying pH.

content of the clam in the soft tissues was found to be depleted insignificantly ($p > 0.01$) with increase in the period of exposure in all experiments. The percentage variations in the ascorbic acid contents of the clam under different experimental conditions were very little (14.09 to 4.21) compared to other biochemical parameters.

The ascorbic acid contents of the test specimen decreased with decrease in pH compared to that at pH 7.6 and almost similar depletion was observed at pH 9.0 compared to that at pH 5.5. The maximum depletion in the ascorbic acid content was observed at pH 5.5 and the minimum was at pH 7.6 in presence of H^+ and Al^{3+}. The interactive effects of trace metals Cu, Cd, Hg and Al were found to be very little. The depletion in the ascorbic acid content was found to be significant with alteration in the pH in the presence of Al in different experimental designs employed. The presence of Al-1 and Al-2 lowered the ascorbic acid content and the percentage depression varied between 10.31 at pH 5.5 and 5.21 at pH 7.6 in presence of Al-1 and 10.14 at pH 5.5 and 5.22 at pH 6.5 in presence of Al-2. Increase in the concentration of Al will not alter the ascorbic acid content of the clam significantly. The lowering of ascorbic acid content by the presence of copper was significant ($p < 0.01$) at different pH and the variation of ascorbic acid was found to be significant with period of exposure in the presence of copper under different experimental conditions except during Cu-1+Al-2, Cu-2 and Cu-2+Al-1 . The maximum variation was observed at pH 9.0 in the presence of copper under different experimental criteria except in the case of Cu-1 where the maximum was observed at pH 6.5 but the interactive effect of copper in lowering the ascorbic acid content was nil compared to the effect of H^+ and Al^{3+}. A similar trend was observed in the presence of Cd and Hg.

The lowering of ascorbic acid content of the clam by the presence of low Cd concentration was not significant ($p > 0.01$) except during Cd-1+Al-1 and was significant ($p < 0.01$) during high

Cd concentration. The period of exposure has no significant effect ($p > 0.01$) on the variation in the ascorbic acid content of the clam in the presence of Cd under all conditions tested. Unlike Cu, the maximum variation was observed at pH 5.5 in the presence of Cd and the minimum was observed at pH 9.0 except Cd-1 exposure. The percentage reduction in the ascorbic acid content varied between 14.09 at pH 5.5 for Cd-1+Al-2 and 4.35 at pH 7.6 for Cd-1. The alterations in the concentrations of Cd have no appreciable effect on the variation in the ascorbic acid content.

The variation in the ascorbic acid content of the clam in the presence of Hg was not significant ($p > 0.01$) except during Hg-1+Al-1, Hg-2+Al-1 and Hg-2+Al-2 and the period of exposure will not alter the ascorbic acid significantly except during Hg1 and Hg2+Al-2 ($P < 0.05$). The change in the concentration of mercury in the exposure medium had no significant effect on the variation of ascorbic acid. The maximum variation was observed at pH 7.6 for Hg-2 and the minimum at pH 5.5 for Hg-1+Al-2 and the magnitude of percentage reduction was found to be between 11.15 and 4.21. The maximum and minimum percentage reduction in the ascorbic acid contents after 120h from the control are given below:

Exposure Medium	Percentage reduction in ascorbic acid content			
	Maximum		Minimum	
H^+	11.17		4.38	
Al-1	10.31	at pH 5.5	5.21	at pH 7.6
Al-2	10.14		5.22	at pH 8.5
Cu-1	11.14	at pH 8.5	5.15	
Cu-1+Al-1	8.53		6.51	at pH 7.6
Cu-1+Al-2	11.28	at pH 9.0	5.65	
Cu-2	10.06		7.20	
Cu-2+Al-1	9.84	at pH 6.0	8.01	at pH 8.5
Cu-2+Al-2	11.08		8.43	
Cd-1	11.15	at pH 7.6	4.35	at pH 7.6
Cd-1+Al-1	8.67	at pH 6.0	4.77	
Cd-1+Al-2	14.09	at pH 5.5	4.84	
Cd-2	7.37	at pH 6.0 & 8.5	4.52	at pH 9.0
Cd-2+Al-1	11.22		8.58	
Cd-2+Al-2	11.17	at pH 5.5	5.82	
Hg-1	7.15		5.75	at pH 8.5
Hg-1+Al-1	9.53	at pH 7.6	5.76	
Hg-1+Al-2	8.49	at pH 9.0	4.21	at pH 5.5
Hg-2	11.15	at pH 7.6	4.51	
Hg-2+Al-1	7.12	at pH 6.0	5.62	at pH 8.5
Hg-2+Al-2	7.94	at pH 9.0	5.75	at pH 5.5

