STUDIES ON THE EFFECTS OF SELECTED TRACE METALS ON METAPENAEUS DOBSONI (MIERS)

A THESIS SUBMITTED TO
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Under Faculty of Marine Sciences

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TO MY PARENTS

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C E R T I F I C A T E

This is to certify that the Thesis bound herewith is an authentic record of the research carried out by Sri. C.R. Sivadasan, M.Sc., under my supervision and guidance in the Chemical Oceanography Division, School of Marine Sciences, in partial fulfilment of the requirements for the Ph.D. degree of Cochin University of Science and Technology and no part thereof has been presented before for any other degree in any University.

Cochin - 682 016,
July, 1987.

(Dr. P.N.K. NAMBISAN)
Supervising Teacher

D E C L A R A T I O N

I hereby declare that the thesis entitled "Studies on the effects of selected trace metals on Metapenaeus dobsoni (Miers)" is an authentic record of research carried out by me under the supervision and guidance of Dr.P.N.K.Nambisan, Professor and Head, Chemical Oceanography Division, School of Marine Sciences, Cochin University of Science and Technology in partial fulfilment of the requirements of the Ph.D. Degree of Cochin University of Science and Technology and that no part of it has previously formed the basis for the award of any degree, diploma or associateship in any University.

C.R. SIVADASAN.

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C.R. SIVADASAN.

CONTENTS

PREFACE

This thesis is an attempt by the author to assess the suitability of *Metapenaeus dobsoni* (Miers), an economically important crustacean species as a sentinel organism of trace metal pollution. The results of detailed investigations on seasonal variation, bioassay, accumulation and depuration of three metals viz., mercury, copper and zinc are presented and discussed.

The importance of trace metals in the aquatic environment and their present status in the study area - Cochin backwaters, the significance of crustacean fisheries, the species *M. dobsoni* and the objectives of the present studies are described in Chapter 1. The methodology adopted during the investigation is given in Chapter 2. Chapter 3 delineates the seasonal variation of Hg, Cu and Zn in the edible and non-edible parts of *M. dobsoni* collected from Cochin backwaters for a period of one year (June 1984-May 1985). The results of bioassay experiments are given in Chapter 4. Kinetics of accumulation, retention and depuration of trace metals, their biological half-life, the influence of size group and environmental factors are given in Chapter 5. The effect of these metals on the physiological response of *M. dobsoni* viz. oxygen consumption is included in Chapter 6. A summary and list of references are also appended.

The results of the present investigation have been published/are under publication as given below:

i) Acute toxicity of mercury to the prawn *Metapenaeus dobsoni* (Miers). Presented in Second National Seminar on Marine Intertidal Ecology, Waltair, Feb. 14-16, 1985.

ii) Toxicity of mercury, copper and zinc to the prawn *Metapenaeus dobsoni* (Miers), *Curr. Sci.* 55, 337-340 (1986).

iii) Baseline levels of Hg, Cu and Zn in *Metapenaeus dobsoni* (Miers) from Cochin backwaters. (under publication).

iv) Accumulation and depuration kinetics of Hg, Cu and Zn in *Metapenaeus dobsoni* (Miers) (under preparation).

ABBREVIATIONS

BCF	:	bioconcentration factor
^{o}C	:	degree celsius
cm	:	centimetre
d	:	days
D.O.	:	dissolved oxygen
g	:	gram
h	:	hour(s)
ha	:	hectare
IS	:	Indian Standard
kg	:	kilogram
l	:	litre
m	:	metre
mg	:	milligram
min	:	minute(s)
mm	:	millimetre
ug	:	microgram
ND	:	not determined
ng	:	nanogram
P	:	probability factor
ppb	:	parts per billion
ppm	:	parts per million
S (‰)	:	salinity (10^{-3})
SD	:	standard deviation
t	:	tonne
Temp	:	temperature
v	:	volume
wt	:	weight

CHAPTER 1

INTRODUCTION

In his eagerness to urbanize and industrialize mankind has not only destroyed plant cover built up meticulously by nature over millions of years, but also polluted air, water and land so much so that development appears to have become synonymous with deforestation and desertification and industrial progress, with pollution. The indiscriminate use of atmosphere as a gigantic sewer has led to many pollution hazards in the developed and developing countries.

Coastal and estuarine areas tend to be increasingly heavily populated and industrialized. The great majority of estuarine systems today are polluted to some extent with a wide variety of toxicants. It was at one time believed that effluents could be freely discharged into estuaries without let or hindrance.

The hazards caused by trace metal pollution in the aquatic environment have focussed attention on the presence of metals in marine and freshwater organisms. The term trace element is used in the current literature to designate the elements which occur in small concentrations in natural biological systems. For practical purposes, the terms such as "trace metals", "trace inorganics", "heavy metals", "micro elements" and "micronutrients" will be treated as synonymous with the term trace elements (Wittmann, 1983).

Metals such as Fe, Zn, Cu, Mo, Cr, Co and Mn are essential for life but can be toxic in higher doses. Hg, Ni, Sb, Pb and Cd have not yet been shown to be essential to life but are present in human tissues and are toxic in higher doses. Kinne (1984) has grouped Hg and Cd under the "black list" and Cu, Zn, Cr, Pb and Ni under the "grey list" of substances to be banned from being released into the sea.

Since metals are required in life processes, marine organisms commonly accumulate trace metals by concentration factors of 10^3 to 10^5. Hence in polluted waters tissue concentration may attain toxic levels. This can result in biomagnification of heavy metals in higher trophic levels which in turn, may lead to contamination of the food species harvested by man (Darnell, 1971). Potentially dangerous metals in this respect are Hg, Cd and Pb.

Minamata disease caused by the consumption of mercury-contaminated shellfish and finfish from Minamata Bay and with the affliction of 'Itai-Itai' caused by the consumption of foods contaminated by Cd in Japan (Ui, 1972) triggered the current alarm of metal pollution in aquatic environment. A case study of marine copper pollution resulting in serious poisoning of fish off coast of Holland has been recorded by Roskam (1972). In the sea the more abundant zinc and copper may sometimes constitute a greater hazard to marine species than Hg or Cd (Kinne, 1984). Marine products may become tainted or poisonous and hence unusable, or the quality is impaired and the prices fall. Public awareness that some fish and marine products are affected by pollution can lead to a buyers' reaction against all similar products. Moreover, the vulnerability to pollution of lagoons and of other coastal embankments assumes vital importance with the growing need for their use for mariculture.

Statutory authorities concerned with environmental management and industrial concerns increasingly recognize the need for biological effects monitoring to detect changes, either deterioration or improvement in environmental quality (Widdows, 1985). The importance of techniques capable of measuring the biological effects of pollution is well stated in "Comprehensive Plan of the IOC Global Investigation of Pollution in the Marine Environment (GIPME)", published by Intergovernmental Oceanographic Commission (IOC) in 1976. At this moment it would be apt to quote Bayne (1985) that "the very term 'pollution' is now recognized as expressing biological features of the response of environmental systems to contamination". Ecological base line studies also have been recognized as the essential compartment for effective pollution control.

1.1 IMPORTANCE OF CRUSTACEAN FISHERIES

Crustaceans are a large, mostly marine, group of organisms. Of great economic importance is the order decapoda comprising of the lobsters, spiny lobsters, crabs, shrimps, prawns and cray fish. All biological communities contain significant crustacean biomass and diversity with representative species present as predators, parasites, grazers or intermediary links in the food web (Eisler, 1981).

India is one of the leading countries harvesting and exporting shrimps. Among shrimps penaeid group commands more attention than the non-penaeid group for their local and international demands. In India there are about 60 species in the penaeid group, among which 15 species are commercially important at present. *Penaeus indicus*, *P. monodon*, *P. semisulcatus*, *Metapenaeus dobsoni*, *Metapenaeus monoceros*, *Parapenaeopsis stylifera* are the important species heavily exploited in Indian coastal waters (CMFRI, 1986a). Annual marine fish production in the country during the year 1984-85 has been provisionally estimated as 1.62 million tonnes. The landings of penaeid prawns in 1984-85 was 1,30,540 t and Kerala contributed 37,000 t accounting 28.5% of total landings of penaeid prawns in India (CMFRI, 1986b). During the calendar year 1986, the export of marine products from India was 89,283 t earning the value of Rs.4627.1 million (Prime, 1987a). Frozen shrimp contributed 52,153 t in quantity realising Rs.3799.7 million during January-December 1986 (Prime, 1987b). The earnings from export of marine products recorded the all time highest during the year 1986.

Even though the frozen shrimp continued to dominate the export trade during 1985-86, its share in exports has slumped marginally from 64% in 1984-85 to about 60% in 1985-86 in terms of quantity. The fall was mainly due to poor landing of shrimps in the country (MPEDA, 1986). Though there has been a declining trend in the annual production of prawns, the demand of prawns is ever increasing in the world market. To safeguard the production trend against fluctuation or decline, farming of commercially important species of prawns could be adopted as an alternative measure for increasing prawn production (Unnithan, 1985). The prawn farming is being widely experimented along the coastal waters of India especially in Cochin and surroundings.

1.2 *METAPENAEUS DOBSONI* (MIERS, 1878)

The species *Metapenaeus dobsoni* (Miers) is widely distributed in Indian waters-through Malayesia and Indonesia to the Philippines. It is a marine and

brackishwater form. On the southwest coast of India, in Malayalam, the name "thelly chemmeen" is applied to the smaller sizes caught from estuaries and backwaters and "poovalan chemmeen" or "kadal chemmeen" to the bigger sizes caught from the sea. On the east coast of India, on the Bengal coast, this and related species are known as "chingri" (George, 1970). In Indian waters the species is present in the juvenile stages in most of the estuaries and backwaters along the coastline and the adults in inshore areas with muddy bottom. It is more abundant along the southwest (SW) coast of India where it contributes to a major fisheries.

Metapenaeus dobsoni

The life cycle of the species is completed in two types of environment, (i) brackishwater of estuaries and backwaters connected with sea and (ii) the sea. The entire course of larval development is passed in the sea and migration to backwaters commences at postlarval stages. The immature prawns found in backwaters are called juveniles and the mature prawns in the sea as adults. The species rarely exceeds 125 mm in length and maximum length attained in estuarine environment is 70-75 mm. *M. dobsoni* is euryhaline in nature and tolerates a wide range of salinity from nearly freshwater to marine condition as existing in Cochin backwaters. The present investigations were conducted at 5, 15 and 25×10^{-3}S representing the low, medium and high levels of salinity in the study area.

1.3 LOCATION OF THE STUDY

Cochin backwaters ($9^{o}55'$ and $10^{o}05'N$; $76^{o}15'$ and $76^{o}20'E$), a typical tropical estuary is permanently connected to sea and fed by the Periyar and Muvattupuzha rivers at the northern side. The Periyar River is recipient of a variety of industrial effluents at Eloor (Udyogamandal) and the Muvattupuzha River receives effluent discharge from a huge pulp-paper factory. The backwater system is subjected to a very wide range of physico-chemical conditions. During the southwest monsoon the estuary becomes dominant with freshwater or very nearly freshwater at the surface and with a tongue of saline water at the bottom. During premonsoon period the backwater becomes almost an extension of the adjoining sea. It is highly productive and forms a nursery ground for a variety of fish and shellfish. The conditions at the barmouth are also influenced by the discharge of domestic wastes and harbour activity in addition to the industrial effluents. The location of the study and sampling stations are given in Fig. 3.1.

Fish mortality as a result of the discharge of industrial effluents containing heavy metals have been reported in Cochin area (Unnithan *et al.*,1977, Venugopal *et al.*, 1980). Concentration of Cu, Zn, Fe and Mn in the particulate matter from the area covering from marine zone to freshwater zone of Cochin backwaters was found to be at higher levels (Sankaranarayanan and Rosamma Stephen, 1978). Sankaranarayanan *et al.*, (1978) observed high concentrations of Zn, Cu and Fe in *Crassostrea madrasensis* from this area and attributed the same to industrial and domestic pollution. Venugopal *et al.*, (1982) showed significant enrichment of Cu and Zn in sediments collected from the northern arm of Cochin backwaters. Enrichment of Zn was evident down stream of effluent discharge area during post and premonsoon season. Hg pollution in Periyar due to the effluents from Chlor-alkali unit was above the limit laid out by Kerala State Pollution Control Board (Shaheed, 1985). Balchand and Nambisan (1986) reported Hg pollution by the discharge of pulp and paper effluents to Muvattupuzha river. An estimated area of 8100 ha of shrimp fields are facing threat of water pollution caused by the bund in Periyar River (KSPCB, 1983).

Estimated toxic, biomagnified and bioaccumulable substances reaching the water courses and the volume of industrial waste discharges to Periyar River are given in Appendix I and II.

1.4 SCOPE OF THE PRESENT STUDIES

In aquatic pollution monitoring, bioassays are necessary because chemical and physical tests are not sufficient to assess the "biological water quality" - the capacity of water to sustain naturally occuring biological processes. The susceptibility to toxic substances depends on the species and the life stage of the particular organism. Further, their effect widely varies depending on the environmental factors.

Attempts to identify the indicator organisms and the studies to fix the baseline concentration of trace metals in the location of the study is limited except in the case of certain molluscs by Lakshmanan (1982). Hence the present investigation was undertaken to assess the toxic effects of three most toxic trace metals viz., Hg, Cu and Zn on *Metapenaeus dobsoni* an organism which is well within the limits of the guidelines for selecting an indicator organism (Phillips, 1980) given in Appendix IV. Further the metal content in higher crustaceans is of bearing in respect to toxicity and human dietary practices on the one hand, and to the marine food chain on the other, because such crustaceans are on a trophic level higher than the bivalves (Prosi, 1983).

1.5 OBJECTIVES OF THE PRESENT INVESTIGATION

(i) To provide documentation of the background levels of selected trace metals during the whole cycle of the year in *M. dobsoni* which will enable to assess the water quality in terms of baseline concentration.

(ii) To study the toxic effects of Hg, Cu and Zn to different size groups of *M. dobsoni* at various salinities by means of bioassays useful in predicting the safe concentration levels for the effective management of aquatic environmental quality.

(iii) To study the accumulation, distribution and retention kinetics of Hg, Cu and Zn at various salinities and for different size groups to provide information on the transfer of toxic metals from hydrosphere to biosphere and also to explore the possibilities of *M. dobsoni* being used as an indicator organism.

(iv) To assess the influence of these metals at sub-lethal levels on the physiological response of *M. dobsoni*, *viz.*, on oxygen consumption.

CHAPTER 2

MATERIALS AND METHODS

This chapter gives a brief outline of the materials and methods employed in the present investigations.

The glasswares (corning glass) were cleaned by soaking in 1:1 nitric acid for 24 h and washed with copious amounts of deionised distilled water before use.

All the chemicals and acids used were of analytical reagent grade. Acids used for the estimation of trace metals were redistilled. Double glass-distilled water was used in the preparation of reagents and digests used for the estimation of trace metals. Deionised water was used in all other cases.

2.1 SAMPLING PROCEDURE

Mid stream surface water samples were collected once a month for a period of one year (June 1984-May 1985) using a clean plastic bucket. The location of sampling stations are given in Fig. 3.1. Samples of *M. dobsoni* for seasonal variation studies were collected from the stations using cast nets, engaging local fishermen. Samples were processed in the laboratory as in 2.3.

2.2 ROUTINE MEASUREMENTS

Temperature, pH, salinity and dissolved oxygen were determined employing standard methods (Grasshoff, *et al*., 1983). A mercury in glass thermometer ($\pm$ 0.01^{o}C) and an Elico Model L1-10T pH meter (accuracy $\pm$ 0.05 pH units) were used for the measurement of temperature and pH respectively of the water samples. Salinity was determined by the Mohr-Knudsen titrimetric method and dissolved oxygen, by the Winkler method.

2.3 PREPARATION OF TISSUE SAMPLES

Samples were washed with large volume of water to remove sediments and detritus matter, then repeatedly with deionised water and finally with distilled water. They were dissected using clean stainless scissors and forceps either into edible and non-edible parts, or into different body parts. Muscle was taken as the edible part and rest of body parts as non-edible in the seasonal collection. In bioaccumulation and depuration studies body parts were separated as exoskeleton, muscle, gill and the rest of body parts as viscera. For analysis of metals after wet digestion, samples were deep frozen in polythene bags and preserved as such till digestion. Those for dry basis were dried to constant wt at 80^{o}C. The dried samples were stored in desiccator over silica gel until analysis.

2.4 DIGESTION PROCEDURE FOR THE DETERMINATION OF Hg

Wet samples were used for the determination of Hg. Digestion was carried out by wet oxidation under reflux with nitric and sulphuric acids in the ratio 4:1 (v/v) recommended by BITC (1976) using the modified Bethge Apparatus described by Shaw and Panigrahi (1986).

2.5 DIGESTION PROCEDURE FOR THE DETERMINATION OF Cu AND Zn

The digestion procedure is basically that of Martincic *et al* ., (1984). Tissue samples were placed in 100 ml Kjeldahl flask covered with a glass funnel: to 0.1 to 2.0 g wet samples 5-10 ml con. HNO_3 and 0.5 ml $HClO_4$ were added and to dry samples of 0.1 to 1.0 g, 5-15 ml con. HNO_3 and 0.5 to 1 ml $HClO_4$ were added. After preheating digestion for a minimum period of 3-6 h flasks were gently heated initially to avoid bumping and the heating continued until the organic matter was completely destroyed, indicated by a clear solution in the flask. Lower or higher sample weights require less or more HNO_3 and $HClO_4$. The solution was cooled and diluted to specific volume.

2.6 DETERMINATION OF Hg USING MERCURY ANALYSER

Mercury content in samples were determined by cold vapour atomic absorption technique using Mercury Analyser MA 5800A (Electronics Corporation of India Ltd., Hyderabad). The process involved for the analysis were as given in "Analytical Methods for Determination of Mercury with Mercury Analyser MA 5800A" issued by ECIL 1981.

2.7 DETERMINATION OF Cu AND Zn USING ATOMIC ABSORPTION SPECTROPHOTOMETER

Cu and Zn were estimated using Atomic Absorption Spectrophotometer, Perkin-Elmer 2380. The samples were directly aspirated into the flame (Air-Acetylene fuel mixture) and using the concentration mode the corresponding concentration in the digest was determined.

2.8 SEAWATER

Unpolluted seawater with low turbidity and settleable solids having $35x10^{-3}$S was collected from Cochin barmouth during high tide. The water was filtered through a column packed with acid washed gravel (0.5-2.0 mm), fine sand and activated

charcoal and finally through a cotton plug. Filtered seawater was stored in polythene containers. Seawater was diluted with deionised water to prepare water of lower salinities.

2.9 COLLECTION, TRANSPORTATION AND ACCLIMATION OF TEST ANIMALS

M. dobsoni was collected from Cochin backwaters using Chinese dip nets. The catch was transferred to water in a container and sorted for *M. dobsoni* and transported to the laboratory in polythene collection bags filled with water and air. Utmost care was taken to avoid any damage during collection and transportation.

The animals were sorted out into different size groups by measuring the length of the specimen from the tip of the rostrum to the tip of the telson. Distinction between male and female has not been made in any of the experiments.

Test animals brought to laboratory were transferred to plastic pools containing water of ambient salinity. Then they were transferred to acclimation tanks of required salinity. Any change in salinity was made in increments of 5×10^{-3}S at a time. Salinity, pH and D.O. were monitored daily for their constancy. Water was well aerated. Animals were fed with boiled egg. Remnant food particles and fecal matter were removed periodically. Acclimation was continued for a minimum period of 5-7 days.

2.10 TEST CONTAINERS

Bioassays were conducted in specially manufactured dye-free polythene tubs of 10 l capacity and 35 cm diameter. Prior to the experiments, tubs were soaked in hydrochloric acid (1:1) and nitric acid (1:1) for 24 h each and washed with copious amounts of deionised water. Lids were provided to protect from dust and to avoid the escape of animals during the experiment.

2.11 TEST SOLUTIONS

Stock solutions of Hg, Cu and Zn were prepared by dissolving analytical reagent grade $HgCl_2$, $CuSO_4.5H_2O$ and $ZnSO_4.7H_2O$ respectively in distilled water. Test solutions were prepared by adding calculated quantities of stock solutions to experimental tubs containing known volume of seawater of desired salinity.

The concentrations of metals* in test solution in the text refers to the added concentration of the metals and not the effective concentration.

2.12 MODE OF BIOASSAY

Static renewal bioassay renewing 50% seawater once in 24 h was conducted following the guide-lines given by Ward and Parrish (1982) and APHA (1980). A minimum of six concentrations and a control in duplicate were used in each set of experiment. Test concentrations were decided after performing range finding tests. Eight or ten animals were exposed to each concentration. The animals were transferred from the acclimation tank to the experimental tubs 24 h prior to the commencement of the experiment using a scoop net without causing any damage to the test animals. Loading of the organisms in the test container was at the rate of$\leq$0.8 g l^{-1} of seawater. Seawater was saturated with air before transferring to the test containers. Temperature, salinity, pH and D.O. content were monitored daily. The D.O. content was always maintained above 60% saturation. Animals were fed with boiled egg during bioassay to avoid cannibalism. Mortality in each concentration was recorded and dead animals were removed every 12 h. Criterion for death was the cessation of movement even after gentle prodding. Fecal matter uneaten food particles and molt, if any, were removed while renewing the seawater with the help of a siphon. Experiment was continued for 96 h.

2.13 PROCESSING OF DATA

The data of bioassay experiments were analysed according to the method of Litchfield and Wilcoxon (1949) using logarithmic probability paper.

The significance of the results in other experiments were analysed statistically by the method given by Scnedcor and Cocharan (1968).

2.14 PROCEDURE FOR BIOACCUMULATION AND DEPURATION STUDIES

Bioaccumulation studies were conducted by exposing *M. dobsoni* to sub-lethal concentrations of the metals. 80-100 animals of the required size group was exposed in test solutions at desired salinity in plastic pools of 40 l capacity. Three-fourth of the test solution was renewed every day. Prior to the commencement of the experiment, 10 animals were sampled as control. Samples

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*Metal refers to metal in solution.

of 10 animals each were collected from the test solution at intervals of 24 h. The experiment was continued for 96 h.

Depuration studies were conducted by transferring the animals exposed to test solutions for 96 h to clean seawater. The experiment was conducted in a constant flow seawater recirculatory system designed as shown in Fig. 2.1. The system consists of lower and upper reservoirs of 175 l and six rearing tanks made of perspex, each of 20 l capacity. The water was automatically pumped into the upper reservoir through a foaming tower by means of a float system. Water was aerated and renewed once in a week.

Filtered seawater of desired salinity was filled in the reservoir and rearing tanks. Animals exposed in test solutions for 96 h were carefully collected by means of scoop net, washed repeatedly in fresh seawater of test salinity and transferred to the rearing tanks each tank holding 10-20 animals. 10 animals prior to the commencement of depuration experiment and subsequent samples of 10 animals each were collected from the rearing tanks at the end of 3, 6, 12 and 18 days. The animals sampled from bioaccumulation and depuration experiments were processed as in 2.3 and analysed for their metal content as in 2.4, 2.5, 2.6 and 2.7 respectively.

During the above experiments, procedures 2.8, 2.9 and 2.11 were adhered to. Animals were fed with clam meat* during acclimation and bioaccumulation and depuration experiments. Clam meat was collected from unpolluted areas.# Water quality was checked daily with respect to salinity, temperature, pH and D.O. Dead animals, fecal matters, food particles and molts if any, were removed periodically in both experiments.

2.15 PROCEDURE FOR THE DETERMINATION OF OXYGEN CONSUMPTION

To study the effect of trace metals on the oxygen consumption of *M. dobsoni*, test animals were simultaneously exposed to a series of sub-lethal metal concentrations along with a control as in the case of bioaccumulation studies. Oxygen consumption of these animals at 0, 24, 48, 72 and 96 h was determined as detailed below.

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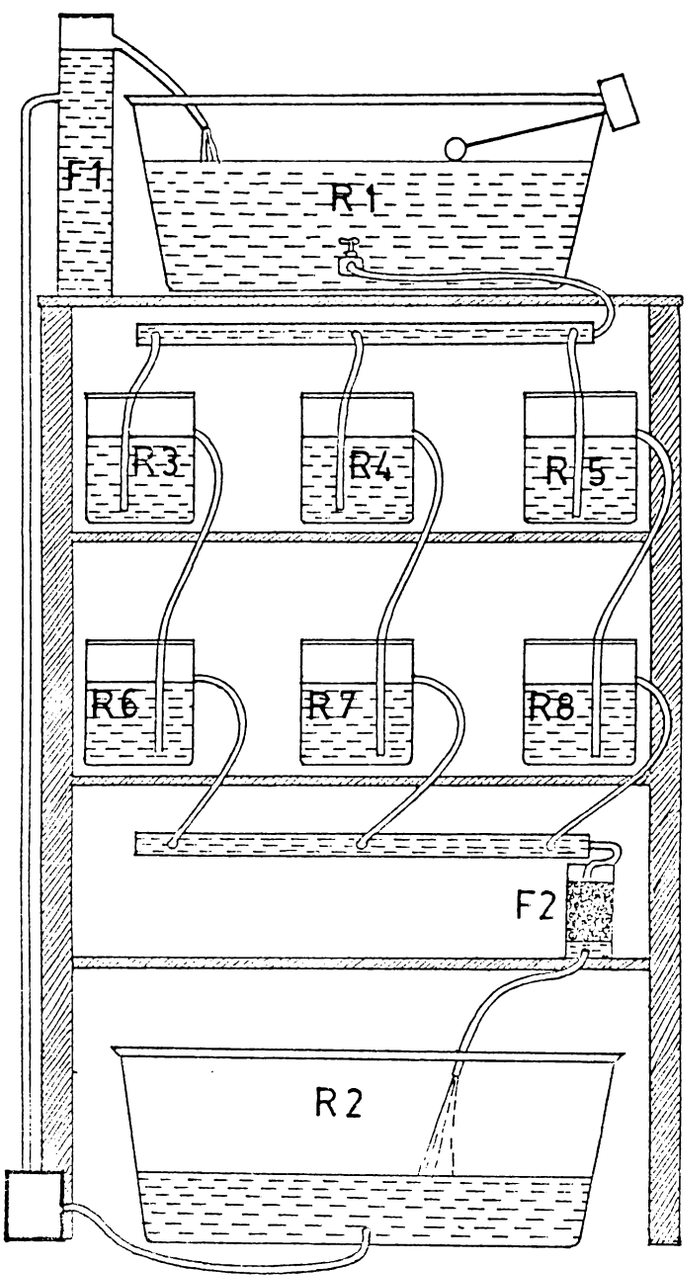
*During accumulation and depuration studies clam meat was fed to avoid nutrient deficiency, since the experiment is continuous for one month. In bioassay studies boiled egg was preferred since clam meat spoils the water quality.

#The trace metal content on analysis was found to be within the range reported from unpolluted areas.



R_1-Upper reservoir — F_1-Foaming tower
R_2-Lower reservoir — F_2-Filter.
R_3-R_8 Rearing tanks — S Float switch.

FIG. 2.1. CONSTANT FLOW SEAWATER RECIRCULATORY SYSTEM.

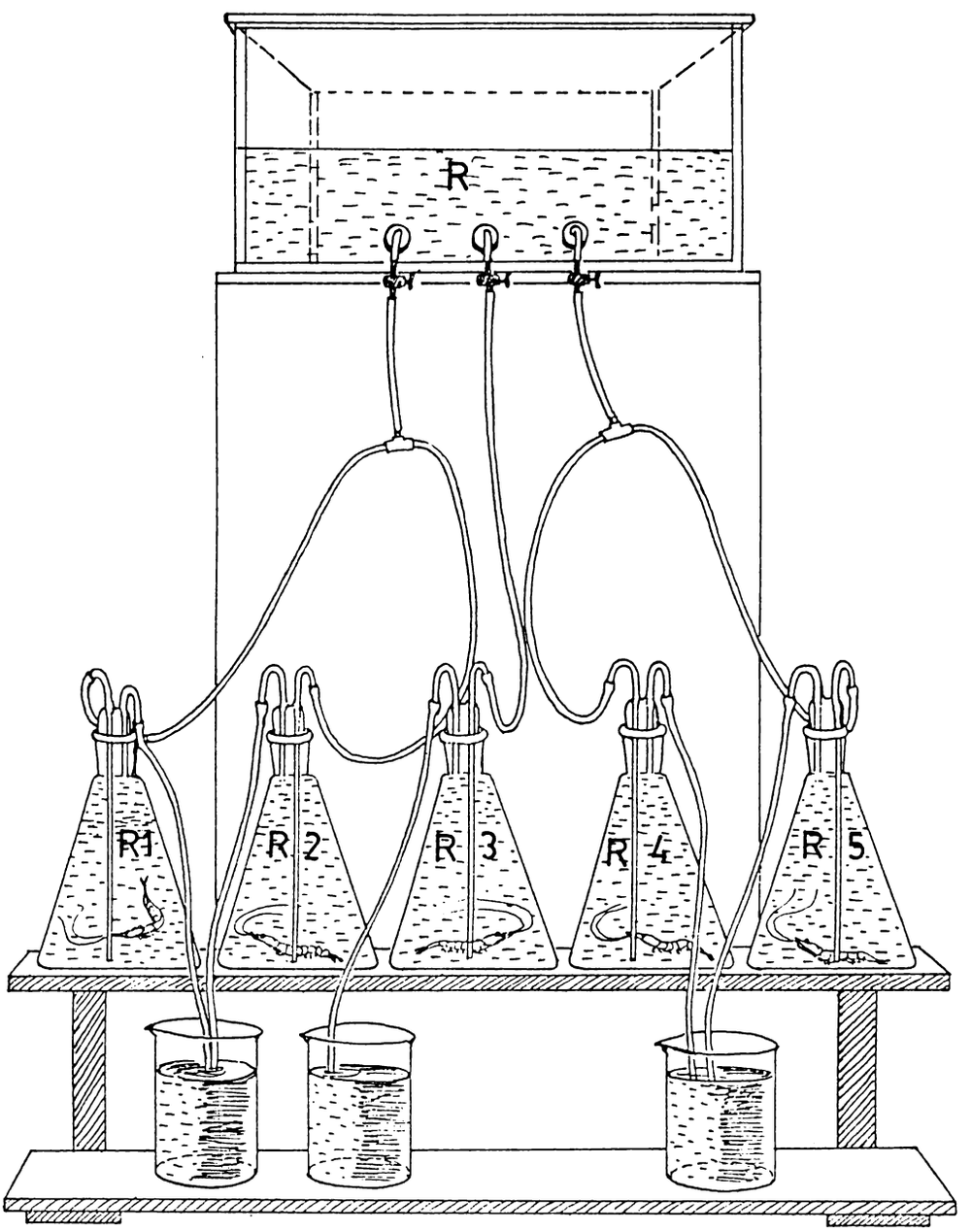
The experimental set up designed for the determination of oxygen consumption is shown in Fig. 2.2. Reservoir is made of perspex and the respiratory chamber of 500 ml capacity made of corning glass with ground glass neck and provided with an inlet and outlet with standard ground glass joints as shown in Fig. 2.2. Respiratory chambers were covered with black polythene sheets.

After the initial set up the reservoir is filled with the test solution. By opening the tap respiratory chambers are allowed to fill with the test solution. Test animals were carefully withdrawn from the exposure medium with scoop net and transferred to the respiratory chambers one in each chamber. Respiratory chambers were closed air tight without trapping air bubbles inside the chamber. Test solution was allowed to run through the respiratory chambers at a constant rate for a minimum period of 15 minutes to acclimate the animal inside the chamber. After the acclimation period the outgoing solution was collected in a 30 ml D.O. bottle taking the precautions for determining D.O. and the D.O. was determined. The inlet and outlet tubes were closed air tight and time was noted as the initial time. After one hour, outlet tube was opened and the inlet was disconnected from the reservoir and brought below the level of respiratory chamber allowing the water inside the chamber to flow through it. The water was collected in 30 ml D.O. bottles and D.O. was fixed.

D.O. content at the beginning and close of the experiment was determined as in 2.2 and the difference in oxygen content gives the amount of oxygen consumed by the test animal. The volume of respiratory chambers and that of the animals by displacement method were determined. Test animals were dried at $80^{o}C$ to constant weight.

Procedures 2.8, 2.9, 2.11 and 2.14 for exposing the animals in test solution were followed. The filtered seawater used for the determination of oxygen consumption was aerated to saturation and allowed to stand for 24 h to maintain the equilibrium condition. Animals were not fed from 24 h prior to the commencement of the experiment till its completion. While conducting the experiment a control without animal was simultaneously run with each set for correcting the oxygen consumption by the microorganisms if any in the test solution.

For determining the 'b' value for oxygen consumption of *M. dobsoni* another set of experiment was conducted using animals of different size group ranging from 0.0716 to 0.1954 g dry wt. Test solution was seawater at salinity $15x10^{-3}$.



R - Reservoir. R_1-R_5 Respiratory chambers.

FIG.2.2. EXPERIMENTAL SET UP FOR THE DETERMINATION OF OXYGEN CONSUMPTION.

CHAPTER 3

SEASONAL VARIATION OF MERCURY, COPPER AND ZINC IN *METAPENAEUS DOBSONI*

The industrially developed and developing countries the world over are concerned about the maintenance of water quality in estuaries. In order to preserve the flora and fauna, water quality criteria must be established which relate the capacity of the biota to tolerate the materials resulting from man's activities. The most important aspects of the contamination of marine environment are the health hazards to man caused through consuming contaminated fishery products and the damage inflicted on marine organisms and ecosystem. The risk of consuming contaminated fishery products can be estimated by comparing the 'tolerable intake' of toxic substances with the amount actually ingested by eating food of marine origin. The greater the difference between 'tolerable intake' and the actual intake, the smaller the contamination hazard to man (Benhard and Zattera, 1975).

Among the inorganic substances Hg is considered the most toxic both for man and marine organisms, followed by Zn and Cu which are relevant pollutants only to marine organisms. The epidemics in Minamata and Niigata, the observed high

levels of Hg in fish from polluted waters of Sweden and North Africa and the fact that there is no effective therapy for mercury poisoning have prompted the Governments in setting up standards and guidelines for tolerance levels of Hg in fish intended for human consumption (MPEDA, 1985).

The seasonal variation of pollutant concentration in organisms is a major source of inference in pollution monitoring surveys. Bryan (1984) suggested the use of biological indicators, since biological availability is one of the prerequisites for pollution. The determination of potentially toxic substances in polluted seawater presents great difficulties and is of doubtful practical interest, since the levels of analytes are extremely low and vary considerably in space and time. Conversely marine organisms by accumulating the impurities at least a thousand fold through the food chain, exhibit concentrations much higher and more representative of average situation in the whole of the locality (Satsmadjis and Voutsinou-Taliadouri, 1983).

As a part of baseline studies, metal concentration in marine organisms from various parts of the world have been estimated. A number of annotated bibliographies (Eisler, 1973; Eisler and Wapner, 1975; Eisler *et al.*, 1978, 1979) and review papers (Holden, 1973; Benhard and Zattera, 1975; Bryan, 1976b; Shiber, 1981) and recently the compilation by Eisler (1981) provide a wealth of information in this respect. Hall (1974) has estimated the Hg content in several brands of commercial canned seafood products. Trace metal levels in crustaceans are presented by authors like Bryan (1968), Knauer (1970), Bertine and Goldberg (1972), Boon (1973), Won (1973), Andersen and Neelakantan (1974), Leatherland and Burton (1974), Horowitz and Presley (1977), Luoma (1977), Ishii *et al.* (1978), Renzoni (1980), Cuadras *et al.* (1981), Sanders (1984), Sandler (1984), Dean *et al.*, (1986) and Medina *et al.* (1986).

Determination of trace metal concentration in Indian species are limited to fishes in the field collection (Somayajulu and Rama, 1972; Tejam and Haldar, 1975; Kureishy *et al.*, 1979; Kureishy *et al.*, 1981) and to oysters (Bhatt *et al.*, 1968; Zingde *et al.*, 1976). Desai *et al.* (1975) and Ramamurthy (1979) determined Hg levels in commercial prawns and marine food fishes from Indian Coasts. Matkar *et al.* (1981) determined Zn, Cu, Mn and Fe content in organisms including prawns and crabs from Bombay Harbour Bay. Seasonal variation of trace metals was studied by Sankaranarayanan *et al.* (1978) and Lakshmanan and

Nambisan (1983) in *Villorita cyprinoides*, *Meretrix casta* and *Perna viridis*. Patel *et al.* (1985) studied the distribution pattern of trace metals in the blood clam *Anadara granosa* and gobiid mudskipper *Boleophthalmus boddaerti* over the period 1976-1980.

No attempt has so far been made to study the seasonal variation of trace metal content in crustaceans from Indian waters and thereby to arrive at their baseline concentrations. Further the location selected for the study, Cochin backwaters is a typical tropical estuary subjected to heavy metal pollution caused mainly by the nearby industrial establishments. Hence it was felt relevant to undertake the present study.

3.1 MATERIALS AND METHODS

The location of sampling stations are given in Fig. 3.1. Sampling of seawater and *M. dobsoni* are detailed in 2.1. Routine measurements and tissue preparation are given in sections 2.2 and 2.3 respectively. Tissue samples were separated into edible and non-edible parts. Sections 2.4, 2.5, 2.6 and 2.7 were followed for the analysis of Hg, Cu and Zn.

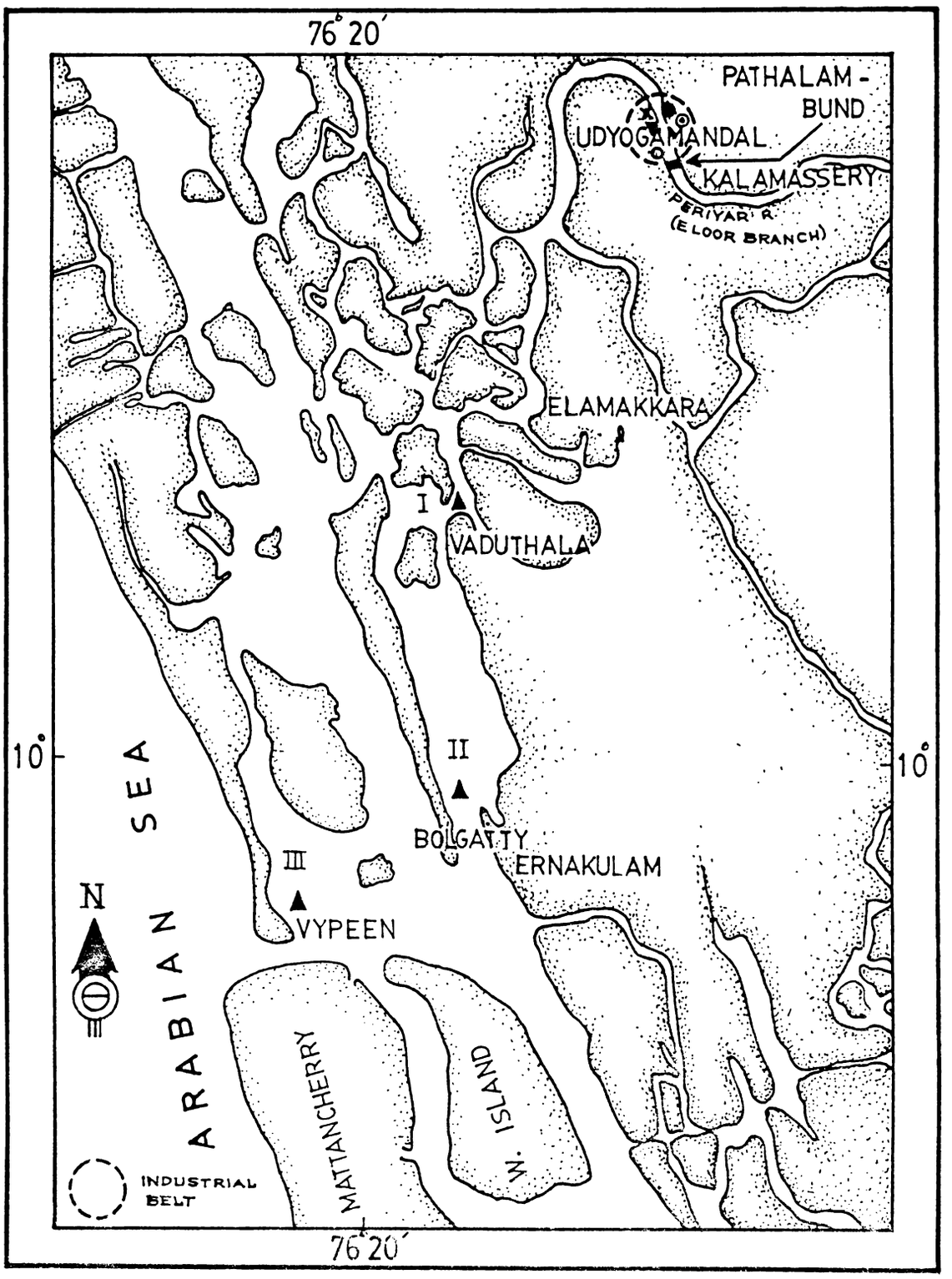
Note: All the water samples collected from the three stations could not be analysed for their trace metal content. Since the data is incomplete in drawing up some concrete conclusions on the seasonal variation of the trace metal content, the same is not included in the thesis.

