PHYSIOLOGICAL EFFECTS OF SOME HEAVY METALS ON *PERNA VIRIDIS* (LINNAEUS)

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

DOCTOR OF PHILOSOPHY
IN
MARINE BIOLOGY

UNDER THE FACULTY OF MARINE SCIENCES

By

P. K. KRISHNAKUMAR, M. Sc.

SCHOOL OF MARINE SCIENCES
COCHIN UNIVERSITY OF SCIENCE AND TECHNOLOGY
COCHIN - 682 016

NOVEMBER - 1987

D E C L A R A T I O N

I hereby declare that the thesis entitled, "PHYSIOLOGICAL EFFECTS OF SOME HEAVY METALS ON *PERNA VIRIDIS* (LINNAEUS)", is an authentic record of research carried out by me under the supervision and guidance of Dr. R. Damodaran in partial fulfilment of the requirements of the Ph.D. Degree in the Faculty of Marine Sciences of the 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.

Cochin 682016,

5th December 1987.

P. K. KRISHNAKUMAR

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 work carried out by Mr. P.K. Krishnakumar, M.Sc., in the Division of Marine Biology and Marine Microbiology, School of Marine Sciences, Cochin 682 016, under my supervision, in partial fulfilment of the requirements for the degree of Doctor of Philosophy of the Cochin University of Science and Technology and further that no part thereof has been presented before for any other degree.

Cochin 682 016,
5th December 1987.

Dr. R. Damodaran
(Supervising Guide).

A C K N O W L E D G E M E N T S

I wish to express my deep sense of gratitude to Dr. R. Damodaran (Professor, Division of Marine Biology and Marine Microbiology, School of Marine Sciences) for his inspiring guidance and constant encouragement throughout the course of this investigation.

I also thank Dr. P. N. K. Nambisan (Professor and Head, Division of Chemical Oceanography, School of Marine Sciences) for his keen interest and valuable suggestions during the course of this work. I am grateful to Dr. C. V. Kurian (Former Head of the School of Marine Sciences) for critically going through the manuscripts. I am also grateful to Dr. N. R. Menon (Professor and Head, Division of Marine Biology and Marine Microbiology, School of Marine Sciences) for helpful discussions and to Dr. Y. L. Dora (Director, School of Marine Sciences) for providing me with the facilities. I wish to acknowledge the valuable assistance and useful discussions with Mr. C. R. Sivadasan (Cardamom Research Board) and Mr. V. K. Pillai (Scientist, C.M.F.R.I., Cochin).

I thank the Alexander von Humboldt Foundation, Bonn (West Germany) for the gift of the Atomic Absoption Spectrophotometer (to Dr. P. N. K. Nambisan) in which the metal analysis were carried out.

I also thank Mr. T.M. Sankaran (Associate Professor, Fisheries College, Panangad) and Mr. Krishna Iyer (Scientist, C.I.F.T.) for their help in statistical analysis of the data.

The work presented here was carried out as a Senior Research Fellow in a I. C. A. R sponsored Scheme. The financial assistance of the I. C. A. R. is gratefully acknowledged.

I would like to express my sincere thanks to Mr. James Kalassery for, Word processing this thesis. I wish to acknowledge my sincere thanks to my teachers and friends in School of Marine Sciences, for their valuable help.

The work described in this thesis has been published/or are under publication as indicated below :

1. Effect of body size on the physiology of green mussel *Perna viridis* (Linnaeus) (1986) In : Proceedings of the National Seminar on Mussel Watch, Vol.I, Cochin University of Science and Technology, pp 77-85.

2. Carotenoid content and tolerance of green mussel *Perna viridis* to Acute toxicity of Mercury and Zinc. (1987). Indian J. Mar.Sci.16, 155-157.

3. Acute toxicity of selected heavymetals to *Perna viridis*. (1987)• Indian J. Mar. Sci. (accepted).

4. Carotenoid concentration as an index of heavy metal stress in green mussel *Perna viridis*. (Under publication).

LIST OF ABBREVIATIONS

B.C.F.	=	Bio-concentration Factor.
B.C.I.	=	Body Condition Index.
B.P.	=	Boiling Point.
°C	=	Degree(s) Celsius.
cm	=	Centi meter(s)
Conc.	=	Concentration
$D.O_2$	=	Dissolved Oxygen
EC50	=	Median Effective Concentration
Fig.	=	Figure.
g	=	Gram(s).
h	=	Hour(s).
LC50	=	Median Lethal Concentration
l	=	Liter(s)
mg	=	Milli gram(s)
min	=	Minute(s)
ml	=	Milli liter(s)
mm	=	Milli meter(s)
µg	=	Micro gram(s)
µl	=	Micro liter(s)
N	=	Number
Nos.	=	Numbers
N.R.	=	Not Reported.
N.S.	=	Not Significant.
nm	=	Nano meter(s)
O.D.	=	Optical Density
O:N	=	Oxygen to Nitrogen ratio
ppb	=	Parts per billion
ppm	=	Parts per million
S.D.	=	Standard Deviation.
TB/2	=	Biological half life or time
Temp	=	Temperature.
Wt	=	Weight.
Yr	=	Year.

CONTENTS

CHAPTER - 1
GENERAL INTRODUCTION

CHAPTER - 2
PHYSIOLOGY OF GREEN MUSSEL *PERNA VIRIDIS* (LINNAEUS)

C H A P T E R - 3

ACUTE TOXICITY OF COPPER, MERCURY AND ZINC

C H A P T E R - 4

BIOACCUMULATION OF COPPER, MERCURY AND ZINC

C H A P T E R - 5

DEPURATION OF COPPER, MERCURY AND ZINC

C H A P T E R - 6

TOXIC EFFECTS OF HEAVY METALS ON RESPIRATION AND FILTRATION RATE

C H A P T E R - 7

CHRONIC TOXICITY OF COPPER ON PHYSIOLOGY

C H A P T E R - 8

CAROTENOID CONCENTRATION AS A BIOCHEMICAL INDEX OF HEAVY METAL STRESS

CHAPTER - 1

GENERAL INTRODUCTION

Human involvement in the marine ecosystem is so deep that the health of the ocean is important for his survival. Ocean and coastal waters are one of the major resources for a variety of human activities like fisheries, aquaculture, navigation, oil and mineral exploration and waste disposal (IMCO *et al*., 1982). But in the recent years man made sources have substantially increased the flux of many pollutants to the marine environment via atmosphere, surface run off and direct discharge. Concern over heavy metal, pesticide, oil, radioactive waste, sewage and detergent pollution have received greater attention. Most of these pollutants are potentially harmful to many organisms at some level of exposure and absorption and so they will affect man and other organisms which are occupying higher trophic levels. Today pollution and protection of the marine environment are the most essential themes of ocean management as it stems from ethical, scientific, social and economic attitudes of man (Kinne, 1984).

1. HEAVY METALS

Heavy metals form a dangerous group of potentially hazardous pollutants, particularly in estuaries and nearshore waters (Bryan, 1984). Heavy metals have been defined as those with a specific gravity grater than 4 or 5, located from atomic numbers 22 to 34 and 40 to 52 on

the periodic table (as well as the lanthanide and actinide series), and having specific biological response (Murphy & Spiegel, 1983).

About half of the heavy metals are biologically essential for the correct functioning of biochemical process and this group comprises Fe, Cu, Zn, Co, Mn, Mo, Sc, Cr, Ni, V, As, and Sn (Dasilva, 1978). These essential metals are frequently found at the active site of enzymes or are involved in enzyme activation. The non-essential metals are Ag, Al, Be, Cd, Hg, Pb, Sb, Ti, and Tl, without any established biological function, include the more important contaminants in the aquatic environment (Dasilva, 1978). Toxicity and biological effects of heavy metals have been recently reviewed by Bryan (1984) and Akberali & Trueman (1985).

The Oslo and Paris commission has classified the contaminants in the marine environment to 'black' and 'grey' lists. The release of black list substances to the marine environment is completely banned and it includes heavy metals such as Hg and Cd and their compounds. The inputs of grey list substances into the sea are subject to special permission and it includes heavy metals such as As, Cr, Cu, Pb, Ni and Zn and their compounds.

In the present study three heavy metals Cu, Hg and Zn were selected since they are widely recognised as the most common pollutants in the marine environment. Here the metals Cu, Hg and Zn were used in the form of $CuSO_4 \cdot 5H_2O$, $Hg\ Cl_2$ and $ZnSO_4 \cdot 7H_2O$ respectively.

a) Copper (Cu):

Cu is one of the heavy metals that is required by living systems for normal metabolism and it is also a metal that can have harmful effects at high concentration (INCRA, 1982). Cu is important for molluscs - firstly as a part of respiratory pigment, secondly in the cytochrome system of electron transfer and finally in the enzyme systems involved in removing the toxic side products of aerobic metabolism (Simkiss *et al.*, 1982).

Cu production exceeds 7.5 million tonnes yr^{-1} and largely from the sulphide ores. Cu reaches the marine environment from Cu mines and a large number of industrial processes like artificial fibre production, agriculture chemicals, wood preservatives, wall paper manufacture, anti-fouling and anticorrossion paints etc. Cu is present in all sewage, being derived from food and due to mixing with industrial wastes (IMCO *et al.*, 1982).

Average Cu content in the open sea is 2 $\mu g\ l^{-1}$ (IMCO *et al.*, 1976) and in the Arabian Sea is 4.9 $\mu g\ l^{-1}$ (Sengupta *et al.*, 1978). The Indian standard for the maximum tolerance limit for Cu in the industrial effluents discharged to marine coastal area is 3 $mg\ l^{-1}$ (IS, 7968, 1976). The acute and sublethal toxicity of Cu has been recenty reviewed by INCRA (1982).

b) Mercury (Hg):

Mercury is probably the most important pollutant both with regards to its effects on marine organisms and its potential hazard to human (Taylor,1979; Bryan, 1984). The total world production of Hg is

about 8.8 K. tonnes Yr^{-1} and out of this 10^5 to 10^6 tonnes yr^{-1} discharged into oceans through human activities (Goldberg, 1976). The most important single use of Hg is in the chlor-alkali industry and others like agricultural chemicals, pharmaceuticals, mercury vapour lamps, extraction of gold and silver, antifouling paints, dentistry, dyeing industry and catalysts in production of acetaldehyde and vinyl chloride (IMCO *et al*, 1982). Chaudhuri (1980) emphasised that 180 tonnes of Hg are introduced in to the Indian environment every year of which 166 tonnes come from 38 caustic soda plants, including 23 units of mercury cell electrolyses of seawater.

The concentration of dissolved Hg vary in seawater from 0.001 to 0.01 $\mu g l^{-1}$ (IMCO *et al*, 1982). The concentration of total dissolved Hg in the Arabian Sea is 0.013 - 0.407 $\mu g l^{-1}$ (Singbal *et al*, 1978). The Indian standard for the maximum tolerance limit for Hg in the industrial effluents discharged to marine coastal area is 0.01 $mg\ l^{-1}$ as Hg (IS, 7968, 1976).

Hg is mainly discharged into water as divalent inorganic mercury or as phenyl mercuric ion. Regardless of its state of entry into the marine environment, mercury compounds are liable to be converted into methyl mercury under anaerobic conditions (IMCO *et al*, 1976). The order of toxicity of different forms of Hg are ionic Hg < organic Hg < propyl mercuric chloride < ethyl mercuric chloride < methyl mercuric chloride (IMCO *et al*, 1976). The lethal and sublethal effects of Hg on aquatic life have been reviewed by Taylor (1979).

c) Zinc (Zn) :

Zn is an essential element and more than 90 Zn containing enzymes and proteins have been discovered, and the addition of Zn increases the activity of many other enzymes (Vallee, 1978). Zn production is largely from sulphide minerals and world production is around 5 million tonnes yr^{-1} (IMCO *et al.*, 1976). Large amount of Zn are discharged to rivers in chemical wastes, e.g. from some artificial fibre manufacturing processes. The normal concentration of Zn in open seawater is 3 $\mu g l^{-1}$ and in coastal waters 2.5 $\mu g\ 1^{-1}$ (IMCO *et al.*, 1976). the average concentration of dissolved Zn in the Arabian Sea is 19.2 $\mu g\ 1^{-1}$ (Sengupta *et al.*, 1978). The Indian standard for the maximum tolerance limit for Zn in the industrial effluents discharged to marine coastal area is 5.0 $mg\ 1^{-1}$ (IS, 7968, 1976).

The essential metals like Cu and Zn are essential at low concentration and marine animals are having mechanisms for absorbing them either from their food or water in which they live. But these metals at slightly higher concentrations become inhibitory and toxic, as in the case of non essential metals.

2. BIOLOGICAL MONITORING OF POLLUTION

The impact of pollutants on the marine environment can be assessed in various ways. The physical aspects include the distribution of potential pollutants in the environment and the chemical aspects include the level and chemical form of pollutant found with in the biotic and abiotic components of ecosystem. Since ultimate concern is

the capacity of the sea to support life, it is desirable to measure pollution in terms of biological response (Bayne *et al.*, 1985).

The biological monitoring can be defined as the systematic use of biological responses to evaluate the changes in the environment with the intent to use this information in a quality control programme (Matthews *et al.*, 1982). There is an increasing realisation of the need to monitor environmental quality in biological terms (Matthews *et al.*, 1982; Herricks & Cairns Jr, 1982; Buikema Jr *et al.*, 1982;Cherry & Cairs, 1982; Bayne *et al.*, 1985).

The response of marine biota to a pollutant can be detected at different levels of organisation and responses. At the highest level of impact the effects are acute and lethal to all biota and such catastrophic effects are easily recognised (Buikema Jr *et al.*, 1982). At the next level of effects the most sensitive species or fauna are eliminated and a proliferation of relatively tolerant species may occur. All species are tolerant to a certain amount of environmental variation and near and beyond the tolerable limits, characteristic biochemical and physiological responses related to the ultimate survival or death of the individual are elicited (Blackstock, 1984; Bayne *et al.*, 1985). These biological responses include physiological, biochemical, morphological, genetic and behavioural responses of organisms to stress (Widdows, 1985). This concept is the basis of biological monitoring of pollutant effects and much discussed utilization of certain organisms as indicators of various degree of impact of pollutants in marine system

(Bayne, 1985). Many reviews and guidelines are available on biological responses related to stress in the marine environment (IMCO *et al*, 1980; Topping, 1983; Blackstock, 1984; Akberali & Trueman, 1985; Bayne *et al*, 1985).

a) Indicator species:

Some organisms by their presence or by their behavioural, physiological or biochemical responses indicate the changes in the environment. The use of bioindicators in monitoring aquatic pollution began some 20 yrs ago with the studies of radionuclide abundance in marine ecosystem (Phillips & Segar, 1986). The use of indicator species or sentinel organism as indicator of the state of pollution in coastal waters is a concept which has received wide acclaim all over the world (Phillips, 1980). According to Sprague (1971) biological indicators when used alone, are superior to chemical and physical measurements used alone. The underlying principle of using indicator organisms or sentinel organisms in pollution monitoring is that vast majority of chemical pollutants exhibiting a biological effect must be first taken up by the organism. Such indicators provide a direct *time-integrated* picture of pollutant *bio-availability* rather than pollutant abundance in the environment (Phillips & Segar, 1986). Phillips (1980) and Simkiss *et al*, (1982) reviewed the assumption and concepts involved in the use of organisms as bioaccumulators of heavy metals and other pollutants.

The most direct and satisfactory approach, however is to look for the effect in the field namely biological effect monitoring

(McIntyre, 1984). This is not concerned merely with the use of organisms in monitoring the residue levels or bioassay type studies, but aim rather to detect and measure response of organism to the contaminant and to provide some assessment of resulting impact on the ecosystem (McIntyre, 1984). As a result of such studies a wide range of possible techniques have been proposed for monitoring biological effect and a stategy has been developed for applying them (IMCO et al., 1980; Bayne *et al.*, 1979, 1985).

The capacity of bivalve molluscs to accumulate potentially toxic pollutants like heavy metals in their tissues, far in excess of environmental level is well known and has become the focus of an increasing number of studies (Bryan, 1984). Because of their world wide distribution, sedentary, filter feeding habit, availability at all seasons, wide salinity tolerance, metabolism, growth, reproduction and tolerance reaction, mussels are now receiving considerable attention and have been proposed as suitable indicators for monitoring pollution in the marine environment both globally and locally as in 'Mussel Watch' programme (Goldberg, 1975; Goldberg *et al.*, 1978; Davies & Pirie, 1980). The bivalve molluscs of the genus *Mytilus* has been subjected to the co-ordinated, multidisciplinary studies to find out their physiological and biochemical mechanisms and changes induced by pollutants (Bayne *et al.*,1979; 1985)

b. The Indian mussel *Perna viridis*:

Perna viridis is having wide spread distribution all along

both east and west coasts of India (Jones, 1968). This is an important bivalve mollusc exploited for human consumption in India and in several other parts of the Indo-pacific region (Rao *et al.*, 1977). This species is ecologically and economically important with outstanding potential for use as biological indicator of marine environmental quality (Hawkins *et al.*, 1986; Krishnakumar & Damodaran, 1986).

Perna viridis is found in the rocky open coast and harbours, and at the mouth of estuaries and rivers.

They occur from intertidal zone to a depth of 15 m attached to rocks and pilings and other hard objects. This species is found to be tolerating wide fluctuations in salinity (Qasim *et al.*, 1977).

The ability of *Perna viridis* to accumulate heavy metals from the environment has been investigated both in the field and in the laboratory (D'silva & Kureishy, 1978; Lakshmanan & Nambisan, 1979; 1983; 1986; Pillai *et al.*, 1986). Acute toxicity of some of the heavy metals to *P. viridis* has been studied (Nambisan & Lakshmanan, 1986). Effect of some of the metals on oxygen consumption and filtration rate is known (Mathew & Menon 1983; 1984; Prabhudeva & Menon, 1985; 1986). However little is known about physiological and biochemical responses of *P. viridis* to natural variables and pollutants (Shaffee, 1976; Hawkins *et al.*, 1986; Krishnakumar & Damodaran, 1986) in comparison with its related temperate species *Mytilus edulis*. So far no systematic approach has been made, based on physiological and biochemical effect studies to assess

the suitability of *P. viridis* as a biological indicator of stress in the marine environment.

3. SCOPE OF THE STUDY

No serious attempt has so far been made in India to make use of the 'Mussel Watch' concept as a useful tool for pollution monitoring of the marine and estuarine environment. The recently conducted 'National seminar on mussel watch' by the Cochin University of Science and Technology (13-14 Feb, 1986) discussed the technical aspects related to mussel watch programme and the application of sentinel organism concept to the coastal areas of India.

It is well known that the biological and physiological characteristics of the organism inhabiting tropical waters such as those prevailing in India, and the ecological as well as the environmental characteristics of temperate areas, where mussel watch programmes are already in existence differ greatly. So it is essential to adopt the techniques and standards developed for temperate species to the situations and conditions in India. In this context it is a prerequisite to collect information on physiology and other biological indices of stress of possible sentinel organisms like *P.viridis*.

In consideration of the above, *P. viridis* which is a potential sentinel organism, is selected for the present study.

4. OBJECTIVES OF THE STUDY

1) To collect baseline information on some of the physiological indices of stress in *P. viridis* which are necessary for

monitoring biological effect.

2) To study the acute and chronic toxic effect of Cu*, Hg* and Zn* on some of the physiological indices of stress.

3) To understand the bioaccumulation, distribution and depuration of the above heavy metals in the different body parts, and the effect of intrinsic and extrinsic factors on it.

4) To investigate the usefulness of carotenoid concentration in the body of *P. viridis* as a general biochemical index of heavy metal stress.

It is proposed that the above informations will be useful to assess the suitability of the Indian mussel *P. viridis* as a biological indicator of heavy metal stress, in the marine environment.

---

*In the text Cu, Hg and Zn stand for their respective ions (Cu^{2+}, Hg^{2+} and Zn^{2+}).

CHAPTER - 2

PHYSIOLOGY OF GREEN MUSSEL *PERNA VIRIDIS* (LINNAEUS)

1. INTRODUCTION

Study of physiological ecology of animals has great significance since it will reveal the physiological flexibility of an organism in relation to the environmental demand. Most of the marine animals are capable of some degree of compensation for environmental changes within its zone of tolerance and zone of resistance (Blackstock, 1984; Akberali and Trueman, 1985). But prolonged unfavourable environmental conditions may excert stress on the animal and chances of survival are significantly reduced. Bayne (1975) offered the following definition of stress as applied to bivalve molluscs, "stress is a measurable alteration of a physiological (behavioural, biochemical or cytological) steady state which is induced by an environmental change, and which renders the individuals (or the population) more vulnerable to further environmental change". Such alterations in the functional state may result in the improvement of an organism's fitness or a deterioration in well being (Bayne *et al.*, 1985).

Working with in this frame work of functional responses IMCO *et al.* (1980) and Bayne *et al.* (1985) have given three categories of measurements for assessing the effect of environmental changes or stress. (1) A *general*, non specific stress response to the total environmental stimuls. (2) A *specific response* to a particular contaminant. (3) The *change in fitness* that results from these responses

to environmental stimuli.

Above physiological responses in the animal can (1) represent an integration of the many cellular and biochemical processes that can alter in response to changes in the environment. (2) They represent non-specific responses to the sum of environmental stimuli which are complementary to more specific responses at biochemical level. (3) They are capable of reflecting deterioration in the environment before effect manifest themselves in the population (Widdows, 1985a).

After considering ecological significance, quantitative aspects, specificity, reversibility, sensitivity, response rate and precision the following physiological responses were proposed for routine use in environmental monitoring (Bayne, 1975; Bayne *et al.*, 1979; 1985; IMCO *et al.*, 1980).

(1) Respiration - a measure of oxygen uptake of the organism.

(2) Oxygen nitrogen ratio (O:N) - a measure of the balance between catabolic processes.

(3) Scope for growth - a measure of the energy status of an organism.

(4) Growth efficiency- the efficiency with which an individual converts food into body tissue.

(5) Body condition index-indicative of alterations in the nutritional status of the animal.

Most of these physiological responses have been developed and evaluated in the field and lab condition using the mussel *(Mytilus edulis)* as the indicator species. The measurement of the physiological

responses in sessile marine bivalves can provide a valuable indication of environmental impact (See review, Akberali and Trueman, 1985; Bayne *et al.*, 1985). Identifying suitable organisms for this purpose and obtaining baseline information for such species are vital in such an approach. Initial experiments (Hawkins *et al*, 1986; Krishnakumar and Damodaran, 1986) indicate that the green mussel *Perna viridis* is a species suitable for such purpose as it occurs along the coastal waters of India. *P. viridis* is reported to be accumulating contaminants far above the environmental level in their body (Lakshmanan and Nambisan, 1983; Pillai *et al.*, 1986). But we have little information regarding the animal's basic physiology (Shaffee, 1976, 1979; Mohan and Cheriyan, 1980; Davenport, 1983) and the impact on it by a wide range of intrinsic (such as size, age, reproductive and nutritional status) and extrinsic factors (such as temperature, salinity, ration, dissolved oxygen, suspended and particulate pollutants) (Hawkins *et al.*, 1986; Krishnakumar and Damodaran, 1986).

So the main objective of this study is to collect baseline information on some of the physiological indices like oxygen consumption, ammonia excretion, O:N ratio, filtration rate and body condition index and the effect of body size on these indices.

2. MATERIALS AND METHODS

Mussels were collected from an unpolluted natural population attached to the seawall near Narakkal, Cochin from November to December 1985 for this study. Mussels were immediately brought to the laboratory

in a polythene bag filled with the seawater taken from the same site. Mussels were cleaned and acclimated for 2 days in a plastic basin of 50 l capacity having a biological filter for water purification (Fig.2.1A). The biological filter was constructed using washed coarse sand, gravel and crushed clam shells (Spotte, 1970). Clean tiles (Fig. 2.1A) were kept in the system as substratum for attachment, and ammonia, nitrate, dissolved oxygen and pH were maintained optimum. Algal food was not added but the mussels were maintained in unfiltered natural seawater (Salinity = 33.5 x 10^{-3}, temp = 28±0.5°C; pH = 7.85 ± 0.05 and dissolved oxygen > 90% saturation).

For the experiment natural, seawater filtered through 42 Whatman filter paper was used. The physico-chemical parameters of the experimental sea water were Salinity = 33.5 x 10^{-3}, temp = 28±0.5°C; pH = 7.8 ± 0.05 and dissolved oxygen > 90% saturation.

a) Measurement of oxygen consumption:

A closed respiratory chamber of 1 litre capacity made up of glass was used for determining oxygen consumption of mussels (Fig. 2.1B). It consists of an airtight lid, an inlet (A) connected to the overflow tank and an outlet (B), the opening on the lid, (C) closed airtight with the rubber cork.

