Interactive Effects of Binary Mixtures of Metals on an Estuarine Clam — Assessment and Kinetics

A THESIS SUBMITTED TO
THE COCHIN UNIVERSITY OF SCIENCE AND TECHNOLOGY
IN PARTIAL FULFILMENT OF THE REQUIREMENTS
FOR THE DEGREE OF

Philosophiae Doctor
in
MARINE ENVIRONMENTAL CHEMISTRY
Under the Faculty of Marine Sciences

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JULY 1992

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CERTIFICATE

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

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July, 1992

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The corrections suggested by the examiners have been incorporated.

Certified that the corrections suggested by the examiners have been incorporated wherever necessary.

Geetha 11/12/1992

PREFACE

Although the clarion call for a judicious utilization of the planet's resources was sounded as early as 1972, it took not less than a score of years for the world nations to come to grip with the environmental issues and to realize the immediate necessity of ensuring a sustainable development. The Stockholm Conference was attended by merely two Heads of Governments. In sharp contrast, at Rio de Janeiro 130 Heads of States and delegates from more than 160 nations assembled in an unprecedented effort, to discuss and debate on grave environmental realities that stared the human race in their face.

Eco-accidents — fish diseases, massive fish-kills, artificial eutrophication of lakes and reservoirs, coastal zone pollution, climatic upheavals, even nuclear reactor failures — have all come to be the order of the day rather than being rare occurrences. No doubt man is in imminent danger of serious environmental hazards unless he wakes up from his slumber and puts his might into efforts aimed at alleviating and curbing anthropogenic pollution and stops indiscriminate thoroughfare of the environment around him.

Estuaries and coastal zones are regions of high fertility which have catered to and nurtured the development of human habitats all along their shores. Burgeoning population and unprecedented rates of urbanization/industrialization, especially in the Third World areas have tremendously increased human activities and have caused pronounced alterations in the features of these aquatic environments. Conventional development has been blind to the requirement of maintaining the quality and the richness of life in the ecosystem.

The increasing public concern about chemicals that threaten human or environmental health has induced national and international authorities to take steps towards formal assessment of the potential hazard of new and existing chemicals. Although, simplified procedures for a first preliminary assessment of various chemicals have been established, it is now time to evaluate the relevance and predictive power of these simplified schemes and to take further steps towards the development of a more in-depth assessment in which interactive effects, rather than individual toxicity measurements, form the major emphasis.

The work described in this thesis represents an attempt made in such a direction and is presented in the following six Chapters.

Chapters I is an introduction to the main theme of the thesis and gives an insight to the assessment of pollution, to the development of "Mussel Watch" concept, to the interactive effects of metals on indicator organisms and to the importance of the sublethal studies.

The Chapter II describes the collection site of the bivalve and the models used to predict the interactive effects. The methods for the determinations of the biochemical components (glycogen, lactic acid, lipid, protein) and metabolic rate as well as for the analysis of the metals are included in this Chapter, besides all other experimental details.

The results of the acute toxicity studies employing the metals copper, cadmium, mercury, arsenic, and selenium individually and in binary combinations are discussed in Chapter III. Attempts were made to predict the interactive

effects of metals from their individual and combined toxicity data employing the models proposed by Marking & Dawson (1975) and by Konemann (1981).

Chapter IV discusses the kinetics of uptake and depuration of the metals (Cu,Cd,Hg,As,Se) individually and in binary combinations (Cu+Cd, Cu+Hg, Cu+As, Cu+Se, Cd+Hg, Cd+As, Cd+Se, HG+As, Hg+Se, As+Se) at two levels of concentrations. Two metabolic indices `α' and `β' with respect to depuration and uptake respectively were defined and calculated for all the individual metals.

The results of the effects of metals and thier binary combinations on metabolic rate and on the bichemical components glycogen, lipid, lactic acid and protein are presented and discussed in Chapter V.

Chapter VI summarizes the salient features of the present study.

CONTENTS

Chapter I

CHAPTER I

INTRODUCTION

The earth condensed from a cloud of interstellar gas and dust about 4.6 billion years ago along with the rest of our solar system. The most unique feature of the earth is its water cover — the oceans — which interacts intimately with the atmosphere, the sea floor and the continents, from all of which it receives enormous inputs. The coastal margins of continents as well as the banks of rivers/estuaries (e.g. the Ganges, the Nile, the Rhine, the Thames) by virtue of their favourable conditions (fertility, climate, accessibility, etc.) have witnessed the sprouting up of many an ancient civilizations and have, thus, remained the hub of all human activities — navigation, recreation, agriculture, industry, etc. Man's inseparable association with the sea began with his early exploratory voyages around the globe. Increasing human population, expanding urbanisation and rapid industrialization during recent years have contributed substantially to the fluxes of innumerable chemical and biological materials into the coastal aquatic environment. Estuarine/coastal areas, because of their intimate contact with the terrestrial environment and of their particular physico-chemical features, trap significant quantities of these materials and, therefore, are the most disturbed segment of the aquatic environment.

Contaminants which produce deleterious effects on the resident biota are termed pollutants. "Marine pollution" has been defined (GESAMP, 1985) as:

"Introduction by man, directly or indirectly, of substances or energy into the marine environment

(including estuaries) resulting in such deleterious effects as harm to living resources, hazards to human health, hindrance to marine activities including fishing, impairing of quality for use of seawater and reduction of amenities".

The pollutants occurring conjointly with the chemical and biological materials which are frequently disposed into the aquatic environment have been broadly categorized (Preston, 1989) as:

(1) halogenated hydrocarbons (including PCBs and organo-chlorine pesticides such as DDT),
(2) petroleum hydrocarbons and its derivatives,
(3) other organic chemicals such as marine bio-toxins and detergents,
(4) nutrient chemicals (such as those found in domestic sewage or agricultural wastes),
(5) inorganic chemicals, particularly metals such as mercury, cadmium and lead,
(6) suspended solids,
(7) radio-active substances and
(8) thermal wastes.

Trace metals are the most persistent pollutants whose residence times in the aquatic environment show wide variations. The term "trace metals" or "heavy metals" enlists a wide range of elements such as Be, Al, Si, Ti, V, Cr, Mn, Fe, Co Ni, Cu, Zn, As, Se, Mo, Ag, Cd, Sn, Te, Hg, and Pb which may even include metalloids and which may not often be quite "heavy" as the name seems to convey (Goldberg, 1976). The trace metals which have been identified as posing significant hazards to the marine environment and which appear on the 'black lists' of various international conventions, arranged in the decreasing order of their acute toxicities to marine organisms (Preston, 1989) include

$Hg^{2+} > Ag^{2+} > Cu^{3+} > Zn^{2+} > Ni^{2+} > Pb^{2+} > Cd^{2+} > As^{2+} > Sn^{3+} > Fe^{2+} > Mn^{+} > Al^{2+} > Be^{2+} > Li^{2+}$

In order to quantify the risks associated with the pollutants, environmental base-line data pertaining to inputs of contaminants including information on the distribution, sources and quantities, chemical and hydrographical parameters, interactions between the contaminant and the marine environment, concentration levels of contaminants in various compartments of the environment, etc., obtained through comprehensive and systematic investigations are essential pre-requisites. Recent advances in instrumentation techniques (GC/MS, AAS, ASV, HPLC, etc.) have vastly enhanced the analytical capabilities and have thus enabled the establishment of precise base-line data and the identification of a number of "hot spots".

Aquatic organisms concentrate contaminants to several orders of magnitude higher than their environmental levels. Hence biomonitoring of these aquatic organisms has been proposed as another strategy for assessing the risks associated with the pollutants. A contaminant invites concern only if it produces a deleterious effect on organisms at lethal, sublethal, organismal or at community level. Hence biological monitoring employing living organisms are complementary to chemical monitoring in assessing the hazard produced as a result of pollution.

General guidelines have been proposed for the selection of organisms intended for biological monitoring. The salient features of these guidelines require that the organism should,

i) accumulate the pollutant,
ii) be sedentary in order to be representative of the study area,
iii) be abundant through out the study area,
iv) be of reasonable size giving adequate tissue for analysis,

v) be very easy to be sampled and be hardy enough to survive in the laboratory

and that there should exist a simple correlation between the pollutant content in the tissue and the average pollutant concentration in the surrounding medium (Phillips, 1976 and 1977a).

Toxicity tests constitute the most important stage in the development of any biomonitoring strategy as they facilitate a primary laboratory assessment of the response of an organism to toxicant stress. The objective of a toxicity test is to define the concentrations at which a test material is capable of producing some deleterious response in a population of test organisms. Two main types of toxicity tests are applied — acute and chronic. The acute toxicity of a substance in the aquatic environment is normally expressed as the concentration of the substance in water which produces a harmful effect in 50% of a batch of the test organisms in a short period of exposure usually four days duration or less. Chronic toxicity test is that resulting from much longer periods of exposure usually of several weeks duration depending on the life-span of the organism. Toxicity tests are also classified, on the basis of the effect, into lethal and sublethal tests. Lethal toxicity tests are characterised by the death of the organism whereas sublethal toxicity studies include effects on behaviour, growth, reproduction, biochemistry, physiology, etc. Thus, toxicity tests comprise of acute-lethal, acute-sublethal, chronic-lethal and chronic-sublethal experiments.

The experimental set up for the toxicity tests are designed according to the specific need and the purpose (APHA, 1985).

i) Static tests:- These are tests in which solutions and the test organisms are maintained undisturbed for the entire duration of the experiment.

ii) Recirculation tests:- These are static tests in which the test solution is constantly recirculated among the different test chambers, the water quality being maintained by aeration, filtration, sterilization, etc.

iii) Renewal tests:- These are static tests in which the test organisms are periodically (usually at 24h intervals) exposed to fresh test solutions of the same composition. This is accomplished by transferring the test organism or by replacing the test solution.

iv) Flow-through tests:- In flow-through tests, continuous replacement (not recirculation) of the test solution in the test chambers is effected.

Toxicity tests are usually conducted by exposing the test organisms to a series of test solutions of different concentrations. One or more control sets are also maintained to provide a measure of the response of the test organisms to extraneous factors such as dilution water, test conditions, handling procedures, etc.

Algae, being at the base of the aquatic food web and being immediately affected by environmental stress, were widely employed in biomonitoring programmes (Rosko and Rachlin, 1975; Conway and Williams, 1979; Li, 1980; De Filippis *et al.*, 1981; Rebhun and Ben-Amtoz, 1988). Various species of shrimps, fishes, crustaceans, etc. which constitute a major proteinaceous food for the human beings have also been used in toxicity studies (Sprague, 1969; Ahsanullah, 1976; Negilski *et al.*, 1981; Hunn *et al.*, 1987; Krishnaja *et al.*, 1987).

The sedentary, filter-feeding, widely distributed bivalves which satisfied most of the requirements of indicator/sentinel organisms soon emerged as the preferred group of aquatic

organisms for toxicity investigations. Convinced of their exceptional features, Goldberg (1975) initiated a "Mussel Watch" and advocated the establishment of a global effort, on similar lines, to provide a basis for assessing the impact of pollution upon public health. Eisler (1971), Calabrese *et al.* (1973), Phillips (1976), Davenport and Manley (1978), Eknath (1978), Kumaraguru *et al.* (1980), MacInnes (1981), Amiard-Triquet *et al.* (1986) and others conducted toxicity studies employing the bivalves.

Most of these approaches were aimed at assessing metal toxicities and at elucidating the effects of single metal in order to determine the median lethal/effective concentration of a toxicant for a designated period of time. Realising the inadequacies of such single metal toxicity investigations, which would have very little relevance in the aquatic environment which is a conglomerate of diverse pollutants, the emphasis soon shifted to investigations on lethal toxicities of metal combinations. The interactive effects of binary combinations of copper and cadmium were investigated by Sprague and Ramsay (1965), Eaton (1973), D' Agostino and Finney (1974), Moulder (1980), de March (1988) and others. The antagonistic effect of manganese, or zinc to cadmium or copper was reported by Sunda *et al.* (1981) and Rebhun and Ben-Amotz (1988). Another keenly investigated interactive effect was that of selenium with mercury, cadmium and copper. Magos and Webb (1980) stated that at least one of its chemical form might have the property to alleviate the trace metal toxicity. Huckabee and Griffith (1974), Lucu and Skremblin (1981), Gostis (1982), Winner and Whitford (1987) and others investigated the interactive effects of selenium with the trace metals copper or cadmium or mercury.

Lethal toxicity studies are important for comparing the toxicities of different toxicants and also for ascertaining the concentrations to be used in combined toxicity studies.

However, sublethal studies, which consider kinetics of uptake and depuration, metabolic changes, changes in various biochemical constituents etc. only can provide a deeper insight into the various physiological/biochemical changes occurring in the organism which might result in such deleterious effects which can be propagated to the next generation and/or to its predators.

Bioaccumulation of aquatic organisms, which are constantly exposed to a large variety of toxicants in the aquatic system, would occur when the rates of accumulation exceed the rates of depuration. Mechanisms controlling the distribution and elimination of toxicants are, thus, of vital importance in regulating the phenomenon of bioaccumulation. The rates of accumulation and depuration define the bioconcentration factor of the toxicants while the rates of depuration determine the half-life of toxicants within the organism.

Reports on the linear dependence of accumulation of trace metals with time are numerous in the literature in various aquatic organisms(Bryan, 1976; Phillips, 1977b; Roesijadi, 1982; Martincic *et al.*, 1984; Devineau and Amiard-Triquet, 1985; Amiard-Triquet *et al.*, 1986; King and Davis, 1987; Chan, 1988; Marigomez and Ireland, 1989). Hydrographic parameters such as salinity, temperature, hardness of water, pH etc. influence the bioavailability and govern the rates of accumulation of trace metals by the aquatic organisms (Phillips, 1977b; Denton and Burden-Jones, 1981; Winner and Gauss, 1986). Low salinity, hardness, elevated temperatures, etc. have been reported to cause significant enhancement of accumulation rate. Besides, the interactive effects caused by the presence of other metals in the medium can alter the uptake rates synergistically or antagonistically. Eisler and Gardner (1973), Moulder (1980), MacInnes (1981), Bjerregaard (1985; 1988), Elliot *et al.*(1986), Cuvin and Furness (1988) and Boisson *et al.* (1989) investigated

the interactive effects of trace metals during accumulation under controlled laboratory conditions.

Investigations on the rates of loss or depuration of toxicants in aquatic organisms exposed to toxicants are relatively few. Cunnigham and Tripp (1973), Scholz (1980), Howell (1983), and Lakshmanan and Nambisan (1989) reported on the depuration of metals whereas the interactive effects of metals on the depuration were studied by Cuvin and Furness (1988).

Sublethal studies aimed at assessing the physiological/ biochemical responses of aquatic organisms under toxicant stress include monitoring of changes in metabolic rate, filtration rate and tissue content of the biomolecules (glycogen, protein, lipid, etc.). Gills, the respiratory organ of aquatic animals, are the route of uptake of food and water and are the most severely affected organ. Metal-induced variations in gill structure have been reported by Skidmore (1970) Morris *et al.* (1982) and Reid and Mac Donald (1991). These changes in gill structure affect the metabolic rate, enzymatic and hormonal activities, and lead to partial/total transformation to anaerobic metabolism which result in utilization of the stored-up energy reserves such as glycogen, protein and lipid. Thus responses of an organism to toxicant-stress are fundamentally biochemical. Metal-induced variations in the physiological responses (oxygen consumption, filtration, etc.) were reported by Scott and Major (1972), Brown and Newell (1972), Watling and Watling (1982), Manley (1983), Davenport and Redpath (1984), Abraham *et al.* (1986) and others. The variations in biochemical constituents of fishes have been studied extensively (Shaffi, 1978 a and b; Gluth and Hanke, 1984;Ram and Sathyanesan, 1985; Abdullah and Ireland, 1986; Hilmy *et al.*, 1987a; Kedarnath and Nishithkumar, 1987 and 1988;

Agarwal and Nair, 1989) though such studies are relatively few in the case of bivalves (Viarengo *et al.*, 1980).

Scope of the present study

Bivalves of the Cochin Estuarine System have been the subject of several toxicity studies directed mainly at estimating the lethal toxicities, besides their physiological responses (metabolic rate, filtration rate, ammonia excretion etc.). The only reports pertaining to changes in the biochemical constituents of bivalves sampled from the Cochin Estuarine System comprise of studies carried out by Lakshmanan (1982), Sathyanathan *et al.*, (1988), Suresh (1988), and Katticaran and Salih (1992).

The present study markedly deviates from conventional toxicological investigations and attempts to provide a stronger scientific basis for the interpretation of toxicant induced responses of bivalves by attempting to view the biochemical changes in a broader perspective of joint/interactive effects of metals present in binary combinations and directing its emphasis to the evaluation of the bioaccumulation process in terms of the kinetics of uptake and depuration.

Villorita cyprinoides var. *cochinensis* was the bivalve chosen for the present investigation. The metals copper, cadmium, mercury, arsenic and selenium were chosen for the study. Their physiological relevance and toxicological implications are listed along with their sources and physico-chemical properties in Table 1.1. Copper and selenium were essential metals while mercury, cadmium and arsenic were acknowledged to be predominantly toxic heavy metals. Arsenic and selenium, though occupying neighbouring positions in the periodic table differ widely in their physico-chemical properties such as ionic radius, oxidation state, etc. Selenium

Table 1.1 General Charecteristics and Sources of the Trace Metals, Copper, Cadmium, Mercury, Arsenic and Selenium

Metal*	At.No.*	Common* Oxdn. States	Coordn.* Number	Ionic* Radii	Biological* Functions	Toxicity*	Levels in Natural Waters			Sources
							Ocean Waters	Fresh Waters	Cochin Estuary	
Copper	29	+1 +2	2 4 6 4 5 6	60 74 91 71 79 87	Essential to all organisms, constituents of redox enzymes and oxygen transport pigments	Very toxic to most plants, highly toxic to invertebrates & moderately so to mammals	0.03-0.23 ug/l (Bowen, 1985)	Dissolved 1.5 ug/l Particulate 100 ug/g (Martin & Meybeck, 1979)	Dissolved (1.4-9.5) Particulate (0.75-8) ug/l (Babukutty, 1991)	Smelting, mining, industrial (metal plating, steel works, refineries, domestic and municipal sewages and sludges; applications of fertilizers, molluscides & fungicides. (BOWEN, 1985)
Cadmium	48	+2	4 5 6 7 8	92 101 109 117 124	None known	Moderately toxic to all organisms, a cumulative poison in mammals causing renal failure & linked with hypertension in man	0.01-0.1 ug/l (GESAMP, 1985)	Dissolved .02 ug/l Particulate i ug/g (Martin & Meybeck, 1979)	Dissolved (0.2-2.3) ug/l Particulate (0.2-1.8) ug/l (Babukutty, 1991)	Agricultural runoff, zinc mining wastes municipal sewages, sludges including those of domestic origin (GESAMP, 1985).
Mercury	80	+1 +2	3 6 2 4 6 8	111 113 83 110 116 128	None known	Very toxic to fungi and green plants and to mammals	2-3 ng/l (GESAMP, 1986)	Total 0.0005-0.0017 mg/l (Balchand & Nambisan, 1986)	Dissolved 40-280 ng/l Particulate 2-36 ug/g (Ouseph, 1990)	Ore deposits (mercuric as well as non-mercuric), industries (caustic soda, paper production fertilizers, coal (GESAMP, 1986)
Arsenic	33	+3 +5	6 4 6	72 47.5 60	None known	Moderately toxic to plants, highly toxic to mammals	1.3-2.6 ug/l (GESAMP, 1986)	Dissolved 1.7 ug/l Particulate 5 ug/g (Martin & Meybeck, 1979)		Application of pesticides, smelting and roasting of sulphide minerals, combustion of fossil fuels, leaching of wastes from mining activity and erosion of land. (GESAMP, 1986)
Selenium	34	-2 +4 +6	6 6 4 6	184 64 42 56	Essential to mammals and some higher plants	Moderately toxic to plants, highly toxic to mammals	22-130 ng/l (GESAMP, 1986)	.07-.39 ug/l (GESAMP, 1986)		Application of selenium in photoelectric cells, rectifiers various industries (glass, steels, alloys, petroleum rubber etc.) as pesticides (GESAMP, 1986)

* (Hubee, 1978)

was included in this study in order to probe into the nature of its toxicological interaction with the other metals, (essential and non-essential) in the light of its well documented ability to alleviate trace metal toxicity.

It is hoped that the studies described in this thesis, besides providing a much wanted data-base on interactive effects of metals, would provoke the initiation of a more elaborate and vigorous kinetic approach in addressing chemotoxicology of interactions of metals with biological systems.

Chapter II

CHAPTER II

MATERIALS AND METHODS

This investigation has been concerned with studies designed to assess the toxic effects (lethal as well as sublethal) of individual and binary mixtures of copper, cadmium, mercury, arsenic and selenium on an estuarine clam, *Villorita cyprinoides* var. *cochinensis*. The details of the materials used and the experimental methodologies employed are presented in this chapter.

2.1. TEST ANIMAL

Species

Villorita cyprinoides var. *cochinensis* is an important species of backwater clam, which occurs conjointly with other species of *Villorita* along the west coast of India, from Goa to south Kerala (Prashad, 1921). Extensive and dense beds of this species are found in all the major back waters of Kerala, *viz.* Vembanad, Kayamkulam and Ashtamudi, where the annual variation of salinity is 0-20 x 10^{-3}. The optimum habitat salinity of the clam has been reported as $\simeq$ 13 x 10^{-3}(Nair and Shynamma 1975). *Villorita cyprinoides* var. *cochinensis* provides a cheap source of protein-rich food and a raw material for the manufacture of cement and lime. Though the erstwhile Government of Travancore-Cochin had listed it as a State property and an article of public utility by an order passed in 1952, the swelling demand of lime shell has led to an uncontrolled exploitation of the species (Nair, 1975).

Collection, Transportation and Acclimatization

Specimens, *Villorita cyprinoides* var. *cochinensis* (20-22mm length) were collected from Kumbalam, a place about 8km south east of the Cochin barmouth (Fig. 2.1) which is considered to be a relatively pristine area. The specimens from the collection site were transported to the laboratory in polyethene bags filled with water of habitat salinity, subjecting them to the least possible stress. In the laboratory, the collected animals were cleaned free of sediment and of other detrital matter and then transferred into large perspex tanks of 50 litre capacity and were then acclimatized to the laboratory conditions (T = 30 ± 2^{0}C, D.O. >80% saturation) by aeration for 48h. The animals were fed daily with the blue green algae *Synechocystis salina* prior to 24h of the commencement of the experiment when the feeding was stopped and the animals were transferred to the experimental salinity (10×10^{-3}).

2.2. TEST MEDIUM

Seawater used in the study was collected from the Arabian sea, 8-10 km off Cochin barmouth. The water was transported to the laboratory in large plastic carbouys of 50 litre capacity and kept in total darkness for about a week for ageing. Before the commencement of the experiment, the seawater was filtered through glass wool and its salinity was determined argentometrically by Knudsen's method (Grasshoff, 1983). Appropriate dilutions of the seawater were made using deionized water to obtain the required salinity (10×10^{-3}) and this was aerated to saturation prior to use. pH, dissolved oxygen and temperature were monitored daily.

2.3. TEST CONTAINERS

Toxicity tests were conducted in specially manufactured

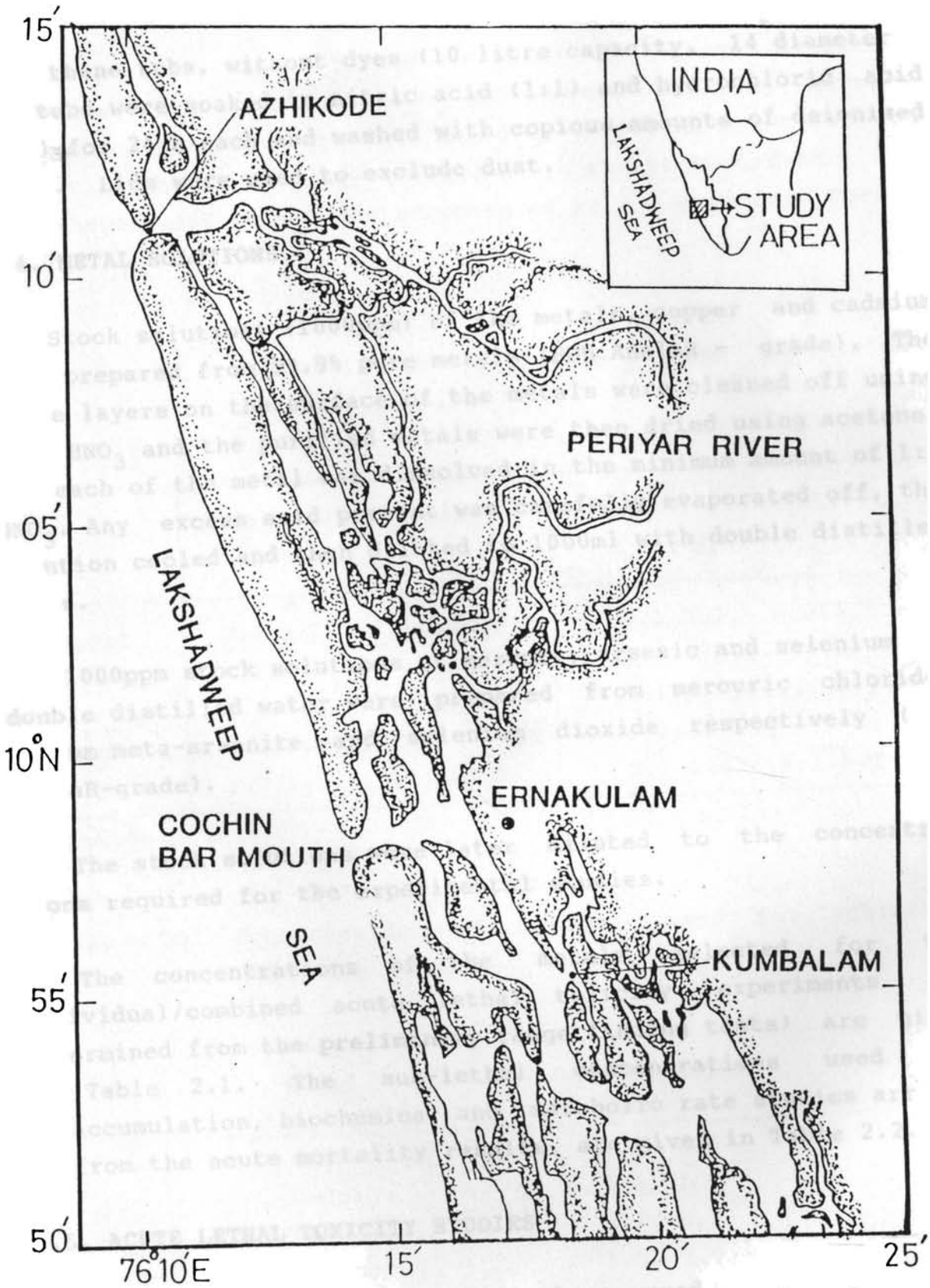


Fig.2.1 Map of the Cochin estuary showing the location of sampling site.

polyethene tubs, without dyes (10 litre capacity, 14" diameter). The tubs were soaked in nitric acid (1:1) and hydrochloric acid (1:1) for 24 h each and washed with copious amounts of deionised water. Lids were used to exclude dust.

2.4. METAL SOLUTIONS

Stock solutions (1000ppm) of the metals, copper and cadmium 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. 1 g each of the metal was dissolved in the minimum amount of 1:1 HNO_3. Any excess acid present was carefully evaporated off, the solution cooled and then diluted to 1000ml with double distilled water.

1000ppm stock solutions of mercury, arsenic and selenium in double distilled water were prepared from mercuric chloride, sodium meta-arsenite and selenium dioxide respectively (BDH AnalaR-grade).

The stock solutions were later diluted to the concentrations required for the experimental studies.

The concentrations of the metals selected for the individual/combined acute lethal toxicity experiments (as determined from the preliminary range finding tests) are given in Table 2.1. The sub-lethal concentrations used for bioaccumulation, biochemical and metabolic rate studies arrived at from the acute mortality results, are given in Table 2.2.

2.5. ACUTE LETHAL TOXICITY STUDIES

Acute toxicity studies have been used to quantify the stress response within a designated period of time. In acute

mortality studies death is considered as the chief criterion. The median lethal concentrations of copper, cadmium, mercury, arsenic and selenium were determined by following short-term (96 h) static renewal bioassay technique in strict accordance with the standardised procedures recommended by Ward and Parrish (1982).

Table. 2.1. Concentration of metals used for lethal toxicity studies

Metal	Individual Toxicity (ppm)	Combined Toxicity (ppm)
Copper	0.4,0.8,1.2,1.4,1.6	0.1,0.2,0.4,0.8,1.2
Cadmium	1,2,4,8,12	0.4,0.8,1.6,3.2,4.8
Mercury	0.4,0.8,1.2,1.4,1.6	0.1,0.2,0.4,0.8,1.2
Arsenic	4,6,8,10,12	1,2,4,8,12
Selenium	1.3,1.6,1.9,2.2,2.5	0.2,0.4,0.8,1.6,2

Table 2.2 Concentration of metals used for sublethal toxicity studies

Metal	Concentration (ppm)
Copper	0.1, 0.3
Cadmium	0.5, 1.5
Mercury	0.1, 0.3
Arsenic	1.0, 3.0
Selenium	0.2, 0.6

Individual Toxicity Studies

Ten animals of the required size group (20 $\pm$ 2mm) were maintained in 5 litre of filtered seawater (salinity 10×10^{-3}, pH 7.2 $\pm$ 0.2, T 30 $\pm$ 2°C, D.O.> 80% saturation) in specially manufactured, dye-free polyethene troughs of 10 litre capacity. Calculated volumes of metal solutions were added individually to maintain the required concentrations of the toxicants. A minimum of five test-concentrations of the metals were set up in each experiment along with a set of control. The test medium was renewed daily, with seawater, the salinity, temperature, pH and dissolved oxygen of which were initially monitored. The troughs were neither aerated nor the animals fed during the experiments. The mortality of the animals were noted every 12h (an animal was considered dead when the valve gap was at least 5 mm and when there was no response to gentle prodding) and the 96 h LC_{50} values were calculated from the cumulative percentage mortalities using the log-probit method (Litchfield and Wilcoxon, 1949).

Combined Toxicity Studies

After determining the 96h LC_{50} values of the individual metals, varying ratios of the individual 96h LC_{50} values were used in binary combinations to study the interactive effects of the above metals. The basis for this selection was that the different concentrations of the various metals employed in binary combinations would contribute a definite percentage of their individual median lethal concentrations to the combined toxicity, according to the toxic unit concept. The acute mortality tests were then conducted as explained in section 2.5.

2.6. ACUTE SUBLETHAL TOXICITY STUDIES

Sublethal toxicity studies were carried out to quantify the

toxic effects that would become manifested in animals subjected to sublethal concentrations of toxicants and thereby to predict the safe concentrations of pollutants. These studies would yield an understanding of the adaptability of the animals to environmental stress of pollutants.

Accumulation and Depuration Studies

In order to delineate the interactive effect of a metal on the accumulation and depuration of another metal, in a binary combination, acute toxicity studies were conducted in strict accordance with the procedures prescribed by Ward and Parrish (1982). The trace metals employed were copper, cadmium, mercury, arsenic and selenium.

For accumulation studies, 20 animals of the required size group (20 $\pm$ 2mm) were exposed to the metal concentrations individually and in binary combinations in specially manufactured, white, dye-free polyethene troughs of 10 litre capacities containing 5 litres of filtered seawater (salinity 10×10^{-3}, pH.7.2 $\pm$ 0.2, D.O. >80% saturation, T 30 $\pm$ 2°C) for a duration of 96h. The troughs were not aerated and the animals were not fed during the experiments. The characteristics of the test media (salinity, temperature, dissolved oxygen, and pH) were monitored daily. The animals (6 nos) were sampled at 24h intervals for a period of 96h. A set of 20 animals were maintained in seawater at the experimental conditions (control) and the whole tissues were sampled periodically at 24h intervals along with the dosed animals in order to test the significance of accumulation.

Depuration studies were conducted by transferring the animals after 96h accumulation into filtered seawater of salinity 10×10^{-3}, (pH = 7.2 $\pm$ 0.2, D.O.> 80 % saturation, T 30 $\pm$ 2°C) for a period of 96h. The whole tissues of the animals were

sampled periodically at 24h intervals along with that of the control animals for a period of 96h.

In both accumulation and depuration studies, the soft tissues of the sampled animals were dissected out, and pressed between the folds of the filter paper and dried to constant weight at 80°C. The dried material was powdered and stored in a desiccator till the analyses of the metals were done.

Digestion procedure for copper, cadmium, mercury, arsenic, and selenium

The dried tissues were powdered and a definite weight (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). After preheating, the samples were digested for about 5h. The solutions were cooled and made upto 25ml using double distilled water.

For the determination of mercury, the dried samples were refluxed with conc. HNO_3 and conc. H_2SO_4 in the ratio 4:1 (v/v) as recommended by BITC (1976), using the Bethge apparatus described by Shaw and Panigrahi (1986). The solutions were cooled and made upto 25ml using 0.6N HNO_3.

Estimation of copper, cadmium, mercury, arsenic and selenium

Copper and cadmium contents of the digested solutions were estimated using an Atomic Absorption Spectrophotometer (Perkin Elmer-2380) by directly aspirating the sample into the air-acetylene flame.

Mercury, arsenic and selenium were 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 the hydrides were then directed to the AAS for the estimation of the respective metal contents.

Determination of Metabolic Rate

Three test animals, pre-exposed to the selected sublethal concentrations of the metals for 24h, 48h, 72h and 96h, were kept in conical flasks of 1 litre capacity containing 1000ml filtered seawater of salinity 10×10^{-3}, the oxygen content of which was measured initially. Gas exchange from the atmosphere was prevented by sealing the flasks with inert liquid paraffin. The duration of the experiment was 3h and frequency of the sampling was 1h. After the experiment, the test media were siphoned out using a flexible polyethene tube for the determination of the dissolved oxygen content. The dissolved oxygen, present initially (O_i) and both in the control and in the dosed media (O_c and O_d respectively) was estimated in triplicate by Winkler's (Grasshoff, 1983) method. The oxygen consumed by the clams, (both control and the experimental) was determined as follows:

Oxygen consumed by the control animals = $O_i - O_c$

Oxygen consumed by the experimental clams = $O_i - O_d$

After the experiments, soft tissues of the clams were scooped out, cleaned in distilled water, dried at 70-80°C for 48h and then weighed to constancy. The oxygen consumed is expressed as ml O_2 h^{-1} g^{-1}(dry wt.).

Biochemical Analyses

In order to assess the interactive effect of a metal in producing biochemical changes in binary combination with another metal, sublethal toxicity studies were conducted in strict accordance with the procedures prescribed by Ward and Parrish (1982). The trace metals employed were copper, cadmium, mercury, arsenic and selenium.

For biochemical studies, 20 animals of the required size group (20 $\pm$ 2mm) were exposed individually and in binary combinations to the metal concentrations, in specially manufactured, white, dye-free, polyethene troughs of 10 litre capacities containing 5 litre of filtered seawater (salinity 10 x 10^{-3} pH 7.2 $\pm$ 0.2, D.O.>80% saturation, T 30 $\pm$ 2°C) for 96h duration. The troughs were neither aerated nor the animals fed during the experiments. The characteristics of the test media (salinity, temperature, dissolved oxygen, and pH) were monitored daily. A set of control animals (20 nos.) were kept in seawater at the experimental conditions. The whole soft tissues of the bivalves (both the control and the dosed) were sampled at 24h intervals. The biochemical constituents, glycogen, lactic acid, lipid and protein were determined by the following standard procedures.

For the determination of the biochemical constituents, six analyses were done by taking six animals (chosen at random) from both the experimental and the control troughs. The tissues of the groups of the six animals were pooled together separately in order to compensate for any individual effects, homogenised and dried between the folds of filter paper and each of these were later subsampled and then analysed for the biochemical constituents.

Glycogen

The glycogen content of the clams was estimated by the method of Pluger, modified by Hassid and Abraham (1963). A known weight of the bivalve soft tissue was dissolved in potassium hydroxide (3 ml of 30%) and digested by heating the tube in a boiling water bath for 20-30 minutes; saturated sodium sulphate solution (0.5 ml) was added and the glycogen was precipitated by the addition of 95% ethyl alcohol. The glycogen thus obtained was purified by repeated precipitations using ethyl alcohol.

The purified glycogen was hydrolysed to glucose by refluxing with concentrated hydrochloric acid (0.6 N) and the sugar was estimated by Heath and Barnes (1970) method. In order to obtain the concentrations of the glycogen content in the samples, standard graph was prepared using glucose and concentration of the sugar thus obtained was converted to glycogen using the conversion factor 0.93. The glycogen content thus obtained was expressed as mg glycogen g^{-1} of the tissue.

Total Lipid

The sulpho-phosphovanillin method (Barnes and Blackstone, 1973) was used for the estimation of lipid. A known amount of the tissue was extracted thrice with the chloroform-methanol mixture (2:1). The above extract was mixed with sodium chloride (0.2 ml of 0.9 N) in a test tube and was then capped with non-adsorbent cotton and was allowed to equilibrate overnight at 4-5°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 the phosphovanillin reagent (5 ml) 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 tissue.

Protein

The protein content was estimated by the modified Folin-Ciocalteu method (CMFRI, 1982). Protein was extracted from approximately 20mg of the bivalve soft tissue using sodium hydroxide (10 ml of 0.1 N) solution and the extract was made upto 25ml using double distilled water. To 1 ml of the sample, alkaline copper reagent (5 ml) was added followed by potassium antimonyl tartrate (5 ml) and mixed well. The optical density was measured at 500nm after half an hour. 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 tissue.

Lactic Acid

Lactic acid content in the whole soft tissues of the clams 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 animal was dried between the folds of the filter paper, weighed and homogenised with trichloroacetic acid (10 ml 10%) and purified sea-sand. The mixture was centrifuged and the supernatent liquid was treated with copper sulphate solution (1ml 20%). This solution was then diluted to 10 ml, shaken well with calcium hydroxide powder (1 g) and kept aside for 30 minutes and again centrifuged. Duplicate aliquots of the supernatent liquid (1 ml) were pipetted out into clean test tubes and chilled after addition of copper sulphate solution (1 drop of 4%). 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 p-hydroxy diphenyl reagent (2 drops of 1.5%), kept aside for 30 minutes and the absorbance was measured at 560nm. Lithium lactate was used for the preparation of the standard curve and the lactic acid content in whole soft tissue was expressed as mg lactic acid g^{-1} of the tissue.

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.

Chapter III

CHAPTER III

LETHAL TOXICITY STUDIES

3.1. INTRODUCTION

The assessment of pollution has two important strategies of approach i) regular monitoring, which is essential to obtain information on the environmental levels of pollutants and ii) toxicity studies, which serve to assess the hazard or the risk associated with bioaccumulation of toxicants. Thus, monitoring and toxicity studies are complimentary strategies for hazard evaluation of pollutants which is the first step in the efforts aimed at protecting the aquatic resources.

Toxicity tests make use of living organisms to define the nature and degree of harmful effects produced by a toxicant. Acute toxicity studies measure a harmful effect in a batch of the test organisms within a short period of exposure time, usually of four days duration or less. Chronic toxicity studies involve larger periods of exposure, usually several weeks, depending on the life span of the organism. Toxicity tests are further classified on the basis of the effects manifested, i.e. as lethal and sublethal tests. Lethal toxicity studies are characterized by the death of the organism concerned and sublethal studies involve assessment of the toxicant-induced effects on behaviour, growth, reproduction, biochemistry, physiology, etc.

This chapter is concerned with the studies conducted to assess the acute lethal doses of various trace metals to an estuarine clam. Acute lethality (mortality) tests are conducted

to obtain the median lethal concentration of a toxicant at 96h and can foretell the bioavailable fraction of the toxicant in terms of its toxicity towards the indicator organism within a designated period of time. Hence acute lethality studies are widely employed in order to compare the toxicities of different chemicals, to evaluate the relative sensitivity of different aquatic organisms to a particular toxicant, to evaluate the effect of water quality on the toxicity of the test material etc. In acute mortality studies, the organisms are exposed to a series of concentrations of toxicants for designated time intervals and the mortalities in each group are recorded. From these data, the LC_{50} value is determined by probit analysis (Sprague, 1969; Ward and Parrish, 1982; Rand and Petrocelli, 1985).

The organisms selected for the toxicity studies should be indicative of the environmental levels of chemicals. Phillips (1976) proposed a set of guidelines for the selection of indicator/sentinel organisms in the light of the necessities and pre-requisites of toxicity experiments. Bivalves satisfy many of the requirements of sentinel organisms better than any other group of aquatic organisms. They are sedentary, filter-feeding, widely distributed, capable of concentrating pollutants to several orders of magnitude higher than the surroundings and are of reasonable size to get adequate tissues for analyses. These qualities, especially the capacity to magnify and integrate the aquatic pollutants have rendered the various species of bivalves like *Mytilus, Perna,* etc., which are widely distributed in the northern hemisphere, ideally suited for use in environmental monitoring programmes (Goldberg, 1975; Goldberg *et al.,* 1978).

Estuaries and coastal areas, which retain large amounts of land derived inputs due to the filtering capacity, are the most severely polluted regions than any other segment of water. Cochin estuary is no exception and receives a large variety of

pollutants including trace metals, pesticides, insecticides, etc. Systematic studies have been conducted to assess the harmful effects of toxicants on various species of aquatic organisms sampled from the Cochin Estuarine System (Lakshmanan, 1982; Latha, 1986; Baby, 1987; Krishnakumar, 1987; Sivadasan, 1987; Prabhudeva, 1988; Philip, 1990 and others). The studies reported in this chapter are designed to assess the acute lethal doses of copper, cadmium, mercury, arsenic and selenium (when applied individually and in binary combinations) to an estuarine clam, *Villorita cyprinoides* var.*cochinensis* maintained at a salinity 10×10^{-3}.

3.2. MATERIALS AND METHODS

Collection, transportation and acclimatization of the test animals, methods of preparation of the metal solutions, concentration of the metal solutions employed (individually and in binary combinations), experimental procedures adopted for the acute mortality studies, etc. have been described in Chapter II.

3.3. INDIVIDUAL TOXICITY STUDIES

Reports on acute mortality studies employing the metals, copper, cadmium and mercury on various molluscan species like *M.edulis, P.viridis, M.casta,* etc. which are widely distributed in the northern hemisphere are numerous (Lakshmanan and Nambisan, 1977, Davenport and Manley, 1978; Kumaraguru *et al.*, 1980; Martin *et al.*, 1981; Eknath and Menon, 1983; Mathew and Menon, 1983; Mohan *et al.*, 1984; Latha *et al.*, 1985; Amiard-Triquet *et al.*, 1986; Prabhudeva and Menon, 1986; Baby, 1987; Krishnakumar, 1987; Philip, 1990 and others). Only a few reports (Calabrese *et al.*, 1973; Martin *et al.*, 1981) are available on acute mortality studies conducted on bivalves employing arsenic and selenium; important available reports

pertain to other species of aquatic organisms and include those of Alderdice and Brett (1957), Holland *et al.* (1960), Nelson *et al.* (1976), Curtis *et al.* (1979), Ahsanullah and Palmer (1980),Hodson *et al.* (1980), Sato *et al.* (1980), Ward *et al.* (1981), Bryant *et al.* (1985), Cockell and Hilton (1988) and Johnston (1988).