3.2 RESULTS

The results of seasonal variation in water quality characteristics and size groups of *M. dobsoni* collected from the sampling locations are presented in Table 3.1, 3.2 and 3.3. Hg, Cu and Zn concentrations in the edible and non-edible parts of *M. dobsoni* collected for a period of 12 months from June 1984 to May 1985 in three locations are presented in Table 3.4, 3.5 and 3.6. The succeeding paragraphs delineates the significant observations made during the present study.

3.2.1 Mercury

At all three stations an increase in Hg concentration was seen in July over June, both in the edible and non-edible parts with a subsequent fall in concentration during August (Fig. 3.2). In September all the three stations recorded a sudden increase in Hg concentration in the edible part, the values being very high in



o-T.C.C., ▼-F.A.C.T., ×-I.R.E., ⊙-U.C.I., ●-C.B.Z.

FIG.3-1. STATION POSITIONS AND THE AREA OF SAMPLING.

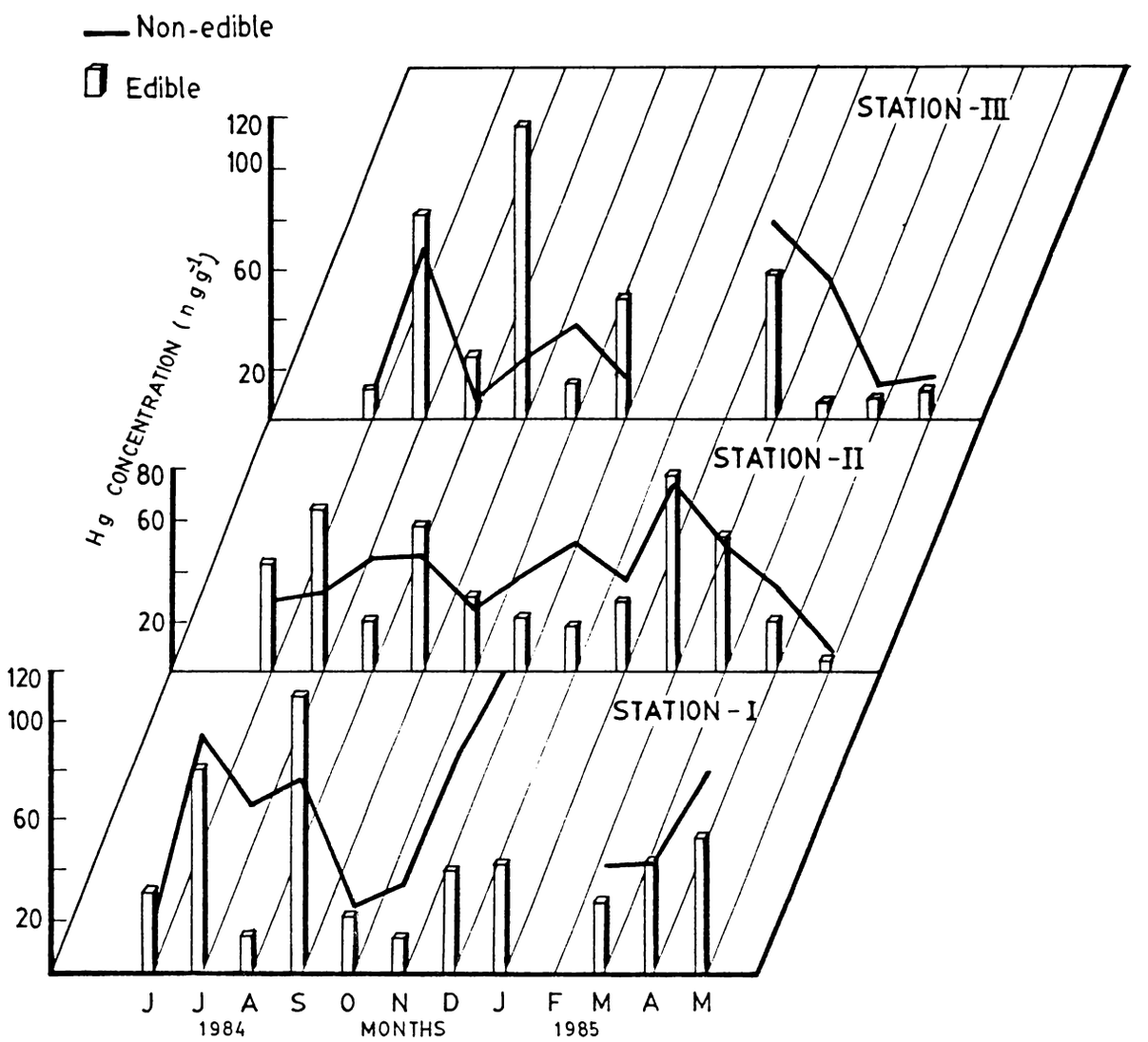


Fig. 3-2. Seasonal variation of mercury in edible and non-edible parts of M. dobsoni.

Table 3.1 Seasonal variation of water quality and size group of M. dobsoni Station-I

Period	Salinity (10^{-3})	pH	D.O. (mg l^{-1})	Size group (mm)	
				Mean	SD
Jun	0.02	7.20	6.96	66.80	3.12
Jul	0.04	6.45	6.84	51.00	10.15
Aug	0.25	6.55	6.44	50.66	10.06
Sep	0.27	6.40	3.37	61.66	10.40
Oct	2.98	6.35	4.17	57.63	4.80
Nov	12.63	6.75	5.07	47.10	2.33
Dec	12.55	6.98	7.70	54.50	3.31
Jan	6.71	6.20	6.36	53.80	4.89
Feb	15.85	6.40	3.35	ND	ND
Mar	21.04	7.36	3.41	53.09	5.45
Apr	14.67	7.14	4.44	47.10	3.21
May	1.05	7.20	2.81	53.38	4.93
Mean				54.22	
SD				6.25	

Table 3.2 Seasonal variation of water quality and size group of M. dobsoni - Station-II

Period	Salinity (10^{-3})	pH	D.O. (mg l^{-1})	Size group (mm)	
				Mean	SD
Jun	0.04	7.60	6.47	51.00	3.92
Jul	0.16	6.50	5.62	45.25	4.57
Aug	1.04	6.10	5.67	55.75	4.64
Sep	6.69	7.55	5.50	58.30	3.12
Oct	14.87	7.05	4.33	51.50	3.21
Nov	14.96	7.00	3.66	48.50	1.58
Dec	17.86	7.20	5.74	53.00	3.97
Jan	18.08	6.45	5.48	52.10	3.03
Feb	22.83	6.55	2.51	50.10	4.31
Mar	22.46	7.26	2.00	46.40	2.78
Apr	21.39	7.13	3.49	44.00	6.26
May	3.32	7.50	4.73	47.40	2.36
Mean				50.32	
SD				4.21	

Table 3.3 Seasonal variation of water quality and size group of M. dobsoni - Station-III

Period	Salinity (10^{-3})	pH	D.O. (my l^{-1})	Size group	
				Mean	SD
Jun	0.07	7.25	6.15	61.45	4.72
Jul	0.75	6.90	4.61	50.00	4.39
Aug	0.52	7.50	6.65	62.60	6.28
Sep	6.82	6.65	2.80	51.80	8.07
Oct	4.81	6.60	4.73	52.60	3.63
Nov	23.91	7.15	3.33	64.00	3.43
Dec	19.25	7.45	7.04	ND	ND
Jan	21.29	6.75	5.65	ND	ND
Feb	25.36	7.00	4.35	51.70	1.57
Mar	27.85	7.20	0.84	45.00	6.20
Apr	21.85	7.56	3.39	47.25	5.39
May	7.06	7.70	4.99	56.88	2.90
Mean				54.33	
SD				6.59	

Table 3.4 Seasonal variation of mercury in the edible and non-edible parts of *M. dobsoni*, concentration ng g^{-1} wet wt (Mean ± SD)

Period	Station-I		Station-II		Station-III	
	Edible	Non-edible	Edible	Non-edible	Edible	Non-edible
Jun	32.39	18.07	42.89	28.58	13.18	10.48
	0.54	1.03	2.59	1.51	0.88	0.54
Jul	81.01	93.68	64.57	30.00	81.09	69.34
	1.84	1.95	1.61	0.97	1.36	1.68
Aug	14.98	66.03	19.24	45.06	26.26	9.26
	1.03	2.21	0.96	1.69	0.12	0.27
Sep	111.30	75.39	57.45	45.79	117.09	25.05
	5.84	3.26	2.37	1.86	4.75	0.66
Oct	22.28	26.20	30.27	25.40	12.89	37.91
	1.28	1.39	0.73	0.36	0.41	0.44
Nov	14.19	33.87	21.36	39.77	47.61	16.82
	0.75	1.52	0.18	0.66	1.86	1.23
Dec	39.97	82.20	18.17	52.20	ND	ND
	1.31	2.03	1.25	1.28	- -	--
Jan	43.50	122.93	29.00	35.57	ND	ND
	1.45	2.68	1.37	1.47	-	-
Feb	ND	ND	79.43	75.73	59.30	77.93
	-	-	4.07	3.04	2.25	2.77
Mar	29.61	42.02	54.78	50.77	5.96	59.32
	0.54	0.80	0.91	0.80	0.25	1.19
Apr	43.87	42.27	18.53	33.77	10.65	12.74
	0.98	0.76	0.50	0.78	0.61	0.78
May	52.65	74.54	4.73	7.64	12.87	16.37
	1.57	2.31	0.21	0.32	0.46	0.48
Mean	44.15	61.56	36.70	39.19	38.69	33.52
SD	29.21	32.04	22.83	16.90	37.18	26.11

Table 3.5 Seasonal variation of copper in the edible and non-edible parts of M. dobsoni, concentration ug g^{-1} wet wt (Mean ± SD)

Period	Station-I		Station-II		Station-III	
	Edible	Non-edible	Edible	Non-edible	Edible	Non-edibl
Jun	2.62	6.79	9.72	32.20	3.22	9.81
	0.30	0.75	0.75	1.06	0.26	0.76
Jul	3.41	13.80	3.97	18.00	2.95	16.64
	0.10	0.34	0.20	1.01	0.05	0.34
Aug	2.35	8.24	6.96	21.98	5.81	22.17
	0.18	0.40	0.23	0.56	0.23	0.74
Sep	7.78	21.89	4.78	13.75	2.39	5.60
	0.55	0.97	0.34	0.93	0.13	0.18
Oct	2.92	8.13	1.77	10.40	6.20	18.32
	0.05	0.72	0.08	0.26	0.30	1.10
Nov	2.19	2.75	2.16	12.53	2.45	9.53
	0.19	0.40	0.32	1.06	0.19	0.80
Dec	7.62	23.51	9.07	20.95	ND	ND
	0.60	1.78	0.55	1.01		
Jan	7.89	26.29	7.08	25.35	ND	ND
	0.75	1.35	0.34	1.35	-	-
Feb	ND	ND	7.42	30.21	25.94	26.22
	-	-	0.40	1.06	1.08	1.74
Mar	2.35	12.74	1.98	5.91	0.72	6.42
	0.15	0.31	0.18	0.20	0.11	0.20
Apr	3.43	13.63	14.79	25.21	4.74	12.87
	0.23	0.60	0.52	0.70	0.21	0.35
May	3.14	12.56	4.04	37.33	3.45	11.38
	0.15	0.55	0.25	1.26	0.25	0.43
Mean	4.15	13.67	6.15	21.25	5.79	13.90
SD	2.36	7.43	3.85	9.48	7.27	6.79

Table 3.6 Seasonal variation of zinc in the edible and non-edible parts of *M. dobsoni*, concentration ug g^{-1} wet wt (Mean ± SD)

Period	Station-I		Station-II		Station-III	
	Edible	Non-edible	Edible	Non-edible	Edible	Non-edible
Jun	10.76	15.29	11.15	20.18	10.74	13.88
	0.51	1.28	0.51	1.26	0.75	0.90
Jul	13.30	18.54	14.30	16.00	15.33	20.71
	0.38	0.47	0.36	0.58	0.82	1.06
Aug	10.82	14.08	10.61	15.84	14.18	23.29
	0.56	0.45	0.48	0.72	0.47	1.34
Sep	12.25	17.65	13.93	18.54	11.67	14.63
	0.48	0.78	0.87	1.21	0.60	0.56
Oct	8.70	15.13	7.50	17.45	13.99	20.66
	0.43	0.78	0.32	0.88	1.04	1.38
Nov	7.38	17.00	6.19	7.44	2.88	10.58
	0.57	1.35	0.43	0.54	0.22	1.04
Dec	13.28	25.07	16.31	21.46	ND	ND
	0.75	1.01	0.76	1.40	-	-
Jan	12.77	21.66	12.41	18.75	ND	ND
	0.86	1.52	0.53	1.15	-	-
Feb	ND	ND	12.39	19.12	42.18	18.63
	-	-	0.36	0.65	1.26	1.24
Mar	8.21	13.79	13.50	18.47	22.73	37.94
	0.24	0.75	0.38	.49	0.75	1.01
Apr	33.55	86.38	80.45	76.68	32.75	47.62
	0.93	1.27	2.56	1.75	0.75	0.65
May	12.71	29.90	18.38	71.38	12.92	19.46
	0.50	1.01	0.42	1.27	0.37	0.50
Mean	13.05	24.95	18.13	26.78	17.94	22.74
SD	7.12	20.97	19.91	22.38	11.59	11.44

Station I and Station III during the course of study. Another significant observation during September was the lower Hg content in non-edible part in all the stations compared to the Hg content in edible part. It is interesting to note similar observations in Stations II and III whenever there is a sudden hike in Hg content in edible part. In all other cases Hg concentration in non-edible part was either high or equal to the concentration in edible part. During October to January Hg concentration was comparatively low in edible part except for the slight increase in Station I during December-January. At the same time Hg concentration in non-edible part was comparatively high, the maximum values during the course of study. Again in February there was a hike in Hg concentration both in edible and non-edible part which gradually decreased in Stations II and III in the subsequent months. But during the same period (March-May), a gradual increase in both parts was recorded in Station I.

Positive correlation was observed between the Hg concentrations in edible and non-edible part, in samples from Station II (Table 3.7); the correlation coefficient (r) being 0.5788 with $P < 0.05$. Corresponding correlation coefficients in other stations were not significant even at 5% level. Hg content in edible and non-edible parts did not give any significant correlation with the ambient salinity in any of the stations (Table 3.8 and 3.9).

3.2.2 Copper

There was not much seasonal variation in the concentration of Cu in the edible part except for February in Station III (Fig. 3.3). In the case of non-edible part two concentration peaks were observed, one during July-August and the other in December-February. The concentration did not vary much during the remaining period. The concentration of Cu in non-edible part was fairly high compared to the edible part (Table 3.5).

Statistically significant positive correlation was obtained between the Cu content in the edible and non-edible parts of *M. dobsoni* (Table 3.7); the correlation coefficients (r) and P values being

$r = 0.9278$ ($P < 0.01$) for Station I and

$r = 0.7633$ ($P < 0.01$) for Station III.

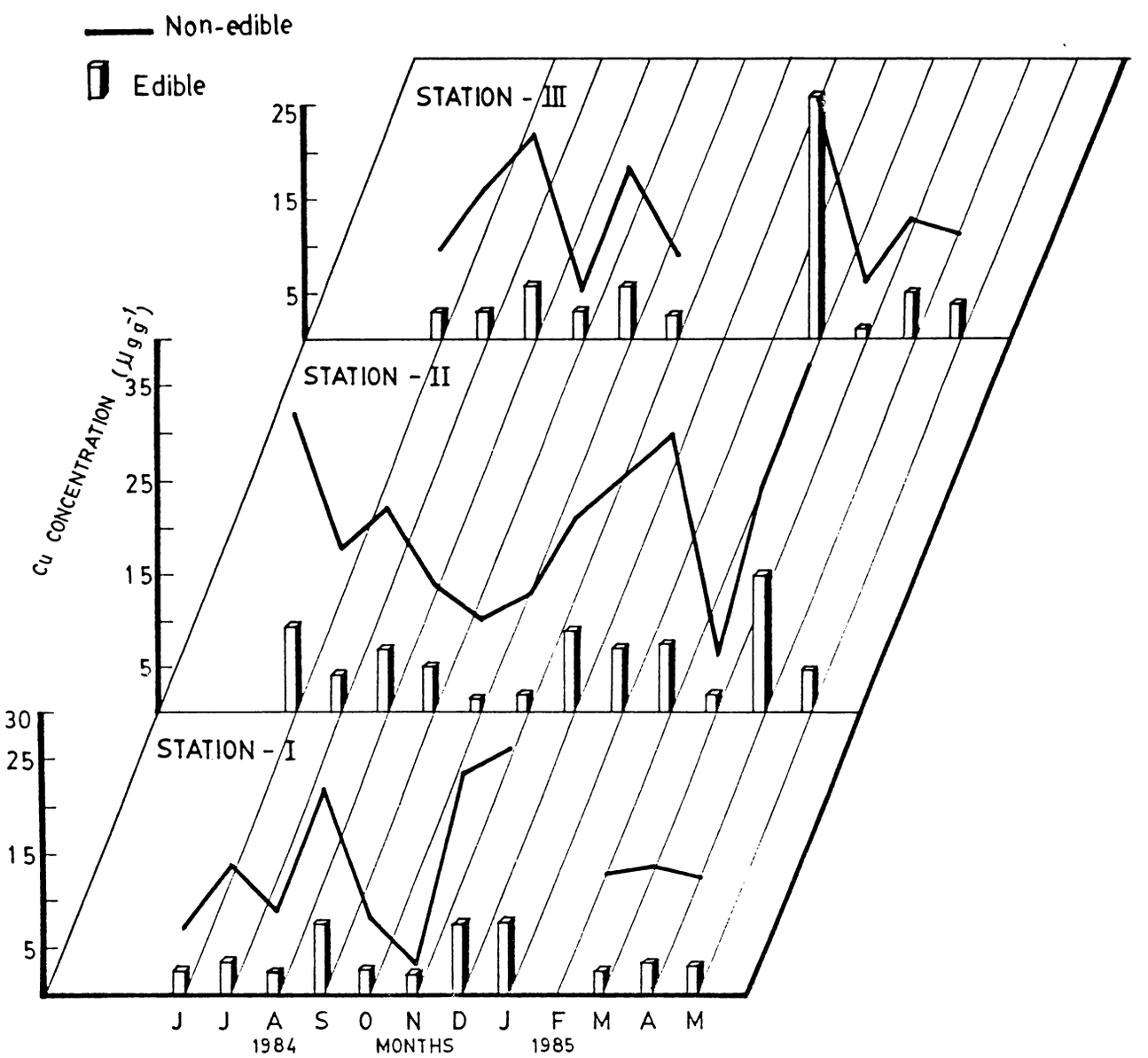


Fig.3-3. Seasonal variation of Cu in edible and non-edible parts of M. dobsoni.

Table 3.7 Metal to metal correlation coefficients (r) of edible and non-edible parts of M. dobsoni

Metal	Station-I	Station-II	Station-III
Hg	0.4669	0.5788*	0.2950
Cu	0.9272**	0.5438	0.7633**
Zn	0.9730**	0.7733**	0.5589

* $P < 0.05$; ** $P < 0.01$

Table 3.8 Correlation coefficients (r) of salinity and trace metal concentrations in edible part of M. dobsoni

Station - I

Hg	-0.3626	-	-
Cu	-0.0520	0.5428	-
Zn	0.2153	0.1807	0.0909
	S ‰	Hg	Cu

Station - II

Hg	0.0948	-	-
Cu	0.1286	-0.1696	-
Zn	0.2956	-0.2486	0.7338**
	S ‰	Hg	Cu

Station - III

Hg	-0.1405	-	-
Cu	0.3044	0.1770	-
Zn	0.5124	-0.0895	0.7331*
	S ‰	Hg	Cu

* $P < 0.05$; ** $P < 0.01$

Table 3.9 Correlation Coefficients (r) of salinity and trace metal concentrations in non-edible part of M. dobsoni.

Station - I

Hg	-0 1923	-	-
Cu	0.0603	0.7865 **	-
Zn	0.3298	-0.0699	0.1077
	S ‰	Hg	Cu

Station - II

Hg	0.5278	-	-
Cu	-0.2745	-0.2184	-
Zn	0.0526	-0.4630	0.5268
	S ‰	Hg	Cu

Station - III

Hg	0.3155	-	-
Cu	-0.0996	0.3591	-
Zn	0.4098	0.0482	-0.0503
	S ‰	Hg	Cu

** $P < 0.01$

The correlation coefficient (r) for Station II was 0.5438; which though not significant at 5% level, was very close to the critical value. The respective regression equation for Stations I and III are

$$Y = 1.5002 + 2.9284\ X \quad (r = 0.9278) \text{ and}$$
$$Y = 9.753 + 0.7159\ X \quad (r = 0.7663);$$

where Y is the Cu content in non-edible part and X, in the edible part. The respective regression lines are given in Fig. 3.5 and 3.6.

The salinity does not seem to have any influence on the metal concentration in both the parts. There was no correlation between salinity and the copper concentration in body parts in any of the stations (Table 3.8 and 3.9).

3.2.3 Zinc

There was no appreciable seasonal variation in Zn content in the edible and non-edible parts. Still, a gradual decrease in Zn content is noticeable in both the parts from July to November. Thereafter Zn content increases and remains almost constant upto March. During April, a sudden hike in Zn content in both the parts was observed which declined in the succeeding month to the same range as in June(Fig.3.4).

The abnormally high value for the edible part obtained during February in Station III does not fit into the general trend observed in the present study. In all other cases Zn content in non-edible part either remained high or almost equal to that in the edible part. This apparent anomaly may be attributed to sample contamination.

The Zn contents in both the parts showed statistically significant positive correlation between them (Table 3.7). The correlation coefficient (r) and P values were

$r = 0.9730$ ($P < 0.01$) for Station I and
$r = 0.7733$ ($P < 0.01$) for Station II.

Station III showed similar relationship even though the correlation coefficient $r = 0.5589$ was not statistically significant. The 'r' value obtained was close to the critical value at 5% level. The corresponding regression lines are

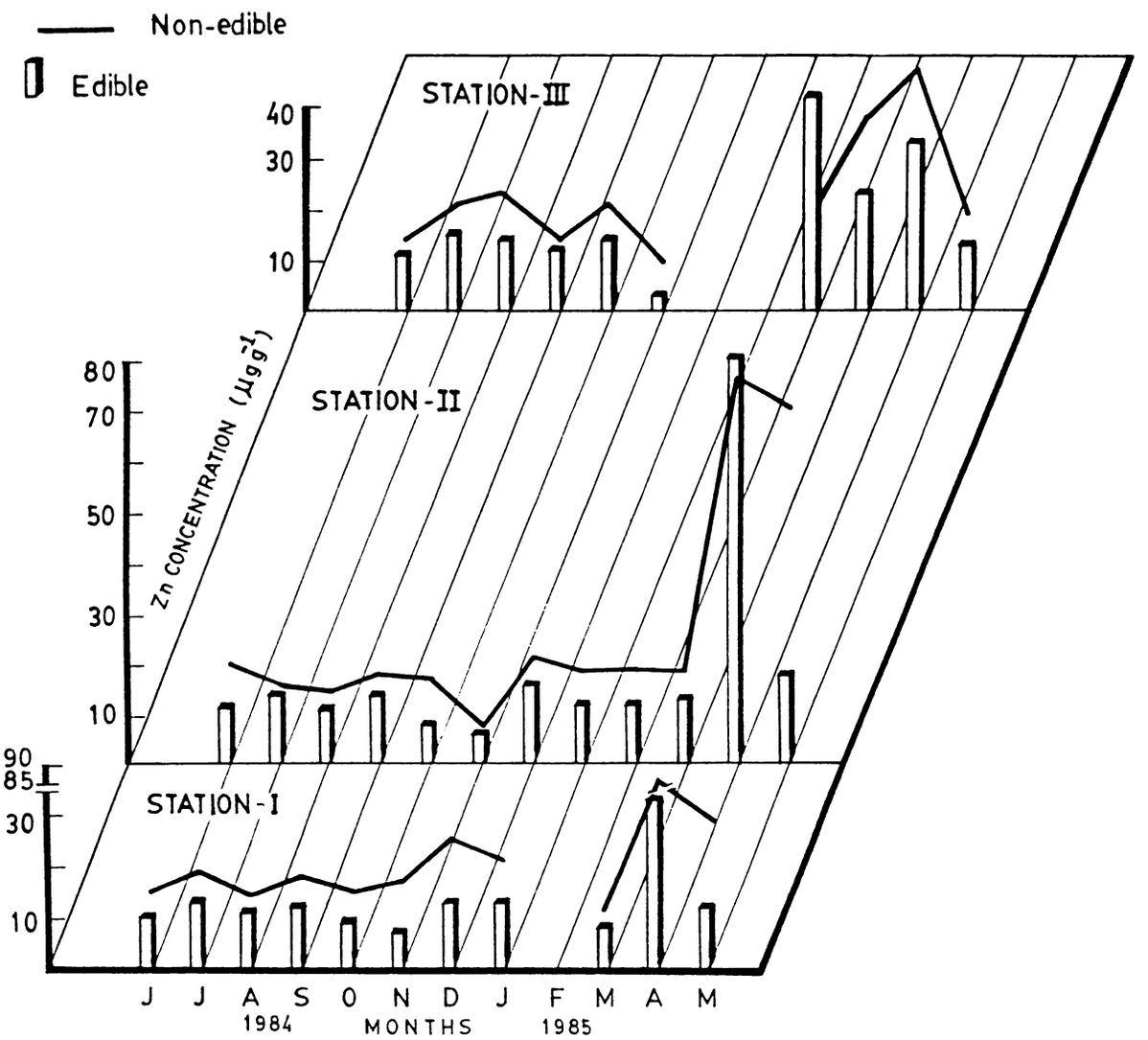


Fig.3-4. Seasonal variation of zinc in edible and non-edible parts of M. dobsoni.

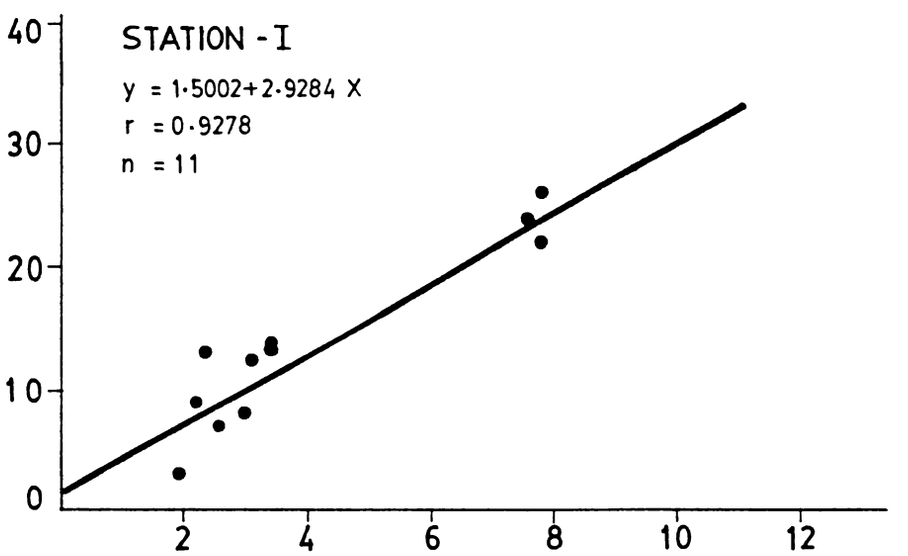


Fig. 3-5. Relationship between Cu content in edible and non-edible parts of M. dobsoni.

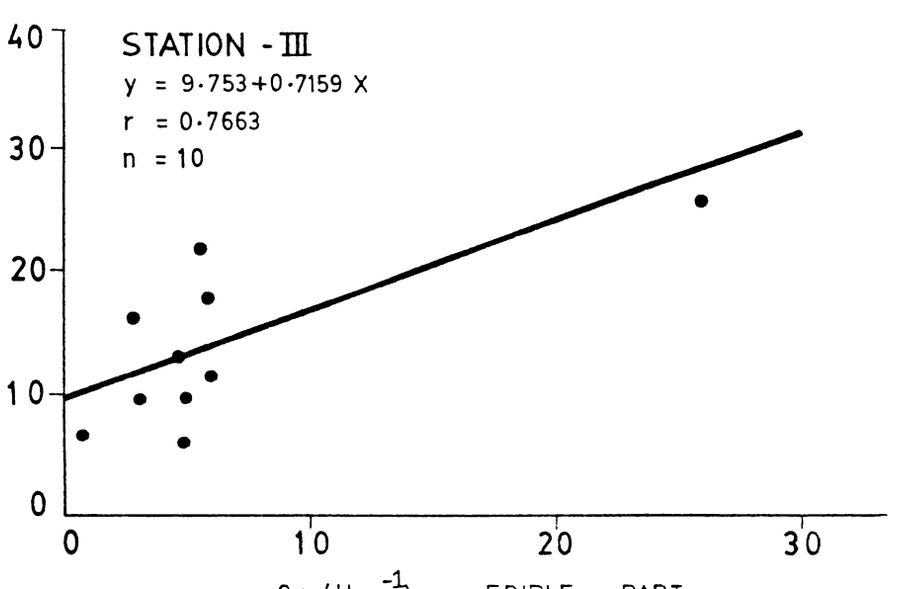


Fig. 3-6. Relationship between Cu content in edible and non-edible parts of M dobsoni.

$$Y = -12.4484 + 2.8665\ X \quad (r = 0.9730) \text{ for Station I}$$

and

$$Y = 11.0233 + 0.869\ X \quad (r = 0.7733) \text{ for Station II}$$

where Y is the Zn content in the non-edible part and X in the edible part. Fig. 3.7 and 3.8 represent the above equations.

The variations in salinity during the course of the year did not affect the Zn content in both the parts. No significant correlation was observed between the Zn content and salinity (Table 3.8 and 3.9).

3.2.4 Influence of Location of Sampling Stations on the Trace Metal Content in *M. dobsoni*

Examining Tables 3.10, 3.11 and 3.12, it can be seen that the whole body, edible and non-edible parts showed maximum Hg content in Station I. Hg content in Station III was low compared to Station II. Cu concentration was relatively high in Station II in whole body, edible and non-edible parts. There was not much difference in Cu content among Stations I and III. All the three stations showed similar trend in Zn concentration as in the case of Cu.

3.3 DISCUSSION

A knowledge of the seasonal variation of concentration of toxic substances in organisms is important in pollution monitoring surveys. Metal concentrations in aquatic organisms may be significantly influenced by temporal variations in metal levels within the ecosystem. Such fluctuations are commonly observed in estuaries (Bryan, 1973).

The concentration of trace pollutants in marine organisms varies considerably as a function of time, as a result of still greater fortuitous changes in the defiled surroundings. The state of pollution of a locality may vary considerably from one week to another. Therefore inspite of slowness of accumulation and depuration processes, analysis carried out on animals gathered at regular intervals of time would generally display large fluctuations.

Recent evidences indicate that free ions are biologically the most 'available' inorganic species of trace metals in seawater (Bryan, 1984), the form generally considered to be of greatest toxicity. The affinity of trace metals for biological

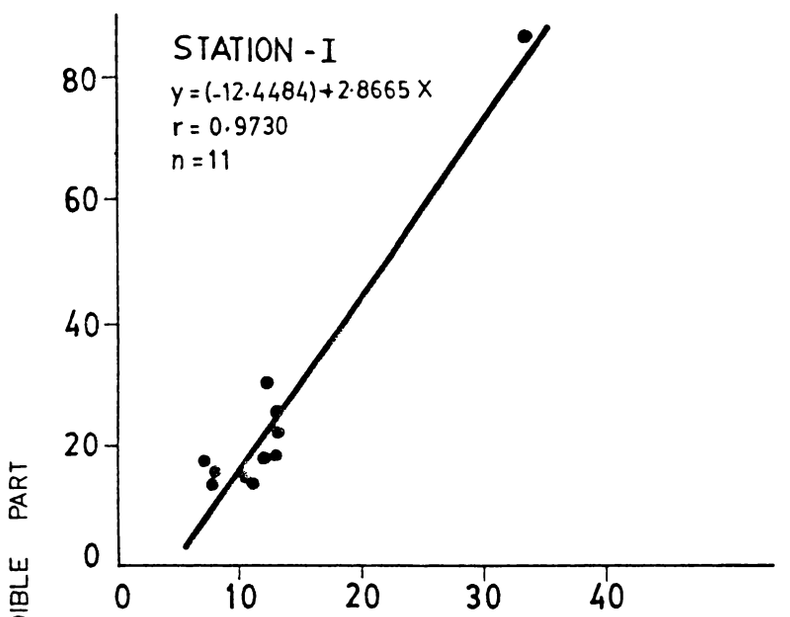


Fig. 3-7. Relationship between Zn content in edible and non-edible parts of M. dobsoni.

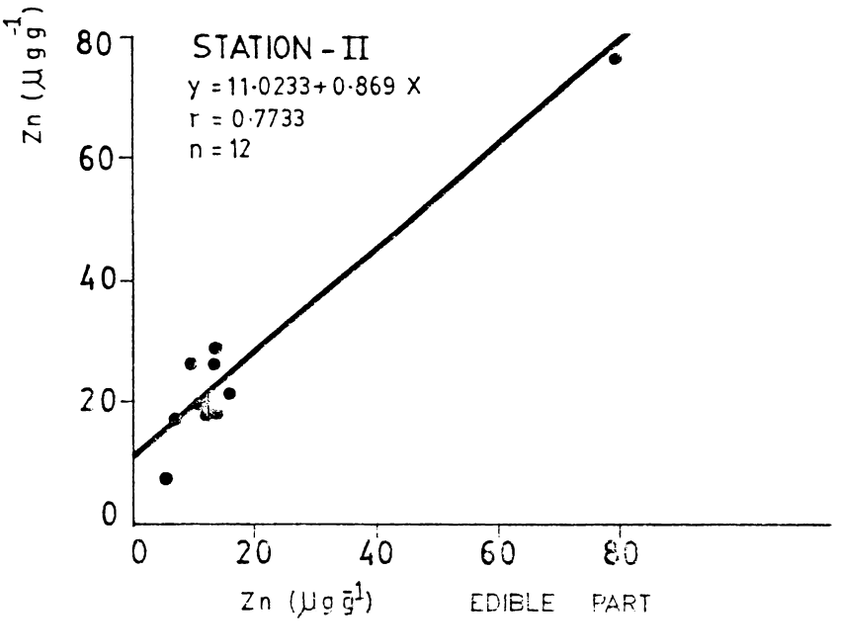


Fig. 3-8. Relationship between Zn content in edible and non-edible parts of M. dobsoni.

Table 3.10 Range, mean and SD of trace metal concentration in the edible part of M. dobsoni, concentration ug g^{-1} wet wt

Metal	Station	Range	Mean	SD	n
Hg	I	0.0142 - 0.1113	0.0442	0.0292	11
	II	0.0047 - 0.0794	0.0367	0.0228	12
	III	0.0060 - 0.1171	0.0387	0.0372	10
Cu	I	2.19 - 7.89	4.15	2.36	11
	II	1.77 - 14.79	6.15	3.85	12
	III	0.72 - 25.94	5.79	7.24	10
Zn	I	7.38 - 33.55	13.05	7.12	11
	II	6.19 - 80.45	18.13	19.91	12
	III	2.88 - 42.18	17.94	11.59	10

Table 3.11 Range, mean and SD of trace metal concentration in the non-edible part of M. dobsoni, concentration ug g^{-1} wet wt

Metal	Station	Range	Mean	SD	n
Hg	I	0.0181 - 0.1229	0.0616	0.0320	11
	II	0.0076 - 0.0757	0.0392	0.0169	12
	III	0.0093 - 0.0779	0.0335	0.0261	10
Cu	I	2.75 - 26.29	13.67	7.43	11
	II	5.91 - 37.33	21.15	9.48	12
	III	5.60 - 26.22	13.90	6.79	10
Zn	I	13.79 - 86.38	24.95	20.97	11
	II	7.44 - 76.68	26.78	22.38	12
	III	10.58 - 47.62	22.74	11.44	10

Table 3.12 Range, mean and SD of trace metal concentration in the whole body of M. dobsoni, concentration ug g^{-1} wet wt

Metal	Station	Range	Mean	SD	n
Hg	I	0.0230 - 0.0717	0.0431	0.0204	5
	II	0.0237 - 0.0543	0.0379	0.0118	7
	III	0.0099 - 0.0428	0.0194	0.0133	5
Cu	I	4.25 - 33.16	13.81	12.53	5
	II	7.87 - 39.28	18.98	10.35	7
	III	5.03 - 19.46	11.33	5.99	5
Zn	I	9.63 - 33.37	17.94	10.77	5
	II	13.39 - 43.94	25.45	13.86	7
	III	11.03 - 50.84	21.06	16.75	5

materials depend not on the lipid-water partition, but on the affinity of the trace metals for subcellular proteins and other molecules. Zn, Cu, Cd, Hg and Ag, a group of metals having similar electronic characteristics; due to differences in atomic number, electrongativity etc., their affinity for biological ligands may vary greatly (Angelici, 1973). Sen Gupta *et al*., (1978) have opined that transition metals, especially those with half filled d-orbitals, have a tendency to attach to donor atoms to undergo bioaccumulation.

Mercury is able to exist in natural environment in contact with water in three oxidation states: as the native metal itself, in the 1+ (mercurous) state and 2+ (mercuric) state. The nature of species which will occur or predominate in solution depends upon the redox potential and pH of the environment, and upon the nature of the anions and other groups present with which Hg can form stable complexes (Gavis and Ferguson, 1972). Cu in marine waters may exist in the form of simple aquated ions (2+ oxidation), metal-inorganic complexes, metal-organic complexes, or it may be adsorbed onto/absorbed into organic and inorganic colloidal material (Van den Berg and Kramer, 1979). The studies have indicated that free aquated Cu ion is the toxic form of the metal (Anderson and Morel, 1978; Jackson and Morgan, 1978). The concentration of ionic Cu^{++} in seawater is thought to be quite low, but it is this form which determines the bioavailability of copper to phytoplanktons (Sunda and Guillard, 1976) and fish eggs (Engel and Sunda, 1979). Zn can exist in seawater in a dissolved state (free ions or dissolved complexes) as solid precipitates, or adsorbed to particle surfaces. The stable species of Zn comprise three different forms; uncomplexed free (i.e., hydrated) ions, inorganic complexes and organic complexes (Young *et al*. 1980).

Mercury content among organisms feeding in contact with sediment is highest among carnivores and lowest in herbivores; a similar pattern exists for organisms feeding above the sediment/water interface (Klemer *et al* ., 1976). Although crustaceans can biomagnify mercury in their tissues through diet, it is reported that accumulation from solutions are more rapid and more pronounced than from food (Luoma, 1976, 1977). The observed maximum total Hg concentration in edible part of *M. dobsoni* among the three stations during the course of the present study was 117.09 ng g^{-1}, the minimum being 4.73 ng g^{-1} fresh wt (Table 3.4). The mean Hg concentration in the edible part in three stations were 44.15, 36.70 and 38.69 ng g^{-1} fresh wt respectively.