The cleaned mussels (size : 10-55 mm) were individually taken in the chamber and a continuous flow of seawater from the overflow tank was maintained for 1 h in order to acclimate the animal to the experimental condition. After collecting a sample of water from the

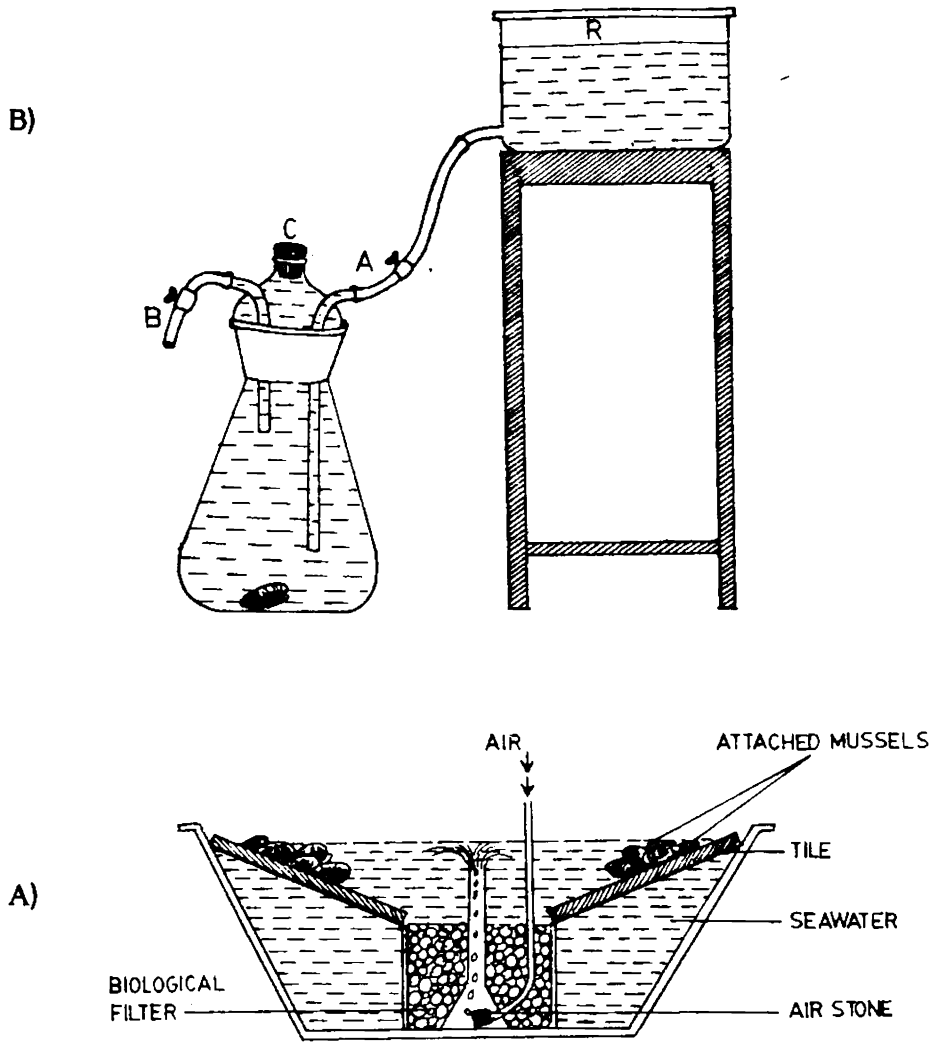


Fig-2.1. A) Acclimation system with biological filter for maintaining mussels in the laboratory.

B) Apparatus used for the measurement of oxygen consumption of mussels.

outlet for the determination of initial O_2 content, the continuous flow of water was cut off and the outlet (B) was closed. The system was kept undisturbed in the room light for 2 h. After 2 h water samples were withdrawn through the outlet by maintaining the flow, to find out the final O_2 content. Water samples were collected using sampling bottles of 60ml capacity and dissolved oxygen content was determined using winkler method (Strickland & Parson, 1968). The difference between the initial and final O_2 content was considered to be consumed by the mussel. Oxygen consumption was calculated in $ml\,h^{-1}$.

b) Measurement of ammonia excretion:

Mussels used in the oxygen consumption experiment were then reconditioned in oxygen saturated seawater for atleast 3 h before estimating ammonia excretion. Same mussels were placed individually in beakers containing 200ml of filtered seawater. An additional beaker containing 200ml of seawater, but with no animal, was kept as control. After 2h incubation water samples were taken in duplicate for ammonia estimation using phenol-hypochlorite method (Solorzano, 1969). Ammonia excretion rate (VNH_4-N, μg NH_4-N h^{-1}) of individual mussels were calculated after substracting the control value.

From the rate of Oxygen consumption (ml O_2h^{-1}) and ammonia excretion (μg NH_4-N h^{-1}) the ratio of oxygen consumed to nitrogen excreted (O:N) was calculated (Widdows, 1985b).

c) Measurement of filtration rate:

The filtration rates of mussels were determined by an indirect

method using neutral red (Cole and Hepper, 1954; Badman, 1975; Abel, 1976). After conducting trial and error expriments the following method was found to be giving optimum and comparable results. The present method is the slight modification of Abel's (1976) method. In order to avoid the disturbance of stirring, mussels were placed individually in 250 ml of filtered seawater already containing $1mgl^{-1}$ neutral red (BDH Ltd) in conical flasks. Mussels were allowed to recover from handling stress in the medium for 10 minutes and first sample of 10ml was removed for finding out the initial dye concentration in the seawater. After 20 minutes a second 10 ml was taken to find out the final concentration. Long time exposure in dye was found to be inhibiting the filtration rate of bivalves (Badman, 1975). So in the present study the overall duration of filtration experiment was restricted to 30 minutes. Dye concentration in the samples was determined spectrophotometrically using Hitachi Spectrophotometer (Model 200-20). Filtration rates were calculated in ml min^{-1}, using the method of Abel (1976) and the values were converted to lh^{-1}.

d) Measurement of body condition index:

To calculate BCI the total displacement volume of the completely closed mussel and the displacement volume of the empty shells were determined. The dissected soft tissue from each mussel was dried at 80°C for 24 h before weighing. The BCI was calculated using the equation (Widdows, 1985b).

$$BCI = \frac{\text{Dry tissue mass (g)}}{\text{Shell cavity volume (ml)}} \times 1000$$

The shell cavity volume is the total displacement volume of a completely closed mussel minus the displacement volume of the shell after opening and removing the soft tissue.

All the physiological measurements were conducted in mussels having a shell length of 10-55 mm. After the experiments the soft tissues dissected from the individual mussels were dried at 80°C for 24h. They were dried to constant weight.

e) Data analysis:

The relationship between body weight and physiological measurements were described by the simple allometric equation after $\log_{10}$ transformation of the values.

$$Y = a \times W^b$$

Where Y = the physiological rate; W = the dry tissue weight, a and b are the intercept and slope of the Y vs W regression respectively. The data were analysed statistically (Snedecor and Cochran, 1968).

Physiological rate of the mussels for a standard animal of 1 g dry weight (Table-2.1) were calculated for comparison using the relationship given by Bayne & Newell (1983).

$$Ys = \left(\frac{Ws}{We}\right)^b \times Ye$$

where Ys is Physiological rate of a standard size animal, Ws is its weight (1g), is the weight of the experimental animal, Ye is the uncorrected physiological rate and b is the corresponding weight exponent.

3. RESULTS

a) Oxygen consumption (VO_2):

The physiological response of the mussels were shown in the table 2.1. The VO_2 of the animal of 1g body weight was found to be 0.7799 mlh $^{-1}$. The relationship between the rate of oxygen consumption and body weight can be represented in the linear form

$$\log VO_2 = \log a + b \log W$$

The regression of log VO_2 against log bodyweight (g) was calculated and shown in the Fig. 2.2a. The *b* value and *a* value estimated are 0.7014 and log 0.4789 respectively.

b) Ammonia excretion (VNH_4-N):

The VNH_4-N of the mussel of 1g body weight was found to be 35.61 µg NH_4-N h^{-1} (Table 2.1). Regression of log µg NH_4-N excreted against log weight were calculated (Fig. 2.2b). This can be represented by the equation

$$\text{Log } NH_4\text{-N} = \log 2.7167 + 0.4155 \times \log w.$$

In the present study ammonia excretion increased significantly ($p<0.001$) with the dry body weight.

c) Oxygen to nitrogen ratio (O:N):

The O :N ratio of the animal of 1g body weight was found to

be 23.09. The regression of log O:N against log body weight was calculated ($p<0.01$) and it is found to be positively correlated with increase in body weight (Fig. 2.2c). The *b* and *a* value were found to be 0.2377 and log 2.8774 respectively.

d) Filtration rate (VF):

Filtration rate of animal of 1g body weight was found to be 0.9123 l h^{-1}. The regression of log VF against log weight was calculated (Fig. 2.3a). Filtration rate was found to be increasing allometrically with body weight. The relationship can be represented by the equation log VF= log 0.9222 + 0.5070 x log W.

e) Body condition index (BCI):

Body condition index of the mussel (1g) was found to be 54.51. The regression of log BCI against log body weight was calculated and found to be not significant (Fig.2.3b). The *b* and *a value were found to* be -0.2237 and log 4.1631 respectively. The BCI was found to be negatively correlated with the increase in body weight.

4. DISCUSSION

The weight specific oxygen consumption (for 1g wt) obtained in the present study (0.7799 ml h^{-1}) is comparable with the earlier study (Shaffee, 1976). Davenport (1983) and Hawkins *et al.* (1986) obtained weight specific oxygen consumption of 1.19 ml h^{-1} and 1.09 ml h^{-1} respectively in *P.viridis*. But for the determinations they used a small number of animals (5 to 6). So the present value seems to be more realistic as it is obtained using a large number of animals. The b value

Table - 2.1. Physiological responses of *Perna viridis* (S‰ = 33.5×10^{-3}; Temp = 28 ± 1°C). Values ($\bar{X}$ ± SD) are calculated for a standard sized (1 g. dry weight) animal

Physiological Responses	No. of determinations	Dry weight (g)	Rate
Oxygen Consumption ($ml\ O_2\ h^{-1}$)	15	0.0693-0.622	0.7799± 0.1661
Ammonia ($\mu g\ NH_4\text{-}N\ h^{-1}$)	23	0.0693-0.622	35.61 ± 4.94
O:N (Atomic equivalent)	21	0.0693-0.622	23.09 ± 5.51
Filtration ($l h^{-1}$)	17	0.0347-0.3956	0.9123± 0.2882
Body condition index	23	0.0679-0.4077	54.51 ± 17.50

of oxygen uptake (0.7014) obtained in the present study falls within the range of 0.4 and 0.9 reported for bivalves (Bayne *et al.*, 1976). And this value is also comparable with the b values reported by Shaffee (1976) and Mohan & Cheriyan (1980) for *P.viridis*. According to Bayne and Newell (1983), the approximate allometric exponent of b in molluscs is 0.7. The weight specific oxygen consumption obtained in *P. viridis* is considerably higher than the mean value of 0.496 ml h^{-1} calculated for 10 species of temperate bivalves (Bayne and Newell, 1983). This high oxygen consumption reflects the metabolic costs associated with the higher growth rate in *Perna* compared to temperate mussels as reported by Shaffee (1979).

Among variety of bivalve species ammonia comprised about 60 to 90% of total measure of nitrogen excretion (Bayne, et al., 1976; Bayne and Newell, 1983). Therefore rate of ammonia excretion may be regarded as reflecting the rate of protein catabolism (Widdows, 1978). The b value obtained for ammonia in the present study (0.4155) is lower than the values reported in other bivalves (Srna and Baggaley, 1976; Bayne and Scullard, 1977). In *M. edulis* the *b* value was found to be varying from 0.482 to 1.48 (Bayne and Scullard, 1977).

The O:N ratio is an index of protein utilization in energy metabolism and a low value of O:N ratio is generally indicative of a stressed condition (Bayne *et al.*, 1976; Bayne and Newell, 1983; Widdows, 1985 a, b, c). For *M.edulis* O:N ratio values above 50 is representative of a healthy mussel and below 30 of stressed mussel (Widdows, 1985a). In

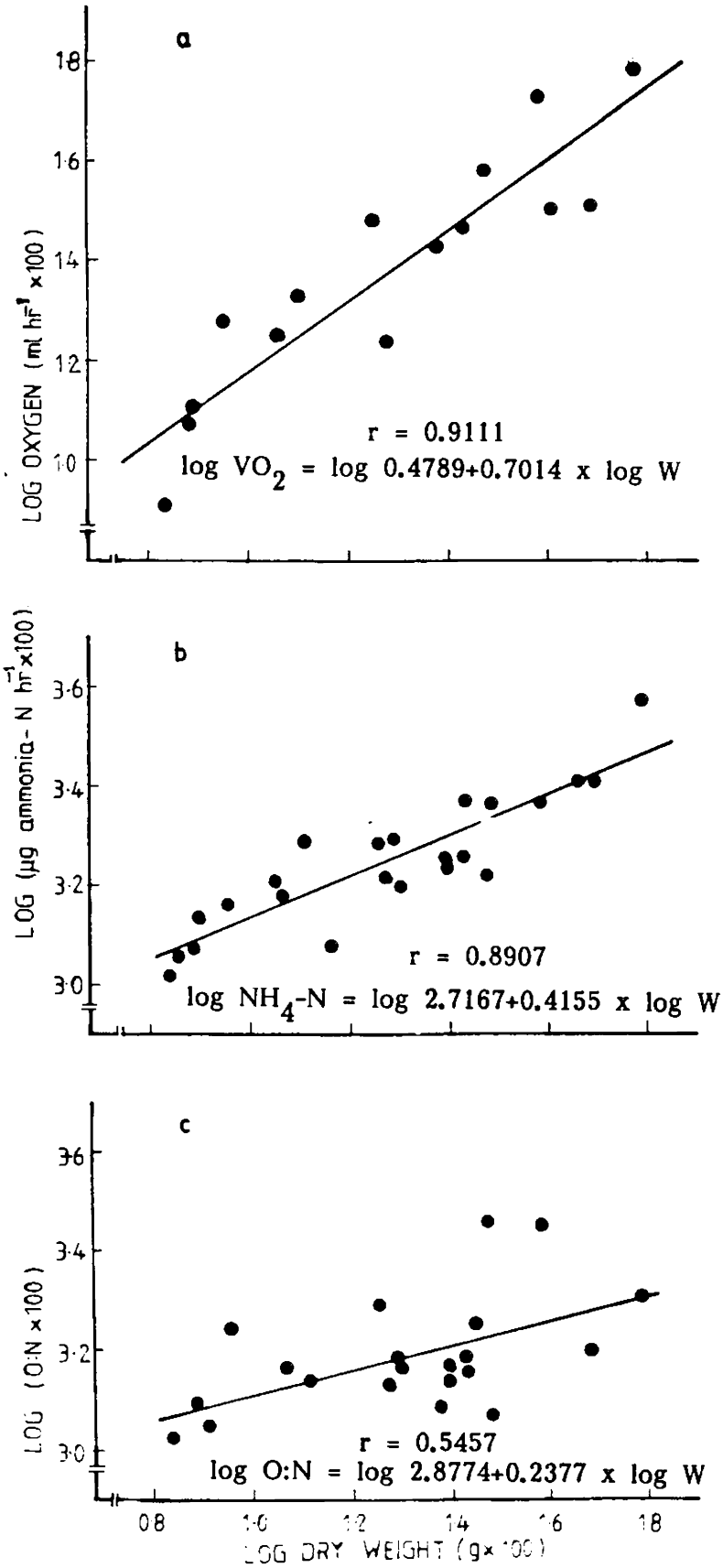


Fig-2.2. a) Rates of Oxygen consumption (ml O_2 h^{-1}). b) Rates of Ammonia excretion (µg NH_4-N h^{-1}). c) O:N ratio in *Perna viridis*

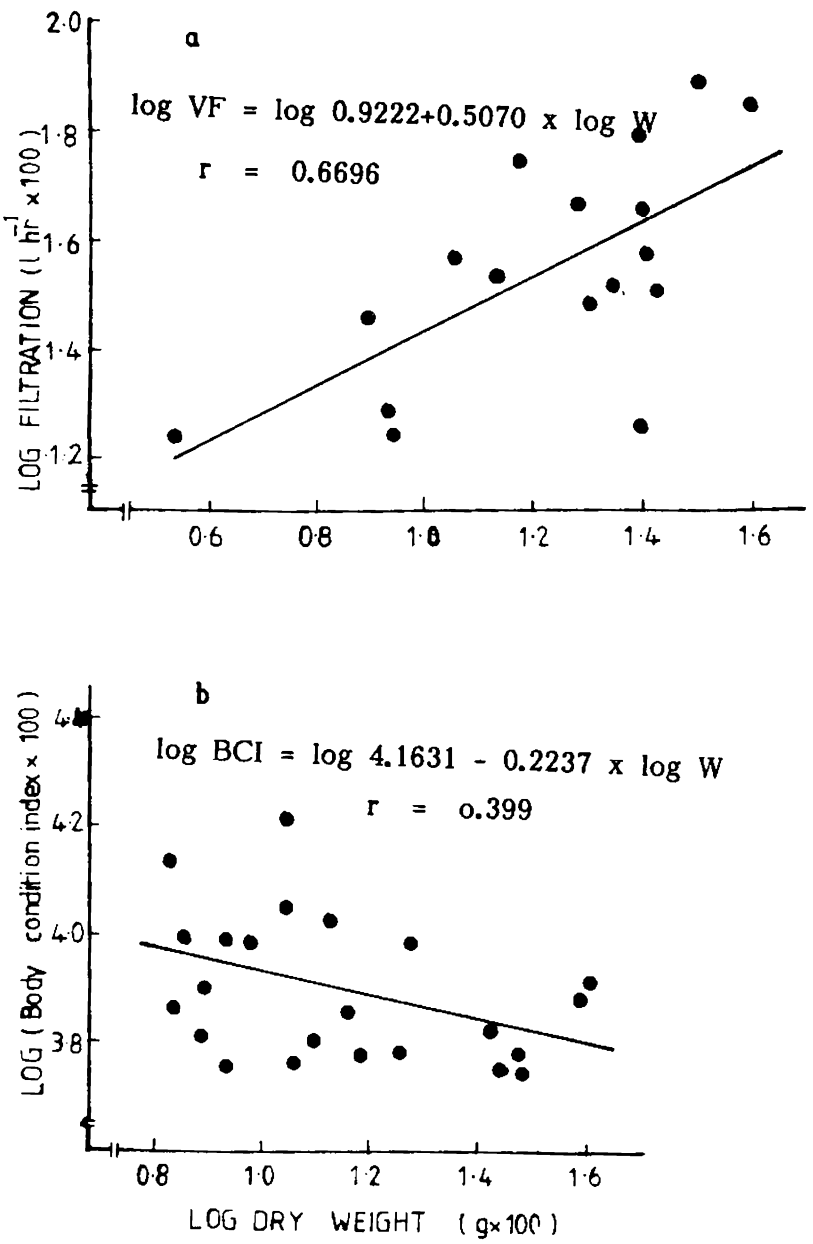


Fig-2.3. a) Filtration rate (l h^{-1}). b) Body condition index.

the present study a comparatively lower O:N ratio (23.09) obtained for *P.viridis* indicates a heavy dependence on protein for energy production in it. The O:N ratio may vary depending upon trophic level, gametogenic cycle, the nature of food and nutrient reserves (Bayne, 1975, Widdows, 1978b, Worral *et al.*, 1983) and so the interpretation of the O:N ratio should be based on relative change rather than on absolute value (Shirely and Stickle, 1982; Widdows, 1985a). The main feature of the seasonal variation in O:N ratio is the marked decline during and immediately after the spawning period (Widdows, 1985a).

Hawkins *et al.* (1986) also reported a very low O:N ratio (8) in *P.viridis*. In *P.viridis* the lipid and carbohydrate reserve were reported to be low from August to December, coinciding with maximum spawning (Qasim *et al.*, 1977; Ajithkumar 1984). Ajithkumar (1984) observed 93% of the mussels in spawning, spent or recovery stage in November. The present study was conducted during this months and so the recorded low O:N value may be due to spawning stress in mature mussels. Still, the low O:N ratio obtained for small, immature mussels (10-15 mm size and 0.06 - 0.01g dry wt) needs further explanation. Utilization of ammonia nitrogen in synthetic pathways or failure to oxidise the carbon skeletons of the amino acids will result in deviation from the theoretical expectations of O:N ratio (Bayne, 1975). This makes it necessary to collect information on this index under different ecological and physiological conditions to obtain base value which can be used as a stress index.

In the present study a positive correlation was obtained (Fig. 2.2c) between O:N ratio and body weight. It is because of the higher exponent for oxygen consumption (0.7014) than ammonia excretion (0.4155) against body weight. In unstarved *M. edulis* a negative correlation was obtained between the O:N ratio and body weight due to high exponent for ammonia excretion than for oxygen consumption and the reverse was obtained in starved mussels (Bayne and Scullard, 1977). In both *Thais lappillus* and *Polinices* (Gastropod) the O:N ratio was not changed with size because of similar exponents for oxygen consumption and ammonia excretion against body weights (Stickle and Bayne, 1982; Mace and Ansell, 1982).

Understanding of the bioenergetics of bivalves requires accurate estimates of filtration rate under defined conditions. Many studies have been carried out on the relationship between filtration rates and body weights of mussels and the reported exponents varies from 0.25 to 0.76 (see review, Bayne & Newell, 1983). The weight exponent obtained in the present study (0.507) is comparable with values obtained for *M. edulis* and *M. californianus* (Winter, 1973).

BCI is the simple index that reflects long term changes in the nutritional state of bivalve molluscs and a reduction in the index reflects either period of stress or spawning (Widdows, 1985a). The BCI and body weight of *P.viridis* showed a negative correlation (Fig. 2.3b). This may be due to reduction in body weight of larger mussels after spawning. Spawning in natural beds of *P.viridis* starts in July and

extends to November (Narasimha, 1983; Ajithkumar, 1984) and so there is a possibility of inclusion of spent mussels in the BCI determinations.

In the present study it has been possible to determine oxygen uptake, ammonia excretion, O:N ratio, filtration rate, BCI and the effect of body size on the above physiological indices of *P. viridis*. Eventhough both *M.edulis* and *P.viridis* live in very similar habitats the latter experiences a more thermaly stable but hotter environment (Shaffee, 1979; Devenport, 1983). So for determining the physiological indices of *Perna viridis* and to use them in the biological monitoring programme, more detailed round the year studies are required.

CHAPTER - 3

ACUTE TOXICITY OF COPPER, MERCURY AND ZINC

1. INTRODUCTION

Toxicity can be defined as the adverse effect caused to a test organism by pollutants. Toxicity is a function of concentration of the pollutant and duration of exposure which will be modified by variables such as temperature, chemical form and bio-availability of the pollutant. Informations obtained from various toxicity tests can be used in pollution management for the purpose of (1) Prediction of environmental effects of a pollutant (2) comparison of toxicants or animals or test conditions and (3) regulation of pollutant discharge (Buikema Jr *et al.*, 1982).

The relatively short term lethal or other effects are called acute toxicity (APHA *et al.*, 1981). In an acute toxicity test, group of aquatic animals are exposed to progressively increasing concentration of toxicants. The primary purpose of the test is to estimate the concentration of the test material that is lethal to 50% of the animals or that is capable of affecting a specific response like respiration, filtration, burrowing, loss of equilibrium etc. within a specific length of time. These concentrations are called median lethal concentration (LC50) and median effective concentration (EC50) respectively (Sprague, 1969, APHA *et al.*, 1981). Various aspects of bioassay methods for acute toxicity, like utility and application were attemped by several workers (Sprague, 1969; Buikema Jr *et al.*, 1982; Ward & Parrish, 1982; Sprague,

1985). Several methods for estimating the LC50 and its associated confidence interval have been proposed in the literature of bioassay (Gelber *et al.*, 1985).

It is recommended that toxicity should be described in terms of the incipient LC50 or lethal threshold concentration. It is the level of the toxicant which is lethal for 50% of individuals exposed for periods sufficiently long that acute lethal action has ceased (Sprague, 1970).

Even after prolonged exposure, LC50 concentration rarely fall within the range of concentrations observed in the contaminated waters (Bryan, 1984). Toxicity of a metal cannot be predicted from knowledge of concentration of total metal alone, since the form in which a metal occurs profoundly affects its bioavailability. Moreover, it is almost impossible to duplicate natural environmental conditions in laboratory experiments with metal and other pollutants. And this observation is a justification for the view that toxicants have a 'laboratory toxicity' and an 'environmental' toxicity and it is difficult to predict the latter from the former (Stebbing, 1985). However acute toxicity tests have proved their ability in environmental management and it remains unsurpassed as a screening technique and a comparative tool (Buikema Jr et al., 1982). From the LC50 values a 'safe level concentration' can be arrived by the use of 'application factors' which vary from 0.1 to 0.0001 (Sprague, 1969; Buikema Jr *et al.*, 1982).

Selection of an organism for acute toxicity test is based on

1) the organism is representative of an ecologically important group. 2) It occupies a position within a food chain leading to man or other importat species 3) the organism is widely available, and easily maintained 4) there is adequate data on the organism (Buikema Jr *et al.*, 1982). LC50 approach to metal toxicity in a wide range of marine species have provided a wealth of information about the effects of different metals on different species and has revealed various factors that modify toxicity (Bryan, 1984). Bivalve molluscs have been widely used as test organisms in acute toxicity tests for various heavy metals(Connor, 1972; Scott & Major, 1972; Olson & Harrel, 1973; Dillon, 1977; Nelson *et al.*, 1977; Lakshmanan & Nambisan, 1977; 1979, Nambisan *et al.*, 1977; Kumaraguru & Ramamoorthi, 1978; D'Silva & Kureishy, 1978; Davenport & Manely, 1978; Kumaraguru *et al.*, 1980; Chung, 1980; Sunila, 1981; Akberali & Black, 1980; Hvilson, 1983; Nambisan & Lakshmanan, 1986). Acute toxicity of some heavy metals have been worked out in *P.viridis* by few workers (D'Silva & Kureishy, 1978; Lakshmanan & Nambisan, 1979; Nambisan & Lakshmanan, 1986). But informations are not available on the sensitivity of different size groups to heavy metals.

During acute tests, in many instances, death is difficult to be determined. In those situations data may be expressed as effective concentration (EC) rather than a lethal concentration (Bukeima Jr *et al., 1982).* Based upon the effect on filtration rate in bivalves, Abel (1976) proposed a simple, quick and economical method for the EC50 determination of heavy metals. But very few works have been reported

using this method (Watling, 1981; Watling & Watling, 1982; Abraham *et al, 1986)* and no such studies were reported in P. viridis.

So the main objectives of these studies are (1) to compare the acute toxicity of Cu, Hg and Zn with the help of LC50 values and toxicity curves (2) to study the sensitivity of smaller mussels to the above metals and (3) to determine the EC50 values for the above metals using filtration rate. Here for the first time in *P. viridis* an attempt has been made to calculate the LC50 and EC50 values with 95% confidence limit and slope function, using standard statistical methods.