Results and Discussion

The cumulative percentage mortalities (96h) of the clam on exposure to various concentrations of copper, cadmium, mercury, arsenic and selenium are given in Table 3.1. The 96h LC_{50} values calculated employing Litchfield and Wilcoxon's (1949) log-probit method are given in Table 3.2, along with the results reported in the literature for the comparison of toxicity.

Based on their lethal toxicity values to *Villorita cyprinoides* var. *cochinensis*, the trace metals employed in this study could be arranged in the following order of decreasing toxicity:

Cu > Hg > Se > Cd > As

As far as the effects of individual metals are concerned, mercury has been reported to be more toxic than copper to many species of aquatic organisms, as for example, *G.duebeni* (Moulder, 1980), *M.dobsoni* (Sivadasan, 1987), *C.magister* zoeae, (Martin *et al.*, 1981), *C.virginica* and *C.gigas*, (Calabrese *et al.*, 1977; Mac Innes and Calabrese, 1978), *M.casta* (Eknath, 1978; Mathew and Menon, 1983). But in the present investigation, a reverse trend was observed and copper was found to be slightly more toxic than mercury. Similar results have been reported in the case of *P.viridis* (Krishnakumar, 1987), *P. indica* (Baby, 1987; Prabhudeva, 1988) and *C. gigas* embryos (Martin *et al.*, 1981).

Table 3.1 Cumulative percentage mortality of V. cyprinoides exposed to metal stress

Metal	Exposure levels(ppm)	Cumulative percentage mortality (96 h)
Copper	0.4	20
	0.8	40
	1.2	60
	1.4	70
	1.6	90
Cadmium	1.0	10
	2.0	30
	4.0	50
	8.0	70
	12.0	80
Mercury	0.4	10
	0.8	40
	1.0	50
	1.2	60
	1.4	80
Arsenic	6.0	10
	8.0	20
	10.0	50
	12.0	60
	14.0	80
Selenium	1.3	0
	1.6	30
	1.9	50
	2.2	70
	2.5	90

Table 3.2 Lethal concentrations of metals to aquatic organisms

Organism	Exposure time	Lethal dose	Reference
		a) **Copper**	
C. gigas	48h LC_{50}	33ppb	Calabrese *et al.*, (1977)
C. virginica (embryos)	48h LC_{50}	103ppb	Calabrese *et al.*, (1973)
C. virginica (embryos)	48h LC_{50}	15.1ppb	Mac Innes and Calabrese (1978)
C. virginica	12d LC_{50}	33ppb	Calabrese *et al.*, (1977)
M. casta	96h LC_{50}	75ppb	Mathew and Menon (1983)
M. casta	96h LC_{50}	570ppb	Kumaraguru *et al.*, (1980)
M. edulis	96h LC_{50}	480ppb	Amiard-Triquet *et al.*, (1986)
M. edulis	10d IC_{50}	90ppb	Davenport and Manley (1978)
M. edulis	48h EC_{50}	5.8ppb	Martin *et al.*, (1981)
P. indica	96h LC_{50}	8.3ppb	Prabhudeva (1988)
P. viridis	96h LC_{50}	32ppb	Mathew and Menon (1983)
P. viridis (15-20mm)	96h LC_{50}	63ppb	Krishnakumar (1987)
(30-40mm)	96h LC_{50}	86ppb	Krishnakumar (1987)
V. cyprinoides	96h LC_{50}	840ppb	Present study

(Contd....)

Table 3.2 contd.....

Organism	Exposure time	Lethal dose	Reference
		b) **Cadmium**	
C. virginica	96h LC_{50}	3.8ppm	Calabrese *et al.*, (1973)
D. spiculum	96h LC_{50}	1.8ppm	Mohan *et al.*, (1984)
M. arenaria	96h LC_{50}	2.2ppm	Eisler (1971)
M. carvalhoi	96h LC_{50}	5.6ppm	Mohan *et al.*, (1984)
M. edulis	96h LC_{50}	1.55ppm	Amiard-Triquet *et al.*, (1986)
M. edulis	96h LC_{50}	1.62ppm	Ahsanullah (1976)
M. edulis	96h LC_{50}	25ppm	Eisler (1971)
M. edulis	96h LC_{50}	1.62ppm	Mohan *et al.*, (1984)
Modiolus sp.	96h LC_{50}	9.2ppm	Mohan *et al.*, (1984)
P. indica	96h LC_{50}	2.212ppm	Baby (1987)
P. viridis	96h LC_{50}	2.5ppm	Mohan *et al.*, (1984)
V. cyprinoides	96h LC_{50}	4.193ppm	Present study

(Contd.....)

Table 3.2 contd.....

Organism	Exposure time	Lethal dose	Reference
		c) **Mercury**	
C. edule	48h LC_{50}	10-33ppb	Connor et al., (1972)
C. gigas	48h LC_{50}	12ppb	Calabrese (1977)
C. virginica (embryos)	48h LC_{50}	5.6ppb	Calabrese et al., (1973)
C. virginica (embryos)	48h LC_{50}	10.2ppb	Mac Innes and Calabrese (1978)
C. virginica	12d LC_{50}	12ppb	Calabrese (1977)
M. carvalhoi	96h LC_{50}	19ppb	Eknath and Menon (1983)
M. casta	96h LC_{50}	42ppb	Eknath (1978)
M. edulis	7d LC_{50}	150ppb	Martin et al., (1975)
P. indica	96h LC_{50}	71.4ppb	Baby (1987)
P. viridis	96h LC_{50}	230ppb	Eknath & Menon (1983)
P. viridis (15-20mm)	96h LC_{50}	125ppb	Krishnakumar (1987)
P. viridis (20-40mm)	96h LC_{50}	155ppb	Krishnakumar (1987)
S. plana (da Costa)	5-7d LC_{50}	50ppb	Akberali and Black (1981)
V. cyprinoides	96h LC_{50}	933.8ppb	Present study

(Contd....)

Table 3.2 contd.....

Organism	Exposure time	Lethal dose	Reference
		d) **Arsenic**	
A. clausi	96h LC_{50}	0.508ppm	GESAMP, (1986)
A. irradians	96h LC_{50}	3.49ppm	Nelson *et al.*, (1976)
C. gigas (larvae)	48h LC_{50}	3.26ppm	Martin *et al.*, (1981)
C. magister zoeae	96h LC_{50}	2.32ppm	Martin *et al.*, (1981)
C. virginica	48h LC_{50}	7.7ppm	Calabrese *et al.*, (1973)
C. volutator	96h LC_{50}	6-60ppm	Bryant *et al.*, (1985)
E. masquinongy (fry)	48h LC_{50}	1.1ppm	Spotila & Paladino (1979)
M. balthica	96h LC_{50}	85ppm	Bryant *et al.*, (1985)
M. edulis	48h LC_{50}	3ppm	Martin *et al.*, (1981)
O. gorbuscha	48hTL_m	11ppm	Alderdice and Brett (1957)
O. keta	7d LC_{100}	9.5ppm	Alderdice and Brett (1957)
P. setiferus	96h LC_{50}	24.7ppm	Curtis *et al.*, (1979)
S. serrata	96h LC_{50}	32ppm	Krishnaja *et al.*, (1987)
V. cyprinoides	96h LC_{50}	10.31ppm	Present study

(Contd....)

Table 3.2 contd.....

Organism	Exposure time	Lethal dose	Reference
		e) **Selenium**	
A. compressa	96h LC_{50}	4.8-6.2ppm	Ahsanullah & Palmer (1980)
Carp	96h LC_{50}	35ppm	Sato et al., (1980)
C. magister	96h LC_{50}	1.04ppm	Glickstein (1978)
C. usitata	96h LC_{50}	6.1ppm	Ahsanullah & Palmer (1980)
C. variegetus	96h LC_{50}	7.4ppm	Ward et al., (1981)
D. magna (adult)	96h LC_{50}	0.58ppm	Johnston (1987)
(juveniles)	48h LC_{50}	0.55ppm	Johnston (1987)
(egg & embryo)	72h LC_{50}	1.4ppm	Johnston (1987)
L. rhomboides	96h LC_{50}	4.4ppm	Ward et al., (1981)
Notocalista sp.	96h LC_{50}	2.9ppm	Ahsanullah & Palmer (1980)
P. aztecus	96h LC_{50}	1.2ppm	Ward et al., (1981)
S. gairdneri	96h LC_{50}	8.1ppm	Hodson et al., (1981)
S. serrata	96h LC_{50}	49ppm	Krishnaja et al., (1987)
V. cyprinoides	96h LC_{50}	2.022ppm	Present study

Trace metal toxicity is primarily the result of the non-specific binding of metals to proteins and other macromolecules (Friedberg, 1974). This non-specific binding can modify the shape and function of macromolecules and adversely affect their biochemical and physiological processes. Most organisms regulate the distribution of the essential and non-essential metals in the tissue, at the cellular and the sub-cellular levels to minimize this non-specific binding (Mason and Simkiss, 1982;Sanders*et al.*, 1983; Mason *et al.*, 1984; Sanders and Jenkins, 1984). This regulation is mediated through soluble and insoluble metal ligands that optimize the specific binding of essential metals to appropriate macromolecules and act as sinks for excessive metals (Simkiss *et al.*, 1982). The ability of a given species to regulate metal metabolism will be a major factor in determining the relationship between metal bioaccumulation and toxicity.

Ochiai (1977) has divided metal-ion toxicity as being caused by the following three mechanisms: i) blocking of essential biological functional groups of biomolecules, ii) displacement of the essential metal-ions in biomolecules, and iii) modifications of active conformation of biomolecules.

Metals are incorporated into the biological systems through the formation of co-ordination compounds between the metal ions and the donor atoms of the ligand molecules (N, O or S). The higher toxicity of copper can be explained on the basis of the following considerations.

Usually copper and mercury exhibit variable oxidation states of +1 and +2 when present in biological systems. According to the Pearson's (1967) classification of metal ions, Cu^{2+} ion is a borderline acid whereas Cu^{+}, Hg^{+}, Hg^{2+} and Cd^{2+} ions are typical examples of soft acids. So also N- and O-containing ligands are either hard bases or borderline bases

whereas S- donor ligands are soft bases. Therefore, Cu^{2+} ions would prefer complexations with borderline bases and Cu^{+}, Cd^{2+}, Hg^{+} and Hg^{2+} ions would yield stable complexes with soft bases.

In any biological system, both the hard and soft bases will be present together and the copper ion (being present as Cu^{+} and/or as Cu^{2+}) can complex with either of them unlike Cd^{2+} or Hg^{2+} and Hg^{+} which would form stable complexes with only soft bases. The existence of Cu(I) as well as of Cu(II) in biological tissues (Birker and Freeman, 1977) lends ample evidence to this view which explains the higher affinity of copper compared with mercury.

The smaller ionic radius and increased ionic charge of Cu(II) ion as compared with those of the Hg^{2+} ion make the Cu(II) complexes much more stable than those of Hg(II).

In addition, ligand field considerations would confer an extra stabilization energy (LFSE) on the Cu^{2+}ion (a $3d^{9}$ion) by virtue of it being subjected to the Jahn-Teller effect specific to the d^{9} fields (Cotton and Wilkinson, 1972). The Cu^{2+} ion would thus be expected to occupy a distorted octahedral configuration. Cd^{2+} and Hg^{2+} ions on the other hand have d^{10} electronic configurations which are not subjected to the ligand field splitting scheme. Hence in the absence of any extra stabilization due to the LFSE, the Cd^{2+} and Hg^{2+} ions should prefer a tetrahedral configuration, it being the stereochemical arrangement which would experience the least repulsion from the ligand atoms in the coordination sphere.

Selenium toxicity has been observed to be manifested by i) inhibition of protein synthesis, ii) replacement of sulphur by selenium, iii) interactions with sulphhydryl groups of protein or cofactors and iv) depletion of methyl groups (Garberg and Hogberg, 1986).

3.4. COMBINED TOXICITY STUDIES.

Acute toxicity studies using single toxicant provide the framework for a quick and reproducible estimate of the toxic effect of the test material. Nevertheless, acute toxicities of trace metals have been reported to be influenced by various abiotic factors in the aquatic environment. Peterson *et al.*, (1984) observed that the metal toxicities to algae were highly pH dependent. The acute toxicity of arsenic was reported to be influenced by temperature (Bryant *et al.*, 1985). The effects of hardness and of humic acids on the toxicities of copper and cadmium were investigated by Winner and Gauss (1986) while Sivadasan (1987) reported on the effect of salinity on the toxicity of copper, mercury, zinc, etc.

Another significant interference on trace metal toxicity is the presence of other metals in the medium. Although trace metals like copper, zinc, manganese, molybdenum, selenium, etc. are found to be essential for the growth and nutrition of plants and animals, beyond a threshold concentration even some of these essential metals are found to become extremely toxic, the effect being almost comparable to or greater than the non essential metals like cadmium, mercury, etc. The binding sites of the metals in the biomolecules have specific stereo-chemistries dependent on the ligand types and the nature of the metal ions and hence allow only specific metals to occupy such sites. Metal ions having identical charges and sizes, irrespective of whether they are essential or non-essential, can displace these specific metals in biological materials. The interaction of zinc with cadmium, copper or mercury in producing toxicity has been well documented in the literature (Sprague and Ramsay, 1965; Hilmy *et al.*, 1987b and Ahsanullah *et al.*, 1988). The ameleoration of the toxicities of thiol complexes of copper, cadmium, mercury and arsenic caused by substitution of the thiol group by seleno-thiol group has been the subject of

several investigations (Huckabee and Griffith, 1974; Van Puymbroeck *et al.*, 1982; Winner and Whitford, 1987; Johnston, 1988). The interactions between metals may be antagonistic, synergistic or additive in nature. Antagonistic interactions refer to the protective effect of one metal on the toxicity of another and synergistic interactions refer to the enhanced toxicity of one metal in the presence of another. Additive interactions are neither antagonistic nor synergistic and the final toxicity is simply a sum of the individual toxicities.

MacInnes (1981) while discussing with the effects of concentrations of metals used in mixture toxicity studies on *C.gigas* embryos, explained that at low concentrations metal ions might have formed complexes with each other, with other ions and with organic ligands in the medium and consequently resulted in only a depleted amount of the metal ions being available for interaction with the biological system. These interactions could account for a merely additive (simple additive) or even an antagonistic effect, whereas at high concentrations the increased availability of ions might be sufficient to overcome the complexing tendency and also to provide an adequate concentration of the metal ions which can result in a synergistic effect.

Synergistic effects of copper and cadmium were reported by Sprague and Ramsay (1965), Eaton (1973), Eisler and Gardner (1973), D'Agostino and Finney (1974), Negilski *et al.* (1981), de March (1988) and others, whereas Roch and Mc Carter (1984) observed an additive effect of copper and cadmium to the fish *S.gairdneri*. An additive effect of copper-cadmium mixture on*P. viridis* was observed by Philip (1990).

Barnes and Stanbury (1948) identified copper-mercury synergism to the copepod *N. spinipes* whereas Corner and Sparrow (1956) reported synergistic effect only with selective

concentrations in the case of *A. clausi*. An additive effect of copper and mercury was noticed by Philip (1990) in the case of bivalves.

Murakami *et al.* (1976) has reported an additive effect of cadmium and mercury mixture on the developing sea urchin eggs whereas antagonistic effects were reported by Reeve *et al.* (1977) for copepod populations. Synergism between cadmium and mercury was noticed by Stratton and Corke (1979) on *A. inequalis*, Mohan *et al.* (1986a) on *P. viridis* and Baby (1987) on *P. viridis* and *P. indica*. On the other hand, Breittmayer *et al.* (1980) reported antagonistic effect of cadmium and mercury on the mussel *M. edulis* and Vranken *et al.* (1988) also observed cadmium-mercury antagonism on a nematode *M. disjuncta*.

The interactive effects of copper-zinc and cadmium-zinc mixtures have been documented in the literature. A synergistic effect of copper-zinc and cadmium-zinc mixtures was noticed by Sprague and Ramsay (1965), Eaton (1973), Eisler and Gardner (1973), Hilmy *et al.* (1987b) etc. The combinations of zinc-copper (Lloyd, 1961; Brown and Dalton, 1970) and zinc-cadmium (Thorp and Lake, 1974) have been reported to be strictly additive according to the toxic unit concept. Antagonistic effect of copper and zinc as well as of cadmium and zinc was also reported by (Negilski *et al.*, 1981). Manganese was found to ameleorate the toxicity of cadmium to the algae, *D. salina* (Rebhun and Ben-Amotz, 1988). Simple additive effect of mercury and zinc was reported by Baby (1987) for *P. indica*. A similar result was noticed in the case of a nematode *M. disjuncta* by Vranken *et al.* (1988).

The protective effect of selenium on mercury and cadmium toxicity was explored by Heisinger (1981) on the fish *G. lappid* with well developed livers. The interactive effects of selenium on the toxicity of copper, cadmium, mercury and arsenic have

been reviewed by Frost and Lish (1975), Vokal-Borek (1979) and Pelletier (1985). The antagonistic effects of selenium on copper and cadmium have been reported by Winner (1987) and Van Puymbroeck *et al.* (1982) on *D.pulex* and *L. stagnalis* respectively. The interactive effects of selenium deficiency and cadmium stress on *D. magna* were investigated by Winner and Whitford (1987). Selenium-mercury antagonism was reported by Johnston (1988) in the growth of *D. magna* and by Lucu and Skremblin (1981) in the shrimp *P. elegans*. A strong synergistic effect of mercury and selenium towards the carp eggs was observed by Huckabee and Griffith (1974) whereas the effects were antagonistic to the fertilized eggs of the rainbow trout *S. gairdnei* and the lake trout, *S. namaycush* (Klaverkamp *et al.*, 1983).

Several mathematical models have been proposed to quantify the toxic effects of mixtures of toxicants and to predict their interactive effects. Joint action of mixtures were defined by Plackett and Hewlett (1952), Sprague and Ramsay (1965), Anderson and Weber (1975), Marking and Dawson (1975), Konemann (1981), EIFAC (1987), de March (1988), and others. Of these, the models developed by Marking and Dawson (1975) and by Konemann (1981) have been used herein to explain the interactive effects of binary mixtures of copper, cadmium, mercury, arsenic and selenium, on the clam *Villorita cyprinoides* var. *cochinensis*.

Marking and Dawson's (1975) method invoked the use of the toxic unit concept (Sprague and Ramsay, 1965) which is a re-definition of the simple similar action which was based on the presumption that each chemical in a mixture could be replaced by an aquatic concentration of another chemical with simple similar action without changing the toxic response. In mathematical terms, the standard response (probability of survival at exposure to a mixture) would occur at

$$\sum_{i=1}^{n} f_i = 1$$

where, $$f_i = \frac{C_i}{LC_{50}}$$

for a mixture of 'n' chemicals with identical f_i values giving 50% mortality the concentrations of the separate chemical will be LC_{50} i/n.

The sum of biological activity S, according to Marking and Dawsons' methods is given by,

$$S = \frac{A_m}{A_i} + \frac{B_m}{B_i}$$

where A and B are the chemicals, i and m are the toxicities (LC_{50} values) of the individual metals and of the metals in combinations respectively. Combined toxic action is considered to be,

less than additive if	$S > 1$
more than additive if	$S < 1$
and simply additive if	$S = 1$

The additive indices were then derived from the values of S as follows:

For $S < 1.0$, the additive index $= \frac{1}{S} - 1.0$

For $S > 1.0$, the additive index $= S(-1) + 1.0$.

The ranges of the additive indices were calculated by substituting the values of the 95% confidence intervals in the formula for S. The lower limit of the individual toxicant (A_i and B_i) and the upper limit of the mixture (A_m and B_m) were substituted for LC_{50} values to determine the lower limit of the index. Similarly, the upper limit of the individual toxicant and the lower limit of the mixture were substituted into the formula to determine the upper limit of the index. If the limit overlapped zero, the combined effect could be considered to be simple additive. The 95% confidence interval influences the significance of the additive index which in turn was influenced by the number of concentrations and the number of test organism per concentration. Therefore, well planned toxicity tests which would yield narrow confidence intervals were the most useful in determining the effects of chemical mixtures (Marking, 1985).

Konemann (1981) in his Mixture Toxicity Index (MTI)scale, selected the conditions of "no addition" (where the toxicity of the mixture did not exceed that of the compound present at the highest toxic concentration) and of "concentration addition" (where the sum of the ratios of the concentration of individual chemical in the mixture to their LC_{50} values was equal to one) as the two reference points (Fig.3.1) for predicting the

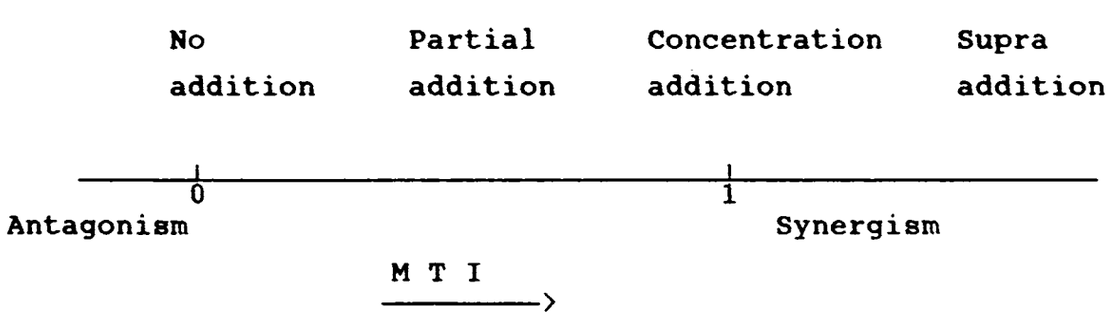


Fig.3. 1 Mixture toxicity index scale

interactive effects of toxicants. These reference points had constant values - independent of the number of compounds in the mixtures and the scale had a log form because of the log- normal distributions of LC_{50}. For equitoxic mixtures the MTI is defined as,

$$MTI = 1 - \frac{\log M}{\log n}$$

where n = number of toxicants in the mixture

M = fi

$f_i = C_i/LC_{50}$

C_i = Concentration of the component "i" in the mixture causing 50% mortality.

MTI values of zero and one represented "no addition" and "concentration addition" respectively. Values less than zero (negative) showed antagonistic effects, those between zero and one indicated partial addition and those above one referred supra-addition (synergistic effect) (Van Leeuwen and Hermens, 1988).

Results and Discussion

The cumulative percentage mortalities (96h) of the animals during exposure to binary mixtures of copper, cadmium, mercury, arsenic and selenium are given in Table 3.3. The 96h LC_{50} values with confidence limits, the additive indices along with their ranges and the MTI values of the binary mixtures are given in Table 3.4. Fig. 3.2 depicts the comparison of individual and combined toxicities of the metals.

As observed from Table 3.4, the negative additive indices with negative ranges (both lower and upper) obtained according to the method of Marking and Dawson (1975) in the case of binary mixtures of copper with cadmium and with selenium indicated

Table 3.3 Cumulative percentage mortality of V. cyprinoides exposed to metal stress

Metals	Exposure levels (ppm)	Cumulative percentage mortality (96 h)
Copper + Cadmium	0.1 + 0.4	0
	0.2 + 0.8	10
	0.4 + 1.6	20
	0.8 + 3.2	40
	1.2 + 4.8	50
Copper + Mercury	0.1 + 0.1	0
	0.2 + 0.2	10
	0.4 + 0.4	50
	0.8 + 0.8	70
	1.2 + 1.2	90
Copper + Arsenic	0.1 + 1.0	0
	0.2 + 2.0	10
	0.4 + 4.0	20
	0.8 + 8.0	70
	1.2 +12.0	90
Copper + Selenium	0.1 + 0.2	0
	0.2 + 0.4	0
	0.4 + 0.8	10
	0.8 + 1.6	30
	1.2 + 2.0	50
Cadmium + Mercury	0.4 + 0.1	0
	0.8 + 0.2	20
	1.6 + 0.4	50
	3.2 + 0.8	70
	4.8 + 1.2	80

Contd.....

Table 3.3 Contd....

Metals	Exposure levels (ppm)	Cumulative percentage mortality (96 h)
Arsenic + Cadmium	1.0 + 0.4	0
	2.0 + 0.8	0
	4.0 + 1.6	10
	8.0 + 3.2	40
	12.0 + 4.8	50
Arsenic + Mercury	1.0 + 0.1	0
	2.0 + 0.2	0
	4.0 + 0.4	20
	8.0 + 0.8	40
	12.0 + 1.2	70
Arsenic + Selenium	1.0 + 0.2	0
	2.0 + 0.4	0
	4.0 + 0.8	10
	8.0 + 1.6	20
	12.0 + 2.0	50
Selenium + Cadmium	0.2 + 0.4	0
	0.4 + 0.8	0
	0.8 + 1.6	30
	1.6 + 3.2	60
	2.0 + 4.8	80
Selenium + Mercury	0.2 + 0.1	0
	0.4 + 0.2	0
	0.8 + 0.4	10
	1.6 + 0.8	20
	2.0 + 1.2	40

Table 3.4 Quantification of interactive effects

	Metals	LC50 (Confidence Limits)	S	Additive Index (Range)	MTI
Cu+Cd	Cu	1.19 (1.09- 1.29)	2.54	-1.54 (-2.86-0.99)	-0.35
	Cd	4.74 (4.32-5.21)			
Cu+Hg	Cu	0.48 (0.4058-0.5619)	1.08	-0.08 (-0.49-0.27)	0.89
	Hg	0.48 (0.41-0.56)			
Cu+As	Cu	0.56 (0.45-0.69)	1.2	-0.2 (-0.72-0.19)	0.74
	As	5.56 (4.49-6.88)			
Cu+Se	Cu	1.22 (1.15-1.30)	2.52	-1.52 (-0.93-2.30)	-0.33
	Se	2.1542 (1.74-2.68)			
Cd+Hg	Cd	1.83 (1.62-2.06)	0.92	0.08 (-0.14-0.33)	1.11
	Hg	0.46 (0.41-0.51)			

Contd.....

Table 3.4 Contd.....

	Metals	LC50 (Confidence Limits)	S	Additive Index (Range)	MTI
Cd+As	Cd	4.45 (3.54-5.6)	2.13	-1.13 (-1.64-⁻0.57)	-0.09
	As	10.61 (8.90-12.66)			
Cd+Se	Cd	2.49 (2.33-2.66)	1.18	-0.1844 ^(0.23-⁻0.03)	0.76
	Se	1.19 (1.01-1.42)			
Hg+As	Hg	0.85 (0.68-1.06)	1.73	-0.731 (-1.32-⁻0.29)	0.21
	As	8.52 (6.83-10.62)			
Hg+Se	Hg	1.75 (1.10-2.77)	2.68	-2.3575 (-4.22-⁻0.38)	-0.75
	Se	3.01 (1.53-5.90)			
As+Se	As	11.68 (9.86-15.87)	2.17	-1.17 (-2.73-⁻0.42)	-0.12
	Se	2.12 (1.08-4.14)			

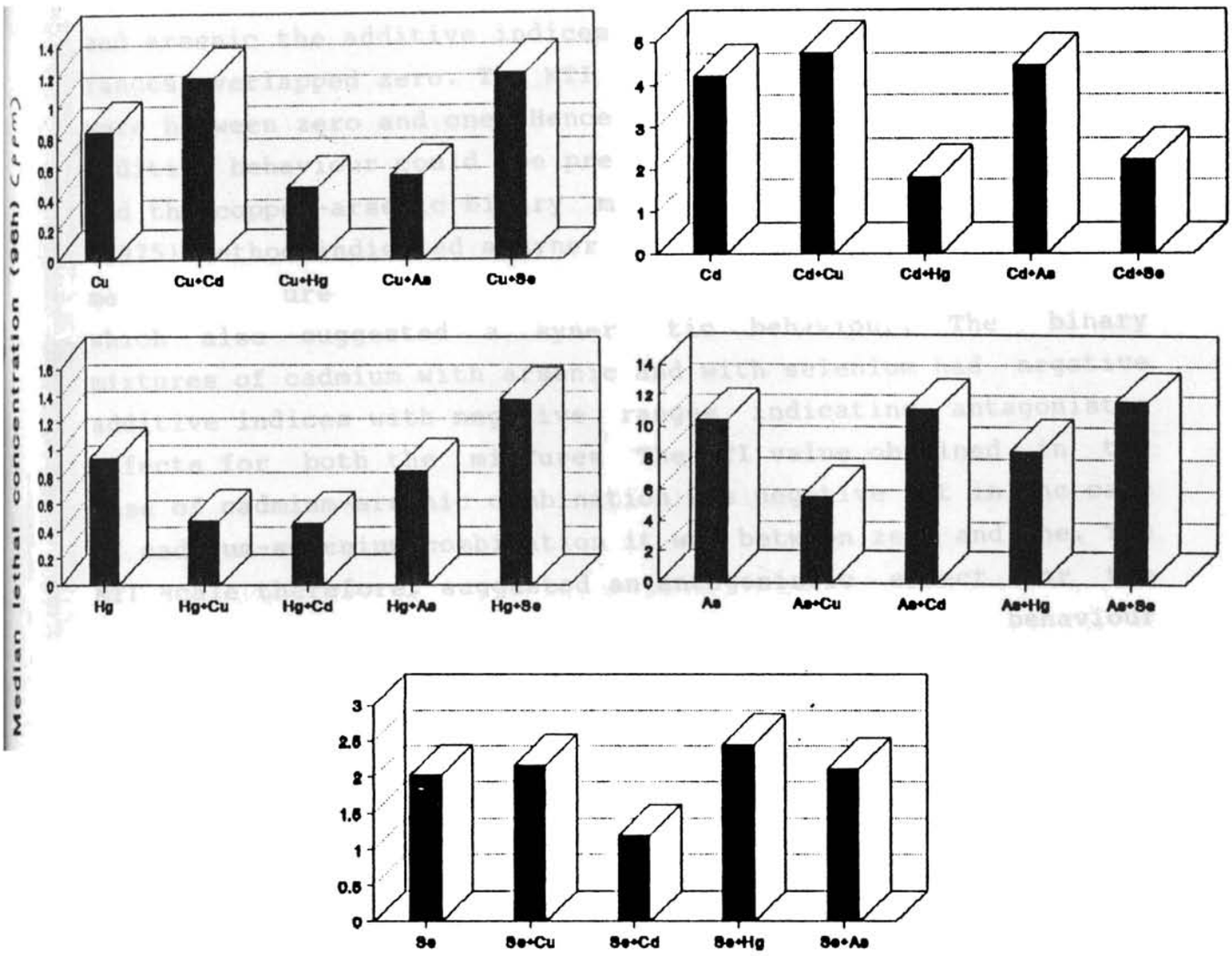


Fig.3.2 Interactive effects of metals on lethal toxicities

antagonistic effects for the above two mixtures. The negative MTI values, according to Konemann's (1981) method confirmed the antagonistic effect of copper-cadmium and copper- selenium mixtures. In the case of binary mixtures of copper with mercury and arsenic the additive indices obtained were negative and the ranges overlapped zero. The MTI values obtained in both cases were between zero and one. Hence according to both models an additive behaviour could be predicted for the copper- mercury and the copper-arsenic binary mixtures. Marking and Dawson's (1975) method indicated a synergistic effect for cadmium and mercury mixture and the MTI value obtained was higher than one, which also suggested a synergistic behaviour. The binary mixtures of cadmium with arsenic and with selenium had negative additive indices with negative ranges indicating antagonistic effects for both the mixtures. The MTI value obtained in the case of cadmium-arsenic combination was negative but in the case of cadmium-selenium combination it was between zero and one. The MTI scale therefore, suggested an antagonistic effect for the cadmium and arsenic mixture and a partial additive behaviour for the cadmium-selenium combination. The additive indices, their ranges and the MTI values were all negative in the case of binary mixtures of mercury with arsenic and selenium. Hence antagonistic effects were indicated for the above mixtures of mercury. The additive index value, their ranges and MTI values were all negative for arsenic-selenium combination also and therefore indicated an antagonistic behaviour.

The antagonistic/ additive effects of the binary mixtures of copper, cadmium and mercury may be due to i) complementary accumulation of the metals onto the binding sites,ii) complex formation between the trace metals in the presence as well as in the absence of organic ligands making the toxicants unavailable to the organisms and alteration of the chemical form of the trace metals into less toxic species in the various tissues, or iii) diversion of the toxic metal to less sensitive organs.

Moulder (1980) ascribed the less than additive effect of copper and mercury observed on *G. duebeni* to the possibility of copper-mercury complex formation in the presence or absence of organic matter, copper occupying the binding sites on the surfaces of the gammarid and copper detoxifying mercury within the tissue, thereby altering the chemical form of mercury to a less toxic chemical species.

Sharma *et al.* (1985) opined that the toxicity of cadmium could be influenced by other metals as a consequence of competition for membrane sites, diversion of cadmium from low molecular weight protein to less sensitive high molecular weight ones and alterations in organ distribution.

Selenium has been reported to displace sulphur in biological tissues and to bind the thiol group in various biomolecules. Hence formation of complexes between the selenothiol- group and the trace metal ions may be one of the reasons for the reduced toxicity of binary mixtures of selenium with copper, cadmium and mercury. The insolubility of the selenides of copper, mercury and cadmium might be another reason for their reduced toxicity. Metallic selenides thus sequester both selenium and the trace metals. Formation of water soluble complexes by the reaction of inorganic mercury with selenium in the presence of glutathione was reported by Imura and Naganuma (1978), Naganuma *et al.* (1982), Naganuma and Imura (1983), etc. This water soluble black complex was unstable and decomposed to give an inorganic water insoluble residue which led to the regeneration of glutathione (GSH), the mechanism of which could be represented as.

$$SeO_3^{2-} \xrightarrow{GSH} GS\ Se\ SG \underset{}{\overset{Hg^{+2}}{\rightleftharpoons}} (GS\ Hg\ Se\ SG)$$

$$GSSG + Hg\ Se$$

A similar mechanism was also put forward by Ridlington and Whagner (1981).

A complementary accumulation of selenium and mercury by saturation of the available sites or by replacement of mercury without toxic effects was suggested by Leonzio *et al.* (1982) to account for the toxicological antagonism of selenium in presence of mercury.

Arsenic has since been used successfully to alleviate selenium poisoning in pigs, dogs, chicks and cattle. Reciprocal inhibitory uptake of arsenic and selenium by the respiring mitochondria was suggested as the reason for counteraction of the above elements in uncoupling oxidative phosphorylation (Underwood, 1979). Enhanced loss of selenium from the body induced by arsenic might be responsible for the observed protective effect against selenosis. Perhaps, arsenic react with selenium *in vivo,* possibly as Se^{-2} to form $As^{+}S$ complex. According to the concept of Hill and Matrone (1970), trace elements with similar electronic structures such as Se^{4+}, As^{3+} are likely to be biological antagonists due to reciprocal inhibition of uptake.

Chapter IV

CHAPTER IV

KINETICS OF UPTAKE AND DEPURATION

4.1. INTRODUCTION

Bioassays are necessary in aquatic pollution evaluations because chemical and physical tests alone are not sufficient to assess the potential effects on aquatic biota. An aquatic bioassay is a procedure in which the response of aquatic organisms are used to detect or measure the presence or effect of one or more substances, wastes, or environmental factors, alone or in combinations (APHA, 1985). Bioassays are classified according to the duration, the method of adding test solutions and the purpose and include lethal toxicity tests, assessment of uptake/depuration rates as well as the measurement of the biochemical/physiological responses of the test organism.

Bioaccumulation can occur only if the rate of uptake of a chemical by an organism exceeds its rate of elimination. Although several biochemical processes are involved in xenobiotic transport, the general modes of uptake occur from water, sediment and food. After chemicals have been taken up, mechanisms controlling their distribution in tissue and elimination become important in bioaccumulation process. The case of metals and their binding to metallothioneins is an example where retention by inducible but metabolically inactive biochemical moieties markedly influences accumulation. Accumulation and retention of metals continues as along as metallothionein synthesis occur. When exposure to metals ceases, elimination of excess burdens of many, but not all occurs. If a chemical can be eliminated rapidly, residues will not accumulate and tissue damage is unlikely. The toxicity and the

bioaccumulation potential of a foreign compound are thus significantly controlled by the rates of uptake and depuration. Based upon the rate of loss of a metal, its biological half-life can be determined. The biological half-life may serve as a warning about the persistence, and potential for cumulative biological effects of a chemical (Buikema *et al.*, 1982). Hence accumulation/depuration studies are particularly important and are integral part of bioassays.

Studies on the accumulation of trace metals have been well documented in the literature. Accumulation of metals as a linear function of time over the period of investigation was reported (Sandholm *et al.*, 1973; Bryan, 1976; Fowler and Benayoun, 1976a and b; Phillips, 1977b; D'Silva and Kureishy, 1978; Lakshmanan and Nambisan, 1979 and 1989; Scholz, 1980; Ritz *et al.*, 1982; Poulsen *et al.*, 1982; Fraizer and George 1983; Tan Yanxiang, 1984; Martincic *et al.*, 1984; Amiard-Triquet *et al.*, 1986; Luten *et al.*, 1986; King and Davis, 1987; Chan, 1988; Giles, 1988; Holwerda *et al.*, 1989; Marigomez and Ireland, 1989; Ringwood, 1989; Van Haren *et al.*, 1990).

The possibility of interaction during accumulation is a major source of uncertainty in the "Mussel Watch" approach to environmental monitoring programme. The trace metals like copper, zinc, selenium, molybdenum, etc. are essential for the growth and metabolism of animals and are present at the active centres of various biomolecules, proteins, enzymes, etc. The incoming metals having identical charge, size, and ligand specificity of the essential metals may replace or displace the essential metals, resulting in distortion conformation of the biomolecules which may ultimately result in toxicity. These interactions may result in stimulation or decrement in the uptake of metals. The bioavailability of the concerned metal may be influenced by the presence of other metals, due to difference in reactivity, complexation in the presence or absence of

organic ligands in the medium etc. The interactive effects of metals during accumulation has been well documented (Eisler and Gardner, 1973; Blaylock *et al.*, 1974; Fowler and Benayoun 1976c; Moulder, 1980; Carpene and George, 1981; MacInnes, 1981; Bjerregaard, 1985, 1988; Baby, 1987; Cuvin and Furness, 1988; Prabhudeva, 1988; Boisson *et al.*, 1989;).

The loss or elimination of trace metals from aquatic organisms has been investigated by Cunningham and Trip (1973), Scholz (1980), Denton and Burden-Jones (1981), Howell (1983), Luten *et al.* (1986), Cuvin and Furness (1988) and Lakshmanan and Nambisan (1989).

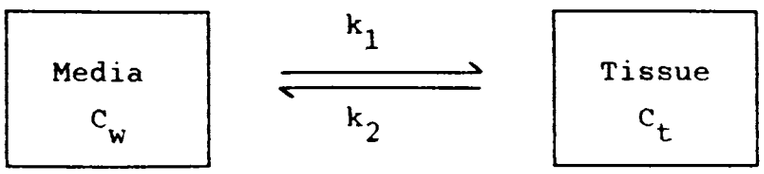
Various abiotic factors such as salinity, temperature, hardness, etc. are reported to influence the accumulation of the trace metals (Denton and Burdon-Jones, 1981; Winner and Gauss, 1986; Winner and Whitford, 1987). Low salinity and hardness and elevated temperatures, all tend to maximise the bioconcentration of metals in aquatic animals.

This chapter reports the results of studies designed to assess and quantify the process of uptake (from water) and depuration of the trace metals —copper, cadmium, mercury, arsenic and selenium- applied individually and in binary combination on an estuarine clam, *Villorita cyprinoides* var. *cochinensis* at salinity $10x10^{-3}$ for a period of 96h. In view of the uneven accumulation of trace metals in the different organs of the animals, whole soft tissue analyses were employed in this study.

4.2. MATERIALS AND METHODS

The materials used and the experimental procedures employed for the accumulation/depuration studies are described in Chapter II.

The bioaccumulation process under unfed laboratory conditions can be described kinetically as a concurrent, two-compartmental uptake and depuration (Bruggeman, 1988) which can be expressed as



$$\frac{dC_t}{dt} = k_1 C_w - k_2 C_t \quad .....(1)$$

where Cw = concentration in water
C_t = concentration in tissue
k_1 = 1st order uptake rate constant
k_2 = 1st order depuration rate constant

During the depuration period, the concentration in the water is virtually zero. The depuration rate is then only a function of the accumulated metal concentration C_t

i.e. $$\frac{dC_t}{dt} = -k_2 C_t \qquad \text{at } C_W = 0$$

$$\frac{dC_t}{C_t} = -k_2\, dt \quad .....(2)$$

Integrating the above equation,

$$\log C_t = -k_2 t + \text{a constant} .....(3)$$

Therefore, a plot of log C_t vs t would yield a straight line, the slope of which would be k_2.

Integration of equation (1) is possible only when k_1, k_2 and C_w

are taken to be constants. Equation (1) would therefore become

$$C_t = \frac{k_1}{k_2} C_w (1-e^{-k_2 t}) \quad .....(4)$$

k_1 can now be evaluated by substituting the value of k_2 obtained from equation (3).

The rate of accumulation would be dependent on the laboratory conditions, the metal concentration in the experimental medium and the metabolic condition of the animal which is exposed to the metal stress. Under controlled laboratory conditions (of temperature, pH, salinity, dissolved oxygen etc.), the uptake process would be defined mainly by the latter two. At any instant of time, the accumulation process would be associated with concomitant depuration as well and hence, the metabolic condition of the animal would have contribution from not only the external stress (induced by applied metal concentration) but also from the internal stress (induced by the metal accumulated within the tissue).

For an organism which has been transferred from a metal-containing medium to a metal-free medium, the external stress is absent and the metabolic condition of the animal during this depuration stage would mainly be a function of the internal stress. The contribution of the applied metal concentration to the metabolic condition which is manifested during the accumulation phase is referred to as M_{CA} and the contribution of the internal stress to the metabolic condition, in the depuration process is denoted as M_{CD}. M_{CA} would be a function of the external metal stress, C_w, whereas M_{CD} would be a measure of the metal concentration within the tissue, which would in turn be a proportional to the applied metal concentration, i.e.,

$$M_{CA} \propto C_W$$

and $$M_{CD} \propto C_t \propto C_W$$

The bioaccumulation process can now be quantified as

$$\frac{dC_t}{dt} = k_1' M_{CA} C_W - k_2' M_{CD} C_t \quad \ldots\ldots (5)$$

Comparing equations (1) and (4)

$$k_1 = k_1' M_{CA} \quad \text{and} \quad k_2 = k_2' M_{CD}$$

By definition, $M_{CA} \propto C_w$ and $M_{CD} \propto C_t \alpha C_w$

Therefore $k_1 \propto C_w$ and $k_2 \propto C_w$

$$k_1 = \beta C_w + k_1^0 \quad \text{and} \quad k_2 = \alpha C_w + k_2^0$$

where k_1^0 and k_2^0 represent the rate coefficients at ideal conditions. (i.e. when $M_{CA} = 0$ and $M_{CD} = 0$ respectively) and " α " and " β " are constants for a specific metal and for a specific species, which define the nature and extent of metal interactions in the manifestations of metal toxicities. The slope of the plot of k_2 vs C_w would be α and the slope of the plot of k_1 vs C_w would yield β.