The reported total Hg concentrations for prawns and shrimps in edible (muscle) part (ng g^{-1} fresh wt) are : *Crangon crangon*, 186 (De Clerck *et al.*, 1974); *Penaeus aztecus*, 60; *Penaeus californiensis*, 120; *Penaeus setiferus*, 40 (from Veracruz, Mexico); 30 (from Ciudad del Carmen, Mexico), (Reimer and Reimer, 1975); *Penaeus orientalis* upto 40 (Doi and Ui, 1975); *Penaeus kerathurus*, 90-260 (Establier, 1977); prawns 20 (Sorentino, 1979); *Parapenaeus longirostris* 520 (from Valencia); 617 and 713 (from Castellon during Sep. and Dec.), (Medina *et al.*, 1986); shrimp, 51-100 (MPEDA, 1985); grass shrimp, 110 and 260 (Santoro and Koepp, 1986).

The values observed during the present study are well below the concentrations reported earlier. The present samples were collected from Cochin backwaters which is prone to Hg pollution with an estimated annual discharge of 2000 kg of Hg from the Chlor-alkali manufacturing concern (KSPCB, 1982). Slightly higher values than the present observations are reported by MPEDA (1985) for the commercial prawn samples. It is quite probable that the samples were contaminated during handling, processing and storage using potable water.

The mean Hg concentration in non-edible part (exoskeleton) of *M. dobsoni* in three stations were 61.56, 39.19 and 33.52 ng g^{-1} fresh wt, with the minimum and maximum being 7.64 and 122.93 ng g^{-1} fresh wt (Table 3.4). Won (1973) has reported 20-50 ng Hg g^{-1} fresh wt for the shrimp shell (exoskeleton). Comparatively low Hg concentration in exoskeletons of samples with high Hg content in edible parts (Fig. 3.2) may be due to the ability of the organism to reduce the Hg load by moulting.

Whole body Hg concentration varied from 9.9 to 71.7 ng g^{-1} fresh wt and the mean values in the three stations were 43.1, 37.9 and 19.4 ng g^{-1} fresh wt (Table 3.12). The whole body Hg concentrations reported by the earlier workers in ng g^{-1} fresh wt basis are : *Crangon crangon*, 120-300 (Zauke, 1977); *Palaemon debilis*, 5-302 (Luoma, 1977); shrimp, 30-90, (Johnson and Braman, 1975) from Gulf of Mexico; 240 (Parveneh, 1977) from Persian Gulf; 40-180, (Anon, 1978) from North Sea and prawns, 4-93, (Desai *et al.*, 1975); 5-30, (Ramamurthy, 1979). The values obtained in the present study are lower than many of the values reported earlier but lies within the range reported from Indian waters.

Copper is unique from both the regulatory and toxicology standpoints. From the regulatory standpoint Cu is a normal component of all the tissues and fluids in all animal species. However, the levels vary greatly from tissue to tissue and species to species. In the present study the lowest and highest Cu concentration observed in edible part were 0.72 and 25.94 ug g^{-1} wet wt, respectively. The mean values in three stations were 4.15, 6.15 and 5.79 ug g^{-1} wet wt (Table 3.5). Cu concentrations reported in ug g^{-1} wet wt for the muscle in the field collections are : *Crangon vulgaris*, 4.0; *Palaemon serratus*, 3.5; *Palaemonetes varians*, 7.9 (Bryan, 1968); shrimp, 2.4-4.5 (Won, 1973); *Pandalus jordani*, 3.4-3.7 (Bernhard and Zattera, 1975); *Ascelis indicus*, 11.2 (Matkar *et al*., 1981); deep sea prawn, 3.25 (Kureishy *et al*., 1981).

The mean concentration of Cu in the exoskeleton of *M. dobsoni* were 13.67, 21.15 and 13.90 ug g^{-1} wet wt for Stations I, II and III respectively with minimum and maximum values of 2.75 and 37.33 ug g^{-1} wet wt (Table 3.5). Earlier workers have reported the Cu concentration in ug g^{-1} wet wt in exoskeleton as: *Carcinus maenas*, 0.6, *Palinurus vulgaris*, 3.0, *Cancer pagurus*, 0.5 (Bryan, 1968); crabs, 6.44; shrimp, 4.9-10.7 (Won, 1973).

The mean whole body concentration of Cu were 13.81, 18.98 and 11.33 ug g^{-1} wet wt with 4.25 and 39.28 ug g^{-1} wet wt as the lower and upper limits (Table 3.12). Reported Cu concentrations in ug g^{-1} wet wt are: *Carcinus maenas* 15.4-31.4, *Crangon vulgaris*, 18.5, (Wharfe and Van Den Broek, 1977), crustaceans 6.8-10.9 (Wright, 1976).

The Cu concentration in the muscle of *M. dobsoni* in the present study lies well within the range reported earlier, the mean value ranging from 4.15 to 6.15 ug g^{-1} wet wt. Exoskeleton of *M. dobsoni* showed slightly elevated values than the crustacean shell/exoskeleton already reported. The probable reason for the same may be that the samples analysed in the present study were as non-edible part, i.e., all the parts except the muscle. Most of the whole body concentrations reported are on dry wt basis. The data available on wet wt basis are comparable to that for *M. dobsoni*.

The transition metal Zn is known to be involved in a number of enzyme systems in aquatic and terrestrial organisms (Vallee, 1959; Coombs, 1972), making it an essential micronutrient. According to Pequegnat *et al*. (1969), Zn is not limiting

to normal life processes in the marine environment and is accumulated in excess of the organism's immediate needs, at least on the basis of enzymatically-bound Zn. The Zn concentration in the muscle of *M. dobsoni* varied from 2.88 to 80.45 and the mean in three stations were 13.05, 18.13 and 17.94 ug g^{-1} wet wt (Table 3.6). In the non-edible part Zn concentration varied from 7.44 to 86.38, and the mean for the three stations were 24.95, 26.78 and 22.74 ug g^{-1} wet wt (Table 3.6). The whole body concentration varied from 9.63 to 50.84 and the mean for the three stations were 17.94, 25.45 and 21.06 ug g^{-1} wet wt (Table 3.12).

The Zn concentrations in ug g^{-1} wet wt basis reported earlier are : *Crangon vulgaris*, 14; *Palaemon serratus*, 10; *Palaemon varians*, 14 (Bryan, 1968); crustaceans 8 spp. 10-20 (Hall *et al.*, 1978), deep sea prawn, 11.96 (Kureishy *et al.*, 1981); *Ascelis indicus*, 11.15 (Matkar *et al.*, 1981) all for the muscle; *Atelecyclus septemdentatus*, 7; *Cancer pagurus*, 3; *Carcinus maenas*, 3; *Homarus vulgaris*, 5; *Palinurus vulgaris*, 16 (Bryan, 1968) all for exoskeleton and crustaceans, 23.7-28.9 (Wright, 1976); *Crangon vulgaris*, 34, *Palaemon serratus*, 21; *Palaemon squilla*, 30; *Palaemonetas varians*, 20, (Bryan, 1968); all for the whole body.

Observed Zn concentration in the muscle of *M. dobsoni* is well within the range reported for prawns. The concentration in exoskeleton was higher than the values reported for other crustaceans. The higher concentration is again probably due to the fact that exoskeleton in the present study refers to all the body parts except muscle. The whole body concentrations reported are almost comparable with the present values in *M. dobsoni*.

From Table 3.4 it can be seen that the mean Hg concentration is maximum in Station I for both edible and non-edible parts and lowest in Station III. Station I lies close to the industrial establishments including a Chlor-alkali unit, and Station III is near the barmouth. Laboratory studies, field evidence and simulation model were all consistent with minimal uptake of Hg from food (sediment) by the shrimp *Palaemon debilis* (Luoma, 1977). It is stated that the total concentration of Hg in shrimp appeared to be primarily governed by the periodic presence of elevated concentrations of soluţe Hg. The frequency

and amplitude of Hg concentration in *M. dobsoni* can be attributed to the presence of biologically available Hg in the study area. The higher concentration during July may be due to the presence of Hg from both natural and anthropogenic sources. In September the SW monsoon recedes and the river inflow is comparatively low allowing the organism exposed much to the biologically available Hg. September recorded highest concentration in the muscle during the course of the study both for Stations I and III and very high value in Station II. The low Hg concentration in exoskeleton during the same period may be attributed to occasional moulting as a depuration mechanism to reduce the Hg load. The fresh exoskeleton may contain comparatively low concentration of Hg.

The hike in Hg concentration during February follows the cessation of northeast monsoon. Thereafter the Hg concentration decreases in Stations II and III. Stations II and III are much exposed to the dilution and mixing action by seawater owing to the proximity of the stations to the sea. At the same time Station I recorded gradual increase in Hg concentration in both the edible and non-edible parts, indicating the availability of Hg from the effluent release under comparatively low saline conditions. Mercury does not appear to be regulated in any species and in contaminated areas is the only metal for which enhanced concentrations are usually most obvious (Bryan, 1984).

The dual nature of Cu and Zn both as essential trace elements and potential toxins at extremely low levels of exposure would demand the organisms strictly to regulate these materials at internal levels suitable for metabolic requirements. The lack of seasonal variation in the muscle may be due to the capacity of *M. dobsoni* to regulate the Cu content. The observed concentration peaks in the non-edible part during July-August and December-February may be due to the displacement of Cu to exoskeleton as a part of depuration process during the two monsoon periods, when the copper availability is more favoured by the low saline conditions existing in the estuary.

The gradual decrease in Zn concentration both in the edible and non-edible parts during July-November is due to the high dilution factors during the SW and NE monsoons. As the monsoon subsides, the Zn content increases. The sudden hike during April may be due to the pre-monsoon showers which mobilize the Zn from industrial and domestic sources to the receiving estuary. The observed maximum value in Station II is quite reasonable in that the station is adjacent

to the main vent for sewage disposal from Cochin City. Sankaranarayanan and Rosamma Stephen (1978) observed high concentration of particulate Zn during March in Cochin backwaters. Sankaranarayanan *et al.* (1978) observed lower concentrations of Zn in *Crassostrea madrasensis* collected from a locality between Stations II and III of the present study during June-November and increasing values from December onwards with a maximum during March/April. Summer maxima of Zn in *Acartia* were associated with proximity to anthropogenic discharges (Pearcy and Osterberg, 1967). Sanders (1984) has observed that the crab muscle from locations nearest to domestic and industrial activities have the highest Cu and Zn concentrations. It is estimated that 10,095 kg of Zn is discharged annually from the industrial belt at Eloor to Periyar River (KSPCB, 1982).

Biota of lower estuary would be expected to exhibit higher rates of trace metal uptake during the season of high run-off. In real situations, it is known that the concentrations of trace metal in estuaries fluctuate widely over both the short and long term and that parameters other than run-off may be responsible for many of these perturbations. Phillips (1977) included stratification of waters, tides and currents and the intermittent flow of industrial effluents as additional factors which may elicit changes in trace metal levels in estuarine or coastal waters. The amounts of trace metals maintained in the water column in estuaries are commonly found to be a simple function of salinity (Phillips, 1980). Thus several authors have published profiles describing the decrease in concentration of trace metals in river or estuarine waters with increase in salinity. If the salinity dependence of metal concentration is linear the metal is said to behave conservatively and the process may be adequately described by the rate of mixing of freshwater with relatively uncontaminated marine receiving waters. However, if departure from linearity occurs, the metal must be responsive to additional events other than freshwater - saltwater mixing.

The earliest report to suggest accumulation of trace metals by biota is salinity dependent, is that of Rucker and Valentine (1961). Other authors have observed direct effects of salinity on the rate of net uptake of trace metals by marine, estuarine or brackishwater biota (Wright, 1977; Vernberg *et al.*, 1977; Bryan and Hummerstone, 1973). By contrast no effect of salinity was noted on ^{203}Hg uptake from solution by the shrimp *Palaemon debilis* (Luoma, 1977).

The present study did not show any significant correlation between salinity and trace metal concentrations either in the edible or non-edible parts (Table 3.8 and 3.9). This observation is of great significance to the environmental status of Cochin backwaters. The flow of Periyar River is obstructed by a seasonal bund constructed during summer at "Pathalam" near Eloor just above the industrial belt to control the intrusion of seawater to the riverine system (KSPCB, 1983). The construction of the bund was necessitated when the natural course of Periyar River was obstructed by a number of hydel projects including the Idukki Hydel Project, one among the ten highest dams of the world. Hence, as pointed out by Phillips (1977), stratification of water and the intermittant industrial effluent discharge play the major role in controlling the trace metal concentration in Cochin backwaters which is more significant than the seasonal variation of salinity. The estimated discharge of trace metals (non seasonal) and the industrial waste discharge into the river by the major industrial establishments to Periyar River are given in Appendices I and II (KSPCB, 1982).

Except for certain areas impacted by anthropogenic wastes, such as Minamata Bay, all Hg concentrations in the field collection of crustaceans from various locations of the world fall well below the U.S. Food and Drug Administration guideline of 0.5 mg total Hg kg^{-1} fresh wt. Hg content in *M. dobsoni* in the present study does not exceed this limit and the maximum observed value in the edible portion is 0.1171 mg kg^{-1} (Table 3.4). So there is no risk in consuming *M. dobsoni* from Cochin backwaters which forms a major fishery resource throughout the year.

The legal limits of Hg, Cu and Zn laid out by various countries are given in Appendix III (Nauen, 1983). Indian Standard Institution has laid out specification for crab meat (IS 7582 - 1975) and for prawns/shrimps canned in brine (IS 2236 - 1968) as 10 ppm maximum for Cu and 50 ppm maximum for Zn. All the samples except two in the present study showed Cu concentrations within the limit (Table 3.5). One sample recorded above the tolerable limit for Zn (Table 3.6). However, the baseline concentrations of the trace metals in *M. dobsoni* determined during the present investigation are of significance. Though the present state of affairs seem to be within the limits laid out by Governmental agencies, the actual state of trace metal pollution is likely to be on the increase because of increasing industrial activity and diminishing river inflow due to hydel projects, which requires effective control measures and more public awareness.

CHAPTER 4

BIOASSAY OF TRACE METALS FOR *METAPENAEUS DOBSONI*

The history of toxicity testing dates back to 2000 years when Aristotle placed freshwater animals in seawater and observed their response. The beginning of the 20th century saw the synthesis of a variety of chemicals which necessitated testing their dual potential of benefits and drawbacks as a drug or poison. Although the possibility of adverse effects of materials on organisms were known earlier, the deleterious effects of chemicals and wastes on organisms such as fish were not recognized till about 1945. Hart *et al.* (1945), Doudoroff *et al.* (1951), Henderson and Tarzwell (1957), Sprague (1969, 1970, 1971) and others advocated and demonstrated the utility of the exposure of fish to industrial wastes for predicting potential damage.

The objective of a toxicity test is to define the concentrations at which the test material is capable of producing some selected response, usually deleterious, on a population under controlled conditions of exposure (Ward and Parrish, 1982). It provides informations such as (a) suitability of environmental conditions for aquatic life, (b) favourable and unfavourable concentrations of environmental factors such as D.O., pH, temperature, salinity or turbidity for aquatic life, (c) toxicity of waste to a test species, (d) effect of environmental factors on waste toxicity, (e) relative sensitivity of aquatic organisms to an effluent or toxicant, (f) effectiveness of waste treatment methods, (g) permissible effluent discharge rates and (h) compliance with water quality standards (APHA, 1980). Since fifties, the acute toxicity tests became the "work horse" in monitoring pollution effects.

In acute toxicity test, response to a toxicant is usually measured as mortality or lethality. It is expressed as lethal concentration (LC) which affects a specified portion of the population within a defined period of time. LC50 would be the concentration that theoretically kills 50% of the population, i.e., the median tolerance or median lethal concentration. This critical concentration is estimated by exposing organisms to a graded logarithmic series of concentrations of waste and then observing their responses. Most acute studies have been conducted for 96 h as recommended by APHA (1971), Portmann (1972) and NAS/NAE (1973). Bryan (1976a) has pointed out that, there is no doubt that the LC50 approach to metal toxicity in marine species has provided a wealth of information about the effects of different metals on different species and has revealed many factors - chemical, biological and environmental - which modify toxicity.

With increasing legal requirement in environmental management, toxicity test methods found their best application to reach specified management goals (Macek *et al.*, 1978; Anon, 1980; Cairns, 1980). Information generated from various toxicity tests can be of use in the management of pollution for the purpose of (a) prediction of environmental effects of a waste, (b) comparison of toxicants or animals or test conditions and (c) regulation of discharge (Buikema *et al.*, 1982).

It is felt that the lethal threshold concentration may be a more appropriate quantity to characterize toxicity, since it represents a common point of physiological response (Wuhrmann, 1952; Sprague, 1969; Brown, 1973). Lethal

threshold concentration, therefore, serves as a better concentration from which relative sensitivities of different species to a toxicant can be determined than LC50 values based on an arbitrary time.

A group of scientists evaluated various types of toxicity tests that have been used to assess effects of chemicals to aquatic life (Brungs and Mount, 1978; Macek *et al.*, 1978). Of the 15 types of tests considered, the highest rated tests included acute mortality, embryo-larval and reproductive impairment (chronic) tests. Acute tests were considered ecologically significant, most scientifically and legally defensible, modest in predictive capability, most simple and cost effective and were considered to have greatest utility.

As with most other groups, larval and juvenile crustaceans usually are significantly more sensitive than adults and therefore are preferred for short-term tests (APHA, 1980). The 'Red Book' (USEPA, 1976) and others have suggested the use of indigenous species for establishing a toxicological data base. Use of indigenous species would be ideal because the organisms are acclimated to ambient conditions for the purpose of deriving site specific toxicity information. Availability of sufficient test organisms of same size or age, throughout the year, is the other practical consideration to be taken into account while selecting test organism. *M. dobsoni* complies with all the above requirements (Menon, 1955a; Jones, 1969; Kuttyamma, 1978) and can be considered as a sentinel organism for coastal waters of India especially for Cochin backwaters.

Lethal concentrations of Hg(II), Cu(II) and Zn(II) to either the larval, post larval or adult stages of some marine crustaceans have been determined by Clarke (1947); Pyefinch and Mott (1948); Corner and Sparrow (1956); Portmann (1968, 1972); Connor (1972); Shealy and Sandifer (1975); Green *et al.*(1976), Ahsanullah (1976, 1982); Canto (1977); Eisler and Hennekey (1977); Ahsanullah and Arnott (1978); Arnott and Ahsanullah (1979); Johnson and Gentile (1979); Curtis *et al.* (1979); Chung (1980); Jacob *et al.* (1980); Trieff (1980); Ahsanullah *et al.*(1981); Martin *et al.* (1981); Denton and Burdon-Jones (1982) and Ahsanullah and Florence (1984).

Bioassays for the trace metal toxicity with crustaceans in Indian waters are very few in number. Ram Murti and Shukla (1984) and Sarojini and Victor, (1985) have conducted the studies in freshwater prawns and the studies in

marine crustaceans are limited to Kulkarni (1983); Mary Carmel *et al.* (1983); Veena and Lakshmy Ammal (1983); Perumal and Subramanian (1985); Ajmalkhan *et al.*(1986) and Sivadasan *et al.* (1986).

In general most studies on toxicity of trace metals to estuarine organisms are acute bioassays of adults under constant salinity and temperature conditions. Estuaries are characterized by fluctuating conditions of salinity and temperature to the extent that both are considered 'ecological master factors', which may act either singly or in concert to modify the structure, function or distribution of estuarine organisms (Kinne, 1971; Alderdice, 1972). The present investigations are aimed at determining LC50 values of Hg(II), Cu(II) and Zn(II) in relation to changes in ambient salinity for different size groups of *M. dobsoni* which form the major share of fisheries in Cochin backwaters throughout the year and liable to trace metal pollution by the nearby industrial establishments.

4.1 MATERIALS AND METHODS

Static renewal bioassay of Hg, Cu and Zn was conducted for *M. dobsoni* as detailed in sections 2.8, 2.9, 2.10, 2.11 and 2.12. Two size groups 30-50 mm and 50-70 mm were tested under three salinities 5, 15 and $25x10^{-3}$. The lower size group is referred in the text as the juvenile and the higher size group as maturing stage. 10 animals each were exposed in the lower size group and 8 in the case of higher size group. The experiment was repeated whenever the mortality in the control exceeded 10%. Details of test concentrations and water quality characteristics are given in Table 4.1. Data was analysed as in 2.13.

4.2 RESULTS

The results of acute toxicity tests with *M. dobsoni* to Hg(II), Cu(II) and Zn(II) are presented in the following subsections.

While conducting the tests no immediate behavioural response was observed when the prawns were exposed to test solutions. Prior to death, they began swimming with a spiraling motion before coming to lie on their sides on the bottom of the tub. Upon death their bodies turned white.

Table 4.1 Metal concentrations, water characteristics and number of animals used for the determination of lethal concentrations

Metal	Size group (mm) Mean ± SD	Metal concentration in mg l^{-1}						Salinity 10^{-3}	Water characteristics	
		1	2	3	4	5	6	Mean ± SD	pH	Temp (°C)
Hg(II)	44.6 ± 2.8	0.005	0.01	0.02	0.04	0.06	0.08	4.9 ± 0.1	7.1 ± 0.1	28.0 ± 0.5
	55.9 ± 2.7	0.005	0.01	0.02	0.04	0.06	0.08	4.8 ± 0.2	7.2 ± 0.1	28.0 ± 0.5
	46.7 ± 2.3	0.01	0.02	0.03	0.04	0.06	0.08	15.0 ± 0.1	6.9 ± 0.2	29.5 ± 0.5
	55.8 ± 3.1	0.01	0.03	0.05	0.07	0.09	0.11	15.2 ± 0.2	7.4 ± 0.1	27.5 ± 0.5
	43.3 ± 2.5	0.01	0.03	0.05	0.07	0.09	0.11	24.6 ± 0.4	7.1 ± 0.1	28.5 ± 0.5
	56.2 ± 1.8	0.02	0.05	0.08	0.11	0.14	0.17	24.8 ± 0.2	7.1 ± 0.1	28.5 ± 0.5
Cu(II)	42.3 ± 5.3	0.1	0.2	0.4	0.6	0.8	1.0	5.1 ± 0.2	7.1 ± 0.2	26.7 ± 0.8
	56.9 ± 4.3	0.1	0.2	0.4	0.6	0.8	1.0	5.2 ± 0.2	7.0 ± 0.1	26.5 ± 0.5
	41.5 ± 5.1	0.25	0.5	1.0	2.0	3.0	5.0	15.0 ± 0.2	7.6 ± 0.2	29.5 ± 0.5
	55.3 ± 2.4	0.3	0.6	0.9	1.2	1.5	2.1*	14.8 ± 0.2	7.3 ± 0.2	26.2 ± 1.0
	40.6 ± 9.8	0.1	0.2	0.5	1.0	3.0	6.0	25.0 ± 0.5	7.7 ± 0.1	29.5 ± 0.5
	55.2 ± 1.4	0.1	0.5	1.0	3.0	5.0	7.0	24.8 ± 0.3	7.7 ± 0.1	27.0 ± 0.5

(Contd.)

Table 4.1 (contd)

Metal	Size group (mm) Mean ± SD	Metal concentration in mg l						Salinity 10^{-3} Mean ± SD	Water characteristics	
		1	2	3	4	5	6		pH	Temp (°C)
Zn	42.3 ± 5.3	0.25	0.5	1.0	1.5	2.0	3.0	5.1 ± 0.2	6.9 ± 0.1	26.7 ± 0.8
	56.9 ± 4.3	0.50	1.0	2.0	3.0	4.0	5.0	5.2 ± 0.2	7.2 ± 0.2	26.5 ± 0.5
	44.4 ± 4.3	0.25	0.5	1.0	2.0	3.0	4.0	15.5 ± 0.1	7.6 ± 0.1	28.5 ± 0.5
	54.8 ± 3.2	0.25	0.5	1.0	2.0	3.0	5.0	15.2 ± 0.3	7.5 ± 0.2	27.0 ± 0.5
	47.0 ± 1.7	0.25	0.5	1.0	2.0	3.0	4.0	25.3 ± 0.2	7.9 ± 0.1	29.0 ± 0.5
	52.6 ± 1.8	0.25	0.5	1.0	2.0	3.0	4.0 **	25.4 ± 0.1	7.9 ± 0.1	29.0 ± 0.5

DO % saturation kept always above 60

No. of animals : 10 for lower size group 8 for the higher

* Concentrations 2.7 and 3.3 mg l^{-1} and ** 5.0 and 7.0 mg l^{-1} were also included

4.2.1 Mercury

The results of acute toxicity tests with Hg(II) at $5x10^{-3}$S for the two size groups are presented in Table 4.2. LC16, LC50 and LC84 values for 48, 60, 72, 84 and 96 h and 95% confidence limits for LC50 values and slopes were determined. In the lowest concentration of 0.005 mg l^{-1}, 10% and 12.5% mortality was noticed by the end of 96 h for the juveniles and maturing stages respectively. By 48 h 100% mortality occurred for both the size groups in 0.08 mg l^{-1}, the highest concentration tested. Mucus secretion was observed in all the concentrations tested.

Toxicity values with 95% confidence limits for LC50 and slopes determined at $15x10^{-3}$S for the two size groups are given in Table 4.3. By the end of 96 h 20% mortality occurred in the lowest concentration (0.01 mg l^{-1}) and 100% mortality in 0.06 mg l^{-1} for lower size group. Higher size group did not record any mortality in 0.01 mg l^{-1} but 100% mortality in 0.09 mg l^{-1} at the end of 96 h. Mucus secretion was observed at concentrations of 0.03 mg l^{-1} and above and the quantity of mucus secreted was higher at higher concentrations.

LC50 values and slopes with 95% confidence limits for the two size groups determined at $25x10^{-3}$S are presented in Table 4.4. 30% mortality was noticed in 0.01 mg l^{-1}, the lowest concentration tested and 100% in 0.09 mg l^{-1} at the end of 96 h for the lower size group. Higher size group recorded 12.5% mortality in 0.02 mg l^{-1} and 100% in 0.08 mg l^{-1} at the end of 96 h. Mucus secretion was observed in concentrations above 0.03 mg l^{-1}.

Fig. 4.1 and 4.2 show the changes with time in LC50 values. For the higher size group toxicity plots are represented by concave curves tending to be asymptotic (Fig. 4.2) and convex curves for the lower size group at 5 and $25x10^{-3}$S (Fig. 4.1), the slopes of which increases with time.

4.2.2 Copper

At $5x10^{-3}$S, LC16, LC50, LC84 and 95% confidence limits for LC50 and slopes of Cu(II) determined for 30-50 mm and 50-70 mm size groups are given in Table 4.5. The mortality at the end of 96 h in 0.1 mg l^{-1} for lower size group was 30% and in the same concentration all the prawns of higher size group were alive. 100% mortality was recorded in 0.6 and 0.8 mg l^{-1} for the lower

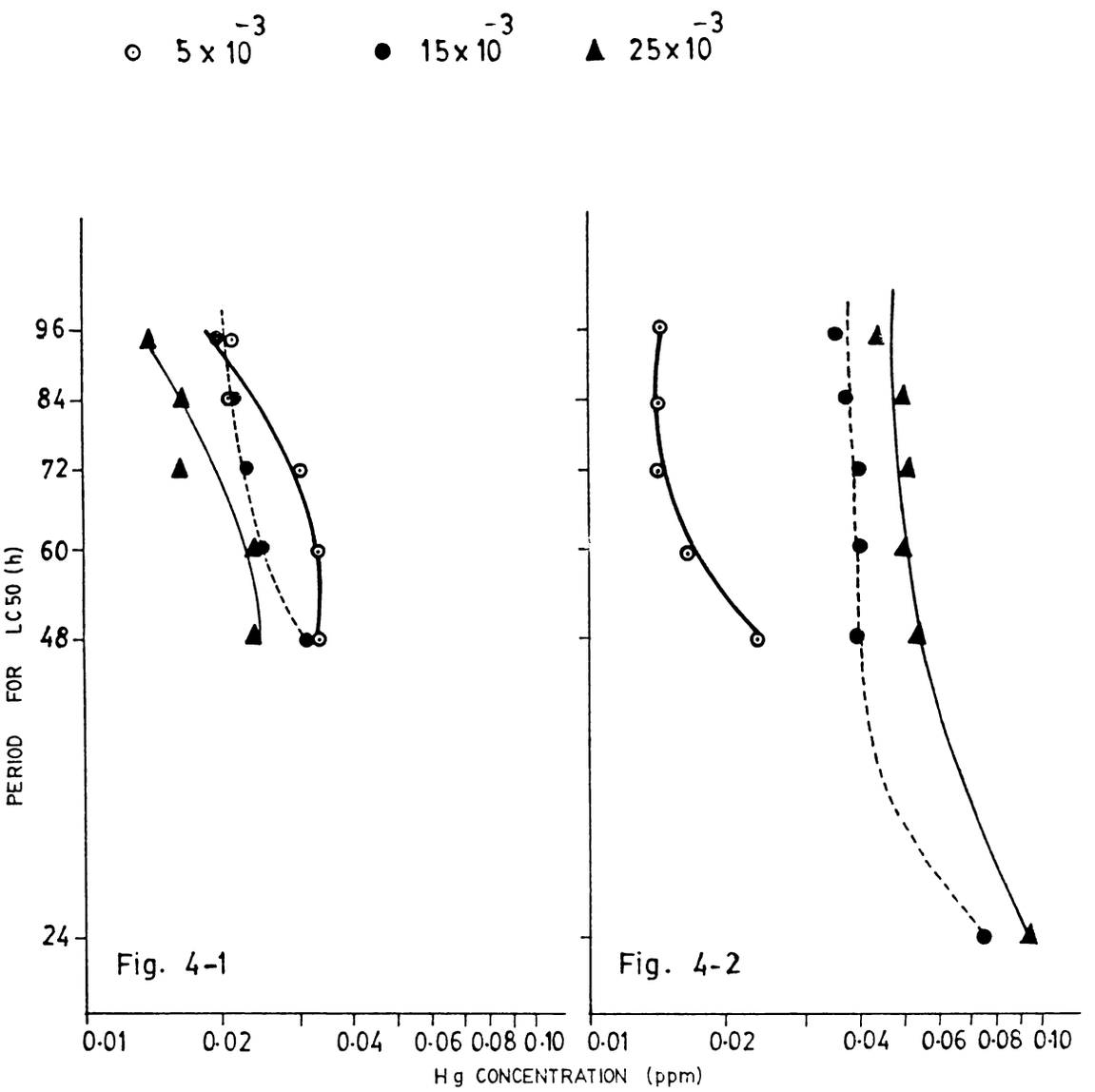


Fig. 4-1. Toxicity curves of Hg (II) to 30-50mm size group at 5,15 and 25 x 10^{-3} S.

Fig. 4-2. Toxicity curves of Hg (II) to 50-70mm size group at 5,15 and 25 x 10^{-3} S.

Table 4.2 Lethal concentrations of Hg (II) to M. dobsoni at $5x10^{-3}$ S

Time (h)	LC16 (mg l^{-1})	LC50 (mg l^{-1}) and 95% confidence limits	LC84 (mg l^{-1})	Slope and 95% confidence limits
		30-50 mm size group		
24	*			
48	0.011	0.033 (0.0190-0.0570)	0.096	2.9545 (1.6215-5.3749)
60	0.011	0.033 (0.0190-0.0570)	0.096	2.9545 (1.6215-5.3749)
72	0.010	0.030 (0.0187-0.0480)	0.086	2.9333 (1.6848-5.1068)
84	0.0078	0.021 (0.0128-0.0342)	0.054	2.6318 (1.6106-4.3003)
96	0.0078	0.021 (0.0128-0.0342)	0.054	2.6318 (1.6106-4.3003)
		50-70 mm size group		
24	*			
48	0.0115	0.024 (0.0146-0.0393)	0.048	2.0434 (1.0398-4.0152)
60	0.009	0.017 (0.0109-0.0263)	0.032	1.8855 (1.1026-3.2242)
72	0.007	0.0145 (0.0087-0.0239)	0.03	2.0701 (1.23-3.4839)
84	0.007	0.0145 (0.0087-0.0239)	0.03	2.0701 (1.23-3.4839)
96	0.007	0.0145 (0.0087-0.0239)	0.03	2.0701 (1.23-3.4839)

* No significant (< 65%) mortality

Table 4.3 Lethal concentrations of Hg(II) to M.dobsoni at $15x10^{-3}S$

Time (h)	LC16 (mg l^{-1})	LC50 (mg l^{-1}) and 95% confidence limits	LC84 (mg l^{-1})	Slope and 95% confidence limits
		30-50 mm size group		
24	*			
48	0.019	0.032 (0.0257-0.0399)	0.052	1.6546 (1.38-1.997)
60	0.015	0.0255 (0.0203-0.032)	0.0425	1.680 (1.363-2.071)
72	0.012	0.023 (0.0166-0.0317)	0.043	1.893 (1.32-2.715)
84	0.012	0.022 (0.0169-0.0284)	0.039	1.803 (1.388-2.343)
96	0.010	0.020 (0.0149-0.0267)	0.038	1.950 (1.40-2.718)
		50-70 mm size group		
24	0.064	0.074 (0.067-0.082)	0.086	1.1592 (1.124-1.196)
48	0.029	0.040 (0.0318-0.0502)	0.056	1.3896 (1.2553-1.538)
60	0.029	0.040 (0.0318-0.0502	0.056	1.3896 (1.2533-1.538)
72	0.029	0.040 (0.0318-0.0502)	0.056	1.3896 (1.2553-1.538)
84	0.026	0.038 (0.0291-0.0496)	0.056	1.4676 (1.326-1.625)
96	0.0215	0.035 (0.0251-0.0487)	0.056	1.614 (1.323-1.968)

* No significant (< 65%) mortality

Table 4.4 Lethal concentrations of Hg(II) to M.dobsoni at $25x10^{-3}$S

Time (h)	LC16 ($mg\ l^{-1}$)	LC50 ($mg\ l^{-1}$) and 95% confidence limits	LC84 ($mg\ l^{-1}$)	Slope and 95% confidence limits
		30-50 mm size group		
24	0.0150	0.032 (0.022-0.0465)	0.066	2.0979 (1.4410-3.0542)
48	0.0110	0.024 (0.0162-0.0355)	0.052	2.1740 (1.4932-3.1650)
60	0.0110	0.024 (0.0162-0.0355)	0.052	2.1740 (1.4932-3.1650)
72	0.0074	0.0165 (0.0101-0.0269)	0.036	2.2057 (1.2894-3.7731)
84	0.0070	0.015 (0.0093-0.0242)	0.033	2.1714 (1.2693-3.7143)
96	0.0066	0.014 (0.0087-0.0223)	0.03	2.1320 (1.3333-3.4089)
		50-70 mm size group		
24	0.060	0.094 (0.0751-0.1176)	0.150	1.5812 (1.3927-1.7951)
48	0.038	0.054 (0.0425-0.0686)	0.076	1.4140 (1.2676-1.5772)
60	0.036	0.051 (0.0368-0.0706)	0.070	1.3946 (1.1949-1.6276)
72	0.036	0.051 (0.0368-0.0706)	0.070	1.3946 (1.1949-1.6276)
84	0.035	0.049 (0.0354-0.0678)	0.068	1.3938 (1.1942-1.6266)
96	0.030	0.043 (0.0306-0.0604)	0.060	1.4143 (1.1543-1.7328)

Table 4.5 Lethal concentrations of Cu(II) to M. dobsoni at $5x10^{-3}$ S

Time (h)	LC16 (mg l^{-1})	LC50 (mg l^{-1}) and 95% confidence limits	LC84 (mg l^{-1})	Slope and 95% confidence limits
		30-50 mm size group		
24	0.185	0.56 (0.3674-0.8534)	1.60	2.93 (1.5685-5.4732)
48	0.205	0.40 (0.3007-0.5320)	0.76	1.92 (1.478-2.4940)
60	0.205	0.38 (0.2814-0.5130)	0.68	1.81 (1.4074-2.3276)
72	0.200	0.35 (0.2653-0.4616)	0.60	1.73 (1.2609-2.3735)
84	0.190	0.31 (0.2299-0.4178)	0.50	1.62 (1.1481-2.2858)
96	0.190	0.30 (0.2277-0.3951)	0.46	1.56 (1.1598-2.0982)
		50-70 mm size group		
24	0.21	0.50 (0.3263-0.766)	1.20	2.3904 (1.4186-4.0278)
48	0.18	0.42 (0.2937-0.6006)	0.92	2.2619 (1.4861-3.4426)
60	0.18	0.42 (0.2937-0.6006)	0.92	2.2619 (1.4861-3.4426)
72	0.16	0.32 (0.2163-0.4732)	0.64	2.000 (1.3368-2.992)
84	0.13	0.26 (0.1609-0.4199)	0.52	2.000 (1.2202-3.278)
96	0.115	0.23 (0.1424-0.3714)	0.46	2.000 (1.2202-3.278)

and higher size group respectively at the end of 96 h. No blue precipitate of copper compounds was observed in any of the concentrations tested.

Table 4.6 provides the toxicity values and slopes of Cu(II) for the bioassay conducted at $15x10^{-3}S$. Mortality was 40% in 0.25 mg l^{-1} for lower size group whereas no mortality was observed in 0.3 mg l^{-1} for the higher size group at the end of 96 h. Percentage mortality was 90 in 5.0 mg l^{-1} and 62.5 in 3.3 mg l^{-1} for the lower and higher size group respectively. A visible blue precipitate of copper presumably, carbonate and hydroxide, was formed in concentrations above 2.0 mg l^{-1} which settled at the bottom of the tub.

Results of bioassays of Cu(II) conducted at $25x10^{-3}S$ are given in Table 4.7. By 96 h, 90% mortality was observed in 6.0 mg l^{-1} for lower size group and 62.5% in 7.0 mg l^{-1} for the higher size group. Large quantities of blue precipitate was formed in concentrations of 3.0 mg l^{-1} and above.

Mucus secretion was observed in all concentrations of Cu(II). Prawns exposed to Cu(II) developed black spots on gills by 96 h. The blackened lesions were absent in control.

Toxicity curves for the two size groups at $25x10^{-3}S$ (Figs. 4.3 and 4.4b) and for lower size group at $5x10^{-3}S$ (Fig. 4.3) are represented by straight lines. For higher size group at 5 and $15x10^{-3}S$ toxicity curves are represented by convex curve (Fig. 4.4a) and nearly straight convex curve (Fig. 4.4b) respectively. At $15x10^{-3}S$ the lower size group show a nearly straight concave curve (Fig. 4.3).

4.2.3 Zinc

Median lethal concentrations and slopes with 95% confidence limits for the two size groups of *M. dobsoni* at 5, 15 and $25x10^{-3}S$ are presented in Tables 4.8, 4.9 and 4.10 respectively. Above 1.5 mg l^{-1} secretion of the mucus was noticed in all the experimental tubs. Changes with time in LC50 values are represented by Figs. 4.5 and 4.6. Lower size group at 5 and $15x10^{-3}$ and higher size group at $25x10^{-3}S$ are represented by convex curves and others showed concavity for the toxic curves.