2. MATERIALS AND METHODS

Animals were collected and acclimated as explained in Chapter - 2. Mussels were acclimated for one week before the experiment (Salinity =35 x 10^{-3}, Temp = 29.5±0.5°C; pH = 7.85±0.1 and dissolved O_2> 90% saturation). Algal feeding was stopped during and two days before the experiment.

a) Test containers:

Bioassays were conducted in specially manufactured, colourless polythene tubs of 10 l capacity and 14" diameter. The tubs were soaked in Nitric acid (1:1) and Hydrochloric acid (1:1) for 24h each and washed with copious amounts of deionized water. Lids were provided for the tubs to protect from dust during the experiment.

b) Test Solution:

100 ppm stock solutions of Cu, Hg and Zn were prepared by dissolving analytical grade $CuSO_4 \cdot 5H_2O$, $HgCl_2$ and $ZnSO_4 \cdot 7H_2O$ respectively

in double distilled water, calculated quantities of the solution were added to experimental tubs containing a known volume of seawater to obtain the required test concentrations.

c) Bioassay for LC50 determination:

Static bioassay procedure given by APHA *et al.*(1981) and Ward & Parrish (1982) were followed in the experiment. For the study filtered, unpolluted seawater was used (Salinity = 35×10^{-3}; Temp = 29±1.0°C, pH = 7.8±0.1 and dissolved Oxygen > 90% saturation). Test animals of two size groups, 15-20 mm and 30-40 mm were sorted and cleaned for the experiment. After range finding tests seven concentrations were used for each metal (Table - 3.1).

Each tub contained 5 l seawater and 10 mussels. One tub was kept as control without metal solution and duplicates were run for each metal concentration. Mussels were inspected every 12 h for mortality and water was renewed every 24 h. Mussels were considered dead if they gaped the shell wide and showed no response to tactile stimulus. Dead mussels were removed and recorded every 12 h. The experiments were conducted in two size groups of mussels separately.

d) Bioassay for EC50 determination:

Filtration rates were determined using mussels of size group 30-40 mm. The procedure given by Abel (1976) was followed as explained in Chapter - 2. After range finding experiments concentrations of metals as shown in Table - 3.3 were used. Controls were kept for each metal and duplicates were maintained for each concentration. 500 ml of test

medium (Salinity = 33.5 x 10^{-3}; Temp = 28.5±1.0°C; pH = 7.8±0.05; dissolved Oxygen >90% saturation) with 5 animals were used for each metal concentration in one 1 conical flasks. Filtration rates were calculated in ml min^{-1} (Abel, 1976).

e) Data analysis:

Cumulative percentage mortality was determined for each metal concentration of the mortality study experiment. This is latter plotted in a log-probit paper and the concentration of metal killing 50% of the test organisms (LC50) during 48, 60, 72, 84 and 96 h exposure together with 95% confidence limit and slope function were calculated following the methods of Litchfield & Wilcoxon, (1949).

From the filtration rate, % reduction of filtration in comparison with control were determined for each metal concentration (Table - 3.3). Then as in LC50, metal concentration reducing 50% of the filtration rate (EC50) together with 95% confidence were calculated.

3. RESULTS

a) Behavioural responses:

Mussels except in control and lower concentration of metals, remained with their valves tightly closed. Some mussels in higher concentration of Cu, Hg and Zn did not withdraw their foot when closing but left them between the valves after 48h exposure. Higher concentration of all the metals decreased byssus thread production and increased mucus secretion. Spawning was noticed among some of the mussels of larger size group in higher metal concentration. Mussels in

the higher metal concentration, after losing their byssal attachment, were found to be unable to fasten again, while mussels in the control remained attached to the sides of the tub and behaved normally through out the experiment.

b) Median lethal concentrations (LC50s):

The cumulative % mortality in different concentrations of Cu, Hg and Zn are given in the Table - 3.1 and LC50 values are summarised in the Table - 3.2.

For larger mussels (30-40mm) at 96 h the highest concentration of Cu at which mortality was not observed and lowest concentration at which 100% mortality was observed were 0.04 and 0.12 ppm respectively. 96 h LC50 for Cu in larger size group was 0.086ppm. But in smaller size group (15-20mm) it was 0.063 ppm.

For Hg at 96 h the highest concentration at which mortality was not observed and the lowest concentration at which 100% mortality was observed were 0.05 and 0.4 ppm respectively for both the size groups. LC50, 96 h for Hg in smaller and larger size groups were 0.155 and 0.125 ppm respectively.

For Zn at 96 h the highest concentration at which mortality was not observed and the lowest concentration at which 100% mortality was observed were 1.0 and 7.0 ppm respectively in larger mussels. 96 h LC50 for Zn in smaller and larger size groups were 2.85 and 3.9 ppm respectively. In both the size groups, mussels were found to be more sensitive to Cu and Hg. The order of toxicity of the metals are Cu>Hg>Zn.

Table - 3.1. Cumulative % mortality of *P. viridis* exposed in Cu, Hg and Zn for 96h.

Metal	Conc. ppm	Exposure Time (Hours)															
		12		24		36		48		60		72		84		96	
		a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b
Cu^{2+}	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0.04	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0.06	0	0	0	0	5	0	5	10	10	20	15	20	30	20	40	20
	0.08	0	0	0	0	10	0	10	10	15	20	40	30	75	40	90	40
	0.10	0	0	5	0	20	0	25	10	40	30	65	60	80	70	100	70
	0.12	0	0	10	0	35	0	60	10	70	20	85	60	90	100	100	100
	0.14	10	0	40	0	50	30	55	80	75	80	85	80	95	90	100	100
	0.16	15	0	40	0	60	40	80	80	100	100	100	100	100	100	100	100
Hg^{2+}	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0.05	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0.10	0	0	0	0	10	0	20	0	20	0	30	0	40	10	40	10
	0.20	0	0	0	0	10	0	40	20	50	40	60	60	70	80	80	80
	0.40	0	0	10	0	45	0	70	30	100	40	100	90	100	100	100	100
	0.60	0	0	10	0	45	0	75	50	100	90	100	100	100	100	100	100
	0.80	0	0	0	0	35	0	80	50	100	80	100	90	100	100	100	100
	1.00	0	0	10	0	40	0	90	40	100	80	100	100	100	100	100	100
Zn^{2+}	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0	10	0	10	0	30	20
	3	0	0	0	0	0	0	0	0	20	0	40	10	40	10	50	30
	4	0	0	0	0	0	0	0	0	10	0	30	10	60	20	70	50
	5	0	0	0	0	0	0	0	0	20	20	40	40	70	40	80	70
	6	0	0	0	0	0	0	10	0	30	20	60	40	80	60	100	90
	7	0	0	0	0	0	0	10	10	30	20	50	50	80	70	100	100

a = 15 - 20 mm size
b = 30 - 40 mm size

Table - 3.2. Lethal concentrations of Cu, Hg and Zn to *Perna viridis*.

Metal	Duration of exposure (h)	Size Group (mm)	LC84 (mg/l)	LC50 and 95% Confidence limits(mg/l)	LC16 (mg/l)	Slope
Cu^{2+}	48	15-20	0.165	0.125(0.109-0.143)	0.088	1.370
	60	15-20	0.160	0.105(0.086-0.128)	0.064	1.582
		30-40	0.170	0.112(0.093-0.135)	0.072	1.537
	72	15-20	0.130	0.088(0.074-0.104)	0.060	1.472
		30-40	0.135	0.100(0.083-0.120)	0.075	1.342
	84	15-20	0.105	0.074(0.061-0.090)	0.049	1.465
		30-40	0.125	0.086(0.071-0.104)	0.059	1.456
	96	15-20	0.076	0.063(0.056-0.070)	0.053	1.203
		30-40	0.125	0.086(0.071-0.104)	0.059	1.456
Hg^{2+}	48	15-20	0.80	0.270(0.177-0.410)	0.092	2.949
	60	15-20	0.45	0.202(0.134-0.303)	0.090	2.236
		30-40	0.50	0.295(0.108-0.803)	0.180	1.667
	72	15-20	0.38	0.16 (0.093-0.275)	0.066	2.400
		30-40	0.34	0.225(0.157-0.322)	0.150	1.506
	84	15-20	0.31	0.135(0.077-0.237)	0.051	2.485
		30-40	0.22	0.155(0.133-0.212)	0.108	1.427
	96	15-20	0.21	0.125(0.09- 0.171)	0.074	1.685
		30-40	0.22	0.155(0.133-0.212)	0.108	1.427
Zn^{2+}	72	15-20	10.45	6.20(4.63-8.31)	2.80	1.950
		30-40	10.40	7.00(5.67-8.64)	4.00	1.678
	84	15-20	6.65	3.60(2.85-4.56)	2.00	1.824
		30-40	8.0	5.40(4.44-6.56)	3.70	1.470
	96	15-20	5.60	2.85(2.14-3.80)	1.50	1.933
		30-40	6.20	3.90(3.17-4.80)	2.40	1.607

Changes in LC50 values with time are given in Fig. 3.1. In both the size groups Cu and Hg were found to be having steeper slopes, which is an indication of acutely acting toxicity. The shallower slopes obtained for Zn shows its slow toxic action. After 84 h exposure to Cu & Hg toxicity curves turned asymptoticaly with the time axis in larger animals. This point in time is the threshold concentration, beyond which there would not be any significant increase in mortality. In the present study threshold concentrations observed in larger animals were 0.086 and 0.155 ppm for Cu and Hg respectively.

Animals of the smaller size group (15-20 mm) were found to be more sensitive to Cu, Hg and Zn in comparison with larger size group (Table -3.2). This is also evident from the toxicity curve (Fig. 3.1).

c) Median effective concentrations (EC50s):

Filtration rate of mussels under different metal concentrations and the calculated EC50 values are given in the Table - 3.3. EC50 values for Cu, Hg and Zn were 0.135, 0.074 and 4.1 ppm respectively. Of the three metals tested Hg was found to be highly toxic. The EC50 curves of metals are shown in fig. 3.2 and from the slopes, metal toxicity affecting filtration rate can be compared. Based upon filtration rate effect study, the order of metal toxicity is Hg > Cu > Zn.

4. DISCUSSION

High Cu, Hg and Zn concentrations increased mucus secretion and decreased byssus production in *P. viridis*. Earlier workers also

Table - 3.3. Effect of Cu, Hg and Zn on filtration rate and median effective concentration in *P. viridis*.

	$CuSO_4 \cdot 5H_2O$			$HgCl_2$			$ZnSO_4 \cdot 7H_2O$		
S. No.	Conc. (ppm)	Filtration rate ml/min.	% reduction	Conc. (ppm)	Filtration rate ml/min.	% reduction	Conc. (ppm)	Filtration rate ml/min.	% reduction
1	0	4.49	0	0	6.541	0	0	4.2394	0
2	0.02	3.39	24.5	0.01	7.350	0	0.5	4.0338	4.85
3	0.04	5.52	0	0.025	4.565	30.21	1.0	3.1451	25.81
4	0.08	4.35	3.12	0.05	5.383	17.70	1.5	3.1451	25.81
5	0.10	3.64	18.93	0.075	2.300	64.84	2.0	3.3969	19.87
6	0.12	2.68	40.32	0.10	1.980	69.73	2.5	3.9126	7.71
7	0.14	2.40	55.46	0.15	0.200	96.94	3.0	4.4898	0
8	0.16	2.92	34.97	--	--	--	4.0	3.1064	26.93
9	0.18	1.09	75.72	--	--	--	5.0	2.0603	51.40
10	0.25	0.24	94.65	--	--	--	6.0	1.3562	68.01
EC16	0.092			0.05			1.2		
EC50	0.135(0.166-0.110)			0.074(0.097-0.057)			4.1 (7.35-2.29)		
EC84	0.195			0.105			12.0		
Slope	1.4559			1.4495			3.1717.		

reported similar observations in bivalves (Scott & Major, 1972; D'Silva & Kureishy, 1978; Lakshmanan & Nambisan, 1979; Sunila, 1981; Hvilson, 1983). Spawning noticed in *P. viridis* exposed to higher concentrations may be due to the stress effect of metals on ripe mussels. As observed in the present study valve closure mechanism in bivalves were reported as a common avoidance response to pollutant stress (see review, Akberali & Trueman, 1985).

In order to have an approximate comparison of the sensitivity of various bivalves to Cu, Hg and Zn, the reported LC50 values are summarised in Table - 3.4. It is however difficult to have a comparison from this data, since experiments were carried out under different time intervals and under varying physio-chemical conditions. Reported data indicate that *P.viridis* is highly sensitive to Cu, Hg and Zn than other bivalves. This shows the usefulness of *P.viridis* as a sensitive test organism for heavy metal toxicity tests. From the Table - 3.4 it can be seen that, for the same species different LC50 values were reported. But LC50 may vary from one population of mussels to other, due to their adaptation to higher heavy metal load or due to genetic factors (Sunila, 1981). Low level pre-exposure was found to be improving the ability of some bivalves to survive acute metal toxicity (Dillon, 1977).

Hg is considered as the most toxic heavy metal to marine animals in general (see reviews, Taylor, 1979; Bryan, 1984). Cu was reported as an important toxic heavy metal to bivalve molluscs (Scott & Major, 1972; Olson & Harrel, 1973; D'Silva & Kureishy, 1978; Kumaraguru

Table - 3.4.Acute toxicity data, LC50 published for Cu, Hg and Zn to marine bivalve molluscs.

Metal	Species	Size (mm)	LC50 (mg/1)	Temp. (°C)	$S‰_{x10^{-3}}$	Source
Copper	*Mytilus edulis*	30-50	0.28 (96 h)	12	N.R*	Abel (1976)
	M.edulis	40-80	0.1-0.2(168 h)	10	N.R	Scott & Major (1972)
	M.edulis	21-30	0.2-0.5(168 h)	7.5±1	16.5	Hvilson (1983)
	M.edulis	10-25	0.4 (48 h)	6.0	7.0	Sunila (1981)
	Crassostrea rhizophorae	19-30	40 (96 h)	23.5	36.0	Chung (1980)
	Meretrix casta	30-32	2.188 (96 h)	27-30	25.0	Nambisan & Lakshmanan (1986)
	M. casta	25-42	0.072 (96 h)	27.0±0.5	25.0	Kumaraguru & Ramamoorthy
	Anadara granosa	29-44	0.06 (96 h)	27.0±0.5	25.0	" " (1978)
	C. madrasensis	31-115	0.088 (96 h)	27.0±0.5	25.0	" "
	Rangia cuneata	N.R	7.4 (96 h)	24.0	22.0	Olson & Harrel (1973)
	Perna viridis	15-16	0.14 (48 h)	29±1	33±1	D'Silva & Kureishy (1978)
	P. viridis	55-60	0.174 (96 h)	28-31	25.0	Nambisan & Lakshmanan (1986)
	P. viridis	15-20	0.063 (96 h)	29±1	35.0	Present study
	P. viridis	30-40	0.086 (96 h)	29±1	35.0	Present study
Mercury	*Meretrix casta*	30-32	3.25 (96 h)	29-31	17.0	Nambisan & Lakshmanan (1986)
	Modiolus curvalleri	12-14	0.19 (96 h)	30±5	33.0	Ekanath & Menon (1983)
	R. cuneata	N.R	8.7 (96 h)	24.0	22.0	Olson & Harrel (1973)
	R. cuneata	N.R	0.058 (96 h)	N.R.	15.0	Dillon (1977)
	R. cuneata	N.R	0.122 (96 h)	N.R.	2.0	" "
	P. viridis	20-24	0.23 (96 h)	30±5	33.0	Ekanath & Menon (1983)
	P. viridis	55-60	0.35 (96 h)	29-30	25.0	Lakshmanan & Nambisan (1979)
	P. viridis	15-20	0.125 (96 h)	29±1	35.0	Present study
	P. viridis	30-40	0.155 (96 h)	29±1	35.0	Present study
Zinc	*M. edulis*	30-50	7.8 (96 h)	12	N.R	Abel (1976)
	C. rhizophorae	19-30	80.0 (96 h)	23.5	36.0	Chung (1980)
	M. casta	30-32	6.67 (96 h)	29-31	25.0	Nambisan & Lakshmanan (1986)
	P. viridis	15-16	2.31 (48 h)	29±1	33±1	D'Silva & Kureishy (1978)
	P. viridis	55-60	3.00 (96 h)	30-32	25.0	Nambisan & Lakshmanan (1986)
	P. viridis	15-20	2.85 (96 h)	29±1	35.0	Present study
	P. viridis	30-40	3.90 (96 h)	29±1	35.0	Present study.

* N. R - NOT REPORTED.

& Ramamoorthy, 1978; Davenport & Manely, 1978; Martin, 1979; Kumaraguru et al., 1980; Chung, 1980; Akberali & Black, 1980; Sunila, 1981; Hvilson, 1983; Nambisan & Lakshmanan, 1986; Latha, 1986). Therefore copper salts are commonly used as molluscicides. In the present study LC50 values showed that Cu is slightly more toxic than Hg (Table - 3.3). Similar situation was reported in the case of *Rangia cuneata* (Olson & Harrel, 1973). Low LC50 values were reported in tropical bivalves like *P.viridis & Villorita cyprinoides* (Nambisan and Lakshmanan, 1986; Abraham *et al.*, 1986) for Cu in comparison with Hg. From the Table - 3.4, tropical species like *P.viridis*, *Meretrix casta*, *Anadora granosa* and *Crassostrea madrasensis*, were found to be more sensitive to Cu than the temperate species. But for *M. edulis* Hg was found to be more toxic than Cu (Stromgren, 1982).

Animal's sensitivity to pollutants varies with age and larval stages (Bryan, 1984). In the present study animals of smaller size group (15-20) were found to be more sensitive to Cu, Hg and Zn than larger size group (30-40mm). This may be because of the higher accumulation of the metals by the younger individuals per mass unit as reported by earlier workers (Boyden, 1977; Fowler *et al.*, 1978, Simpson, 1979; Cossa et al., 1980; Riisgard *et al.*, 1985; Cossa & Rondean, 1985 and chapter - 4 of this study). Similarly younger individuals of *M.edulis* (Sunila, 1981) and *Perna* sp. (Watling & Watling, 1982) were found to be more sensitive to heavy metals.

The EC50 value obtained in the present study for Cu (0.135ppm)

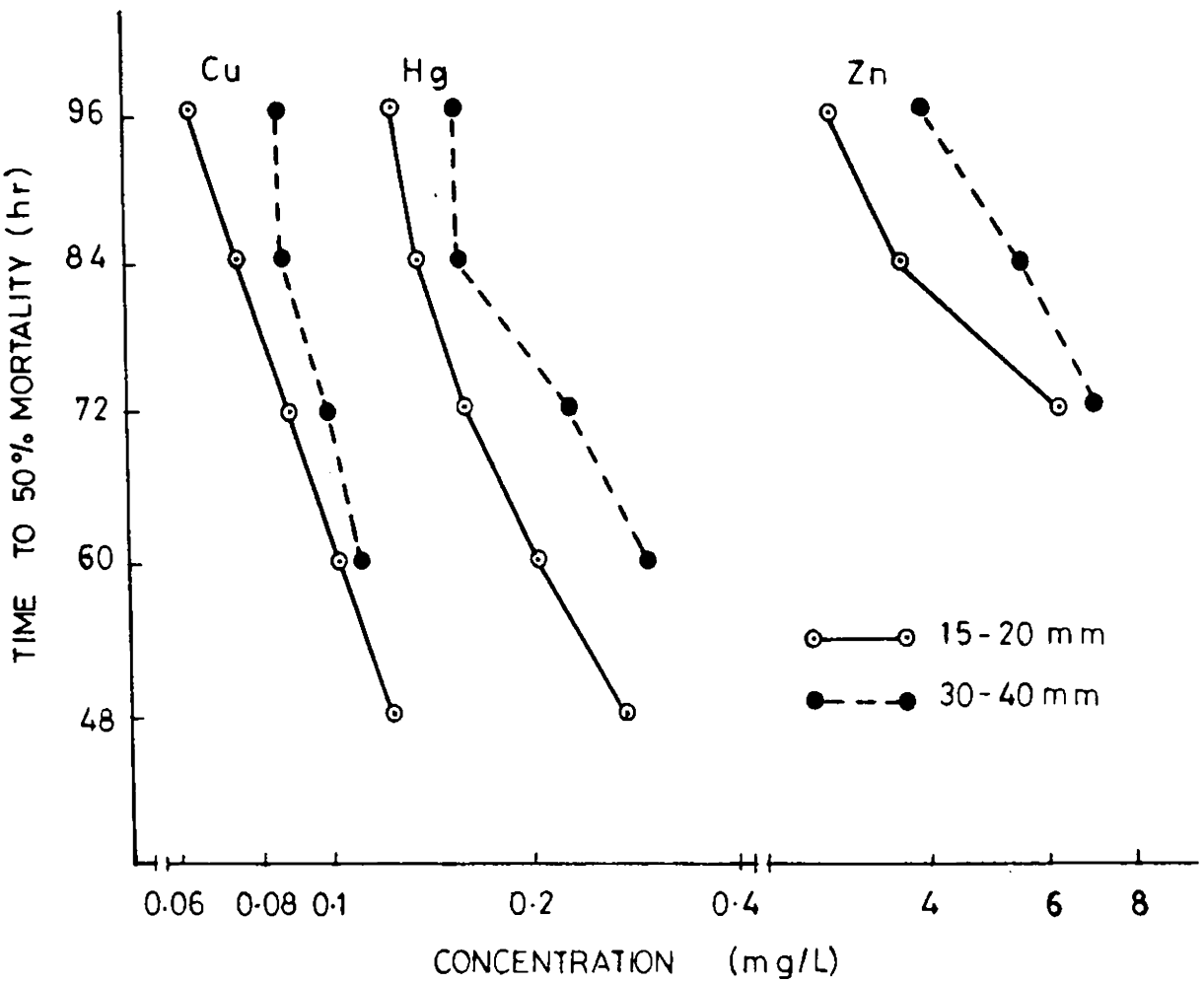


Fig-3.1. Toxicity curve for Cu, Hg and Zn in *Perna viridis* of smaller and larger size groups.

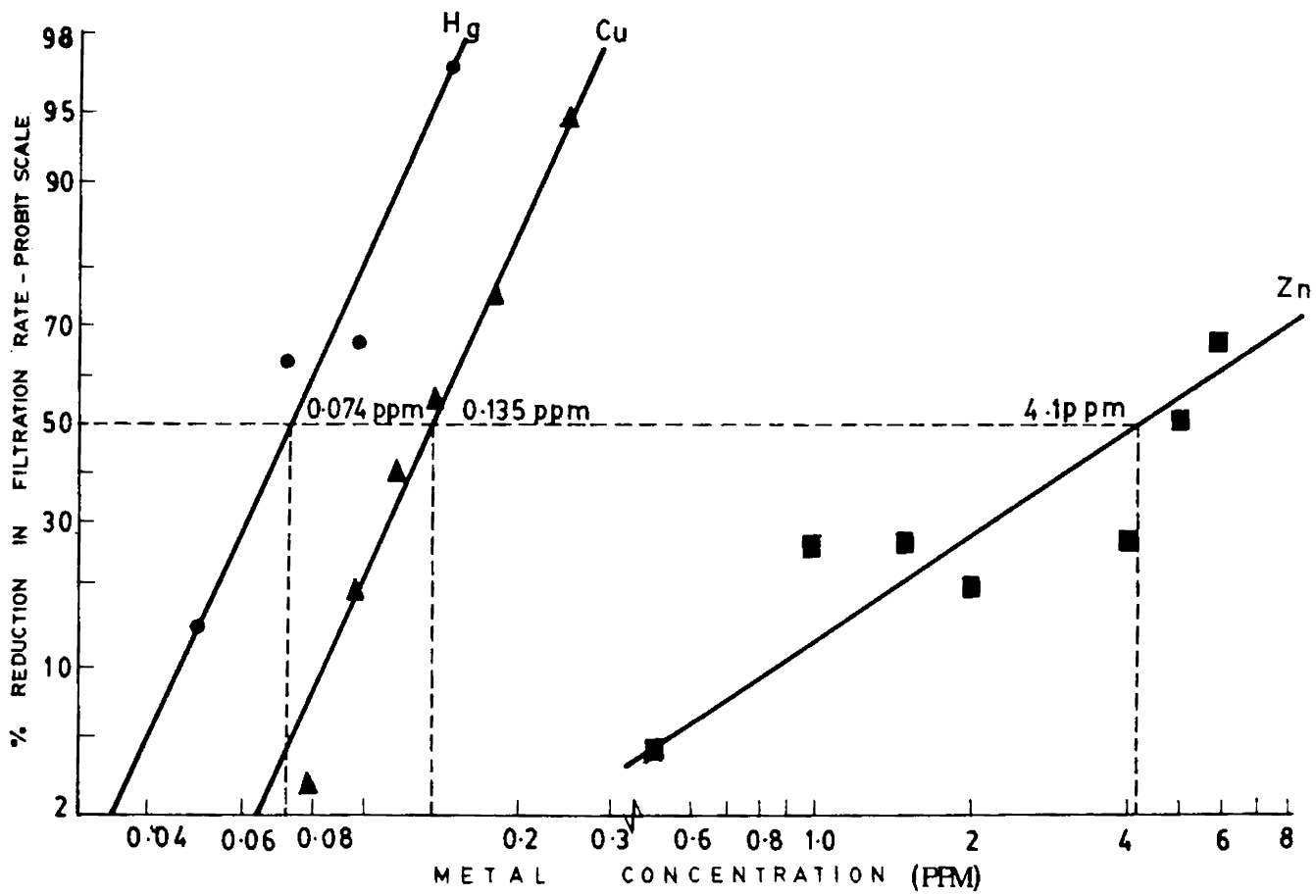


Fig-3.2. EC50 curves for Cu, Hg and Zn.

is comparable with the values reported in *M.edulis* (Abel,1976) and *Perna perna* (Watling, 1981). However the EC50 values obtained for Hg (0.074 ppm) and Zn (4.1 ppm) are higher than the values reported by Abel (1976) and Watling & Watling (1982). But EC50 values based on filtration rate were reported to vary with both intrinsic and extrinsic factors (Abel, 1976; Watling & Watling, 1982).