Again, from equation (1) ,

At equilibrium, $\frac{dC_t}{dt} = 0$

Therefore, $k_1 C_W = k_2 C_t$ or

$$K_{bc} = \frac{C_t}{C_W} = \frac{k_1}{k_2}$$

where K_{bc} represents the **bioconcentration factor.**

Half-life, which is the time taken for depuration of half of the accumulated metal content i.e., $t_2 - t_1 = t_{1/2}$ can be

obtained by applying the limits C_t at time t_1 and C_{t_2} at time t_2. Since $C_{t_1} = 2\ C_{t_2}$,

$$t_{1/2} = \frac{\ln 2}{k_2}$$

4.3. RESULTS AND DISCUSSION

Metal uptake or bioaccumulation is determined by processes occurring at the environmental interface of an organism and these include (i) characteristics of the interface (ii) reactivity of the metal species (iii) presence of other metals or major cations which may antagonize or stimulate metal uptake and (iv) abiotic factors such as temperature which affect the rate of biological or chemical reactions (Luoma, 1983).

i) Characteristics of the environmental interface.

Most trace metals traverse the environmental interface of organisms *via* diffusion processes. The mechanism of this carrier facilitated uptake could include (a) transport *via* carrier specific molecules for nutritionally essential cations (e.g. Ca, Mo, Zn) (b) non-specific complexation of metal forms with carrier molecules, which could result in either "accidental" transport across the interface or immobilisation at the external interface, (c) transport of metals complexed with essential nutrients (e.g. amino acids and proteins) or carrier specific molecules for the nutrients, or (d) transport of nutritionally essential metal complexes. Metal forms which are lipid soluble or of reduced polarity could cross membranes *via* diffusion. Metal uptake by endocytosis in molluscs has also been documented (Luoma, 1983; Simkiss and Mason, 1984).

ii) Reactivity of the metal forms with the biological interface.

The effects of methylation on metal availability are best known for mercury and arsenic. Methylated mercury is reported to be several times more bioavailable and toxic to biota than inorganic forms of mercury. Although metallo-organic forms of arsenic may also be more bioavailable than its inorganic forms, organic arsenic is found to be less toxic than inorganic arsenic.

The oxidation state of the metal is another factor that affects the availability of mercury, selenium and chromium. In general metallic mercury (Hg^0) is more available than Hg^{2+} because of the lipid solubility of the former. Se^{2+} and Cr^{6+} are more available to mussels than Se^{6+} and Cr^{3+}.

Temperature also affects the quantity of metal accumulated by an organism since biological processes typically double in rate with every $10^{o}C$ increment in temperature. Redox potential and pH are other factors which strongly affect the metal bioavailability because of their high influence on metal partitioning and speciation.

iii) Presence of other metals and major cations.

Interactive effects among metals may be a very important factor influencing metal availability to organism. Several types of interactive influences are possible: (a) enhancement of uptake (b) competitive displacement on metal specific binding protein in tissue and (c) competitive inhibition of uptake at the environmental interface.

Stimulation of the uptake of one metal by exposure to another may occur, if the first metal induces synthesis of

binding sites (e.g. metallothionein-like proteins) which affect the accumulation of both metals. Enhancement of uptake may also occur at high exposures to some metals if membrane integrity is disrupted. Antagonism of availability is commonly observed in exposures to more than one metal. Antagonism among metals depends both upon the characteristics of the ligands binding the metals and on the ratio of the metal concentrations to the ligand concentrations. The dependence of metal uptake on reactions with ligands on carrier molecules suggest that major cations also should influence availability. The intensity of calcium and magnesium binding to organic ligands is similar to that of most trace metals. Thus calcium and magnesium should inhibit at least some metal-organic interactions.

Since this investigation was carried out under controlled laboratory conditions (*vide* Chapter II) the influence of the media was limited to the stress provided by the dosed metal concentrations.

Accumulation studies

The concentrations of trace metals accumulated in the soft tissue of bivalves observed at designated time intervals of the accumulation phase of the experiment (24h, 48h, 72h, and 96 h), are given in Tables 4.1 - 4.5. The tissue concentration of the metals (C_t) was taken as the difference between the concentration at time "t" (C) and that at zero time (C_o) i.e. control.

$$\text{i.e.} \qquad C_t = C - C_0$$

The plots of C_t vs $1 - e^{-K_2 t}$ are depicted in Fig. 4.1 - 4.5 The rate constants of accumulation are given in Table 4.11.

Table 4.1 Tissue concentration of copper (µg/g dry weight) in V. cyprinoides exposed to copper and its binary combinations (Accumulation phase)

Exposure levels (ppm)	Time (h) n = 6							
	24		48		72		96	
	µg/g	SD	µg/g	SD	µg/g	SD	µg/g	SD
Control	8.86	0.75	9.19	1.01	8.53	0.98	10.05	1.12
Cu (0.1)	13.23	1.82	18.09	1.65	26.18	2.31	31.28	3.18
Cu (0.1) +Cd (0.5)	10.42	1.01	14.64	1.23	16.76	1.56	22.46	2.03
Cu (0.1) +Hg (0.1)	11.95	1.09	13.03	1.23	14.12	1.34	18.76	1.46
Cu (0.1) +As (1.0)	10.79	1.11	12.10	1.19	15.42	1.43	17.57	1.56
Cu (0.1) +Se (0.2)	10.28	1.18	15.94	1.29	17.99	1.47	20.24	1.54
Cu (0.3)	16.34	1.91	20.34	1.83	28.10	2.42	44.78	3.58
Cu (0.3) +Cd (1.5)	14.51	1.28	16.68	1.46	24.64	1.98	33.53	2.49
Cu (0.3) +Hg (0.3)	14.55	1.51	18.63	1.63	21.53	2.13	24.10	2.04
Cu (0.3) +As (3.0)	15.76	1.38	17.62	1.49	23.62	1.51	28.98	2.56
Cu (0.3) +Se (0.6)	14.61	1.37	15.42	1.53	18.64	1.79	22.34	1.99

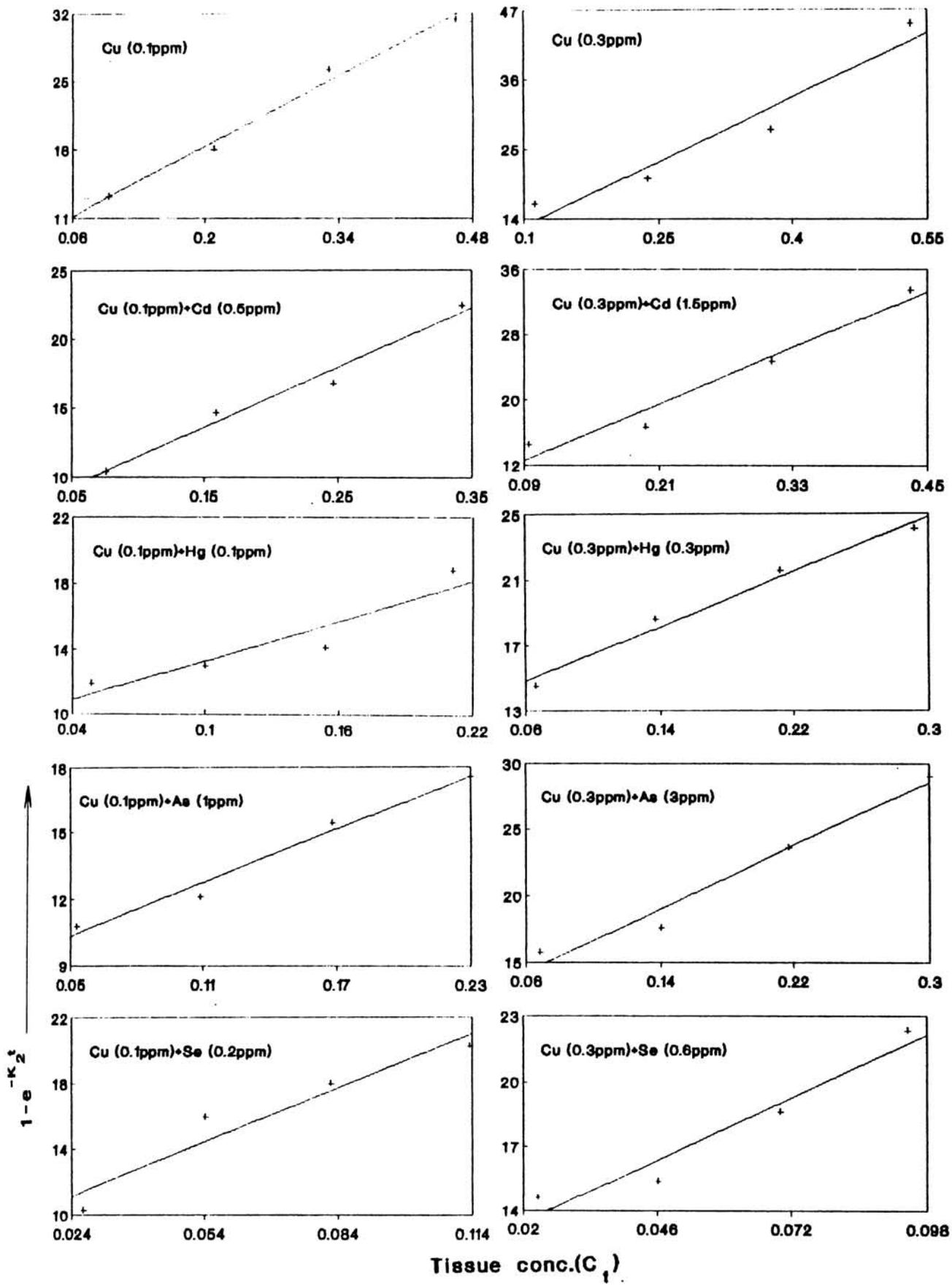


Fig.4.1 Plot of C_t *vs* $1-e^{-K_2 t}$

Table 4.2 Tissue concentration of cadmium (µg/g dry weight) in V. cyprinoides exposed to cadmium and its binary combinations (Accumulation phase)

Exposure levels (ppm)	Time (h) n = 6							
	24		48		72		96	
	µg/g	SD	µg/g	SD	µg/g	SD	µg/g	SD
Control	5.98	0.09	5.57	0.09	6.44	0.07	6.13	0.06
Cd (0.5)	16.86	1.98	22.72	1.97	30.11	2.46	33.29	3.18
Cd (0.5) +Cu (0.1)	4.08	0.91	10.81	1.03	17.24	1.64	22.68	2.18
Cd (0.5) +Hg (0.1)	8.76	0.99	16.11	1.56	20.07	2.11	26.81	2.48
Cd (0.5) +As (1.0)	10.98	0.91	15.37	1.31	18.84	1.45	22.04	1.76
Cd (0.5) +Se (0.2)	13.76	1.43	23.95	1.46	27.92	2.14	38.49	2.23
Cd (1.5)	23.79	2.41	47.54	3.76	68.26	4.85	71.24	4.23
Cd (1.5) +Cu (0.3)	6.99	0.93	12.50	1.18	23.75	2.16	38.02	3.32
Cd (1.5) +Hg (0.3)	18.77	1.84	23.76	2.43	42.39	3.95	50.48	4.52
Cd (1.5) +As (3.0)	23.73	1.13	33.18	1.98	36.44	2.31	48.64	3.23
Cd (1.5) +Se (0.6)	32.73	2.98	47.39	3.56	51.90	4.12	59.48	3.42

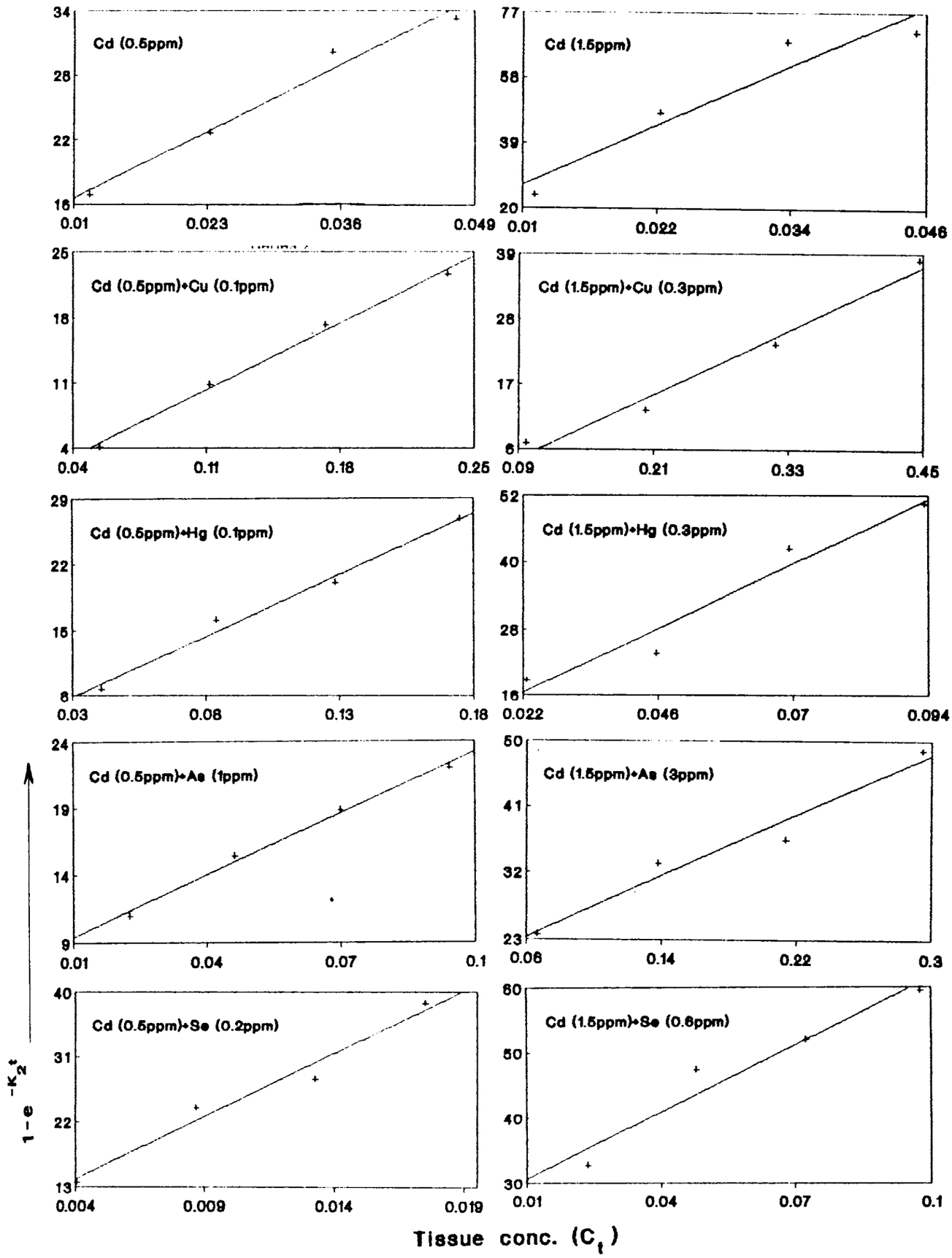


Fig.4.2 Plot of C_t *vs* $1-e^{-K_2 t}$

Table 4.3 Tissue concentration of mercury (µg/g dry weight) in V. cyprinoides exposed to mercury and its binary combinations (Accumulation phase)

Exposure levels (ppm)	Time (h) n = 6							
	24		48		72		96	
	µg/g	SD	µg/g	SD	µg/g	SD	µg/g	SD
Control	0.15	0.03	0.16	0.02	0.13	0.02	0.14	0.01
Hg (0.1)	10.71	1.01	15.48	1.31	24.81	1.48	30.03	1.72
Hg (0.1) +Cu (0.1)	8.19	0.93	15.50	1.23	18.27	1.84	22.44	1.98
Hg (0.1) +Cd (0.5)	10.21	1.12	16.65	1.48	20.23	1.90	29.54	1.44
Hg (0.1) +As (1.0)	8.56	0.48	10.88	1.16	21.06	1.45	27.06	1.56
Hg (0.1) +Se (0.2)	7.97	0.56	12.47	1.41	15.13	1.48	23.86	1.93
Hg (0.3)	16.48	1.44	33.22	2.14	40.80	3.21	58.91	3.91
Hg (0.3) +Cu (0.3)	12.16	1.14	16.54	1.24	21.78	1.35	29.07	1.48
Hg (0.3) +Cd (1.5)	15.76	1.08	20.69	2.11	32.39	2.48	42.29	2.33
Hg (0.3) +As (3.0)	15.33	1.43	21.52	1.99	29.49	2.41	35.20	2.95
Hg (0.3) +Se (0.6)	9.59	0.99	23.02	1.44	25.55	2.15	39.47	1.99

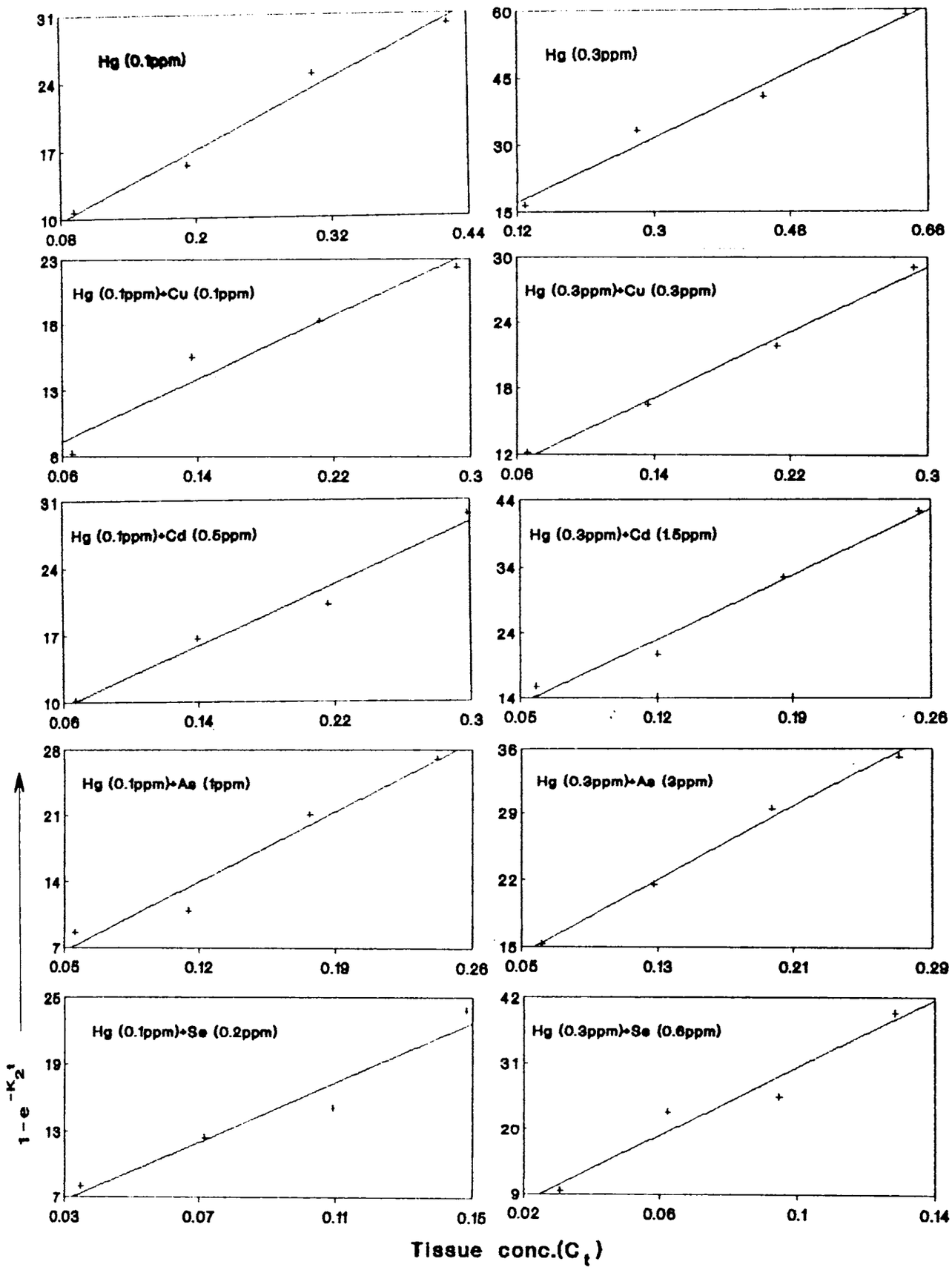


Fig.4.3 Plot of C_t *vs* $1-e^{-K_2t}$

Table 4.4 Tissue concentration of arsenic (µg/g dry weight) in V. cyprinoides exposed to arsenic and its binary combinations (Accumulation phase)

Exposure levels (ppm)	Time (h) n = 6							
	24		48		72		96	
	µg/g	SD	µg/g	SD	µg/g	SD	µg/g	SD
Control	2.41	0.35	2.46	0.28	2.11	0.23	2.06	0.39
As (1.0)	5.78	0.41	7.65	0.68	8.42	0.64	10.20	0.99
As (1.0) +Cu (0.1)	5.98	0.51	8.05	0.78	9.74	0.87	16.75	1.58
As (1.0) +Cd (0.5)	6.82	0.58	10.01	1.03	10.81	0.99	12.42	1.13
As (1.0) +Hg (0.1)	8.11	0.95	9.83	1.01	16.97	1.65	18.27	1.56
As (1.0) +Se (0.2)	5.93	0.51	7.43	0.93	12.72	1.18	13.03	1.46
As (3.0)	8.09	0.65	9.78	0.79	11.57	0.88	15.63	1.11
As (3.0) +Cu (0.3)	6.16	0.67	10.55	1.56	12.34	1.43	20.93	1.21
As (3.0) +Cd (1.5)	9.99	0.86	12.83	1.14	14.43	1.23	16.81	1.45
As (3.0) +Hg (0.3)	9.85	0.81	11.70	1.11	22.32	1.86	27.08	1.78
As (3.0) +Se (0.6)	11.96	0.98	12.01	1.18	16.54	1.78	21.96	1.46

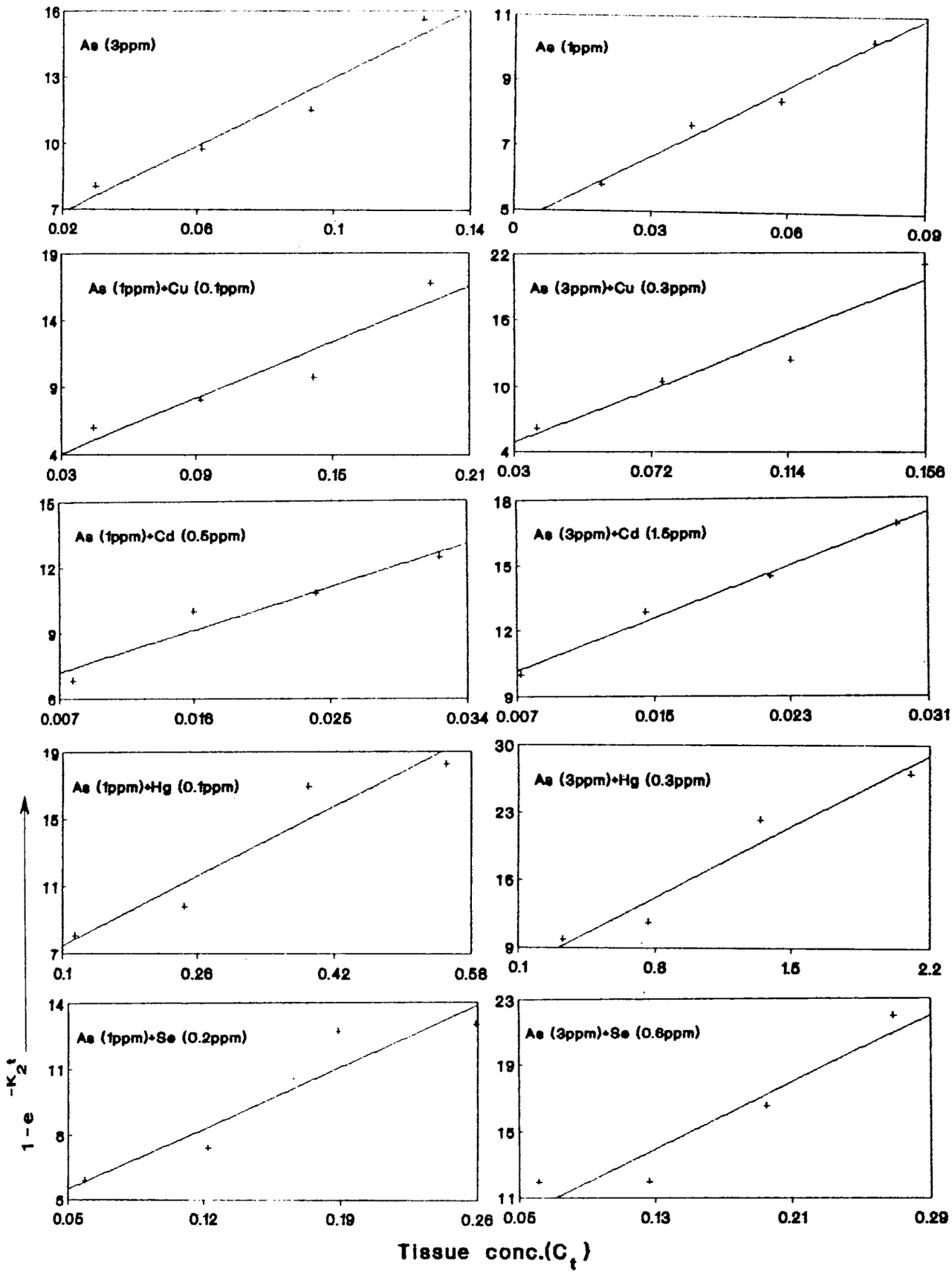


Fig.4.4 Plot of C_t *vs* $1-e^{-K_2 t}$

Table 4.5 Tissue concentration of selenium (µg/g dry weight) in V. cyprinoides exposed to selenium and its binary combinations (Accumulation phase)

Exposure levels (ppm)	Time (h) n = 6							
	24		48		72		96	
	µg/g	SD	µg/g	SD	µg/g	SD	µg/g	SD
Control	1.108	0.015	0.086	0.011	0.082	0.013	0.095	0.021
Se (0.2)	0.186	0.040	0.325	0.050	0.341	0.030	0.431	0.030
Se (0.2) +Cu (0.1)	0.114	0.009	0.124	0.010	0.164	0.008	0.252	0.018
Se (0.2) +Cd (0.5)	0.165	0.020	0.194	0.020	0.380	0.030	0.381	0.040
Se (0.2) +Hg (0.1)	0.202	0.010	0.291	0.043	0.389	0.021	0.568	0.043
Se (0.2) +As (1.0)	0.284	0.023	0.307	0.028	0.345	0.019	0.399	0.032
Se (0.6)	0.256	0.020	0.361	0.030	0.558	0.040	0.685	0.070
Se (0.6) +Cu (0.3)	0.130	0.032	0.172	0.018	0.375	0.015	0.537	0.011
Se (0.6) +Cd (1.5)	0.216	0.020	0.351	0.040	0.435	0.040	0.537	0.060
Se (0.6) +Hg (0.3)	0.294	0.050	0.380	0.030	0.672	0.030	0.786	0.020
Se (0.6) +As (3.0)	0.360	0.031	0.439	0.028	0.512	0.021	0.625	0.041

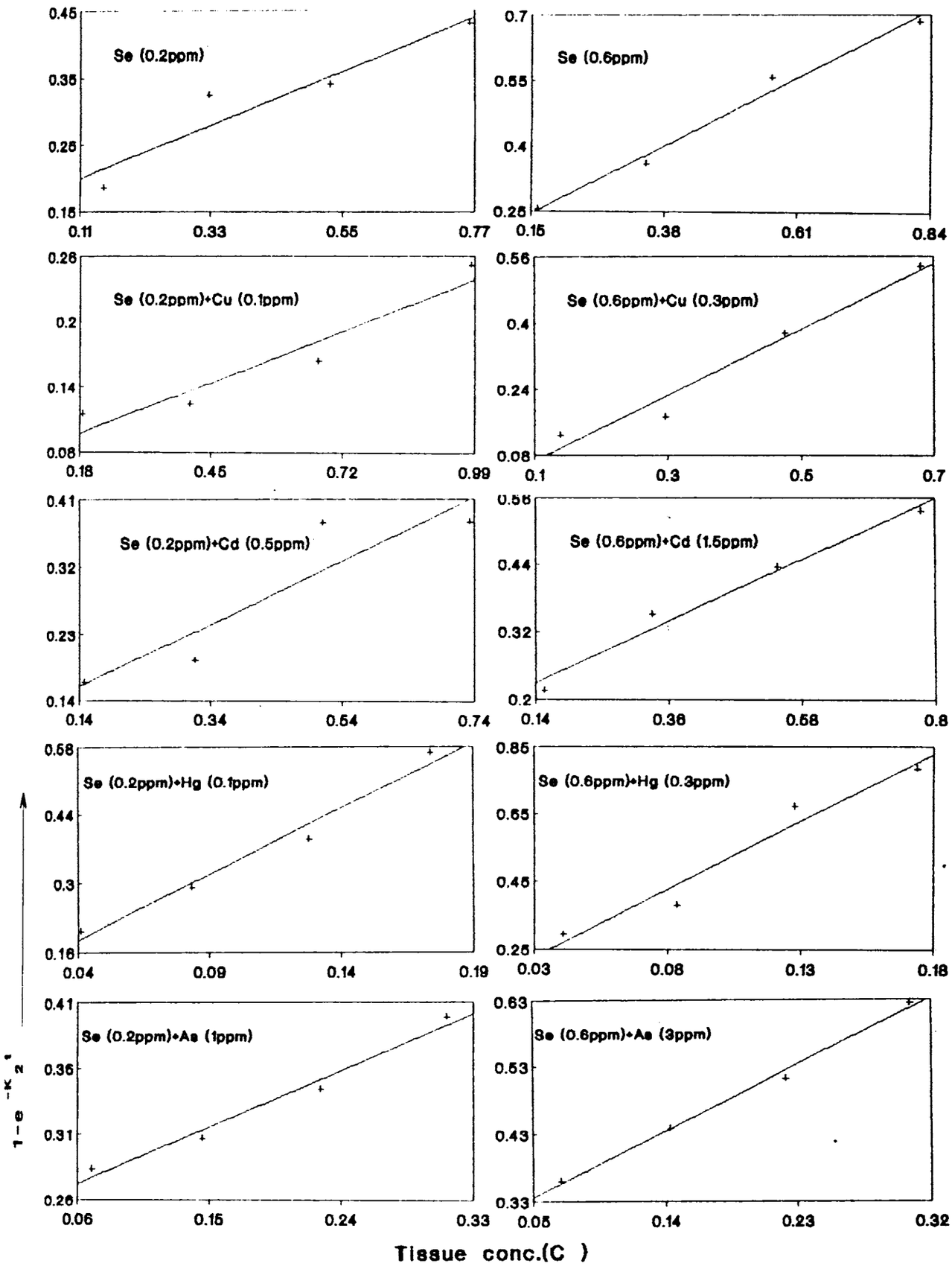


Fig.4.5 Plot of C_t *vs* $1-e^{-K_2 t}$

Individual metals

At both the levels of concentration employed (*vide* Chapter II) metal accumulation in soft tissues of the bivalves was observed to be linearly correlated with time. The rate constants of accumulation were found to be lower at higher concentrations and were observed to decrease in the order

Hg > Cu > Cd > As > Se.

Binary combinations of Copper

The uptake rate of copper was depressed by 27-66% by other metals present in the binary combinations. At the lower level of concentrations, (0.1ppm Cu + 0.5 ppm Cd/ 0.1ppm Hg/ 1ppm As/ 0.2ppm Se), mercury produced a maximum of 61% depression in the rate constant of accumulation of copper whereas at higher levels of concentrations (0.3ppm Cu + 1.5ppm Cd/ 0.3ppm Hg/ 3ppm As/ 0.6ppm Se), both mercury and selenium produced comparable effects (62 and 66% depression respectively). Cadmium produced the least depression (27 and 34%) in the rate constant of accumulation of copper at the two levels of concentration.

Binary combinations of Cadmium

In the case of lower concentration of the binary mixtures of cadmium, (0.5ppm Cd + 0.1ppm Cu/ 0.1ppm Hg/ 1ppm As/ 0.2ppm Se), copper and mercury did not exhibit any significant influence on the rate of accumulation of cadmium. Arsenic, however, produced a depression of 37% . Selenium on the other hand was found to enhance the rate constant of accumulation of cadmium by 40% . At the higher level of binary combinations of cadmium (1.5ppm Cd + 0.3ppm Cu/ 0.3ppm Hg/ 3ppm As/ 0.6 ppm Se) all metals produced depressions in the range of 32 - 58% in the rate constant of accumulation of cadmium. The observed

minimum depression of 32% was produced by mercury and the maximum depression of 58% was caused by arsenic.

Binary combinations of Mercury

All metals present in the binary combinations negatively influenced the rate of accumulation of mercury at both levels of concentration. At the lower levels of concentration, (0.1ppm Hg + 0.1ppm Cu/ 0.5ppm Cd/ 1ppm As/ 0.2ppm Se), the minimum depression in the rate constant of accumulation (2.7%) was caused by cadmium and at the higher level of concentrations (0.3ppm Hg + 0.3ppm Cu/ 1.5ppm Cd/ 3ppm As/ 0.6ppm Se), the least depression (14%) was produced by arsenic. At both levels of concentrations the maximum depression (28 and 52%) was produced by copper.

Binary combinations of Arsenic

The rate of accumulation of arsenic at both levels of concentrations was augmented by the presence of other metals in the binary combinations. At the lower level of concentrations of binary mixtures (1ppm As+ 0.1ppm Cu/ 0.5ppm Cd/ 0.1ppm Hg/ 0.2ppm Se) a minimum of 17% increment in the rate constant of accumulation was caused by cadmium and a maximum enhancement of 129% was produced by copper. At the higher level of concentrations of binary combinations, (3ppm As+ 0.3ppm Cu/ 1.5ppm Cd/ 0.3ppm Hg/ 0.6ppm Se) cadmium caused the minimum increment (5%) in the rate constant of accumulation and the maximum elevation of 133% was produced by mercury.

Binary combinations of Selenium

In the lower level of concentrations of binary mixtures of selenium (0.2ppm Se + 0.1ppm Cu/ 0.5ppm Cd/ 0.1ppm Hg/ 1ppm As) copper and arsenic depressed the rate constant of accumulation

of selenium (42 and 38% respectively) whereas cadmium and mercury augmented the rate constant of accumulation of selenium (12 - 109% respectively). At the higher level of concentrations (0.6ppm Se + 0.3ppm Cu/1.5ppm Cd/ 0.3ppm Hg/ 3ppm As), copper and mercury enhanced the rate constant of accumulation of selenium (2 and 59%) and arsenic as well as cadmium depressed the rate constant (27 and 29% depression).

From the results reported above it could be seen that the rate constants of accumulation of the metals decreased in the order,

Hg > Cu > Cd > Se > As

which reflected a decrease in the soft acid character of the metals concerned.

The linear type of accumulation of metals, treated individually under constant laboratory conditions, with respect to the exposure time and/or concentration of exposure are numerous (e.g. Klockner, 1979; Lakshmanan and Nambisan, 1979 and 1989; Scholz, 1980; Ahsanullah *et al.*, 1981; Frazier and George, 1983; Howell, 1983; Amiard-Triquet *et al.*, 1986; Coleman *et al.*, 1986; Luten *et al.*, 1986; Wang Chuseng and Yin Weiping, 1987; Baby, 1987; Sivadasan, 1987; Prabhudeva, 1988; Marigomez and Ireland, 1989; Krishnakumar *et al.*, 1990). However, the levels of certain essential metals like copper, zinc, manganese, etc. were observed to be regulated to some extent (White and Rainbow, 1982;Bryan, 1984; Devineau and Amiard-Triquet, 1985; Amiard *et al.*, 1987).

In *V. cyprinoides* var. *cochinensis* the accumulation rate constant of copper was observed to be depressed by the presence of other metals in binary combinations; the maximum depression was offered by mercury at the lower levels and by selenium at

the higher levels of concentrations. Elliot *et al.* (1986) reported that the accumulation of copper by *M. edulis planulatus* was augmented by the presence of cadmium or zinc in the binary mixtures of copper whereas in the case of *M. edulis*, Phillips (1976) reported a reduction in the accumulation of copper in the presence of cadmium and/or zinc. While investigating the effect of salt variations and interactive effects of copper and silver on *P.indica*, Prabhudeva (1988) noticed that, the accumulation of copper (as copper sulfate or as copper nitrate) was reduced by silver (present as silver sulfate or silver nitrate) upto a particular level beyond which the accumulation was found to increase. Antagonistic effect on the accumulation of copper and zinc was reported by Hilmy *et al.* (1985) in the case of the fish *C. lazera.*

The accumulation of cadmium by the teleost *F. heteroclitus* was observed to be enhanced by the presence of copper (Eisler and Gardner, 1973) whereas, no significant effect was produced by copper in the accumulation of cadmium by the shrimp *C. australiensis* (Ahsanullah *et al.*, 1981). Carpene and George (1981) reported that neither zinc, copper, mercury nor iron affected the uptake of cadmium through the gills of *M. edulis*. Detailed investigations were carried out by Elliot *et al.*, (1986) on the interactive effects of metals during accumulation by *M. edulis planulatus* and revealed that at high external zinc concentrations (200 μg/l), cadmium accumulation was decreased whereas low experimental zinc concentration (100 μg/l) caused an enhancement of the cadmium uptake. Cadmium uptake in the presence of copper, was found to be reduced at higher external cadmium concentration (20 μg/l) whereas at lower external cadmium concentration (10 μg/l), the uptake was observed to be enhanced. Copper and zinc interacted significantly to depress cadmium accumulation at high levels (Cd-20 μg/l, Zn-200 μg/l and Cu-20 μg/g). Baby (1987) recorded that while the presence of cadmium in the tissue did not have any influence on the uptake

of mercury in *P.indica*, the presence of mercury increased the uptake of cadmium. Similar result of reduced accumulation of cadmium in the presence of mercury was reported earlier by Breittmayer *et al.*(1980). In the present study the rate constant of accumulation of cadmium was not significantly influenced by copper or mercury, but was depressed by arsenic and was enhanced by selenium at the lower level of concentrations. At higher level of concentrations the rate constant of accumulation of cadmium was depressed by all other metals; the maximum effect was that of arsenic and the minimum effect was of mercury. The interactive effects of cadmium and selenium during accumulation were investigated by Bjerregaard (1982; 1988) on various species of aquatic organisms. Higher concentrations of selenium augmented cadmium uptake in sea stars, whereas lower concentration had little or only marginal effects on cadmium uptake. The uptake of cadmium was not affected by selenium in the case of *L. littorea* and *A. marina*. Cadmium uptake rate in the adductor muscle of *M.edulis* was reduced upon exposure to lower selenium concentrations whereas exposure to higher selenium concentrations increased the cadmium uptake rate in mantle and foot. The significant positive interaction observed in the crab *C. maenas* between selenium concentration in the medium and contact time was attributed to inhibition of the efflux of cadmium to an increase in the binding site of cadmium in cells Bjerregaard (1982). Thus the augmenting effect of selenite on cadmium uptake was dependent on the metabolic conversion of Se (IV) to Se (-II) resulting in the formation of a protein-stabilized Cd-Se complex with a molar ratio Cd : Se close to 1. The augmentation of cadmium uptake in the presence of selenium was also noticed by Boisson *et al.*, (1989) in the case of marine macro-algae.

Other metals present in the binary combinations depressed the rate constant of accumulation of mercury, the maximum depression being produced by copper at both the levels and

minimum depression at lower level was that of cadmium and at higher level the depressive effect was produced by selenium. Mercury accumulation by *G. duebeni* was studied by Moulder (1980) and observed a reduction in the accumulation in the presence of copper. In an extensive field study, Turner and Rudd (1983) observed that the accumulation of mercury was reduced in the presence of selenium in a concentration dependent manner and proposed the addition of low concentration of selenium as beneficial to the treatment of aquatic ecosystems contaminated with mercury, both by reducing the accumulation of mercury and by rendering it less toxic to fish consumers.

The rate constant of accumulation of selenium was augmented by cadmium and mercury at the lower levels of concentrations and copper and mercury at the higher levels of concentrations. Depression of the corresponding rate constant was observed with copper and arsenic at the higher levels and with cadmium and arsenic at the lower levels of concentrations. A slight though insignificant, reduction in selenium accumulation in the presence of mercury was observed by Fowler and Benayoun (1976b) in the mussels. Similarly, a decreased uptake of selenium with increasing mercury concentrations, though insignificant, was observed in the case of minnows, *P.phoxinus* (Cuvin and Furness, 1988). However, Blaylock *et al.* (1974) reported an enhancement of selenium uptake in the presence of elevated mercury concentrations in mosquito-fish. Pelletier (1986) also observed an augmented uptake rate of selenium in the presence of mercury although selenium had no obvious effect on the rate of accumulation of mercury. The rate of accumulation of arsenic was augmented by other metals present in the binary combinations. The enhancement was maximum in the presence of copper at the lower level of concentrations whereas at the higher level of concentrations it was a maximum in the presence of mercury. The minimum effect was that of cadmium.

Depuration studies

The tissue concentrations of the metals (analysed at 24h, 48h, 72h, 96h) in the bivalves pre-exposed to the metals, during the depuration phase of the experiments are given in the Tables 4.6 - 4.10. The tissue concentration (C_t) of the metals in bivalves is taken as the difference between the concentrations at time 't' (C) and that at zero time (C_o) i.e., control.

$$\text{i.e.,} \quad C_t = C - C_0$$

The plotsof log C_t vs t are given in Figs. 4.6 - 4.10 and the rate constants of depuration are given in Table 4.11.

Individual Metals

The ratesof depuration of all the metals from the soft tissue of the bivalves pre-exposed to the metals (individually and in binary combinations), were found to be linearly correlated with time. The rate constants of depuration of the metals, copper, mercury and arsenic were found to be greater at the higher level of concentration, whereas a reverse trend was observed in the case of cadmium and selenium.