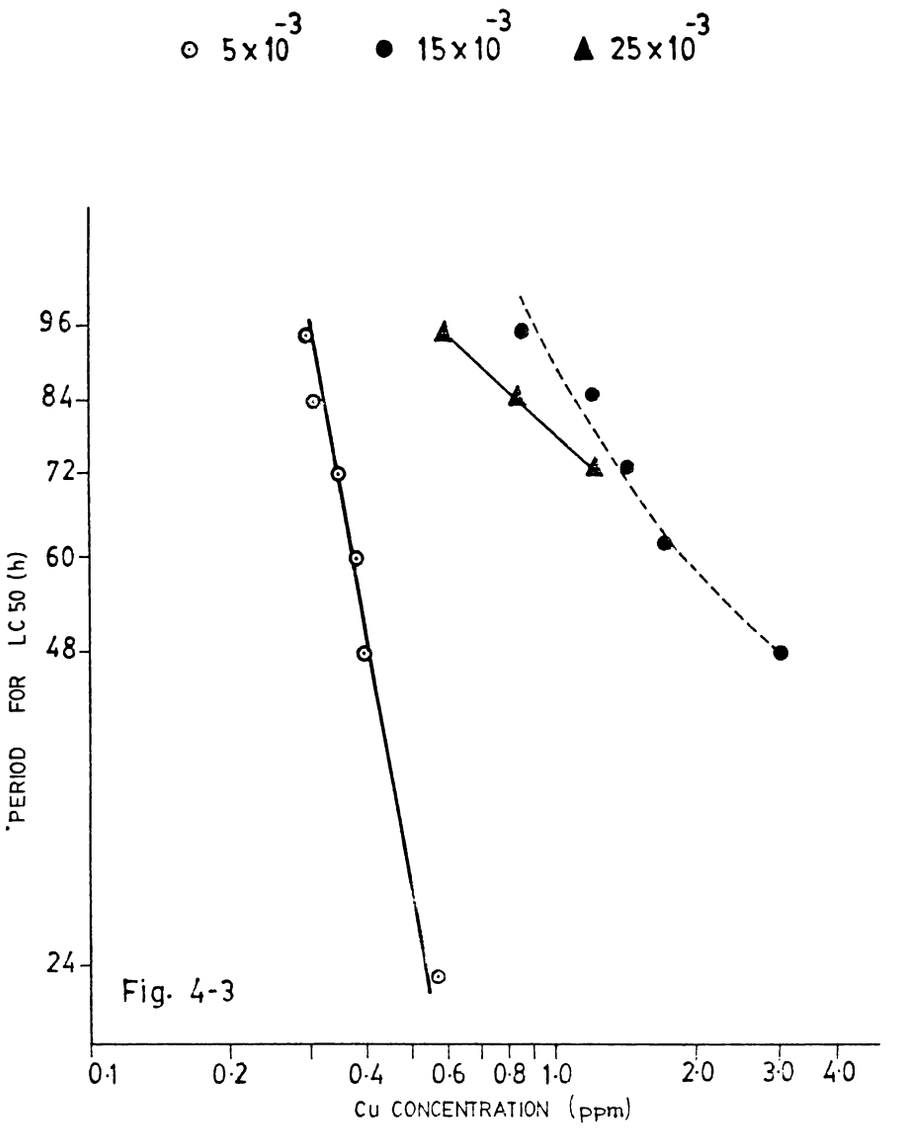


Fig. 4-3. Toxicity curves of Cu(II) to 30-50mm size group at 5,15 and 25×10^{-3} S.

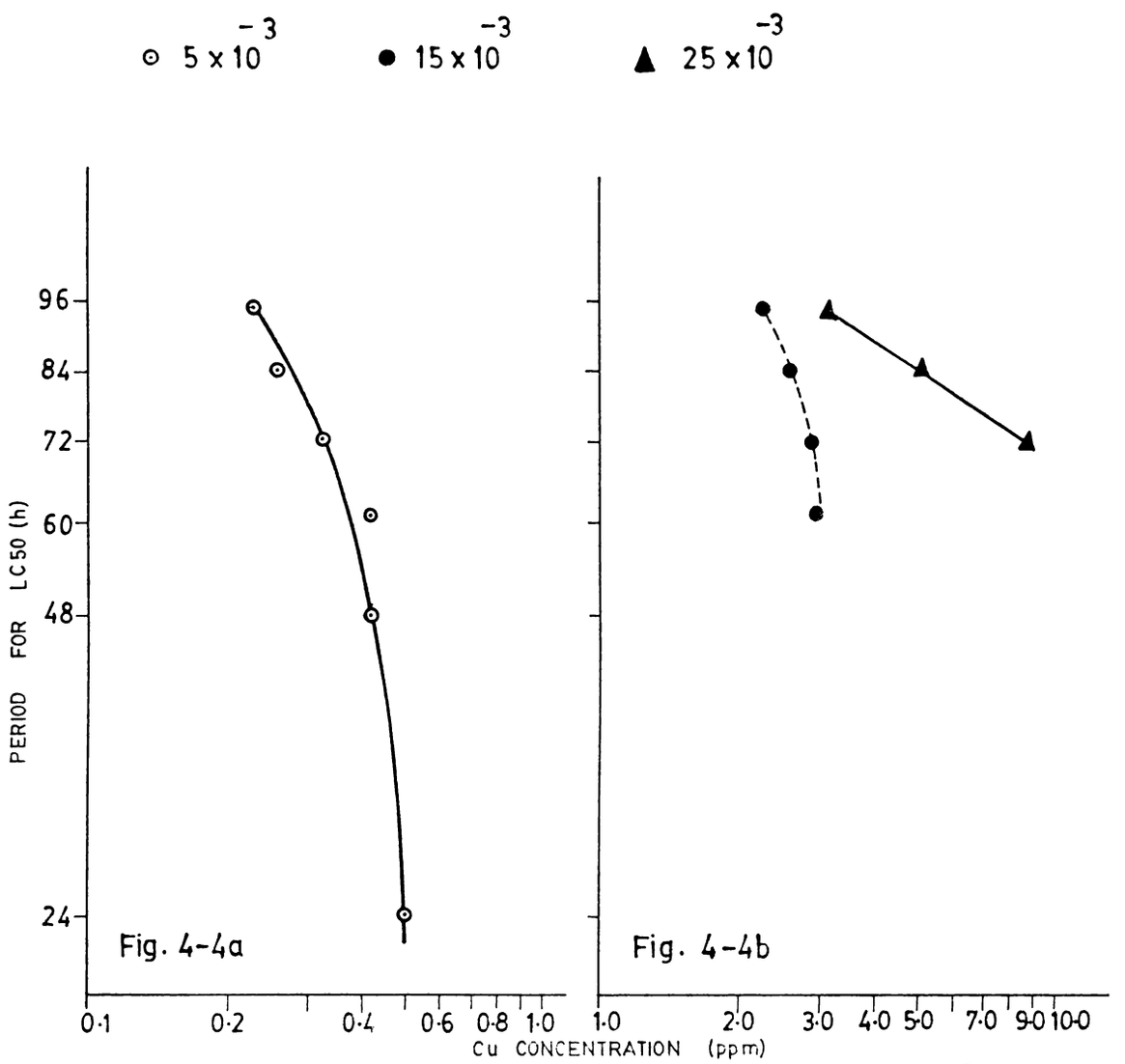


Fig. 4-4a. Toxicity curve of Cu (II) to 50-70mm size group at 5×10^{-3} S.

Fig. 4-4b. Toxicity curves of Cu (II) to 50-70mm size group at 15 and 25×10^{-3} S.

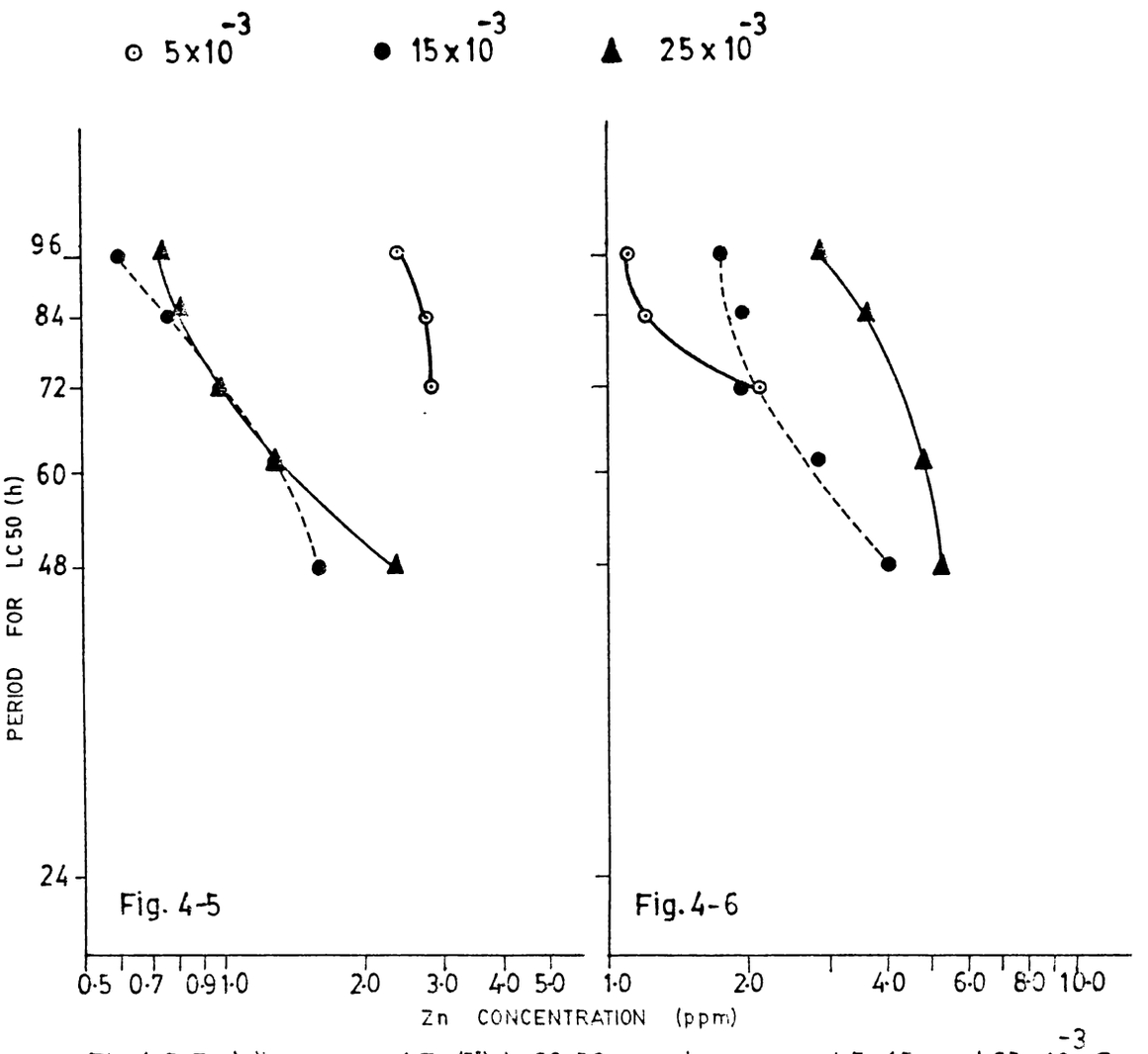


Fig.4-5. Toxicity curves of Zn(II) to 30-50mm size group at 5, 15 and 25×10^{-3} S.

Fig.4-6. Toxicity curves of Zn(II) to 50-70mm size group at 5, 15 and 25×10^{-3} S.

Table 4.6 Lethal concentrations of Cu(II) to M. dobsoni at 15×10^{-3} S

Time (h)	LC16 (mg l^{-1})	LC50(mg l^{-1}) and 95% confidence limits	LC84 (mg l^{-1})	Slope and 95% confidence limits
		30-50 mm size group		
24	*			
48	0.78	3.00 (1.8206-4.9434)	10.0	3.589 (1.795-7.175)
60	0.50	1.70 (1.0591-2.7287)	5.6	3.347 (1.850-6.040)
72	0.42	1.40 (1.6802-2.3522)	4.5	3.270 (1.760-6.076)
84	0.36	1.20 (0.7083-2.0332)	4.0	3.333 (1.721-6.443)
96	0.23	0.84 (0.4856-1.4527)	2.8	3.493 (1.670-7.304)
		50-70 mm size group		
24	*			
48	*			
60	1.05	3.00 (1.8250-4.9314)	8.0	2.760 (1.459-5.220)
72	1.00	2.85 (1.8380-4.4175)	7.4	2.720 (1.501-4.929)
84	0.88	2.55 (1.6390-3.9650)	6.6	2.740 (1.449-5.182)
96	0.78	2.25 (1.4389-3.5189)	6.0	2.775 (1.467-5.249)

* No significant (< 65%) mortality

Table 4.7 Lethal concentrations of Cu (II) to M. dobsoni at 25×10^{-3} S

Time (h)	LC16 (mg l^{-1})	LC50 (mg l^{-1}) and 95% confidence limits	LC84 (mg l^{-1})	Slope and 95% confidence limits
		30-50 mm size group		
24	*			
48	*			
60	*			
72	0.38	1.20 (0.5980-2.4082)	3.6	3.0789 (1.7417-5.4425)
84	0.28	0.82 (0.4215-1.5951)	2.4	2.9277 (1.7115-5.0081)
96	0.19	0.58 (0.3332-1.0095)	1.7	2.9918 (1.8429-4.3568)
		50-70 mm size group		
24	*			
48	*			
60	*			
72	1.40	8.6 (2.4930-29.6674)	50.0	5.9784 (1.1809-30.2656)
84	0.96	5.0 (1.9676-12.7055)	26.0	5.2042 (1.5494-17.4797)
96	0.66	3.1 (1.3071-7.3523)	14.0	4.6065 (2.1825-9.7224)

* No significant ($<$ 65%) mortality

Table 4.8 Lethal concentrations of Zn (II) to M. dobsoni at 5 x 10^{-3} S

Time (h)	LC16 (mg l^{-1})	LC50 (mg l^{-1}) and 95% confidence limits	LC84 (mg l^{-1})	Slope and 95% confidence limits
		30-50 mm size group		
24	*			
48	*			
60	*			
72	0.72	2.80 (1.5864-4.942)	9.6	3.66 (1.5237-8.7913)
84	0.72	2.80 (1.5864-4.942)	9.6	3.66 (1.5237-8.7913)
96	0.64	2.40 (1.3793-4.176)	8.0	3.54 (1.4205-8.8216)
		50-70 mm size group		
24	*			
48	*			
60	*			
72	0.70	2.10 (1.1335-3.8907)	6.2	2.9761 (1.2102-7.3190)
84	0.50	1.20 (0.7230-1.9917)	3.0	2.450 (1.3495-4.4481)
96	0.46	1.10 (0.6120-1.9772)	2.5	2.332 (1.2554-4.3319)

* No significant (< 65%) mortality

Table 4.9 Lethal concentrations of Zn (II) to M. dobsoni at 15×10^{-3} S

Time (h)	LC16 (mg l^{-1})	LC50 (mg l^{-1}) and 95% confidence limits	LC84 (mg l^{-1})	Slope and 95% confidence limits
		30-50 mm size group		
24	*			
48	0.66	1.60 (1.0277-2.4910)	3.80	2.3996 (1.537-3.746)
60	0.57	1.30 (0.9036-1.8703)	3.00	2.2942 (1.624-3.241)
72	0.41	1.00 (0.6331-1.5796)	2.50	2.4695 (1.582-3.855)
84	0.36	0.76 (0.4584-1.260)	1.55	2.0753 (1.371-3.140)
96	0.29	0.60 (0.4103-0.8769)	1.30	2.1178 (1.527-2.937)
		50-70 mm size group		
24	*			
48	2.10	4.00 (2.586-6.1872)	7.4	1.8774 (1.493-2.360)
60	1.40	2.80 (1.7544-4.4688)	5.4	1.9642 (1.440-2.679)
72	0.86	1.95 (1.3148-2.892)	4.3	2.2363 (1.591-3.143)
84	0.86	1.95 (1.3148-2.892)	4.3	2.2363 (1.591-3.143)
96	0.78	1.77 (1.1689-2.4723)	3.6	2.1486 (1.565-2.950)

* No significant (< 65%) mortality

Table 4.10 Lethal concentrations of Zn (II) to M. dobsoni at $25x10^{-3}$ S

Time (h)	LC16 (mg l^{-1})	LC50 (mg l^{-1}) and 95% confidence limits	LC84 (mg l^{-1})	Slope and 95% confidence limits
		30-50 mm size group		
24	*			
48	0.80	2.40 (1.6367-3.5193)	6.8	2.9167 (1.8202-4.6736)
60	0.27	1.30 (0.7510-2.2503)	5.8	4.6382 (1.9859-10.8327)
72	0.23	0.98 (0.5628-1.7065)	3.9	4.1202 (1.7186-9.8773)
84	0.23	0.82 (0.5025-1.3380)	2.8	3.4899 (1.7457-6.9767)
96	0.22	0.76 (0.4502-1.2831)	2.4	3.3062 (1.7798-6.1415)
		50-70 mm size group		
24	*			
48	2.50	5.2 (3.1636-8.5472)	10.5	2.0496 (1.2127-3.4638)
60	2.30	4.8 (2.9057-7.9291)	9.8	2.0643 (0.8658-4.9216)
72	1.80	3.6 (2.5647-5.0893)	7.4	2.0278 (1.0969-3.7483)
84	1.80	3.6 (1.5647-5.0893)	7.4	2.0278 (1.0969-3.7483)
96	1.45	2.8 (2.0386-3.8458)	5.3	1.9119 (1.1059-3.3050)

* No significant (< 65%) mortality

4.2.4 Effect of Size Group on Toxicity

At $5x10^{-3}$S 30-50 mm size group showed more resistance to Hg(II), Cu(II) and Zn(II), except the slight discrepancy at 48 h and 60 h LC50 values of Cu(II) (Fig. 4.7). At the same time 30-50 mm size group was more sensitive to all the three metals tested at 15 and $25x10^{-3}$S (Fig. 4.8 and 4.9).

4.2.5 Effect of Salinity on the Toxicity

The toxicity of Hg(II) was seen to be increasing with increase in salinity for the 30-50 mm size group (Fig. 4.10). For the higher size group the result was exactly the reverse showing more sensitivity to Hg(II) at lower salinities (Fig. 4.11).

Cu(II) was more toxic to both size groups of *M. dobsoni* at $5x10^{-3}$S (Figs. 4.12 and 4.13). For 30-50 mm size group the order of toxicity was $25x10^{-3} > 15x10^{-3}$S (Fig. 4.12). In 50-70 mm size group sensitivity to Cu(II) was more at $15x10^{-3}$ than at $25x10^{-3}$S (Fig. 4.13).

The order of Zn(II) toxicity to 50-70 mm size group of *M. dobsoni* at different salinities was $5x10^{-3} > 15x10^{-3} > 25x10^{-3}$ (Fig. 4.15). Lower size group showed maximum tolerance in $5x10^{-3}$S and there was not much difference in toxicity at $15x10^{-3}$ and $25x10^{-3}$S (Fig. 4.14).

4.2.6 Estimation of Relative Potency and Order of Toxicity

The potency ratios and their 95% confidence limits of Hg(II), Cu(II) and Zn(II) examined in pairs for the two size groups of *M. dobsoni* at 5, 15 and $25x10^{-3}$S were calculated by the method of Litchfield and Wilcoxon (1949) and given in Tables 4.11, 4.12 and 4.13 respectively.

Hg(II) was 16 times more toxic than Cu(II) and 75 times than Zn(II) and Cu(II) was 5 times toxic than Zn(II) to the higher size group at $5x10^{-3}$S. For the lower size group at the same salinity Hg(II) was 14 times toxic than Cu(II) and 114 times toxic than Zn(II) and Cu(II) was 8 times toxic than Zn(II) (Table 4.11). Hence the order of toxicity for both the size group is Hg >> Cu > Zn and the elements differ significantly in their potency.

In $15x10^{-3}$S Hg(II) was 64 times toxic than Cu(II), 49 times toxic than Zn(II) and Zn(II) 1.3 times toxic than Cu(II) for the higher size group and Hg(II) 42 times toxic than Cu(II), 30 times toxic than Zn(II) and Zn(II) 1.4 times toxic than Cu(II) for the lower size group (Table 4.12). Here Cu(II) and Zn(II) do not differ significantly in potency. The order of toxicity may be taken as Hg >>Zn = Cu.

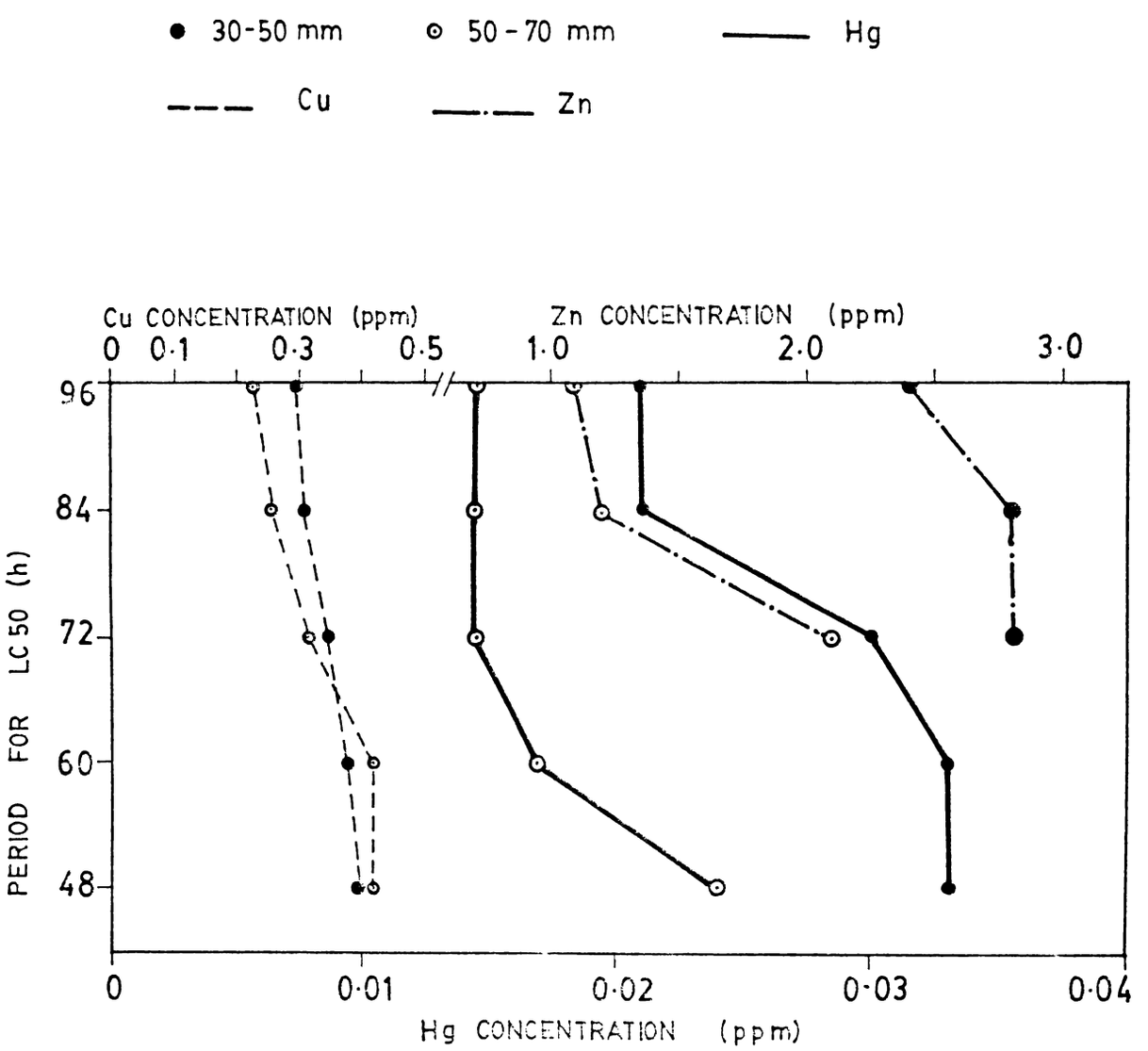


Fig. 4-7 · Effect of size group on toxicity of Hg(II), Cu(II) and Zn(II) at 5×10^{-3} S.

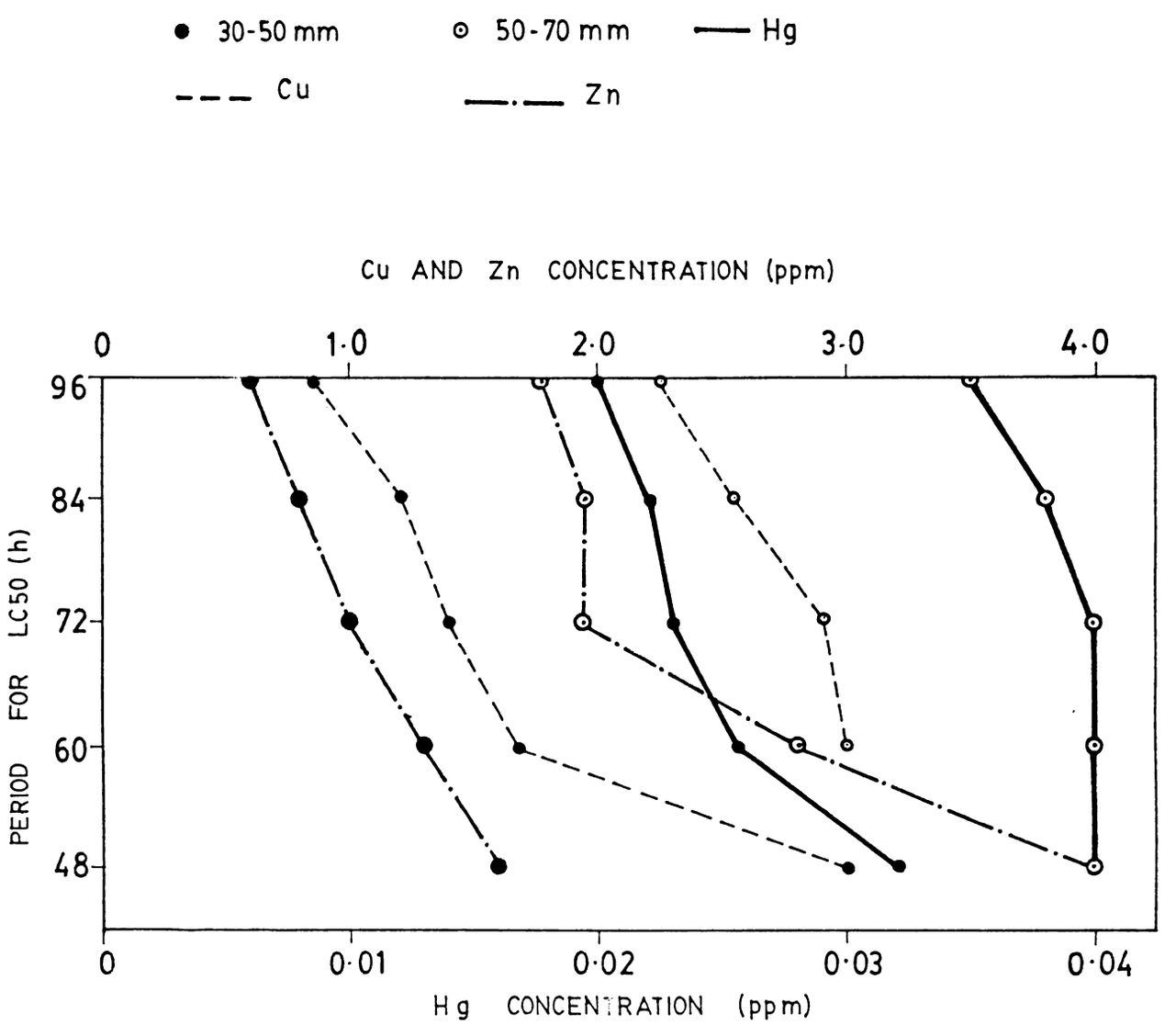


Fig. 4-8. Effect of size group on toxicity of Hg(II), Cu(II) and Zn(II) at 15×10^{-3} S.

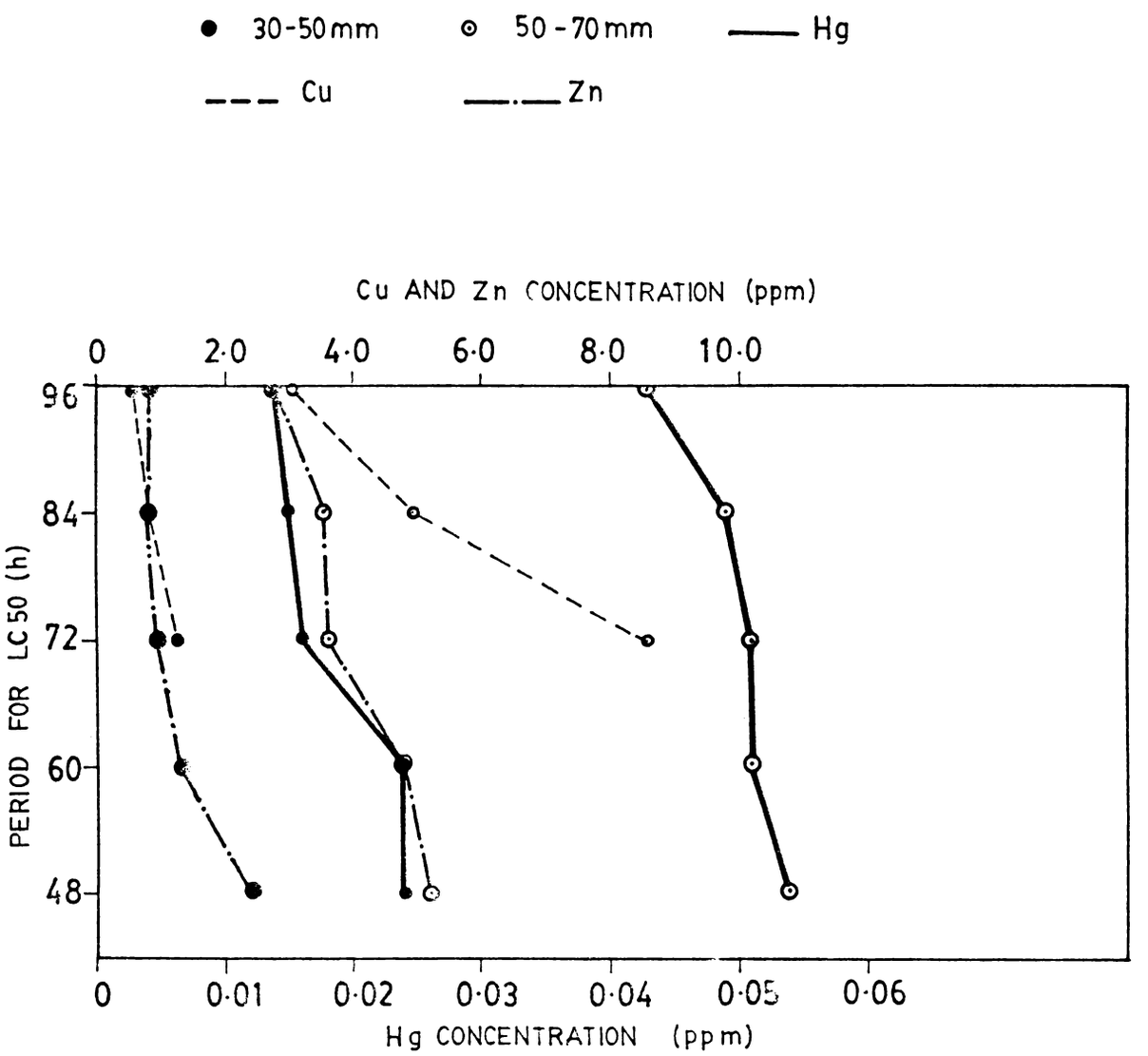


Fig. 4-9. Effect of size group on toxicity of Hg (II), Cu (II) and Zn (II) at 25×10^{-3} S.

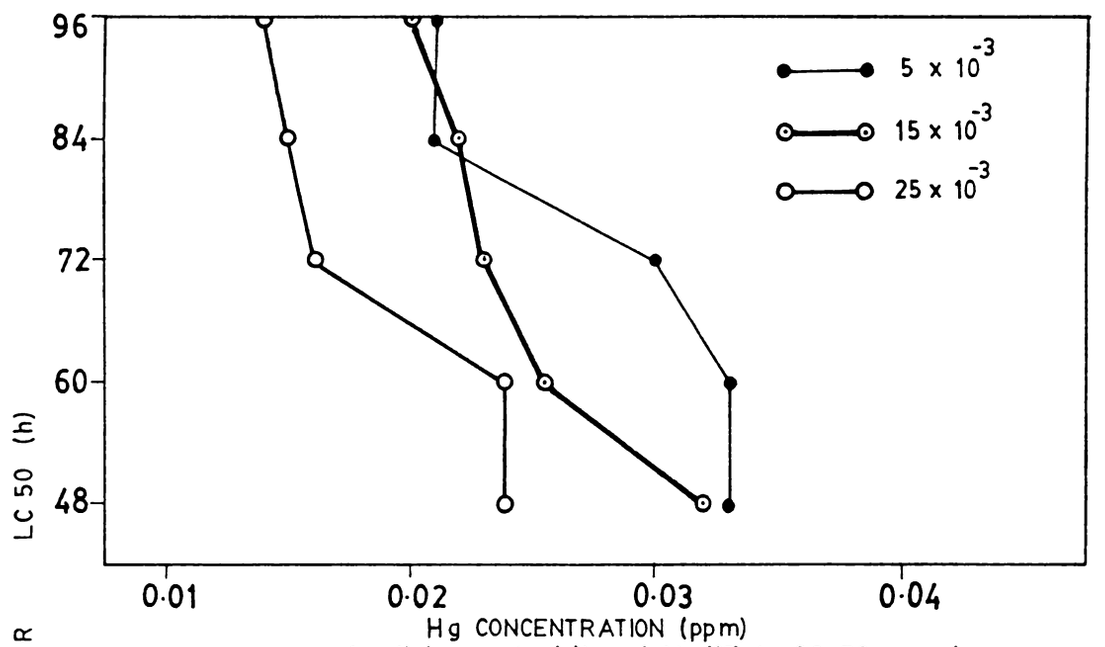


Fig. 4-10. Effect of salinity on toxicity of Hg (II) to 30-50 mm size group.

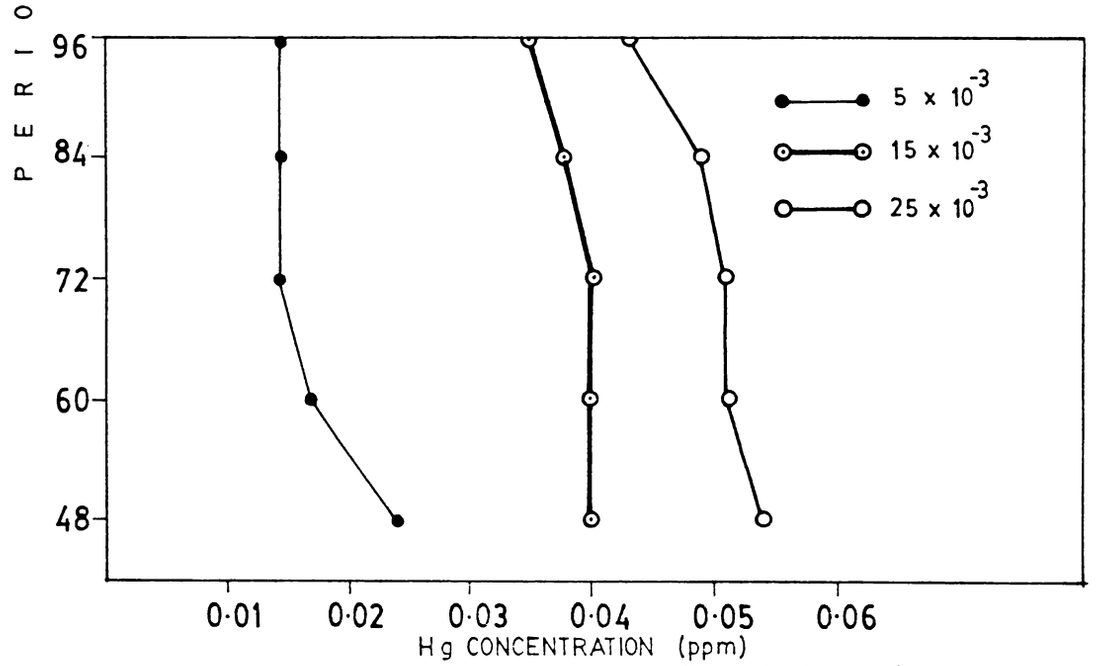


Fig. 4-11. Effect of salinity on toxicity of Hg (II) to 50-70 mm size group.

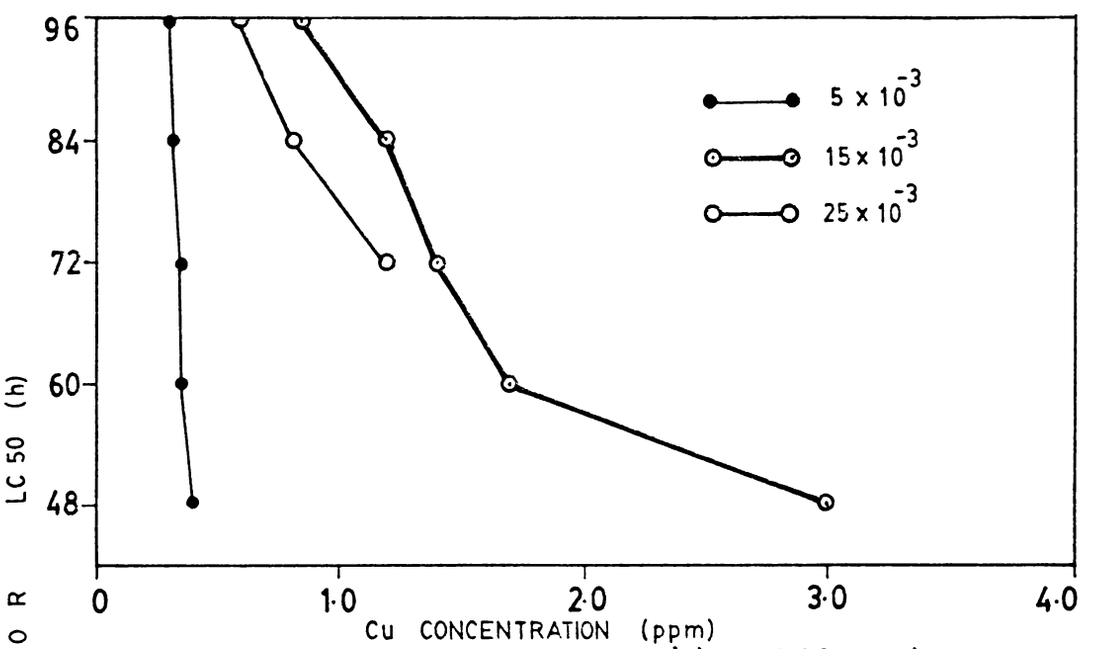


Fig.4-12.Effect of salinity on toxicity of Cu(II) to 30-50mm size group.

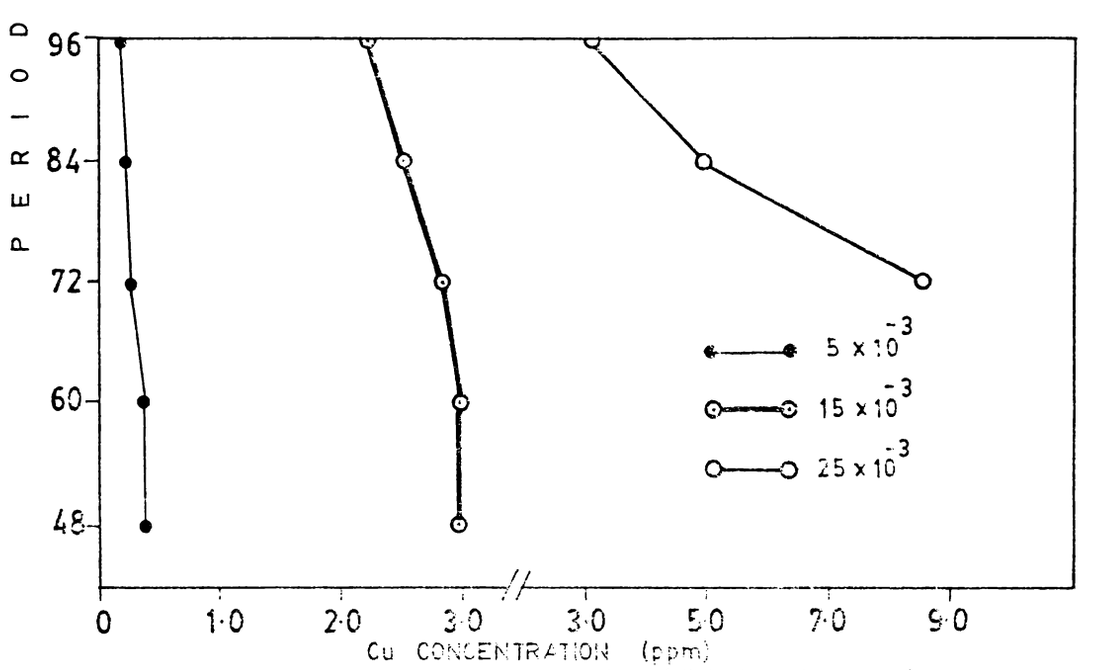


Fig. 4-13.Effect of salinity on toxicity of Cu(II) to 50-70mm size group.