The EC50 values obtained in the present study show that Hg is more toxic than Cu and Zn (Fig. 3.2 and Table - 3.3). Earlier workers also reported that Hg is highly toxic than Cu based up on filtration study (Abel, 1976; Watling & Watling, 1982; Abraham *et al.*, 1986). These observations are in contradiction with the LC50 values obtained in the present study. Similar contradictory LC50 and EC50 values were reported for Hg and Cu in *Villorita cyprinoides* (Abraham *et al.*, 1986). According to Buikema Jr *et al.* (1982) EC50 is not comparable with LC50 because the measured responses are different. According to them EC50 values will be lower than LC50, because impairment of functions occur before mortality of the test organism. Results reported by Abel (1976) and Abraham *et al.*(1986) are in agreement with this. In the present study it is found to be applicable for Hg. But Cu and Zn were found to be having higher EC50 value in comparison with LC50.

Similarly high EC50 value (activity of the cilia) were reported for Cu in M.edulis in comparison with its 24 h LC50 value (Sunila, 1981). Hughes *et al.*(1987), reported high EC50, value for Cu (burying behaviour) in comparison with 96 h LC50 in *Polynices sordidus*.

This may be due to differences in the duration of EC50 and LC50 experiments. Another reason is that EC50 values, based on certain pysiological functions like filtration rate may vary with the mode of toxic action of the heavy metals. So the EC50 test proposed by Abel (1976) cannot be used as a sensitive test in case of metals like Cu which do not have any drastic effect on filtration rate. This is further investigated in Chapter - 6 by studying the O_2 consumption and filtration rate in *P.viridis* exposed to same concentration of Cu and Hg, and Hg was found to be drastically inhibiting filtration rate in comparison with Cu, as an indication of its well known neurotoxic effect, which impairs ciliary activity. This may be the reason for the low EC50 value obtained for Hg in comparison with Cu and Zn.

The EC50 values are generally calculated based on single responses like filtration, valve closure, respiration, byssus production etc. While LC50 values are depandent on the mortality rate of the animal, which is the cumulative effect of all the physiological impairment caused by the heavy metals. Even though mortality is explained as a very crude index of stress (Akberali & Trueman, 1985), as seen in the present study 96 h LC50s are sensitive and reliable, and they are in agreement with the results of sublethal effect study (See Chapters - 6 and 8).

The results indicate that EC50 tests are very sensitive and time saving in case of metals like Hg, which are highly toxic to certain physiological functions like filtration rate, even at very low

concentration. So both LC50 and EC50 tests can be used simultaneously in animals like *P.viridis*, and lowest values can be taken for further use.

CHAPTER - 4

BIOACCUMULATION OF COPPER, MERCURY AND ZINC

1. INTRODUCTION

Bioaccumulation is the process of concentrating some toxicants, particularly complex organics, in the tissues of aquatic organisms at concentrations many times higher than of ambient waters (Buikema Jr *et al.*, 1982).

Marine molluscs are well-known for their ability to accumulate pollutants from their environment and have been widely used for biological monitoring of metal contamination. Because of their world-wide distribution, sedentary, filter feeding habits and potential as indicators, species of *Mytilus* have become the subject of various monitoring programmes of the 'Mussel Watch' type (Goldberg *et al.*, 1978; Davies and Pirie, 1980). The accumulation of abnormal concentrations of metals by the tissues of marine organisms is a matter of great concern today. This is because the accumulation of highly toxic metals by commercial species may become harmful to man and other vertebrates. Apart from the above it may also affect the productivity of the estuarine and marine environment (Bryan, 1984).

Mussels are included in various marine pollution monitoring programmes as bio-indicators of heavy metal pollution. The biological monitoring programmes have many advantages such as (1) metals are usually concentrated within the organisms to levels that make them easier to detect. (2) The accumulated doses are by definition, only

those which are biologically available to organisms. (3) The body load of metals represent the sum of the environmental changes that have occured in the recent history of a particular ecosystem (Simkiss *et al.*, 1982).

Bivalve molluscs have been widely used to monitor heavy metals in the marine environment by several authors. (Phillips, 1976 a & b; 1977 a & b; Luoma, 1977a; Goldberg, 1975; Goldberg *et al.*, 1978; Eganhouse and Young 1978; Davies & Pirie, 1978; Simpson, 1979; Cossa *et al.*, 1980; Cotter *et al.*, 1982; Martincic *et al.*, 1984; Riisgard *et al.*, 1985; King & Davies, 1987). The bioaccumulation capacity of marine animals has been reviewed by Bryan (1979;1984); Phillips(1980) and Simkiss *et al.* (1982).

The metal ions in the marine environment have been classified into two main types (Ahrland, 1975) (1) Class A Type such as Na, K, Ca and Mg which are basically electrostatic in behaviour and in saline solution form hydrophilic charged ions.(2) Class B Type metals such as Cu, Zn and Ni; which form covalent compounds and therefore less likely to exist as free ions in solution.Toxicity of any pollutant is related to its lipid solubility and thus to its ability to pass through the lipoprotein membrane of the cell (Nelson & Donkin, 1985). The transport of these pollutants across the membrane depends not only on lipid solubility but also on various carrier species (ligands) in the animal body(Simkiss & Mason, 1984; Nelson and Donkin, 1985).

Based on the metal ligand interactions Simkiss & Mason (1984)

put toward four models for the uptake of metals into the cells. (1) The cell contains an excess of suitable ligands on to which the metal becomes bound. (2) The cell contains a limited amount of suitable ligand but the system is inducible and more ligands can therefore be synthesised. (3) The ligands limited but inducible as in (2). In addition there is a turn over of metal ligand complex which subsequently leaves the cell. (4) The cell contains ligands but there is competition between different metals for binding on to these molecules.

The uptake system acts as if they have first order kinetics, so that the rate at which the metals enter the organism is proportional to the level in the ambient seawater (Delhaye and Cornet, 1975; Nambisan *et al.*, 1977; Lakshmanan & Nambisan 1977 ; 1979; D'Silva & Qasim, 1979; Martincic *et al.*, 1984; Amiard *et al.*, 1986; Simkiss & Mason, 1984). The retention of the metal on the ligands with in the organisms determines the value of an animal as an accumulator of environmental metals. (Simkiss *et al.*, 1982, Simkiss & Mason, 1984). Once the metal has entered the cells they undoubtedly become bound to a variety of ligands and it is the metabolism of these complexes that determine the subsequent fate of the metal and the final body load (Simkiss & Mason, 1984).

Based on some X-ray microporbe analysis studies on *Littorina littorea*, the specific cells of metal accumulation have been identified (Simkiss & Mason, 1984). They are (1) Specific cells which contain high capacity ligands that will bind a variety of metals (basophil cells).

(2) Cells which have ligands that are inducible (ctenidia). (3) Cells which accumulate specific metals only (Porecells). (4) Other cells which contain variety of metals (nephrocytes).

Some of the recent studies concluded that metals are taken into the body of molluscs by atleast three independent means, (1) they may diffuse across the lipid membrane as metallochloride complexes (Carpene & George 1981). (2) as chelated complexes (Coombs and George, 1978) and (3) they may parasitize other ion pumps (Roesijadi, 1982). The common features of all these metal uptake mechanisms are that uptake depends up on the concentration of particular molecular species.

Most of the recent investigations revealed that different types of detoxification mechaisms exist with in the bivalve molluscs. These include binding to specific low molecular weight proteins of the metallothionein type (Olafson *et al.*, 1979; Viarengo *et al.*, 1985; Livingstone, 1985 a & b) and immobilization by intracellular compartmentation in tertiary lysosomes (George, 1983 a & b) and in membrane limited vesicles of amoebocytes (Pirie *et al.*, 1984). George *et al.* (1978) reported immobilisation of Cu and Zn in the amoebocytes of *Ostrea edulis*, with Cu being in association with sulphur. This type of detoxifying mechanism probably protects tissues during accumulation.

Metal levels found in mussels may be influenced by factors other than pollution and these factors need to be considered in any monitoring programme (Phillips, 1980). Such parameters include size, age, sex, season, height of the water column, salinity, chemical species

and interactions with other pollutants (Brayan, 1979, 1984). Information about the influence of these parameters on bioaccumulation is essential for the meaningful interpretation of the results of monitoring programmes like 'Mussel Watch' (Phillips & Segar, 1986, Segar & Stamman, 1986). The trace metal cycling in the marine environment is connected to the salinity of the medium (Bryan, 1971) and therefore, the effect of salinity on the uptake of trace metals by indicator organisms is very important (Phillips, 1977a). Many workers have studied the effect of salinity on the bio-concentration of heavy metals by molluscs (Phillips, 1976a, 1977a, Luoma, 1977b; Denton & Burdon-Jones, 1981, Latha, 1986).

The intrinsic factors which influence metal concentration in mussels are size, sex and reproductive condition and seasonal variation (Bryan, 1979; 1984; Phillips, 1980). Boyden (1977) showed that concentration of trace metal is generally higher in smaller animals. Similar observations were reported by other investigators in mussels (Fowler *et al.*, 1978 ; Cossa *et al.*, 1979; 1980; Simpson, 1979; Riisgard *et al.*, 1985; Cossa, & Rondean, 1985).

Kinetics of heavy metal accumulation have been studied in *P.viridis* (Nair *et al.*, 1977; D'Silva & Kureishy, 1978, Lakshmanan & Nambisan, 1979). But no study has been reported on the effect of salinity and size group on bioaccumulation of heavy metals. So the main objectives of these studies are (1) to understand the kinetics of metal accumulation in the different body parts (2) to study the effect of salinity on bioaccumulation. (3) to study the effect of size group on

accumulation.

2. MATERIALS AND METHODS

a) Experimental procedure:

Mussels were collected, cleaned and acclimated for 5 days in the laboratory as explained earlier (Chapter-2). One batch of mussel was acclimated in the experimental salinity of 35 x 10^{-3} and the other in 25 x 10^{-3} salinity. Temp = 28.5°C; pH = 7.8; dissolved oxygen >4 ml l^{-1}). Mussels were fed with algal culture. Feeding was stopped during and two days before the experiment.

Mussels (35-45 mm size) were exposed to Cu, Hg, and Zn in separate plastic tubs containing 15 l of filtered sea water. Stock solutions of metals were prepared and added to the medium as described earlier (Chapter - 3). For each metal two concentrations were used. The concentrations of different metals used and physio-chemical parameters of the experimental system are given in the table-4.1. The test medium was changed every day and 6 to 8 mussels were removed from each tub after every 24, 48, 72 and 96 h of exposure for metal analysis.

In the second set of experiment, two size groups of animals were selected. Smaller (15-20mm) and larger (30-40mm) mussels were exposed as in the first experiment. The concentration of metals used and the physio-chemical parameters are given in the table-4.1. Animals were removed for the metal analysis as in the first experiment. Mussels were removed from the control of both the experiments to determine the background concentration of metal in the tissue.

Table - 4.1. Metal concentration and physio-chemical parametres of the seawater used for the bioaccumulation experiments.

Metal	Conc. (ppm)	Length of the animal (mm)	Salinity (x 10^{-3})	Temp. (°C)	Dissolved oxygen ml/l	pH
Cu^{2+}	0.02	35-45	35	29.5±.5	>4.0	7.91
	0.04	35-45	35	29.5±.5	>4.0	7.80
Hg^{2+}	0.0375	35-45	35	29.5±.5	>4.0	7.58
	0.075	35-45	35	29.5±.5	>4.0	7.73
Zn^{2+}	0.75	38-48	35	28.5±.5	>4.0	7.73
	1.50	38-48	35	28.5±.5	>4.0	7.80
Cu^{2+}	0.02	35-45	25	28.5±0.5	>4.0	7.80
	0.04	35-45	25	28.5±0.5	>4.0	7.80
Hg^{2+}	0.0375	35-45	25	28.5±0.5	>4.0	7.75
	0.075	35-45	25	28.5±0.5	>4.0	7.75
Zn^{2+}	0.75	35-45	25	28.5±0.5	>4.0	7.80
	1.50	35-45	25	28.5±0.5	>4.0	7.80
Cu^{2+}	0.02	15-20	33.5	28.5±1.0	>4.0	7.8±0.1
		30-40	33.5	28.5±1.0	>4.0	7.8±0.1
	0.04	15-20	33.5	28.5±1.0	>4.0	7.8±0.1
		30-40	33.5	28.5±1.0	>4.0	7.8±0.1
Hg^{2+}	0.0375	15-20	33.5	28.5±1.0	>4.0	7.8±0.1
		30-40	33.5	28.5±1.0	>4.0	7.8±0.1
	0.075	15-20	33.5	28.5±1.0	>4.0	7.8±0.1
		30-40	33.5	28.5±1.0	>4.0	7.8±0.1
Zn^{2+}	0.75	15-20	33.5	28.5±1.0	>4.0	7.8±0.1
		30-40	33.5	28.5±1.0	>4.0	7.8±0.1
	1.50	15-20	33.5	28.5±1.0	>4.0	7.8±0.1
		30-40	33.5	28.5±1.0	>4.0	7.8±0.1

Mussels from the first set of experiment were opened and soft tissues were dissected out using clean stainless steel scissors and forceps in to gills, mantle, adductor muscle + foot and the remaining body parts as viscera. 6 to 8 animals were dissected to pool the body parts. Mussels taken from the second set of experiment were opened and the whole soft tissues were taken. The tissue samples were gently washed with distilled water and all the samples except those used for mercury analysis were dried at 80°C for 24 h.

b) Digestion procedure for Cu and Zn:

The samples were wet-oxidized using the procedures given by FAO (1973) and Martincic *et al.* (1984). The dried samples were weighed and taken in microkjeldhl flasks. 5 ml of con. HNO_3 and 0.5 ml of $HClO_4$ were added per 1 g of the sample. A clean funnel was kept in the flask and keeping the flask in slanting position, it was first heated gently and then strongly but cautiously. The digestion was continued until all the organic matter was destroyed and the digest became colourless. Blanks were prepared in the same way without the samples. Samples were made upto 25 ml using distilled water in a volumetric flask.

c) Digestion procedure for Hg:

The procedure given by BITC (1976) and Pentreath (1976) were followed for mercury digestion. The wet tissue samples were blotted dry, weighed and taken in the oxidation flask of Bethge apparatus. 2.5 ml of a cold mixture of con. HNO_3 and con H_2SO_4 in the ratio 4:1 (V/V) was added to the flask. It was heated cautiously at first, collecting the

distillate in the reservoir. When the mixture started darkening, a little of the distillate was run from the reservoir to the flask. The procedure was continued maintaining a slight excess of HNO_3 in the oxidation flask, until the solution ceased to darken and fumes of H_2SO_4 were evolved. The solution was allowed to cool and transferred to a volumetric flask and made upto 50 ml using distilled water.

d) Instrumental determination of metals:

The Cu and Zn concentrations were determined in a Perkin Elmer 2380 model Atomic Absorption spectrophotometer using air acetylene flame. The samples were aspirated directly into the flame and the corresponding readings were noted. Standards of metal solutions were prepared using AR grade $CuSO_4 \cdot 5H_2O$ and $ZnSO_4 \cdot 7H_2O$.

Mercury was analysed by cold vapour atomic absorption technique. For this a mercury analyser (Model MA-77 Ser No. 005, designed by Analytical chemistry division, BARC, Bombay) was used, $SnCl_2$ was used as reducing agent and air, free from Hg was used as a carrier gas. A standard graph was prepared from Standard $HgCl_2$ solution.

From the readings the metal concentrations in the tissue samples were calculated and are expressed in $\mu g\ g^{-1}$ tissue weight. From these values the bio-concentration factor (CF) is calculated using the relationship,

$$CF = \frac{\text{96 h tissue concentration - 0 h tissue concentration}}{\text{Concentration in the seawater}}$$

e) Data analysis:

To test the linearity of metal uptake, regression analysis was carried out using a micro computer (UPTRON, S-850). The linear relationship between the exposure period and tissue content of metals can be represented by the equation $Yt = a + bt$, where Yt = metal concentration in the animal at time t, bt = the slope of the line which is equal to rate of uptake of metal and a = natural metal concentration in the mussels.

3. RESULTS

a) Distribution of metal in different bodyparts:

Mussels exposed to 0.04 ppm Cu, 1.5 ppm Zn and 0.075 ppm Hg at 35 x 10^{-3} salinity showed frequent valve closure after 72 h of exposure. The details of metal distribution is shown in the table - 4.2. After 96 h exposure in 0.02 and 0.04 ppm of Cu, gills attained the maximum Cu concentration of 55.921 and 139.654 $\mu g\ g^{-1}$ dry wt respectively. It was followed by viscera, mantle and muscele + foot. Gills of mussels exposed to 0.0375 and 0.075 ppm of Hg attained the maximum concentration of 79.92 and 45.48 $\mu g\ g^{-1}$ wet wt respectively. It was followed by viscera, mantle and musscle + foot as in the case of Cu exposed mussels. In mussels exposed to 0.75 and 1.5 ppm Zn, viscera accumulated maximum amount of 351.46 and 416.02 $\mu g\ g^{-1}$ dry wt of Zn respectively. In all experiments adductor muscle and foot showed comparatively minimum concentration of metal. The tissue metal concentration followed the order given below :

Table - 4.2. Concentration of Cu, Hg & Zn in different body parts of *P.viridis* in 35 x 10^{-3} salinity (each value for a pooled sample of 6-8 animals).

Metal	Conc. (ppm)	Time (h)	Metal concentration µg/g tissue weight			
			Gills	Viscera	Mantle	Muscle + Foot
Cu^{+2}	0.02	0	9.278	8.889	6.230	3.254
		24	17.727	13.234	11.837	4.370
		48	27.831	12.980	10.810	5.112
		72	28.841	22.635	14.304	6.295
		96	55.921	43.979	28.592	13.683
	0.04	0	8.194	9.088	5.638	2.888
		24	36.785	19.373	19.762	16.367
		48	52.616	81.535	42.167	18.574
		72	50.867	108.219	31.595	27.759
		96	139.655	98.221	96.084	40.456
Hg^{+2}	0.0375	0	0.530	0.154	0.059	0.561
		24	29.42	2.62	1.75	1.74
		48	59.22	11.18	3.42	2.50
		72	117.64	59.77	5.99	2.79
		96	79.22	20.10	9.92	8.34
	0.075	0	0.107	0.486	0.192	0.057
		24	23.930	5.40	3.13	0.47
		48	29.040	6.09	5.40	2.67
		72	35.100	8.09	4.61	4.84
		96	45.480	17.57	8.97	3.72
Zn^{+2}	0.75	0	125.00	202.82	126.48	80.34
		24	236.67	302.07	144.22	156.74
		48	238.14	422.10	236.05	185.75
		72	319.01	337.15	180.39	174.46
		96	247.95	351.46	264.33	142.57
	1.5	0	130.09	177.57	109.80	81.39
		24	254.37	304.15	196.04	115.99
		48	253.63	393.64	255.02	73.98
		72	266.55	429.01	241.82	145.88
		96	379.57	416.02	255.88	132.80

Table - 4.3. Concentration of Cu, Hg & Zn in different body parts of *P.viridis* in 25 x 10^{-3} salinity (each value for a apooled sample of 6-8 animals).

Metal	Conc. (ppm)	Time (h)	Metal concentration µg/g tissue weight			
			Gills	Viscera	Mantle	Muscle + Foot
Cu^{2+}	0.02	0	7.99	9.170	5.92	3.15
		24	46.08	54.770	89.22	21.07
		48	67.20	61.030	34.90	29.80
		72	78.30	44.290	28.72	23.01
		96	196.34	122.330	123.09	31.78
	0.04	0	8.34	10.16	6.86	4.19
		24	41.98	66.93	42.53	22.52
		48	255.28	287.88	60.12	83.59
		72	177.82	118.23	77.84	66.21
		96	268.24	254.59	149.36	167.39
Hg^{2+}	0.0375	0	0.432	0.372	0.168	0.369
		24	1.964	0.428	0.719	0.183
		48	6.570	0.978	0.154	0.332
		72	0.933	0.925	0.647	0.465
		96	1.520	2.290	0.702	0.522
	0.075	0	0.473	0.402	0.034	0.170
		24	1.300	0.643	0.153	0.401
		48	2.590	0.974	0.175	0.482
		72	6.170	0.992	0.959	0.981
		96	2.710	1.030	0.487	0.954
Zn^{2+}	0.75	0	128.36	104.77	112.87	80.84
		24	236.11	128.25	126.78	98.14
		48	389.48	150.49	135.85	189.40
		72	439.33	291.28	178.96	143.36
		96	697.80	437.90	149.36	167.39
	1.50	0	102.39	112.11	101.98	78.47
		24	83.16	191.40	110.89	129.34
		48	250.32	248.86	220.41	141.13
		72	381.43	250.56	149.79	164.75
		96	404.97	421.54	163.39	262.62

0.02 & 0.04 ppm Cu - Gills > Viscera > Mantle > Muscle + foot

0.0375 & 0.075 ppm Hg - Gills > Viscera > Mantle > Muscle + foot

0.75 ppm Zn - Viscera > Mantle > Gills > Muscle + foot

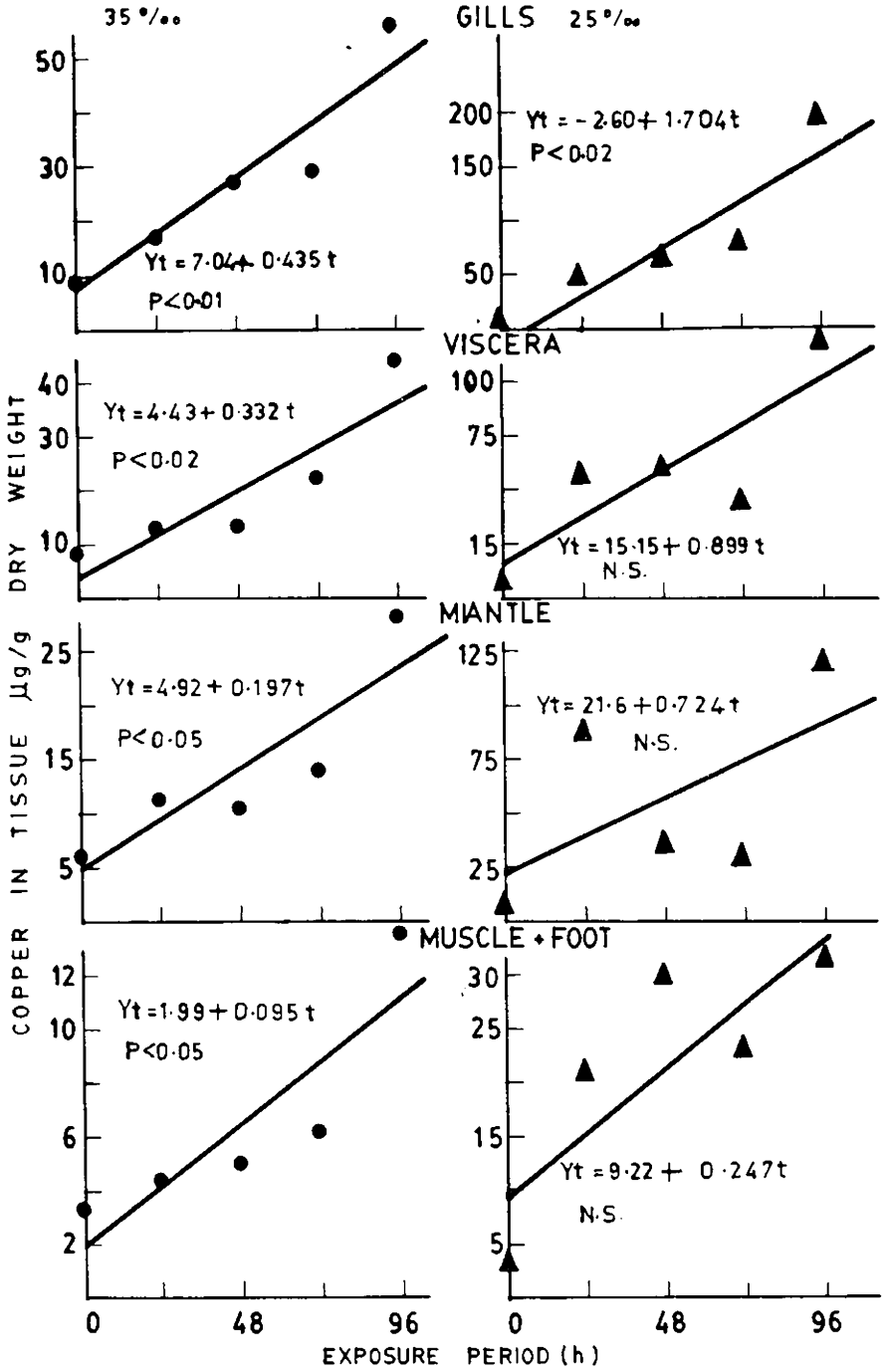
1.50 ppm Zn - Viscera > Gills > Mantle > Muscle + foot

b) Rate of metal accumulation in 35 x 10^{-3} salinity:

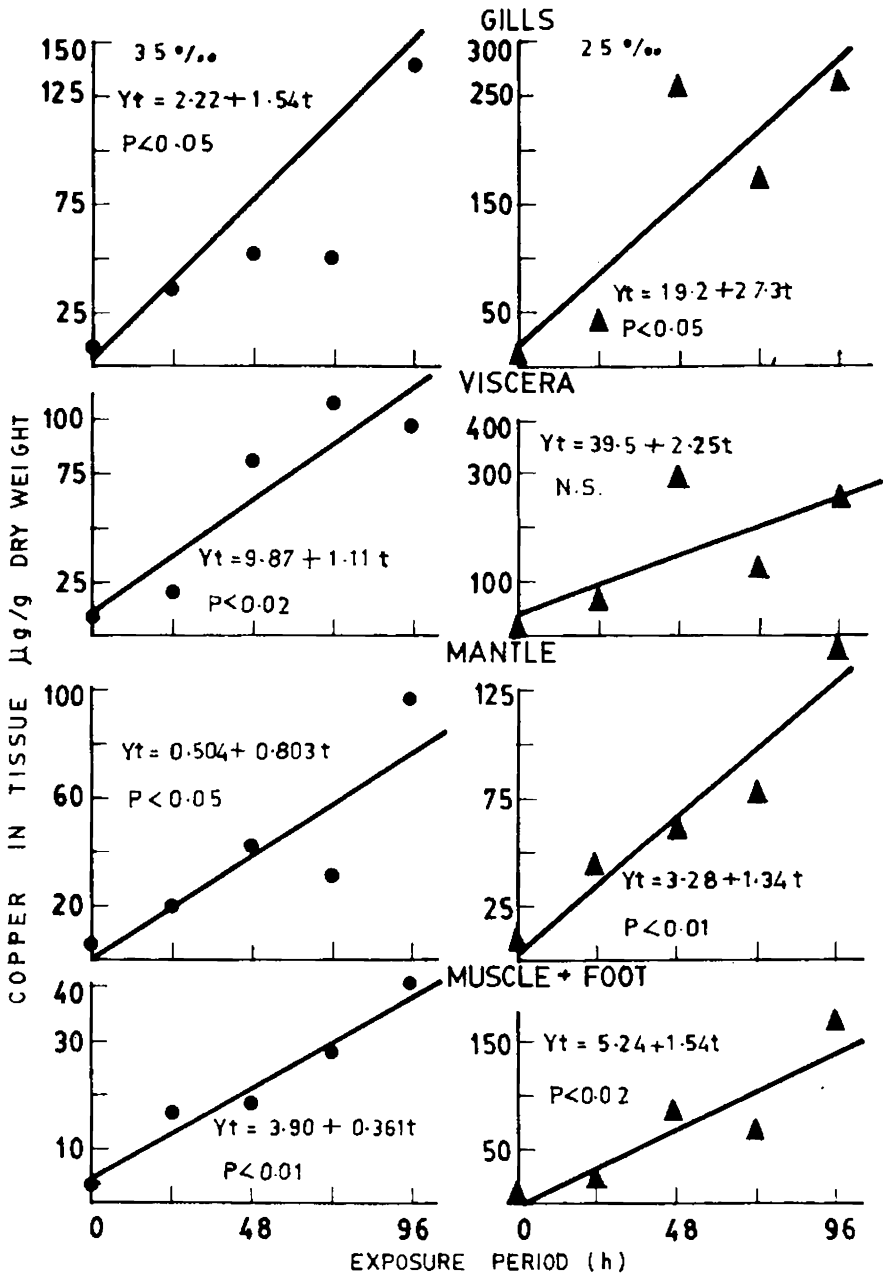
The accumulation of metal in the different body parts of mussels was found to be linear (Figs. 4.1 to 4.6). Body parts of mussels exposed to 0.02 and 0.04 ppm of Cu showed significant increase in Cu concentration (Figs. 4.1 and 4.2). In Cu exposed mussels the rate of accumulation was found to be maximum in gills followed by viscera, mantle and muscle + foot (Fig. 4.8). Hg exposed mussels also showed significant linear accumulation with progress of time in all body parts. (Figs. 4.3 & 4.4). The rates of accumulation in gills were maximum follo-wed by viscera, mantle and muscle + foot (Fig. 4.9). Viscera of Zn exposed mussels showed maximum rate of accumulation followed by gills, mantle and muscle + foot (Fig. 4.10). In both Cu and Zn exposed mussels, rate of accumulation increased with the increase in external metal concentration (Figs. 4.8 & 4.10). But in Hg exposed mussels the rate of accumulation showed inverse relationship with metal concentration (Fig. 4.9). Based upon the rate of accumulation of metals in the different body parts the following order was seen Zn > Cu > Hg.

c) Bio-concentration factors (CF) in 35 x 10^{-3} salinity:

The bio-concentration factors of different body parts of mussels exposed to Cu, Zn and Hg are presented in figs. 4.11 to 4.13. Cu



- 4.1. Accumulation of Cu in the different body parts of Perna viridis exposed to 0.02 ppm of Cu .(●)- 35 x 10^{-3}, (▲)- 25 x 10^{-3} .



Accumulation of Cu in the different body parts *Perna viridis* exposed to 0.04 ppm of Cu.(● - 35×10^{-3}, ▲ - 25×10^{-3}).

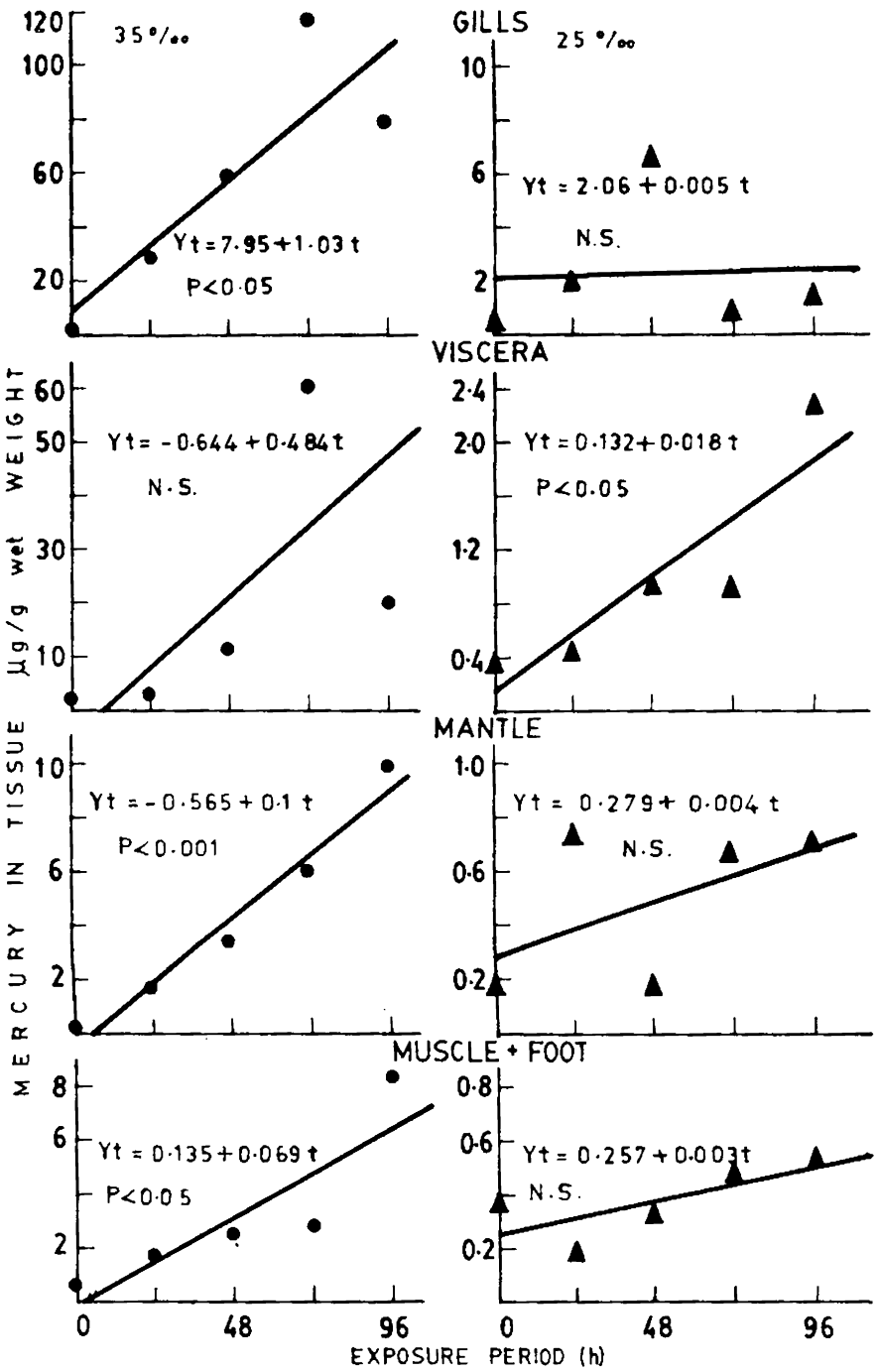
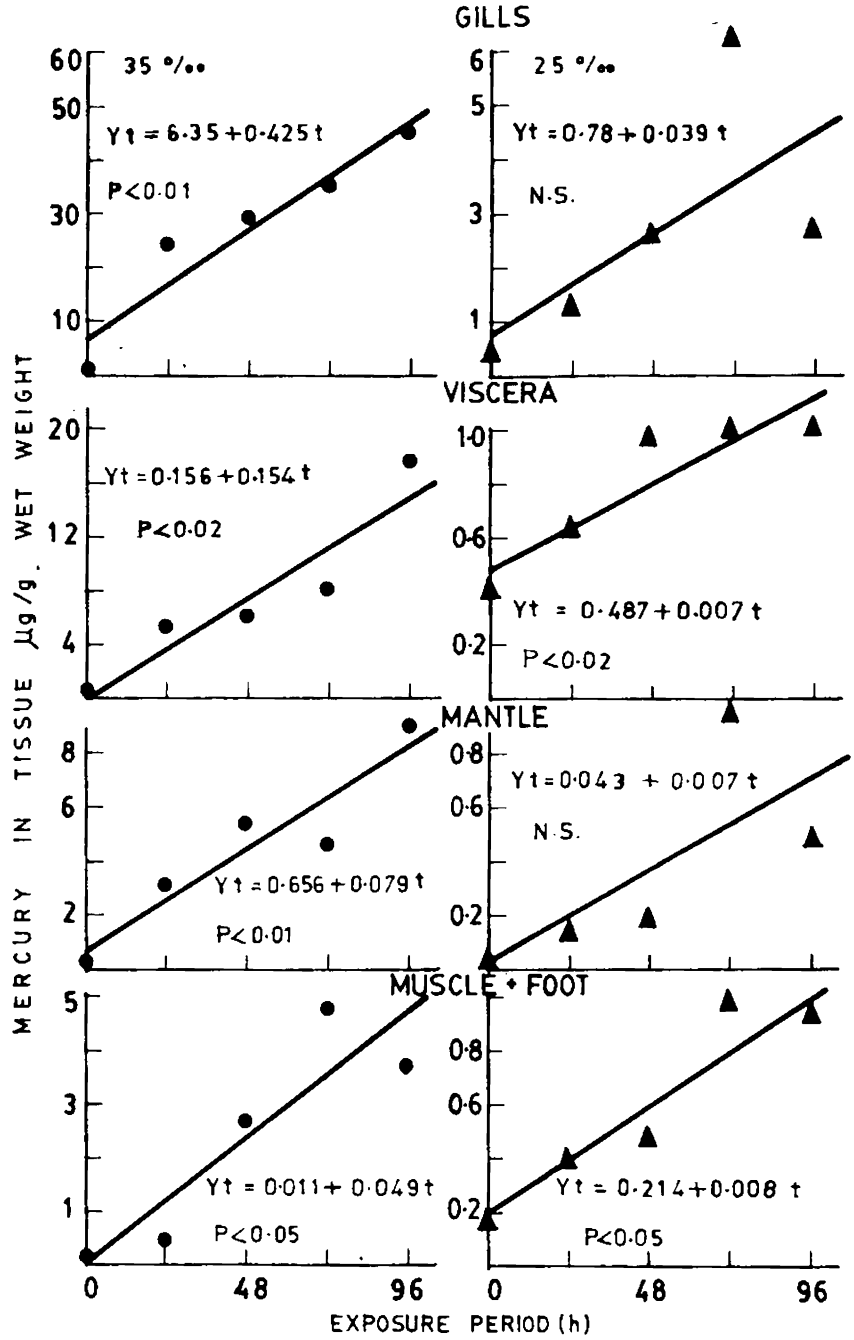


Fig - 4.3. Accumulation of Hg in different body parts of Perna viridis exposed to 0.0375 ppm of Hg. (● -35 x 10^{-3} ▲ - 25 x 10^{-3}).



g - 4.4 . Accumulation of Hg in different body parts of Perna viridis exposed to 0.075 ppm of Hg. (● -35 x 10^{-3}, ▲ - 25 x 10^{-3}).

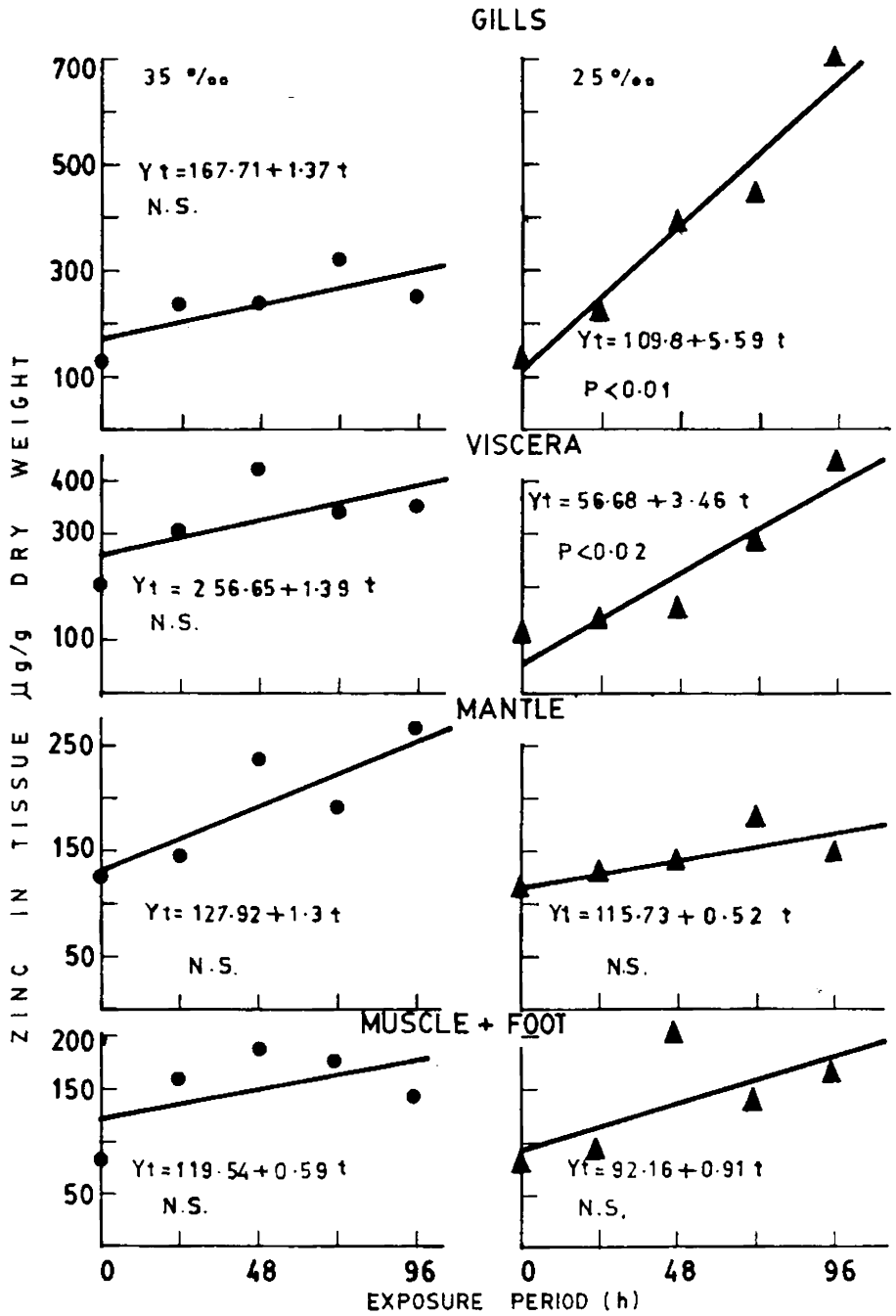


Fig - 4.5. Accumulation of Zn in different body parts of Perna viridis exposed to 0·75 ppm of Zn. (● - 35 x 10^{-3}, ▲ - 25 x 10^{-3}).

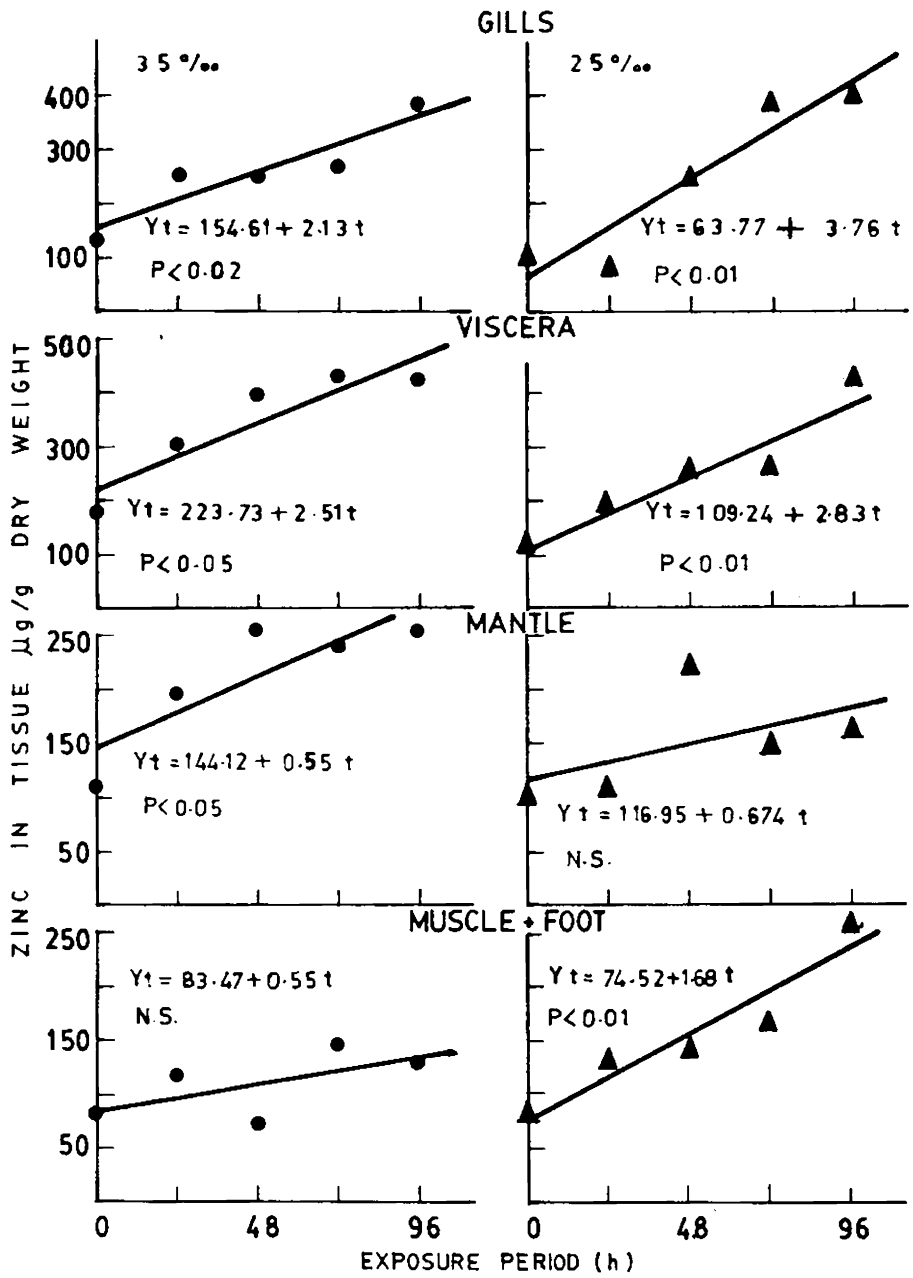


Fig - 4.6. Accumulation of Zn in different body parts of Perna viridis exposed to 1.5 ppm of Zn. (● - 35×10^{-3}, ▲ - 25×10^{-3}).

exposed animals showed highest CF followed by Hg and Zn. After 96 h exposure to 0.02 and 0.04 ppm Cu, gills registered CF of 2332 and 3286 respectively. Gills of mussels exposed to 0.0375 and 0.075 ppm of Hg showed CF of 2117 and 604 respectively. Gills of mussels exposed to 0.75 and 1.5 ppm of Zn registered CF of 163 and 166 respectively. The CF in the different body parts of mussels after 96 h exposure followed the order given below:

0.02 ppm Cu - Gills > Viscera > Mantle > Muscle + foot

0.04 ppm Cu - Gills > Mantle > Viscera > Muscle + foot

0.0375 ppm Hg - Gills > Viscera > Mantle > Muscle + foot

0.075 ppm Hg - Gills > Viscera > Mantle > Muscle + foot

0.75 ppm Zn - Viscera > Mantle > Gills > Muscle + foot

1.50 ppm Zn - Gills > Viscera > Mantle > Muscle + foot.

In all the body parts of animals exposed to Hg, CF decreased with the increase in concentration of the exposure medium (Fig. 4.12). So based upon CF in the different body parts metals can be arranged in the order Cu > Hg > Zn.

d) Effect of Salinity:

At lower salinity (25×10^{-3}) accumulation of Cu showed a clear cut increase with progress of time in all the body parts in comparison with higher salinity (35×10^{-3}) as shown in Figs. 4.1 & 4.2. The Zn exposed animals also showed a definite increase in accumulation with progress of time in all the body parts except in mantle at 25×10^{-3} salinity (Figs. 4.5 & 4.6). But Hg exposed animals showed a clear

decrease in accumulation with progress of time in all the body parts at 25 x 10^{-3} salinity in comparison with 35 x 10^{-3} salinity (Figs. 4.3 and 4.4, Table - 4.3).

Rate of accumulation in all the body parts of Cu-exposed animals and in all body parts except the mantle of Zn-exposed animals showed clear increase at 25 x 10^{-3} salinity in comparison with 35 x 10^{-3} salinity (figs. 4.8 and 4.10). The rate of accumulation in the body parts of Hg exposed animals showed a clear decrease at low (25 x 10^{-3}) salinity (Fig.4.9).

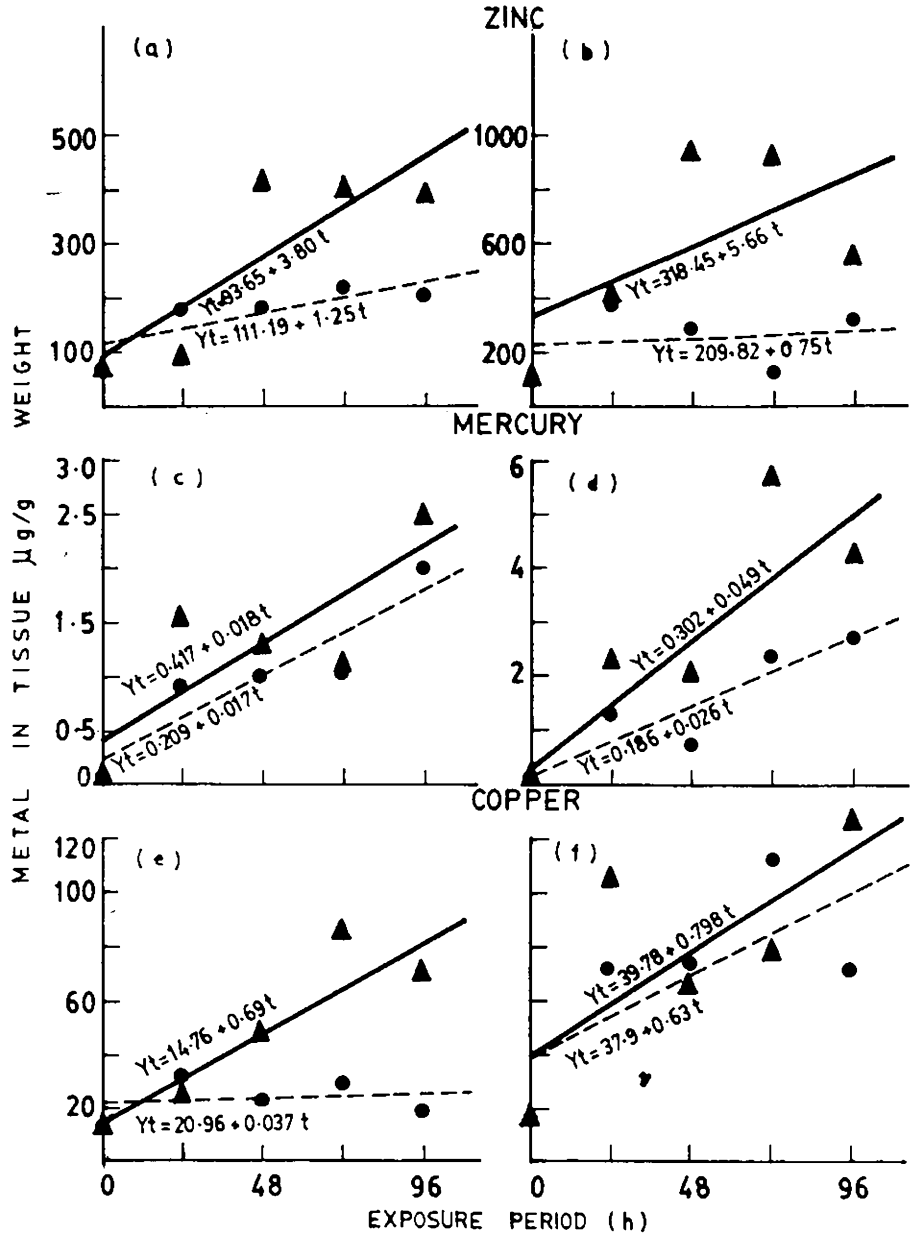
CF in all the body parts of Cu exposed and in all except mantle of Zn exposed mussels increased at lower salinity (Figs. 4.11 & 4.13). But CF in all the body parts of Hg exposed mussels decreased with decrease in salinity (Fig. 4.12).

e) Effect of body size:

Animals of smaller size group (15-20mm) showed an increase in accumulation with the progress of time in comparison with larger size group (30-40mm) exposed to Cu, Hg, and Zn (Fig. 4.7). The concentration of Cu, Hg, and Zn in the whole tissue of small (15-20mm) and larger (30-40mm) mussels after 96 h exposure is given in the table - 4.4. The rate of accumulation was comparatively high in animals of smaller size group for Cu, Hg and Zn (Fig. 4.14). Similiarly CF was high for animals of smaller size group (15-20mm) after 96 h exposure in comparison with larger (30-40mm) size group (Fig.4.15).