Binary combinations of Copper

The rate of depuration of copper was found to be negatively influenced by other metals present in binary combinations. Cadmium was found to cause the least depression (15 and 23%) at both levels of concentrations whereas the highest depression was produced by selenium (72 and 79%) at both levels of concentrations. On the basis of their ability to alleviate the rate of depuration of copper, the metals could be arranged in the order :

Table 4.6 Tissue concentration of copper (µg/g dry weight) in V. cyprinoides exposed to copper and its binary combinations (Depuration phase)

Exposure levels (ppm)	Time (h) n = 6							
	24		48		72		96	
	µg/g	SD	µg/g	SD	µg/g	SD	µg/g	SD
Cu (0.1)	31.28	2.56	20.71	2.21	17.34	1.58	12.41	1.13
Cu (0.1) +Cd (0.5)	24.38	2.12	15.80	1.21	14.98	0.96	10.42	1.05
Cu (0.1) +Hg (0.1)	16.82	1.42	14.79	1.51	13.75	1.24	11.96	1.25
Cu (0.1) +As (1.0)	16.01	1.54	14.32	1.33	12.85	1.31	10.78	1.12
Cu (0.1) +Se (0.2)	20.24	2.32	19.32	1.54	16.91	1.47	16.24	1.39
Cu (0.3)	30.39	2.98	28.32	2.43	19.38	1.76	16.42	1.49
Cu (0.3) +Cd (1.5)	25.17	2.18	20.79	2.09	18.52	1.46	13.82	1.24
Cu (0.3) +Hg (0.3)	20.24	2.1	17.05	1.79	15.78	1.48	13.05	1.29
Cu (0.3) +As (3.0)	25.86	2.43	22.13	2.18	19.59	1.45	15.64	1.32
Cu (0.3) +Se (0.6)	22.11	2.15	20.78	1.96	19.44	1.87	18.23	1.91

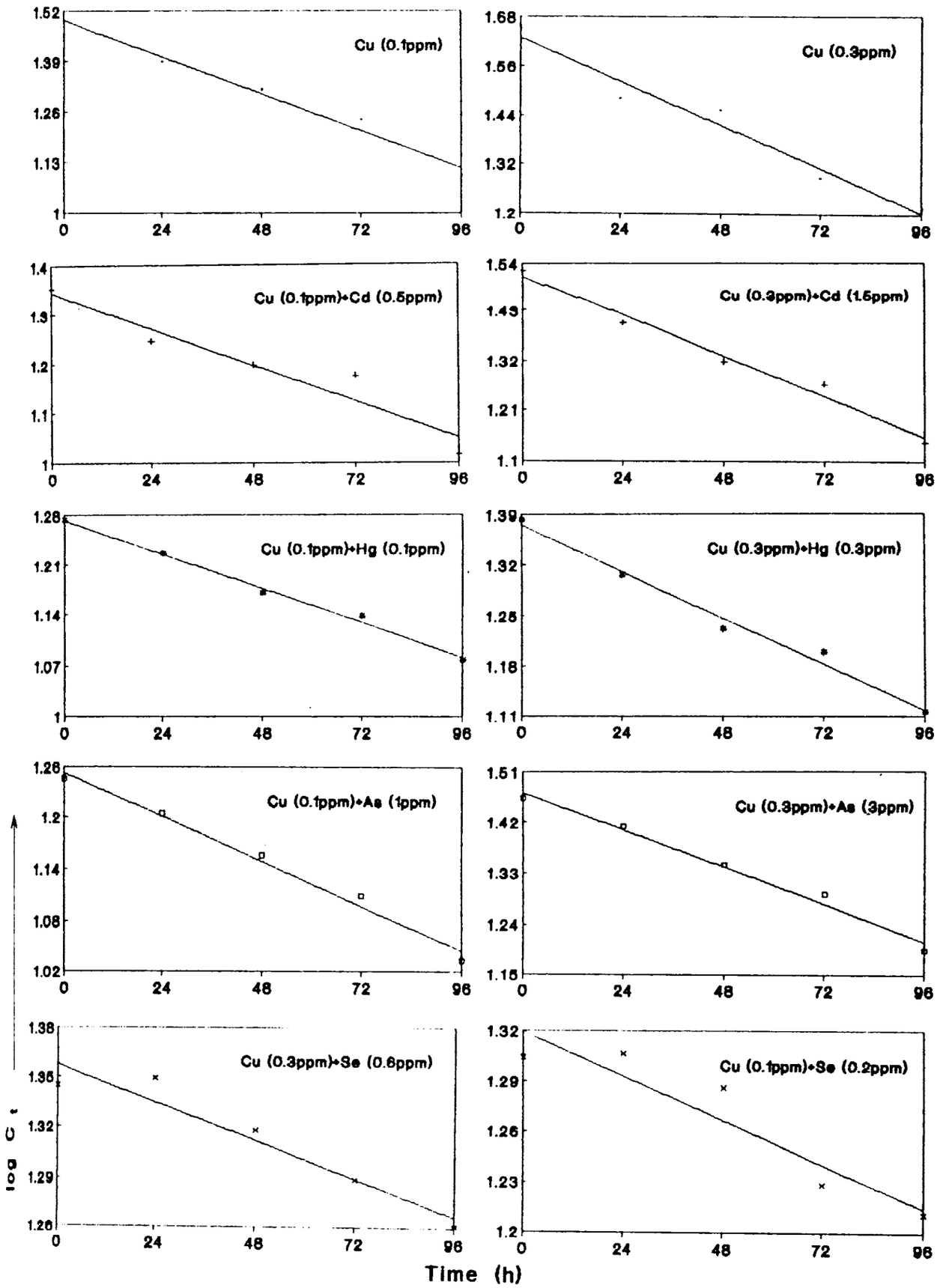


Fig.4.6 Plot of log C_t *vs* t

Table 4.7 Tissue concentration of cadmium (µg/g dry weight) in V. cyprinoides exposed to cadmium and its binary combinations (Depuration phase)

Exposure levels (ppm)	Time (h) n = 6							
	24.00		48.00		72.00		96.00	
	µg/g	SD	µg/g	SD	µg/g	SD	µg/g	SD
Cd (0.5)	32.84	2.64	32.58	2.75	30.80	2.86	30.03	2.55
Cd (0.5) +Cu (0.1)	18.93	1.67	16.14	1.75	14.56	1.23	14.02	1.41
Cd (0.5) +Hg (0.1)	22.56	1.18	20.78	1.03	19.07	1.21	18.29	1.31
Cd (0.5) +As (1.0)	21.08	1.93	19.64	1.45	18.73	1.25	18.01	2.12
Cd (0.5) +Se (0.2)	36.49	1.96	33.58	1.87	30.67	2.11	28.87	2.24
Cd (1.5)	69.92	5.93	68.34	5.85	65.18	5.87	64.84	6.11
Cd (1.5) +Cu (0.3)	24.39	2.19	19.26	1.86	18.05	1.79	15.25	1.32
Cd (1.5) +Hg (0.3)	35.32	2.71	33.65	2.43	28.82	1.98	26.56	1.87
Cd (1.5) +As (3.0)	45.21	3.14	43.64	1.89	40.78	1.31	39.54	1.08
Cd (1.5) +Se (0.6)	56.78	3.11	52.86	2.98	49.64	1.87	48.57	1.88

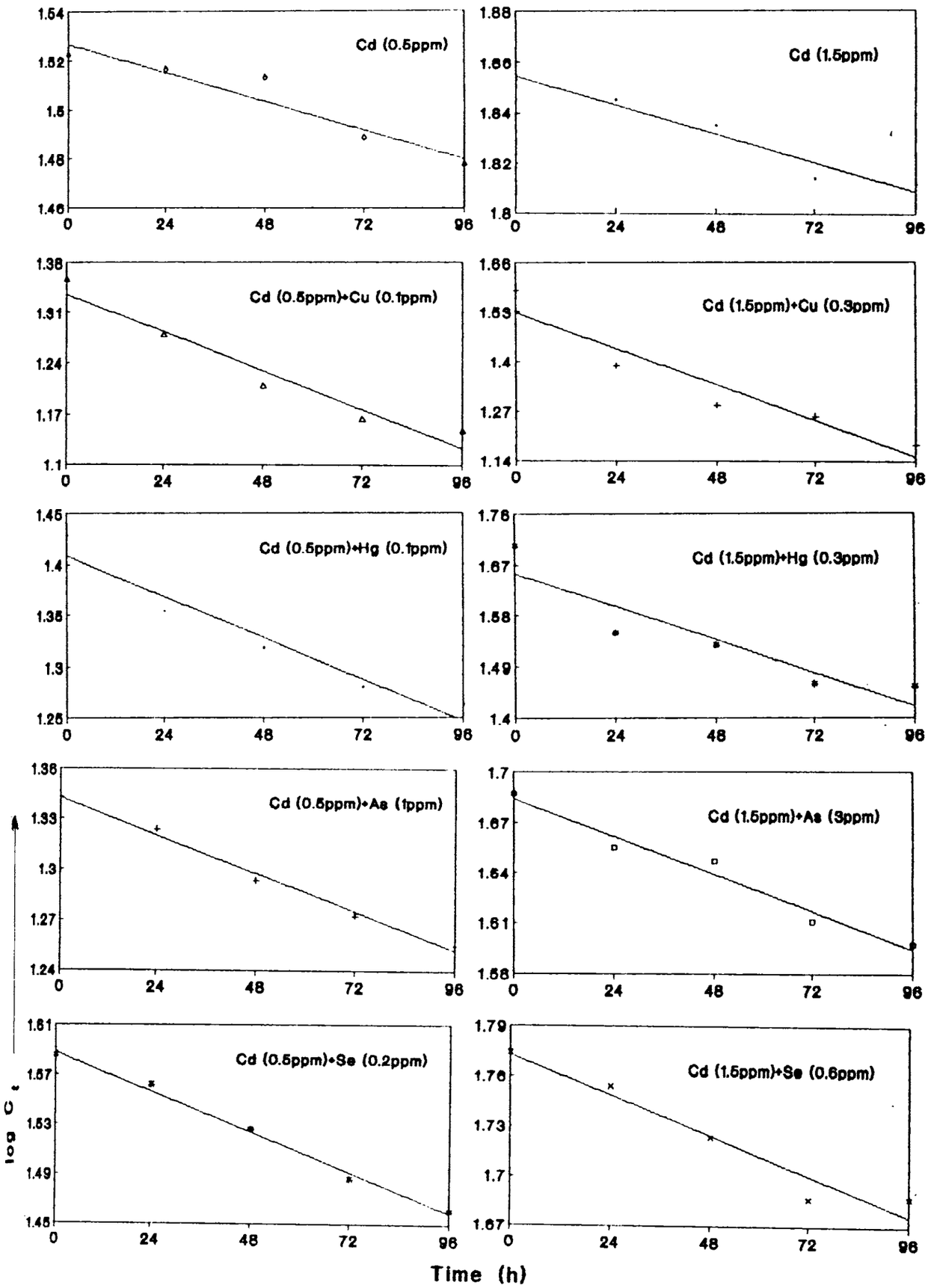


Fig.4.7 Plot of log C_t *vs* t

Table 4.8 Tissue concentration of mercury (µg/g dry weight) in V. cyprinoides exposed to mercury and its binary combinations (Depuration phase)

Exposure levels (ppm)	Time (h) n = 6							
	24.00		48.00		72.00		96.00	
	µg/g	SD	µg/g	SD	µg/g	SD	µg/g	SD
Hg (0.1)	22.37	1.11	18.47	1.41	14.36	1.32	13.57	1.28
Hg (0.1) +Cu (0.1)	21.24	1.13	19.41	1.01	14.28	1.23	13.05	1.31
Hg (0.1) +Cd (0.5)	27.43	2.70	26.38	1.89	20.94	1.97	15.93	1.78
Hg (0.1) +As (1.0)	25.94	1.51	21.44	1.86	19.34	2.13	16.76	1.54
Hg (0.1) +Se (0.2)	22.66	1.93	20.83	2.01	19.57	1.78	16.76	1.55
Hg (0.3)	35.65	2.18	29.23	1.93	21.56	1.98	18.45	1.99
Hg (0.3) +Cu (0.3)	28.54	1.93	23.55	1.98	19.52	1.87	16.82	1.69
Hg (0.3) +Cd (1.5)	40.84	3.11	34.52	3.08	28.50	1.96	26.31	2.11
Hg (0.3) +As (3.0)	29.57	2.64	26.78	3.52	23.46	2.81	19.79	2.54
Hg (0.3) +Se (0.6)	37.57	2.55	35.45	1.75	33.56	1.89	29.79	1.91

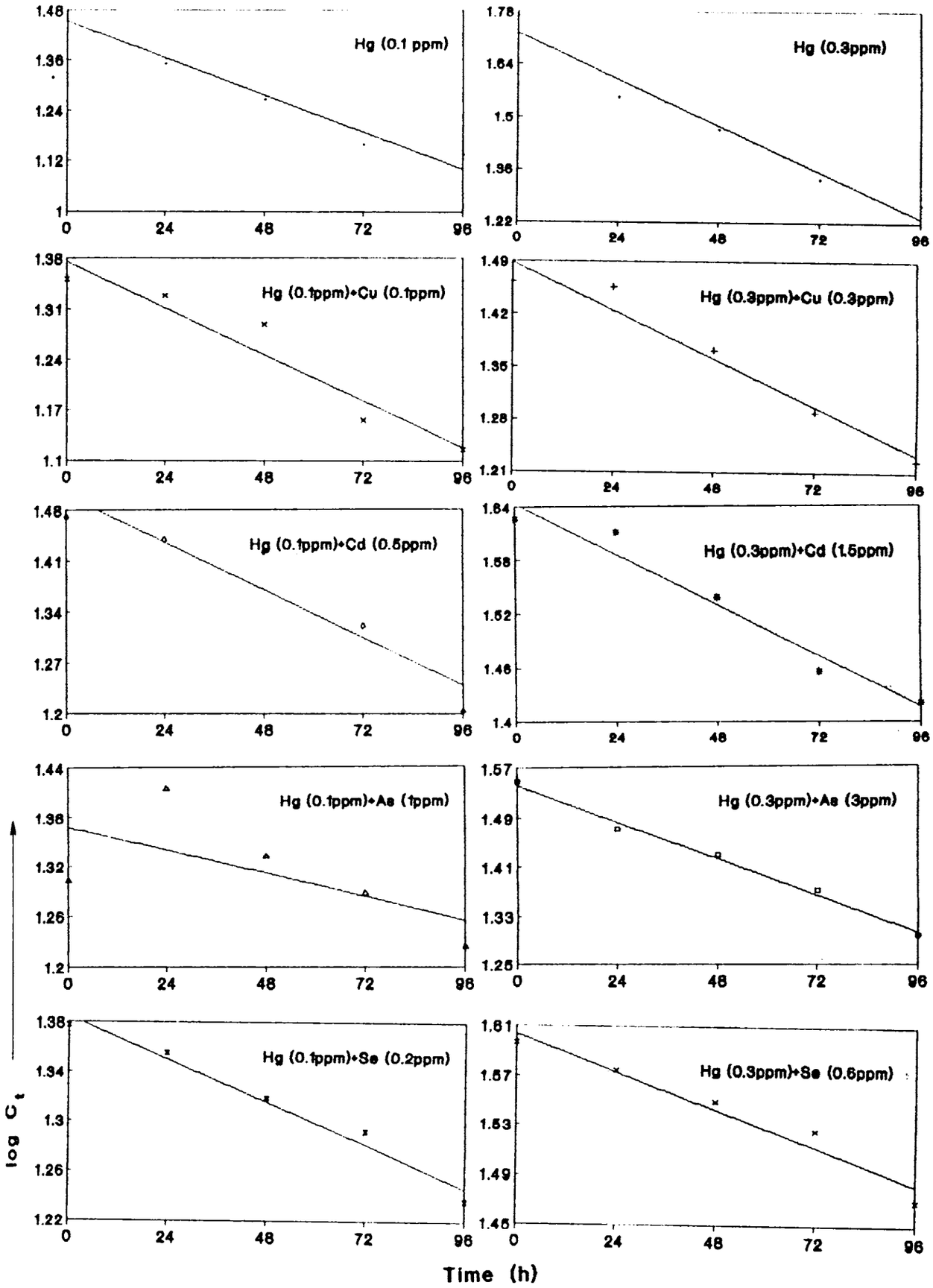


Fig.4.8 Plot of log C_t *vs* t

Table 4.9 Tissue concentration of arsenic (µg/g dry weight) in V. cyprinoides exposed to arsenic and its binary combinations (Depuration phase)

Exposure levels (ppm)	Time (h) n = 6							
	24.00		48.00		72.00		96.00	
	µg/g	SD	µg/g	SD	µg/g	SD	µg/g	SD
As (1.0)	9.87	0.78	9.02	0.55	8.85	0.66	8.66	0.86
As (1.0) +Cu (0.1)	15.36	1.31	14.83	1.41	12.86	1.28	11.01	0.88
As (1.0) +Cd (0.5)	12.27	1.26	12.02	1.25	11.84	1.23	11.53	1.09
As (1.0) +Hg (0.1)	15.27	1.46	12.55	1.32	9.78	1.01	6.45	1.08
As (1.0) +Se (0.2)	12.04	1.14	10.41	1.21	9.24	0.98	7.66	0.91
As (3.0)	14.86	0.96	13.56	0.95	12.45	1.45	12.09	0.88
As (3.0) +Cu (0.3)	18.46	1.66	16.89	1.46	15.64	1.44	14.96	1.43
As (3.0) +Cd (1.5)	16.70	1.10	16.52	1.48	15.98	1.34	15.78	1.32
As (3.0) +Hg (0.3)	25.78	2.33	22.56	2.42	19.78	1.31	14.57	1.43
As (3.0) +Se (0.6)	18.51	1.47	16.71	1.53	13.76	1.36	12.80	1.75

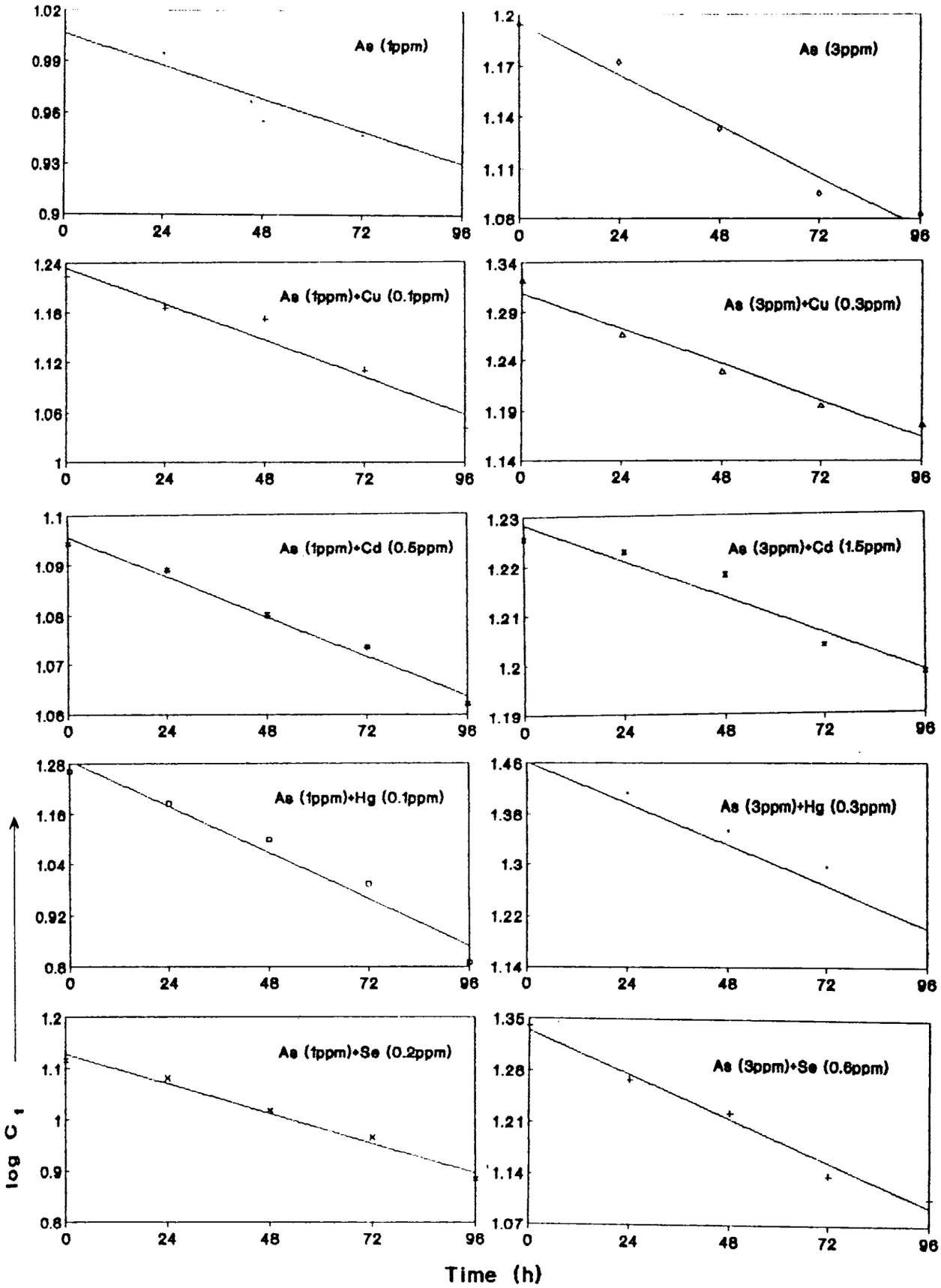


Fig.4.9 Plot of log C_t *vs* t

Table 4.10 Tissue concentration of selenium (µg/g dry weight) in V. cyprinoides exposed to selenium and its binary combinations (Depuration phase)

Exposure levels (ppm)	Time (h) n = 6							
	24		48		72		96	
	µg/g	SD	µg/g	SD	µg/g	SD	µg/g	SD
Se (0.2)	0.272	0.030	0.175	0.010	0.139	0.008	0.118	0.010
Se (0.2) +Cu (0.1)	0.127	0.011	0.097	0.008	0.074	0.006	0.046	0.003
Se (0.2) +Cd (0.5)	0.237	0.020	0.231	0.021	0.150	0.013	0.098	0.008
Se (0.2) +Hg (0.1)	0.448	0.030	0.439	0.031	0.398	0.020	0.379	0.022
Se (0.2) +As (1.0)	0.276	0.014	0.259	0.020	0.229	0.019	0.200	0.018
Se (0.6)	0.341	0.020	0.244	0.025	0.208	0.018	0.156	0.016
Se (0.6) +Cu (0.3)	0.359	0.014	0.244	0.019	0.210	0.030	0.157	0.040
Se (0.6) +Cd (1.5)	0.330	0.022	0.242	0.023	0.182	0.019	0.138	0.010
Se (0.6) +Hg (0.3)	0.648	0.041	0.615	0.042	0.577	0.030	0.524	0.020
Se (0.6) +As (3.0)	0.488	0.035	0.459	0.026	0.381	0.030	0.328	0.021

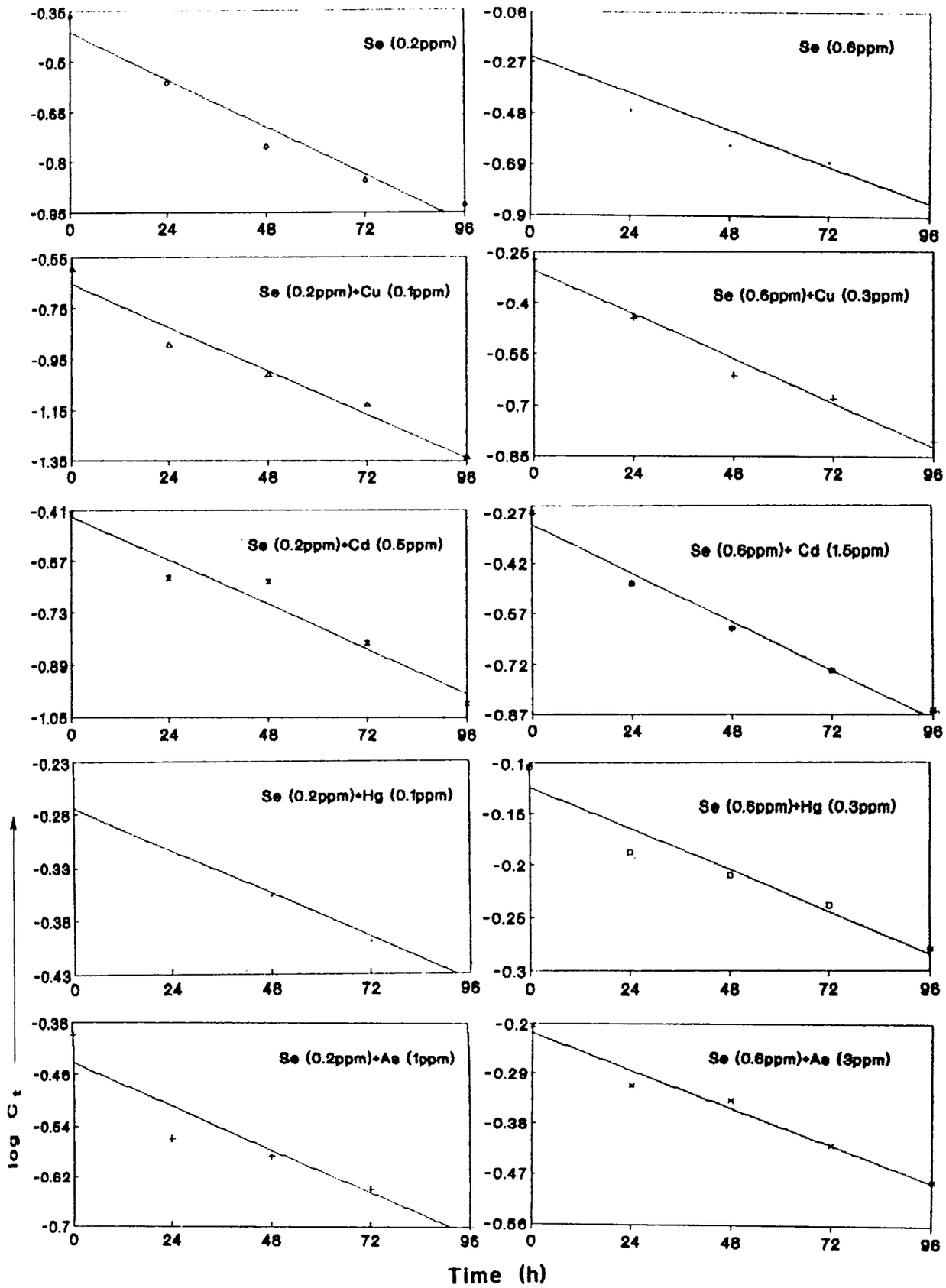


Fig.4.10 Plot of log C_t *vs* t

Table.4.11 Quantification of the parameters of uptake and depuration

Metal	Exposure levels (ppm)	K_2 x 1000	K1	BCF	Half-life
Cu	0.1	3.96	2.04	514.20	175.00
	0.3	4.44	1.00	224.93	156.08
Cu+Cd	0.1+0.5	3.07	1.33	431.70	225.73
	0.3+1.5	3.76	0.72	192.63	184.31
Cu+Hg	0.1+0.1	1.99	0.80	402.50	348.24
	0.3+0.3	2.67	0.37	139.10	259.55
Cu+As	0.1+1.0	2.16	0.87	401.10	320.83
	0.3+3.0	2.73	0.54	197.73	253.85
Cu+Se	0.1+0.2	1.1	1.24	1128.60	630.00
	0.3+0.6	0.94	0.35	369.63	737.23

Contd.....

Table.4.11 Quantification ofthe parameters of uptake and depuration

Metal	Exposure levels (ppm)	K_2 x 1000	K1	BCF	Half-life
Cd	0.5	0.48	0.46	955.06	1443.75
	1.5	0.46	0.44	956.56	1506.52
Cd+Cu	0.5+0.1	2.21	0.45	205.62	313.57
	1.5+0.3	3.85	0.23	59.93	180.00
Cd+Hg	0.5+0.1	1.68	0.44	260.26	412.50
	1.5+0.3	0.93	0.30	321.45	745.16
Cd+As	0.5+1.0	0.94	0.29	306.78	737.23
	1.5+3.0	2.69	0.18	68.54	257.62
Cd+Se	0.5+0.2	0.19	0.64	3389.26	3647.37
	1.5+0.6	0.97	0.22	228.49	714.43

Contd.....

Table.4.11 Quantification of the parameters of uptake and depuration

Metal	Exposure levels (ppm)	K_2 x 1000	K1	BCF	Half-life
Hg	0.1	3.67	2.24	610.80	188.83
	0.3	5.11	1.37	268.00	135.62
Hg+Cu	0.1+0.1	2.67	1.61	601.20	259.55
	0.3+0.3	2.66	0.66	249.43	260.53
Hg+Cd	0.1+0.5	2.72	2.18	801.80	254.78
	0.3+1.5	2.36	1.10	466.93	293.64
Hg+As	0.1+1.0	2.26	1.93	851.80	306.64
	0.3+3.0	2.5	0.81	322.43	277.20
Hg+Se	0.1+0.2	1.44	1.93	1338.50	481.25
	0.3+0.6	1.26	1.19	941.77	550.00

Contd.....

Table.4.11 Quantification of the parameters of uptake and depuration

Metal	Exposure levels (ppm)	K_2x1000	K1	BCF	Half-life
As	1.0	0.79	0.06	70.61	877.22
	3.0	1.24	0.03	25.44	558.87
As+Cu	1.0+0.1	1.84	0.13	69.34	376.63
	3.0+0.3	1.51	0.06	38.84	458.94
As+Cd	1.0+0.5	0.3	0.07	217.77	2310.00
	3.0+1.5	0.33	0.03	100.10	2100.00
As+Hg	1.0+0.1	4.57	0.12	25.86	151.64
	3.0+0.3	2.72	0.07	27.04	254.78
As+Se	1.0+0.2	2.4	0.10	39.82	288.75
	3.0+0.6	2.48	0.04	16.79	279.44

Contd.....

Table.4.11 Quantification of the parameters of uptake and depuration

Metal	Exposure levels (ppm)	K_2x1000	K1	BCF	Half-life
Se	0.2	6.24	0.083	13.27	111.06
	0.6	5.9	0.013	2.26	117.46
Se+Cu	0.2+0.1	7.13	0.006	0.88	97.19
	0.6+0.3	5.41	0.007	1.32	128.10
Se+Cd	0.2+0.5	5.73	0.012	2.11	120.94
	0.6+1.5	5.99	0.005	0.83	115.69
Se+Hg	0.2+0.1	1.67	0.023	13.50	414.97
	0.6+0.3	1.67	0.011	6.65	414.97
Se+As	0.2+1.0	2.83	0.007	2.38	244.88
	0.2+3.0	2.78	0.005	1.84	249.28

Binary combinations of Cadmium

The rate of depuration of cadmium was augmented by other metals present in the binary combinations, except at the lower concentration of selenium. The maximum positive influence in the rate constant of depuration was offered by copper at the two levels of concentrations (360 and 737%). At lower level of concentrations of binary mixtures, the least positive influence was that of arsenic (96%) and at the higher levels of concentrations of binary mixtures, mercury offered the least positive influence (102%). The lower level of selenium depressed the depuration rate constant of cadmium by 60% .

Binary combinations of Mercury

All other metals present in binary combinations produced depressions in the rate of depuration of mercury. At both the levels of concentrations of binary combinations of mercury, the maximum depression in the depuration rate constant was produced by selenium (61 and 75%) at both levels of concentration. The least depression in the depuration rate constant of mercury was produced by cadmium (26%) at the lower level of concentrations and at the higher level, the least depression was produced by copper.

Binary combinations of Arsenic

Except cadmium all other metals at both levels of concentrations of binary mixtures, enhanced the rate constant of depuration of arsenic. The maximum effect was that of mercury (≈ 479 and 119%) respectively at both levels of concentrations and the least effect was that of copper (≈ 21 and 133%) respectively at both the levels concentrations. Cadmium at both levels of concentrations depressed the rate constant of depuration (62 and 74%) of arsenic.

Binary combinations of Selenium

Except the lowest concentration of copper, all other metals at both levels of concentration depressed the rate constant of depuration of selenium. A minimum depression (2 and 4%) at both levels of concentrations was produced by cadmium and the maximum depression of 71 and 73% at both levels of concentration was caused by the binary mixtures of mercury.

The rate constants of depuration of the trace metals employed in this study was found to decrease in the following order :

Cu > Hg > Se > As > Cd

The same sequence was observed for higher concentrations also except for a reversal of mercury and copper; i.e.,

Hg > Cu > Se > As > Cd

The rate constants of depuration were $\approx 10^{-2}$ times lower than the rate constants of accumulation. The very slow elimination rates of metals may be indicative of the immobilization of the metals within the tissues as a result of formation of strong metal complexes with the tissue components like metalloproteins (Riisgard *et al.*, 1985; Viarengo, 1985; Lakshmanan and Nambisan, 1989).

D' Silva and Kureishy (1978) observed that the rate of loss of copper was far more closely correlated to the internal copper concentration of the oyster, *C.viriginica*. The rate of loss of cadmium in the marine prosobranch, *L. littorea* was observed to follow an exponential pattern (Marigomez and Ireland, 1989); a similar trend was reported for *M. edulis* (Scholz, 1980; Luten *et al* 1986). Peden *et al.* (1973) reported that there was no elimination of this metal when *C. maenas*, *P. vulgata* and *N.*

lapillus individuals, which had accumulated cadmium under contaminated field conditions (Cd 0.01 mg l^{-1}) were transferred to an uncontaminated estuary in which the cadmium content was .0002mg l^{-1}.

As far as binary mixtures were concerned the rate constant of depuration of copper was depressed by other metals, the maximum depression being produced by selenium, whereas the rate constant of depuration of cadmium was augmented by other metals present in the binary mixtures and the maximum effect was that of copper. The rate constant of depuration of mercury was depressed by other metals and the maximum enhancement was caused by selenium. Except cadmium, all other metals were found to enhance the depuration rate constant of arsenic, the maximum effect was that of mercury. Except at lower concentration cf copper, all other metals depressed the depuration rate constant of selenium; the maximum effect was produced by mercury.

Reports on the interactive effects of metals during depuration are scarce. Selenium has been shown to decrease the rate of elimination of mercury in the shrimps (Lucu and Skremblin, 1981). The presence of mercury inhibited the elimination of selenium by minnows (Cuvin and Furness, 1988). Fowler and Benayoun (1976c) observed that in the case of mussels and shrimps, the rate of elimination of selenium was not directly linked to the mercury present in the medium.

α and β

α and β were introduced earlier as constants which being attributes of k_2 and k_1, define the depuration and the accumulation processes respectively. This study has been the first attempt at quantifying the interactive effects of metals in terms of the kinetics of metal uptake and depuration.No rigorous interpretation of the significance of these constants

was attempted with the limited data generated in this investigation. Nevertheless, it was felt worthwhile to indicate some of the general trends which became visible in a closer analysis of the results.

Metal	α	β
Copper	2.4×10^{-3}	-5.19
Cadmium	-2.0×10^{-5}	-0.018
Mercury	7.2×10^{-3}	-4.35
Arsenic	2.3×10^{-4}	-0.012
Selenium	-8.5×10^{-4}	-0.17

Based on the decreasing β values, the metals could be arranged in the order Cu > Hg > Se > Cd > As, which was found to be the same as the order of decreasing toxicity of the metals (Cu > Hg > Se > Cd > As). Hitherto no comparable assessments were possible of the accumulation rates of different metals from a knowledge of the respective LC_{50} values (cf. the order of lethal toxicities (page no. 26) and that of accumulation rates arrived at on the basis of their absolute values page no. 48). The inability to relate lethal toxicities to accumulation rates is mainly because different metal accumulation experiments are conducted at different dosed metal concentrations which, therefore, renders any such comparison unsound. However, since β values bring about a normalization with respect to the dosed metal concentration it has now emerged as a more realistic entity for quantification. This proves that the rates of accumulation would have to be normalized with respect to the dosed metal concentration for any meaningful interpretation of the nature of the accumulation process. Any attempt to compare the accumulation rates of metals based on their absolute values would only lead to erroneous judgments.

It was found possible to segregate the metals into two groups on the basis of their α values— one with positive values and the other with negative values. Metals which yielded positive α values depurated faster at higher dosed concentrations whereas for metals with negative α values, the rates of depuration were found to be lower at higher concentrations. The negative α values obtained for cadmium and selenium, thus indicated a metabolic condition which favoured a preferential retention of the metals at higher concentrations; however, copper, mercury and arsenic exhibited positive α values which indicated a higher depuration rate and consequently a lower retention at higher concentrations.

Evaluation of the interactive effects of metals (based on the MTI concept) indicated that copper-cadmium, copper-selenium, cadmium-arsenic,mercury-selenium, arsenic-selenium combinations acted antagonistically and copper-mercury, copper-arsenic, cadmium-selenium, mercury-arsenic acted as partialadditives to *V. cyprinoides* var. *cochinensis*. A comparison of the interactive effects at the lethal levels with the classification of the metals made with their α values revealed that binary combination of metals belonging to the same group produced additive effects whereas binary combinations of metals belonging to different groups behaved antagonistically. The cadmium-mercury combination, however, remained an exception to these general trends.

Bioconcentration Factor (B.C.F.)

The BCF values of the metals and their binary combinations are given in Table 4.11.

Individual metals

The BCF values of the trace metals employed individually were found to be inversely related to the external metal

concentrations and the following decreasing order of BCF values was observed at the both the levels of concentrations tried.

Cd > Hg > Cu > As > Se.

Binary combinations of Copper

BCF value of copper was found to be depressed by the presence of cadmium, mercury and arsenic (12-39%). At the lower levels of binary combinations, the minimum depression of 16% was produced by cadmium and the maximum depressions of 22% was produced by arsenic and mercury. At the higher levels of concentrations the maximum depression of 39% was produced by mercury and the minimum depression of 12% was caused by arsenic. Selenium, however augmented the BCF of copper (120 and 64%) at both levels of concentrations.

Binary combinations of Cadmium

Except the lower level of selenium, all other metals at both the levels of concentrations in binary mixtures depressed the BCF of cadmium. At both levels of concentrations, copper produced the maximum depressions (78 and 94%). At lower level, the minimum depression was produced by arsenic and at higher level the minimum depression was produced by mercury. The lower concentration of selenium remarkably augmented the BCF of cadmium (255%).

Binary combinations of Mercury

All other metals except copper present in the binary combinations of mercury, at both levels of concentrations enhanced the BCF of mercury. The maximum enhancement was produced by selenium (119 and 251%) at both levels of concentrations. The least effect was that of cadmium at the

lower level of concentrations and of arsenic at the higher level of concentrations. Copper had only a less significant negative influence (2 and 7%) at both levels of concentration in the BCF of mercury.

Binary combinations of Arsenic

Cadmium at both the levels of concentrations in the binary mixtures enhanced the BCF of arsenic (208 and 293%). The higher concentrations of copper and mercury also enhanced the BCF (53 and 6%) of arsenic. The lower concentrations of mercury and copper depressed the BCF of arsenic by 63% and 2% respectively. Selenium at both the levels of concentrations in the binary mixtures depressed the BCF of arsenic (44 and 34%).

Binary combinations of Selenium

The BCF value of selenium was depressed by other metals, except mercury at both levels of concentrations. The maximum effect at the lower concentration level was that of copper and at the higher concentration level was that of cadmium. At both levels the minimum effect was that of arsenic. Mercury caused an elevation in BCF value of selenium 1% and 195% at both levels of concentrations.

Bioconcentration factor is the ratio of the pollutant concentration in an organism to that in the ambient environment when the concentration in the organism is in a steady state. (Connel and Miller, 1984).

In the case of the clam, *Villorita cyprinoides* var. *cochinensis*, the increasing order of BCF at lower concentration was

Cd > Hg > Cu > Se > As

and that at the higher concentration was the same except a reversal in trend of copper and mercury i.e.

Cd > Cu > Hg > Se > As

The BCF of the metals were observed to be lower at the higher levels of concentrations. Similar trend i.e. decreased BCF values with increasing exposure concentrations were also reported (Wang Chuseng and Yin Weiping, 1987; Lakshmanan and Nambisan, 1989). The order of BCF for the metals copper, cadmium and lead in the case of the clam, *A. granosa* was Cd > Cu > Pb (Wang Chuseng and Yin Weiping, 1987). In the case of *P. viridis* Lakshmanan and Nambisan (1989) observed the following trend for the trace metals

Cu > Hg > Pb.

The BCF of copper was observed to be depressed by cadmium, mercury or arsenic. The maximum depression was produced by mercury at the higher levels of concentration. Selenium, at both levels of concentration elevated the BCF of copper.

Except the lower concentration of selenium, all metals depressed the BCF of cadmium; the maximum depression being produced by copper at both levels of concentrations. The lower level of concentration of selenium remarkably enhanced the BCF of cadmium.

BCF value of mercury was enhanced by all other metals (except copper) at both levels of concentrations. The maximum enhancement was produced by selenium. Copper had only a marginal elevating effect on the BCF of cadmium.

At both levels of concentrations cadmium enhanced and selenium depressed the BCF value of arsenic. The lower levels

of copper and mercury enhanced the BCF of arsenic whereas, at the higher levels the BCF of arsenic was depressed.

The BCF of selenium was depressed by all the other metals except mercury at both levels of concentrations.

Biological Half life

The $t_{1/2}$ values of the metals and their binary combinations are given in Table 4.11.

Individual metals

The $t_{1/2}$ values of copper, mercury and arsenic were observed to be lower at higher concentrations whereas a reverse effect was noticed in the case of cadmium and selenium. The following sequence was observed in the $t_{1/2}$ values at the lower levels of concentrations of the metals.

$$Se < Cu < Hg < As < Cd.$$

At the higher levels of concentrations, the increasing trend observed in the $t_{1/2}$ values was

$$Se < Hg < Cu < As < Cd.$$

Binary combinations of Copper

The $t_{1/2}$ value of copper was enhanced by 18-372%, in the presence of other metals in binary combinations. At both the levels of concentrations the following sequence of metals was observed in producing the enhancement in the $t_{1/2}$ value of copper

$$Cd < As < Hg < Se.$$

Binary combinations of Cadmium

Except the lowest concentration of selenium, all other metals depressed the $t_{1/2}$ value of cadmium. A minimum depression of 49% in $t_{1/2}$ value of cadmium was offered by arsenic at the lower level of concentrations and at the higher level of concentrations the minimum depression of 51% was offered by mercury. At both levels of concentrations the maximum depression was produced by copper (78 and 88%).

Binary combinations of Mercury

The $t_{1/2}$ value of mercury was augmented by all other metals present in the binary combinations. The maximum augmenting effect was that of selenium (154 and 306%) at both the levels of concentrations. Cadmium caused a minimum elevation of 35% at the lower level of concentrations and at the higher level of concentrations a minimum of 92% elevation of the $t_{1/2}$ value of mercury was produced by copper.

Binary combinations of Arsenic

Except cadmium all other metals present in binary combinations depressed the $t_{1/2}$ value of arsenic. At both levels of concentrations, the maximum depression was produced by mercury and the minimum by copper. Cadmium however, elevated the $t_{1/2}$ value of arsenic at both levels of concentrations (163 and 276%).