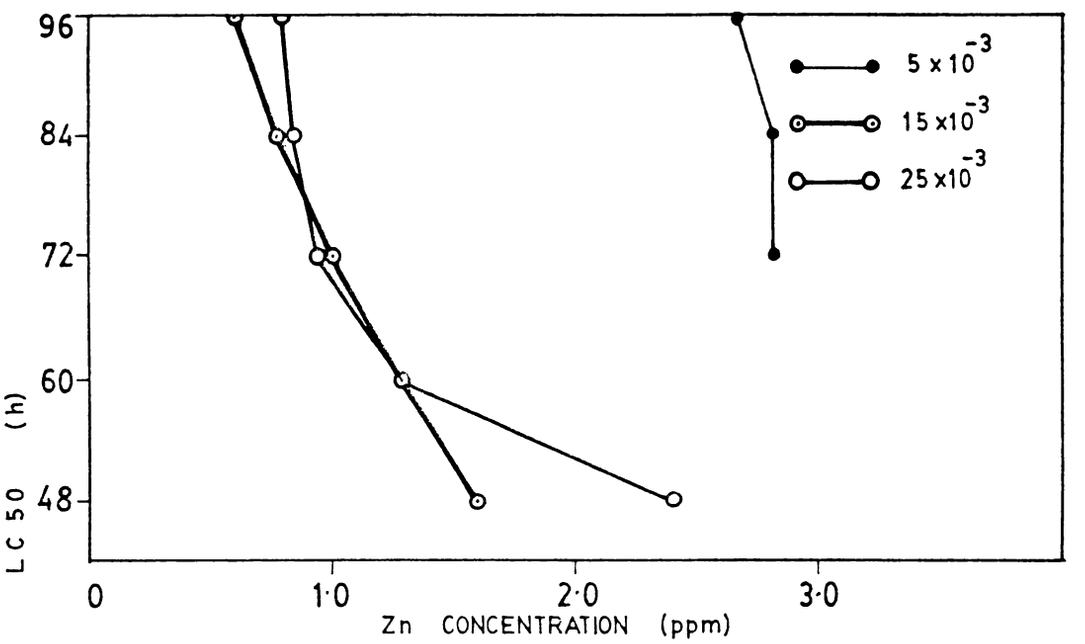


Fig. 4-14. Effect of salinity on toxicity of Zn(II) to 30-50mm size group.

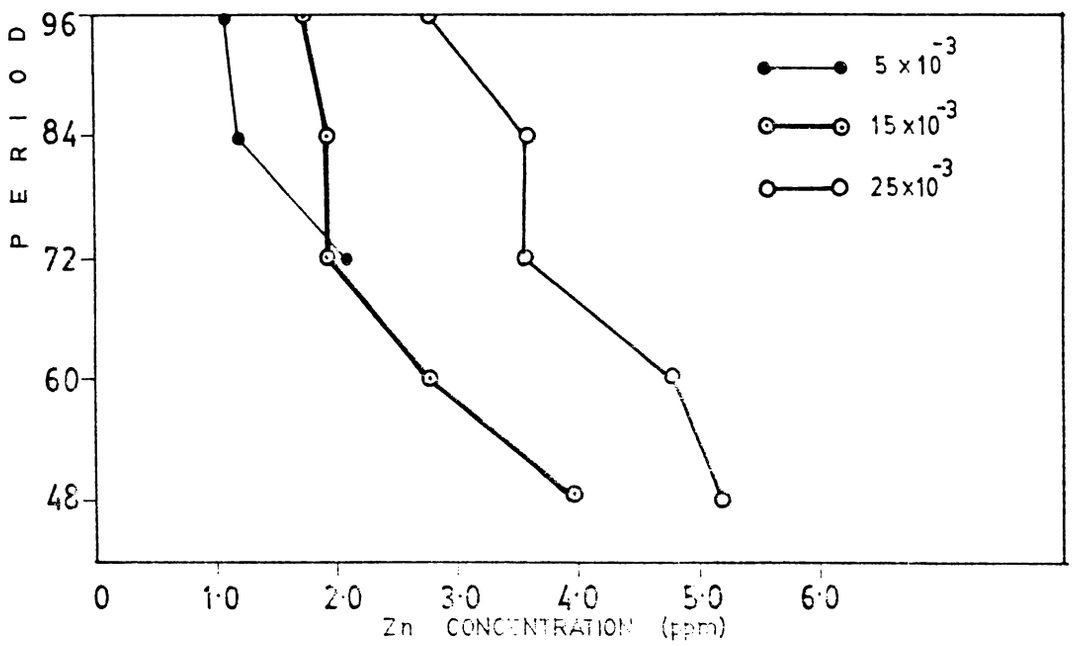


Fig. 4-15 Effect of salinity on toxicity of Zn (II) to 50-70mm size group.

Table 4.11 Potency ratios of metals in pairs at $5x10^{-3}S$

Metals in pairs	Size Group (mm)	Potency ratio	95% confidence limits	
			Lower	Upper
Cu/Hg	50-70	15.862	7.931	31.724
Cu/Hg	30-50	14.286	8.163	25.001
Zn/Hg	50-70	75.862	35.285	163.103
Zn/Hg	30-50	114.286	54.422	240.001
Zn/Cu	50-70	4.782	2.277	10.042
Zn/Cu	30-50	8.00	4.32	14.80

Table 4.12 Potency ratios of metals in pairs at $15x10^{-3}S$

Metals in pairs	Size group (mm)	Potency ratio	95% confidence limits	
			Lower	Upper
Cu/Hg	50-70	64.29	36.735	112.50
Cu/Hg	30-50	42.00	22.105	79.80
Zn/Hg	50-70	48.57	29.437	80.142
Zn/Hg	30-50	30.00	18.630	48.300
Cu/Zn	50-70	1.32	0.744	2.357
Cu/Zn	30-50	1.40	0.718	2.730

Table 4.13 Potency ratios of metals in pairs at 25×10^{-3} S

Metals in pairs	Size group (mm)	Potency ratio	95% confidence limits	
			Lower	Upper
Cu/Hg	50-70	72.093	28.837	180.232
Cu/Hg	30-50	41.428	20.208	84.927
Zn/Hg	50-70	65.116	41.475	102.232
Zn/Hg	30-50	54.285	27.007	109.113
Cu/Zn	50-70	1.107	0.443	2.768
Zn/Cu	30-50	1.310	0.609	2.817

Hg(II) was found to be 72 times toxic than Cu(II), 65 times toxic than Zn(II) for the higher size group and 41 times toxic than Cu(II), 54 times toxic than Zn(II) for the lower size group at $25x10^{-3}$S (Table 4.13). Cu(II) and Zn(II) do not differ significantly in potency for the two size groups under study. The order of toxicity is found to be Hg $>>$ Zn = Cu.

4.2.7 Estimation of ET50

Time course in cumulative mortality for all the concentrations tested for Hg(II), Cu(II) and Zn(II) at three salinities for the two size groups were plotted and ET50 (effective time for 50% mortality) values determined inall the cases are presented in Table 4.14.

4.3 DISCUSSION

Median lethal concentration in mg l^{-1} of Hg(II) for 96 h for 30-50 mm and 50-70 mm size group varied from 0.014 to 0.021 for the former and 0.0145 to 0.043 for the latter as the salinities varied from 5 to $25x10^{-3}$ (Tables 4.2 to 4.4). These values are in good agreement with the values reported for some other marine crustaceans. Green *et al.* (1976) obtained the 96 h LC50 value as 0.017 mg l^{-1} for the post larval white shrimp *Penaeus setiferus*. In addition he found that there was no significant difference in 96 h LC50 values for the two size groups (7-13 mm and 15-25 mm) of this species. Grass shrimp *Palaemonetes pugio* behaved slightly differently to two forms of Hg(II) as mercuric acetate and mercuric thiocyanate with 96 h LC50 values 0.06 and 0.09 mg l^{-1} (Curtis *et al.*, 1979). 48 h LC50 was 5.7 and 0.01 mg l^{-1} for the adult and larvae of *Crangon crangon* (Portmann, 1968; Connor, 1972). Shealy and Sandifer, (1975) obtained the 48 h LC50 as 0.0156 mg l^{-1} for the larvae of *Palaemonetes vulgaris* and 48 h LC50 values of adult *Carcinus maenas* and *Pandalus montagui* has been reported as 1.0 mg l^{-1} by Portmann (1968).

The range of 96 h LC50 values of Cu(II) for the lower and higher size groups of *M. dobsoni* were 0.3 to 0.84 and 0.23 to 3.1 mg l^{-1} in that order (Tables 4.5 to 4.7). The 48 h LC50 values of Cu(II) reported earlier for marine crustaceans in mg l^{-1} are 0.6 for the larvae (Connor, 1972) and 109.0 for the adult *Carcinus maenas*; 0.2 for the adult *Pandalus montagui* ; 30.0 for the adult *Crangon crangon* (Portmann, 1968); 0.33 and 0.01 for the larval stages of *Crangon crangon* and *Homarus gammarus* (Connor, 1972). 96 h LC50 values in mg l^{-1} ranged from

Table 4.14 ET50 values of M. dobsoni at 5, 15 and 25x10^{-3} S for various concentrations Hg(II), Cu(II) and Zn(II)

Size Group (mm)	Hg(II)		Cu(II)		Zn(II)	
	Con. mg l^{-1}	ET50 (h)	Con. mg l^{-1}	ET50 (h)	Con. mg l^{-1}	ET50 (h)
			5x10^{-3}S			
30-50	0.02	103	0.4	70	2.0	111
	0.04	53	0.6	24	3.0	76
	0.06	42	0.8	22	-	-
50-70	0.02	60	0.2	110	1.0	91
	0.04	43	0.4	53	3.0	60
			15x10^{-3}S			
30-50	0.02	83	1.0	93	1.0	71
	0.03	58	2.0	50	2.0	45
	0.04	38	3.0	43	3.0	33
	0.06	32	5.0	30	-	-
50-70	0.03	133	2.1	90	2.0	106
	0.05	47	2.7	73	3.0	60
	0.07	37	3.3	53	5.0	40
			25x10^{-3} S			
30-50	0.01	130	0.5	96	1.0	78
	0.03	42	1.0	73	2.0	46
	0.05	30	6.0	66	3.0	35
	-	-	-	-	4.0	30
50-70	0.05	67	3.0	146	4.0	93
	0.08	43	5.0	56	5.0	65
	0.11	23	7.0	41	7.0	40

0.048 for the larval stage-I of *Homarus americanus* (Johnson and Gentile, 1979); 0.11 for the juvenile and 0.5 for the adult *Allorchestes compressa* (Ahsanullah and Florence, 1984) and 0.17 for the larvae of *Paragrapsus quadridentatus* (Ahsanullah and Arnott, 1978). Mary Carmel *et al.* (1983) reported the 120 h LC50 of Cu(II) in mg l^{-1} for three size groups (15-25 mm, 25-45 mm and 45-65 mm) of *Penaeus indicus* as 0.41, 0.37 and 0.3 at salinity $15x10^{-3}$ and 0.47, 0.5 and 0.55 at salinity $25x10^{-3}$. Except for the values reported by Portmann (1968) for *Carcinus maenas* and *Crangon crangon*, *M. dobsoni* was found to be relatively less sensitive to Cu(II) especially for maturing stages at higher salinities.

LC50 in mg l^{-1} for 96 h for the juvenile and maturing stages of *M. dobsoni* were 0.6 to 2.4 and 1.1 to 2.8 at the salinities tested (Tables 4.8 to 4.10). Portmann (1968) reported the 48 h LC50 in mg l^{-1} for the adults *Carcinus maenas*, *Crangon crangon* and *Pandalus montagui* as 14.5, 100.0 and 10.0 respectively and larvae of *Carcinus maenas* as 1.0 (Connor, 1972). 96 h LC50 in mg l^{-1} for *Allorchestes compressa*, *Palaemon* sp. and adult *Paragrapsus quadridentatus* were 0.58, 11.3 and 11.0 (Ahsanullah, 1976); and *Pagurus longicarpus* 0.4 (Eisler and Hennekey, 1977); adult *Callianasa australiensis* 10.2 (Ahsanullah *et al.*, 1981) and the larvae of *Paragrapsus quadridentatus* 1.23 (Ahsanullah and Arnott, 1978). The present study revealed that *M. dobsoni* is more sensitive to Zn(II) than the observations made earlier.

Toxicity curve gives an overall picture of toxicity test progress and indicate when acute toxicity has ceased. The concavity of the curve means that toxicity during the early hours of exposure is larger than that from later hours. The convexity means the reverse. The curves with steeper slopes show an acutely acting toxicity and curves with shallower slopes means a slowly acting one. The cessation of toxicity is indicated by the curve becoming asymptotic to the time axis (Sprague, 1970).

Toxicity curves of Hg(II) are concave and for maturing stage the curve becomes asymptotic by 96 h. For Zn(II) the maturing stage shows asymptotic trend at 5 and $15x10^{-3}$S. Cu(II) at lower salinities show convex curves for maturing stages and straight line with higher slopes for juveniles at $5x10^{-3}$S and straight line with low slopes for both size groups at $25x10^{-3}$S. In general it can be concluded that 96 h exposure of maturing stages of *M. dobsoni* would be sufficient to arrive at incipient LC50 and larger duration may be required for juveniles. Sprague (1970) recommended an exposure time of 96-168 h for determining asymptotic LC50 for

most macro invertebrates and fish.

Body size is a critical factor influencing the effective dose of toxins. This is applicable both for different size ranges of the same organism and in general for unrelated organisms from the same habitat. The observed high sensitivity of juveniles to Hg(II), Cu(II) and Zn(II) at high salinity and for maturing stages at low salinity have much bearing to the behaviour of *M. dobsoni*.

In the life cycle, large population of the young specimen enter the backwaters before they reach a length of about 7 mm (Menon, 1955a). Kemp (1915) recorded that specimens from Chilka Lake examined by him did not exceed 75 mm in length. The migration back to sea takes place after this size is attained. Gunter *et al.* (1964) pointed out that the adaptation to low salinity is highly developed in younger stages of *Penaeus indicus* and Panicker (1968) indicated that osmoregulation in dilute media is less effective in larger individuals. Thus juveniles are much adapted to lower salinities and the maturing stages prefers high salinities. At this point it is worthwhile to note that Cochin backwaters which provide the habitat for the test animals for the present studies is subjected to a salinity regime varying from the nearby marine conditions during summer to near freshwater conditions during monsoon. The juveniles being more adapted to low salinities, under stressful conditions of high salinity become more susceptible to the toxic action of the metals. Similarly the maturing stages adapted to high salinities become more sensitive to the metals when exposed at low salinity. Mary Carmel *et al.* (1983) while studying the toxic effect of Cu(II) on juvenile *Penaeus indicus* encountered with similar observations, i.e., lower size groups were less sensitive to Cu(II) at 15 and 20×10^{-3}S whereas the trend was reversed at 25 and 30×10^{-3}S.

McKenney and Neff (1979) have observed that the resistance adaptation of developing larvae of *Palaemonetes pugio* to salinity and temperature was progressively less with exposure to increasing zinc levels, indicating that larvae were more resistant to zinc toxicity under optional conditions of salinity and temperature. Adult crab *Uca pugilator* can tolerate relatively high concentrations of Hg and Cd for long periods of time when temperature and salinity are optimal, but under stressful temperature-salinity regimes, survival time is considerably shortened (Vernberg and Vernberg, 1972b). Authors like Brown and Ahsanullah (1971), Connor (1972), DeCoursey and Vernberg (1972) and Ahsanullah

and Arnott (1978) have pointed out that larvae were found to be more sensitive to heavy metals than the adults in all cases where comparative marine crustacean data are available.

Toxicity of Hg(II) in relation to salinity revealed the pronounced adaptability of different size groups of *M. dobsoni* to adapt to optimal salinity. Copper showed maximum toxicity to both the size groups at salinity $5x10^{-3}$. Cu(II) toxicity at higher salinities and Zn(II) toxicity for both size groups at three salinities were reflective of their preferential adaptation to optimum salinity conditions.

The interaction of salinity and/or temperature to reduce the resistance of estuarine crustaceans to heavy metal toxicity have been thoroughly discussed by Vernberg and Vernberg (1972a), Jone (1973, 1975a, b), Vernberg *et al.* (1974), Roesijadi *et al.* (1974) and Rosenberg and Costlow (1976). Low salinities tend to have a greater capacity to maintain metals in water column in either solution or suspension (Bryan and Hummerstone, 1973) and consequently the availability to estuarine organism is potentially greater than that to marine species. The observed higher toxicity of Cu(II) to *M. dobsoni* in the present studies at $5x10^{-3}$S may be due to the better availability of Cu(II) in dissolved form. At higher salinities Cu(II) was precipitated as a visible blue precipitate and the availability of Cu(II) may be less to induce toxic effects. The observed highest 96 h LC50 among the metals tested was recorded by Cu(II), (3.1 mg l^{-1}) for the higher size group at $25x10^{-3}$S. Bryan (1976a, b) suggests that increased toxicity of heavy metals to estuarine organisms at lower salinities is reflective of the increased rates of heavy metal absorption at reduced salinities. Uptake rates and resultant concentrations of cadmium in adult blue crab *Callineetes sapidus* increased at low salinities (Hutcheson, 1974). Lower salinities enhanced the toxic effects of mercury on the porcelain crab *Petrolisthes armatus* (Roesijadi *et al.*, 1974).

There are instances where the toxic action of trace metals are low at lower salinities. The amphipod *Gammarus duebeni* shows improved tolerance of methyl mercury in 2% seawater relative to that in 100% seawater (Lockwood and Inman, 1975). Salinity over the range of $20\text{-}30x10^{-3}$ did not affect the toxicity of copper to american lobster *Homarus americanus* (McLeese, 1974). Hence decreased survival at salinities in the presence of toxins is not however universal. In general, areas of optimal salinity and temperature conditions exists for each species and in some cases, for each developmental stage (McKenney and Neff,1979).

Studies on the mechanism of metal toxicity is limited to a few species of fishes and molluscs. Much emphasis has been given on the gill region in fishes which is the most affected organ by the metal toxicants. Corner and Sparrow (1956) working with the brine shrimp *Artemia salina*, the copepod *Acartia clausi* and the barnacle *Elminius modestes* concluded that the mode of action of copper is specific in its effect on respiratory mechanism. The lethality of heavy metals has been ascribed to the coagulation of mucus (i.e. precipitation of insoluble metal protein compounds) on gill surfaces which results in respiratory failure and death by suffocation (Doudoroff and Katz, 1953, Plonka and Neff, 1969). Deposition of coagulant mucus on the gills of the fish appears to alter the gas exchange process and as a consequence creates hypoxia (Burton *et al.*, 1972a). Brown *et al.*(1968) suggest that metals act largely on gill surfaces where they cause thickening of epithelial walls resulting in death by suffocation as oxygen transfer is impeaded. Histological investigations have shown that copper and zinc poisoning resulted in a separation of gill epithelium from the basement membrane (Lloyd, 1960; Skidmore and Torrell, 1972). Ghate and Mulherkar (1979) observed distortion og gill plates, vacuolation and necrosis in the gill tissue of two species of freshwater prawns *Caridina* and *Macrobrachium* after chronic exposure to copper sulphate. They further stated that as crustacean gills are important in respiration as well as osmoregulation, cellular damage resulting in disorganization of gill tissue would have serious consequences.

During the exposure of *M. dobsoni* to Hg(II), Cu(II) and Zn(II) mucus secretion was observed except in a few very low concentrations. Further the development of black spots on the gill tissues of *M. dobsoni* exposed to Cu(II) clearly indicate that gills are severely affected on exposure to toxic metals and death might have happened due to hypoxia. Similar blackened branchial lesions were observed by Nimmo *et al.*(1977) on *Penaeus duorarum* exposed to cadmium in acute and subacute tests. Profuse secretion of mucus in gill region of *Macrobrachium lamarrie* has been observed by Ram Murti and Shukla (1984).

Eventhough the exact mechanism that accounts for zinc toxicity is not known, Pagenkopf (1980) identified a qualitative relationship between the Lewis acid character of H, Ca, Mg and Zn ions and the variation in toxicity. If the gills possess Lewis base character these four cations can conceivably co-ordinate or at least absorb on the gill surface. Zinc complex is 10 times more stable than calcium and magnesium complexes and displaces them from the gill surface.

Most of the studies on toxicity of heavy metals to aquatic organism have shown that the free (hydrated) metal ion is the most toxic form (Florence, 1983). Oehme (1972) found that ionic mercury forms covalent bonds with enzymes and other biological proteins. Mercury exhibits a strong preference in proteins for sulphur centres over nitrogen or oxygen centres (Vallee and Ulmer, 1972; Ukita, 1972). Methyl mercury derivatives and inorganic Hg^{2+} ions interact with —SH and —S—S— group in biological molecules (Albert, 1973; Vallee and Ulmer, 1972). Since the functional —SH and —S—S— groups are ubiquitous and crucial to the integrity of proteins or the functioning of enzymes, this binding preference of mercury provides the biochemical basis for much of its toxicity (Rabenstein, 1978a, b).

Ochiai (1977) has divided the mechanism of metal ion toxicity into the following three categories: (i) blocking of the essential biological groups of biomolecules, (ii) displacing the essential metal ions in biomolecules and (iii) modifying the active conformation of biomolecules. Thus, though the metal-ion toxicity is evidently complex, certain metal ions consistently induce greater damage than the others (Nieboer and Richardson, 1980). At this instant it is worth to quote Simkiss, (1983) : "The reactivity of the metals such as Cu, Hg, Zn and Cd with protein in terms of enzyme function and toxicology raises problems about the ways in which an organism may protect its physiological ligands from such permanent metals".

Cu(II) at 15 and 25×10^{-3}S precipitated to a noticeable extent and it is somewhat unrealistic to describe LC50 in terms of the added concentration in water. However, by the movement of the test animals the precipitates were kept in suspension to a large extent. Suspended particles may come in contact with gill surfaces causing similar damage as described by Brown *et al.* (1968). If such damage did not occur, sufficient precipitate would possibly gather around the gills and ultimately cause suffocation (Portmann, 1972).

Among the inorganic substances Hg(II) was considered the most important pollutant both with regard to its effect on marine organisms and its potential hazard to man followed by Cu(II) and Zn(II) (Benhard and Zattera, 1975). The studies on crustacea revealed the orders of toxicity as Hg > Cu > Zn (Wisely and Blick, 1967; Portmann, 1972). Other authors have also indicated that copper is among the more toxic metals to aquatic animals or at least that copper is more toxic than zinc (Portmann, 1968; Pickering and Henderson, 1966; Sprague, 1964; Reish *et al.*,

1976; Bryan, 1971; Ahsanullah and Arnott, 1978; Arnott and Ahsanullah, 1979 and Ahsanullah *et al.*,1981). Exceptions in the unique position of Hg(II) as the most toxic element have also been reported in *Perna viridis*, *Villorita cyprinoides* and *Meretrix casta* by Lakshmanan (1982). The order of toxicity for the three species was Cu > Hg > Zn.

The present study has clearly shown Hg(II) as the most toxic metal to *M. dobsoni*. Between Cu(II) and Zn(II) except at $15x10^{-3}$S for both size groups and at $25x10^{-3}$S for the maturing stage Cu(II) is more toxic than Zn(II). Further their potency ratios show that Cu(II) and Zn(II) do not differ significantly (Tables 4.12 and 4.13) and it is concluded that Cu(II) and Zn(II) are of the same order of toxicity. Comparatively higher toxicity of Zn(II) to Cu(II) is in agreement with the results of Eisler and Hennekey (1977) that "crustaceans appear to be one of the most sensitive group to zinc". Thus, for *Metapenaeus dobsoni* the order of toxicity is Hg >> Cu = Zn.

The main objective in the design and use of toxicity tests in biomonitoring is to enable us to predict with known accuracy a concentration that will not harm an entire system and to make this prediction in a responsible and cost effective manner. The use of application factors (AF) applied to acute toxicity tests in water quality criteria is recognised as a temporary solution to the problem of pollution by toxicants (NAS/NAE, 1973). These factors vary from 0.1 to 0.0001. LC50 is multiplied by AF to obtain 'safe' concentration which presumably has no sublethal or chronic effects. The Ohio River Sanitation Commission (1955) recommended an arbitrary factor of 0.1 to the 48 h TLm values. Warner (1967) states that in Holland, Germany and Switzerland, AF of 0.1 or 0.05 of 20 day LC50 are widely accepted. The maximum values recommended are 0.1 or 0.05 toxic units for non-persistent chemicals and 0.1 to 0.01 toxic units for the persistent chemicals and pesticides (Sprague, 1971). AF of 0.01 of 96 h LC50 has been recommended by EPA (1979).

United States Environmental Protection Agency has explored many ways to use toxicity data to derive water quality criteria for the protection of aquatic life (USEPA, 1979, 1980) and many of these methods have been criticized for being scientifically indefensible (Water Pollution Control Federation, 1981). However, the use of application factors applied to acute toxicity tests in water quality

criteria is recognised as a temporary solution in the absence of long-term chronic tests to determine the relative susceptibility of adults and most sensitive stages in the life cycle of different taxa, and to relate lethal concentrations for both adults and larvae with experimentally determined 'safe' levels.

CHAPTER 5

KINETICS OF BIOACCUMULATION AND DEPURATION

Bioaccumulation of trace metals and organochlorines by marine organisms offers a method of monitoring the presence of contaminants in living marine resources. Among the many substances regarded as environmental contaminants, elements such as Cu and Zn at natural concentrations* are essential for life while others

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* The normal concentration of metals in seawater ranges from 0.01-0.2 ug l^{-1} total Hg, around 1 ug l^{-1} Cu, around 10 ug l^{-1} Zn and 0.01-0.1 ug l^{-1} Cd (Benhard and Zattera, 1975).

such as Hg and Cd which occur naturally at low levels are not essential. Whether essential or not, inorganic or organic, many potentially toxic substances possess properties which make them readily available for accumulation by marine organisms which is the basis for much of our concern about pollution hazards.

Two processes are involved in bioaccumulation: (i) bioconcentration, is the uptake of toxicants by an organism directly from water; and (ii) biomagnification, is the uptake of toxicants through the food. The process of bioaccumulation is not likely to be a health hazard to man if the organisms are living in an unpolluted environment, where only the natural background levels of the metals are present. In areas where the concentrations of trace metals have increased as a result of man's activities, the possibility of these metals being concentrated at each trophic levels in the aquatic food chain (Stewart and Schulz-Baldes, 1976) may result in fish and shellfish having concentrations of metals which may be harmful to man. This is illustrated by the well documented 'Minamata disease' in Japan in the fifties (Ui, 1972).

An increased ambient supply of a metal may lead to more rapid uptake of that metal by biota. The changes occurring in biota when a decrease in ambient level of metal availability occurs, are also of importance. The events which take place during this period depend to a large extent on the metal half-life in the organism. The organisms exposed to bioconcentrate are transferred to clean water to determine biological half-life, i.e., the time required for tissue concentrations to decline to half of their steady state concentration (Hamelink, 1977). Half-life of the metals varies considerably between species and within species (Phillips, 1980). Some metals may persist long enough after episodic increases in their uptake rates to obviate the possibility of their being excreted *in toto* prior to the next influx. Such a situation was described by Luoma (1977, 1977a) for Hg kinetics in the shrimp *Palaemon debilis* and polychaete *Nereis succinea*.

The temporal change in levels of metals in aquatic biota is often highly dependent on the changes occurring in the ambient water mass. Knowledge of the factors that influence the uptake, storage and elimination of metals is essential for developing predictive models, which will greatly assist in the structuring of realistic pollution control programmes. Hence bioaccumulation studies are an important complement to chronic toxicity tests in the estimation

of potential for environmental harm (Buikema *et al.*, 1982). Further the metal accumulation studies has stimulated the possibility of using marine organisms as pollution indicators (Huggett *et al.*, 1973; Clarke *et al.*, 1976).

Most of the studies on accumulation and depuration of trace metals have been conducted using radiotracer techniques. Studies on depuration of the metals by transplanting the animals from contaminated areas to clean water bodies have also been tried. Accumulation and depuration kinetics of trace metals in crustaceans have been conducted by a number of authors : Bernard and Lane (1961), Bryan (1964, 1967, 1968), Cross *et al.* (1968), Hannerz (1968), Vernberg and O'Hara (1972), Miettinen *et al.* (1972), Bryan and Hummerstone (1973), Renfro *et al.* (1974a), Small *et al.* (1974), Solan *et al.* (1974), Ray and Tripp (1976), Fowler *et al.*(1978), Icely and Nott (1980), Ahsanullah *et al.* (1981a), White and Walker (1981), Baudin (1982), Crecelius *et al.* (1982), White and Rainbow (1982), Chang *et al.*(1983), Lyon *et al.*(1984), Sanders and Costlow (1984), White and Rainbow (1984), Devineau and Amiard-Triquet (1985), Riisgard and Famme (1986).

In India the studies in this regard is very few in number and that too restricted to the bivalve molluscs (Nambisan *et al.*, 1977, D'Silva and Kureishy, 1978; D'Silva and Quasim, 1979; Kumaraguru and Ramamoorthy, 1979; Lakshmanan and Nambisan, 1979; Lakshmanan and Nambisan, 1986; Latha Thampuran, 1986). No attempt seems to have been made for such studies on crustaceans from Indian waters. The present investigations are aimed at revealing the scope of such studies on crustaceans and thereby assessing their utility as an indicator of trace metal pollution.

5.1 MATERIALS AND METHODS

The procedure for bioaccumulation and depuration studies are given in 2.14. Metal concentration in the exposure medium, size group of the animals and water quality parameters in accumulation and depuration studies are given in Table 5.1a. To find out the effect of size group on accumulation of the metals in *M. dobsoni* three size groups, 30-40, 40-50 and 50-60 mm were exposed to Hg, Cu and Zn. Metal concentrations and water quality characters of experiment are given in Table 5.1b. Effect of size group in the rate of depuration was not studied.

Table 5.1a Metal concentrations, water quality characteristics, average length and weight of M. dobsoni used in the accumulation and depuration studies (Mean ± SD)

Metal con. ($mg\ l^{-1}$)	Salinity (10^{-3})	pH	Temp (°C)	Average	
				Length (mm)	Wt (g)*
Hg					
0.005	5.13	7.55	28.00	52.25	0.605
	0.31	0.05	0.82	3.15	0.045
	14.92	7.57	29.80	41.50	ND
	0.67	0.23	1.15	5.16	-
	25.03	7.25	28.50	50.00	0.423
	0.18	0.15	0.50	1.73	0.038
0.01	5.13	7.55	28.00	52.25	0.627
	0.31	0.05	0.82	3.15	0.012
	15.67	7.68	29.08	44.46	ND
	0.21	0.17	0.86	4.27	-
	25.03	7.25	28.50	50.00	0.525
	0.18	0.15	0.50	1.73	0.069
Cu					
0.25	5.20	7.50	28.00	50.63	0.165
	0.20	0.15	1.00	4.17	0.019
	15.45	7.61	29.00	43.42	ND
	0.39	0.15	0.50	4.27	-
	24.87	6.95	28.62	51.33	0.161
	0.32	0.20	0.51	1.15	0.018
0.50	5.20	7.50	28.00	50.63	0.181
	0.20	0.15	1.00	4.17	0.048
	15.45	7.61	29.00	43.42	ND
	0.39	0.15	0.50	4.27	-
	24.87	6.95	28.62	51.33	0.161
	0.32	0.20	0.51	1.15	0.019

Contd...

Table 5.1a contd.....

Metal con. (mg l^{-1})	Salinity (10^{-3})	pH	Temp (°C)	Average	
				Length (mm)	Wt g*
Zn					
0.2	5.14	7.45	27.33	50.70	0.205
	0.22	0.10	0.57	5.48	0.027
	15.47	7.58	29.12	43.00	0.114
	0.28	0.06	0.63	4.48	0.022
	25.24	6.84	28.53	50.50	0.157
	0.16	0.31	0.48	0.57	0.020
0.4	5.14	7.45	27.33	50.70	0.206
	0.22	0.10	0.57	5.48	0.060
	15.47	7.58	29.12	43.00	0.115
	0.28	0.06	0.63	4.48	0.003
	25.24	6.84	28.53	50.50	0.152
	0.16	0.31	0.48	0.57	0.014

* Wet wt for Hg and dry wt for Cu and Zn

Table 5.1b Metal concentrations (mg l^{-1}), water quality characteristics, average length and weights of M. dobsoni used in the accumulation studies (Effect of size group) (Mean ± SD)

Size Group (mm)	Salinity (10^{-3})	pH	Temp (°C)	Average Length (mm)	Wt (g)*
			Hg : 0.005 mg l^{-1}		
30-40	5.08	7.05	28.00	34.85	0.185
	0.12	0.13	0.50	2.16	0.034
40-50				47.38	0.502
				1.80	0.053
50-60				56.13	0.822
				1.64	0.037
			Cu : 0.2 mg l^{-1}		
30-40	5.04	7.25	26.66	34.70	0.048
	0.07	0.20	0.76	2.36	0.004
40-50				42.92	0.083
				2.97	0.020
50-60				53.08	0.184
				1.78	0.023
			Zn : 0.25 mg l^{-1}		
30-40	5.10	7.10	26.50	35.10	0.046
	0.16	0.10	0.71	2.31	0.004
40-50				46.07	0.118
				2.35	0.012
50-60				51.90	0.157
				2.18	0.010

* Wet wt for Hg and dry wt for Cu and Zn

Relationship between length, body wt and percentage of muscle in *M. dobsoni* used in the studies are given below.

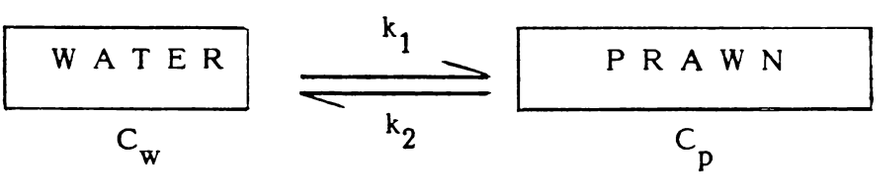
Length (mm)		Total body wet wt (g)		% of muscle (wet wt)	
Mean	SD	Mean	SD	Mean	SD
38.2	2.68	0.269	0.07	56.74	2.43
43.8	1.07	0.391	0.02	56.35	1.65
48.6	1.17	0.551	0.06	58.26	3.14
55.0	2.78	0.801	0.15	60.13	2.24
63.0	2.82	1.156	0.14	60.32	1.20
68.2	2.10	1.270	0.18	60.57	2.43

5.2 RESULTS

An attempt was made to suggest a kinetic model for accumulation and depuration of trace metals in *M. dobsoni* and results of the same are given in detail in the following paragraphs.

5.2.1 Kinetic Model for Mercury and Copper

The measured tissue concentration of Hg and Cu in *M. dobsoni* and the concentration of the exposed media were interpreted in terms of the first order two compartment exchange as proposed by Branson *et al.* (1975) and Bruggeman *et al.* (1981) to compare the accumulation and depuration rates and the bioconcentration factors.



For the model the accumulation rate is given as

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

where

C_w = concentration of metal in water

C_p = concentration of metal in prawns

k_1, k_2 = rate constants for accumulation from water and depuration (time^{-1})

Integrating equation (1)

$$C_{p(t)} = \frac{k_1}{k_2} C_w (1 - e^{- k_2 t}) \quad .. \quad (2)$$

The depuration rate

$$\frac{dC_p}{dt} = - k_2 C_p \quad .. \quad (3)$$

Integrating equation (3)

$$C_{p(t)} = C_{p(o)} e^{-k_2 t}$$

or

$$\ln C_{p(t)} = \ln C_{p(o)} - k_2 t \quad .. \quad (4)$$

$$\ln C_{p(t)} \propto t \quad .. \quad (5)$$

k_2 is given by the slope of the plot of $\ln C_{p(t)}$ *versus* t

Substituting the value of k_2 in equation (2), k_1 can be calculated

Slope of the plot of $C_{p(t)}$ *versus* $(1 - e^{-k_2 t})$

will be equal to $\frac{k_1}{k_2} C_w$

Hence the rate of accumulation $k_1 = \frac{\text{Slope} \times k_2}{C_w}$ .. (6)

At equilibrium, the ratio between prawn and water concentrations is represented by the bioconcentration factor K_b.

$$K_b = \frac{C_{p(\infty)}}{C_w} = \frac{k_1}{k_2} \quad .. \quad (7)$$

5.2.2 Kinetic Model for Zn

The depuration of Zn from the whole body and body parts of *M. dobsoni* was found to be very slow. The plot of ln $C_{f(t)}$ *versus* t as in the case of Hg and Cu gave a straight line running almost parallel to the time axis (Fig. 5.10). Hence, considering the rate of depuration as negligible, the following equation is suggested :

$$\frac{dC_p}{dt} = k_1 C_w \quad .. \quad (8)$$

Rearranging (8)

$$dC_p = k_1 C_w dt \quad .. \quad (9)$$

Integrating (9)

$$C_p = k_1 C_w t + C \quad .. \quad (10)$$

Plotting C_p *versus* t :

$$\text{Slope} = k_1 C_w$$

$$k_1 = \frac{\text{Slope}}{C_w}$$

where k_1 = the rate constant for accumulation of Zn.

5.2.3 Accumulation of Mercury

The rate constants for accumulation (k_1) for Hg according to the kinetic model suggested are given in Table 5.2. The rate constants for accumulation for muscle and gill tissue are on the increase when the concentration of the exposure medium increased. But the reverse was observed in the case of exoskeleton + viscera and whole body. The rate of accumulation of Hg in muscle, gill and exoskeleton + viscera of the animals exposed to lower concentration of Hg (0.005 mg l^{-1}) increased with the increase in salinity of the exposure medium. At higher concentration (0.01 mg l^{-1}) of exposure medium exoskeleton + viscera followed similar pattern, whereas in muscle and gill the rate of uptake decreased with increase in salinity.

Table 5.2 Rate constants for accumulation (k_1), depuration (k_2) and bioconcentration factor (K_b) for Hg

Salinity (10^{-3})	Hg concentration in exposure medium mg l^{-1}	k_1 (h^{-1})	k_2 (h^{-1})	$K_b = \frac{k_1}{k_2}$
		Muscle		
5	0.005	0.3915	0.00208	188.22
	0.01	0.7812	0.00521	149.94
15	0.005	0.5911	0.00416	141.87
	0.01	0.6943	0.00416	166.65
		Gill		
5	0.005	3.5712	0.00372	960.00
	0.01	5.0496	0.00473	1067.57
15	0.005	3.7496	0.00468	801.20
	0.01	3.5000	0.00312	1121.79
		Exoskeleton + viscera		
5	0.005	0.2604	0.00521	49.98
	0.01	0.1040	0.00208	50.00
15	0.005	0.2700	0.00810	33.33
	0.01	0.1560	.00156	100.00
		Whole body		
5	0.005	1.0000	0.00625	160.00
	0.01	0.3541	0.00208	170.24

The observed values of Hg accumulated in whole body and body parts of *M.dobsoni* exposed at 0.005 and 0.01 mg l^{-1} Hg at 5, 15 and $25x10^{-3}$S are presented in Table 5.3 to 5.8. After the initial uptake a tendency to reduce the Hg load during accumulation is noticed in whole body and body parts in both the salinities tested. A regular pattern is discernable in all the cases. The rate of accumulation was found to depend on the concentration of the metal in the exposure medium but the depuration took place independent of the concentration of the metal in the medium. The tissues attained a 'threshold concentration' depending on the salinity of the medium. At $5x10^{-3}$S the threshold concentration in ng g^{-1} wet wt were 300-350 for whole body and exoskeleton + viscera, and 150-170 for muscle. The threshold concentration for the gill tissue has a wide range from 1200-2800 ng g^{-1} wet wt (Fig. 5.1).