Table - 4.4 · Concentration of Cu, Hg & Zn in whole soft tissue of smaller and larger size groups *P. viridis* ($\bar{x}$ ± SD; N=6)

Metal	Conc. (ppm)	Exposure time (h)	Metal in whole tissue µg/g weight	
			Smaller (15-20mm)	Larger (30-40mm)
$Cu2^{+}$	0.02	0	14.13±1.221	13.23±1.433
		24	26.41±1.295	29.90±1.227
		48	47.24±2.189	23.59±1.852
		72	83.74±8.444	28.61±2.408
		96	68.65±9.648	18.26±8.694
	0.04	0	16.34±2.208	15.00±2.206
		24	104.37±4.610	71.30±8.108
		48	66.64±7.867	73.00±7.299
		72	77.59±4.432	112.19±5.662
		96	125.49±6.877	71.12±3.557
Hg^{2+}	0.0375	0	0.077±0.002	0.069±0.003
		24	1.521±0.187	0.931±0.084
		48	1.286±0.087	0.992±0.044
		72	1.115±0.159	1.017±0.081
		96	2.475±0.957	2.015±0.884
	0.075	0	0.077±0.002	0.083±0.001
		24	2.264±0.462	1.325±0.324
		48	0.975±0.089	0.717±0.077
		72	5.723±0.968	2.346±0.621
		96	4.226±0.344	2.687±0.475
Zn^{2+}	0.75	0	82.49±4.62	77.15±5.42
		24	97.71±26.15	174.81±13.14
		48	413.81±54.58	180.80±23.28
		72	398.41±47.91	217.92±14.15
		96	388.46±22.53	205.91±17.91
	1.50	0	105.03±7.56	108.03±5.38
		24	426.11±26.69	378.24±21.32
		48	924.62±66.24	290.25±39.90
		72	914.58±74.73	131.41±16.74
		96	539.68±62.10	321.71±28.66



ig - 4.7. Effect of body size (▲ - 15 - 20 mm); (● - 30 - 40 mm) on the accumulation of Cu, Hg and Zn in Perna viridis
a) 0.75 ppm b) 1.5 ppm c) 0.0375 ppm d) 0.075 ppm
e) 0.02 ppm f) 0.04 ppm .

4. DISCUSSION

The metal accumulation pattern observed in different body parts were found to be independent from each other (Figs. 4.1 to 4.6). This may be due to differences in the physiological functions these tissues perform (Eganhouse & Young, 1978). High Hg concentrations were reported in the gills of bivalves by several authors (Unlu *et al.*, 1972; Dillon & Neff, 1978; Lakshmanan & Nambisan, 1979; Denton & Burdon-Jones, 1981). Similarly highest concentration of Cu in gills and lowest in adductor muscles were reported in bivalves. (Nambisan *et al.*, 1977; Lakshmanan, 1982; Latha, 1986). This type of high metal uptake in the gills may be due to their functional activity and position relative to incoming ambient water (Dillon & Neff, 1978). Gill tissues come in direct contact with the ambient water containing heavy metal for a longer duration than any other part. Moreover the absorption of the metal ions by the mucus sheets may also add to the greater concentration in the gill tissue as reported by Smith *et al.* (1975).

In the present study maximum Zn accumulation was observed in viscera. In mussels exposed to Cu & Hg, viscera showed high metal concentration next to the gills. Lobel (1985) concluded that kidney plays a major role in the storage of Zn in the body of M. edulis. George *et al.* (1982) and George (1983 b) reported that heavy metals are detoxified and stored in the kidney of mussels and oysters. In the present study kidney was not separated for metal analysis, but taken together with the remaining body parts as viscera. That may be the

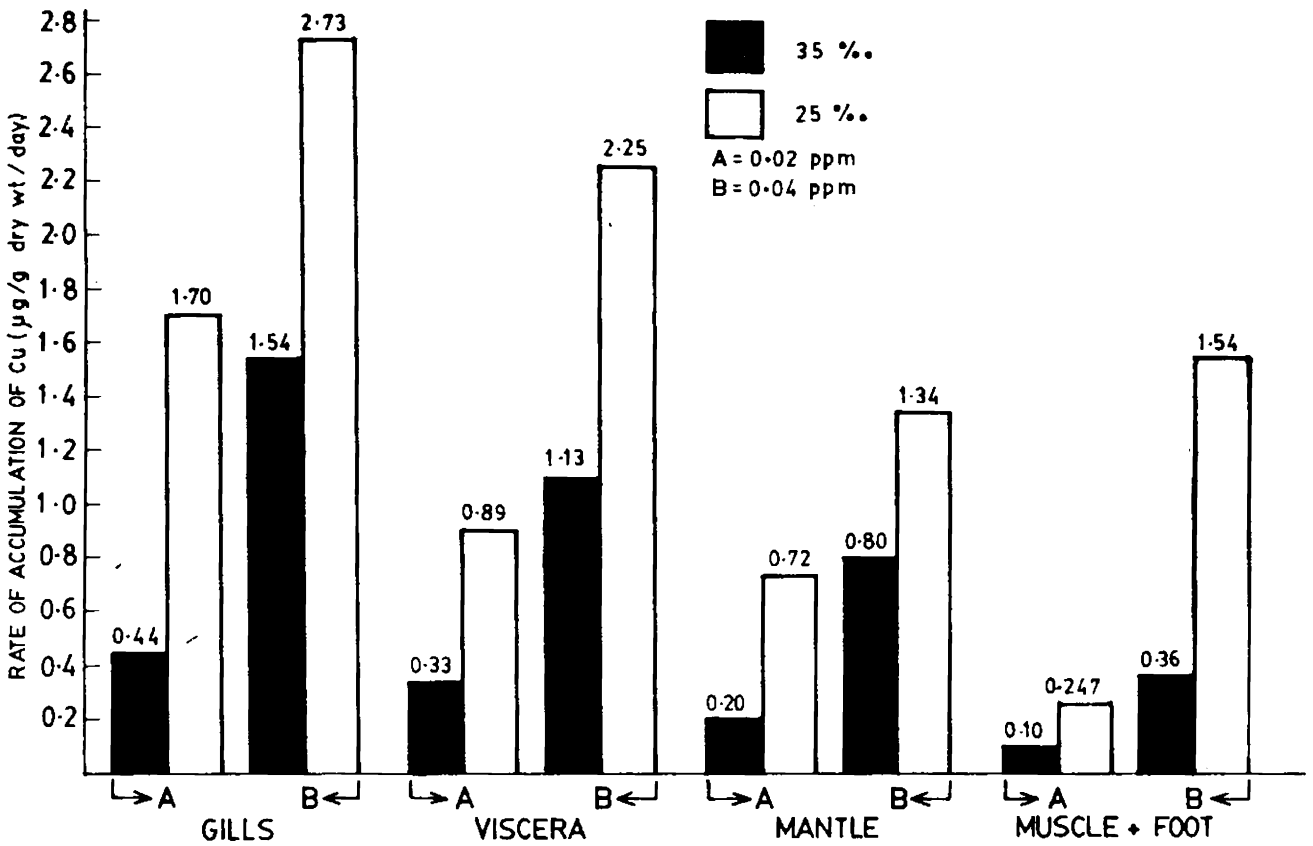


Fig - 4.8. Rate of accumulation of Cu in the different body parts of Perna viridis after 96 h exposure.

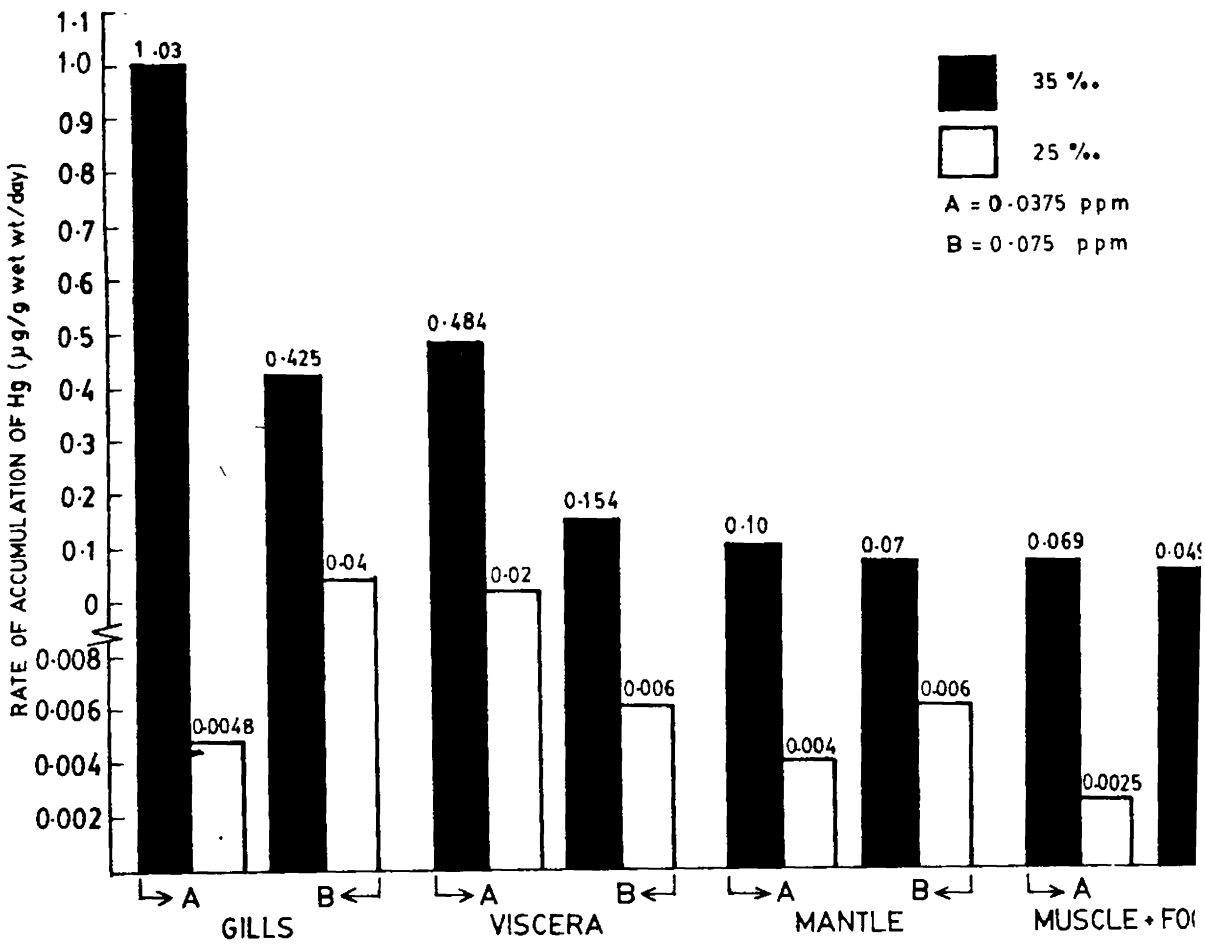


Fig - 4.9. Rate of accumulation of Hg in the different body parts of Perna viridis after 96 h exposure.

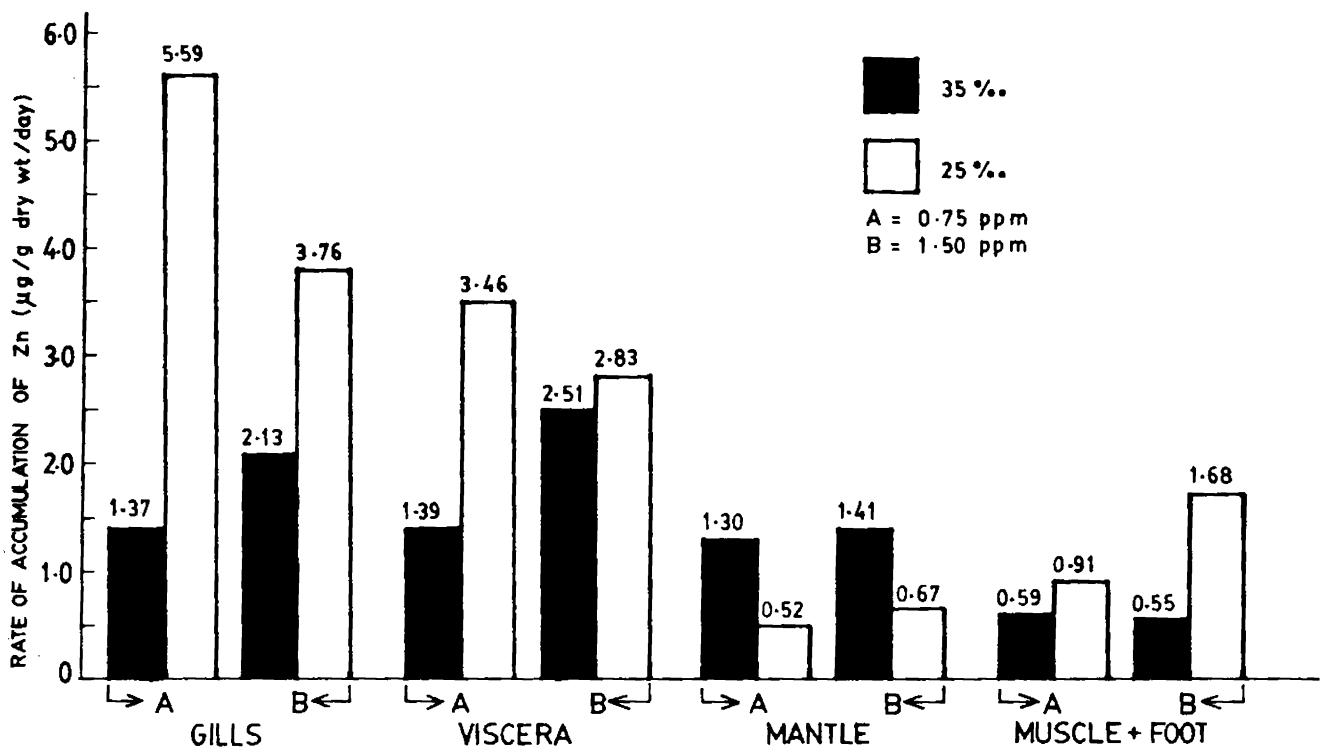


Fig - 4.10 . Rate of accumulation of Zn in the different body parts of Perna viridis after 96 h exposure.

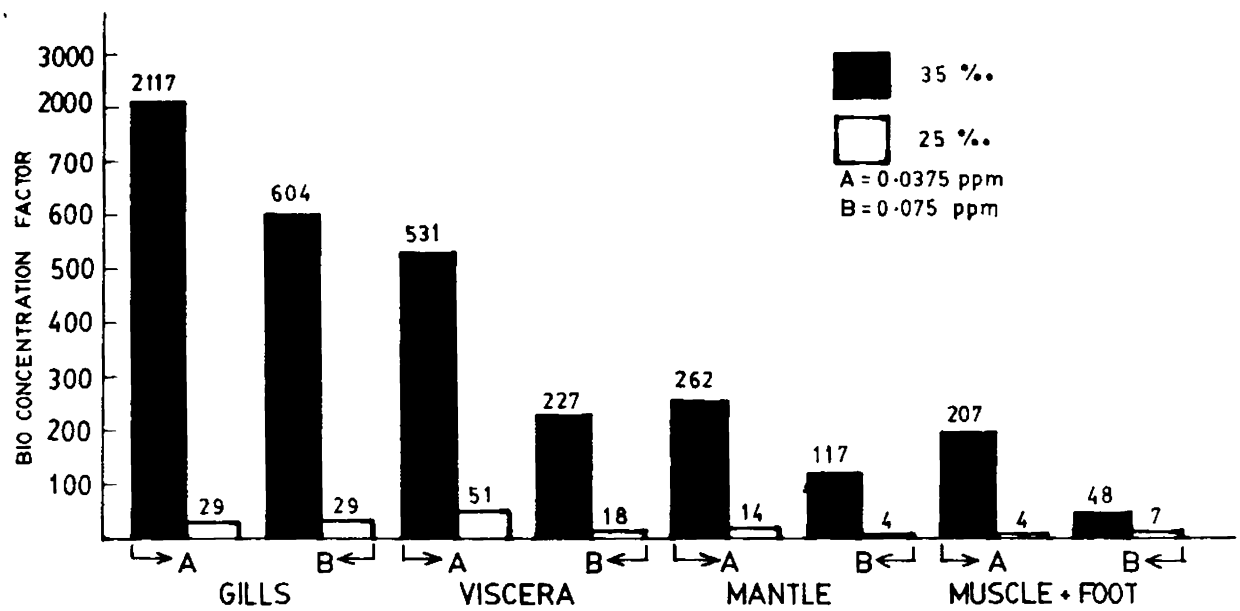


Fig - 4.12. Bio-concentration factor (CF) for Hg in the different body parts of Perna viridis after 96 h exposure.

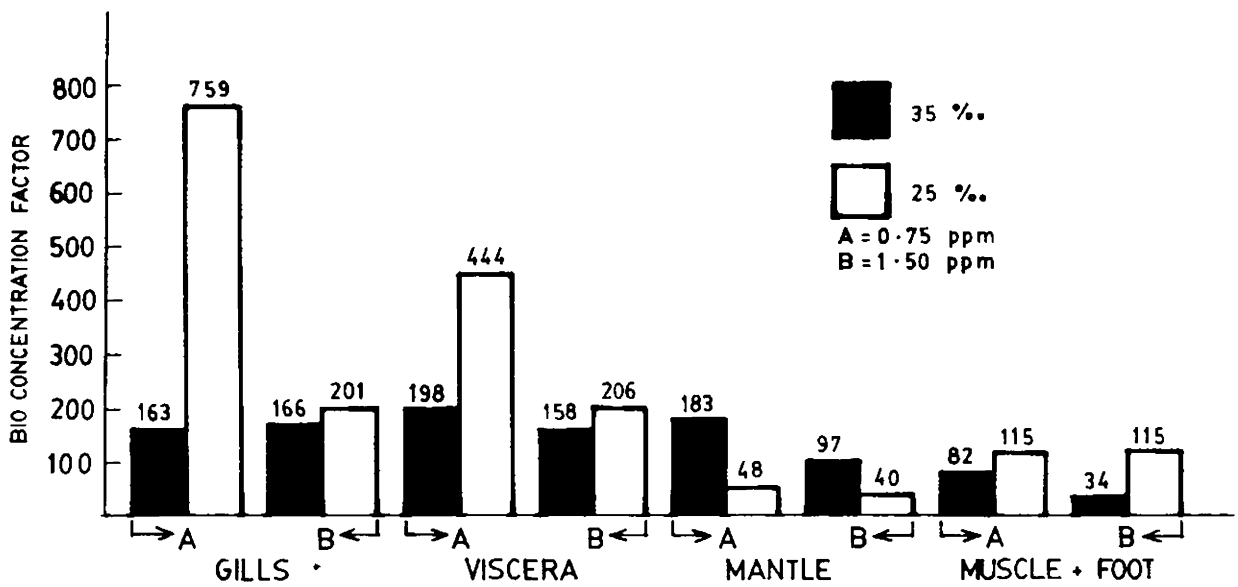


Fig - 4.13. Bio-concentration factor (CF) for Zn in the different body parts of Perna viridis after 96 h exposure.

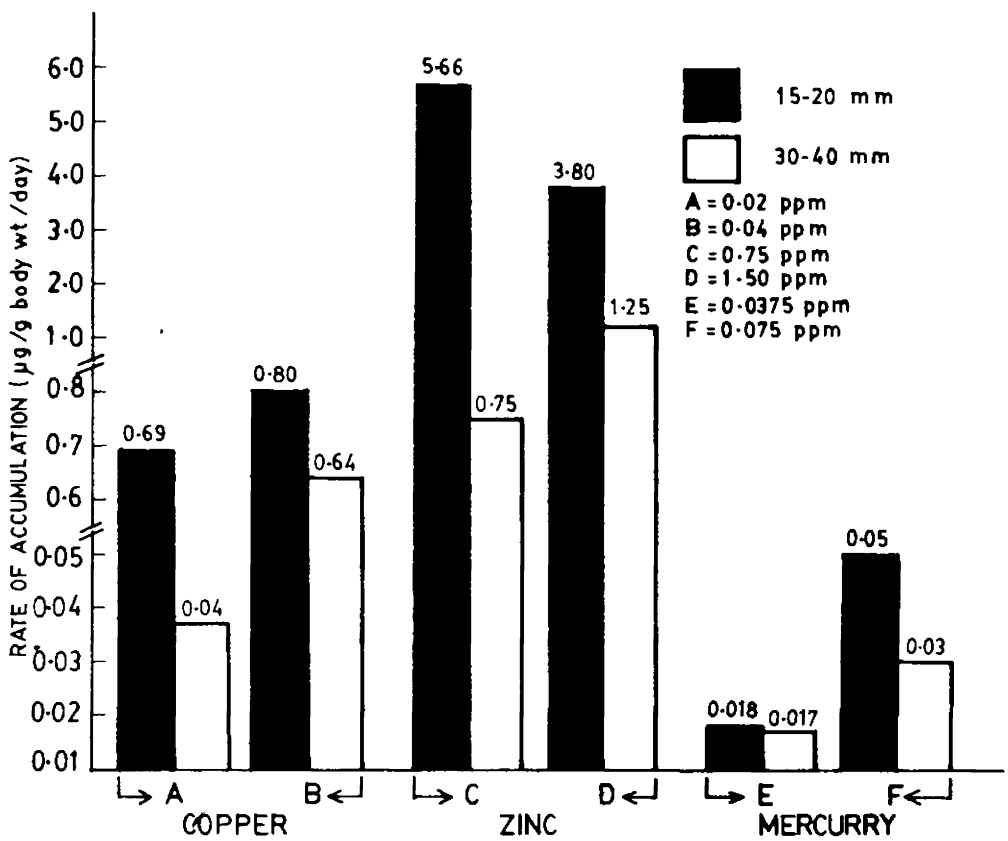


Fig - 4.14. Effect of body size on the rate of accumulation of Cu, Hg and Zn in the tissue of Perna viridis after 96 h exposure.

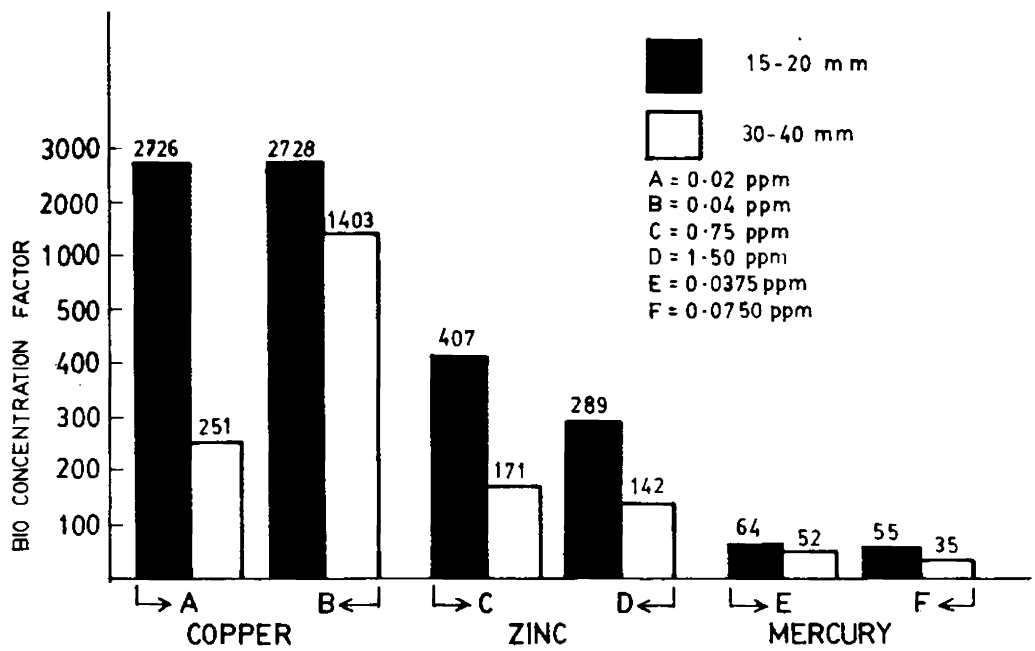


Fig - 4.15. Effect of body size on the Bio-concentration factor of Cu, Hg and Zn in the tissue of Perna viridis after 96 h exposure.

reason for high metal concentration observed in viscera. After 96 h exposure, Cu, Hg, and Zn distribution in the different body parts of *P. viridis* indicate that the ability of animal to accumulate metals vary from organ to organ as reported in *M.edulis* (Amiard *et al.*, 1986).

The main entry of metals in to the animals is (1) directly from the solution and (2) through the food (Bryan, 1979; Simkiss *et al.*, 1982). In the present study mussels were not fed during the experiment and so the only source of entry of metals is direct uptake from the medium. *P.viridis* showed a time integrated linear accumulation of metals. (Figs. 4.1 to 4.6). Such linear metal accumulation was reported in *P.viridis* (Nair *et al.*, 1977; D'Silva & Kurieshy, 1978; Lakshmanan & Nambisan, 1979; Lakshmanan & Nambisan 1986).