Binary combinations of Selenium

Except the lower concentration of copper and higher concentration of cadmium, all other metals elevated the $t_{1/2}$ value of selenium at both levels of concentrations. The maximum elevation was produced by mercury 274% and 253% at both levels

of concentrations and the minimum elevation was caused by cadmium at the lower level and by copper at the higher level. The lower concentration of copper however depressed the $t_{1/2}$ value of selenium by 17% and the higher concentration of cadmium caused only 1.5% elevation.

The biological half-life ($t_{1/2}$) of a metal is defined as the time required for half the accumulated trace metal to be lost as a result of biological processes. Since the depuration of metals from tissue obeys first order kinetics, based upon the rate of loss of a metal its biological half-life can be determined. The dosage, duration of exposure to a specific metal, duration of depuration, physiological condition of the animals, etc. may affect the half-life of the heavy metal (Unlu *et al.*, 1972; Cunningham and Tripp, 1973). Cunningham and Tripp (1973) identified three mechanisms of metal release by bivalves *viz.* i) decrease in biological half-life with an increase in body burden of trace metal ii) stable biological half-life when an equilibrium is maintained by a proportionate increase and iii) increase in biological half-life with increase in body burden of trace metals.

In the present study, the biological half-lives of copper, mercury and arsenic decreased with increase in the body burden. The biological half-life of selenium however remained almost stable as consequence of proportionate increase/ decrease in the rates of accumulation/ depuration. The biological half-life of cadmium increased with increase in the body burden. The increasing order of half-lives of the metals were

$$Se < Cu < Hg < As < Cd.$$

at the lower levels of concentrations and it was the same at the higher levels of concentrations also, except for a reversal in the positions of copper and mercury. i.e.

Se < Hg < Cu < As < Cd.

In binary combinations, other metals enhanced the half-life of copper; the maximum enhancement was that of selenium. The half-life value of cadmium was depressed by other metals (except at the lower concentration of selenium) and the maximum depression was produced by copper. The half-life value of mercury was enhanced by all other metals present in binary combinations and the maximum enhancement was by selenium. Except for cadmium, all other metals depressed the half-life of arsenic and the maximum depression being produced by mercury. Except at the lower concentration of copper, all other metals enhanced the half-life of selenium and the maximum effect was by mercury.

Okazaki and Panietz (1981) investigated the depuration of twelve trace metals, treated individually, and observed that the biological half-lives of manganese in *C. gigas* and copper, zinc and nickel in *C. virginica* increased with increase in body burden whereas cadmium, mercury, silver and iron in *C. gigas* decreased with increase in body burden. The half-life value observed for mercury in the case of *C. gigas* was 23.3 d and for *C. virginica* was 133.5 d and for cadmium were 40 d and 85 d respectively for the two species. Denton and Burden-Jones (1981) reported values in the range of 32-42 d for mercury in varying salinities in the case of the oyster *S. echinata* whereas Cunningham and Tripp (1973) reported values in the range of 35-45 d at different ranges of temperatures.

Chapter V

CHAPTER V

BIOCHEMICAL STUDIES

An organism is the sum of its parts and its activities are the sum of the activities of its component cells. Just as properties of molecules differ from those of their component atoms, tissues/organs have properties different from those of the cells and an organism has properties different from its component organ systems. Nevertheless, the capabilities of a tissue or organ or organ systems are ultimately dependent upon the functions of its cells (Karp, 1984). Metabolism — the sum total of the biochemical reactions occurring within a cell — leads to the production and utilization of chemical energy within the cell, and is catalyzed by enzymes. Most of the enzymes are conjugated proteins and contain the protein component — apoenzymes — and the non-protein component — cofactors. The cofactors may be inorganic (called the prosthetic group and include simple metal ions or complexed metal ions) or organic (coenzymes) (Karp, 1984; Huhee, 1978). Many of the transition elements occur in trace amounts in the prosthetic group of various enzymes (e.g. Fe, Cu, Zn, Co, Mn, Mo, Se, Cr, Ni, V, As and Sn) and are absolutely necessary because they act as catalysts of the biological life processes. They occupy the active sites of enzymes which are directly involved in interaction with the substrates (e.g., Fe in haemoglobin, Cu in haemocyanin, Mg in chlorophyll, Co in cyanocobalamine, etc.). An element is considered essential when

(i) its importance is recognized in all healthy, living tissues within a zoological family,

ii) deficiency syndromes develop with depletion or removal of an element, and disappear on resupply, and when

iii) the deficiency syndromes are attributable to a distinct biochemical effect (Overhoff and Forth, 1978).

Metal ions having no known biological functions are considered non-essential (e.g., Cd, Hg, Pb, etc). Such of these metal ions which have sizes, charges and geometries similar to the biologically essential ones can replace them at their binding sites, lead to alteration/distortion of conformation of the biomolecule, and prove to be toxic to the organism. The replacement of zinc ions in biological tissues by copper, cadmium and mercury ions is well known. Even the essential elements become toxic when their concentrations exceed those required for the nutritional responses, by factors varying between 40-200 fold (Venugopal and Luckey, 1975).

Aquatic animals bioaccumulate metals, both essential and non-essential and maintain their "internal milieu" more or less constant by means of a variety of regulatory mechanisms (e.g. induction of metallothioneins, distribution to less toxic sites, elimination, etc.). When the assimilating capacity of the organism is exceeded, the above regulatory mechanisms fail and result in drastic physiological/biochemical changes which might even prove to be fatal to them. The impacts of stress on aquatic organisms can be assessed by lethal and sublethal effects. Sublethal concentrations affect the behaviour, growth, physiology and reproduction of organisms (Bayne *et al.*, 1981; Calabrese *et al.*, 1984). The ultimate test of significance of a sublethal concentration is whether or not it has an impact on the propagation of a species, or on its population (Bayne *et al.*, 1979). Lethal concentrations, on the other hand, only lead to the death of the organisms. Impacts of elevated levels are therefore best understood by monitoring changes brought about by exposure of the organism to sublethal concentrations of the metals concerned.

Assessment of the variations in the chemical constituents or in the chemical processes occurring within the cell and the consequent physiological alterations are the focus of all

sublethal studies. Responses to an environmental change, at whatever level of cellular organisation, are fundamentally biochemical and these biochemical indices provide the earliest warning of a decline in animal condition.

For a particular biochemical response to be acceptable as an index of stress, the measurable change in the biochemical process must result from or be a response to a change in the environmental factors and it must be possible to demonstrate that the change in the biochemical process will be detrimental to growth, reproduction or survival of the organism (Livingstone, 1985). Certain biochemical systems may respond only to a specific type of environmental stressor, and may aid in its identification in a complex environmental situation, thus leading to a broad categorization of biochemical indices into "specific indices of stress" and "general indices of stress". Table 5.1 lists some of the general and specific indices of stress.

Table 5.1 General and specific indices of stress

General Indices	Specific Indices
Taurine:glycine ratio (Bayne,1976; Roesijadi and Anderson, 1979; Widdows *et al.*,1981)	Mixed function oxygenase (Pani *et al.*, 1976; Yoshida *et al.*, 1976)
Adenylate energy charge (Atkinson, 1977; Skjoldal and Bakke, 1978; Ivanovici, 1980)	Metallothioneins Piotrowsky *et al.*,1973; Roesijadi, 1982; Viarengo *et al.*, 1985;
Enzymatic activity (Gabbot *et al.*, 1979; Livingstone, 1981 ; Cyriac, 1990; Suresh and Mohandas, 1990).	

When aquatic animals are exposed to toxicants such as trace metals, their primary responses like avoidance behaviour, valve closure, etc., result in lowered oxygen supply to the different organs which in turn cause various physiological, metabolic, enzymatic and hormonal changes in the body. Metabolic rate, which depicts the immediate physiological response of stress of an organism, is the most widely used index of sublethal stress to evaluate the changes in metabolism due to environmental alteration (Hawkins, *et al.*, 1986). Metabolic rate is also an index of energy expenditure to meet the demands of environmental alteration, and is measured in terms of oxygen consumption of the animal.

Gills are the primary sites of attack of toxicants. Metal-induced deformations in gill structure of aquatic animals have been reported (Skidmore, 1970; Reid and Mc Donald, 1991) to result in changes in oxygen consumption of the animals. In the case of bivalves, oxygen consumption is dependent mainly on the ventilation volume, and the efficiency of gas exchange (Mohan *et al.*, 1986a). Metal-induced variations in oxygen consumption of bivalves have been reported, among others by Brown and Newell (1972), Scott and Major (1972), Waldichuk (1974), Mathew and Menon (1983), Mohan *et al.* (1986a and b) Prabhudeva and Menon (1986), Krishnakumar (1987), Philip (1990).

The body can develop resistance against the toxicants upto a threshold level by producing substances like glutathione, metallothionein and others in order to sequester and remove the toxicants which result in increased energy demands which impose severe restrictions on the energy budget and forces the organism to respire anaerobically by utilising the stored biochemical components *viz.* glycogen, lipid, protein etc. Reports on metal-induced variation in the energy reserves, carbohydrates, glycogen, lipid, and protein contents of aquatic organisms are numerous in the literature (Shaffi, 1978 a and b; Rao and Rao,

1979 and 1981; Coglianese and Neff, 1982; Ramalingam and Ramalingam, 1982; Bhaskar *et al.*, 1984; Tort *et al.*, 1984; Ram and Sathyanesan, 1985; Abdullah and Ireland 1986; Bhaskar and Govindappa, 1986; Hilmy *et al.*, 1987 a and b; Rajyalakshmi and Reddy, 1988; Ramalingam, 1988; Agarwal and Nair, 1989; Vijayram and Vasugi, 1989; Cyriac, 1990).

However, reports on the effects of toxicants on the above biomolecules are scarce in the literature in the case of bivalves. Lakshmanan and Nambisan (1985) and Sathyanathan *et al.*, (1988) have reported on the effect of trace metals on the glycogen contents of the bivalves, *P. viridis* and *V. cyprinoides* var. *cochinensis*. Lane (1986) investigated the effect of starvation on the biochemical constituents (carbohydrate, protein and lipid) of the clam *R. cuneata*.

The shift from aerobic to anaerobic metabolism under toxicant induced stress is well established in bivalves. Various end products of anaerobic metabolism were also reported in the case of bivalves. Succinate, lactate and alanine (Stokes and Awapara, 1968; de Zwaan and Zandee, 1972; Gade, 1980; Zurberg and Ebberink, 1981) have been reported as the end products of anaerobic metabolism in addition to the opine compounds (Nichitta and Ellington, 1983). Studies on lactic acid as an end product have been carried out at the tissue level by Lakshmanan and Nambisan (1985) and Sathyanathan *et al.* (1988). Suresh (1988) investigated the effect of copper on the serum lactic acid content of two bivalves. Very recently Katticaran and Salih (1992) have reported on the effect of copper on the lactic acid content in the adductor muscle and digestive gland of the clam *S. scripta*. No reports were available on attempts to elucidate the relevance of lipids and proteins as indices of metal stress.

This chapter describes the studies conducted to assess the influence of the trace metals copper, cadmium, mercury, arsenic and selenium (applied individually and in binary combinations) on the metabolic rate, on the macromolecules (glycogen, lipid and protein) and on the lactic acid content of the clam, *Villorita cyprinoides* var. *cochinensis*.

5.2. MATERIALS AND METHODS

Details of the materials used and the methodologies adopted are presented in Chapter II.

5.3. RESULTS AND DISCUSSION

Metabolic Rate

The metabolic rates of the clam exposed to the metals at two levels of concentration for a period of 96h and recorded at 24h intervals are presented in Table 5.2. The rate of oxygen consumption i.e., the metabolic rate recorded by the control animals was 1.943 ml O_2 $h^{-1}g^{-1}$ (dry weight). Both levels of concentration of copper, cadmium, mercury, and arsenic when applied individually and in binary combinations, caused reductions in the oxygen consumption rate of the clam, whereas, lower level of selenium and its binary combinations recorded slight enhancement in the metabolic rate.

The percentage variations from the control value were used to compare and contrast the effects of toxicants on the metabolic rate of the clam and are depicted in Figs. 5.1 - 5.5.

Copper, cadmium and mercury when treated individually and in binary combinations caused a sudden depression in the metabolic rate of the clam at 24h; thereafter, however, the metabolic rate recorded a gradual increase. The percentage

Table 5.2 Metal induced variations in the metabolic rate of V. cyprinoides var. cochinensis (ml/ O_2 /h/g (dry wt.)

a). Individual metals

Control value: 1.94 ml/ O_2 $h^{-1}g^{-1}$ (dry wt.)

Metals and exposure levels	Time (h) n = 6			
	24	48	72	96
Cu(0.1)	1.00 ±0.03	1.34 ±0.04	1.52 ±0.03	1.59 ±0.02
Cd(0.5)	1.14 ±0.06	1.49 ±0.05	1.63 ±0.04	1.65 ±0.05
Hg(0.1)	1.06 ±0.06	1.21 ±0.05	1.51 ±0.06	1.63 ±0.08
As(1.0)	1.66 ±0.07	1.52 ±0.06	1.48 ±0.08	1.33 ±0.05
Se(0.2)	2.45 ±0.09	2.32 ±0.08	2.24 ±0.07	2.20 ±0.09
Cu(0.3)	0.82 ±0.04	0.99 ±0.05	1.01 ±0.03	1.08 ±0.06
Cd(1.5)	1.00 ±0.03	1.11 ±0.06	1.24 ±0.07	1.29 ±0.04
Hg(0.3)	0.86 ±0.03	1.12 ±0.06	1.15 ±0.05	1.19 ±0.07
As(3.0)	1.40 ±0.05	1.27 ±0.07	1.20 ±0.08	1.02 ±0.06
Se(0.6)	1.76 ±0.07	1.53 ±0.06	1.48 ±0.08	1.36 ±0.05

Contd.....

Table 5.2 Metal induced variations in the metabolic rate of V. cyprinoides var. cochinensis (ml/ O_2/h/g (dry wt.)

b). Binary combinations

Control value: 1.943 ml/ O_2 h^{-1} g^{-1} (dry wt.)

Metals and exposure levels	Time (h) n = 6			
	24	48	72	96
Cu(0.1)+Cd(0.5)	0.96 ±0.04	1.21 ±0.02	1.34 ±0.05	1.47 ±0.03
Cu(0.1)+Hg(0.1)	1.24 ±0.07	1.53 ±0.06	1.61 ±0.05	1.73 ±0.04
Cu(0.1)+As(1.0)	1.25 ±0.04	1.31 ±0.03	1.40 ±0.05	1.45 ±0.08
Cu(0.1)+Se(0.2)	2.35 ±0.08	2.29 ±0.05	2.16 ±0.07	2.05 ±0.04
Cd(0.5)+Hg(0.1)	1.32 ±0.07	1.47 ±0.06	1.55 ±0.08	1.61 ±0.07
Cd(0.5)+As(1.0)	1.35 ±0.04	1.49 ±0.03	1.52 ±0.05	1.56 ±0.08
Cd(0.5)+Se(0.2)	2.26 ±0.08	2.18 ±0.09	2.11 ±0.06	2.05 ±0.07
Hg(0.1)+As(1.0)	1.35 ±0.04	1.49 ±0.05	1.52 ±0.05	1.56 ±0.07
Hg(0.1)+Se(0.2)	2.38 ±0.05	2.24 ±0.03	2.14 ±0.04	2.05 ±0.05
As(1.0)+Se(0.2)	2.40 ±0.08	2.32 ±0.09	2.24 ±0.07	2.11 ±0.05
Cu(0.3)+Cd(1.5)	0.69 ±0.06	0.85 ±0.05	0.90 ±0.07	0.95 ±0.04
Cu(0.3)+Hg(0.3)	0.84 ±0.09	1.14 ±0.05	1.24 ±0.04	1.33 ±0.05
Cu(0.3)+As(3.0)	1.02 ±0.03	1.10 ±0.06	1.15 ±0.08	1.20 ±0.09
Cu(0.3)+Se(0.6)	1.36 ±0.09	1.32 ±0.08	1.29 ±0.04	1.20 ±0.09
Cd(1.5)+Hg(0.3)	1.12 ±0.08	1.32 ±0.05	1.40 ±0.07	1.42 ±0.08
Cd(1.5)+As(3.0)	1.12 ±0.06	1.20 ±0.08	1.24 ±0.05	1.26 ±0.04
Cd(1.5)+Se(0.6)	1.00 ±0.05	1.20 ±0.03	1.26 ±0.04	1.39 ±0.05
Hg(0.3)+As(3.0)	1.12 ±0.05	1.20 ±0.07	1.24 ±0.06	1.26 ±0.04
Hg(0.3)+Se(0.6)	1.01 ±0.06	1.18 ±0.07	1.27 ±0.05	1.33 ±0.04
As(3.0)+Se(0.6)	1.82 ±0.09	1.71 ±0.07	1.62 ±0.06	1.49 ±0.08

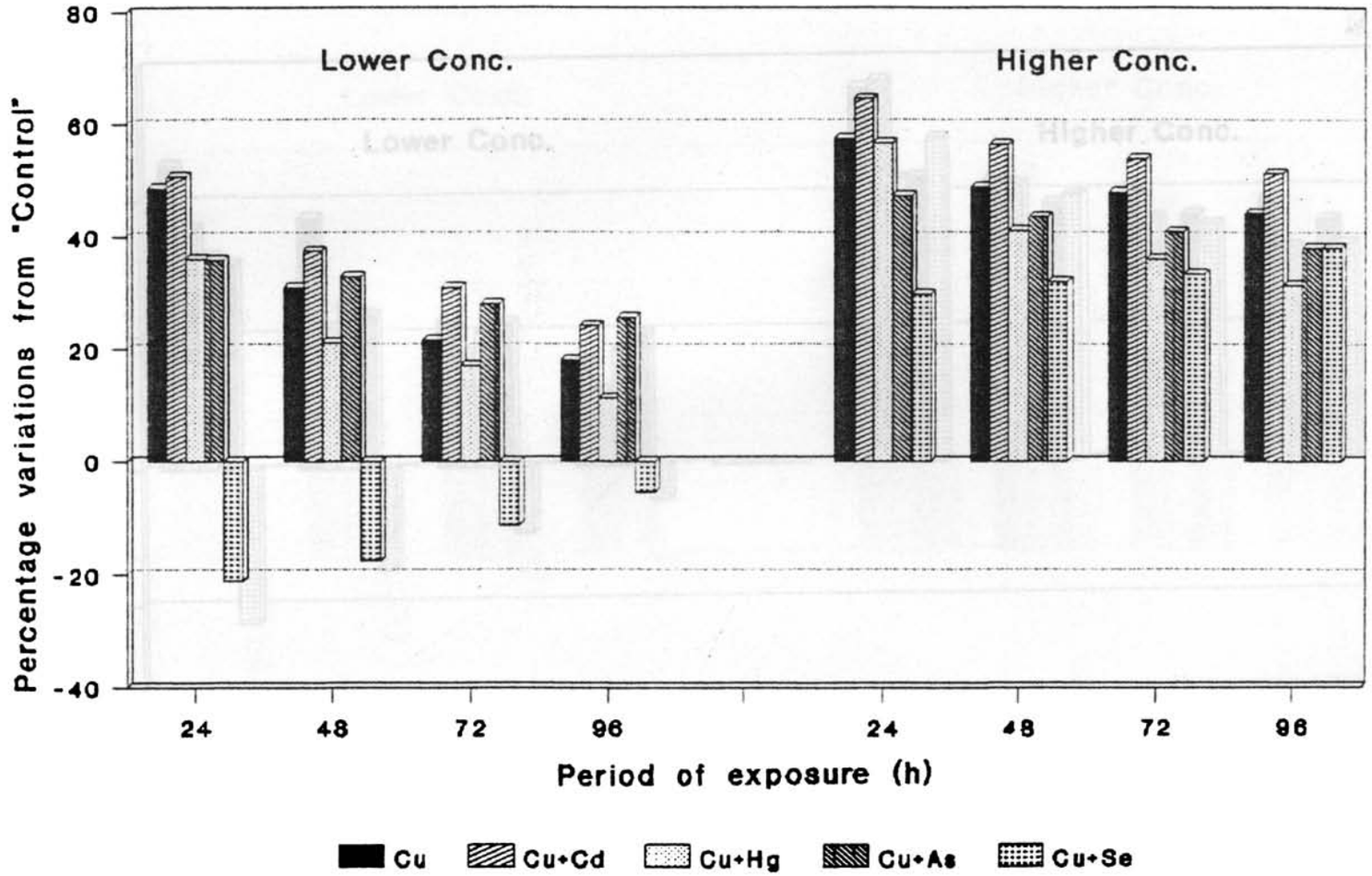


Fig.5.1 Metal-induced variations in the metabolic rate

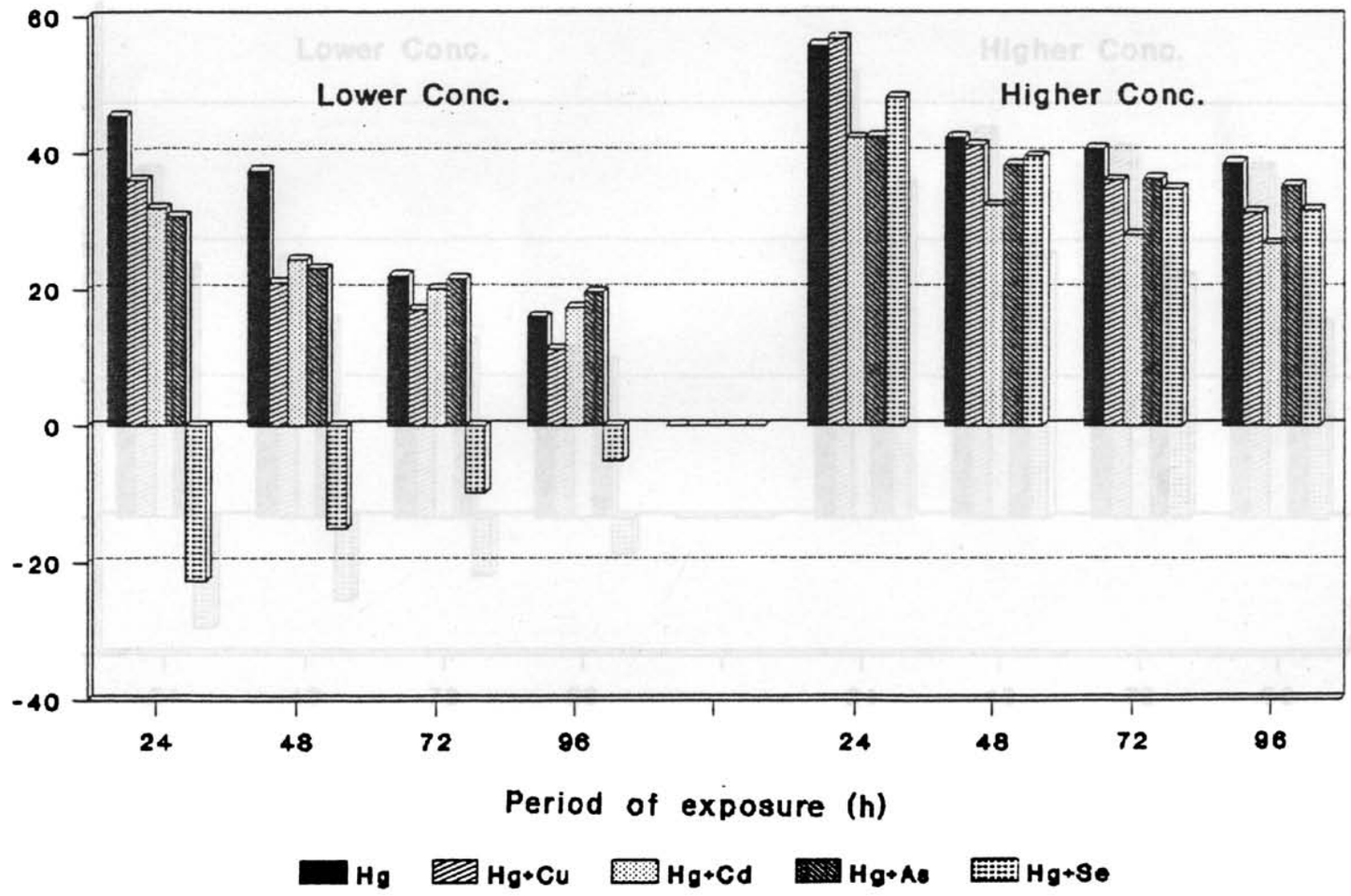


Fig.5.2 Metal-induced variations in the metabolic rate

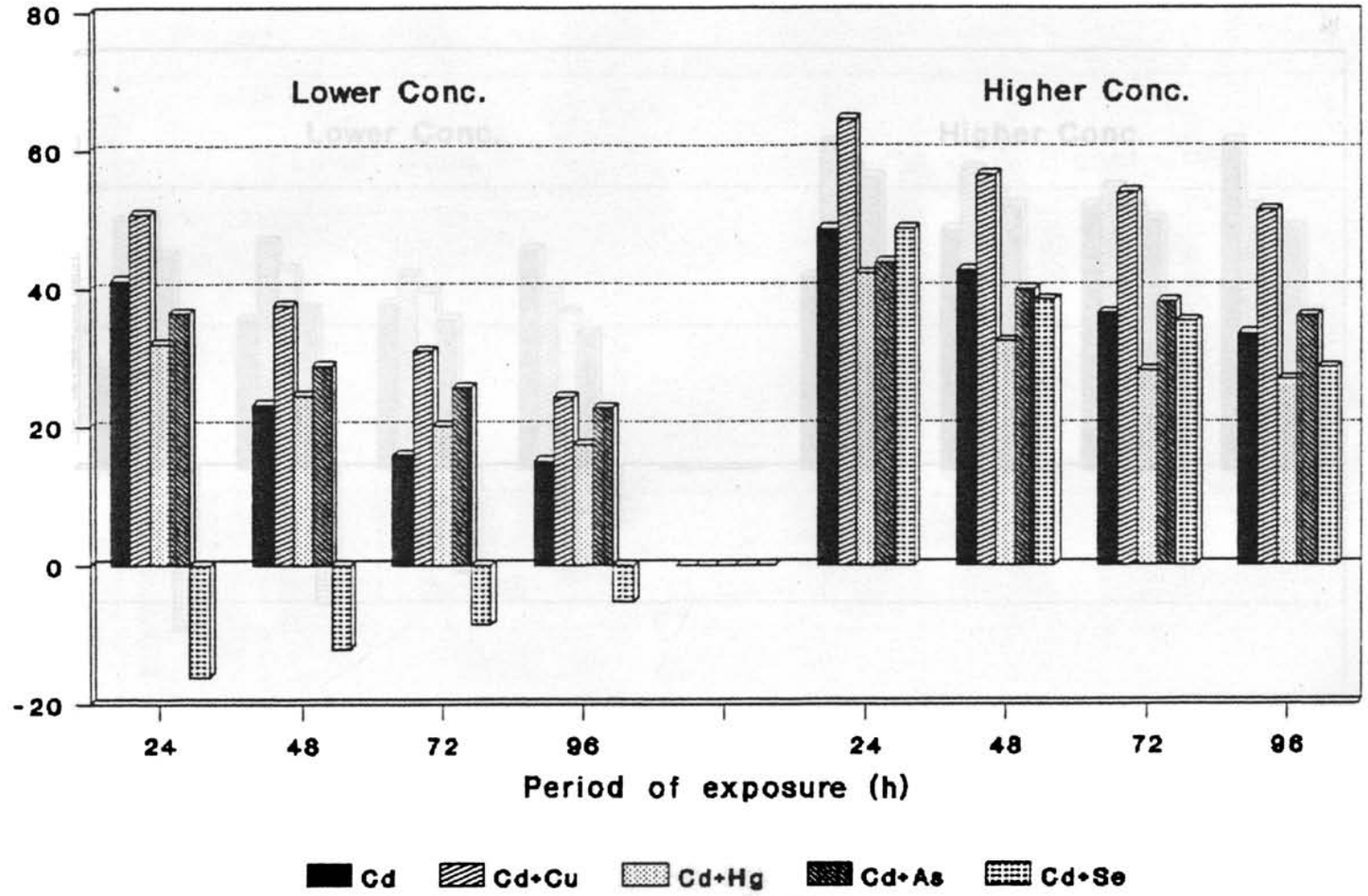


Fig.5.3 Metal-induced variations in the metabolic rate

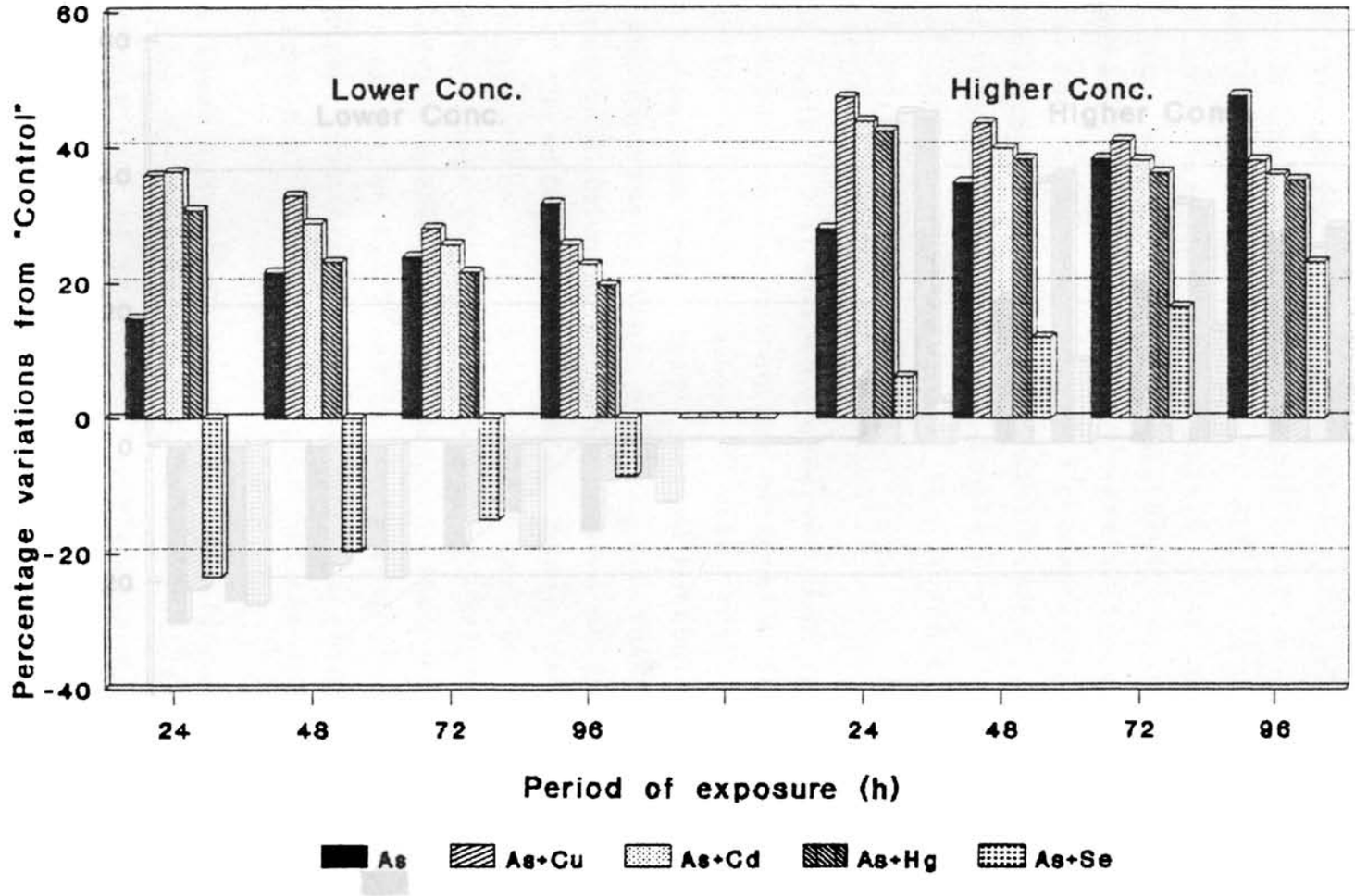


Fig.5.4 Metal-induced variations in the metabolic rate

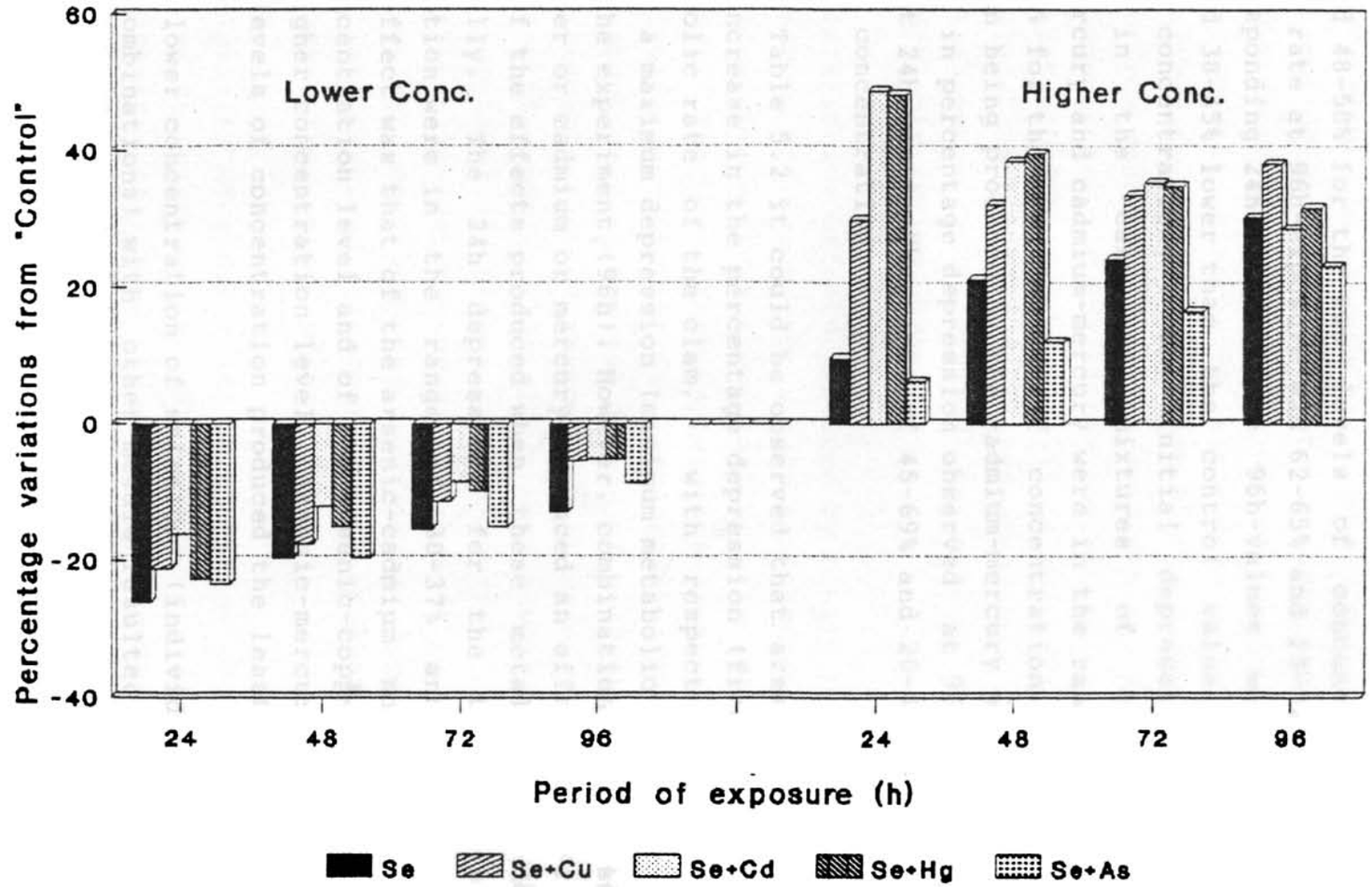


Fig.5.5 Metal-induced variations in the metabolic rate

variation from control observed at 24h was in the range of 41-49% and 48-58% for the two levels of concentration. The metabolic rate at 96h-exposure was 62-65% and 23-32% higher than the corresponding 24h-values. The 96h-values were thus only 16-19% and 38-45% lower than the control value for the two levels of concentration. The initial depressions (at 24h) observed in the case of mixtures of copper-cadmium, copper-mercury and cadmium-mercury were in the range of 31-51% and 42-64% for the two levels of concentration, the highest depression being produced by the cadmium-mercury mixtures. The decrement in percentage depression observed at 96h with respect to that at 24h was in the range of 45-69% and 20-45% for the two levels of concentration.

From Table 5.2 it could be observed that arsenic produced a gradual increase in the percentage depression (from control) of the metabolic rate of the clam, with respect to time and effected a maximum depression (minimum metabolic rate) at the end of the experiment (96h). However, combinations of arsenic with copper or cadmium or mercury produced an effect which is an average of the effects produced when these metals were dosed individually. The 24h depressions for the two levels of concentration were in the range of 30-37% and 43-48%; the maximum effect was that of the arsenic-cadmium mixture at the lower concentration level and of the arsenic-copper combination at the higher concentration level. Arsenic-mercury combinations at both levels of concentration produced the least effect.

The lower concentration of selenium (individually and in binary combinations) with other metals resulted in elevating the metabolic rate of the clam. The elevations were maximum at 24h, and thereafter decreased marginally towards the end of the experiment (96h). At 24h, 0.2ppm selenium caused an elevation of 26% and combinations involving the lower level of selenium effected elevations in the range of 16-24% from the control

value. The selenium-arsenic and selenium-cadmium combinations caused the maximum and minimum elevations. The higher level of concentrations of selenium and its binary combinations with copper and arsenic effected 6-30% depression in the metabolic rate at 24h. Continued exposure resulted in further decrease of the metabolic rate which finally (at 96h) recorded around 23-38% depression from control. The binary mixtures selenium-cadmium and selenium-mercury, at the higher concentration level however, produced initial (24h) depressions (from control) of 49% and 48% respectively, but thereafter effected an increase upon subsequent exposure. At 96h, the percentage deviations from control were only 29% and 32% .

The interactive effects produced by binary combinations of copper, cadmium and mercury have been analysed on the basis of the variations produced in the metabolic rate observed at the 24h-exposure. The percentage depressions produced by copper at both levels of concentration were enhanced by cadmium by 4.3% and 16% respectively at the two levels of concentration, whereas the lower concentration of mercury was observed to mitigate the toxic effect of copper. This effect was, however, not recorded at the higher level of copper and mercury. The effect of arsenic at both levels of concentration was to alleviate the toxic effect of copper and the percentage depression in the metabolic rate produced by copper-arsenic mixture was 26% and 18% lower than the copper-induced value.

The toxicity of cadmium, at both levels of concentration, was observed to be augmented by copper whereas mercury and arsenic mitigated the same. Cadmium-copper combinations at the two levels of concentrations caused 18% and 33% higher depressions than the cadmium-induced value. Cadmium-mercury and cadmium-arsenic combinations, at the lower concentration levels produced respectively 23% and 12% lower depressions than the

cadmium-induced value. The depressions at the higher concentration level were 14% and 10%.

The effects of copper (at the lower concentration level), of cadmium and arsenic (at both concentration levels) and of selenium (at higher concentration level) were to alleviate the toxic effect of mercury. The copper-mercury combination at the lower concentration level produced 29% depression in the mercury-induced value, whereas at the higher concentration level the effect was not predictable. The depressions produced by the mercury-cadmium and the mercury-arsenic combinations at the lower concentration levels were 30% and 32% of mercury-induced value whereas at the higher concentration levels they were only 25% and 24% respectively.

The effect of other metals on the toxicity of arsenic was to further reduce the metabolic rate, exception noted was in the case of selenium, where the arsenic-selenium combinations recorded larger metabolic rates than arsenic alone.

The enhancement in metabolic rate noticed when selenium was used individually at lower concentration, was found to get depressed with the addition of other metals. At higher level of selenium, the effect of other metals was to further depress the metabolic rate.

Oxygen consumption in bivalves represents the product of two factors, ventilation volume and efficiency of gas exchange (Mohan, *et al.*, 1986a). Behavioural responses like valve closure, siphonal activity, filtration rate or gill irrigation, etc. influence the rate of oxygen uptake. The rate of filtration is known to be influenced by several endogenous and exogenous factors such as salinity, temperature, concentration of suspended matter, etc. (Abel, 1976; Watling and Watling, 1982). The rate of filtration, was reported to be impaired by

the presence of heavy metals (Abel, 1976; Watling and Watling, 1982; Mathew and Menon, 1983; Abraham *et al.*, 1986). Reports on the interactive effects of metals on the filtration rate or on the oxygen consumption are scarce except for those of Watling and Watling (1982), Mohan *et al.*, (1986b), Baby (1987) and Philip (1990). Gills are the primary sites of attack of toxicants and the structural disturbances produced on the gills can significantly alter the rate of gill irrigation and thereby the of the rate of absorption of oxygen.

The weight-specific oxygen consumption observed for the clam, *Villorita cyprinoides* var. *cochinensis* was considerably higher than the value (0.496 ml O_2h^{-1}) reported for ten species of temperate bivalves (Bayne and Newell, 1983). This indicated that the metabolic costs associated with rates of growth were higher in *V. cyprinoides* var. *cochinensis* than in the other species of mussels studied earlier (Shafee, 1979; Hawkins *et al.*, 1986).

In the case of *V. cyprinoides* var. *cochinensis*, copper, cadmium, mercury, arsenic and selenium (except the lower concentration of selenium) were generally found to act as respiratory depressants. The depressive effect of copper, cadmium and mercury on respiration in other animals have already been reported (Brown and Newell, 1972; Scott and Major, 1972;Waldichuk, 1974; Mathew and Menon, 1983; Prabhudeva and Menon, 1986; Mohan *et al.*, 1986a; Baby, 1987;Krishankumar, 1987; Sivadasan, 1987; Philip, 1990), and the results from the present study proved the same in the case of *V. cyprinoides*. However, copper, cadmium and mercury were peculiar in that the initial (24h) sudden depression was followed by a gradual increase in the metabolic rate along with increase in the exposure time. Comparing the depressions produced at 24h the metals could be arranged in the following order of toxicity :

Cu > Hg > Cd.

The observed initial decrease could be a reflection of two classical stages of stress response *viz.*, the alarm reaction and the stage of resistance that have been found to be typical of the above metals (Donaldson and Dye 1975; Sathyanathan *et al.*, 1988). However, silver, zinc and lead were reported to be respiratory stimulants (Mathew and Menon 1983; Prabhudeva and Menon, 1986; Krishnakumar, 1987; Sivadasan, 1987; Philip, 1990) as in the case of selenium observed in the present study. These results indicated that such physiological responses in marine organisms were dependent on the specific metal toxicant.