With the increase in salinity the threshold values also increased. $15x10^{-3}$S recorded the threshold concentration of 700-800 (ng g^{-1} wet wt) for exoskeleton + viscera and 250-300 (ng g^{-1} wet wt) for muscle. Threshold concentrations were almost twice than that at $5x10^{-3}$S. Threshold concentration was not achieved in gill tissues in both the exposure concentrations at $15x10^{-3}$S during the course of the study (Fig. 5.1).

The bioconcentration factor K_b calculated using the kinetic model (Table 5.2) increased with the increase in concentration of exposure media under each salinity in whole body and all other parts except in the case of muscle at $5x10^{-3}$S. With the increase in salinity K_b decreased at lower exposure concentration but was on the increase at higher exposure concentration.

The bioconcentration factor (BCF)* were calculated by dividing the maximum tissue concentration after subtracting the mean concentration in the control by the exposure concentration (Tables 5.3 to 5.8). The BCF increased with increase in salinity in all the cases irrespective of the metal concentration in exposure medium. Further BCF are higher at lower exposure concentrations and independent of salinity of the medium except for exoskeleton in $25x10^{-3}$S.

Based on the BCF values at $25x10^{-3}$S the order of accumulation in different body parts of *M. dobsoni* is :

Gill $\gg$ Viscera $>$ Exoskeleton $>$ Muscle

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*Hereinafter the bioconcentration factor calculated using the kinetic model will be described as K_b and that calculated as above will be denoted as BCF in the text.

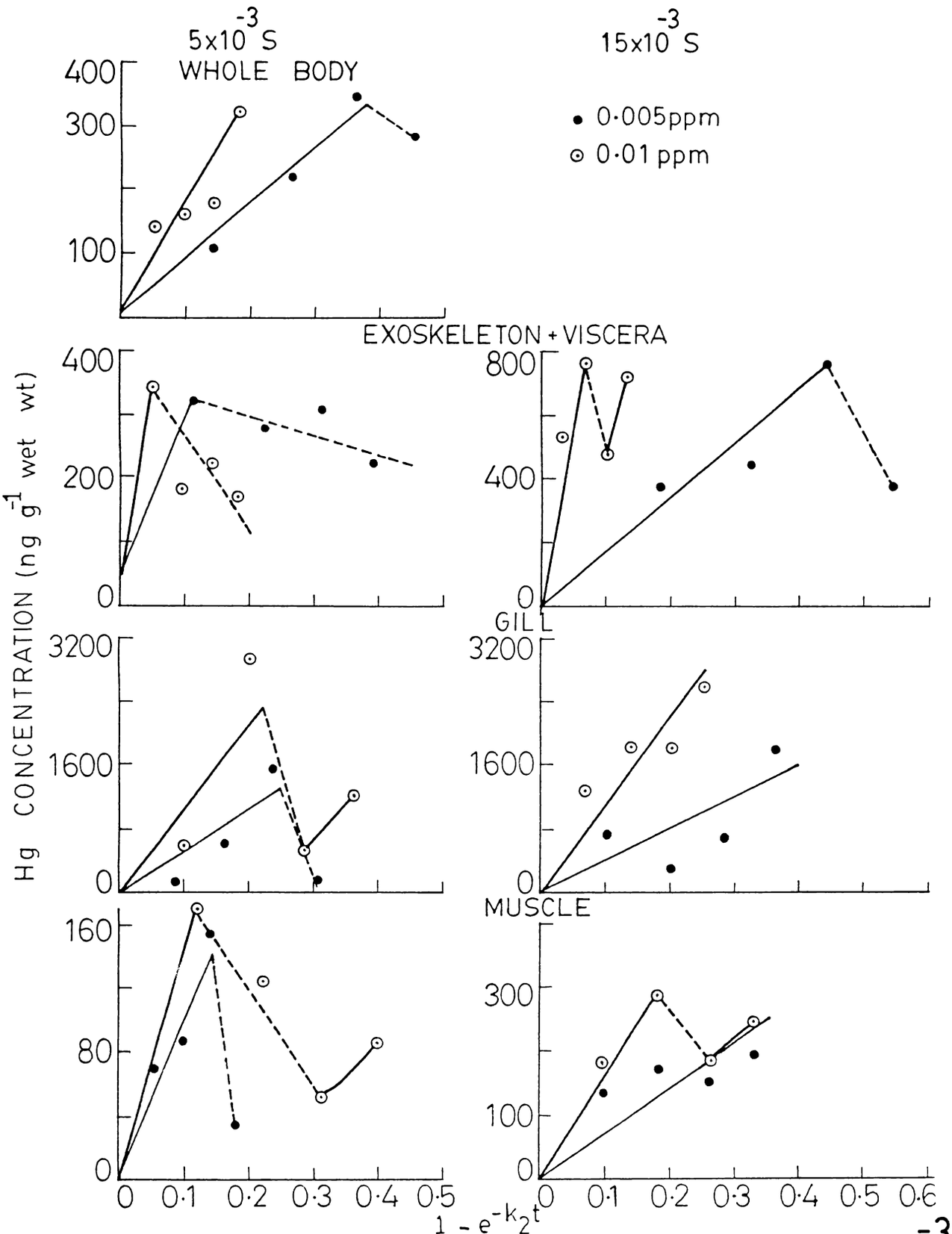

Fig.5-1. Accumulation of Hg in M. dobsoni at 5 and 15×10^{-3} S.

Table 5.3 Accumulation of Hg in M. dobsoni exposed to 0.005 ppm Hg at $5x10^{-3}S$

Time (h)	Hg concentration ug g^{-1} wet wt (Mean ± SD)			
	Muscle	Gill	Exoskeleton + viscera	Whole body
Control	0.0041	0.0000	0.0415	0.0079
	0.0001	-	0.0022	0.0002
24	0.0706	0.1388	0.3197	0.1051
	0.0043	0.0071	0.0204	0.0064
48	0.0871	0.6456	0.2805	0.2181
	0.0030	0.0245	0.0108	0.0094
72	0.1551	1.5592	0.3069	0.3448
	0.0045	0.0278	0.0113	0.0210
96	0.0361	0.1762	0.2205	0.2849
	0.0032	0.0081	0.0154	0.0105
BCF	30.2	311.84	55.64	67.38

Table 5.4 Accumulation of Hg in M. dobsoni exposed to 0.01 ppm Hg at $5x10^{-3}$S

Time (h)	Hg concentration ug g^{-1}wet $_{wt}$ (Mean ± SD)			
	Muscle	Gill	Exoskeleton + viscera	Whole body
Control	0.0041	0.0000	0.0415	0.0079
	0.0001	-	0.0021	0.0002
24	0.1708	0.5662	0.3463	0.1397
	0.0106	0.0192	0.0132	0.0094
48	0.1246	2.9442	0.1796	0.1601
	0.0048	0.1030	0.0062	0.0056
72	0.0535	0.5305	0.2248	0.1727
	0.0018	0.0164	0.0065	0.0055
96	0.0842	1.4096	0.1646	0.3236
	0.0052	0.535	0.0057	0.0197
BCF	16.67	294.42	30.48	31.57

Table 5.5 Accumulation of Hg in M. dobsoni exposed to 0.005 ppm Hg at $15x10^{-3}$ S

Time (h)	Hg concentration ug g wet wt (Mean ± SD)		
	Muscle	Gill	Exoskeletor + viscera
Control	0.0032	0.0000	0.0289
	0.0001	-	0.0018
24	0.1370	0.7468	0.375
	0.0047	0.0287	0.0171
48	0.1713	0.3091	0.4482
	0.0055	0.0197	0.0144
72	0.1485	0.6948	0.7581
	0.0050	0.0211	0.0245
96	0.1921	1.7871	0.3789
	0.0080	0.0603	0.0102
BCF	37.78	356.34	145.84

Table 5.6 Accumulation of Hg in M. dobsoni exposed to 0.01 ppm Hg at 15×10^{-3} S

Time (h)	Hg concentration ug g^{-1} wet wt (Mean ± SD)		
	Muscle	Gill	Exoskeleton + viscera
Control	0.0066	0.0000	0.0273
	0.0009	-	0.0012
24	0.1817	1.2882	0.5221
	0.0027	0.0680	0.0077
48	0.2772	1.8104	0.7571
	0.0052	0.0769	0.0136
72	0.1848	1.8032	0.4783
	0.0056	0.0667	0.0066
96	0.2416	2.5726	0.7207
	0.0057	0.0705	0.0080
BCF	27.06	257.26	72.98

Table 5.7 Accumulation of Hg in M. dobsoni exposed to 0.005 ppm Hg at 25×10^{-3} S

Time (h)	Hg concentration ug g^{-1} wet wt (Mean ± SD)					
	Muscle	Gill	Exoskeleton	Viscera	Exoskeleton + viscera	Whole body
Control	0.0319	0.1586	0.3951	0.2119	0.0869	0.0598
	0.0019	0.0096	0.0146	0.0086	0.0039	0.0020
24	0.1723	1.1111	0.4746	0.7300	0.7058	0.4540
	0.0137	0.0677	0.0180	0.0452	0.0437	0.0158
48	0.1309	0.9553	0.4267	0.8300	1.0923	0.3799
	0.0055	0.0362	0.0161	0.0539	0.0393	0.0181
72	0.2626	2.2066	0.6915	0.7726	0.9244	0.4568
	0.0097	0.0104	0.0317	0.0726	0.0619	0.0392
96	0.1519	1.3773	0.5331	0.4701	0.5127	0.4111
	0.0069	0.0115	0.0405	0.0374	0.0194	0.0275
BCF	46.14	409.6	59.28	123.62	201.08	79.4

Table 5.8 Accumulation of Hg in M. dobsoni exposed to 0.01 ppm Hg at 25×10^{-3}S

Time (h)	Hg concentration ug g^{-1} wet wt (Mean ± SD)					
	Muscle	Gill	Exoskeleton	Viscera	Exoskeleton + viscera	Whole body
Control	0.0319	0.1586	0.3951	0.2119	0.0869	0.0598
	0.0019	0.0096	0.0146	0.0080	0.0039	0.0020
24	0.3255	3.9301	1.2426	1.1965	1.4153	0.7845
	0.0198	0.1375	0.0844	0.0454	0.0721	0.0266
48	0.2640	1.5636	0.4924	0.5967	0.6822	0.4140
	0.0113	0.0453	0.0172	0.0226	0.0313	0.0215
72	0.3042	2.5907	0.4246	1.3590	1.2668	0.8469
	0.0088	0.0637	0.0182	0.0571	0.0544	0.0313
96	0.3529	1.5533	0.6347	1.0569	0.4606	0.4235
	0.021	0.0947	0.0133	0.0686	0.0271	0.0283
BCF	32.1	377.15	84.75	114.71	132.84	78.71

5.2.4 Accumulation of Copper

Accumulation rate constants for Cu provided by the kinetic model are given in Table 5.9. The rate constant k_1 for whole body and for other parts were in the same range irrespective of salinity and metal concentration of the exposure medium except in the muscle at lower salinity and exposure concentration. Gill tissue recorded abnormally high values for k_1 at $5x10^{-3}$S which increases with the exposure concentration. Exoskeleton + viscera and whole body concentrations were not affected either with change in salinity and/or exposure concentration.

Tables 5.10 to 5.15 provide the observed mean concentrations of Cu accumulated in whole body and body parts of *M. dobsoni* exposed to 0.25 and 0.5 mg l^{-1} Cu at 5, 15 and $25x10^{-3}$S. Gradual increase in tissue concentration was observed in all the cases (Fig. 5.2).

K_b values are given in Table 5.9. Except for the muscle and whole body at $5x10^{-3}$S K_b increased with the increase in concentration of exposure medium. Muscle and gill at lower exposure concentration exhibited higher K_b values at $5x10^{-3}$S and reverse at higher exposure concentration. Exoskeleton + viscera recorded higher K_b values with the increase in salinity in both exposure concentrations. BCF values increased as the salinity decreased and higher values were obtained at lower exposure concentrations of Cu (Tables 5.10 to 5.15).

The order of accumulation of Cu in different body parts of *M. dobsoni* at $25x1-^{-3}$S is

Gill > Viscera > Exoskeleton > Muscle

5.2.5. Accumulation of Zinc

Accumulation rate constants k_1 for Zn are given in Table 5.16. The rate constant was found to be higher in muscle and whole body at $15x10^{-3}$S. Gill tissue recorded the maximum value of k_1 in higher concentration at $25x10^{-3}$S. The rate constant was higher at higher concentration except in the case of gill and muscle at $15x10^{-3}$S and for muscle at $25x10^{-3}$S.

Concentration of Zn accumulated in whole body and body parts of *M. dobsoni* exposed to 0.2 and 0.4 mg l^{-1} Zn at 5, 15 and $25x10^{-3}$S and BCF are presented in Tables 5.17 to 5.22. The accumulation pattern of Zn is represented graphically in Fig.5.3 to 5.6. BCF values at lower concentration for muscle and whole body decreased as the salinity increased and for the gill tissue BCF increased with increase in salinity and the value remained constant for exoskeleton + viscera. At higher concentration accumulation in the gill tissue decreased with the increase in salinity and in exoskeleton + viscera the value increased with increase in salinity. The maximum value of BCF for muscle and whole body at higher exposure concentration was recorded at $15x10^{-3}$S.

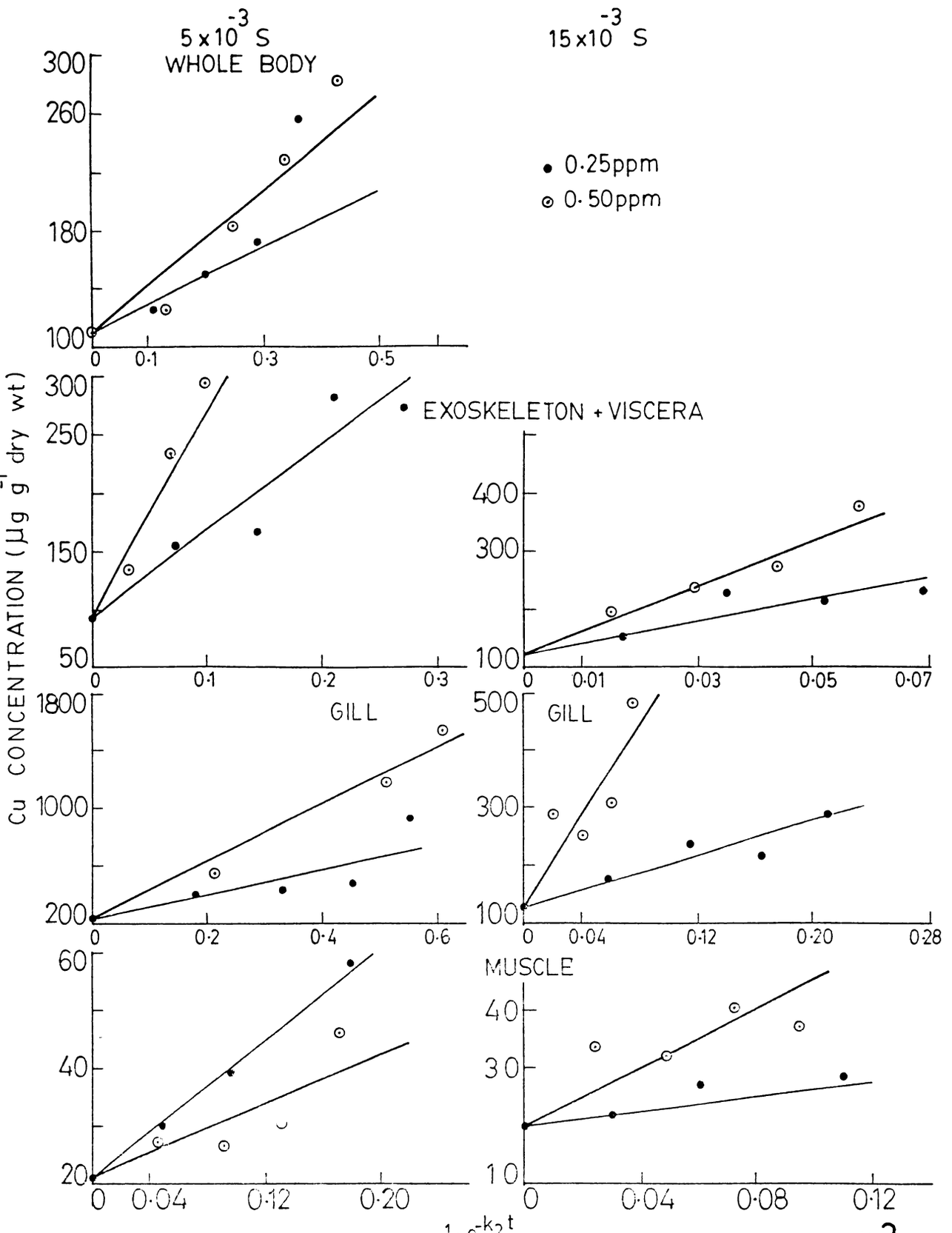

Fig:5-2. Accumulation of Cu in M.dobsoni at 5 and 15 x 10^{-3} S.

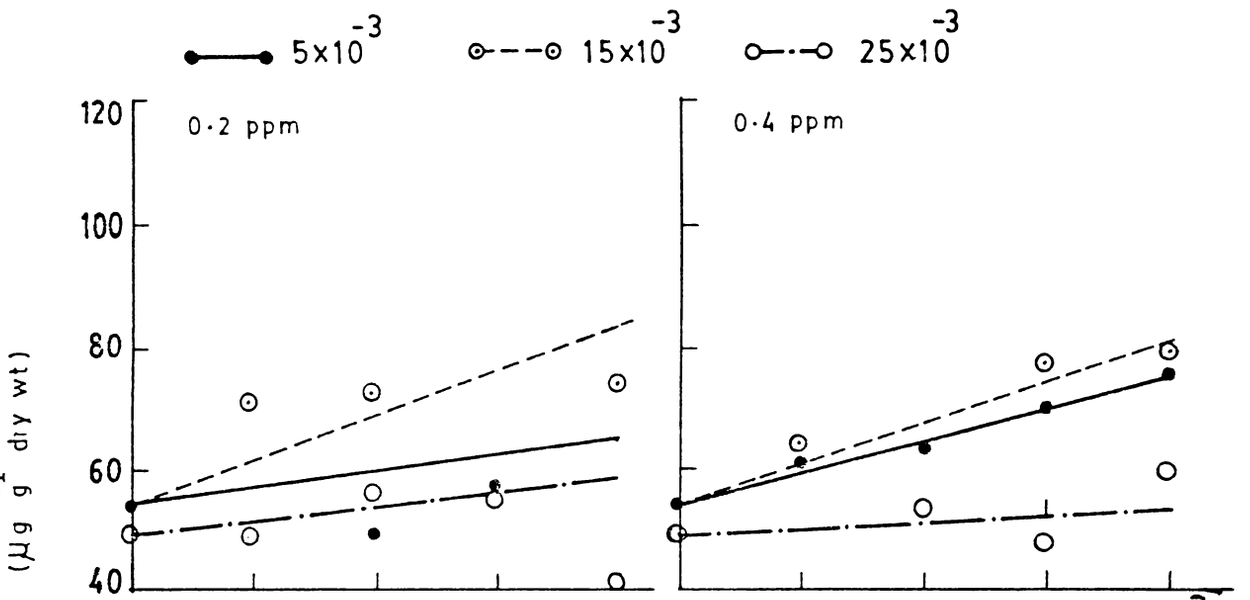

Fig.5-3. Accumulation of Zn in muscle of M.dobsoni at 5,15 and 25x10^{-3} S.

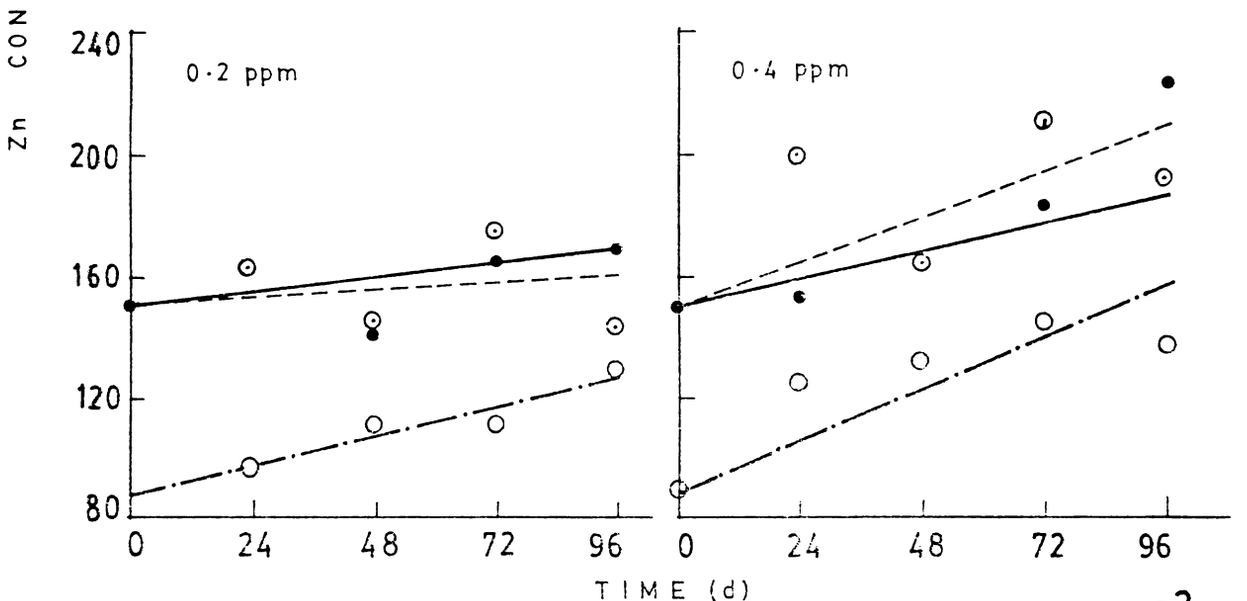

Fig.5-4. Accumulation of Zn in gill of M.dobsoni at 5,15 and 25x10^{-3} S.

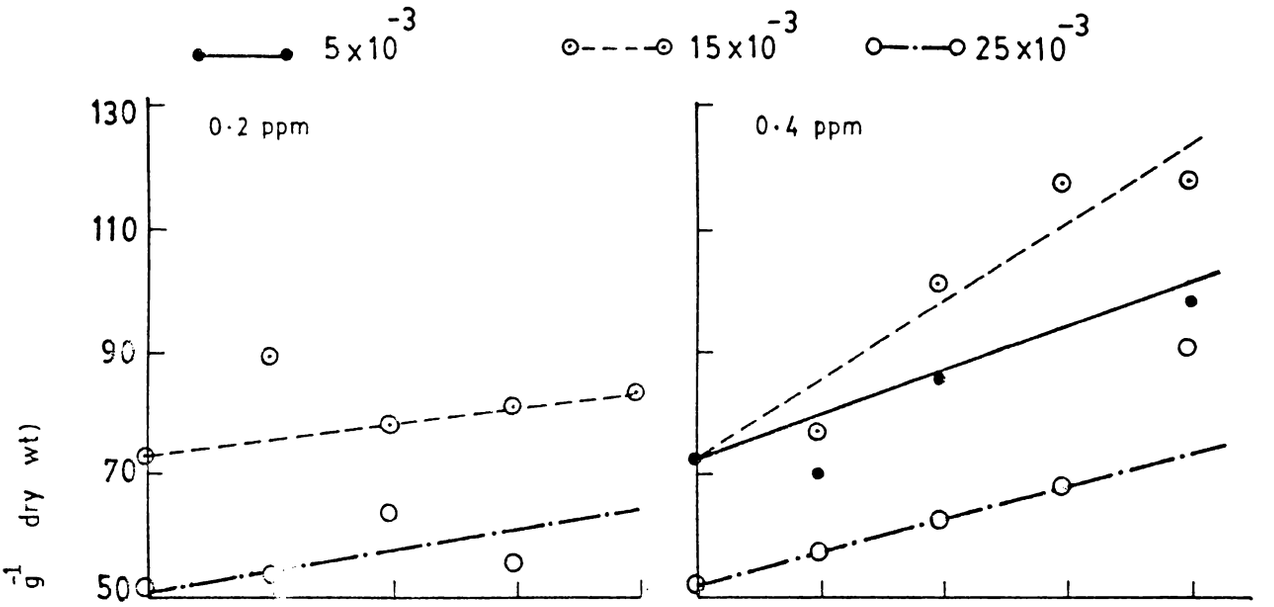

Fig.5-5. Accumulation of Zn in whole body of M. dobsoni at 5,15 and 25×10^{-3} S.

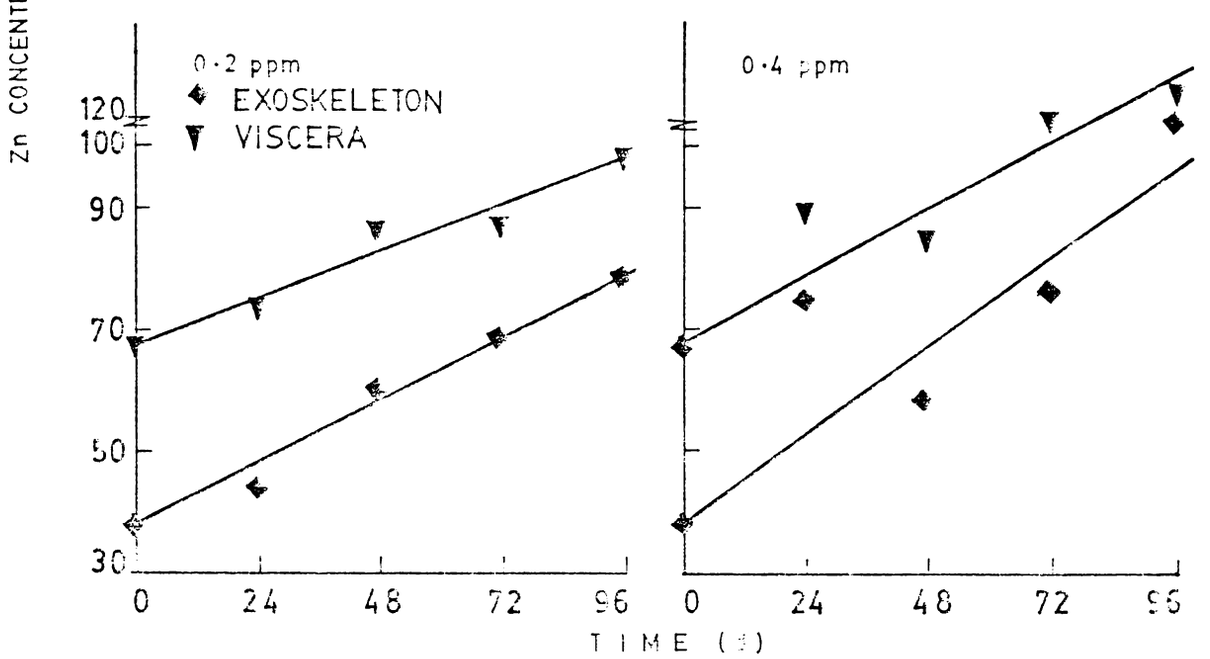

Fig.5-6. Accumulation of Zn in exoskeleton and viscera of M. dobsoni at 25×10^{-3} S.

Table 5.9 Rate constants for accumulation (k_1), depuration (k_2) and bioconcentration factor (K_b) for Cu

Salinity (10^{-3})	Cu concentration in exposure medium mg l^{-1}	k_1 (h^{-1})	k_2 (h^{-1})	$K_b = \frac{k_1}{k_2}$
		Muscle		
5	0.25	1.649	0.00208	793.08
	0.50	0.392	0.00196	200.00
15	0.25	0.260	0.00130	200.31
	0.50	0.520	0.00104	500.48
		Gill		
5	0.25	26.656	0.00833	3200.00
	0.50	40.000	0.01	4000.00
15	0.25	7.500	0.0025	3000.00
	0.50	6.240	0.0008	7800.00
		Exoskeleton + viscera		
5	0.25	3.625	0.00325	1115.38
	0.50	5.467	0.00156	3504.49
15	0.25	5.952	0.00074	8043.24
	0.50	5.000	0.00062	8064.52
		Whole body		
5	0.25	3.787	0.00473	800.63
	0.50	3.740	0.00586	638.23

Table 5.10 Accumulation of Cu in M. dobsoni exposed to 0.25 ppm Cu at $5x10^{-3}$S

Time (h)	Cu concentration ug g^{-1} dry wt (Mean ± SD)			
	Muscle	Gill	Exoskeleton + viscera	Whole body
Control	21.11	231.35	92.29	108.91
	0.84	17.32	5.52	7.29
24	29.85	389.12	153.83	126.41
	1.52	25.09	11.46	5.19
48	39.06	424.94	162.79	147.59
	2.08	23.91	7.59	13.52
72	35.14	464.37	281.83	171.31
	2.17	34.80	14.65	9.74
96	58.23	927.50	272.98	254.69
	3.71	31.52	25.84	17.27
BCF	148.48	2784.60	758.16	583.12

Table 5.11 Accumulation of Cu in M. dobsoni exposed to 0.50 ppm Cu at $5x10^{-3}$ S

Time (h)	Cu concentration ug g^{-1} dry wt (Mean ± SD)			
	Muscle	Gill	Exoskeleton + viscera	Whole body
Control	21.11	231.35	92.29	108.91
	0.84	17.32	5.52	7.29
24	27.02	546.02	134.47	125.44
	1.05	31.12	8.57	7.62
48	26.61	ND	232.13	182.43
	1.06	-	14.84	10.92
72	30.29	1181.92	296.29	228.10
	0.75	45.47	11.45	8.55
96	46.80	1539.47	208.52	282.56
	1.67	34.92	7.42	18.11
BCF	51.38	2616.24	408.0	347.3

Table 5.12 Accumulation of Cu in *M. dobsoni* exposed to 0.25 ppm Cu at 15×10^{-3} S

Time (h)	Cu concentration ug g^{-1} dry wt (Mean ± SD)		
	Muscle	Gill	Exoskeleton + viscera
Control	19.86	127.64	124.20
	1.10	4.40	5.00
24	21.70	173.39	152.82
	0.68	5.64	13.89
48	26.97	233.97	227.82
	1.23	13.75	16.08
72	43.84	214.49	212.39
	1.06	14.42	18.49
96	25.68	284.69	278.89
	0.95	10.36	13.29
BCF	95.92	625.44	618.76

Table 5.13 Accumulation of Cu in M. dobsoni exposed to 0.50 ppm Cu at 15x10^{-3}S

Time (h)	Cu concentration ug g^{-1} dry wt (Mean ± SD)		
	Muscle	Gill	Exoskeleton + viscera
Control	19.86	127.64	124.20
	1.10	4.40	5.00
24	33.7	283.43	192.26
	1.61	14.22	17.45
48	32.44	250.75	236.23
	1.75	12.40	17.50
72	40.50	304.82	275.42
	1.31	12.12	12.28
96	36.62	482.56	378.80
	2.06	11.63	20.70
BCF	41.28	709.84	509.20

Table 5.14 Accumulation of Cu in M. dobsoni exposed to 0.25 ppm Cu at 25×10^{-3}S

Time (h)	Cu concentration ug g^{-1} dry wt (Mean ± SD)					
	Muscle	Gill	Exoskeleton	Viscera	Exoskeleton + viscera	Whole body
Control	28.35	163.78	84.34	108.77	93.87	60.39
	1.79	10.43	4.87	6.91	4.46	4.98
24	28.54	206.12	97.05	120.36	117.84	106.05
	1.93	11.74	6.30	8.28	10.06	9.96
48	33.65	193.62	156.22	172.08	127.80	102.87
	2.89	12.93	11.85	16.68	15.24	6.32
72	51.50	343.00	148.14	221.11	150.89	124.34
	3.77	31.21	12.43	9.06	8.10	7.56
96	35.77	278.14	152.56	298.73	166.13	129.21
	1.01	12.51	5.62	16.98	11.12	8.64
BCF	92.60	716.88	287.52	759.84	289.04	275.28

Table 5.15 Accumulation of Cu in M. dobsoni exposed to 0.5 ppm Cu at $25x10^{-3}$S

Time (h)	Cu concentration ug g^{-1} dry wt (Mean ± SD)					
	Muscle	Gill	Exoskeleton	Viscera	Exoskeleton + viscera	Whole body
Control	28.35 1.79	163.78 10.43	84.34 4.87	108.77 6.91	93.87 4.46	60.39 4.98
24	28.51 1.71	216.67 17.41	117.72 7.13	137.17 7.12	121.17 7.74	115.48 10.58
48	53.01 3.44	346.89 21.45	155.59 8.21	212.70 13.35	174.79 10.78	161.25 6.11
72	56.49 7.43	352.36 21.82	159.10 6.20	245.39 16.66	190.82 15.58	144.09 9.79
96	70.73 1.96	396.59 22.96	188.14 12.78	270.02 18.09	208.89 7.90	179.34 11.45
BCF	84.76	465.62	207.60	322.50	230.04	237.90

Table 5.16 Rate constants for accumulation (k_1) for Zn

Body part	Zn con. in exposure medium mg l^{-1}	Rate constant for accumulation k_1 (h^{-1})		
		$5x10^{-3}$S	$15x10^{-3}$ S	$25x10^{-3}$ S
Muscle	0.2	0.125	0.292	0.098
	0.4	0.222	0.277	0.042
Gill	0.2	0.208	0.333	0.392
	0.4	0.370	0.309	0.714
Exoskeleton	0.2	-	-	0.416
	0.4	-	-	0.583
Viscera	0.2	-	-	0.313
	0.4	-	-	0.458
Whole body	0.2	-	0.166	0.125
	0.4	0.292	0.542	0.208

Table 5.17 Accumulation of Zn in M. dobsoni exposed to 0.2 ppm Zn at $5x10^{-3}$S

Time (h)	Zn concentration ug g^{-1} dry wt (Mean ± SD)			
	Muscle	Gill	Exoskeleton + viscera	Whole body
Control	54.29	149.14	76.86	73.79
	3.54	9.71	2.85	8.83
24	102.49	165.67	104.98	102.57
	8.16	14.91	9.36	8.16
48	48.98	140.00	76.77	49.01
	3.36	15.40	9.12	5.88
72	57.22	165.62	96.11	84.31
	3.42	9.90	5.76	6.72
96	74.16	168.37	105.37	66.79
	6.68	15.12	7.35	8.58
BCF	241.00	96.12	142.55	143.9

Table 5.18 Accumulation of Zn in M. dobsoni exposed to 0.4 ppm Zn at $5x10^{-3}$ S

Time (h)	Zn concentration ug g^{-1} dry wt (Mean ± SD)			
	Muscle	Gill	Exoskeleton + viscera	Whole body
Control	54.29	149.14	76.86	73.79
	3.54	9.71	2.85	8.53
24	61.43	153.12	91.98	70.68
	4.88	12.85	6.37	3.99
48	63.65	164.02	81.05	86.17
	3.59	15.41	5.42	7.48
72	70.69	184.08	116.56	117.41
	6.51	12.64	10.15	11.18
96	76.64	223.27	125.57	98.18
	4.48	27.57	8.59	8.08
BCF	55.87	185.33	121.78	109.05

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Table 5.19 Accumulation of Zn in M. dobsoni exposed to 0.2 ppm Zn at 15×10^{-3}S

Time (h)	Zn concentration ug g^{-1} dry wt (Mean ± SD)			
	Muscle	Gill	Exoskeleton + viscera	Whole body
Control	54.29 3.54	149.14 9.71	76.86 2.85	73.79 8.83
24	71.37 2.65	162.01 10.89	100.72 5.43	89.01 2.18
48	72.04 5.66	154.63 9.26	104.29 6.85	78.27 8.43
72	45.16 4.46	175.58 8.15	95.48 11.69	81.52 5.32
96	73.61 3.68	141.52 9.00	81.66 7.65	83.77 8.62
BCF	96.60	132.20	137.15	76.10

Table 5.20 Accumulation of Zn in M. dobsoni exposed to 0.4 ppm Zn at $15x10^{-3}S$

Time (h)	Zn concentration ug g^{-1} dry wt (Mean ± SD)			
	Muscle	Gill	Exoskeleton + viscera	Whole body
Control	54.29 3.54	149.14 9.71	76.86 2.85	73.79 8.83
24	64.09 4.96	199.24 16.47	113.46 8.74	77.01 4.85
48	53.12 3.54	163.65 9.68	76.63 5.63	101.02 9.67
72	77.35 5.63	210.79 18.74	135.20 9.65	118.48 7.28
96	79.20 5.43	190.28 13.45	130.61 6.50	118.17 8.46
BCF	62.27	154.125	145.85	111.73

Table 5.21 Accumulation of Zn in M. dobsoni exposed to 0.2 ppm Zn at 25×10^{-3}S

Time (h)	Zn concentration ug g^{-1} dry wt (Mean ± SD)					
	Muscle	Gill	Exoskeleton	Viscera	Exoskeleton + viscera	Whole body
Control	49.33	87.01	38.82	67.66	49.14	51.42
	1.33	4.17	1.44	3.81	3.28	3.82
24	49.27	96.70	45.95	73.19	59.24	53.24
	3.13	8.06	3.24	4.89	5.54	3.55
48	55.10	109.43	59.66	85.24	74.82	63.49
	2.09	7.30	2.30	5.69	6.43	3.71
72	54.41	110.23	69.35	87.62	67.40	55.60
	3.05	10.34	4.02	4.17	5.62	2.09
96	40.98	128.15	78.58	98.26	77.64	ND
	2.98	8.57	3.58	6.66	5.15	--
BCF	28.85	205.70	198.80	153.00	142.50	60.35

Table 5.22 Accumulation of Zn in M.dobsoni exposed to 0.4 ppm Zn at $25x10^{-3}S$

Time (h)	Zn concentration ug g^{-1} dry wt (Mean ± SD)					
	Muscle	Gill	Exoskeleton	Viscera	Exoskeleton + viscera	W hole body
Control	49.33	87.01	38.82	67.66	49.14	51.42
	1.33	4.17	1.44	3.81	3.28	3.82
24	20.53	123.85	76.12	88.94	62.88	57.01
	1.68	11.19	6.38	7.30	4.52	2.39
48	52.88	130.73	58.71	84.81	87.29	62.76
	2.65	7.93	3.54	2.85	3.30	4.15
72	47.46	144.68	76.03	103.94	73.96	68.29
	1.73	9.64	1.90	6.38	4.96	3.87
96	59.99	135.51	120.06	122.94	112.22	91.93
	6.49	12.69	14.88	7.19	7.50	6.09
BCF	26.65	144.18	203.10	138.20	157.70	101.28

The order of accumulation of Zn in different body parts of *M. dobsoni* is :

Gill > Exoskeleton > Viscera > Muscle

5.2.6 Effect of Size Group on the Accumulation of Hg, Cu and Zn in *M. dobsoni*.

Hg, Cu and Zn accumulated in different size groups of *M. dobsoni* at $5x10^{-3}S$ on exposure to 0.005, 0.25 and 0.2 mg l^{-1} of Hg, Cu and Zn respectively are graphically represented in Fig. 5.7. The accumulation was maximum in the lower size group (30-40 mm) for all the metals and minimum in the case of higher size group (50-60 mm) in that order. Hg exhibited depuration during accumulation especially in the lower size group as observed in the previous experiments. The accumulation of Zn decreased after the initial uptake and came close to a steady state. Cu was accumulated gradually and found to be on the increase during the course of the experiment.