The interactive effect of Al^{3+} with Cu, Cd and Hg in the depletion of ascorbic acid content was negligibly small. The decrease observed in the ascorbic acid content may be due to the protective role of ascorbic acid as reported earlier by Verma *et al.* (1986) for due to heavy metal toxicity. Vitamin C level of diet was sufficient for noble growth and for prevention of deficiency sign such as lordosis scoliosis and a reduction in bone collagen formation in channel cat fish, *Ictalurus punctatus* (Durve and Lovell, 1982). Abrupt fall in the ascorbic acid in common cirripedes during copulation was observed by Barnes and Barnes (1982). In studies on other species, it is generally observed that

ascorbic acid produces a significant ameliorative effect against excessive levels of heavy metals (Chou *et al.*, 1987).

It has been suggested that animals have an increased ascorbic acid requirement during certain types of stress (Baker, 1967). Chatterjee, (1973) theorized that all invertebrates and teleosts are unable to synthesis ascorbic acid which lends support to the hypothesis that ascorbic acid of *Neanthes virens* becomes depleted during the periods of increased demands such as pollutant exposure. The result of the present investigation didn't support this hypothesis. After a chronic exposure to PCB, the ascorbic acid levels of *Neanthes virens* were significantly higher than that of the controls (Carr and Neff, 1981). In the present investigation, after 5 days of exposure of the test specimen to different concentrations of Cu, Cd, Hg and Al, the tissue level ascorbic acid variation was found to be less than that due to change in H^+. These results indicated that ascorbic acid is not a limiting factor during the trace metal stress whereas, it is clearly established the depletion of ascorbic acid content with change in H^+ in the exposure medium.

SUMMARY

Contamination of coastal and estuarine waters by point and non-point pollution sources is a severe water quality problem that has attracted the attention of environmentalists. This interest is based largely on the increased awareness of the presence of contaminants such as trace metals, pesticides and radionuclides as well as of their effects on aquatic ecosystems and their possible toxicological implications to humans.

Acidification of natural waters and trace metal pollution are two important environmental problems facing the world today. Recent investigations on the impact of acid precipitation (acid rain) on aquatic ecosystems have demonstrated that sublethal effects seem to be of greater ecological significance than even large-scale fish kills. An important consequence of acidification is the mobilisation of aluminium from the edaphic to the aquatic environment. These elevated Al levels in acidic waters may be toxic to aquatic biota. The mechanism by which low pH water and combinations of low pH water and elevated Al concentrations become toxic to aquatic organisms are not fully understood. It is the paucity of information on the interactive effects of H^+ and Al^{3+} on the bioavailability of trace metals that initiated this study.

Estuaries form a buffer zone between freshwater and the sea. Over the ages, shores of estuaries have been favoured sites for human settlement. Some of the world's highest population densities are found adjacent to major estuarine systems. Estuaries have been used as convenient conduits for the disposal of a broad spectrum of wastes generated by industries and for sewage disposal. As a consequence of these activities, estuaries in industrialised nations commonly are highly stressed environments. Trace metals constitute a serious threat to the stability of the estuarine

ecosystem. An appreciation of the role that these substances play on the health of an estuary requires a thorough understanding of their chemical, physical and biological dynamics in the system.

The objectives and scope of the present study along with the considerations for the selection of the metals Cu, Cd, Hg and Al are detailed in Chapter I. The suitability of *Villorita cyprinoides* var *cochinensis* as the bioindicator is also discussed.

The different experimental designs employed for both acute and sublethal studies and the methods for the determination of filtration rate, metabolic rate, bioaccumulation and depuration of trace metals and various biochemical constituents (glycogen, lactic acid, lipid, protein and ascorbic acid) are given in Chapter II.

The location of the sampling sites in the Cochin estuary, the speciation scheme used for the determination of different forms of Al and of the methods of determination of hydrographical parameters and Al are presented in Chapter III.

The temporal and spatial variations of various hydrographical parameters like salinity, pH, DOC, POC, DO along with the different forms of Al in the Cochin estuary were measured. The correlations of Al fractions to different hydrographical parameters were dependent on seasons and the correlations varied with sampling periods. Particulate aluminium, total dissolved aluminium, total monomeric aluminium and filterable cation exchange aluminium were found to be negatively correlated to pH, salinity and suspended matter whereas, these forms were positively correlated to particulate organic carbon. The correlation between dissolved organic carbon and different fractions of Al varied with season. Labile aluminium and colloidal aluminium fractions were negatively correlated to suspended matter whereas they were positively correlated to dissolved organic carbon during post-monsoon. Labile and colloidal aluminium were found to be uncorrelated to salinity or particulate organic carbon. All fractions of Al were positively

correlated to dissolved oxygen during all seasons. There was no well-defined relationship between the temperature and Al in this estuary.