Cadmium and mercury were respiratory depressants to *P. viridis*, but a combination of both exhibited an immediate reduction at lower concentration, a temporary elevation at medium concentration followed by a decline at higher concentrations with both the combinations. Oxygen consumption was reduced to a greater extent than with either of the metals individually, at lower concentrations. Similarly in the case of *M. carvalhoi, Modiolus* sp. and *D. spiculusm* also a more than additive interaction of cadmium and mercury was observed (Mohan *et al.*, 1986a and b). Baby (1987), however, observed that mercury and cadmium acted as simple additives to *P. indica* whereas Watling and Watling (1982) reported that mercury and selenium acted antagonistically on the filtration rate of *P.viridis*.

Glycogen

Glycogen content of the bivalve was estimated after 24h, 48h, 72h and 96h exposures to the metals (both individually and in binary combinations). The results are presented in Table 5.3 and Figs. 5.6 - 5.10. Analyses and intercomparison of the metal-induced effects have been based on the 96h glycogen content.

Table 5.3 Metal Induced Variations in the Glycogen content of V.cyprinoides var. cochinensis (mg/g)

a). Individual Metals

Control value: 3.07 ±0.031 mg/g

Metals and exposure levels	Time (h) n = 6			
	24	48	72	96
Cu(0.1)	2.70 ±0.033	2.57 ±0.024	2.54 ±0.046	2.43 ±0.052
Cd(0.5)	2.80 ±0.042	2.69 ±0.074	2.54 ±0.063	2.39 ±0.052
Hg(0.1)	2.78 ±0.056	2.70 ±0.072	2.43 ±0.054	2.27 ±0.041
As(1.0)	2.90 ±0.058	2.80 ±0.062	2.55 ±0.046	2.49 ±0.054
Se(0.2)	2.96 ±0.053	2.86 ±0.037	2.75 ±0.054	2.55 ±0.046
Cu(0.3)	2.39 ±0.054	2.04 ±0.045	1.93 ±0.064	1.82 ±0.062
Cd(1.5)	2.63 ±0.062	2.53 ±0.054	2.34 ±0.063	2.04 ±0.074
Hg(0.3)	2.50 ±0.034	2.30 ±0.035	2.02 ±0.046	1.86 ±0.073
As(3.0)	2.65 ±0.038	2.48 ±0.054	2.31 ±0.053	1.91 ±0.062
Se(0.6)	2.64 ±0.062	2.42 ±0.073	2.32 ±0.029	1.92 ±0.038

Contd.....

Table 5.3 Contd.....

b). Binary combinations

Control value: 3.07 ±0.031 mg/g

Metals and exposure levels	Time (h) n = 6			
	24	48	72	96
Cu(0.1)+Cd(0.5)	2.98 ±0.036	2.86 ±0.048	2.78 ±0.038	2.69 ±0.027
Cu(0.1)+Hg(0.1)	2.75 ±0.052	2.60 ±0.037	2.50 ±0.028	2.48 ±0.046
Cu(0.1)+As(1.0)	2.90 ±0.043	2.70 ±0.054	2.57 ±0.062	2.46 ±0.037
Cu(0.1)+Se(0.2)	2.87 ±0.039	2.81 ±0.048	2.77 ±0.056	2.73 ±0.045
Cd(0.5)+Hg(0.1)	2.72 ±0.048	2.62 ±0.046	2.53 ±0.054	2.48 ±0.035
Cd(0.5)+As(1.0)	2.90 ±0.029	2.87 ±0.056	2.77 ±0.038	2.70 ±0.044
Cd(0.5)+Se(0.2)	2.86 ±0.043	2.74 ±0.032	2.70 ±0.053	2.63 ±0.062
Hg(0.1)+As(1.0)	2.86 ±0.041	2.75 ±0.025	2.54 ±0.032	2.44 ±0.043
Hg(0.1)+Se(0.2)	2.97 ±0.023	2.90 ±0.033	2.87 ±0.027	2.77 ±0.019
As(1.0)+Se(0.2)	2.93 ±0.029	2.87 ±0.039	2.73 ±0.046	2.69 ±0.045
Cu(0.3)+Cd(1.5)	2.80 ±0.026	2.56 ±0.037	2.53 ±0.048	2.33 ±0.035
Cu(0.3)+Hg(0.3)	2.37 ±0.046	2.12 ±0.037	1.97 ±0.036	1.86 ±0.058
Cu(0.3)+As(3.0)	2.59 ±0.061	2.56 ±0.038	1.94 ±0.059	1.90 ±0.035
Cu(0.3)+Se(0.6)	2.77 ±0.026	2.64 ±0.038	2.47 ±0.058	2.39 ±0.063
Cd(1.5)+Hg(0.3)	2.38 ±0.044	2.20 ±0.036	1.92 ±0.054	1.89 ±0.046
Cd(1.5)+As(3.0)	2.80 ±0.053	2.77 ±0.034	2.65 ±0.046	2.43 ±0.055
Cd(1.5)+Se(0.6)	2.75 ±0.06	2.68 ±0.04	2.47 ±0.03	2.33 ±0.05
Hg(0.3)+As(3.0)	2.75 ±0.025	2.63 ±0.036	2.53 ±0.045	2.27 ±0.038
Hg(0.3)+Se(0.6)	2.69 ±0.028	2.58 ±0.035	2.50 ±0.046	2.43 ±0.034
As(3.0)+Se(0.6)	2.75 ±0.037	2.60 ±0.026	2.40 ±0.043	2.28 ±0.053

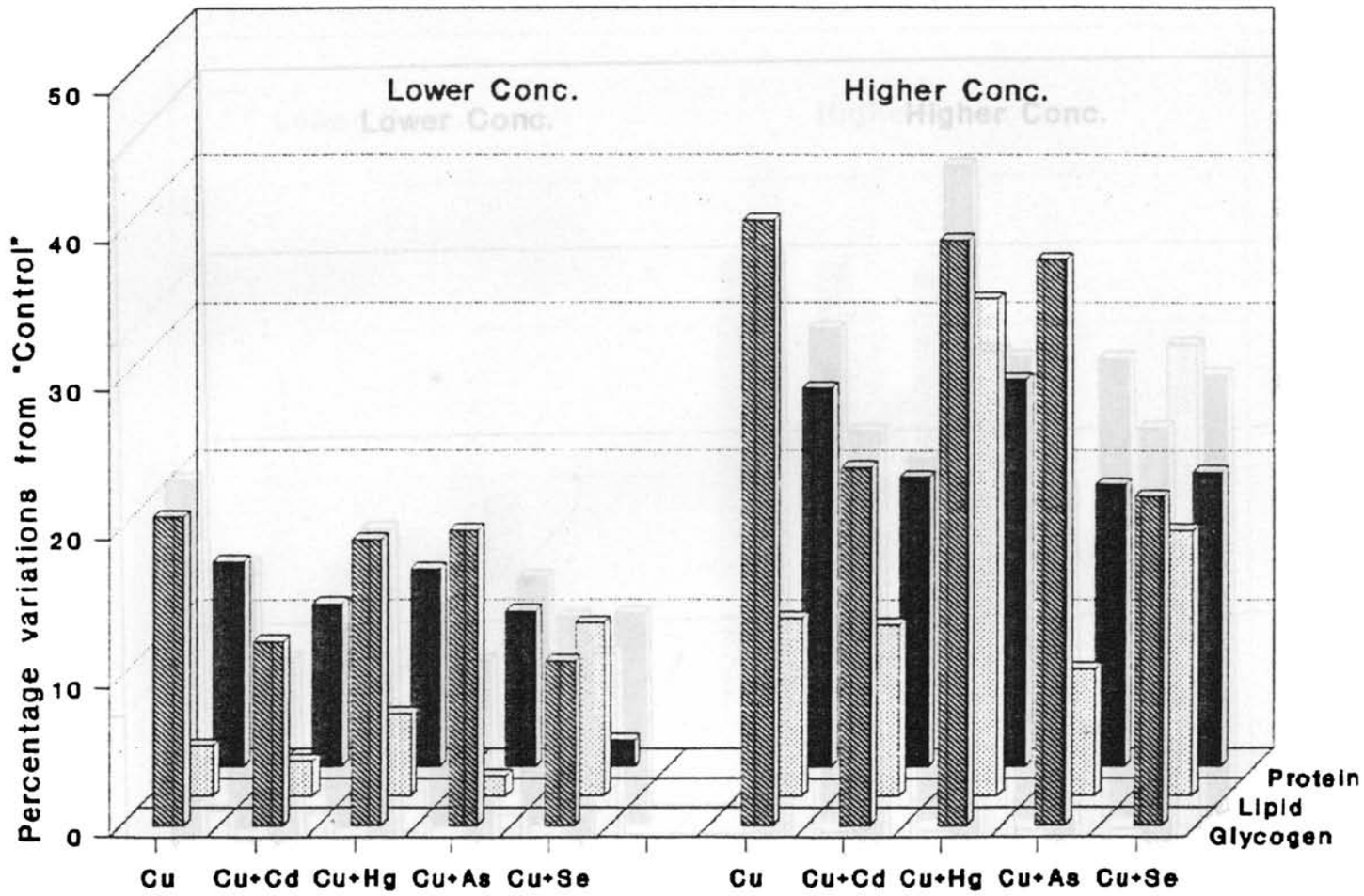


Fig.5.6 Metal-induced variations in the energy reserves (Glycogen, lipid, protein)

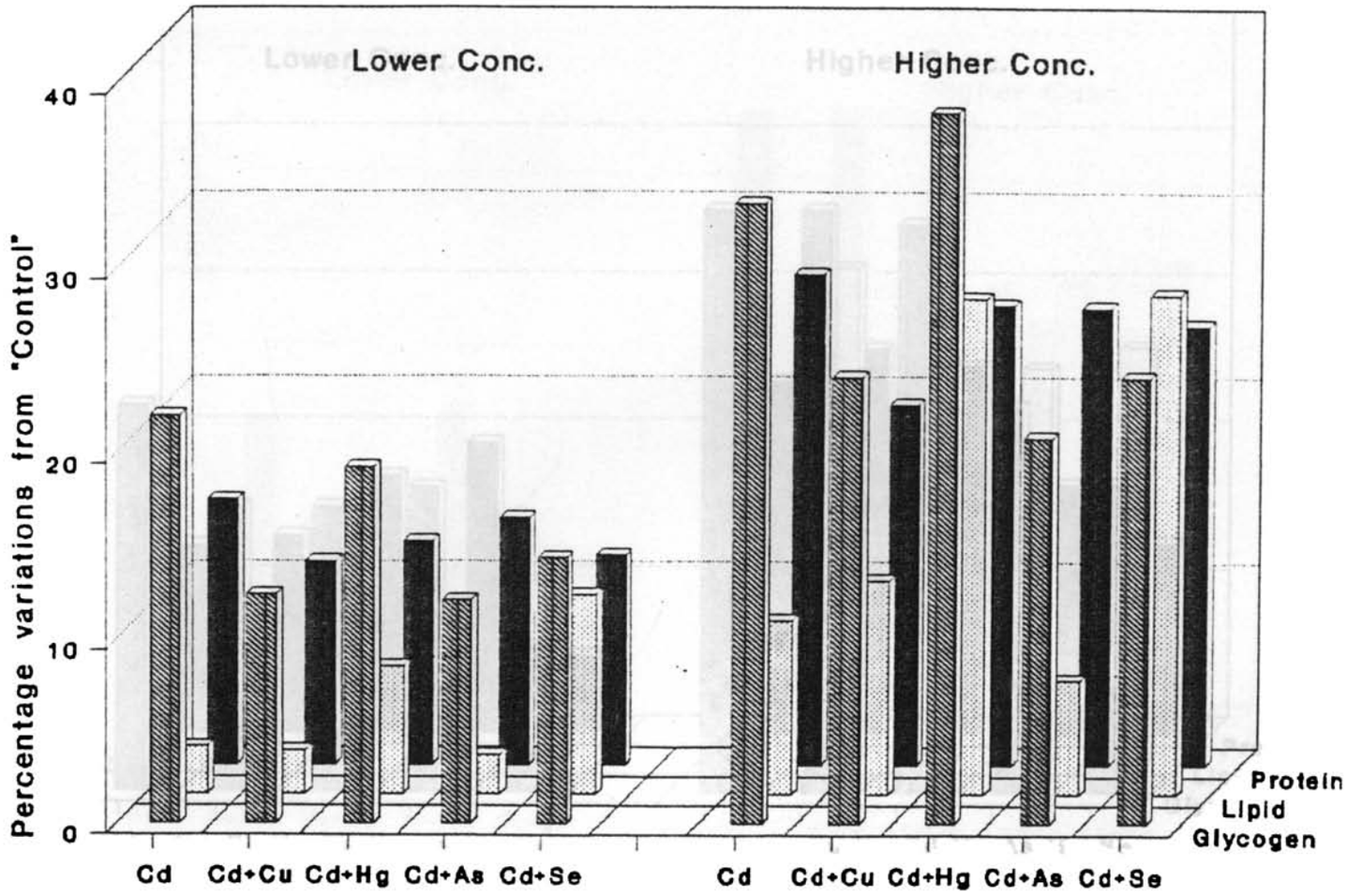


Fig.5.7 Metal-induced variations in the energy reserves (Glycogen, lipid, protein)

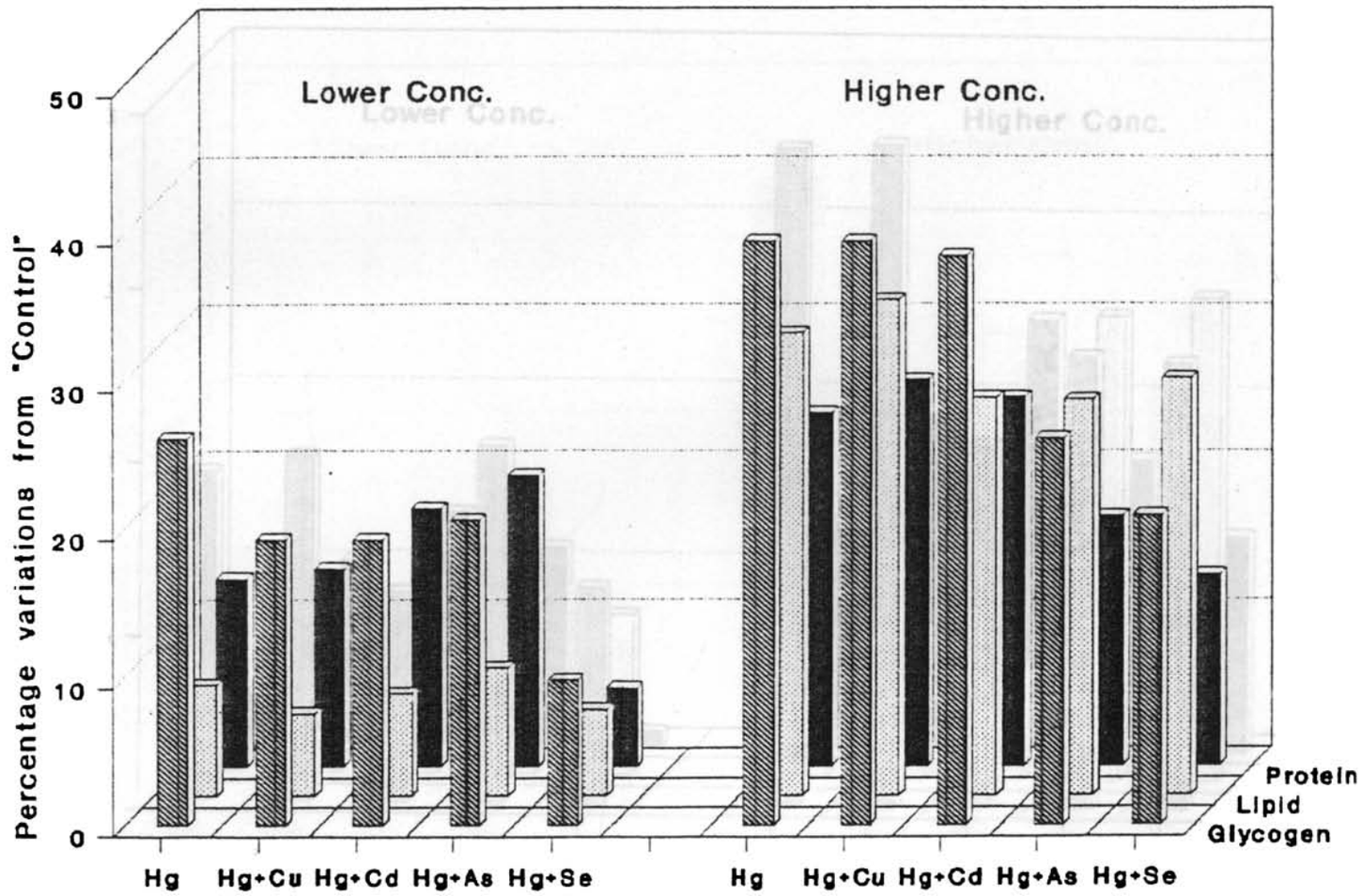


Fig.5.8 Metal-induced variations in the energy reserves (Glycogen, lipid, protein)

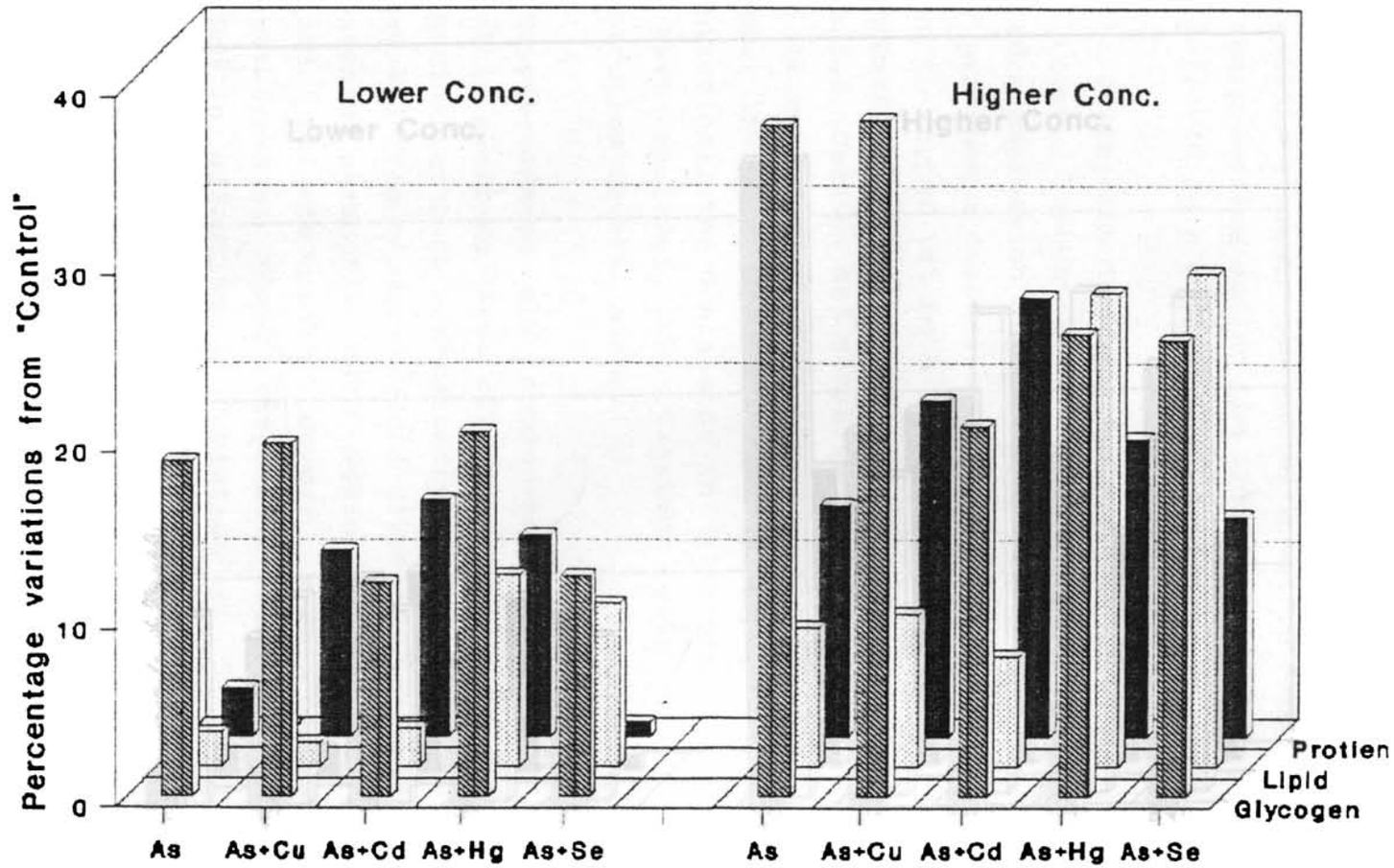


Fig.5.9 Metal-induced variations in the energy reserves (Glycogen, lipid, protein)

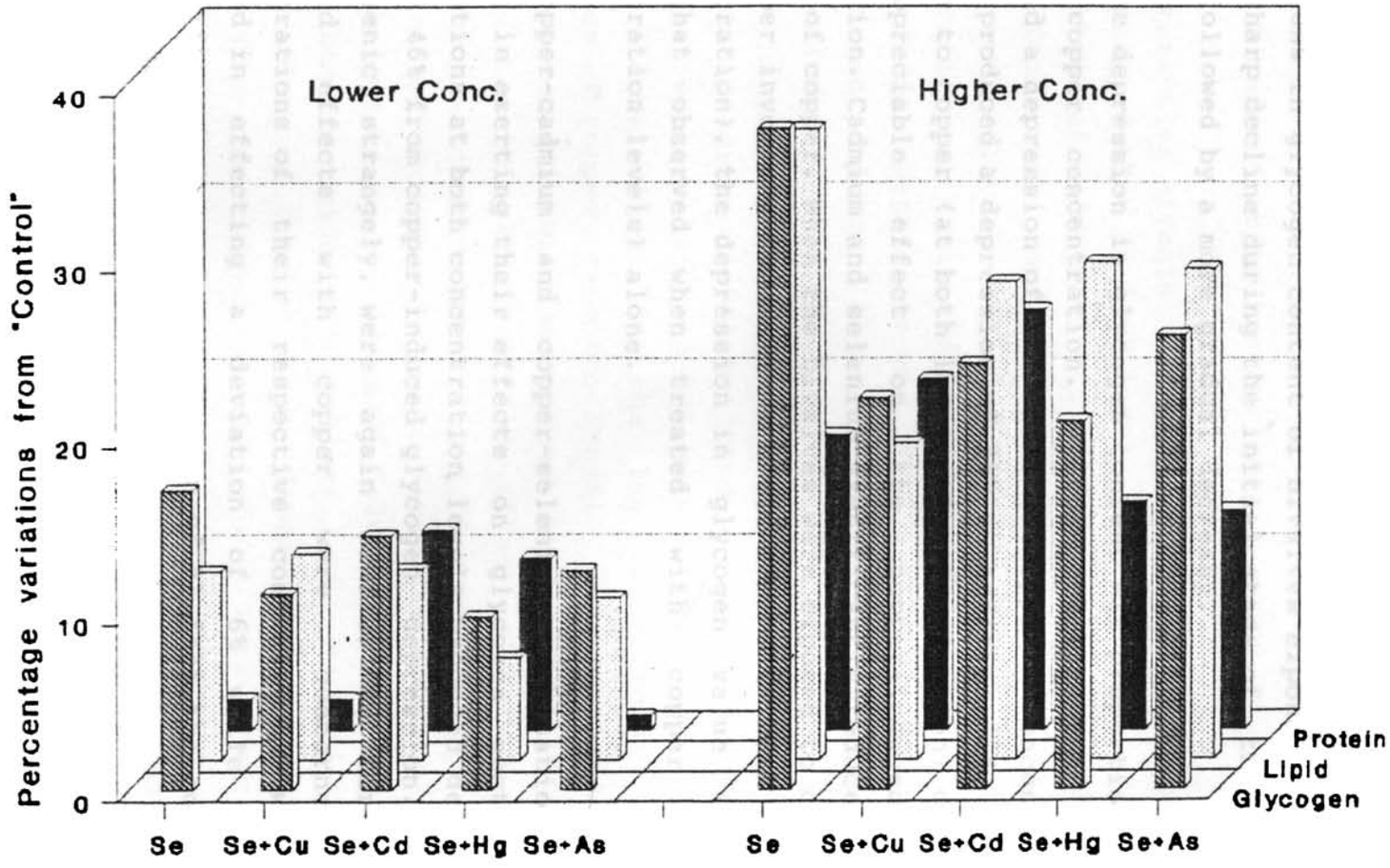


Fig.5.10 Metal-induced variations in the energy reserves (Glycogen, lipid, protein)

Glycogen content of the control animals did not undergo any significant variation during the period of the experiment and recorded a value of 3.07 ± 0.058 mg g^{-1}. A general feature of the variations in glycogen content of bivalves exposed to the metals was a sharp decline during the initial stage of the experiment (24h) followed by a more gradual decrease.

The depression in glycogen content was proportional to the dosed copper concentration. 0.1ppm concentration of copper produced a depression of 21% while 0.3ppm concentration of copper produced a depression of 41% . Addition of mercury or arsenic to copper (at both levels of concentration) did not have any appreciable effect on the copper-induced glycogen depression. Cadmium and selenium significantly reduced the toxic effect of copper. When the bivalves were exposed to combinations of copper involving cadmium or selenium (at both levels of concentration), the depression in glycogen value was smaller than that observed when treated with copper (at both concentration levels) alone.

Copper-cadmium and copper-selenium combinations were similar in exerting their effects on glycogen content. Their combinations at both concentration levels produced deviations of 41% and 46% from copper-induced glycogen depression. Mercury and arsenic, strangely, were again similar as far as their combined effects with copper were concerned. Lower concentrations of their respective combinations with copper resulted in effecting a deviation of 6% while the higher concentrations produced a deviation of 5% from the corresponding copper-induced values.

The depression in glycogen content produced by the lower concentration of cadmium was 22%; a threefold increase in the concentration of cadmium further increased the depression of glycogen content to 34% of the control value. However, binary

combinations of cadmium with copper, mercury, arsenic and selenium were observed to bring about less reductions than that produced by cadmium acting individually. These reductions were in the range of 12-15 % and 20-24%, at the lower and higher levels of concentration respectively.

Interactive effects of cadmium with other metals were interesting in that at each level of concentration of binary combinations of cadmium with copper, arsenic and selenium, the toxic effects were comparable. Mercury seemed to have a dual action in modifying cadmium toxicity —— at the lower concentrations it mitigated cadmium toxicity (deviation from cadmium-induced depression was 13%), at the higher concentration it was found to enhance the toxic effect of cadmium (deviation from cadmium-induced depression was 15%).

The depression of 26% produced by the lower concentration of mercury was increased to 39% upon a threefold increment in mercury concentration. At the low concentration level, the influence of copper, cadmium, arsenic and selenium acting in binary combination with mercury on the glycogen content of the bivalve was to alleviate the effect of mercury applied individually. Lower concentrations of copper, cadmium, arsenic and selenium, thus, were able to limit the depressions in the glycogen content to the range of 9-20% of the control value. Higher concentration combinations of mercury-arsenic and mercury-selenium brought about depressions in the range of 20-26% (from the control value), which were lower than that induced by the higher concentration of mercury, whereas copper and cadmium produced no noticeable effect on mercury-induced glycogen depression. The low toxicity of selenium had a strong influence in moderating the toxicity of mercury and the result is in agreement with the established antagonistic effect of mercury-selenium combinations. Yet another striking similarity was observed between the effects produced by the mercury-arsenic

(lower concentration) and the mercury-selenium (higher concentration) combinations. Both these combinations effected a depression of 21% from the control value.

The higher concentration of arsenic (3ppm) effected a depression in glycogen content (from control) of 38% while at the lower concentration the effect was almost halved. Lower concentration of binary combinations of arsenic-copper and arsenic-mercury did not produce any appreciable change on the arsenic-induced value of the glycogen content. Effects of arsenic-cadmium and arsenic-selenium combinations (lower concentrations) on the glycogen content were similar and smaller in magnitude than that produced by arsenic alone (12.21 $\pm$ 0.23). Higher concentrations of arsenic-cadmium, arsenic-mercury, and arsenic-selenium were observed to be less toxic than arsenic itself and effected glycogen depressions of 21%, 26% and 26%, respectively from the control value. The deviations observed from the arsenic-induced glycogen depression for the low concentration combinations of arsenic-cadmium and arsenic-selenium were 36% and 34% . Arsenic-copper combinations at the lower and higher levels and arsenic-mercury combination at the lower level produced 5%, 1% and 9% higher percentage depressions in the glycogen content respectively than that induced by arsenic alone.

The low toxicity of selenium was clearly reflected in the poor glycogen depressions produced by almost all concentrations of selenium and its binary combinations. Exposure to the lower concentration of selenium caused a 17% depression in the glycogen levels (of control animals) whereas the higher concentration produced 38% lowering of the glycogen content. The toxic effect of selenium was mitigated by the presence of a second metal. Glycogen levels observed for the lower concentrations of combinations, ranged between 9 and 15% while

that for higher concentrations, ranged between 20 and 26% of the control value.

Exposure of the bivalves to copper, cadmium, mercury or arsenic in presence of selenium had markedly reduced the selenium-induced glycogen value. At the lower level of concentration, combinations of selenium with copper, cadmium, mercury and arsenic produced deviations in the range of 15-43% from selenium-induced value. Binary combinations of selenium at the higher levels of concentrations produced rather comparable effects and the deviations from selenium-induced glycogen depression ranged between 31-45% ; the maximum effect being that of mercury and the minimum effect being that of arsenic.

Aquatic animals under toxicant-induced stress conditions control their energy requirement by chilling their activities and the stored form of carbohydrate, (glycogen) is the immediate source of energy in such circumstances. Glycogenolysis involves a number of enzymes and is hormone induced. The primary effects of environmental stress induce the endocrine glands to release large amounts of hormones which increase glycogenolysis to meet the increased energy demand. To maintain the blood glucose level relatively high, despite the negative influence of stressors, glycogenolysis is essential. The elevation in blood glucose may form a part of the restorative process in which glucose is mobilized from glycogen reserves (Grant and Mehrle, 1973; Wardle, 1978; Gluth and Hanke, 1984; Cyriac, 1990). The accumulation of metabolic end products in the serum might lead to changes in the pH of blood and thus interfere with the buffering action of organs which might favour the process of glycogenolysis of tissues. Lane (1986) observed that under starvation-induced stress the bivalve *R. cuneata* utilized the energy reserves, *viz.*, carbohydrates, protein and/or lipid in a sequential manner. An alternative scheme for anaerobic metabolism, in which the redox balance was maintained by the

simultaneous utilization of both carbohydrate and protein (aspartate and glutamate) was proposed by Hochackka and Moustafa (1972) and Hochachkka *et al.* (1973).

Utilization of glycogen in fishes and other aquatic organisms under the sublethal stress of heavy metals has been well documented (Shaffi, 1978a; Abdullah and Ireland, 1986; Kedarnath and Nishithkumar, 1987; 1988; Hilmy, *et al.*, 1987a; 1987b; Ramalingam, 1988; Cyriac, 1990). Manganese and chromium intoxication of the fresh water teleost *C. faciatus* resulted in depletion of liver glycogen content and in the elevation of blood glucose levels (Kedarnath and Nishithkumar, 1987; 1988). Ramalingam,(1988) investigated the effect of DDT, Malathion and mercury on *S.mossambicus* and observed a depletion of glycogen content in the liver and muscle and a concomitant increase in the serum carbohydrate and the free sugars. An inverse relationship was found between cadmium concentration and glycogen content in the liver, muscle and brain of the different species of fishes *L. rohita, O. punctatus and C. batrachus* (Shaffi, 1978a). The effect was similar to that observed with copper as a toxicant to the above three species of fishes (Shaffi, 1978b). However, reports on the effects of toxicants on carbohydrate metabolism of bivalves are relatively few (Lakshmanan and Nambisan, 1985; Sathyanathan *et al.*, 1988) and the present study clearly established that the glycogen content of *V. cyprinoides* get reduced under sublethal levels of metal toxicants.

Enhanced activity of the rate limiting enzymes such as glucose- 6 - phosphatase, fructose-1,6- diphosphatase, pyruvate carbohydrase, etc. was suggested as the pathway for glycogenolysis in cadmium treated rats (Viarengo, 1985). Increased phosphorylase activity in red muscle of *T. mossambica* acclimatized to alkaline media was suggested by Bhasker and Govindappa (1986) and inhibition of the enzymes of the TCA cycle

(*viz.* pyruvate dehydrogenase, succinate dehydrogenase, and malate dehydrogenase) was put forward by Cyriac (1990) in the case of *T. mossambica* under copper and mercury stress as the cause for increased glycogenolysis which was taken as an indication of the shift from aerobic to anaerobic metabolism under pollutant stress.

Total Lipid

Lipid content of the metal-exposed bivalves was estimated at 24h intervals for a period of 96h. Exposure of bivalves to metal stress caused a decrease in their lipid content resulting from utilization of this energy reserve. The results are presented in Table 5.4 and the percentage deviations from control value are depicted in Figs. 5.6 - 5.10. Lipid content of the control animals was 0.884 mg g^{-1} and the metal-induced effects have been interpreted on the basis of the 96h-exposure results.

Arsenic and selenium respectively produced the minimum (2% and 8%) and the maximum (11% and 36%) depression in the lipid content of the clam, from the control value at both levels of concentration.

At the lower level of concentration, the variations in lipid content effected by exposure to copper, cadmium and arsenic individually and in binary combinations were not significant. At the higher concentration level, the metal stress was more keenly felt by the bivalves which responded by enhanced usage of its lipid reserves. The percentage depressions varied between 7 and 36.

Exposure of the bivalves to the lower concentration of copper-mercury combination produced a decrease of about 6% (from the control) in the lipid content. The higher concentration of

able. 5.4. Metal Induced Variations in the Lipid content of V. cyprinoides var. cochinensis (mg/g)

a) Individual metals

Control value: 0.884 ±0.011 mg/g

etals and xposure evels	Time (h) n = 6			
	24	48	72	96
u(0.1)	0.868 ±0.051	0.863 ±0.034	0.858 ±0.048	0.854 ±0.056
d(0.5)	0.879 ±0.044	0.865 ±0.046	0.863 ±0.039	0.861 ±0.055
g(0.1)	0.856 ±0.027	0.841 ±0.037	0.828 ±0.032	0.818 ±0.022
s(1.0)	0.879 ±0.053	0.878 ±0.032	0.869 ±0.042	0.867 ±0.043
e(0.2)	0.865 ±0.011	0.823 ±0.016	0.812 ±0.024	0.79 ±0.023
u(0.3)	0.853 ±0.023	0.842 ±0.014	0.801 ±0.024	0.778 ±0.019
d(1.5)	0.848 ±0.026	0.832 ±0.023	0.823 ±0.016	0.801 ±0.017
g(0.3)	0.725 ±0.021	0.675 ±0.018	0.645 ±0.017	0.608 ±0.024
s(3.0)	0.858 ±0.022	0.851 ±0.019	0.83 ±0.018	0.815 ±0.025
e(0.6)	0.708 ±0.021	0.658 ±0.011	0.628 ±0.024	0.568 ±0.019

Contd.....

Table 5.4 Contd.....

b). Binary combinations

Control value: 0.884 ±0.011 mg/g

Metals and exposure levels	Time (h) n = 6			
	24	48	72	96
Cu(0.1)+Cd(0.5)	0.869 ±0.034	0.868 ±0.025	0.867 ±0.022	0.863 ±0.032
Cu(0.1)+Hg(0.1)	0.878 ±0.044	0.869 ±0.033	0.848 ±0.023	0.835 ±0.026
Cu(0.1)+As(1.0)	0.875 ±0.033	0.874 ±0.028	0.873 ±0.032	0.872 ±0.034
Cu(0.1)+Se(0.2)	0.819 ±0.031	0.801 ±0.022	0.799 ±0.026	0.781 ±0.034
Cd(0.5)+Hg(0.1)	0.865 ±0.026	0.853 ±0.033	0.832 ±0.034	0.823 ±0.032
Cd(0.5)+As(1.0)	0.881 ±0.016	0.878 ±0.028	0.87 ±0.036	0.865 ±0.034
Cd(0.5)+Se(0.2)	0.855 ±0.033	0.812 ±0.026	0.793 ±0.025	0.789 ±0.031
Hg(0.1)+As(1.0)	0.858 ±0.034	0.843 ±0.032	0.832 ±0.029	0.808 ±0.031
Hg(0.1)+Se(0.2)	0.879 ±0.025	0.869 ±0.021	0.86 ±0.032	0.833 ±0.022
As(1.0)+Se(0.2)	0.859 ±0.043	0.849 ±0.033	0.823 ±0.022	0.803 ±0.021
Cu(0.3)+Cd(1.5)	0.849 ±0.023	0.831 ±0.022	0.804 ±0.016	0.782 ±0.022
Cu(0.3)+Hg(0.3)	0.686 ±0.015	0.659 ±0.021	0.633 ±0.019	0.588 ±0.033
Cu(0.3)+As(3.0)	0.848 ±0.023	0.828 ±0.031	0.815 ±0.024	0.808 ±0.025
Cu(0.3)+Se(0.6)	0.844 ±0.031	0.818 ±0.021	0.786 ±0.014	0.726 ±0.024
Cd(1.5)+Hg(0.3)	0.766 ±0.025	0.708 ±0.033	0.678 ±0.032	0.647 ±0.042
Cd(1.5)+As(3.0)	0.847 ±0.043	0.841 ±0.032	0.835 ±0.031	0.829 ±0.012
Cd(1.5)+Se(0.6)	0.804 ±0.026	0.799 ±0.016	0.756 ±0.021	0.645 ±0.013
Hg(0.3)+As(3.0)	0.759 ±0.029	0.701 ±0.025	0.689 ±0.016	0.648 ±0.015
Hg(0.3)+Se(0.6)	0.784 ±0.021	0.768 ±0.019	0.708 ±0.025	0.635 ±0.022
As(3.0)+Se(0.6)	0.768 ±0.021	0.728 ±0.019	0.665 ±0.022	0.638 ±0.018

copper, its binary combination with cadmium (copper-cadmium) and the lower concentration of the copper-selenium combination were similar in their effects on the lipid content (12% depression). The effects of copper-mercury and copper-selenium combinations on the lipid content underwent a drastic reversal in trend. While at the lower concentration level, the copper-mercury combination was half as toxic as copper-selenium combination, at the higher concentration it was the other way around. Mercury or selenium in combination with copper was observed to enhance the toxic effect of copper and copper-mercury and copper-selenium combinations produced deviations of 63 and 240% at the lower level and 179 and 49% at the higher level from the respective copper-induced lipid contents.

Cadmium produced a depression of 2% at the lower concentration level and 10% at higher concentration level. The toxic effect of cadmium was augmented by the presence of mercury or selenium. At higher level of concentrations, cadmium-mercury and cadmium-selenium combinations produced very similar effects (about 27% depression, from the control value) while at the lower level of concentration, the cadmium-selenium combination surpassed the cadmium-mercury combination in its toxic effects. While these combinations produced deviations of 165% and 313% respectively from the cadmium-induced value at the lower concentration levels and the deviation at the higher concentration level in both cases was 185% .

While 0.1ppm of mercury produced a depression of 7% (from the control value) in the lipid content, a three-fold increase in concentration of mercury was found to effect a much more conspicuous change (31% depression) in the lipid content. Lower concentration combinations of mercury with copper, cadmium and selenium and higher concentration combinations of mercury with cadmium, selenium and arsenic brought about a reduction in the mercury-induced depression. The influence of cadmium and

selenium on mercury toxicity underwent a reversal in trend between lower concentrations (selenium was less toxic than cadmium) and higher concentrations (selenium was more toxic than cadmium).

Binary combinations of mercury with copper, cadmium and selenium produced deviations of 7-26% from the mercury-induced value at the lower concentrations whereas the deviations were in the range of 9-15% at the higher concentrations. Arsenic (at the lower concentration level) and copper (at the higher concentration level) in combination with mercury was, however, observed to enhance the toxic effect of mercury and produced 15% and 6% deviations from mercury-induced value.

Only the binary combinations of arsenic with mercury and selenium and the higher concentration of arsenic applied singly produced any significant effects on the lipid content. The percentage depressions (from the control value) produced by mercury and selenium at their lower concentrations were more or less comparable to the depressions caused by higher concentration of arsenic and were in the range of 7-10% . Higher concentration of arsenic-mercury and arsenic-selenium, drastically reduced the lipid contents which deviated from the arsenic-induced value (at high concentration level) by 242% and 260% respectively.

Exposure of the bivalves to selenium produced 11% depression for the lower concentration and 36% depression for the higher concentration. Copper, cadmium and arsenic did not have any noticeable effect on the selenium-induced lipid content at lower concentration level. Lower concentration of mercury, however, brought about a depression of 6% in the lipid content. Higher concentrations of copper, cadmium, mercury and arsenic were conspicuous by their interactive effects which resulted in depressing the lipid content by 17 to 28% .

Lower concentration of mercury with selenium produced a 50% deviation in selenium-induced value. 21-50% deviation from the selenium-induced depression in lipid content was brought about by the binary combinations of selenium with other metals at the higher concentration level.

Lipids are classified as simple lipids (esters of fatty acids with various alcohols), complex lipids (esters of fatty acids containing groups in addition to an alcohol and a fatty acid) and precursor or derived lipids (Murray *et al.*, 1988). No attempt was made in this study to distinguish between the lipids and therefore the results reported in this investigation refer to total lipid contents.

Next to carbohydrates, fats are the best energy producers of the body, and this can be used to meet the endogenous energy requirements. Changes in the lipid content of various aquatic animals under stressed laboratory conditions have been reported (Rao and Rao 1981; Coglianese and Neff, 1982; Morris *et al.*, 1982; Orr and Downer, 1982; Bhaskar *et al.*, 1984; Abdullah and Ireland, 1986; Hilmy *et al.*, 1987b; Rajyalakshmi and Reddy, 1988; Agarwal and Nair, 1989).

Agarwal and Nair (1989) observed a significant decrease in total phospholipids which play a vital role in bioelectrical conductance in the brain of Malathion-exposed *S. mossambicus*. Depletion in the lipid content of hepatopancreas, pedipalpal muscle, haemolymph and embryos on exposure to sublethal doses of carbaryl and lindane reported by Rajyalakshmy and Reddy (1988), and this depletion was attributed to increased demands of energy. Reduction of total lipids and phospholipids and concomitant increases in fatty acids and cholesterol were reported by Rao and Rao (1979) in various tissues of methyl parathion-exposed *S. mossambicus*. A change in the saturated/unsaturated ratio of the structural gill lipid was

suggested as possible adaptive process by which G. duebeni combated pollutant-stress (Morris *et al.*, 1982). The observed depletion in lipids in *V. cyprinoides* was attributed to the increased energy demand under stress caused by the sublethal levels of metal toxicants.

Protein

Protein content of the bivalve soft tissues was estimated at an interval of 24h and for a period of 96h, after exposing them to the metals (individually and in binary combinations). A control was also maintained. The results are presented in Table 5.5 and Figs. 5.6 - 5.10. The protein content registered by the control animals was 3.573 mg g^{-1}. In the case of the treated animals, the protein content was found to be decreased with respect to exposure time as well as to exposure concentrations. The 96h results were analysed to compare and contrast the metal-induced effects.