5.2.7 Biological Half-Life of Trace Metals

Depuration of metals in clean water (Cw=0) follows an exponential decay curve

$$\frac{dC_p}{dt} = -k_2 C_p \qquad .. \quad (3)$$

Integrating

$$C_{p(t)} = C_{p(o)}\, e^{-k_2 t}$$

t = time after transfer to clean water.

The plot of log C_p *versus* t will give a straight line, when first order kinetics for release from one compartment is followed.

The biological half-life ($t_{\frac{1}{2}}$) of the metals were calculated by the equation

$$t_{\frac{1}{2}} = \frac{\ln 2}{k_2} = \frac{0.69}{k_2} \qquad .. \quad (11)$$

where k_2 is the depuration constant.

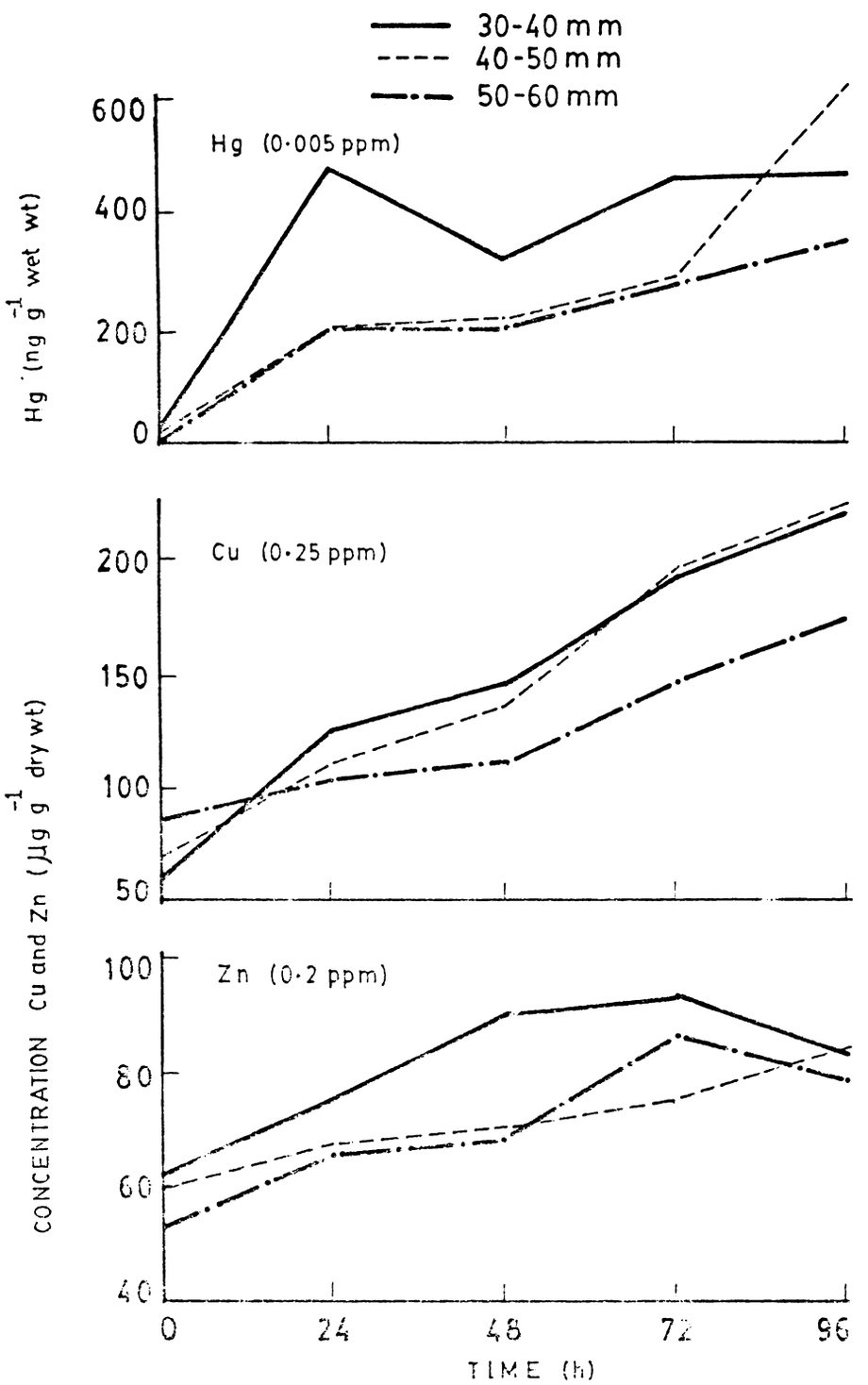

Fig.5-7. Accumulation of Hg, Cu and Zn in different size group of M. debsoni at 5×10^{-3} S.

5.2.8 Depuration of Mercury

Rate constant k_2 calculated using the kinetic model are given in Table 5.2. The rate constants for depuration in the muscle exposed to 0.005 and 0.01 mg l^{-1} Hg were almost same at 5 and $15x10^{-3}$S except for a low value in the lower concentration at $5x10^{-3}$S. Depuration rate was same in gill tissue at the two exposure concentrations in two salinities. In exoskeleton + viscera and whole body, low values were observed in higher exposure concentrations at the salinities tested.

The depuration pattern of Hg in *M. dobsoni* is graphically represented in Fig. 5.8. In the muscle at $5x10^{-3}$S accumulation trend persisted for 3 days even after changing the animals to clean water. Similar observation was noticed in exoskeleton + viscera at lower salinity. After 12 days of depuration reaccumulation of Hg in muscles and exoskeleton + viscera was noticed.

Two stage depuration mechanism was clearly observed in the case of gill tissue in both the salinities. A rapid depuration followed by reaccumulation and afterwards a slow release of accumulated Hg. All the body parts exposed to higher concentration of Hg at $15x10^{-3}$S followed the same two component depuration mechanism. The values of k_2 and $t_{\frac{1}{2}}$ were calculated based on the depuration of slow release component.

Biological half-life of Hg in muscle was about 6.4 d for slow release component (Table 5.23). In low exposure concentration at $5x10^{-3}$S $t_{\frac{1}{2}}$ was 13.82 d for the muscle. Gill tissues at both salinities and exposed Hg concentrations showed an average half-life of 7.29 d. Average $t_{\frac{1}{2}}$ for exoskeleton + viscera and whole body was 4.55 d for the animals exposed to 0.005 mg l^{-1} Hg and 15.35 d for 0.01 mg l^{-1} Hg at the salinities tested.

5.2.9 Depuration of Copper

Depuration rate constants of Cu in *M. dobsoni* exposed to 0.25 and 0.5 mg l^{-1} Cu at 5 and $15x10^{-3}$S are presented in Table 5.9. The values did not vary much in the muscle. Relatively high values of k_2 for gill tissues at $5x10^{-3}$ was due to the fact that k_2 was calculated based on the fast releasing component of depuration whereas in all other cases k_2 was calculated based on the slow release component. This was necessitated because there was no change in the rate of depuration of the slow release component. For comparison k_2 for 0.25 mg l^{-1}

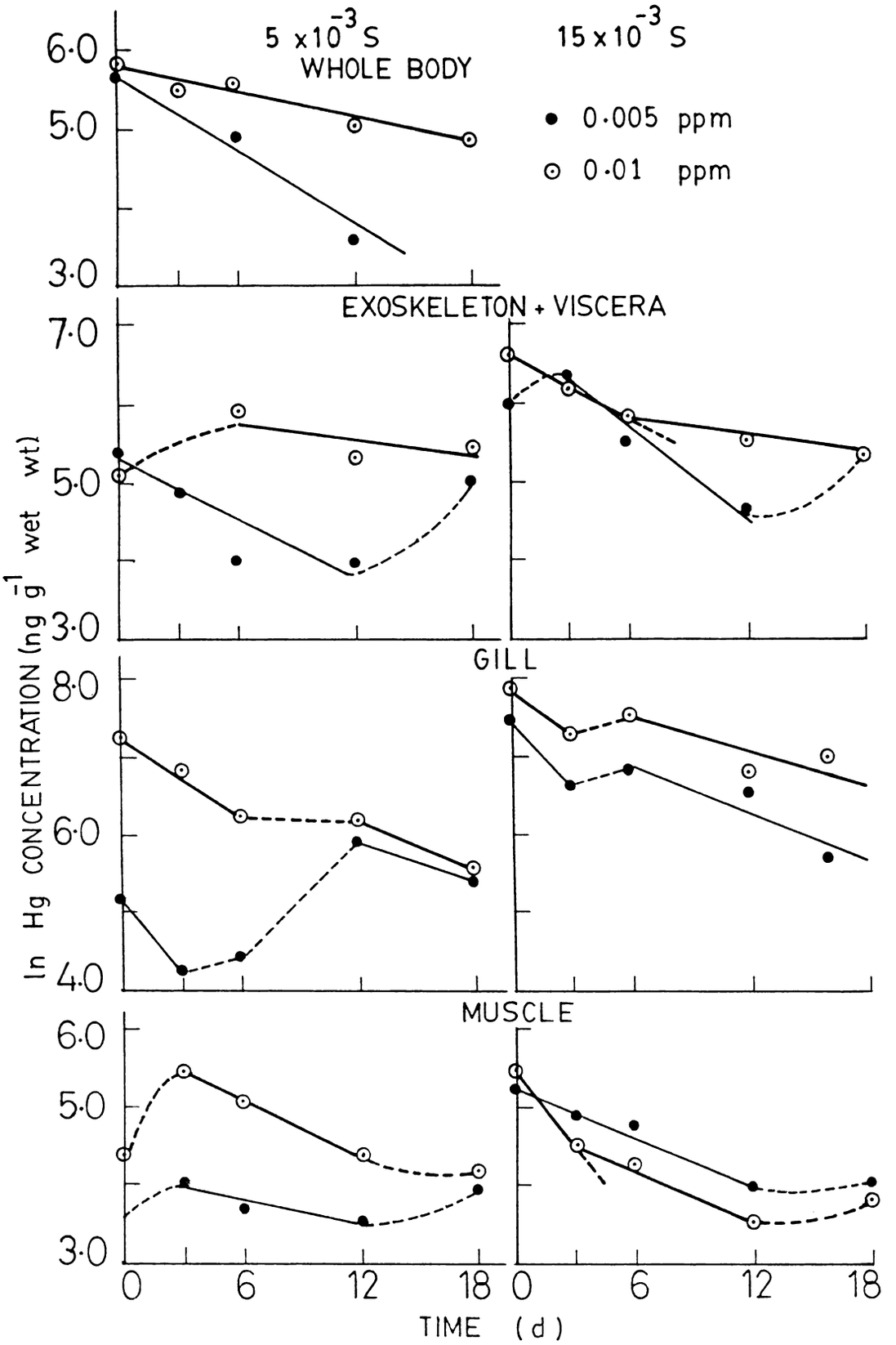

Fig.5-8. Depuration of Hg in M.dobsoni at 5 and 15×10^{-3} S.

Table 5.23 Biological half-life of Hg in M. dobsoni

Salinity (10^{-3})	Hg con. in exposure medium mg l^{-1}	k_2 (h^{-1})	$t_{\frac{1}{2}} = \frac{0.69}{k_2}$ (h)	$t_{\frac{1}{2}}$ (d)
		Muscle		
5	0.005	0.00208	331.73	13.82
	0.01	0.00521	132.44	5.51
15	0.005	0.00416	165.63	6.90
	0.01	0.00416	165.63	6.90
		Gill		
5	0.005	0.00372	185.48	7.73
	0.01	0.00473	145.87	6.08
15	0.005	0.00468	147.44	6.14
	0.01	0.00312	221.15	9.21
		Exoskeleton + viscera		
5	0.005	0.00521	132.44	5.52
	0.01	0.00208	331.73	13.82
15	0.005	0.00810	85.18	3.55
	0.01	0.00156	442.30	18.42
		Whole body		
5	0.005	0.00625	110.40	4.60
	0.01	0.00208	331.73	13.82

was also taken for the fast releasing component. The rate constants for exoskeleton + viscera and whole body at the exposure concentrations and salinities are in the same range (Table 5.9).

Muscle in both exposure concentrations and salinities exhibited accumulation after the commencement of depuration experiment (Fig. 5.9). Average biological half-life for the muscle was 14.25 d and 24.88 d respectively at 5 and $15x10^{-3}S$ irrespective of the concentration of the exposed media during accumulation (Table 5.24). The average half-life for the fast component in gill tissue at $5x10^{-3}S$ was 3.17 d and that of the slow releasing component at $15x10^{-3}S$ was 11.5 d and 35.93 d respectively for 0.25 and 0.5 mg l^{-1} exposure concentration during accumulation. Exoskeleton + viscera exhibited $t_{\frac{1}{2}}$ of 8.85 d and 18.43 d at $5x10^{-3}S$ and 38.85 d and 46.37 d at $15x10^{-3}S$ respectively for 0.25 and 0.5 mg l^{-1} exposure concentration. The average $t_{\frac{1}{2}}$ for whole body was 5.5 d at $5x10^{-3}S$.

5.2.10 Depuration of Zinc

The depuration pattern of Zn in whole body and body parts in *M. dobsoni* exposed to 0.2 and 0.4 mg l^{-1} Zn at 5 and $15x10^{-3}S$ are represented in Fig. 5.10. It can be seen that except for the fast release of a small amount of Zn in a few cases, the depuration of Zn was very slow so that the calculation of depuration rate constant k_2 became impracticable within the duration of the experiment. Hence the calculation of k_1, K_b and $t_{\frac{1}{2}}$ were not feasible in the case of Zn.

5.3 DISCUSSION

In the process of bioaccumulation, the chemical state as well as the kinetics of transformation between species of dissolved trace metals in seawater are of prime importance (Raspor, *et al.*, 1978; Allen *et al.*, 1980; Nurnberg, 1982). Other important factors are those relating to the state of an organism such as its age, size, stage in life history and so on. Many of the factors that influence rates of absorption are those which have been recognized as having an important influence on acute toxicity of pollutants (Bryan, 1976b).

The primary barrier between an organism and its environment is the cell membrane. Therefore, the toxicity of many agents is related to their lipid solubility and thus to their ability to pass through the lipoprotein membrane of

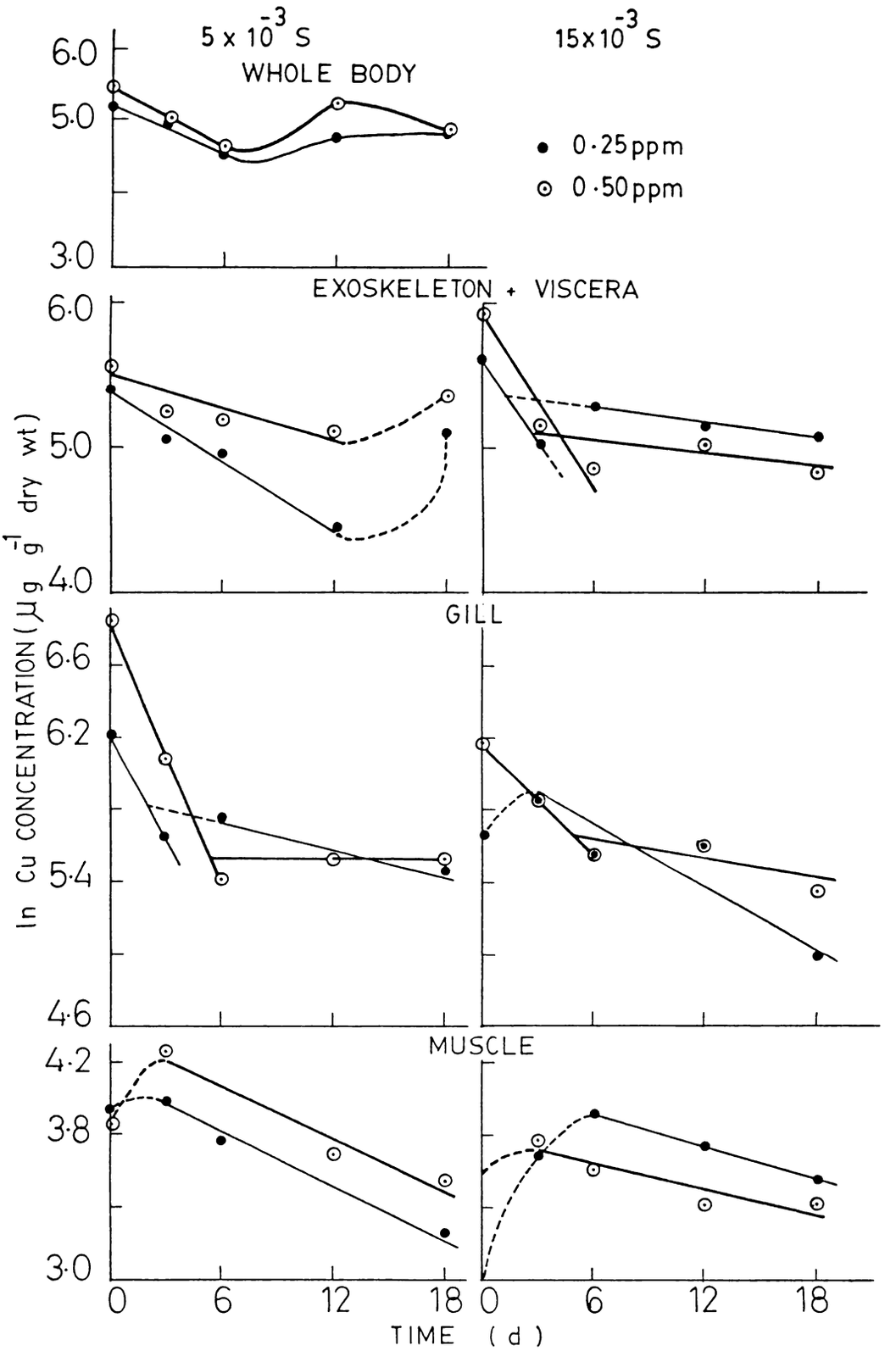

Fig.5-9.Depuration of Cu in M.dobsoni at 5 and 15x10^{-3} S.

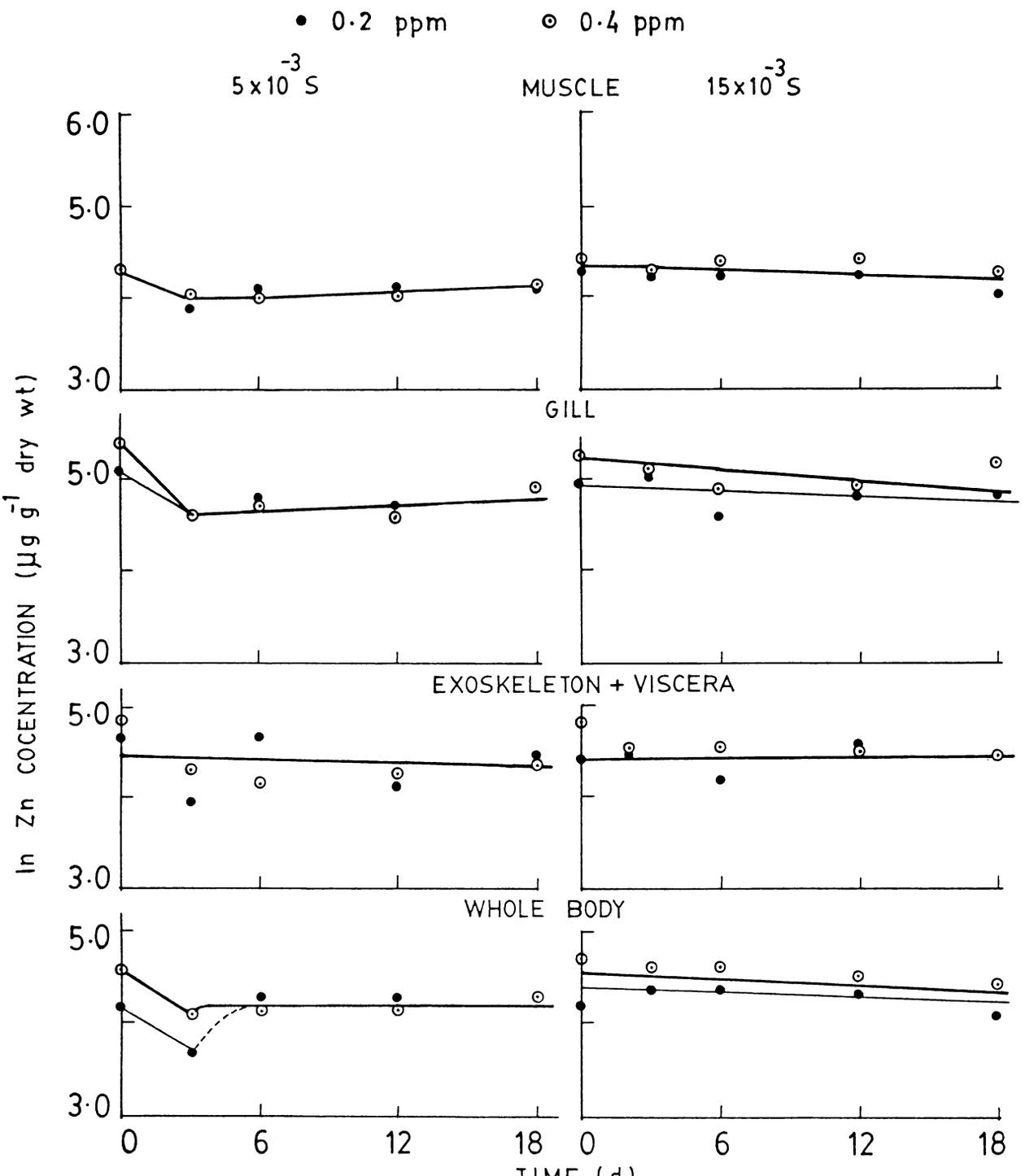

Fig. 5-10. Depuration of Zn in M. dobsoni at 5 and 15×10^{-3} S.

Table 5.24 Biological half-life of Cu in M. dobsoni

Salinity (10^{-3})	Cu con.in exposure medium mg l^{-1}	k_2 (h^{-1})	$t_{\frac{1}{2}} = \frac{0.69}{k_2}$ (h)	$t_{\frac{1}{2}}$ (d)
		Muscle		
5	0.25	0.00208	331.73	13.82
	0.50	0.00196	352.04	14.67
15	0.25	0.0013	530.76	22.12
	0.50	0.00104	663.46	27.64
		Gill		
5	0.25	0.00833	82.83	3.45
	0.50	0.01	69.00	2.88
15	0.25	0.0025	276.00	11.50
	0.50	0.0008	862.50	35.93
		Exoskeleton + viscera		
5	0.25	0.00325	212.31	8.85
	0.50	0.00156	442.31	8.43
15	0.25	0.00074	932.43	38.85
	0.50	0.00062	1112.90	46.37
		Whole body		
5	0.25	0.00473	145.88	6.08
	0.50	0.00586	117.75	4.91

the cells (Nelson and Donkin, 1985). Since the lipid soluble species tend to be retained in the membranes and fatty tissues of the organisms, bioavailable compounds are very often accumulated. Many fundamental processes like respiration, nerve conduction and muscle contraction take place on membrane surfaces. As a result, the toxicity of lipid soluble compounds often depend on their interference with these mechanisms.

Membrane transport depends not only on lipid solubility but various carrier species in the membrane also serve to facilitate the passage of molecules or ions. These carriers may be proteins in the membrane or phospholipids themselves (George, 1982). A metal which exhibits a higher binding affinity for a carrier protein may also be toxic due to its interference with various enzyme systems e.g., Hg (Simkiss, 1983). Zn and Cu associated with the soft tissues of the oyster *Ostrea edulis* are in two forms: one a relatively solubilized component and the second a component firmly bound to tissue residues, probably cell membrane residues (Coombs, 1974). The soluble complexes can act as a freely available mobile reserve of metal to ensure a constant saturation of metal-dependent enzyme systems operating under adverse environments.

The rates of uptake and excretion of a pollutant in an organism determine, to a great extent, the degree of time integration of ambient pollutant levels exhibited by that organism (Phillips, 1980). *M. dobsoni* was found to accumulate Cu and Zn as the time advances, although there was difference in the rate of accumulation between the metals. A steady state was not achieved during 96 h of exposure in the whole body or body parts (Fig. 5.2 to 5.6). Hg behaved altogether differently that even during the accumulation, *M. dobsoni* was found to shed the Hg load after reaching the threshold concentration exhibited by the whole body and body parts depending on the salinity of the exposure medium as described in 5.2.3. Fowler *et al.* (1975) has pointed out that uptake and excretion rates of pollutants in laboratory animals may vary with the dosage, route and length of exposure period.

The process by which pollutants cross the absorptive surfaces of marine organisms is not well understood. Coombs and George (1978) discussed the possibilities of carrier mediated heavy metal transport in larger organisms. Most of the evidence for metals points to uptake being passive, although this does not exclude some sort of carrier mediation. In some species there is direct proportionality between

rates of absorption and external concentration, but in other species possibly owing to adsorption characteristics of the metal, the rate increases rather more slowly than the external concentration (Bryan, 1979). At lower salinity accumulation of Hg and Zn in *M. dobsoni* increased with increase in ambient metal concentration. At higher salinities rate constant either remained constant or the trend was reversed. In *Callianassa austreliensis* Zn and Cu concentration increased in whole body in a quadratic fashion with the increase in concentration of the water (Ahsanullah *et al.*, 1981a). Bryan (1976b) showed that in most tissues the Zn content increased markedly only after exposure to dissolved Zn concentrations above 0.2 mg Zn l^{-1}. It has been observed that in decapod crustaceans such as crab *Carcinus maenas*, the concentration of Zn remains comparatively independent of that in the environment both in the laboratory and in the field (Bryan, 1971, 1976a).

Rate constant for Cu at $5x10^{-3}$S in gill tissue increased with increase in concentration of the medium. Except for the muscle in other cases rate constant was not affected by exposure concentration. The higher rate of uptake in the muscle in lower concentration may be attributed to the high bioavailability of Cu at lower concentrations. It may be recalled that in the toxicity tests irrespective of size group Cu was found to be more toxic at $5x10^{-3}$S (Chapter 4).

The effect of salinity on the uptake of trace metals by biota is more direct in nature. The higher rate constants for accumulation of Cu at lower salinities in muscle and gills of *M. dobsoni* indicate the presence of more bioavailable Cu at lower salinities. It may be noted that at 15 and $25x10^{-3}$S Cu was precipitated which lessens the availability of Cu for bioaccumulation. Hg and Zn at lower exposure concentrations showed increase in k_1 values in muscle and gill with increase in salinity. At higher concentrations the trend was reversed showing greater accumulation at lower salinities that the metal is made more available at lower salinities. During toxicity tests Hg and Zn showed more dependency on size group and salinity (Cahpter 4).

In decapod crustaceans the absorption of Zn and probably several other metals from solution is almost certainly a passive process involving adsorption on the cuticle of the gills and inward diffusion probably attached to organic molecules (Bryan, 1971). In *M. dobsoni* gill was found to be the major site of accumulation for Hg, Cu and Zn. The rate constant k_1 is maximum in the case of Cu and the least for Zn. K_b values of Cu for gill varied from 3000 to 7800 and for Hg 800-1100 depending on the exposure salinity and concentration of the

medium. k_1 values ranged from 6.24 to 40.0 h^{-1} for Cu and 3.5 to 5.05 h^{-1} for Hg and 0.2 to 0.7 h^{-1} for Zn (Table 5.2, 5.9 and 5.16). Accumulation of high amounts of Hg in gills were reported in oysters *Crassostrea gigas*, *Crassostrea virginica* (Cunningham and Tripp, 1975b; Okazaki and Panietz, 1981). Gills are reported to be the most important route through which Zn is absorbed when fish is exposed to high concentrations (Hughes and Flos, 1978).

Bjerregaard and Vislie (1986) pointed out that exposure to Cu augments Cu concentration in gills and carapace, and passive absorption probably plays a role in Cu uptake. He further points out that copper taken up in the haemolymph from low ambient concentration is transferred to hepatopancreas, keeping the Cu level in the haemolymph and other organs constant; but at higher concentrations this regulatory mechanism breaks down causing an increase in copper levels in all internal organs. Lyon *et al.* (1984) found that Cu, Cd and Zn injected into haemolymph of the crayfish *Austropotamobius pallipes* was readily removed by uptake in hepatopancreas. A comparable mechanism is found in the shrimp *Palaemon elegans* which regulates its whole body Cu content at exposures upto 0.1 mg l^{-1} for 21 d, while at higher exposure concentrations the whole body content increases linearly with increasing ambient concentrations (White and Rainbow, 1982). Exposure to lethal concentrations disrupt (10 mg Cu l^{-1}) or alter (1 mg Cu l^{-1}) regulation of all the major inorganic ions in the haemolymph of *Carcinus maenas* (Bjerregaard and Vislie, 1986). *M. dobsoni* exposed to 0.2 and 0.4 mg l^{-1} Cu might have lost their regulatory capacity due to the higher exposure concentration and accumulated Cu in gill, muscle and whole body. Although changes in the concentration of inorganic ions in the haemolymph are probably not the direct cause of death, marked effect of lethal copper concentrations or ion regulation may in part be responsible for the higher toxicity of Cu towards euryhaline invertebrates at low salinities (Hunter, 1949; Bryan 1976a).

Zn is transported to the tissues bound to blood proteins, mainly haemocyanins and can be stored in hepatopancreas or excreted via the antenal gland, gut or gills (Bryan 1984). The loss of Zn in the crab *Carcinus maenas* over the gills *Homarus gammarus* through the urine and *Austropotamobius pallipes* through the faeces are important (Bryan, 1967). As a result of those processes excess Zn can to a large extent be eliminated. Similar mechanism may be responsible for the low values of k_1 for Zn in *M. dobsoni* compared to Cu and Hg.

The concentration of trace metals present in an organism can vary with age of the organism. Age dependence may be a function of the period over which an organism has been exposed to a pollutant. Preferences might also exist in preferred food of young and old individuals of a species; which would lead to age dependent variations in the amounts of the trace metal ingested. While Hg concentrations tend to increase with age in aquatic biota (especially finfish), other metals commonly exhibit higher concentrations in younger or smaller individuals, growth being accompanied by decreased levels (Phillips, 1980). Garcia and Fowler (1972) noted higher concentrations of several elements in smaller individuals of *Penaeus californiensis*, samples being derived from the Gulf of California.

The effect of size on mercury uptake from solution and/or food were studied by Hannerz (1968); both pike *Exox lucius* and pike perch were found to exhibit reciprocal relationship between body length and Hg accumulation. The smaller individuals thus accumulated greater concentration of the element in a given exposure period. The most likely explanation suggested by the author was that smaller fish have a larger surface area : volume ratio and therefore would be expected to accumulate Hg faster from solution than would larger individuals. This hypothesis holds good for the present investigations on the accumulation of Hg, Cu and Zn by different size groups of *M. dobsoni* (fig. 5.7).

There are a variety of mechanisms for removing contaminants which are accumulated. For trace metals, storage, at least of a temporary nature, is provided by the general binding capacity of compounds such as proteins (Bryan, 1979). More specific storage proteins of the metallothionein type have been discovered in various marine groups. Cd and Zn metalloproteins have been induced in the hepatopancreas of the crab *Scylla serrata* (Olafson *et al.*, 1979), Cd and Cu metallothioneins in hepatopancreas of wild *Cancer pagurus* (Overnell and Trewhella, 1979) and Cd, Zn and Cu binding proteins in the hepatopancreas of *Carcinus maenas* (Rainbow and Scott, 1979). Granular storage mechanism also exist. Excess Cu is stored as granules in the hepatopancreas of the shrimp *Crangon crangon* (Djangmah, 1970) and in the hepatopancreatic caeca of the amphipod *Corophium volutator* (Icely and Nott, 1980). Detoxification and removal of Cd from heavily contaminated shrimp *Penaeus duorarum* appears to involve incorporation in the gills followed by sloughing off the affected parts (Nimmo *et al.*, 1977). The existence of such a detoxification mechanism often leads to the presence of high metal levels in specific organs such as hepatopancreas and gills. The observed reaccumulation of Hg in gill tissue of *M. dobsoni* during depuration experiment may account for such a mechanism (Fig. 5.8).

Evidence for the regulation of Zn and Cu in decapod crustaceans has been given by Bryan (1968); but there is no evidence for the regulation of nonessential metals such as Cd or Hg. Regulation of Zn content on exposure to dissolved metal has been reported in the shrimp *Pandalus montagui* (Ray *et al.*, 1980a), shrimp *Palaemon elegans* (White and Rainbow, 1982) and *Crangon crangon* (Amiard *et al.*, 1985). *In situ* studies on the latter two species confirmed that Zn concentration in organisms are largely independent of the metal level in the environment (White and Rainbow, 1982). The prawn larvae *Palaemon serratus* exposed to overloads of Zn in seawater (525 ug l^{-1}) the concentration of Zn in the organisms increased slightly. At the concentration of 275 ug l^{-1} the level of Zn in the contaminated larvae was only 1.3 times higher than the control. When the external metal level increased there was only a slight variation in the bioaccumulation of Zn (Devineau and Amiard-Triquet, 1985). The ability to regulate Cu and Mn has been established for various species of molluscs, crustaceans and fishes (Bryan and Ward, 1965; White and Rainbow, 1982), whereas the concentration of Hg, Ag and Pb in the organisms depend on their concentrations in the environment (Bryan, 1971; Vernberg *et al.*, 1974; Ray and Tripp, 1976). All these data support the hypothesis of Bryan (1979), that essential trace elements are regulated whilst nonessential metals are not. But the margin between the threshold exposure levels beyond which internal metal levels are no longer controllable and lethal doses is very narrow.

M. dobsoni in the present experiments were exposed to 0.2 and 0.4 mg l^{-1} Zn and 0.25 and 0.5 mg l^{-1} Cu of added Cu and Zn concentrations to natural seawater may be beyond the threshold exposure levels and the regulation of these metals in *M. dobsoni* might have become impossible. Hence accumulation of Cu and Zn occurred in whole body and body parts in the present experiment (Fig. 5.2 to 5.6). Similar accumulation pattern was also observed in the experiment to determine the effect of size group on the accumulation of these metals (Fig. 5.7).

Crustaceans such as crabs, lobsters and prawns possess a chitinous exoskeleton which must be shed regularly during periods of growth. This process may have importance in determining the temporal changes in metal concentrations present in the animal. Large amounts of either radionucleides or stable metals accumulated by the exoskeleton relative to the total body load in crustaceans have been noted in studies by the authors like Pequegnat *et al.* (1969), Small (1969), Fowler

et al. (1971), Fowler and Benayoun (1974), Jennings and Rainbow (1979b) and Wright and Brewer (1979). If the uptake from the solution predominates the levels of metals in the exoskeleton commonly account for more than half of the total body load of elements and this may be correlated to the capacity of the carapace to adsorb large amounts of metals in a nonspecific fashion (Phillips, 1980). The apparent loss of metals on moulting then depends on the exposure period, as the nonspecific binding of metals to carapace is much more rapid than the metal uptake by the soft parts. The observed high BCF of Zn in exoskeleton of *M. dobsoni* accounts for the heavy load of the metal in the exoskeleton. The order of accumulation of the metals in exoskeleton was found to be Cu> Zn> Hg.

It seems possible that two or more sources of Hg existed in the animal and that the clearing rate from each source might be different. Hypothesis that would explain a fast initial clearing rate and a slow later one, is degenerative pathological change. Destruction or blocking of enzyme pathways in excretory organs could lead to progressively reduced ability to eliminate free Hg/ which would then recycle into the organism (Solan *et al.*, 1974). Further, the relatively fast clearing rates and relatively slow subsequent ones suggested two concurrent processes each with a simple negative exponential clearing rate. Similar two compartment depuration of Hg is observed in the case of *M. dobsoni*, which was more conspicuous in the case of gill tissue (Fig. 5.8). Muscle and exoskeleton + viscera exposed to 0.01 mg l^{-1} Hg at $15x10^{-3}$S followed the same two compartment depuration mechanism. Luoma (1977) showed that ^{203}Hg loss from *Neanthes succinea* and *Palaemon debilis* appeared to follow two compartment kinetics. Most of the Hg in the above two species occurred in the more slowly exchanging of the two physiological compartments. A rapid initial loss of ^{203}Hg (fast component) was noticed in *Serratus scriba*, *Carcinus maenas*, *Tapes decussatus* and *Mytilus galloprovincialis* by Miettinen *et al.* (1972).

Two compartment depuration pattern was also exhibited in the case of Cu. Gill tissue attained higher concentrations showed definite two compartment depuration (Fig. 5.9). Exoskeleton + viscera which accumulated higher Cu concentration exhibited similar process. In the case of Zn there are indications of two compartment depuration at $5x10^{-3}$S, which occurred during the first 3 days for the fast component and the depuration of slow release component was found to be very slow (Fig. 5.10). Renfro (1973) has observed an initial rapid decrease of

^{65}Zn in *Neries diversicolor* during the first 2 to 3 days, than a slow rate of loss. In *Balanus balanoides* maintained in the laboratory over a period of 7 months, after an initial steep fall in the level of incorporated radioisotope, the loss of ^{65}Zn is reduced, indicating a relatively high retention time for the ingested ^{65}Zn tending to remain in the barnacle for a significant period (White and Walker, 1981).

It hjas been shown that in general Hg was lost most rapidly from gill tissue (Cunningham and Tripp, 1975b; Smith *et al.*, 1975; Denton and Burden-Jones, 1981). The sharp decrease in Hg content in gill tissue of *M. dobsoni* during the early days of depuration agrees with the above observations (Fig. 5.8). As in the case of Hg, Cu was depurated at a faster rate from the gill during the initial period of depuration (Fig. 5.9).

For marine crustaceans, several ^{65}Zn biological half-life values are available. Cross *et al.* (1968) reported half-life for the amphipod *Anonyx* Sp. which obtained ^{65}Zn directly from seawater, to range from 90-150 days depending on temperature. Fowler *et al.* (1971) labelled *Euphausia pacifica* by feeding radioactive brine shrimp for 15 days, then measured the rate of ^{65}Zn loss over the next five months. They calculated the biological half-life for ^{65}Zn in the slow compartment to be 140 days. Sand shrimp *Crangon franciscorum* fed on radioactive *Artemia* followed the loss of ^{65}Zn and biological half-life of ^{65}Zn in the slow turnover compartment was 29-34 days. Depuration experiment with *M. dobsoni* was conducted for 18 days and there was no substantial decrease in the body content of Zn (Fig. 5.10). The duration of the experiment was found to be quite insufficient, disabling the calculation of $t_{\frac{1}{2}}$.

The biological half-life of Hg from the slowly exchanging compartment for a remarkable variety of species including polychaetes (22 days), small shrimp (16 days), estuarine poecilid fish (20 days, Luoma 1974); marine crabs (20-25 days, Solan *et al.*, 1974) have been determined. Average $t_{\frac{1}{2}}$ of *M. dobsoni* in the present studies exposed to 0.01 mg l^{-1} Hg was found to be 15.35 d.

It should always be emphasized that a biological half-life of an element in an organism is not a constant, but a variable that changes with environmental conditions, physiology of the organism and even the design of the experiment (Renfro, 1973). The interspecific difference in $t_{\frac{1}{2}}$ may be partially due to the initial total concentration of trace metal (Okazaki and Panietz, 1981). The increase in $t_{\frac{1}{2}}$ has been attributed to the possible break down of a cellular-physiological

mechanism of trace metal disposal and subsequent permanent deposition of the metal in the tissues (Pringle *et al.*, 1968).

Cunningham and Tripp (1975a) have recognized the following categories of trace metal release : (i) increase in $t_{\frac{1}{2}}$ with increase in the body burden of trace metal, (ii) stable $t_{\frac{1}{2}}$ when an equilibrium is maintained by a proportionate increase in the rate of trace metal loss as its body burden increases and (iii) decrease in $t_{\frac{1}{2}}$ with increase in body burden of a trace metal.

For considering an organism as biological indicator Butler *et al.*(1971) suggested some basic prerequisites which have been properly amended by Haug *et al.* (1974) and Phillips (1976, 1977a) (See Appendix IV). Except for its nonsedentary nature, *M. dobsoni* fulfills all the requisites suggested. The use of *Parapenaeus longirostris* moving freely away from the shore has been suggested by Satsmadjis and Voutsinou-Taliadouri (1983) which is more representative of the pollution of whole body of water than the use of molluscs more or less confined to a site.