The metals such as Cu and Zn have shown higher rate of accumulation in comparison with non-essential Hg. It is not clear that such a difference is due to the ability of mussels to regulate the uptake of non-essential metals like Hg with in its tolerance limit. But Hg was reported as a toxic metal having high rate of accumulation in marine animals (Bryan, 1984). The rate of accumulation of inorganic mercury ($HgCl_2$) was found to be lesser than organic mercury (CH_3HgCl_2) in *M.galloprovincialis* (Fowler *et al.*, 1978). The low rate of accumulation of Hg obtained in the present study may be the reason for the low 96 h LC50 recorded for Hg in comparison with Cu (Chapter - 3).

Cu and Zn were found to be accumulated more at low salinity in *P.viridis* (Figs. 4.1 to 4.6). Similiar results were reported by

Philips (1976a) Brayan (1976) and Latha (1986). Generally at low salinity, rate of accumulation of metals in bivalves was found to be high (George *et al.*, 1982;Bryan, 1976; Denton & Burdon Jones, 1981; Phillips, 1980). Bryan and Hummerstone (1971) and Bryan, (1976) reported a higher accumulation of Cu at low salinity in *Nereies diversicolor*. Bryan & Hummerstone (1973 b) reported that *N. diversicolor* exhibited increased rate of Zn uptake at low salinity. Phillips (1976a) suggested that such effects were due to changes in the ratio of major ions to added trace metals. Salinity changes may affect the chemical form and chemical interactions of metal in seawater, which in turn may affect their availability (Denton & Burdon-Jones, 1981). Phillips (1977a) suggested that less saline water is having more capacity to maintain metals either in solution or in suspension. But this fails to expalin the low rate of accumulation observed for some metals in low salinity. the present study *P.viridis* showed a low rate of accumulation of Hg at low salinity. Similiar reduced rate of Hg accumulation was reported in *N.succinea* at salinity lower than 16×10^{-3} (Luoma, 1977a). The bioaccumulation was linear with time under two salinity regimes studied (35×10^{-3} and 25×10^{-3}) for Cu, Hg and Zn. So the difference in total body burden of metals is due to the alteration in rate and not in the capacity of the system. From the various salinity effect studies it is clear that salinity should be viewed as an extremely complex variable, affecting not only the physio -chemical and hydrological characteristics of trace metal cycling in the marine environment but also eliciting any

of the several biological changes in the indicator organisms itself and in its food (Phillips, 1977a;1980).

The present study clearly indicates the ability of smaller size group (15-20mm) mussels to accumulate higher concentration of Cu, Hg and Zn (Fig. 4.7). Many authors have reported that the concentration of trace metal present in an organism can vary with age or size (Boyden, 1979; Simpson; 1979; Cossa *et at, 1980;* Phillips, 1980; Bryan, 1979; 1984). High Hg concentration was reported in the tissues of smaller mussels (Fowler *et al.*, 1978). The uptake of mercury was more rapid in smaller individuals than in larger ones of both oysters and mussels (Cunningham & Tripp, 1975a). The Higher metal uptake in the soft tissues of smaller mussels may be due to greater surface area to volume ratio than larger animals (Fowler *et al.*, 1978). The smaller animals are having high weight specific metabolic rate in comparison with larger animals. So the high accumulation rate of metals in smaller animals may also be related to its high metabolic rate.

The present study clearly shows the effect of the size group and salinity on heavy metal accumulation by *P.viridis*. Phillips and Segar (1986) suggested that these factors will interfere with the production of accurate data showing comparative bioavailabilities of pollutants with space or time in monitoring, using bio-indicators. Based on the results of the present study it can be said that while using *P.viridis* as bio-indicator of heavy metal monitoring, size group of the animal and its habitat salinity should be taken into account.

CHAPTER - 5

DEPURATION OF COPPER, MERCURY AND ZINC

1. INTRODUCTION

It is well known that lamellibranch molluscs and mussels in particular, are able to accumulate heavy metals in their tissues (Phillips, 1976b; Goldberg *et al.*, 1978). The accumulated metals are stored in different parts of the body with the help of organic compounds, especially protein. Potentially toxic metals can be detoxified intracellularly by lysosomes or by binding to metallothionein (Livingstone, 1985b). Metallothioneins are a class of soluble, low molecular weight proteins, characterized by their high affinity for heavy metal cations (Viarengo, 1985). Upon entering the cells many metal ions are bound by metallothioneins. These metallothioneins are widely distributed in animals and shown to be present in tissues of metal exposed fishes (Merafante, 1976) and subsequently reported in metal exposed invertebrates (Roesijadi, 1980). Their synthesis within the animal body can be induced by heavy metals both in the field and laboratory (Viarengo, 1985).

If the concentration of metals taken up into the cell is high and the metal saturate the physiological pool of thioneins, then the excess of cations can stimulate the synthesis of new thioneins (Viarengo, 1985). It is currently assumed that only, if the rate of influx of metals into the cells exceeds the rate of metallothionein synthesis and/or if the maximum value of thionein produced in the cells

is exceeded by the metals, they interact with subcellular components, and cellular toxicity occur (Viarengo, 1985).

Other methods of storage include deposition in skeletal materials such as bone and intracellular deposition (Bryan, 1979). Microscopical investigations have shown that Cu and Zn accumulate in the amoeboid blood cells present in both haemolymph and tissues and that they are detoxified by compartmentation within membrane limited vesicle in these cells (George *et al.*, 1978; George *et al.*, 1982). The recent investigations have shown that the metals are immobilised and detoxified by cellular compartmentation within pigmented electron dense, membrane limited grannules and these are eventually excreted in the urine (George *et al.*,1982). Lysosomes have also been implicated in metal store in *M.edulis* (George, 1983a & b, Viarengo, 1985).

There are a variety of mechanisms for removing contaminants from the body of molluscs, and this process in general is called depuration or self purification. Some losses in the surrounding water will occur over the general body surface by diffusion or in association with secretions such as algal extracellular products or mucus (Bryan, 1979). In the scallop *Chlamys opercularis* Bryan (1973) showed that Zn is removed by incorporation into kidney granules which can be excreted. Kidney is a major site for the excretion of lead in *M.edulis* and losses occur in the form of granules (Coombs & George, 1978).

Depending upon the ability to excrete contaminants, atleast three types of relations are possible (Bryan, 1979). 1) The rate of

excretion from the organism is proportional to the body burden. 2) The organism stores the contaminant rather than excreting it, so that, unless the growth of new tissue is sufficiently rapid, the concentration in the body increases with age. 3) The organism excretes most of any additional input. Once metals, have entered cells they undoubtedly become bound to a variety of ligands and it is the metabolism of these complexes that determines the subsequent fate of the metals and the final body load (Simkiss & Mason, 1984). So the trace metal depuration may be related to the deposition or binding characteristic of each metal.

Based upon the rate of loss of a pollutant, biological half life of a metal can be determined. Biological half life of a metal can be defined as the time required for half the accumulated metal to be lost as the result of biological process (Okazaki and Panietz, 1981; Buikema Jr *et al.*,1982). The biological half life may serve as a warning about the persistence and potential for cumulative biological effects of a chemical (Buikema Jr *et al.*, 1982).

The kinetics of metal loss or depuration in bivalves have been studied by many workers (Unlu *et al.*, 1972, Cunningham & Tripp 1973; Renfro, 1973; Schulz-Baldes, 1974; D'Silva & Qasim, 1979; Simpson, 1979 ; Dillon & Neff, 1978; Okazaki & Panietz, 1981; Moore *et al.*,1984; Viarengo *et al*, *1985*; *Riisgard et al.*, 1985). The biological half lives of various heavy metals in marine animals have been determined (Unlu *et al.*, 1972; Miettinen *et al.*, 1972; Okazaki & Panietz, 1981, Moore *et*

al.,1984; Viarengo *et al.*,1985).

The hydroclimate, dosage and duration of exposure to a specific metal and physiological conditions of an animal undoubtedly are some of the parameters affecting heavy metal depuration (Cunningham & Tripp, 1973). Some of these factors affecting depuration have been studied in bivalves (Simpson, 1979; Dillon & Neff, 1978, Fowler *et al.*, 1978; Denton & Burdon-Jones, 1981).

Very little information is available on the kinetics of depuration in *P.viridis* (Lakshmanan, 1982). The rate of depuration of residual amount of metal in *P.viridis* is of obvious ecological importance. But the mechanism of depuration of metals in *P.viridis* is poorly understood. So here, an attempt has been made to study the kinetics of depuration, of Cu, Hg and Zn.

The main objectives of the studies are (1) To compare the rate of depuration of metals in the different body parts. (2) To determine the biological half lives of Cu Hg, and Zn. (3) To investigate the effect of salinity on depuration.

2. MATERIALS AND METHODS

Mussels exposed 96 h to Cu, Hg and Zn in the experiment explained in chapter-4 were used for the depuration study (Table - 4.1). After 96 h exposure mussels were taken out to determine the concentration of metals in different body parts. The remaining mussels were gently rinsed in pure seawater and transferred to a continuous seawater flow system for depuration study. At a time mussels exposed to

a single metals were transferred to the system for depuration study.

a) Continuous seawater flow system:

For the long term depuration study a seawater system as shown in fig 5.1 is used. It is having 6 numbers of 25 l capacity, perspex made experimental tanks (E) and an upper and lower (UR and LR) fibreglass reservoirs (150 l each). From the lower reservoir seawater is pumped into the upper reservoir through the aerating tower (AT). The seawater is equally distributed into the experimental tank after flowing through the mixing tank (MT). After flowing through the system water is allowed to go out through the outlet (OT) and the flow rate is controlled by taps T1 and T2. Water level in the upper reservoir is always maintained above a particular level with the help of a float system (F) attached to the pump's switch (S). The system is filled with filtered seawater. The incoming filtered seawater is kept in the lower reservoir for pumping. In each experimental tank 20 mussels were maintianed. *Synechocytis* sp. cultured in metal free medium is added to the mixing tank and well mixed with the stirror to maintain a concentration of 3×10^3 cells ml^{-1} in the experimental tanks. Seawater is always aerated in the aerating tower and maintained a water flow rate of 150 ml min^{-1} through the system. Every two days seawater from the outlet is collected for monitoring the physio-chemical parameters (Table - 5.1). Twenty five days long depuration study was conducted in two salinities as shown in the table 5.1. Tissues were digested and Cu, Zn and Hg concentrations were estimated as explained

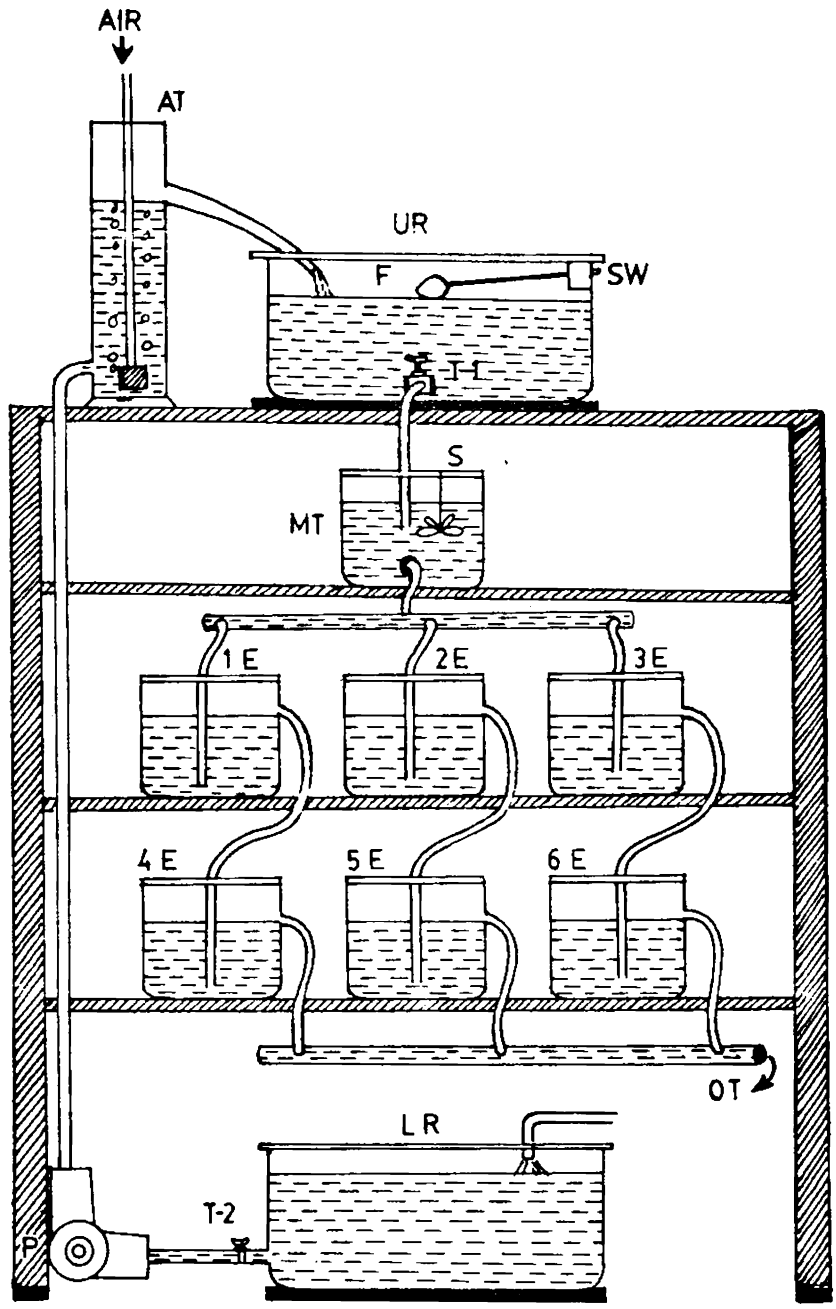


Fig-5.1. Continuous sea water circulatory system used for depuration study in Perna viridis. E - experimental tank, UR - Upper reservoir, LR - Lower reservoir, AT - Aerating tower, MT - Mixing tank, SW - Switch T 1 and T2 - Taps, S - Stirror, P - Pump, F - Float.

Table - 5.1. Physio-chemical parameters of 25 days depuration study in the seawater circulatory system.

Metal	Exposed Conc. (ppm)	Period of depuration (Days)	Salinity ($\times 10^{-3}$)	Temperature °C	pH	Dissolved oxygen ml/l
Cu^{2+}	0.02	25	35	29.0±0.5	7.9±0.05	>3.8
	0.04			29.0±0.5	7.9±0.05	>3.8
Hg^{2+}	0.0375	25	35	28.5±0.5	7.85±0.05	>3.8
	0.075			28.5±0.5	7.85±0.05	>3.8
Zn^{2+}	0.75	25	35	28.5±1.0	7.8±0.10	>3.8
	1.50			28.5±1.0	7.8±0.10	>3.8
Cu^{2+}	0.02	25	25	28.5±1.0	7.8±0.05	>4.0
	0.04			28.5±1.0	7.8±0.05	>4.0
Hg^{2+}	0.0375	25	25	28.5±0.5	7.8±0.10	>4.0
	0.075			28.5±0.5	7.8±0.10	>4.0
Zn^{2+}	0.75	25	25	28.5±1.0	7.8±0.10	>4.0
	1.50			28.5±1.0	7.8±0.10	>4.0

in Chapter - 4.

b) Data analysis:

Metal concentration in different body parts after 96 h was taken as the initial metal concentration (0 day) for the depuration study. Percentage of the initial concentration of metal with the progress of depuration time was calculated. Least-squre lines were fitted through the semi-logarithimic plots of points. The rate of loss of metals from the body parts of mussels are characterized by the equation.

$$\text{Log } Y = a - bx$$

Where x is the time in days; Y = % initial concentration; a = y intercept and *b* = the slope of the least square line. The biological half-lives (TB/2) were determined following the method of Renfro (1973). The slope of the least squre line (*b*) was substituted in the equation

$$TB/2 = \frac{\text{Log } 2}{b}$$

From this TB/2 was calculated for each metal from its two different concentrations. From the TB/2 of different body parts total mean TB/2 for the whole animal were calculated, for each metal.

3. RESULTS

a) Depuration of heavy metals:

Metal concentration in different body parts of mussels maintained for depuration in 35×10^{-3} and 25×10^{-3} salinities were shown in tables - 5.2 and 5.3. Cu showed comparitively rapid depuration

Table - 5.2. Metal concentration in different body parts of *Perna viridis* during depuration in 35 x 10^{-3} salinity (each value obtained for a pooled sample of 6 to 8 mussels).

Metal	Exposed metal conc. (ppm)	Days of Depuration	Metal concentration in tissue (µg/g tissue Wt)			
			Gills	Viscera	Mantle	Muscle + Foot
Cu^{2+}	0.02	0	55.921	43.979	28.592	13.683
		5	25.196	20.325	13.167	7.230
		10	32.200	23.325	18.918	15.894
		15	14.213	21.057	12.358	26.163
		20	15.684	30.743	28.288	10.101
		25	17.847	18.548	7.078	1.596
	0.04	0	139.655	98.221	96.084	40.456
		5	50.416	30.452	34.657	16.961
		10	11.205	46.250	8.388	8.629
		15	54.507	66.112	22.059	14.444
		20	35.424	26.736	9.028	13.171
		25	25.510	30.782	16.586	18.947
Hg^{2+}	0.0375	0	79.92	20.10	9.92	8.34
		5	93.94	17.27	7.58	6.43
		10	58.88	27.01	7.92	6.87
		15	61.17	18.72	7.50	8.21
		20	79.90	17.07	4.32	5.39
		25	40.76	16.22	4.17	4.73
	0.075	0	45.48	17.57	8.97	3.72
		5	49.78	16.53	10.43	4.56
		10	42.76	13.32	5.57	3.49
		15	34.29	15.81	5.91	2.98
		20	21.40	15.01	6.41	3.63
		25	25.51	12.28	4.38	2.66
Zn^{2+}	0.75	0	247.95	351.46	264.33	142.57
		5	157.62	262.25	318.59	120.45
		10	371.38	159.20	110.13	110.39
		15	170.77	435.98	288.86	102.05
		20	215.75	248.41	123.20	126.24
		25	132.34	158.65	108.43	68.29
	1.50	0	379.57	416.02	255.88	132.80
		5	226.01	123.39	91.48	152.16
		10	139.86	343.75	84.78	82.87
		15	105.81	340.27	82.32	87.27
		20	244.21	339.75	242.08	62.68
		25	167.41	142.86	104.92	43.36

Table - 5.3. Metal concentration in different body parts of *P.viridis* during depuration in 25 x 10^{-3} salinity (each value obtained for a pooled sample of 6-8 mussels)

Metal	Exposed metal conc. (ppm)	Days of Depuration	metal Concentration in tissue µg/g tissue Wt			
			Gills	Viscera	Mantle	Muscle + Foot
Cu^{2+}	0.02	0	196.34	122.33	123.09	31.78
		5	49.12	39.58	38.28	35.81
		10	58.65	32.24	12.93	11.35
		15	34.72	25.14	12.55	22.37
		20	31.09	31.62	33.49	26.05
		25	30.66	24.78	18.10	27.55
	0.04	0	268.24	254.59	149.36	167.39
		5	33.38	154.58	18.47	41.96
		10	97.56	107.28	122.78	45.08
		15	102.99	39.68	99.31	31.25
		20	43.32	25.20	41.42	32.07
		25	30.65	53.48	24.94	20.99
Hg^{2+}	0.0375	0	1.52	2.29	0.702	0.522
		5	2.78	0.756	0.835	0.920
		10	1.47	1.634	0.681	0.511
		15	0.82	0.741	0.623	0.294
		20	0.73	0.631	0.532	0.281
		25	0.68	0.867	0.411	0.273
	0.075	0	2.71	1.03	0.487	0.954
		5	2.212	0.911	0.571	1.012
		10	2.034	1.503	0.469	0.931
		15	1.716	0.852	0.414	0.841
		20	1.577	0.712	0.336	0.722
		25	1.391	0.688	0.351	0.611
Zn^{2+}	0.75	0	697.80	437.90	149.36	167.39
		5	220.82	385.65	207.40	113.36
		10	216.64	263.39	146.05	133.78
		15	442.00	268.55	136.61	104.48
		20	204.35	158.87	103.83	102.19
		25	201.64	84.27	61.67	47.99
	1.50	0	404.97	421.54	163.39	262.62
		5	402.67	375.53	141.03	190.35
		10	324.53	337.51	134.11	172.78
		15	293.81	315.96	121.96	137.80
		20	242.47	223.15	108.08	106.80
		25	189.58	136.12	67.27	86.52

from the body parts. After 25 days, in 0.02 Cu group maintained in 35 x 10^{-3} salinity gills showed 68% metal loss and muscle 88%. In viscera the metal loss was comparitively low (58%). In 0.04 Cu group muscle showed 53% loss and viscera 69%. The maximum reduction was in gills and mantle (82% and 83% respectively).

Depuration of Zn was slow compared to Cu. In 0.75 Zn group after 25 days gills lost 47% and viscera, mantle and muscle the loss was around 50 to 60 %. During depuration some of the body parts showed temporary increase in Zn concentration. (Table - 5.2). In 1.5 Zn group muscle and mantle showed maximum reduction of 67% and 59 % respectively.

Hg showed very slow decline in comparison with Cu and Zn. After 25 days 0.0375 Hg group showed only 19% reduction in viscera. Mantle showed maximum reduction of 53%. In 0.075 Hg group maximum reduction (51%) attained in mantle. While in muscle and viscera the metal reduction was very slow (30%).

In 25 x 10^{-3} salinity Cu, Zn and Hg concentration showed a rapid loss in comparison with 35 x 10^{-3} salinity (Table - 5.3). After 25 days in 0.02 Cu groups gill and mantle showed maximum metal loss (84%). But metal loss was slow in viscera (79%) and muscle (13%). In 0.04 Cu group maximum metal loss was from mantle (83%) and gills (89%). In 0.0375 Hg group maximum metal loss was from gills (56%) and viscera (62%). In 0.075 Hg group maximum metal loss was from gill (49%) and mantle (38%). In 25 x 10^{-3} salinity also Hg loss was slow when compared to Cu. In 0.75 Zn group metal loss from the gill was 69% and in 1.5 Zn

group it was 53%.

b) Biological half lives (TB/2):

The linearity of metal loss and biological half lives in different body parts are represented in Figs. 5.2 to 5.7. The mean TB/2 calculated for Cu, Hg and Zn is shown in fig. 5.8 for comparison.

At 35 x 10^{-3} salinity Cu showed longest TB/2 in viscera (29.44 days) followed by muscle (21.63 days). Gills and mantle showed comparatively short TB/2 (15.24 and 17.02 days). The total mean TB/2 for the whole animal was 20.83 days. (Fig. 5.8a). The order of TB/2 in the body parts is:

Viscera > Muscle > Mantle > Gills

Zn showed longer TB/2 in viscera and gills (36.8 and 34.95 respectively). The total mean TB/2 for Zn was found to be 31.93 days (Fig. 5.8c). The order of TB/2 in the body parts is:

Viscera > Gills > Mantle > Muscle

Hg showed longer TB/2 in comparison with Cu and Zn. Viscera showed longest TB/2 (72.71 days), followed by muscle (42.95 days) as shown in fig. 5.8b. The order of TB/2 for Hg in the body parts is:

Viscera > Muscle > Gills > Mantle

The total mean TB/2 for Hg was 41.04 days which is higher than the values obtained for Cu and Zn. The order of TB/2 of the metals studied is: Hg > Zn > Cu

c) Effect of salinity:

At low salinity (25 x 10^{-3}) comparatively more metals were

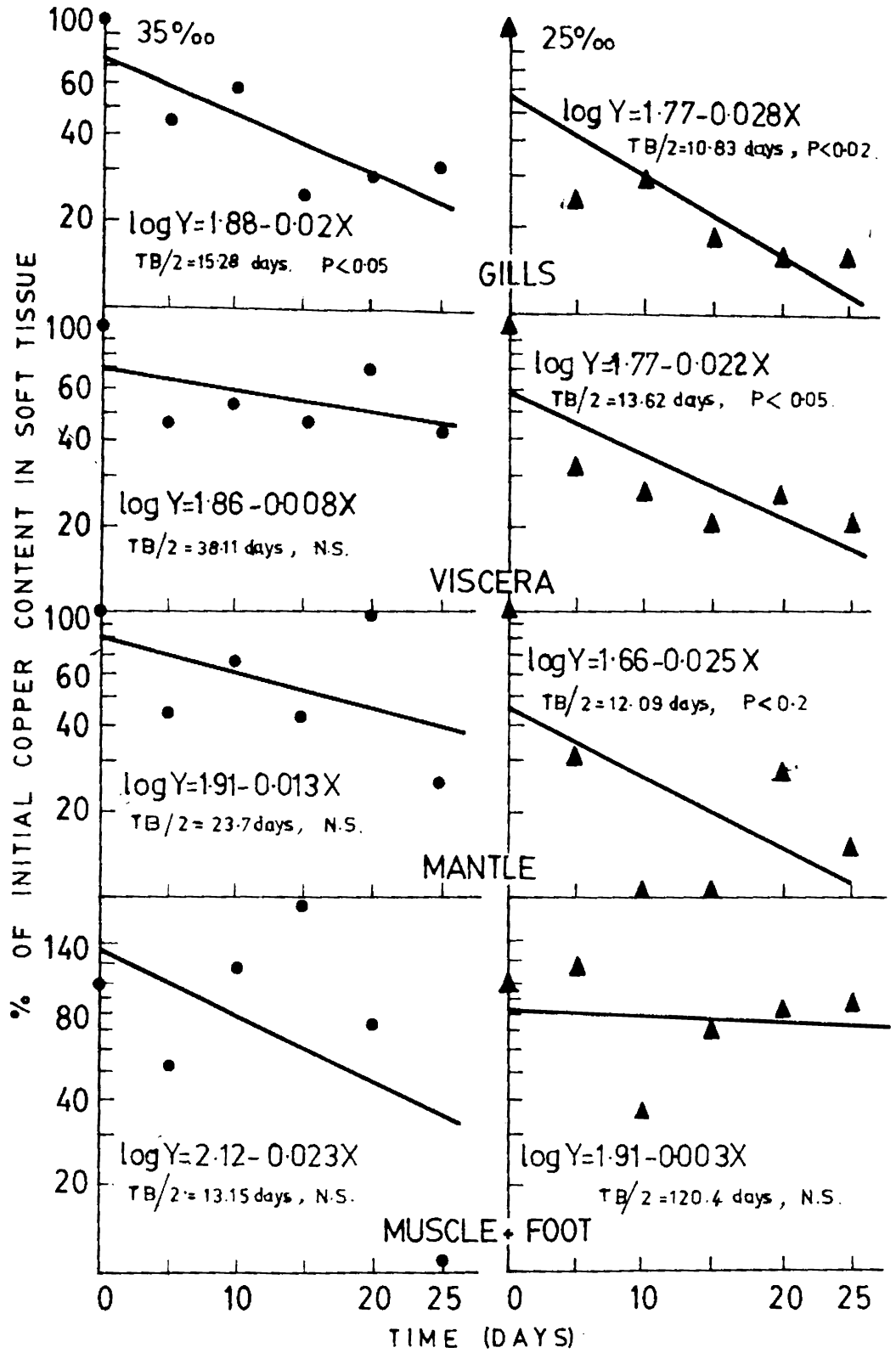


Fig - 5.2 Depuration of Cu from the different body parts of Perna viridis (previously exposed to 0.02 ppm of Cu) in (●)35 x 10^{-3}, &(▲)25 x 10^{-3} salinity.