Based on the depressions recorded in the protein content, under the stress caused by the different metals, following decreasing order of protein utilization could be established, at the lower level tried:

Cd > Cu > Hg > As > Se.

However, at higher level of concentrations tried, a slight change in the order was noticed as given below:

Cd > Cu > Hg > Se > As

Lower concentration of copper produced a depression of 14% which increased to 25% upon a threefold increase in the concentration of copper. At the lower level of concentration,

Table 5.5.Metal Induced Variations in the Protein Content of V. cyprinoides var. cochinensis (mg/g)

a). Individual Metals

Control value: 3.58 ±0.032 mg/g

Metals and exposure levels	Time (h) n = 6			
	24	48	72	96
Cu(0.1)	3.48 ±0.043	3.43 ±0.035	3.32 ±0.028	3.08 ±0.056
Cd(0.5)	3.41 ±0.052	3.38 ±0.049	3.29 ±0.061	3.06 ±0.048
Hg(0.1)	3.50 ±0.043	3.44 ±0.032	3.33 ±0.026	3.12 ±0.036
As(1.0)	3.53 ±0.048	3.51 ±0.056	3.50 ±0.054	3.48 ±0.052
Se(0.2)	3.59 ±0.056	3.60 ±0.046	3.62 ±0.038	3.64 ±0.039
Cu(0.3)	3.40 ±0.048	3.22 ±0.047	3.15 ±0.039	2.66 ±0.051
Cd(1.5)	3.36 ±0.054	3.20 ±0.038	3.10 ±0.042	2.62 ±0.035
Hg(0.3)	3.42 ±0.034	3.28 ±0.026	3.20 ±0.028	2.72 ±0.034
As(3.0)	3.49 ±0.048	3.42 ±0.036	3.35 ±0.045	3.11 ±0.043
Se(0.6)	3.46 ±0.029	3.38 ±0.026	3.33 ±0.035	2.98 ±0.042

Contd.....

Table 5.5 Contd.....

b).Binary combinations

Control value: 3.58 ±0.032 mg/g

Metals and exposure levels	Time (h) n = 6			
	24	48	72	96
Cu(0.1)+Cd(0.5)	3.50 ±0.054	3.49 ±0.047	3.38 ±0.037	3.18 ±0.042
Cu(0.1)+Hg(0.1)	3.47 ±0.043	3.44 ±0.054	3.33 ±0.062	3.10 ±0.043
Cu(0.1)+As(1.0)	3.50 ±0.045	3.45 ±0.043	3.38 ±0.056	3.20 ±0.058
Cu(0.1)+Se(0.2)	3.57 ±0.035	3.55 ±0.022	3.51 ±0.035	3.51 ±0.045
Cd(0.5)+Hg(0.1)	3.46 ±0.044	3.36 ±0.034	3.30 ±0.054	3.14 ±0.046
Cd(0.5)+As(1.0)	3.48 ±0.045	3.42 ±0.047	3.38 ±0.046	3.10 ±0.036
Cd(0.5)+Se(0.2)	3.51 ±0.045	3.49 ±0.047	3.40 ±0.054	3.17 ±0.055
Hg(0.1)+As(1.0)	3.50 ±0.037	3.46 ±0.035	3.38 ±0.042	3.17 ±0.052
Hg(0.1)+Se(0.2)	3.53 ±0.045	3.50 ±0.054	3.48 ±0.052	3.23 ±0.051
As(1.0)+Se(0.2)	3.57 ±0.052	3.56 ±0.041	3.54 ±0.042	3.54 ±0.043
Cu(0.3)+Cd(1.5)	3.45 ±0.029	3.23 ±0.045	3.21 ±0.039	2.88 ±0.047
Cu(0.3)+Hg(0.3)	3.40 ±0.048	3.24 ±0.063	3.18 ±0.054	2.64 ±0.065
Cu(0.3)+As(3.0)	3.35 ±0.057	3.30 ±0.052	3.22 ±0.044	2.90 ±0.033
Cu(0.3)+Se(0.6)	3.46 ±0.033	3.34 ±0.053	3.27 ±0.055	2.87 ±0.045
Cd(1.5)+Hg(0.3)	3.40 ±0.043	3.26 ±0.053	3.13 ±0.046	2.69 ±0.036
Cd(1.5)+As(3.0)	3.37 ±0.038	3.30 ±0.037	3.13 ±0.045	2.69 ±0.035
Cd(1.5)+Se(0.6)	3.40 ±0.054	3.35 ±0.045	3.23 ±0.056	2.72 ±0.043
Hg(0.3)+As(3.0)	3.43 ±0.043	3.30 ±0.053	3.22 ±0.054	2.97 ±0.063
Hg(0.3)+Se(0.6)	3.45 ±0.045	3.34 ±0.053	3.28 ±0.042	3.11 ±0.043
As(3.0)+Se(0.6)	3.49 ±0.045	3.45 ±0.054	3.30 ±0.052	3.13 ±0.051

the binary combinations of copper-cadmium and copper-arsenic were similar in their effects on the protein content; the observed depression was 11%. Copper-mercury combination produced a depression of 13% which was comparable to that produced by copper alone. The copper-selenium binary combination seemed to have no effect on the protein content and produced only 2% depression from the control value. At the higher concentration level, the effects of copper-cadmium, copper-arsenic and copper-selenium on protein content were observed to be identical and the percentage depression observed from the control value was 19%, whereas the effect of copper-mercury combination, as observed at the low concentration level, was not much different from that produced by copper alone (26.02 % depression).

The effect of cadmium, arsenic and selenium was to alleviate the toxic effect of copper when present in binary combination with copper and the percentage deviation from copper-induced effect varied between 20 and 87% at the lower concentration level and between 22 and 26% at the higher concentration level. The maximum effect, at the lower concentration level was that of selenium and at the high concentration level it was that of arsenic. The minimum effect was that of cadmium at both concentration levels.

The depression in the protein content produced by the low concentration of cadmium (14%) increased to 27% with a three-fold increase of the cadmium concentration. The effect of all the other metals in binary combinations with cadmium, at both levels of concentration, was to mitigate the toxic effect of cadmium. The percentage depressions (from the control value) produced by the binary combinations were always lower than that induced by cadmium alone. The deviations from the cadmium-induced value were in the range of 7-24% at the lower concentration level and in the range of 6-27% at the higher

concentration level. At both concentration levels the maximum effect was that of copper and the minimum influence was that of arsenic.

Lower concentration of mercury produced a 13% depression in the protein content which increased to 24% by a three-fold increment in mercury concentration. At the low concentration level the binary combinations of mercury-copper and mercury-cadmium produced percentage depressions (from the control value) which were almost comparable to the mercury-induced depression (13% and 12% respectively) whereas at higher concentration level, the effect was slightly higher than that of mercury. Mercury-arsenic (at higher concentration level) and mercury-selenium (at both concentration levels) produced percentage depressions much below than that induced by mercury alone (11% and 10% respectively at the lower concentration level and 17% and 13% respectively at the higher concentration level). Copper and cadmium at higher concentration level, however, produced percentage depressions higher than that induced by the higher concentration of mercury alone.

The interactive effect of arsenic and selenium to mercury resulted in producing 10-24% lowered depressions than the mercury-induced value at lower concentration level and 29-46% lowered depressions at the higher concentration level; the maximum effect at both concentration levels was that of selenium.

1 ppm arsenic produced a depression of 3% in the protein content (from the control value) and this was increased to 13% at the higher concentration level (3ppm). The binary combinations arsenic-copper, arsenic-cadmium and arsenic-mercury produced depressions (from the control value) which were significantly higher than that produced by arsenic alone at both concentrations. The toxic effect of arsenic was synergistically

modified by the presence of a second metal in combination with it. Several-fold increase (286 - 390%) from the arsenic-induced value was observed at the lower concentration level and at the higher concentration level the increase from the arsenic-induced value was only 29 - 90% . At both concentration levels the maximum effect was produced by cadmium whereas the minimum effect was produced by low concentration of copper and high concentration of mercury.

At the lower concentration level, the effect of selenium and its binary combinations (with copper and arsenic) on the protein content was negligible. Selenium-cadmium and selenium-mercury combinations, however produced 11% and 10% depressions respectively from the control value. At the higher concentration level, selenium produced a depression (from the control value) of 17% in the protein content. The binary combinations, selenium-copper and selenium-cadmium, produced depressions of 20 and 28% respectively from the control value, which were higher than that produced by selenium alone whereas the selenium-mercury and selenium-arsenic combinations produced almost comparable depressions smaller than (12%) the selenium-induced value.

At the higher concentration level copper and cadmium showed a synergistic effect on selenium and produced 53% and 83% higher depressions than the selenium induced value. However mercury and arsenic behaved antagonistically to selenium, and produced smaller percentage-depression values (23% lower than the selenium-induced value) when compared to the selenium induced value.

Proteins are high molecular weight polypeptides and play a central role in cell functions and cell structure. Simple proteins contain only amino acids where as complex proteins

contain additional non-amino acid materials such as heme, vitamin derivatives, lipid or carbohydrates.

In a chronic sublethal study conducted by Ram and Sathyanesan (1985) the protein, RNA and DNA contents in brain, liver and ovary of *C.punctatus* exposed to Cythion (50% Malathion) were found to be reduced as a result of induced alterations in the normal physiological functions which possibly led to impairment of the synthesis and metabolism of protein, RNA and DNA. Studies on the incorporation of the labelled amino acids into the TCA-insoluble material obtained from *M. galloprovinciallis* revealed a 50-70% decrease in the rate of protein synthesis. This decrease in protein metabolism was taken as indicative of the detrimental effect exerted on the mussels exposed to copper. Gluth and Hanke (1984) investigated the effect of temperature on toxicant stress in the carp *C.carpio* and observed that the toxicant-induced reduction in protein (which occurred at the later stage of the experiment was low at $12^{o}C$ and was directly proportional to the temperature. The steady decline observed in the levels of total protein in the liver and muscle after 7 and 15 days respectively, supported the view that proteins were energy reserves and were used up only during continued exposure to the stress. Ramalingam and Ramalingam (1982) investigated the effect of DDT, Malathion and mercury on the protein content of *S. mossambicus* and observed significant decrease in the liver and muscle tissues only after 7 and 15 days respectively. The depletion in protein content observed in *V. cyprinoides* at the later stage of the experiment was attributed to the preferential utilization of carbohydrates followed by proteins under metal-induced stress at sublethal levels.

Lactic Acid

The lactic acid content of the bivalves exposed to the metals was measured periodically at 24h intervals for a period of 96h. Bivalves exposed to the metals registered marked increase in their lactic acid contents as obvious from the results presented in Table 5.6 and Fig. 5.11. The values recorded an almost regular increase which was proportional to the applied metal concentration and to the exposure time. The values peaked at the end of the 96h period. The deviations observed (from the control value) in the lactic acid content after 96h exposures have been used as the basis for comparing and contrasting the metal-induced effects on lactic acid contents.

The percentage elevation in the lactic acid content induced by the lower concentration of copper (289 at 96h) was raised to 419% by a three-fold increment in concentration of copper. Copper-cadmium, copper-mercury, copper-arsenic and copper-selenium combinations produced elevations in lactic acid content (from the control value) which were considerably lower than that induced by copper when applied singly and ranged between 141-261% at the lower concentration level and between 185-407% at the higher concentration level.

In binary combinations with copper, the effect of the other metals was to bring down the copper-induced elevations by 9-51% and by 3-56%, respectively at the two levels of concentrations used. At the lower level of concentration, the effect was maximum with cadmium and at the higher levels it was maximum with selenium. Irrespective of the concentrations used mercury produced the minimum effect.

The lower concentration of cadmium produced an elevation of 190% in lactic acid content which was almost doubled (331%) by a

Table 5.6. Metal Induced Variations in the Lactic Acid Content of V. cyprinoides var. cochinensis (mg/g)

a). Individual Metals

Control value: 0.0289 ± .005 mg/g

Metals and exposure levels	Time (h) n = 6			
	24	48	72	96
Cu(0.1)	0.0612 ±0.002	0.0819 ±0.003	0.0983 ±0.003	0.1123 ±0.002
Cd(0.5)	0.0485 ±0.003	0.0599 ±0.006	0.0725 ±0.004	0.0838 ±0.005
Hg(0.1)	0.0586 ±0.004	0.0826 ±0.006	0.0952 ±0.005	0.1085 ±0.004
As(1.0)	0.0438 ±0.003	0.0586 ±0.005	0.0735 ±0.004	0.0828 ±0.006
Se(0.2)	0.0349 ±0.003	0.0369 ±0.002	0.0389 ±0.004	0.0409 ±0.003
Cu(0.3)	0.0845 ±0.004	0.109 ±0.003	0.1282 ±0.005	0.1501 ±0.004
Cd(1.5)	0.0662 ±0.006	0.0789 ±0.004	0.1046 ±0.005	0.1246 ±0.003
Hg(0.3)	0.0856 ±0.005	0.1038 ±0.004	0.1126 ±0.005	0.1404 ±0.003
As(3.0)	0.0642 ±0.005	0.0746 ±0.003	0.0849 ±0.004	0.1066 ±0.005
Se(0.6)	0.0726 ±0.003	0.0848 ±0.004	0.1146 ±0.003	0.1208 ±0.006

Contd.....

Table 5.6 Contd.....

b). Binary combinations

Control value: 0.0289 ± .005 mg/g

Metals and exposure levels	Time (h) n = 6			
	24	48	72	96
Cu(0.1)+Cd(0.5)	0.0385 ±0.002	0.0426 ±0.004	0.0538 ±0.005	0.0698 ±0.005
Cu(0.1)+Hg(0.1)	0.0608 ±0.007	0.0788 ±0.006	0.0971 ±0.008	0.1043 ±0.018
Cu(0.1)+As(1.0)	0.0483 ±0.002	0.0726 ±0.003	0.0878 ±0.006	0.0973 ±0.005
Cu(0.1)+Se(0.2)	0.0409 ±0.002	0.0553 ±0.004	0.0656 ±0.006	0.0736 ±0.005
Cd(0.5)+Hg(0.1)	0.0568 ±0.004	0.0728 ±0.003	0.079 ±0.006	0.082 +0.008
Cd(0.5)+As(1.0)	0.0425 ±0.003	0.0498 ±0.004	0.0625 ±0.004	0.0758 ±0.005
Cd(0.5)+Se(0.2)	0.0445 ±0.005	0.0529 ±0.004	0.0683 ±0.005	0.0784 ±0.004
Hg(0.1)+As(1.0)	0.0497 ±0.005	0.0625 ±0.006	0.0856 ±0.009	0.0978 ±0.005
Hg(0.1)+Se(0.2)	0.0328 ±0.002	0.0426 ±0.003	0.0517 ±0.005	0.0598 ±0.004
As(1.0)+Se(0.2)	0.0398 ±0.005	0.0476 ±0.004	0.0593 ±0.005	0.0679 ±0.004
Cu(0.3)+Cd(1.5)	0.0459 ±0.005	0.0523 ±0.004	0.0606 ±0.006	0.0874 ±0.004
Cu(0.3)+Hg(0.3)	0.0861 ±0.017	0.1018 ±0.015	0.1098 ±0.009	0.1463 ±0.012
Cu(0.3)+As(3.0)	0.0751 ±0.002	0.0882 ±0.004	0.1035 ±0.007	0.1332 ±0.014
Cu(0.3)+Se(0.6)	0.0523 ±0.006	0.0635 ±0.004	0.0701 ±0.005	0.0825 ±0.006
Cd(1.5)+Hg(0.3)	0.0698 ±0.005	0.0874 ±0.009	0.0955 ±0.008	0.1236 ±0.012
Cd(1.5)+As(3.0)	0.0473 ±0.006	0.0597 ±0.007	0.0703 ±0.005	0.0893 ±0.006
Cd(1.5)+Se(0.6)	0.0601 ±0.005	0.0823 ±0.006	0.0919 ±0.011	0.0998 ±0.009
Hg(0.3)+As(3.0)	0.0738 ±0.006	0.0968 ±0.008	0.0997 ±0.008	0.1285 ±0.015
Hg(0.3)+Se(0.6)	0.0499 ±0.003	0.0561 ±0.003	0.0623 ±0.004	0.0725 ±0.004
As(3.0)+Se(0.6)	0.0595 ±0.002	0.0753 ±0.006	0.0937 ±0.004	0.1045 ±0.005

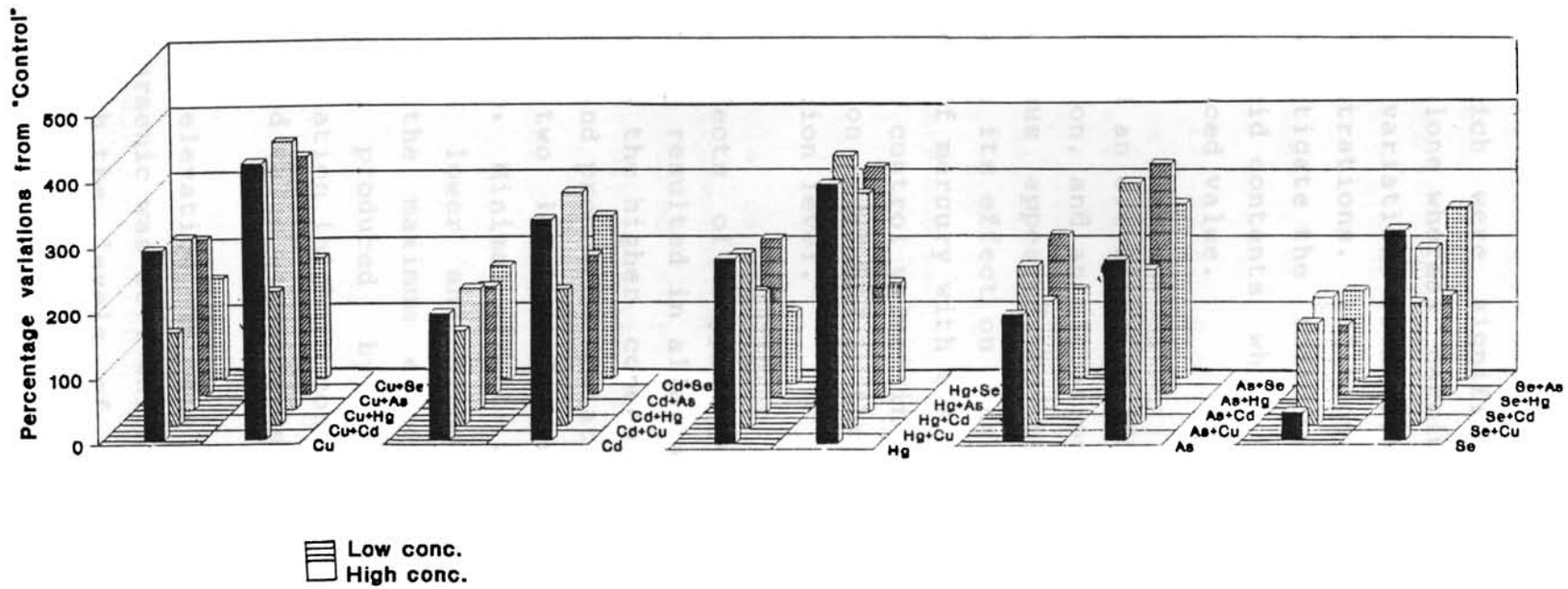


Fig.5.11 Metal-induced variations in the lactic acid content

three-fold rise in concentration of cadmium. Binary combinations of cadmium with copper, arsenic and selenium produced percentage elevations in the lactic acid content (from the control value) which were significantly lower than that produced by cadmium alone whereas cadmium-mercury combinations showed only marginal variations from cadmium induced value, at both levels of concentrations. Addition of a second metal was generally found to mitigate the toxic effect of cadmium and resulted in lactic acid contents which were invariably lower than the cadmium-induced value.

Mercury produced an elevation of 275% (from the control) at its lower concentration, and an elevation of 386% at the higher concentration and thus appeared to be intermediate between copper and cadmium in its effect on the lactic acid content. Binary combinations of mercury with the other metals produced depressions (from the control value) in the range of 106-261% at the lower concentration level and in the range of 150-406% at the higher concentration level.

Interactive effects of copper, cadmium, arsenic and selenium with mercury resulted in alleviating the toxic effect of mercury (except at the higher concentration combination of mercury and copper) and produced deviations 5-62% and 10-61% respectively (at the two levels of concentrations) from the mercury-induced value. Minimal effects were produced by copper and arsenic at the lower and higher concentration levels respectively, while the maximum effect (at both levels of concentrations) was produced by selenium. With the mercury-copper combination (higher concentration) 5% increase beyond mercury-induced value was observed.

The percentage elevation in the lactic acid content produced by 1ppm arsenic was 187, which was raised to 269 by 3ppm arsenic. At both the levels of concentrations arsenic-

copper and arsenic-mercury combinations produced elevations (from the control) in the lactic acid content, which were considerably higher than that produced by arsenic alone. Cadmium or selenium, when present in binary combinations with arsenic, however, produced elevations which were smaller than that produced by arsenic alone.

Lower concentrations of selenium effected an increase in the lactic acid content by 42%, while the higher concentration caused a much steeper increase, to a value of 318% . All the other metals present in binary combination with selenium, at the lower concentration level were, observed to cause elevations in the lactic acid content (from the control value) which were significantly higher than that produced by selenium when applied individually while, at the higher concentration level other metals present in binary combination with selenium were found to produce elevations which were smaller in magnitude than that induced by selenium alone.

At the lower levels of concentrations, copper, cadmium, mercury, and arsenic augmented the lactic acid contents much beyond the selenium-induced value (159-316%). The minimum effect was that of mercury, and the maximum effect was that of copper. At the higher concentrations, however, their effect was to lower selenium- induced effect by 17-53%; minimum effect in this case was that of arsenic and the maximum effect was that of mercury.

Numerous reports are available on the accumulation of lactic acid as an end product of toxicant-induced anaerobic metabolism in the case of fishes (Shaffi, 1978 a and b; Tort *et al.*, 1984; Bhaskar and Govinappa, 1986; Hilmy *et al.*, 1987a). Though there is ample evidence that external stimuli induce transition from aerobic to anaerobic metabolism which draw upon the energy reserves, the pathways of anaerobic metabolism

adopted were found to differ for different species of organisms as well as for the different tissues in the same organism. The different pathways yielded different end products such as aspartate, succinate, propionate, lactate and opine compounds.

Valve closure, production of excess mucus, etc. by bivalves (in order to combat stress) could result in a shift of aerobic to anaerobic metabolism. However, the major end products reported include succinate/alanine during hypoxia/anoxia (Stokes and Awapara, 1968; de Zwaan and Zandee, 1972), lactate/alanine (Gade, 1980; Meinardus and Gade, 1981; Zurberg and Ebberink, 1981) the opine compounds, etc. Accumulation of lactic acid under copper and mercury stress in the bivalves *V. cyprinoides* var.*cochinensis* and *P. viridis* at the whole tissue level was reported by Lakshmanan and Nambisan (1985) and by Sathyanathan *et al.*(1988). Suresh (1988) investigated the effect of copper on the haemolymph lactic acid content of *V. cyprinoides* var. *cochinensis* and *S. scripta* and opined that anaerobic pathway leading to the production of lactic acid operated effectively during the initial days and the production of lactic acid was proportional to the external metal concentration. Katticaran and Salih (1992) studied the effect of copper on the lactic acid content in the adductor muscle and digestive gland of *S.scripta* and reported an elevation in the lactic acid content after 24h of exposure of the clam. Results from the present study corroborated these observations, and established a shift from aerobic to anaerobic respiration in *V. cyprinoides* var. *cochinensis* under stress from sublethal concentrations of metal toxicants.

Chapter VI

CHAPTER VI

SUMMARY

The present chemotoxicological study was undertaken to probe into the interactive effects of the trace metals copper, cadmium, mercury, arsenic and selenium on a widely distributed, and nutritively important species of bivalves, *Villorita cyprinoides* var. *cochinensis* sampled from the Cochin Estuarine System, which like any other estuary is a recipient of a host of agricultural and industrial inputs including trace metals. The above metals were employed in binary combinations to get a clearer picture of metal-metal interactions.

Lethal studies revealed that most of the binary combinations employed i.e., copper-cadmium, copper-selenium, cadmium-arsenic, mercury-selenium, and arsenic-selenium, were antagonistic to *Villorita cyprinoides* var. *cochinensis* whereas copper-arsenic and copper-mercury combinations were partially additive. The joint effects of cadmium-selenium and mercury-arsenic combinations were, however, not very diagnostic, as they did not indicate any definite trend. The cadmium-mercury combination alone showed a synergistic effect to the clam.

The rate constants of accumulation of copper, cadmium, mercury and selenium were depressed by the presence of other metals (exception noticed was in the case cadmium, in combination with selenium at the lower concentration level) while that of arsenic was enhanced by all other metals.

Depuration studies indicated that addition of a second metal to copper or mercury brought about a depression in the rate constants whereas, presence of other metals in binary

combination with cadmium or arsenic effected enhancement of the respective rate constants. The cadmium-selenium combination again behaved differently.

α and β have been introduced as constants which, define the depuration and accumulation processes of the metals. The order of β values were found to reflect the order of lethal toxicity of the metals. Based on the α values obtained for the metals, it could be possible to categorize the metals into two groups.The binary combinations of metals belonging to the same group of α values were observed to act additively and those belonging to different groups were found to be antagonistic to the clam. The cadmium-mercury combination, however, behaved exceptionally.

The BCF values of copper, cadmium and selenium underwent a lowering when a second metal was applied in conjunction with these metals. The BCF value of mercury was enhanced by other metals except copper, where no noticeable effect was observed. Presence of copper or cadmium with arsenic brought about an enhancement, whereas, addition of mercury or selenium to arsenic effected a depression of the same.

The $t_{1/2}$ value of copper, mercury and selenium were generally enhanced, while that of cadmium and arsenic was depressed by other metals.

Studies on the oxygen consumption rate showed that the metals, copper, cadmium, mercury and arsenic were respiratory depressants to *Villorita cyprinoides* var. *cochinensis* whereas selenium, at its lower concentration level (both individually and in binary combination with other metals) acted as a respiratory stimulant. Copper, cadmium and mercury and their binary combination were peculiar in that, the immediate depression in the metabolic rate noticed were found to be

alleviated with further efflux of time. Arsenic and the higher concentration of selenium, however, were observed to bring about greater depression with increase of the exposure period.

The binary combinations produced marked variations from their component metals. However, no combination was effective in producing >50% deviation from the individual metal-induced variation in the metabolic rate.

Exposure to metal stress invariably brought about a depletion in the glycogen content of the clams. The effect of individual metals were generally observed to be depressed in their binary combinations and no combination was effective in producing >50% deviation from that of the respective component metals.

The effect of copper, cadmium, arsenic and their binary combinations on the lipid content of the clam was conspicuous only at the higher concentration levels. Mercury and selenium were observed to cause a marked depletion of the lipid content towards the end of the experiment period.

Mercury and selenium caused considerable enhancement (>50%) in the individual metal-induced effects produced by copper, cadmium or arsenic when applied along with the above metals in binary combinations. The effect of copper, cadmium or arsenic, however, was to reduce the effect of mercury or selenium when present in binary combinations and no combination was effective in producing >50% variation from that induced by mercury or selenium.

The protein content of the bivalves recorded considerable depression upon 96h of exposure. Although, the individual effect produced by copper, cadmium and mercury was depressed by other metals, the effect of arsenic or selenium was observed to

be enhanced by other metals in binary combinations. More than 50% variations were produced by the copper-selenium combination at the lower concentration level, all the binary combinations of arsenic at the lower concentration level and arsenic-cadmium combination at the higher concentration level.

Exposure of the bivalves to the trace metals resulted in continuous increase in the lactic acid content throughout the period of exposure.

The effect of other metals on copper, cadmium and mercury were to reduce their respective individual effects. However, copper or mercury when applied along with arsenic was found to enhance the arsenic-induced elevation. The effect of selenium was enhanced by all other metals at the lower concentration level whereas the same was depressed by the other metals at the higher concentration level.

REFERENCES

Abel, P.D. (1976). Effect of some pollutants on the filtration rate of *Mytilus*. *Mar. Pollut. Bull.*, 7: 228-231.

Abdullah, A. M. and Ireland, M.P.(1986). Seasonal studies on cadmium concentration and toxicity, oxygen consumption, digestive gland glycogen and lipid in dog whelks collected at Aberystwyth, Wales. *Mar. Pollut. Bull.*, 17: 562-566.

Abraham, T.J., Salih, K.Y.M. and Chacko, J. (1986). Effects of heavy metals on the filtration rate of Bivalve *Villorita cyprinoides* (Hanely) var. *cochinensis*. *Indian J. Mar. Sci.*, 15: 195-196.

Agarwal, S. and Nair, N.B. (1989). Physiological effect of malathion on the brain of *Sarotherodon mossambicus*. *Curr. Sci.*, 58: 103-104.

Ahsanullah, M. (1976). Acute toxicity of cadmium and zinc to seven invertibrate species from Western Port, Victoria. *Aust. J. Mar. Freshwat. Res.*27: 187-196.

Ahsanullah, M., Mobley, M.C. and Rankin, P. (1988). Individual and combined effects of zinc, cadmium and copper on the marine amphipod *Allorchestes compressa*. *Aust. J. Mar. Freshwat. Res.*, 39: 33-37.

Ahsanullah, M., Negilski, D.S. and Mobley, M.C. (1981). Toxicity of zinc, cadmium and copper to the shrimp *Callianassa australiensis*. III. Accumulation of metals. *Mar. Biol.*, 64: 311-316.

Ahsanullah, M., and Palmer, D.H. (1980). Acute toxicity of selenium, to three species of marine invertebrates, with notes on a continous flow test system. *Aust. J. Mar. Freshwat. Res.*, 31: 795-802

Alderdice, D.F. and Brett, J.R. (1957). The toxicity of sodium arsenite to young chum salmon. Prog.Rep. Pacific Coast. Stat. *Fish. Res. Board. Can.*, 108: 27-29.

Amiard, J.C., Amiard-Triquet, C., Berthet, B. and Metayer, C. (1987). Comparative study of the pattern of bioaccumulation of essential (Cu,Zn) and non-essential (Cd,Pb) trace metals in various estuarine and coastal organisms. *J. Exp. Mar. Biol. Ecol.*, 106: 73-79.

Amiard-Triquet, C., Berthet, B., Metayer, C. and Amiard, J.C. (1986). Contribution to the ecotoxicological study of cadmium, copper and zinc in the mussel *Mytilus edulis*. II Experimental study. *Mar. Biol.*, 92: 7-13.

Anderson, P.D. and Weber, L.J. (1975) (eds.). The toxicity to aquatic populations of mixtures containing certain heavy metals. In: *International conference on heavy metals in the environment.Vol.2. Part 2.* Toronto, Ontario. 933-954

APHA, (1985). *Standard methods for the examination of water and waste water.* 16th edn. American Public Health Assosciation, American Water Work Assosciation and Water Pollution Control Federation, Washington, D.C.

Atkinson, D.E. (1977).*Energy metabolism and its regulation.* Academic Press. New York. 293pp.

Babukutty, Y. (1991). *Studies on the inter-compartmental exchange of trace metals in an estuarine system.* Ph.D.

Thesis. Cochin University of Science and Technology, Cochin.

Baby, K.V. (1987). *Combined toxicity of heavy metals and petroleum hydrocarbonsa on selceted marine organisms*. Ph.D. Thesis. Cochin University of Science and Technology, Cochin.

Balchand, A.N. and Nambisan, P.N.K. (1986). Effect of pulp-paper mill effluents on the water quality of Muvattupuzha River emptying into Cochin backwaters. *Indian J. Mar. Sci.*, 15: 253-259.

Barker, S.B. (1957). Preparation and colorimetric determination of lactic acid. In: Colowick, S.P. and Kaplan, N.O. (eds.) *Methods in Enzymology*. Academic Press, New York. III:241-245.

Barnes, M. and Blackstock, J. (1973). Estimation of lipids in the marine animals and tissues: Detailed investigation of the sulphophosphovanillin method for "total" lipid. *J. Exp. Mar. Biol. Ecol.*, 12: 103 - 118.

Barnes, H., Stanbury, F.A. (1948). The toxic action of copper and mercury salts both separately and when mixed on the herpacticid copepod *Nitocra spinipes* (Boeck). *J. Exp. Biol.*, 25: 270-275.

Bayne, B.L., Clarke, K.R. and Moore, M.N. (1981). Some practical considerations in the measurement of pollution effects on bivalve molluscs and some possible ecological consequences. *Aquat. Toxicol.* 1: 159-174.

Bayne, B.L., Moore, M.N., Widdows, J., Livingstone, D.R. and Salkeld, P. (1979). Measurement of the responses of individuals to environmental stress and pollution: studies with bivalve molluscs. *Phil. Trans. R. Lond.* B 286: 563-581.

Bayne, B.L. and Newell, R.C. (1983). Physiological energetics of

marine molluscs. In: Saleudinn, A.S.M. and Wilbur, K.M. (eds.). *The Mollusca IV.* Academic Press, New York. 407-515.

Bayne, B.L., Thompson, R.J. and Widdows, J. (1976) (eds.). *Marine Mussels: Thier Ecology and Physiology.* Cambridge University Press, London.

Bhaskar M. and Govindappa, S. (1986). Acclimation to sublethal acidic and alkaline media of *Tilapia mossambica* (Peters): Changes in glycogen metabolism of red muscle. *Bull. Environ. Contam. Toxicol.*, 37: 113-119.

Bhaskar, M.V., Krishnamurthy,V., Shobha Rani, P., Reddanna, P. and Govidappa, S. (1984). Differential pattern of modulations in lipid profiles of red and white muscles of *Tilappia mossambica* (Peters) during acclimation to sublethal alkaline medium. *J. Environ. Biol.*, 5: 149

Birker, P.J.M.W.L. and Freeman, H.C. (1977). Structure, properties and fuctions of a copper(I)-copper(II) complex of D.pencillamine: Pentathallium(I)-μ_8-chloro-dodeca (D-pencillaminato)- octa cuprate(I) hexacuprate (II) n-hydrate. *J. Am. Chem. Soc.*, 99: 6890-6899.

BITC., (1976). The Bureau of International Technique du chlor. Standardisation of methods for the determination of traces of mercury. Part-II Determinations of total mercury in materials containing organic matter. *Anal. Chim. Acta.*, 84: 231-257.

Bjerregaard, P. (1982). Accumulation of cadmium and selenium and their mutual interaction in the shore crab *Carcinus maenas* (L). *Aquat. Toxicol.*, 2: 119-130.

Bjerregaard, P. (1985). Effect of selenium on cadmium uptake in the shore crab *Carcinus maenas (L). Aquat. Toxicol.*, 7: 177-189.

Bjerregaard, P. (1988). Effect of selenium on cadmium uptake in selected benthic invertebrates. *Mar. Ecol. Prog. Ser.*, 48: 17-28.

*

Blaylock, B.G., Curtiss, E.H., Goldstein, R.A., Huckabee, J.W., Matti, C.S., Perryman, S.L. and Witherspoon, J.P. (1974). Ecology of toxic metals. Ecology and Analysis of Trace Contaminants. *Prog. Rep. Oakridge Nat. Lab.*, USA. 140-175.

Boisson, F., Gnassia-Barelli, M., Chiaverini, J. and Romeo, M. (1989). Effect of selenium in the uptake of cadmium by the marine micro algae *Hymenomonas* (cricosphaera) *elongata*. *Mar. Environ. Res.*, 28. 465-469.

Bowen, H.J.M., (1985). The natural environment and the biogeochemical cycles. In: Hutzinger, D.(ed.). *The Handbook of Enviromental Chemistry*.Springer-Verlag. New York. 1-26.

*

Breittmayer, J.P., Guido, R. and Tuncer, S. (1980). Effect du cadmium sur la toxicite du mercure vis-a-vis de la moule *Mytilus edulis (L.)*. *Chemosphere* 9: 725-728.

Brown, V.M. and Dalton, R.A. (1970). The acute toxicity to rainbow trout of mixtures of copper, zinc and nickel. *J. Fish. Biol.* 2: 211- 216.

Brown, B. and Newell, R.C. (1972). The effect of copper and zinc on the metabolism of the mussel *Mytilus edulis*. *Mar. Biol.*, 16: 108-118

Bruggeman, W. (1988). Bioaccumulation. In: de Kruijf, H. A. M., de zwart, D., Ray, P. K. and Viswanathan, P.N. (eds.) *Mannual on Aquatic Ecotoxicology*. Allied Publishers, New Delhi.149-153.

Bryan, G.W. (1976). Heavy metal contamination in the sea. In: R.

Johnston (ed.) *Marine Pollution*, Academic Press, London. 185-302.

Bryan, G.W. (1984). Pollution due to heavy metals and their compounds In: O. Kinne (ed.) *Marine Ecology*, 1289-1431.

Bryant, V., Newbery, D.M., Mc Luskey, D.S. and Campbell, R. (1985). Effect of temperature and salinity on the toxicity of arsenic to three estuarine invertebrates (*Corophium Volutor, Macoma balthica, Tubifix costatus*). *Mar. Ecol Prg. Ser.*, 129-137.

Buikema, A.L.Jr., Niederlehner, B.R. and Cairns, J. Jr.(1982). Biological monitoring. Part IV. Toxicity testing. *Wat. Res.* 16: 239-262.

Calabrese, A., Collier, R.S., Nelson D.A., MacInnes, J.R. (1973). The toxicity of heavy metals to embryos of the American oyster *Crassostrea viriginica*. *Mar. Biol.*, 18: 162-166.

Calabrese, A., MacInnes, J.R., Nelson, D.A. and Miller, J.E. (1977). Survival and growth of bivalve larvae under heavy metal stress. *Mar. Biol.*, 41: 179-184.

Calabrese, A., MacInnes, J.R., Nelson, D.A., Greig, R.A. and Yevich, P.P. (1984). Effects of long term exposure to silver and copper on growth, bioaccumulation and histopathology in the blue mussel *Mytilus edulis*. *Mar. Environ. Res.*, 11: 253-274.

Carpene, E. and George, S.G. (1981). Absorption of cadmium by gills of *Mytilus edulis* (L). *Molec. Physiol.*, 1: 23-34.

Chan, H.M. (1988). Accumulation and tolerance to copper, lead and zinc by the green mussel *Perna viridis*. *Mar. Ecol. Prog.*

Ser., 48: 295-303.

CMFRI Special Publication No.8 (1982). *Mannual of research methods for fish and shellfish nutrition.* Central Marine Fisheries Reseaech Institute, Cochin.

Cockell, K.A. and Hilton, J.W. (1988). Preliminary investigations on the comparative chronic toxicity of four dietary arsenicals to juveniles of rainbow trout (*Salmo gairdneri.* R). *Aquat. Toxicol.*, 12: 73-82.

Coglianese, M.P. and Neff, J.M. (1982). Biochemical responses of the blue crab, *Callinectes sapidus* to pentachlorophenol. In: Vernberg, W.B., Calabrese, A., Thurberg, F.P. and Vernberg, F.J (eds.) *Physiological Mechanisms of Marine Pollutant Toxicity*. Academic Press, New York. 127-143.

Coleman, N., Mann, T.E., Mobley, M. and Hickman, N. (1986). *Mytillus edulis planulatus* an "integrator" of cadmium pollution? *Mar. Biol.*, 92: 1-5.

Connell, D. W. and Miller, J G. (1984). Chemodynamics of Pollutants. In: Connell, D.W. and Miller, J.G.(eds.) *Chemistry and Ecotoxicology of Pollution.* John Wiley and Sons, New York. 7-42.

Connor, P.M. (1972). Acute toxicity of heavy metals to some marine larvae. *Mar. Pollut. Bull.*, 3: 190-192.

Conway, H.L. and Williams, S.C. (1979). Sorption of cadmium and its effect on growth and utilization of inorganic carbon and phosphorus of two freshwater diatoms. *J. Fish. Res. Bd. Can.*, 36: 579-586.

Corner, E.D.S. and Sparrow, B.W. (1956). The modes of action of

toxic agents. I. Observations on the poisoning of certain crustacea by copper and mercury. *J. Mar. Biol. Ass. U.K.*, 35: 531-548.

Cotton, F.A. and Wilkinson, G. (1972). (eds.). *Advanced Inorganic Chemistry*. John Wiley and Sons. New York.

Cunningham, P.A. and Tripp, M.R. (1973). Accumulation and depuration of mercury in the American oyster *Crassostrea virginica*. *Mar. Biol.*, 20: 14 - 19.

Curtis, M.W., Copeland, T.L. and Ward, C.H. (1979). Acute toxicity of twelve industrial chemicals to freshwater and saltwater organisms. *Wat. Res.*, 13: 137-141.

Cuvin, M.L.A. and Furness, R.W. (1988). Uptake and elimination of inorganic mercury and selenium by minnows, *Phoxinus phoxinus*. *Aquat. Toxicol.*, 13: 205-216.

Cyriac, P.J. (1990). *Toxicological studies on* Oreochromis mossambicus *(Peters)*. Ph.D. Thesis, Cochin University of Science and Technology.

D' Agostino, A. and Finney, C. (1974). The effect of copper and cadmium on the development of *Tigriopus japonicus*. In: F.J. Vernberg and W.B. Vernberg (eds.) *Pollution and Physiology of Marine Organisms*. 445-463.

D'Silva, C. and Kureishy, T.W. (1978). Experimental studies on the accumulation of copper and zinc in the green mussel. *Mar. Pollut. Bull.*, 9: 187-190

Davenport, J. and Manley, A. (1978). The detection of hightened sea-water copper concentrations by the mussel *Mytilus edulis*. *J. Mar. Biol. Assoc.* U.K., 58: 843-850.

Davenport, J. and Redpath, K.J. (1984). Copper and the mussel *Mytilus edulis* L. In:(ed.) Bolis, L., Zadunaisky, J. and Giles, R. (eds.) *Toxins, Drugs and Pollutants in Marine Animals*. Springer-Verlag, Berlin. 176-189.

De Filippis, L.F., Hampp, R., Ziegler, H. (1981). The effects of sublethal concentrations of zinc, cadmium and mercury on *Euglena*. II. Respiration, photosynthesis and photochemical activities. *Arch. Mikrobiol.*, 128: 404-411.

de March, B.G.E. (1988). Acute toxicity of binary mixtures of five cations (Cu^{2+}, Cd^{2+}, Zn^{2+}, Mg^{2+} and K^{+}) to the freshwater amphipod *Gammarus lacustris* (Sars): Alternative descriptive models. *Can. J. Fish. Aquat. Sci.*, 45: 625-633.

Denton, G.R.W. and Burdon-Jones, C. (1981). Influence of temperature and salinity on the uptake, distribution and depuration of mercury, cadmium and lead by the black-lip oyster *Saccostrea echinata*. *Mar. Biol.*, 64: 317-326.