Based on the above factors, *M. dobsoni* is recommended as a pollution-indicator. While recommending the species as an indicator organism of trace metal pollution in the National level the following points may be clarified.

(i) Whether the organism would exhibit similar pattern of accumulation trend when exposed to natural levels, since the present investigations were based on the addition of trace metals to natural background levels, that too in relatively higher concentrations.

(ii) The seasonal variation of trace metal contents described in Chapter 3 has not taken into consideration of ambient metal levels. The effect of the same may be verified.

(iii) A compilation of base line concentration of trace metals in *M. dobsoni* from different laboratories would help in assessing its potential use as an indicator organism.

Based on the data of *M. dobsoni* in the present investigation and in comparison with the data for similar organisms elsewhere, it is recommended that *M.dobsoni* may be taken up as an indicator organism at the National level.

CHAPTER 6

EFFECT OF TRACE METALS ON OXYGEN CONSUMPTION OF *METAPENAEUS DOBSONI*

Respirometry is among the simplest means of establishing quantitative assessments of toxicant effects by control and test organism. Although respirometry applied to aquatic organisms cannot be claimed as a technique with any degree of specificity in the identification of toxic mechanisms, it is valuable in detecting signs of metabolic involvements (Cheng and Rodrick, 1974). Oxygen consumption was chosen as a parameter to assess the stress because it is a valuable indicator of energy expended to meet the demands of an environmental alteration. When combined with coincident chemical analysis of contaminants accumulated within tissues, the measurement of physiological responses can identify specific anthropogenic stressors (Widdows *et al.*, 1981; Widdows, 1985).

Several authors have reported altered respiratory rates due to exposure to sublethal levels of heavy metals (Jones, 1942; Corner and Sparrow, 1956; Benhard and Lane, 1963; Vernberg and Vernberg, 1972b; De Coursey and Vernberg, 1972; Thurberg *et al.*, 1973; Coller *et al.*, 1973; Green *et al.*, 1976; Reeve *et al.*, 1977; Saliba and Vella, 1977; Moraitou-Apostolopoulou and Verriopoulos, 1979; Papathanassiou, 1983; Byczkowski and Sorenson, 1984; Depledge, 1984; Hutcheson *et al.*, 1985; Diaz-Mayans *et al.*, 1986).

Among the Indian species respiratory effect of Cu on barnacles *Balanus amphitrite amphitrite* and *B. tintinnabulum tintinnabulum* has been reported by Prabhakara Rao, *et al.* (1985) and Patil and Kaliwal (1983) used freshwater prawn *Macrobrachium hendersodyanum* to examine the influence of Cd, Cu and Zn on oxygen consumption.

The present study was designed to evaluate the sublethal effects of Hg, Cu and Zn on the oxygen consumption of *M. dobsoni*.

6.1 MATERIALS AND METHODS

Procedure for the determination of oxygen consumption is given in 2.15. Metal concentration of the exposure medium, water quality characteristics, wt, length and volume of the animals used for the experiment are given in Table 6.1. Oxygen consumption for a minimim of 10 animals were determined in each set of experiments.

6.2 RESULTS

The allometric relation between body weight (W) and oxygen consumption rate (Y)

$$W = a\ Y^{b}$$

where a and b are fitted parameters, was used in the present study (Sokal and Rohlf, 1969).

The above relation can be represented in the linear form

$$\log W = \log a + b \log Y$$

Table 6.1 Metal concentrations, water quality characteristics, wt, length and volume of *M.dobsoni* used for determination of oxygen consumption (Mean ± SD)

Metal concentration ($mg\ l^{-1}$)	Salinity (10^{-3})	pH	Temp (°C)	*M. dobsoni* Wet wt	Length	Volume
Hg						
0.0025	15.313	6.85	27.5	0.5906	50.00	0.64
0.005	±	±	±	±	±	±
0.01	0.23	0.15	0.5	0.1094	3.25	0.12
Cu						
0.1	15.60	6.90	26.50	0.5429	47.80	0.61
0.25	±	±	±	±	±	±
0.50	0.21	0.10	0.71	0.1186	3.22	0.14
Zn						
0.1	15.25	6.88	27.5	0.5416	48.20	0.58
0.2	±	±	±	±	±	±
0.4	0.13	0.05	0.05	0.0621	2.04	0.10

To minimize the variation due to differences in size, the measured rates of oxygen consumption was transformed for a standard animal of 1 g dry tissue wt using the relationship

$$Y_s = \left(\frac{Ws}{We} \right)^b Y_e$$

where Y_s = rate of oxygen consumption of a standard sized individual

W_s = the weight (1 g)

W_e = the weight of the experimental animal

Y_e = the uncorrected rate of oxygen consumption

b = the corresponding weight exponent

(after Bayne and Newell, 1983).

6.2.1 Oxygen Consumption in Relation to Body wt.

The oxygen consumption of *M. dobsoni* was determined for different body weight varying from 0.0716 g to 0.1954 g, dry wt (Table 6.2). The regression of log wt against log oxygen consumption was calculated and represented in Fig. 6.1. The 'b' value estimated was 0.5828.

6.2.2 Oxygen Consumption of *M. dobsoni* Exposed to Hg

Rate of oxygen consumption (ml g^{-1} dry wt h^{-1}) of *M. dobsoni* exposed to 0.0025, 0.005 and 0.01 mg^{-1} Hg for 96 h at intervals of 24 h are given in Table 6.3. Oxygen consumption decreased in all the concentrations tested. The decrement was found to be statistically significant at the lowest concentration of 0.0025 mg l^{-1}. However, the depression in oxygen consumption did not show direct relation with the concentration of the exposure medium. After the initial depression the rate was found to be enhanced to the level as in control and thereafter a gradual decrease was noted (Fig. 6.2).

6.2.3 Oxygen Consumption of *M. dobsoni* Exposed to Cu

In Table 6.4 the rate of oxygen consumption (ml g^{-1} dry wt h^{-1}) of *M. dobsoni* exposed to 0.1, 0.25 and 0.50 mg l^{-1} Cu are given. In all the concentrations a depression in oxygen consumption was noticed. The depression was highly significant at higher concentration. It appears that impairment on oxygen consumption

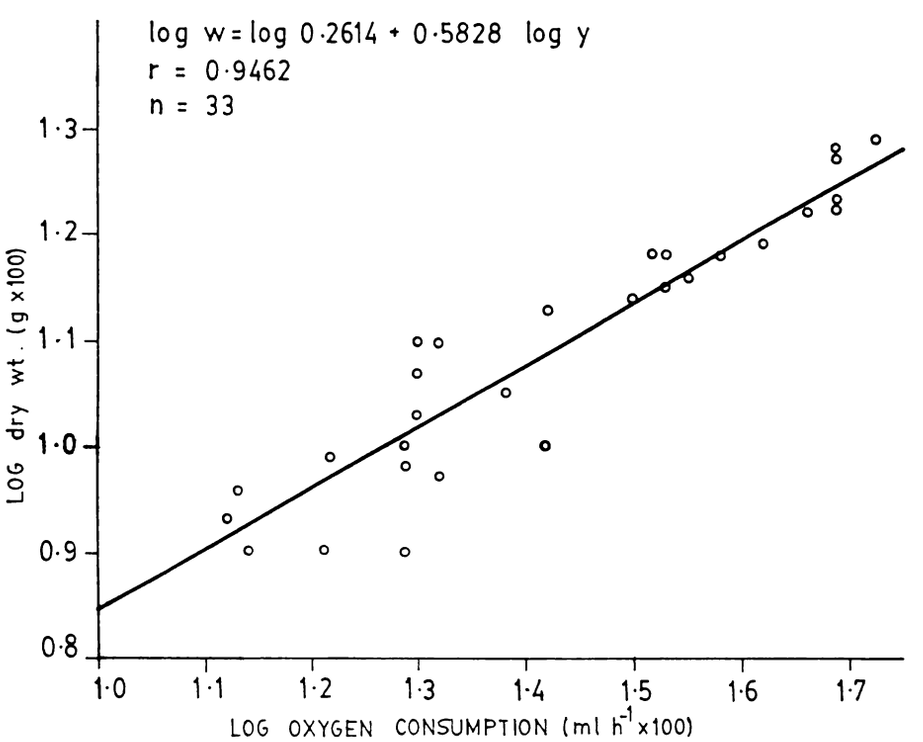

Fig.6-1. Relationship between body weight and oxygen consumption in M. dobsoni.

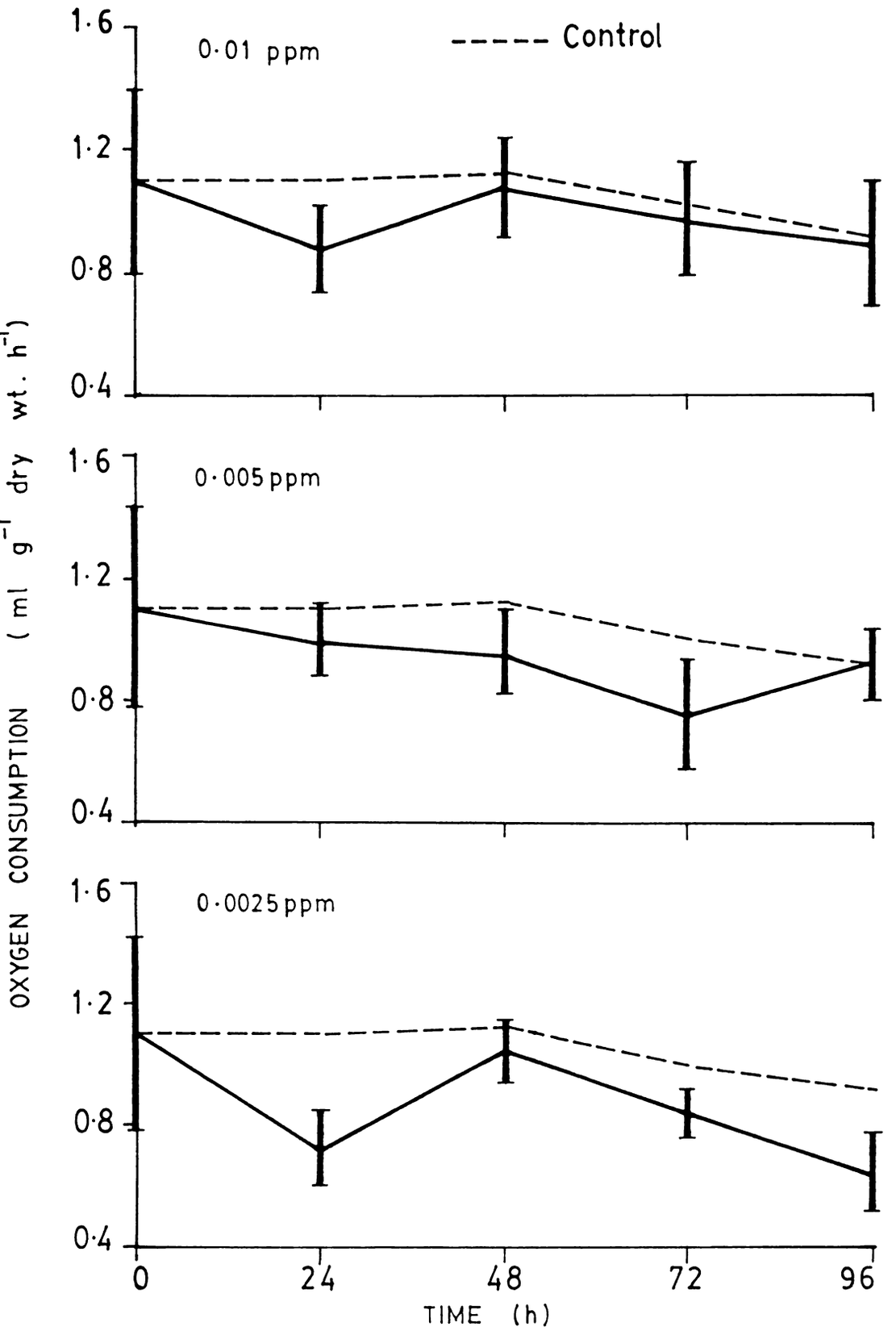

Fig.6-2. Oxygen consumption of M. dobsoni exposed to Hg.

Table 6.2 Oxygen consumption of *M. dobsoni* in relation to body weight

Sl.No.	Body dry wt (g)	Oxygen uptake ml h^{-1}	Sl.No.	Body dry wt (g)	Oxygen uptake ml h^{-1}
1	0.0716	0.1007	18	0.1264	0.2115
2	0.0735	0.1019	19	0.1275	0.2028
3	0.0795	0.1460	20	0.1361	0.2646
4	0.0799	0.1659	21	0.1397	0.3173
5	0.0800	0.1991	22	0.1410	0.3404
6	0.0853	0.1337	23	0.1443	0.3581
7	0.0910	0.1342	24	0.1506	0.3395
8	0.0931	0.2100	25	0.1515	0.3380
9	0.0965	0.1991	26	0.1521	0.3851
10	0.0975	0.1660	27	0.1564	0.4260
11	0.0980	0.1671	28	0.1657	0.4583
12	0.1008	0.1991	29	0.1684	0.4970
13	0.1011	0.1991	30	0.1715	0.4970
14	0.1014	0.2646	31	0.1881	0.4936
15	0.1091	0.2020	32	0.1934	0.4901
16	0.1154	0.2403	33	0.1954	0.5730
17	0.1195	0.2006			

Table 6.3 Rate of oxygen consumption (ml g^{-1} dry wt h^{-1}) of *M. dobsoni* exposed to different concentrations of Hg (Mean ± SD)

Time (h)	Hg concentration (mg l^{-1})			
	0	0.0025	0.005	0.01
24	1.1044	0.7231**	1.0077	0.8974
	0.2729	0.1107	0.1174	0.1409
48	1.1109	1.0231	0.9661	1.0817
	0.2064	0.1056	0.1403	0.1691
72	1.0594	0.8444*	0.7524**	0.9969
	0.1967	0.0884	0.1736	0.1879
96	0.9243	0.6529	0.9215	0.9060
	0.0800	0.1180	0.1374	0.2050

* $P < 0.05$, ** $P < 0.01$

Table 6.4 Rate of oxygen consumption (ml g^{-1} dry wt h^{-1}) of *M. dobsoni* exposed to different concentrations of Cu (Mean ± SD)

Time (h)	Cu concentration (mg l^{-1})			
	0	0.1	0.25	0.50
24	1.2627	1.1094	0.8950**	0.8976*
	0.1904	0.2141	0.0928	0.3914
48	1.2102	1.1411	0.9298*	0.8875**
	0.2091	0.1422	0.0967	0.1223
72	1.1174	0.8836*	0.8122*	0.8210**
	0.2102	0.1686	0.0830	0.1373
96	0.9936	0.8841	0.7195*	0.6408**
	0.1465	0.1076	0.2346	0.0824

* $P < 0.05$, ** $P < 0.01$

increases with concentration of the exposure media. After a rapid initial depression the rate of oxygen consumption was maintained almost in the same level (Fig. 6.3).

6.2.4 Oxygen Consumption of *M. dobsoni* Exposed to Zn

The effect on the rate of oxygen consumption of *M. dobsoni* on exposure to 0.1, 0.2 and 0.4 mg l^{-1} of Zn is given in Table 6.5. The rate was found to be enhanced in all the concentrations tested. The enhanced rate of uptake was statistically significant at 48 h in all the exposure concentrations. At 0.4 mg l^{-1} the effect was highly significant upto 72 h. It can be seen that the rate of oxygen consumption increases gradually in all the cases and after reaching a maximum value of about 1.2 ml g^{-1} dry wt h^{-1}, the rate of uptake was found to be on the decreasing trend coming close to the control level (Fig. 6.4).

6.3 DISCUSSION

The regression of log oxygen consumption on log wt in *M. dobsoni* in the present studies has a slope ('b') of 0.5828. Kuttyamma (1978) working on the same species obtained the 'b' value as 0.5031 in acclimation salinity $30x10^{-3}$. Subrahmaniam (1962) and Kutty (1969) has reported the 'b' value as 0.604 and 0.501 respectively for *Penaeus indicus*. The exponent 0.5828 in the present study is thus seen to be in close agreement with the values reported.

Mercury is known to decrease the crustacean metabolism. In the present study *M.dobsoni* showed reduced rate of oxygen consumption in 0.0025, 0.005 and 0.01 mg l^{-1} Hg at $15x10^{-3}$S. *Carcinus maenas* exposed to 0.1 mg l^{-1} Hg showed depressed respiratory activity (Depledge, 1984). In the crab *Uca pugilator* when larvae or adults are exposed to Hg, respiration was depressed (Vernberg *et al.*,1974). Vernberg and Vernberg (1972b) reported mercury induced changes in respiration of fiddler crab *Uca pugilator* exposed to 0.18 mg l^{-1} Hg for 21 days. On exposure to Hg oxygen consumption was generally lowered in the prawn *Palaemon serratus* when specimens were placed in toxic solutions (Papathanassiou, 1983). Green *et al.*, (1976) observed that the respiratory rates of the post larval white shrimp *Penaeus setiferus* on long term exposure to low levels of Hg (0.5 and 1.0 ug l^{-1} Hg) were not significantly different.

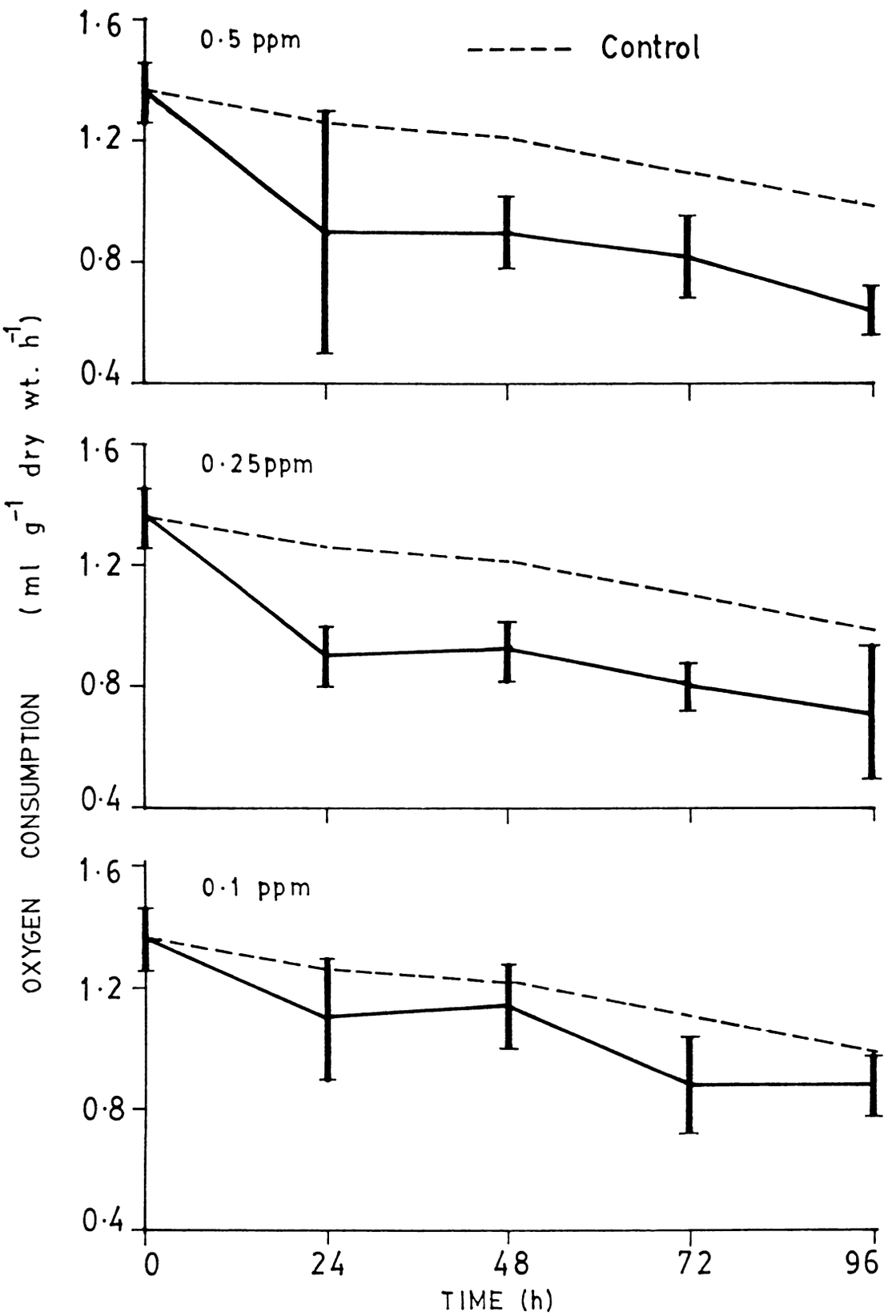

Fig.6-3. Oxygen consumption of M.dobsoni exposed to Cu.

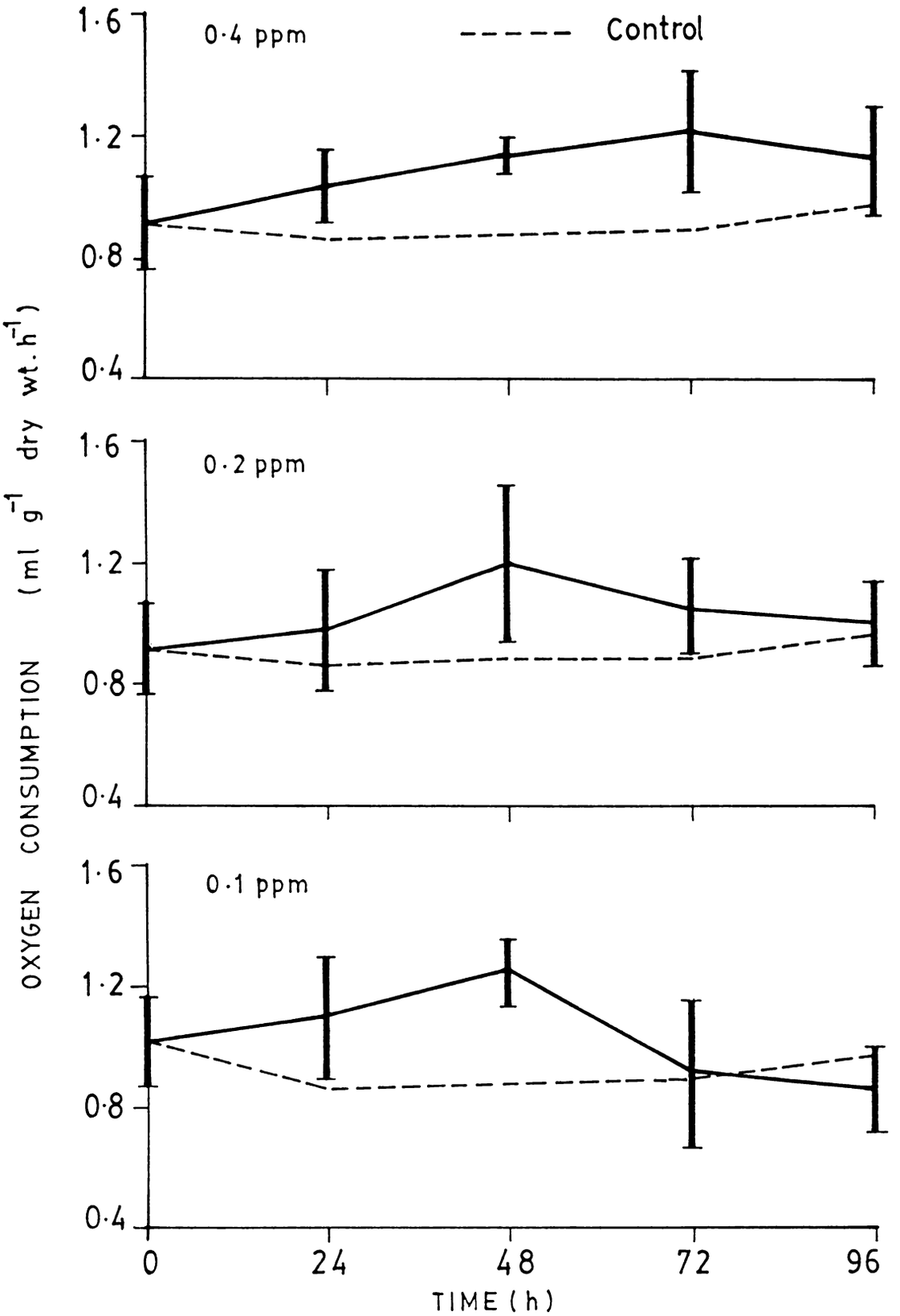

Fig. 6-4. Oxygen consumption of M.dobsoni exposed to Zn.

Table 6.5 Rate of oxygen consumption (ml g^{-1} dry wt h^{-1}) of *M. dobsoni* exposed to different concentrations of Zn (Mean ± SD)

Time (h)	Zn concentration (mg l^{-1})			
	0	0.1	0.2	0.4
24	0.8605	1.1006**	0.9863	1.0419**
	0.1263	0.2060	0.1988	0.1274
48	0.8881	1.2750**	1.2008*	1.1475**
	0.0978	0.1290	0.2680	0.0687
72	0.8974	0.9202	1.0538	1.2289**
	0.1583	0.2554	0.1766	0.1942
96	0.9759	0.8608	1.0149	0.1379
	0.1620	0.1364	0.1369	0.1719

* $P < 0.05$ ** $P < 0.01$

Cu also was found to be a respiratory depressent in *M. dobsoni*. Similar observations have been made in *Artemia* (Corner and Sparrow, 1956), in the freshwater prawn *Macrobrachium hendersodyanum* (Patil and Kaliwal, 1983) and in shore crab *Carcinus maenas* (Depledge, 1984).

The enhanced rate of oxygen consumption on exposure to Cu have been reported by authors like Moraitou-Apostolopoulou and Verriopoulos (1979) in *Acartia clausi*; and Jones (1942) in a few planktonic crustaceans. In the case of *Carcinus maenas* and *Cancer irroratus* Cu has no influence on respiration (Thurberg *et al.*, 1973).

The present investigations recorded statistically significant increase in oxygen consumption of *M. dobsoni* upto 48 h in all the concentrations of Zn tested. Thereafter the rate of oxygen consumption was lowered. Patil and Kaliwal (1983) observed depressed rate of oxygen consumption in freshwater prawn *Macrobrachium hendersodyanum* on exposure to Zn. There are incidences where oxygen uptake was increased on exposure to Zn as reported by Baby and Menon (1986) in *Perna indica*.

The mechanism implied in the change of respiratory activity remain unknown, but is assumed that there exists some damage to membrane and mitochondrial levels (Thurberg et al., 1977; Byczkowski and Sorenson, 1984). Hubschman (1967) found that cell death in presence of Cu ions resulted from the failure of cell repair mechanisms to keep pace with damage. Under these circumstances cell survival is dependent not only on the amount of contaminent reaching the tissues but also on its rate of accumulation. Thus if heavy metal ions accumulate slowly, cellular repair mechanisms are more likely to keep pace with damage than if the ions accumulate rapidly.

In *M. dobsoni* gill was found to be the major site of accumulation for Hg, Cu and Zn. The rate constant for accumulation is maximum for Cu and the least for Zn (Chapter 5). The k_1 values ranged from 6.24 to 40.0 h^{-1} for Cu, 3.5 to 5.05 h^{-1} for Hg and 0.21 to 0.71 h^{-1} for Zn (Table 5.9, 5.2 and 5.16). Accordingly Cu was found to depress the rate of oxygen consumption to the maximum extent and correlate with the increase in concentration of exposure medium. Hg also showed the depressing effect on oxygen uptake. Conversely Zn during the initial period of exposure upto 48 h in 0.1 and 0.2 mg l^{-1}, and

upto 72 h in 0.4 mg l^{-1} functioned as respiratory accelerator. Thereafter the decreasing trend was exhibited and in 0.1 mg l^{-1} the oxygen consumption at 96 h was less than that of the control (Fig. 6.4). These finding are in good agreement with the assumptions of Hubschman (1967).

Further the pattern of oxygen consumption on exposure to Hg (initial accelerated depression, then the increase nearing the control level and the subsequent fall) has high bearing on the observation made during the accumulation studies (Chapter 5). The significance of 'threshold value' of Hg beyond which depuration takes place even during accumulation experiment might be responsible for such uneven trend in oxygen consumption. In the case of Cu a gradual accumulation was observed in gill tissue (Fig. 5.2). Analogous to this finding rate of oxygen consumption significantly decreased gradually in correlation with the concentration of the exposure medium (Fig. 6.3).

Zn was accumulated in gill tissue gradually and the depuration was found to be very slow (Chapter 5). As the depuration process happened to be very slow, the rate of oxygen consumption might have started declining after the initial accelerated uptake of oxygen during the process of accumulation.

Scott and Major (1972) observed that the experimental mussels secreted mucus when exposed to Cu. The secretion of the mucus though a protective mechanism adopted against adverse conditions may also reduce the oxygen uptake by reducing its ability to involve in gas exchange. Mucus secretion was observed in *M. dobsoni* exposed to Hg, Cu and Zn. Further, *M. dobsoni* exposed to 0.25 and 0.5 mg l^{-1} Cu developed black spots on the gills. The spots were visible after 72 h of exposure and were absent in control. Similar blackened foci were observed on the branchial lamalle of *Penaeus duorarum* and *P. vulgaris* exposed to Cd in acute and subacute concentrations (Nimmo *et al.*, 1977). They suggested that it is possible that Cd was collected by the hemocytes and accumulated in the gills which were then walled off and later sloughed off. Whether such a mechanism exist in the case of Cu is not known; and such detailed studies are beyond the scope of the present investigation.

SUMMARY

In view of the increasing importance in biomonitoring the quality of aquatic environment, baseline studies to determine the ranges and natural variations of trace metals in sentinel organisms have caught the attention of environmental scientists the world over. The use of indigenous species as biological indicators to predict the changes in water quality of the pollution-prone areas has also been universally accepted. The present investigation is an attempt to determine the baseline concentrations of Hg, Cu and Zn in *Metapenaeus dobsoni* (Miers) a widely distributed prawn of Indian waters and to study the feasibility of *M. dobsoni* as a biological indicator of trace metal pollution. Salient features are summarized in the following paragraphs.

On studying the seasonal variation of Hg, Cu and Zn in *M. dobsoni* collected from Cochin backwaters for the period June 1984-May 1985, the trace metal content in edible and non-edible parts did not significantly correlate with the ambient salinity. Seasonal variation in Cu and Zn content in both parts were not appreciable but Hg content varied with the season. Copper and zinc content in the edible and non-edible parts showed statistically significant positive correlation.

Mercury content in *M. dobsoni* collected from Station I close to industrial establishments was found to be maximum. Concentrations of Cu and Zn was relatively high in Station II which lies in between the industrial establishments and the main vent of sewage disposal from Cochin city. Stratification of water by obstructing the natural course of river flow and the intermittant industrial effluent discharge seems to play the major role in controlling the tracemetal concentration in the estuary than the seasonal variation of salinity.

Mercury content in *M. dobsoni* does not exceed the limit of 0.5 mg kg^{-1} fresh wt set by United States Food and Drug Administration (U.S. FDA) and at present, there is no harm in consuming *M. dobsoni* which is heavily exploited from Cochin backwaters. Levels of Cu and Zn are also within the specified

limits. The present investigation forms the basis in suggesting the baseline concentration of these three trace metals in *M.dobsoni* from Cochin backwaters.

In acute toxicity tests the adaptability of organisms seem to have more influence on the toxic effects of trace metals. The lower size group adapted to lower salinity showed more resistance to all the metals tested at lower salinity and they became more sensitive at higher salinities. The reverse was true in the case of higher size group.

Mercury was found to be the most toxic out of the three metals tested and the order of toxicity was found to be Hg $\rangle\rangle$ Cu = Zn. The LC50 values determined for the different size groups at various salinities $\overset{ave}{\lambda}$ of great importance in predicting the water quality criteria in pollution monitoring.

The kinetics of accumulation and depuration of trace metals in an organism mainly depend on the concentration in the ambient levels. The present study in *M. dobsoni* suggested a first order two compartment kinetic model for accumulation and depuration of trace metals based on the tissue concentration and the concentration of the exposed media.

During the course of accumulation, *M. dobsoni* was found to shed the Hg load after reaching a threshold concentration depending on salinity of the exposure medium. Gill was found to be the major site of accumulation for all the metals and the order of accumulation in different body parts was gill $\rangle$ viscera $\rangle$ exoskeleton $\rangle$ muscle.

Accumulation of trace metals was found to be the maximum in the lower size group and minimum, in the case of higher size group. The observation is in confirmation of the hypothesis that smaller animals have a large surface area : volume ratio and therefore would accumulate trace metals faster from the solution than the larger ones.

A two stage depuration mechanism exists in the case of Hg, consisting of a rapid depuration followed by reaccumulation and afterwards a slow release of accumulated Hg. A similar two compartment depuration was observed in the case of Cu also. In both cases gill tissue exhibited definite two compartment depuration where they attained higher concentration during accumulation. There were indications of two compartment depuration

in the case of Zn also. Subsequent depuration of Zn in the slow compartment was found to be very slow.

Depuration was at a faster rate from the gills in the case of Hg and Cu during the initial period. Since the depuration of Zn was very slow, the duration of 18 days for the depuration experiment was found to be insufficient in calculating $t_{\frac{1}{2}}$. The half-life values of Hg and Cu in *M. dobsoni* exposed to various concentrations under varying salinities were calculated.

On exposure to Hg and Cu oxygen consumption was decreased while oxygen uptake was enhanced on exposure to Zn. After the initial hike in oxygen consumption the rate of uptake was decreased and came close to the level as in control at the end of 96 h exposure to Zn.

The response of the animal towards oxygen consumption was comparable to the rate constant values (k_1) for accumulation. Copper having the maximum value for rate constant depressed the rate of oxygen consumption to the maximum extent and Zn having the least value for k_1 instead of depressing the rate of oxygen consumption, enhanced the consumption rate. Hg also depressed the oxygen consumption but not as much as Cu. The influence of 'threshold' value during accumulation was reflected in the rate of oxygen consumption on exposure to Hg.

In the light of the above observations METAPENAEUS DOBSONI which conforms to the requirement of a sentinal organism in the estuarine environmental management, is recommended as an indicator organism of trace metal pollution for Indian waters.

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* Not referred to in original.

APPENDIX I

Estimated Hg, Cu and Zn released to the water courses (Non-seasonable)

(KSPCB, 1982)

Name of water body	Industry	Quantity kg/annum		
		Hg	Cu	Zn
Periyar river	Travancore Cochin Chemicals Ltd. (T.C.C)	Roughly 2000 kg/ year		Negligible
	United Catalysts India Ltd. (U.C.I)		327.0	
	Travancore Chemical Manufacturing Co. (T.C.M)			145.0
	Comico Binani Zinc Ltd. (C.B.Z)			7500.0

APPENDIX II

Industrial waste discharges into Periyar River (KSPCB, 1982).

Industries discharging more than 1000 l/day	Waste Water million l/ year (Non-seasonal)	Suspended solids t/year	Average pH
Travancore Rayons Ltd.	5360.7	332.47	5.21
Indian Aluminium Co.	4110.0	102.75	5.90
Travancore Cochin Chemicals Ltd.	3504.0	327.82	7.4-9.4
Fertilizers and Chemicals Travancore Ltd. (F.A.C.T.)	20658.0	1336.06	5.78-7.62
Indian Rare Earths Ltd;	705.1	11.97	3.9-12.0
Hindustan Insecticides Ltd.	65.6	2.04	4.13
Periyar Chemicals	43.2	11.67	5.6
United Catalysts India Ltd.	126.0	57.45	7.62
Cominco Binani Zinc Ltd.	844.9	7.60	-
Travancore Chemical Manufacturing Co. Ltd.	239.1	2.42	6.93-7.05

APPENDIX III

Legal limits for hazardous substances in fish and fishery products (Nauen, C.E. 1983)

Country	Hg standard	Cu standard	Zn standard
Australia	0.5 ppm for fish, crustaceans, molluscs and canned fish	10.0 ppm for fish and fish products	150.0 ppm for fish and fish products
Canada	0.5 ppm for total mercury, edible wt	None	None
France	0.5 ppm in fish, crustacea and molluscs	None	None
Federal Republic of Germany	1.0 ppm in edible part of freshwater and marine fish and fish products	None	None
India	0.5 ppm for total mercury in fish and fish products expressed in mg/kg dry wt *	10.0 ppm for fish and fish products on dry wt basis	50.0 ppm in fish and fish products on dry wt basis
Japan	0.4 ppm for total mercury 0.3 ppm for methyl mercury	-	-
United Kingdom	-	20.0 ppm	50.0 ppm
United States of America	1.0 ppm for total mercury in fish, shell fish, crustaceans, other aquatic animals edible portion only; fresh, frozen or processed	-	-
U.S.S.R	0.5 ppm for fresh fish, tinned fish and products from marine fish		

* mg/kg wet wt (Personal Communication, Marine Products Export Development Authority, Cochin-16)

APPENDIX IV

Basic pre-requisites of an indicator organism

The basic pre-requisites for the organism were suggested by Butler *et al.* (1971) as follows:

(a) The organism should accumulate the pollutant without being killed by the levels encountered in the environment.
(b) The organism should be sedentary in order to be representative of the study area.
(c) The organism should be abundant throughout the study area.
(d) The organism should be sufficiently long-lived to allow the sampling of more than one year-class, if desired.
(e) The organism should be of reasonable size, giving adequate tissue for analysis.
(f) The organism should be easy to sample and hardy enough to survive in the laboratory, allowing defecation before analysis (if desired) and laboratory studies of pollutant uptake.

To these requirements, Haug *et al.* (1974) added the following:

(g) The organism should tolerate brackish water.
(h) A simple correlation should exist between the pollutant content of the organism and the average pollutant concentration in the surrounding water.

Phillips (1976*a*, 1977*b*) amended the final requirement as follows:

(h) All organisms of a given species used in a survey should exhibit the same correlation between their pollutant content and the average pollutant concentration in the surrounding water, at all locations studied, under all conditions.

GLOSSARY

Acute toxicity - a relatively short-term lethal or other effect, usually defined as occurring within 4 days for fish and macroinvertebrates and shorter times for smaller organisms.

Acclimate - to accustom test organisms to different environmental conditions, such as temperature, light and water quality.

Asymptotic LC50 - toxicant concentration at which LC50 becomes a constant for a prolonged exposure time.

Bioassay - determination of the relative strength of a substance by comparing its effect on a test organism with that of a standard preparation.

Bioconcentration - uptake of toxicants by an organism directly from water.

Biomagnification - uptake of toxicants through food

Biological half-life - time required for tissue concentrations to decline to half of their steady state concentration.

Biological water quality - the capacity of water to sustain naturally occurring biological processes.

BCF - $\frac{\text{final tissue concentration}}{\text{concentration in water}}$

Control - test organisms in test chamber under test conditions exposed to the natural water to which they are normally exposed.

Chronic toxicity - long-term effects that may be related to changes in appetite, growth, metabolism, reproduction and even death or mutations.

Dose - amount of toxicant that enters the organism.

ET50 - effective time for 50% mortality

Exposure time - time of exposure of test organism to test solution.

Hypoxia - a condition when oxygen availability is low.

Lethal concentration (LC) - toxicant concentration producing death of test organism. Usually defined as median (50%) lethal concentration, LC50, i.e. concentration killing 50% of exposed organisms in a specific time of observation.

Median tolerance limit (TLm) - test material concentration at which 50% test organisms survive for a specified exposure time.

Response - a measured biological effect of the material tested. In acute toxicity tests this usually is death.

Renewal bioassay - static test with periodic exposure (usually 24 h intervals) of test organisms to fresh test solution of the same composition.

Toxicity - adverse effect to a test organism caused by pollutants.

Static bioassay - test in which solutions and test organisms are placed in test chambers and kept there for the duration of the test.