The maximum value of total dissolved aluminium (63.15 $\mu g\ l^{-1}$) was observed in freshwater zone during monsoon and the minimum value (2.32 $\mu g\ l^{-1}$) was found in the marine region during pre-monsoon. The maximum value of Al observed in the riverine region may be due to the acidification processes occurring in the upper reaches of the river Periyar; the minimum value could be attributed to the active removal mechanisms present in this estuary. Salinity, pH, adsorption, sedimentation, dilution and biological processes are responsible for the removal of Al in this estuary. Because of these active removal mechanisms, Cochin estuary acts as a sink for Al. The marked correlation values between salinity and total dissolved Al and the marked departure from the dilution line suggest that Al behaves non-conservatively in this estuary. Therefore, the input of this estuary to the contribution of Al in the Arabian sea is very little.

Trace metal toxicities and the interactive effects of H^+ and Al^{3+} on the physiological parameters like filtration rate and metabolic rate have been described in Chapter IV. The interactive effects of H^+ on the trace metal toxicities varied and depended on the chemical speciation and on the nature of biological systems. The presence of Al^{3+} increased the toxicities of Cu, Cd and Hg. This may be due to the change in bioavailability of the trace metals in presence of Al as well as to the toxic effect of Al^{3+}. The toxicities of the trace metals used in this study varied (based on LC50 values) in the following sequence:

$$Hg > Cu > Cd > Al$$

Reduction of pH modified the toxic effects of Cu and Cd in a largely protective fashion whereas in the case of Hg the toxicity increased.

Filtration and metabolic rates of the clam were found to be suppressed by trace metals at different pH. The variations could be explained on the basis of production of mucus during the exposure of the clam to different metals at different pH, bioavailability of metals at the gill surface and interactions of H^+ and trace metals with the physiological characteristics like valve-movement and cell-morphology.

The accumulation of trace metals at different pH, the depuration of accumulated metals at pH 7.6 and the interactive effect of Al^{3+} on the bioavailability of Cu, Cd and Hg have been discussed in Chapter V. The accumulation and depuration were found to be linear and the bioavailability was found to be dependent on pH. This may be due to the changes in chemical speciation of metals and competitive interaction of H^+. The changes in physiological parameters like filtration rate and metabolic rate and the changes in the adsorptive ability at the biological surface also appear to have contributed to the variation in accumulation and depuration of trace metals. By comparing BCF (bioconcentration factor) values after 120h of Cu, Cd, Hg and Al, the order of their accumulation in the soft tissues of the test specimen was found to be

$$Hg > Cu > Cd > Al$$

which is in very good agreement with the order of toxicities based on LC50 values.

The depuration of trace metals accumulated at different experimental conditions was compared using biological half life periods ($B_{1/2}$). $B_{1/2}$ values of Cu, Cd, Hg and Al were measured and on their basis these metals could be categorised into two of the three groups suggested by Cunningham and Tripp (1975b). Group I consists of Cd in which $B_{1/2}$ increased with increase in body burden of the metal and Group II consists of Cu and Al where $B_{1/2}$ decreased with increasing body burden of trace metal. Depuration of mercury was found to be a complex one and Hg belonged to Group I

during low body burden and changed to Group II with increase in the initial concentrations of Hg.

In general, the variations in the accumulation and depuration of Cu, Cd, Hg and Al at different pH and the interactive effect of Al^{3+} could be explained based on the competitive interaction of H^{+} for binding sites, the metal chemistry, the enhanced and varied mucus secretion, the variation in the speciation of metals, the several detoxification mechanisms, the alterations in metabolic processes such as metabolic rate and filtration rate as well as other biochemical changes.

Biochemical responses to trace metals have been acknowledged as reliable indices for environmental monitoring purposes. Biochemical constituents like glycogen, lactic acid, lipid, protein and ascorbic acid in the test specimens exposed to sublethal levels of trace metals at different pH were measured. Depletion was observed in the glycogen, lipid, protein and ascorbic acid contents of the clam whereas, an elevation was noticed in lactic acid content. These variations were found to change with pH. The results of this investigation have corroborated the relevance of glycogen, lactic acid, lipid and protein contents as useful indices of stress even in the context of competitive interaction between H^{+} and Al^{3+} ions. Ascorbic acid content however, was not found to be very good index for predicting the trace metal stress.

Considering the magnitude of the environmental acidification problem and the complexities of pH–Al synergism, it is abundantly clear that a holistic appreciation of a particular environmental regime would be possible only after further and detailed investigations on the mechanisms of accumulation at the molecular level are carried out.

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