Denton, G.R.W. and Burdon-Jones, C. (1982). The influence of temperature and salinity upon the acute toxicity of heavy metals to the banana prawn (*Penaeus merguiensis*) de Man. *Chem. Ecol.*, 1: 131-143.

Devineau, J. and Amiard-Triquet, C. (1985). Patterns of bio-accumulation of an essential trace element (zinc) and pollutant metal (cadmium) in larvae of the prawn *Palaemon serratus*. *Mar. Biol.*, 86: 139-143.

de Zwaan, A. and Zandee, D. (1972). Body distribution and seasonal changes in the glycogen content of the common sea mussel *Mytilus edulis*. *Comp. Biochem. Physiol.*, 43A: 53-58.

Donaldson, E.M. and Dye, H.M. (1975). Corticosteroid concentrations in sockeye salmon exposed to low concentrations of copper. *J. Fish. Res. Bd. Can.*, 32: 533-539.

Eaton, J.G. (1973). Chronic toxicity of a copper, cadmium and zinc mixture to the fathead minnow (*Pimephales promelas rafinisque*). *Wat. Res.*, 7: 1723-1736.

EIFAC (1987). European Inland Fisheries Advisory Commission Revised report on combined effects of freshwater fish and other aquatic life of mixtures of toxicants in water. EIFAC (Eur. Inland. Fish. Adv. Comm.) Tech. pap. 75pp.

Eisler, R. (1971). Cadmium poisoning in *Fundulus heteroclitus* (Pisces: Cyprinodontidae) and other marine organisms. *J. Fish. Res. Board Can.* 28: 1225-1234.

Eisler, R. and Gardner, G.R. (1973). Acute toxicology to an estuarine teleost of mixtures of cadmium, copper and zinc salts. *J. Fish. Biol.*, 5: 131-142.

Eknath, A.E. (1978). *Studies in the bioassay of a few marine organisms employing various industrial effluents.* M.F.Sc. Thesis. Univ. of Agricul. Sci. Bangalore.

Eknath, A. E. and Menon, N.R. (1983). Mercury toxicity in two intertidal tropical marine molluscs. *Fish. Technol.*, 20: 84-89.

Elliot, N.G., Swain, R., Ritz, D.A. (1986). Metal interaction during accumulation by the mussel *Mytilus edulis planulatus*. *Mar.Biol.*, 93: 395-399.

Fowler, S.W. and Benayoun, G. (1976a). Accumulation and

distribution of selenium in mussel and shrimp tissues. *Bull. Environ. Contam. Toxiciol.*, 16: 339-347.

Fowler, S. W. and Benayoun, G. (1976b). Selenium kinetics in marine zooplankton. *Mar. Sci. Comm.*, 2: 43-67.

Fowler, S. W. and Benayoun, G. (1976c). Influence of environmental factor on selenium flux in two marine invertebrates. *Mar. Biol.*, 37: 59-68.

Frazier, J.M. and George, S.G. (1983). Cadmium kinetics in oysters - a comparative study of *Crassostera gigas* and *Ostrea edulis*. *Mar. Biol.*, 76: 55-61.

Friedberg, F. (1974). Effects of metal binding on protein structure. *Q. Rev. Biophys.* 7: 1-33.

Frost, D. V. and Lish, P. M. (1975). Selenium in Biology. *Ann. Rev. Pharmacol.* 15: 259-284.

Gabbot, P.A., Cook, P.A. and Whittle, M.A. (1979). Seasonal changes in glycogen synthase activity in the mantle tissue of the mussel *Mytilus edulis* L.: Regulation by tissue glucose. *Biochem. Soc. Trans.*7: 895-896.

Gade, G. (1980). The energy metabolism of the foot muscle of the jumping cockle *Cardium tuberculatum*: Sustained anoxia versus muscular activity. *J. Comp. Physiol.*, 137: 177-182.

Garberg, P. and Hogberg, J. (1986). The role of selenium-oxygen interactions in selenium metabolism. *Ambio.* 15: 354-355.

GESAMP (1985). (IMO/FAO/UNESCO/WMO/WHO/IAEA/UN/UNEP Joint Group of Experts on the Scientific Aspects of Marine Pollution): Cadmium, lead and tin in the marine environment. *UNEP*

Regional Seas Reports and Studies No. 56. UNEP.

GESAMP (1986). (IMO/FAO/UNESCO/WMO/WHO/IAEA/UN/UNEP Joint Group of Experts on the Scientific Aspects of Marine Pollution) Review of Potentially Harmful Substances: Arsenic, Mercury and Selenium: *Rep. Stud. GESAMP*, 28.

Giles, M.A. (1988). Accumulation of cadmium by rainbow trout *Salmo gaidneri* during extended exposure. *Can. J. Fish. Aquat. Sci.*, 45: 1045- 1053.

Glickstein, N. (1978). Acute toxicity of mercury and selenium to *Crassostrea gigas* embryos and *Cancer magister* larvae. *Mar. Biol.*, 49: 113-117.

Gluth, G. and Hanke, W. (1984). A comparison of physiological changes in carp *Cyprinus carpio,* induced by several pollutants at sublethal concentration - II. The dependency on the temperature. *Comp. Biochem. Physiol.*, 79C: 39 - 45.

Goldberg, E.D. (1975). The mussel watch — a first step in global marine pollution monitoring. *Mar. Pollut. Bull.*, 6: 111.

Goldberg, E. D. (1976). *The Health of the Oceans*. UNESCO Press, Paris. 122pp.

Goldberg, E.D., Bowen, V.T., Farringtion, J.W., Harvey, G., Martin, J.H., Parker, P.L., Riseborough, R.W., Robertson, W., Schneider, E. and Gamble, E. (1978). The Mussel Watch. *Environ. Conserv.*, 5: 101-125.

Gostis, O. (1982). Combined effects of selenium/mercury and selenium/copper on the cell population of the alga *Dunaliella minuata*. *Mar. Biol.*, 71: 217-222.

Grant, B.F. and Mehrle, P.M. (1973). Endrin toxicosis in rainbow trout (*Salmo gairdneri*). *J. Fish. Res. Bd. Can.*, 30: 31-40.

Grasshoff, K. (1983). In: Grasshoff, K., Ehrhardt, M. and Kremling, K. (eds.) *Methods in Seawater Analysis*. Verlag Chemie, Weinheim.

Hassid, W. and Abraham, S. (1963). Chemical Procedures for analysis of polysaccharides. In: Colowick, S.P. and Kaplan, N.O. (eds.) *Methods in Enzymology* Vol. III: 34 Academic Press, New York.

Hawkins, A.J.S., Menon, N.R., Damodaran, R. and Bayne, B. L. (1986). Metabolic responses of the mussels *Perna viridis* and *Perna indica* to declining oxygen tension at different salinities. *Comp. Biochem. Physiol.*, 88A: 691-694.

Heath, J.R. and Barnes, H. (1970). Some changes in the biochemical composition with season and during the moulting cycle of of the common shore crab *Carcinus maenas* (L). *J. Exp. Mar. Biol.*, 5: 199-233.

*
Heisinger, J.F. (1981). *Antagonism of selenium and cadmium pretreatments to subsequent embryotoxic doses of mercury and cadmium in fish embryos*. Technical Report Univ. South Dakota.

*
Hill, C.H. and Matrone, G. (1970). *Cited in* Underwood, E.J. (1979). Interactions of trace elements. In: Oehme, F.J. (ed.) *Toxicity of Heavy Metals in the Environment*. Part 2. Marcel Dekker, New York. 641-668.

Hilmy, N. A., El Domaity, Daabees, A. Y. and Abdel Latife, M.A. (1987a). Physiological and biochemical indices of zinc toxicity in two freshwater fishes *Clarias lazera and Tilapia*

zilli. *Comp. Biochem. Physiol.*, 87C: 297-301.

Hilmy, A.M., El Domaity, N.A., Daabees, A.Y. and Alsarha, A. (1987b). The toxicity to *Clarias lazera* of copper and zinc applied jointly. *Comp. Biochem. Physiol.*, 87C: 309-314.

Hilmy, A.M., Shabana, M.B. and Daabees, A.Y. (1985). Bioaccumulation of cadmium: Toxicity in *Mugil cephalus*. *Comp. Biochum. Physiol.*, 81C: 139-143.

Hochachka, P.W., Fields, J. and Moustafa, T. (1973). Animal life without Oxygen. Basic biochemical mechanism. *Am. Zool.*, 13: 543.

Hochachka, P.W. and Moustafa, T. (1972). Invertebrate faculative anaerobiosis. *Science*, 178: 1056 - 1060.

Hodson, P.V., Spry, D.J. and Blunt, B.R. (1980). Effects of rainbow trout *Salmo gairdneri* of a chronic exposure to waterborne Selenium. *Can. J. Fish. Aquat. Sci.*, 37: 233-240.

Holland, A.A., Lasater, J.E., Neumann, E.D. and Eldrige, W.E. (1960). Toxic effects of organic and inorganic pollutants in young salmon and trout. State of Washington, *Dept. of Fish. Res. Bull.*, No.5. 264pp.

Holwerda, D.A., de Knecht, J.A., Hemelraad, J. and Veenhof, P.R. (1989). Cadmium kinetics in freshwater clams. Uptake of cadmium by the excised gill of *Anodonta anatina*. *Bull. Environ. Contam. Toxicol.*, 42: 382-388.

Howell, R. (1983). Heavy metals in marine nematodes: Uptake, tissue distribution and loss of copper and zinc. *Mar. Pollut. Bull.*, 7: 263-268.

Huckabee, J.W. and Griffith, N.A. (1974). Toxicity of mercury and selenium to the eggs of carp *Cyprius carpio*. *Trans. Amer. Fish. Soc.*, 103: 822 - 825.

Hunn, J.B., Hamilton, S.J. and Buckler, D.R. (1987). Toxicity of sodium selenite to rainbow trout fry. *Wat. Res.*, 21: 233-238.

Huheey, J.E. (1978) (ed.). *Inorganic Chemistry: Principles of Structure and Reactivity*. Harper and Raw, New York.

Imura, N. and Naganuma, A. (1978). Interaction of inorganic mercury and selenium in rabbit blood after intravenous administration. *J. Pharmacol. Dyn.*, 1: 67

Ivanovici, A.M. (1980). The adenylate energy charge in the estuarine mollusc *Pyranzinus ebeninus*. Laboratory studies of responses to salinity and temperature. *Comp. Biochem. Physiol.*, 66A: 43-45.

Johnston, P.A. (1988). Acute toxicity of inorganic selenium to *Daphnia magna* (Straus) and the effect of sub-acute exposure upon growth and reproduction. *Aquat. Toxicol.*, 10: 335-352.

Karp, G. (1984). Energy, Enzymes and Metabolism. In: Karp, G.(ed.) *Cell Biology*. McGraw-Hill Book Company. 77-137.

Katticaran, C.M. and Salih, K.Y.M. (1992). Copper induced metabolic changes in *Sunetta scripta* (Bivalvia): Oxygen uptake and lactic acid production. *Bull. Environ. Contam. Toxicol.*, 48: 592 - 598.

Kedarnath and Nishithkumar (1987). Toxicity of manganese and its impacts on some aspects of carbohydrate metabolism of a

freshwater teleost *Colisa fasciatus*. *Sci. Total Environ.*, 67: 257-262.

Kedarnath and Nishithkumar (1988). Hexavalent Chromium: Toxicity and its impacts on certain aspects of carbohydrate metabolism of the freshwater teleost *Colisa fasciatus*. *Sci. Total Environ.*, 72: 175-181.

King, D.G. and Davies, I.M. (1987). Laboratory and field studies of the accumulation of inorganic mercury by the mussel *Mytilus edulis* (L). *Mar. Pollut. Bull.*, 18: 40-45.

Klaverkamp, J.F. MacDonald, W.A., Lillie, W.R. and Lutz, A. (1983). Joint toxicity of mercury and selenium in salmonid eggs. *Arch. Environ. Contam. Toxicol.*, 12: 405-413.

Konemann, A. (1981). Fish toxicity tests with mixtures of more than two chemicals: a proposal for a quantitative approach and experimental results. *Toxicology*, 19: 229-238.

Klockner, K. (1979). Uptake and accumulation of cadmium by *Ophryotrocha diadema* (Polychaeta). *Mar. Ecol. Progr. Ser.*, 1: 71 -76.

Krishnaja, A.P., Rege, M.S. and Joshi, A.G. (1987). Toxic effects of certain heavy metals (Hg, Cd, Pb, As and Se) on the intertidal crab *Scylla serrata*. *Mar. Environ. Res.* 21: 109-119.

Krishnakumar, P.K. (1987). *Physiological effects of some heavy metals on* Perna viridiis *(Linnaeus)*. Ph. D. Thesis. Cochin University of Science and Technology, Cochin.

Krishnakumar, P.K., Damodaran, R. and Nambisan, P.N.K. (1990). Accumulation, distribution and depuration of mercury in the

green mussel *Perna viridis* (Linnaeus). *Proc. Indian Acad. Sci. (Anim. Sci.)*, **99**: 345-352.

Kumaraguru, A.K., Selvi, D. and Venugopalan, V.K. (1980). Copper toxicity to an estuarine clam *(Meretrix casta). Bull. Environ. Contam. Toxicol.*, **24**: 853-857.

Lakshmanan, P.T. (1982). *Investigations on the chemical constituents and trace metal interactions in some bivalve molluscs of the Cochin backwaters.* Ph. D.Thesis. Cochin University, Cochin.

Lakshmanan, P.T. and Nambisan, P.N.K. (1977). Toxicity of copper on the bivalve *Villorita cyprinoides* var. *cochinensis. Indian J. Mar. Sci.*, **1**: 83 - 85.

Lakshmanan, P.T. and Nambisan,P.N.K. (1979). Accumulation of mercury by the mussel *Perna viridis* (Linnaeus). *Curr. Sci.*, **48**: 672-674.

Lakshmanan, P.T. and Nambisan, P.N.K. (1985). Tissue lactic acid and glycogen level of molluscs exposed to Cu and Hg. *Curr. Sci.*, **54**: 478-479.

Lakshmanan, P.T. and Nambisan, P.N.K. (1989). Bioaccumulation and depuration of some trace metals in the mussel, *Perna viridis* (Linnaeus). *Bull. Environ. Contam. Toxicol.*, **43**: 131-138.

Lane, J.M. (1986). Allometric and biochemical studies on starved and unstarved clams, *Rangia cuneata* (Sowerby, 1831). *J. Exp. Mar. Biol. Ecol.*, **95**: 131-143.

Latha, T. (1986). *Physiological effects of Copper (II) on* Sunetta scripta. Ph. D. Thesis. Cochin University, Cochin.

Latha, T., Nambisan, P.N.K. and Damodaran, R. (1985). Studies on *Sunetta scripta* L. salinity tolerance and copper toxicity. *Bull. Dept. Mar. Sci.* University of Cochin. XIII: 46 - 52.

Leonzio, C., Focardi, S. and Bacci, E. (1982). Complementary accumulation of selenium and mercury in fish muscle. *Sci. Total Environ.*, 24: 249-254.

Li, W.K.W. (1980). Cellular accumulation and distribution of cadmium in *Isochrysis galbana* during growth inhibition and recovery. *J. Plankton Res.* 2: 283-294.

Litchfield, J.T. and Wilcoxon, F. (1949). A simplified method for evaluating dose/effect experiments. *J. Pharmac. Exp. Ther.*, 96: 99-113.

Livingstone, D.R. (1981). Introduction of enzymes as a mechanism for the seasonal control of metabolisms in marine invertebrates: Glucose -6- phosphate dehydrogenases from the mantle and hepatopancreas of the common mussel *Mytilus edulis* L. *Comp. Biochem. Physiol.*, 69B: 147-156.

Livingstone, D.R. (1985). Biochemical measurements. In : Bayne, B.L. (ed.) *The Effects of Stress and Pollution on Marine Animals*. Praeger Scientific, New York.

Lloyd, R. (1961). The toxicity of mixtures of zinc and copper sulphates to rainbow trout (*Salmo gairdneri*). *Ann. Biol.* 49: 535-538.

Lucu, C. and Skremblin, M. (1981). Evidence on the interactions of mercury and selenium in the shrimp *Palaemon elegans*. *Mar. Environ. Res.*, 5:265-275.

Luoma, S.N. (1983). Bioavailability of trace metals to aquatic

organisms: a review. *Sci. Total Environ.*, 28: 1-22.

Luten, J.B., Bouquet, W., Burggraaf, M.M., Rauchbaar, A.B. and Rus, J. (1986). Trace metals in mussels (*Mytilus edulis)* from the Waddenzee, coastal North Sea and the estuaries of Ems. Western and Eastern Scheldt.*Bull.Environ.Contam.Toxicol.*,36: 770-777.

MacInnes, J.R. (1981). Response of embryos of the American oyster *Crassostrea virginica* to heavy metal mixtures. *Mar. Environ. Res.*, 4: 217-227.

MacInnes, J.R. and Calabrese, A. (1978). Response of the embryos of the American oyster, *Crassostrea virginica,* to heavy metals at different temperatures. In: MacLusky, D.S. and Berry, A.J. (eds.) *Physiology and Behaviour of Marine Organisms*. Pergamon Press, New York.

Magos, L. and Webb, M. (1980). The interactions of selenium with cadmium and mercury. *C R C. Crit. Rev. Toxicol.*, 8: 1-42.

Manley, A.(1983). The effects of copper on the behaviour, respiration, filtration and ventilation activity of *Mytilus edulis. J. Mar. Biol. Ass.* U.K. 63: 205-222.

Marigomez, J.A. and Ireland, M.P. (1989). Accumulation, distribution and loss of cadmium in the marine prosobranch *Littorina littorea. Sci. Total Environ.*, 78: 1-12.

Marking, L.L. (1985). Toxicity of chemical mixtures. In: Rand, G.M. and Petrocelli, S.R. (eds.) *Fundamentals of Aquatic Toxicology*. Hemispere Publishing corporation, Washington. 164-176.

Marking, L.L. and Dawson, V.K. (1975). Method for assessment of

toxicity or efficacy of mixtures of chemicals. *U.S. Fish.Wildl. Serv.Invest. Fish. Control.*, 67: 1-8.

Martin, J.M. and Meybeck, M. (1979). Elemental mass-balance of material carried by world major rivers. *Mar. Chem.*, 7: 173-206.

Martin, M., Osborn, E.K., Billing, P. and Glickstein, N. (1981). Toxicities of ten metals to *Crassotrea gigas* and *Mytilus edulis* embryos and *Cancer magister* larvae. *Mar. Pollut. Bull.*, 12: 305-308.

Martincic, D., Nurberg, H.W., Stoeppler, M. and Branica, M. (1984). Bioaccumulation of heavy metals by bivalves from Lim Fjord (North Adriatic Sea). *Mar. Biol.*, 81: 177-188.

Mason, A.Z. and Simkiss. K. (1982). Sites of mineral deposition in metal-accumulating cells. *Exp. Cell. Res.*, 139: 383-391.

Mason, A.Z., Simkiss, K. and Ryan, K. (1984). Ultrastructural localization of metals in specimens in *Littorina littorea* (L) from polluted and non-polluted sites. *J. Mar. Biol. Assoc.* U.K., 64: 699-720.

Mathew, R. and Menon, N.R. (1983). Oxygen consumption in tropical bivalves *Perna viridis* (Linn.) and *Meretrix casta* (Chem.) exposed to heavy metals. *Indian J.Mar.Sci.*, 12: 57-59.

Meinardus,G. and Gade, G. (1981). Anaerobic metabolism of the common cockle *Cardium edule*. IV. Time dependent changes of metabolites in the foot and gill tissue induced by anoxia and electrical stimulation. *Comp. Biochem. Physiol.*, 70B: 271-277.

Mohan, C.V., Menon, N.R. and Gupta. T.R.C. (1984). Acute

toxicity of cadmium to six intertidal invertibrates. *Fish. Technol.*, 21: 1-5.

Mohan, C.V., Gupta, T.R.C., Shetty, H.P.C. and Menon, N.R. (1986a). Combined toxicity of mercury and cadmium tn the tropical green mussel *Perna viridis*. *Dis. Aquat. Org.*, 2: 65-72.

Mohan, C.V., Menon, N.R. and Gupta. T.R.C. (1986b). Filtration in some tropical intertidal bivalves exposed to mercury and cadmium mixtures. *Fish. Technol.*, 23: 205-210.

Morris, R.J., Lockwood, A.P.M. and Dawson, M.E. (1982). Changes in the fatty acid composition of the gill phospholipids in *Gammarus duebeni* with degree of gill contamination. *Mar. Pollut. Bull.*, 13: 345-348.

Moulder, S.M. (1980). Combined effect of chlorides of mercury and copper in sea water on the euryhaline amphipod *Gammarus duebeni*. *Mar. Biol.*, 59: 193-200.

Murakami, T.H.M., Hayakawa, T., Fujii, T., Hara,Y., Itami, A., Kishida, A. and Nishida, I. (1976). The effects of heavy metals on developing sea-urchin eggs. *J.Okayama. Med. Soc.*, 88: 39-50.

Murray, R.K., Granner, D.K., Mayes, P.A. and Rodwell, V.W. (1988) (eds.). Harper's Biochemistry (Twenty-first edition). Appleton and Lange, Connecticut.

Naganuma, A. and Imura, N. (1983). Mode of *in vitro* interaction of mercuric mercury with selenite to form high molecular weight substance in rabbit blood. *Chem. Biol. Interact.*, 43: 271-282.

Naganuma, A., Tabata, J. and Imura, N. (1982). A reaction product from mercuric mercury, selenite and reduced

glutathione. *Res. Commun. Chem. Pathol. Pharmacol.*, 38: 291-299.

Nair, S.G. (1975). Studies on the rate of growth of *Villorita cyprinoides* var. *cochinensis* (Hanley) from the Cochin backwaters. *Bull. Dept. Mar. Sci.*, Univ. Cochin, VII: 919-929.

Nair, S.G. and Shynamma, C.S. (1975). Studies on the salinity tolerence of *Villorita cyprinoides* var. *cochinensis* (Hanley). *Bull. Dept. Mar. Sci.*, Univ. Cochin, VII: 537-542.

Negilski, D.S., Ahsanullah, M. and Mobley, M.C. (1981). Toxicity of zinc, cadmium and copper to the shrimp *Callianassa australiensis* II. Effect of paired and triad combinations of metals. *Mar. Biol.*, 64: 305-309.

Nelson, D.A., Calabrese, A., Nelson, B.A., Mac Innes, J.R., Wenzloff, D.R. (1976). Biological effects of heavy metals on juvenile bay scallops *Argopecten irradians* in short-term exposures. *Bull. Environ. Contam. toxicol.*, 16: 275-282.

Nichitta, C.V., and Ellington, W.R. (1983). Energy metabolism during air exposure and recovery in the high intertidal bivalve mollusc *Guekensea demissa granosissima* and the subtidal bivalve mollusc *Modiolus squamosus*. *Biol. Bull.*, 165: 708-722.

Nieboer, E. and Richardson, D.H.S. (1980). The replacement of the nondescript term "Heavy Metals" by a biologically and chemically significant classification of metal ions. *Environ. Pollut*B., 3-26.

Ochiai, E. (1977). *Bioinorganic chemistry: an introduction.* Allyn and Bacon, Boston.

Okazaki, R.K. and Panietz, M.H. (1981). Depurtion of twelve trace metals in tissues of the oysters *Crassostrea gigas* and *Crassostrea virginica*. *Mar. Biol.*, 63: 113-120.

Orr, G.L. and Downer R.G.H. (1982). Effect of lindane (γ-Hexacholorocyclohexane) on carbohydrate and lipid reserves in the American Cockroach, *Periplaneta* L. *Pest Biochem. Physiol.*, 17: 89-95.

Ouseph, P.P. (1990). Dissolved, particulate and sedimentary mercury in the Cochin estuary, south west coast of India. In: Michaelis, W. (ed.) *Estuarine Water Quality Management*, Springer- Verlag, Berlin. 461-465.

*
Overhoff, H. and Forth, W. (1978). Biologisch essentielle Elemente (" spurenelemente") Deut. Arzteblatt 301-305.

Pani, P. Sanna, A., Brigaglia, M.I., Columbano,A. and Congiu,L. (1976). Early investigations on the effect of methyl mercuric chloride upon DMN-acute hepatotoxicity. *Experientia*. 32: 1449-1451.

*
Pearson, R.G. (1967). *Chem. Brit.* 3: 103.

Peden, J.D., Crothers, J.H., Waterfall, C.E. and Beasley, J. (1973). Heavy metals in Somerset marine organisms. *Mar. Pollut. Bull.* 4: 7-9.

Pelletier, E. (1985). Mercury - selenium interactions in Aquatic organisms. A rieview. *Mar. Environ. Res.*, 18: 111 - 132.

*
Pelletier, E. (1986). Modification de la Bioaccumulation du selenium chez. *Mytilus edulis* en presence du mercure organique et inorganique. *Can. J. Fish. Aquat. Sci.*, 43: 203-210.

Pelletier, E. (1988). Acute toxicity of some methyl mercury complexes to *Mytilus edulis* and lack of selenium protection. *Mar. Pollut. Bull.*, 19: 213 - 219.

Peterson, H.G., Healey, F.P. and Wagemann, R. (1984). Metal toxicity to algae: a highly pH dependent phenomenon. *Can. J. Fish. Aquat. Sci.*, **41**: 974-979.

Philip, M. (1990). *Sub-lethal effects of heavy metals on* Perna indica *(Kuriakose and Nair) and* Donax incarnatus *Gmelin.* Ph. D. Thesis, Cochin University of Science and Technology, Cochin.

Phillips, D.J.H. (1976). The common mussel *Mytilus edulis* as an indicator of pollution by zinc, cadmuim,lead and copper. I. Effects of variables on uptake of metals. *Mar. Biol.*, **38**: 59-69

Phillips, D.J.H. (1977a). The use of biological indicator organisms to monitor trace element pollution in marine and estuarine environments: a review. *Environ. Pollut.*, **13**: 281-317.

Phillips, D.J.H. (1977b). Effects of salinity on the uptake of zinc by the common mussel *Mytilus edulis* . *Mar. Biol.*, **41**: 79-88.

Piotrowsky,J.K.,Bolanowska,W.and Sapota,A.(1973). Evaluation of metallothionein content in animal tissues. *Acta Biochim. Pol.*, **20**: 207-215.

Placket R.L. and Hewlett P. S. (1952). Quantal response of mixtures of poisons. *J. Roy. Stat. Soc.*, B **14**: 141- 143.

Poulsen E., Riisgard, H.U. and Mohlenberg, F. (1982). Accumulation of cadmium and bioenergetics in the mussel *M. edulis*. *Mar. Biol.*, **68**: 25-29.

Prabhudeva K.N. (1988). *Toxicity, accumulation and depuration of heavy metals in the brown mussel,* Perna indica. Ph. D. Thesis, Cochin University of Science and Technology, Cochin.

Prabhudeva K.N and Menon N.R. (1986). Toxicity of metal mixtures to *P. viridis* (Mollusca, Pelecypoda) In: Maclean, J.L., Dizon, L.B. and Hosillos, L.V. (eds.) The first Asian Fisheries Froum. Asian Fisheries Sciety, Manila, Philippines.

Prashad, B. (1921). Notes on Lamellibranch. *Rec. Ind. Mus.*, 22: 111-119.

Preston, M.R. (1989). Marine Pollution. In: (ed.) J.P. Riley. *Chemical Oceanography*, Volume 9. Academic Press, London. 53-196.

Rajyalakshmi, A. and Reddy, G.T. (1988). Effect of carbaryl and lindane on the lipid content of the maternal tissues and embryos of the viviparous scorpion, *Heterometrus fulvipes* (Koch). *J. Environ. Biol.*, 9: 97-105.

Ram, R.N. and Sathyanesan A.G. (1985). Organophosphate-induced biochemical changes in the brain, liver and ovary of the fish *Channa punctatus* (Bloch). *Indian Natn. Sci. Acad.*, B 51: 537-542.

Ramalingam, K. (1988). Toxic effects of DDT, malathion and mercury on the tissue carbohydrate metabolism of *Sarotherodon mossambicus* (Peters). *Proc. Indian Acad. Sci.* (Anim. Sci.), 97: 443-448.

Ramalingam, K. and Ramalingam, K.C. (1982). Effects of sublethal levels of DDT, malathion, and mercury on tissue proteins of *Saratherodon mossambicus* (Peters). *Proc. Indian Acad. Sci.* (Anim. Sci.), 91: 501 - 505.

Rand, G.M. and Petrocelli, S.R. (1985) (eds.). *Fundamentals of aquatic toxicology*. Hemisphere Publishing Company. Washington.

Rao, K.S. and Rao, K.V.R. (1979). Effect of sublethal concentration of methyl parathion on selected oxidative enzymes and organic constituents in the tissues of the fresh water fish *Tilapia mossambica* (Peters). *Curr. Sci.*, **48**: 526-528.

Rao, K.S. and Rao, K.V.R. (1981). Lipid derivatives in the tissues of the freshwater teleost *Saurotherodon mossambicus (*alias *Tilapia mossambica)* (Peters) -Effect of methyl parathion. *Proc. Ind. Natnl. Sci. Acad.*, **B47**: 53-57.

Rebhun, S. and Ben-Amotz, A. (1988). Antagonistic effect of manganese to cadmium toxicity in the alga *Dunaliella salina*. *Mar. Ecol. Prog. Ser.*, **42**: 97-104.

Reeve, M. R., Walter, M.A., Darcy, K. and Ikeda, T. (1977). Evaluation of potential indicators of sublethal toxic stress on marine zooplankton (feeding, fecunding, respiration and excretion). *CEPEX Bull. Mar. Sci.*, **27**: 105-113.

Reid, S.D. and McDonald, D.G. (1991). Metal binding activity of the gills of rainbow trout (*Oncorhynchus mykiss*). *Can. J. Fish. Aquat. Sci.*, **48**: 1061-1068.

Ridlington, J.W. and Whanger, P.D. (1981). Interactions of selenium and antioxidants with mercury, cadmium and silver. *Fundam. Appl. Toxicol.*, **1**: 368-375.

Riisgard, H.U., Kiorboe, T., Mohlenberg, F., Draback, I., and Pheiffer Madsen, P. (1985). Accmulation, elimination and chemical speciation of mercury in the bivalves *Mytilus edulis* and *Macoma balthica*. *Mar. Biol.*, **86**: 55-62.

Ringwood, A.H. (1989). Accumulation of cadmium by larvae and adults of an Hawaiian bivalve *Isognomon californicum* during chronic exposure. *Mar. Biol.*, 102: 499-504.

Ritz, D.A., Swain, R. and Elliot, N.G. (1982). Use of the mussel *Mytilus edulis planulatus* (Lamark) in monitoring heavy metal levels in sea water. *Aust. J. Mar. Freshwat. Res.*, 33: 491-506.

Roch, M. and Mc Carter, J. A. (1984). Hepatic metallothionein production and resistance to heavy metals by rainbow trout (*Salmo gairdneri*) I. Exposed to an artificial mixture of zinc, copper and cadmium. *Comp. Biochem. Physiol.* 77: 71-75.

Roesijadi, G. (1982). Uptake and incorportion of mercury into mercury binding proteins of gills *Mytilus edulis* as a function of time. *Mar. Biol.*, 66: 151-157.

Roesijadi, G. and Anderson, J.W. (1979). Condition index and free amino acid content of *Macoma inquinata* exposed to oil-contaminated marine sediments. In(eds.) Vernberg, W.B., Thurberg, F.P., Calabrese,A. and Nernberg, F.J. *Marine Pollution : Functional Responses.* 69-83.

Rosko, J.J. and Rachlin, J.W. (1975). The effect of copper, zinc, cobalt and manganese on the growth of the marine diatom *Nitzschia closterium. Bull. Torrey bot. Club.* 102: 100- 106.

Sanders, B.M. and Jenkins, K.D. (1984). Relationships between free cupric ion concentrations in sea water and copper metabolism and growth in crab larvae. *Biol. Bull.*, 167: 704-712.

Sanders, B.M., Jenkins, K.D., Sunda, W.G. and Costlow, J.D. (1983). Free cupric ion activity in sea water: Defects of

metallothionein and growth in crab larvae. *Science*, **222**: 53-55.

Sandholm, M., Oksanen, H.E. and Pesonen, L. (1973). Uptake of selenium by aquatic organisms. *Limnol. Oceanogr.*, **18**: 496-499.

Sathyanathan, B., Muraleedharan Nair, S., Jacob Chacko, and Nambisan, P.N.K. (1988). Sublethal effects of copper and mercury on some biochemical constituents of the estuarine clam *Villorita cyprinoides* var. *cochinensis*. *Bull. Environ. Contam. Toxicol.*, **40**: 510-516.

Sato, T., Ose, Y. and Sakai, T. (1980). Toxicological effects of selenium in fish. *Environ. Pollut. Ser.A. Ecol. Biol.*, **21**: 217-224.

Scholz, N. (1980). Accumulation, loss and molecular distribution of cadmium in *Mytilus edulis*. *Helgolander wiss. Meeresunters.* 33: 68-78.

Scott, D.M. and Major, C.M. (1972). The effect of copper (II) on survival, respiration and heart rate in the common blue mussel *Mytilus edulis* L. *Biol. Bull.*, 143: 679-688.

Shafee, M.S. (1979). Ecological energy requirements of the green mussel *Perna viridis* Linnaeus from the Ennore estuary, Madras. *Oceanol. Acta.*, 2: 69-74.

Shaffi, S.A. (1978a). Cadmium intoxication on tissue glycogen content in three fresh water teleosts. *Curr. Sci.*, **47**: 868-871.

Shaffi, S.A. (1978b). Variations in tissue glycogen content, serum lactate and glucose levels due to copper intoxication

in three freshwater teleosts. *Curr. Sci.*, 47: 954-956.

Sharma, A., Mukherjee, A. and Talukder, G. (1985). Modification of cadmium toxicity in biological systems by other metals. *Curr. Sci.*, 54: 539-549.

Shaw, B.P. and Panigrahi, A.K. (1986). Uptake and tissue distribution of mercury in some plant species collected from a contaminated area in India: Its ecological implications. *Arch. Environ. Contam. Toxicol.*, 15: 431-436.

Simkiss, K. and Mason, A.Z. (1984). Cellular responses of molluscan tissues to environmental metals. *Mar. Environ. Res.*, 14: 1-4.

Simkiss, K., Taylor, M. and Mason, A.Z. (1982). Metal detoxification and bioaccumulation in molluscs. *Mar. Biol. Letts.*, 3: 187-201.

Sivadasan, C.R. (1987). *Studies on the effects of trace metals on* Metapenaeus dobsoni *(Miers)*. Ph. D. Thesis. Cochin University of Science and Technology, Cochin.

Skidmore, J.F. (1970). Respiration and osmoregulation in rainbow trout with gills damaged by zinc sulphate. *J. Exp. Biol.*, 52: 481-494.

Skjoldal, H. R. and Bakke, T. (1978). Relationship between ATP and energy charge during lethal metabolic stress of the marine isopod, *Cirolana borealis*. *J. Biol. Che.* 253: 3355-3356.

Spotila, J.R. and Paladino, F.V. (1979). Toxicity of arsenic to developing muskellunge fry, *Esox masquinongy*. *Comp. Biochem. Physiol.*, 62 C: 67-69.

Sprague, J.B. (1969). Measurement of pollutant toxicity to fish. I. Bioassay methods for acute toxicity. *Wat. Res.*, 3: 793-821.

Sprague, J.B. and Ramsay (1965). Lethal levels of mixed copper-zinc solution for juvenile salmon. *J.Fish. Res. Bd. Can.*, 24: 475-492.

Sunda, W.G., Barber, R.T. and Huntsman, S.A. (1981). Phytoplankton growth in nutrient rich seawater : importance of copper-manganese cellular interaction. *J. Mar. Res.*, 39: 567-586.

Stokes, T., and Awapara, J. (1968). Alanine and succinate as end-products of glucose degradation in the clam *Rangia cuneata*. *Comp. Biochem. Physiol.*, 25: 883-892.

*
Stratton, G.W. and Corke, C.T. (1979). *Cited in* Sharma, A., Mukherjee, A. and Talukder, G. (1985). Modification of cadmium toxicity in biological systems by other metals. *Curr. Sci.*, 54: 539-549.

Suresh, K. (1988). *Haematology of some marine and estuarine molluscs of commercial importance* . Ph. D. Thesis Cochin University of Science and Technology, Cochin.

Suresh, K. and Mohandas, A. (1990). Haemolymph acid phosphatase activity pattern in copper stressed bivalves. *J. Invert. Pathol.*, 55: 118-125.

Tan Yanxiang (1984). Accumulation of mercury in blood clam from Bohai Gulf. *Acta Oceanologia Sinica*, 1: 114-122.

Thorp, U.J. and Lake, P.S. (1974). Toxicity bioassay of cadmium on selected freshwater invertebrates and interaction of cadmium and zinc on the freshwater shrimp *Paratya*

tasmaniensis Rick. *Aust. J. Mar. Freshwat. Res.*, 25: 97-104.

Thurberg, F.P., Calabrese, A. and Dawson, M.A. (1974). Effect of silver on oxygen consumption of bivalves at various salinities. In: Vernberg, F.G. and Vernberg, W. (eds.) *Pollution and Physiology of Marine Organisms*. Academic Press, New York.

Tort, L., Torres, P. and Hidalgo, J. (1984). Short-trem cadmium effects on gill tissue metabolism. *Mar. Pollut. Bull.*, 15: 448-450.

Turner, M.A. and Rudd, J.W.M. (1983). The English-Wabigoon River system. III. Selenium in lake enclosures. Its geochemistry, bioaccumulation and ability to reduce mercury bioaccumulation. *Can. J. Fish. Aquat. Sci.*, 40: 2228-2240.

Underwood, E.J. (1979). Interactions of trace elements. In: Oehme, F.J. (ed.) *Toxicity of Heavy Metals in the Environment*. Part 2. Marcel Dekker, New York. 641-668.

Unlu, M.Y., Heyrand, M. and Keckes, S. (1972). Mercury as a hydrospheric pollutant. I. accumulation and excretion of $HgCl_2$ in *Tapes decussatus* L.; In: Ruivo, M. (ed.) *Marine pollution and sea life*. Fishing News Books Ltd., London. 292-295.

Van Haren, R.J.F., Van der Meer, J. and de Vries, M.B. (1990). Cadmium and copper accumulation in the common mussel *Mytilus edulis* in the Western Scheldt estuary. A model approach. *Hydrobiologia*, 105-118.

Van Leeuwen, C.J. and Hermens, J.H. (1988). Quantitative structure activity relationships and mixture toxicity. In: de Kruijf, H. A. M., de zwart, D.,Ray, P. K. and Viswanathan, P.N. (eds.) *Mannual on Aquatic Ecotoxicology*. Allied

Publishers, New Delhi.

Van Puymbroeck, S.L.C., Stips, W.J.J. and VanderBorght, O.L.J. (1982). Antagonism between selenium and cadmium in a freshwater mollusc. *Arch. Environ. Contam. Toxicol.*, 11: 103-106.

Venugopal, B. and Luckey, T.D. (1975). Toxicology of non-radioactive heavy metals and their salts. In: Venugopal, B., Hutcheson, D.(eds.) *Heavy metal Toxicity, Safety and Hormology*. Stuttgart, Thieme, 4-73.

Viarengo, A. (1985). Biochemical effects of trace metals. *Mar. Pollut. Bull.*, 16: 153-158.

Viarengo, A., Palmero, S., Zanicchi, G., Capelli, R., Vaissiere, R. and Orensu, M. (1985). Role of metallothionein in copper and cadmium accumulation and elimination in the gills and digestive glands of *Mytilus galloprovincialis*. *Mar. Environ. Res.*, 16: 23-26.

Viarengo, A., Petrica, M., Mancinelli, G., Capelli, R. and Orensu, M. (1980). Effects of copper on the uptake of aminoacids, on protein synthesis and on ATP content in different tissues of *Mytilus galloprovincialis*. Lam. *Mar. Environ. Res.*, 4: 145-152.

Vijayram, K. and Vasugi, S.R. (1989). Sublethal effects of pulp and paper mill effluents on the biochemistry of a freshwater fish *Rasbora daniconius*. *Indian J. Environ. Hlth.*, 31: 36-42.

*
Vokal-Borek, H. (1979). Selenium. Univ. Stockholm. *Inst. Phys. Rep.*, 79-116.

Vranken, G., Tire, C. and Heip, C. (1988). The toxicity of paired metal mixtures to the nematode *Monhystera disjuncta* (Bastian,

1865). *Mar. Environ. Res.*, 26: 161-179.

Waldichuck, M. (1974). Some biological concern in heavy metal pollution. In: Vernberg, F.J. and Vernberg, W.B. (eds.) *Pollution and Physiology of Marine Organisms*. Academic Press, New York. 1 - 57.

Ward, G.S., Hollister, T.A., Heitmuller, P.T. and Parrish, P.T. (1981). Acute and chronic toxicity of selenium to estuarine organisms. *North East Gulf Sci.*, 4: 73-78.

Ward, G.S. and Parrish,P.R. (1982). Manual of methods in aquatic environment research. Part 6, Toxicity tests. *FAO Fish. Tech. Rep.*, (185), 23pp.

Wardle, C.S. (1978). Non-release of lactic acid from anaerobic swimming muscle of plaice *Pleuronectes platessa L.*, a stress reaction. *J. Exp. Biol.*, 77: 141-155.

Watling, H.R. and Watling, R.J. (1982). Comparative effects of metals on the filtering rate of the brown mussel (*Perna perna*). *Bull. Environ. Contam. Toxicol.*, 29: 651-657.

Wang Chuseng and Yin Weiping (1987). Accumulation of heavy metals in *Arca granosa*. *Acta Oceanologica Sinica*, 3: 420-427.

White, S.L. and Rainbow, P.S. (1982). Regulation and accumulation of copper, zinc and cadmium by the shrimp *Palaemon elegans*. *Mar. Ecol. Prog. Ser.*, 8: 95-101.

Widdows, J., Phelps, D.K. and Galloway, W. (1981). Measurement of physiological condition of mussels transplanted along a pollution gradient in Narrangansett Bay. *Mar. Environ. Res.*4: 181-194.

Winner, R.W. and Gauss, J.D. (1986). Relationship between chronic

toxicity and bioaccumulation of copper, cadmium and zinc as affected by water hardness and humic acid. *Aquat. Toxicol.*, 8: 149-161.

Winner, R.W. and Whitford, T.C. (1987). The interactive effects of a cadmium stress, a selenium deficiency and water temperature on the survival and reproduction of *Daphnia magna* Straus. Aquat. Toxicol., 10: 217-224.

Yoshida, T., Ito, Y., Suzuki, Y. (1976). Inhibition of hepatic drug metabolising enzyme by cadmium in mice. *Bull. Environ. Contam. Toxicol.*, 15: 402-405.

Zurburg, W. and Ebberink, R.H.M. (1981). The anaerobic energy demand of *Mytilus edulis*. Organ specific differences in ATP-supplying processes and metabolic routes. *Mol. Physiol.*, 1: 153-164.

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* Not referred in original