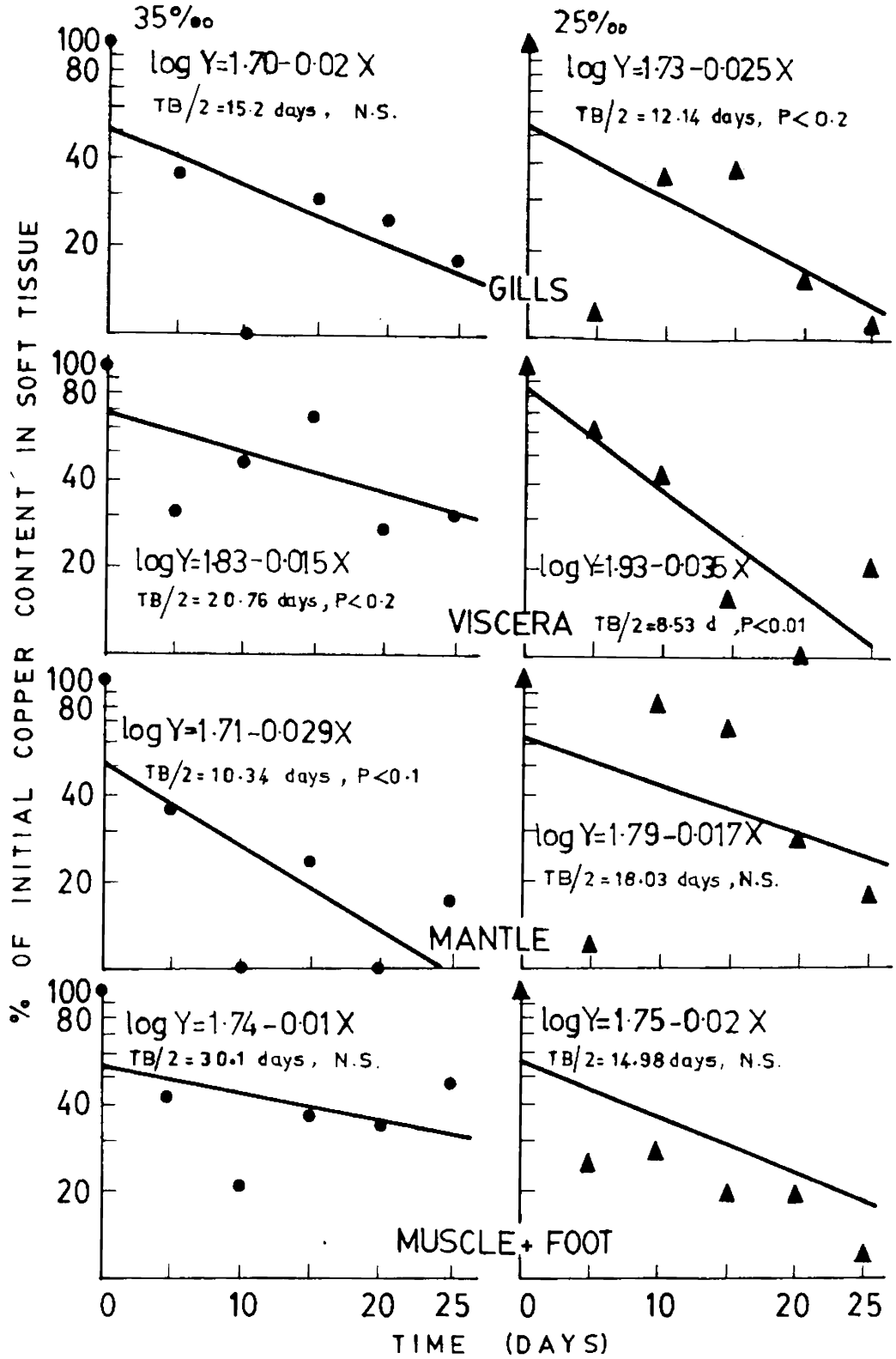


Fig - 5.3. Depuration of Cu from the different body parts of Perna viridis (previously exposed to 0.04 ppm of Cu) in (●) 35 x 10^{-3}, &(▲)25 x 10^{-3} salinity.

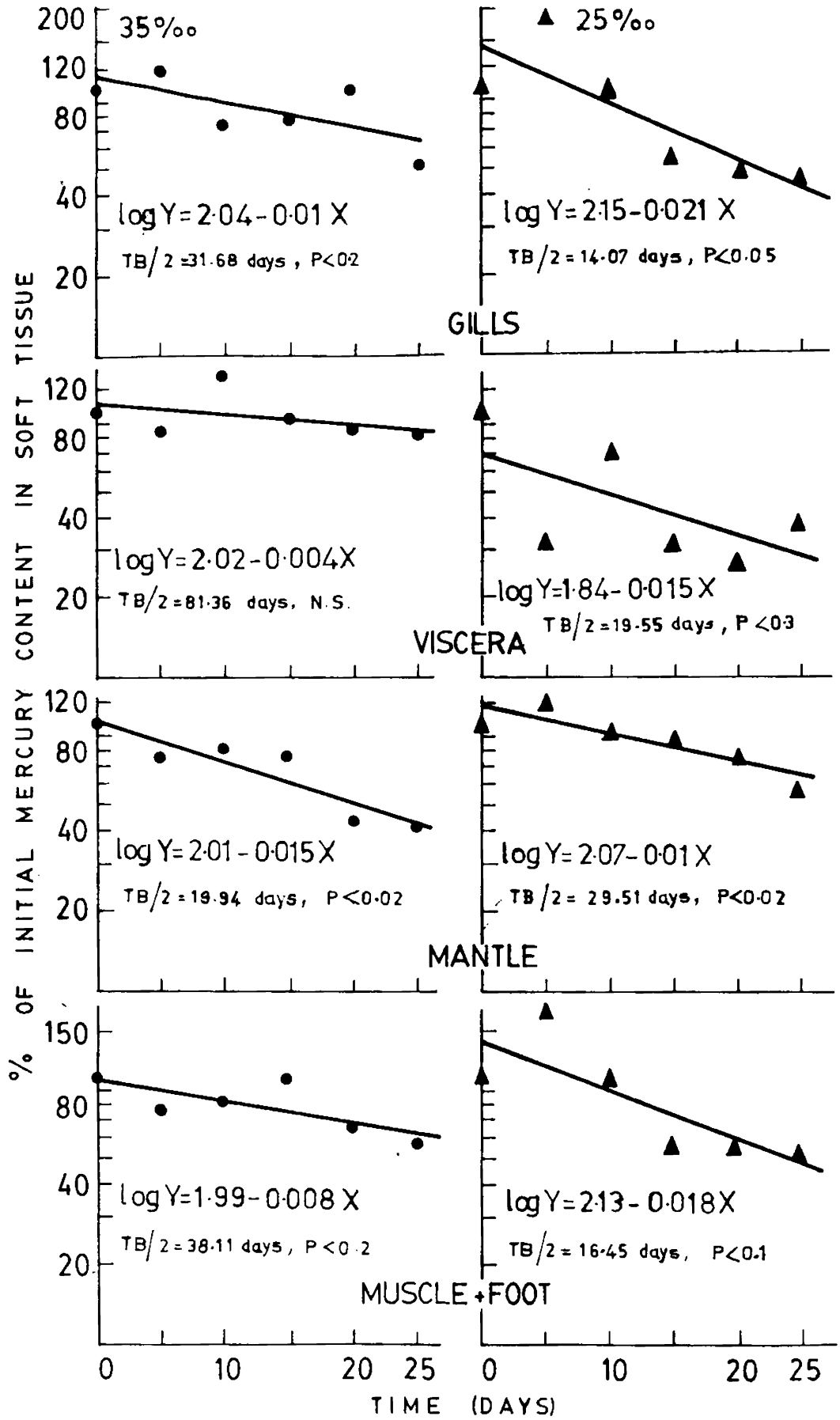


Fig - 5.4. Depuration of Hg from the different body parts of Perna viridis (previously exposed to 0.0375 ppm

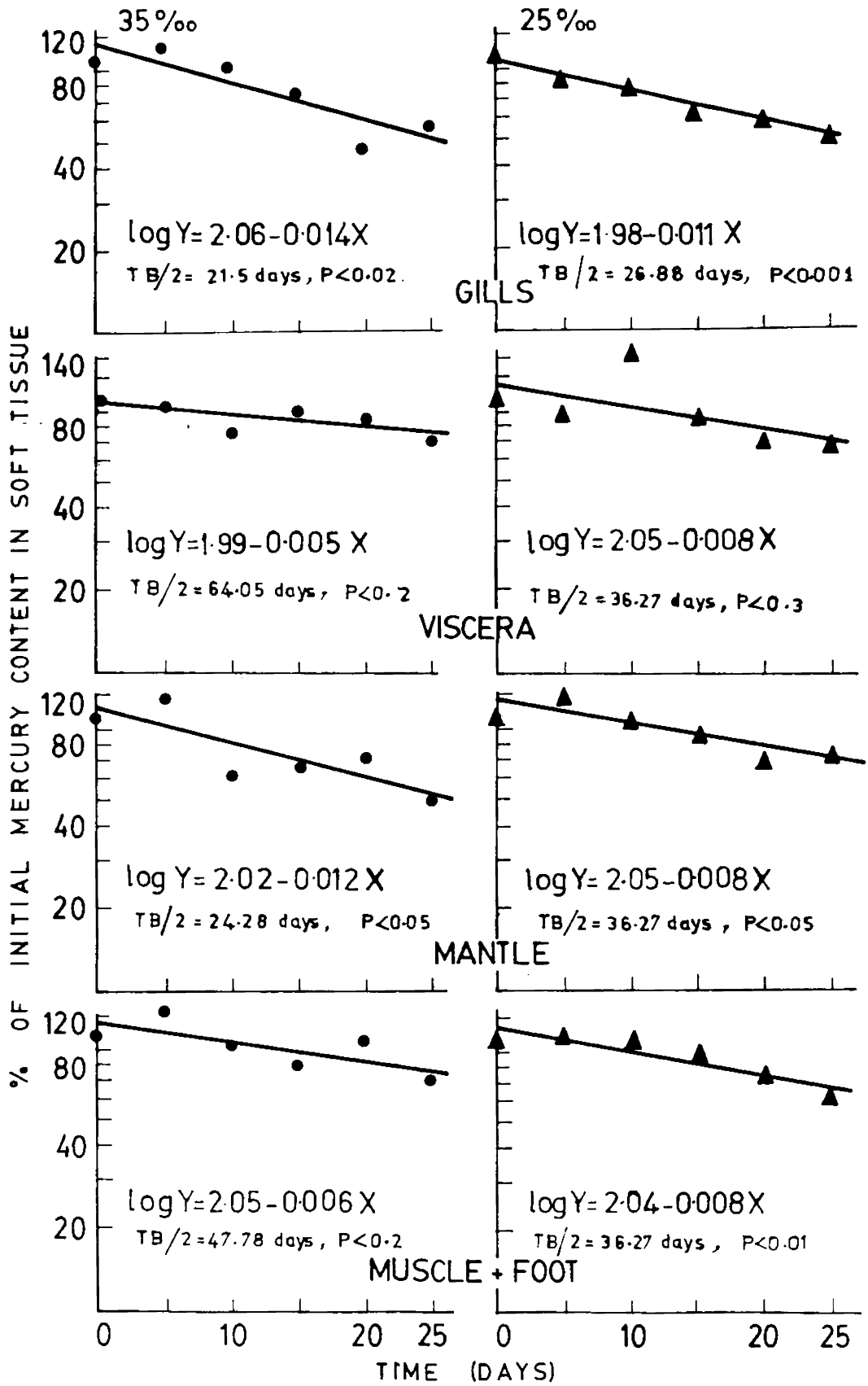


Fig - 5.5. Depuration of Hg from the different body parts of Perna viridis (previously exposed to 0.075 ppm of Hg) in(●) 35 x 10^{-3} & (▲) 25 x 10^{-3} salinity.

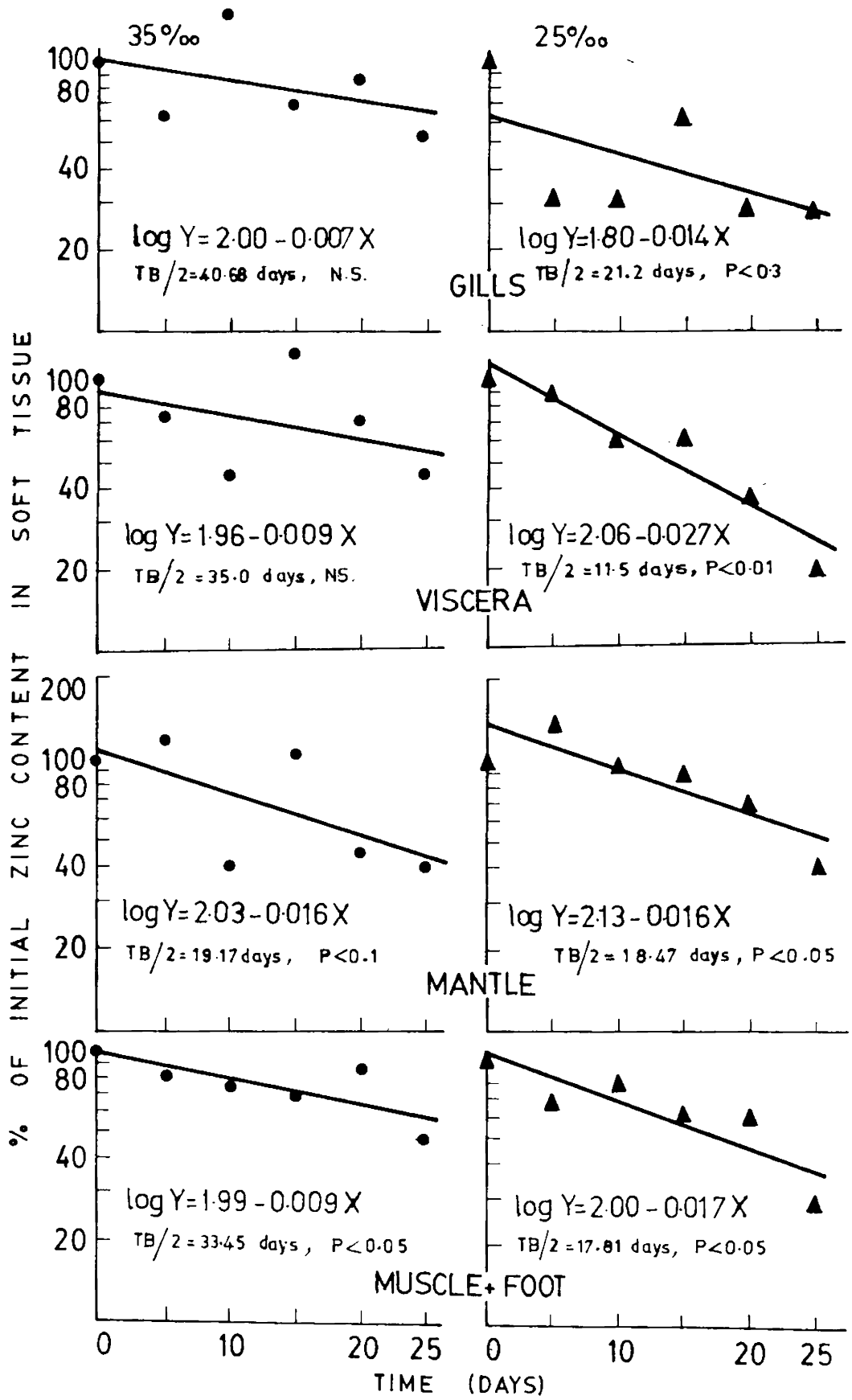


Fig - 5.6. Depuration of Zn from the different body parts of Perna viridis (previously exposed to 0.75 ppm of Zn) in (●) 35 x 10^{-3} &(▲)25 x 10^{-3} salinity.

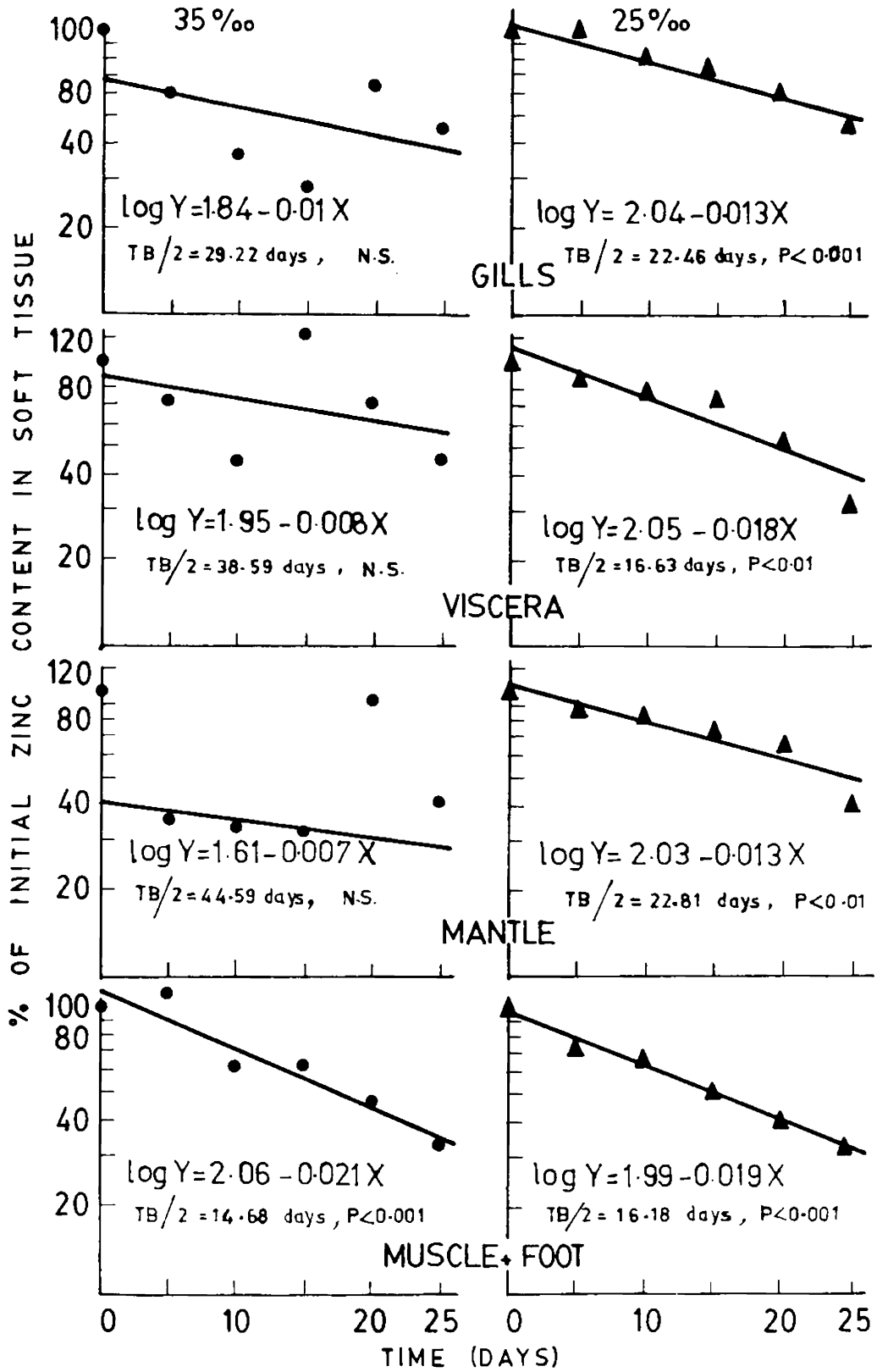


Fig - 5.7. Depuration of Zn from the different body parts of Perna viridis (previously exposed to 1.5 ppm of Zn) in (●)35 x 10^{-3} &(▲)25 x 10^{-3} salinity.

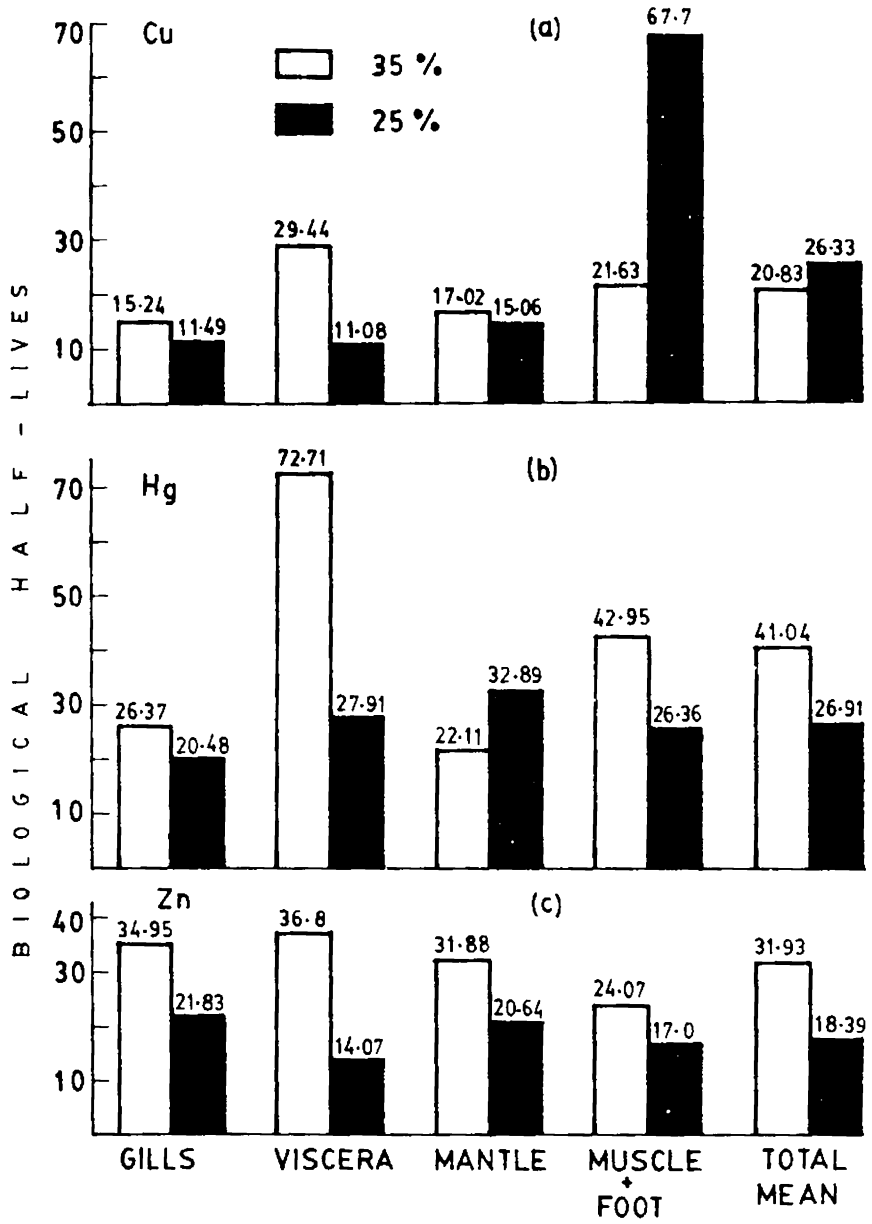


Fig - 5.8. Biological half lives of Cu, Hg and Zn in the different body parts of Perna viridis after 25 days of depuration.

depurated (Table - 5.3) This is clear from the slopes of metal loss obtained in 25 x 10^{-3} salinity for Cu, Hg and Zn (Fig. 5.2 to 5.7).

At low salinity Cu showed short TB/2 in all the body parts except in muscle + foot. Due to this the total mean TB/2 in low salinity (26.33 days) was slightly higher than the TB/2 obtained in higher salinity (Fig. 5.8a). But Zn showed low TB/2 in all the body parts at low salinity (Fig. 5.8c). Similarly the total mean TB/2 for Zn at low salinity (18.39 days) was shorter than the TB/2 at higher salinity. Hg also showed shorter TB/2 at low salinity in all the body parts except in mantle (Fig. 5.8b). The result as a whole showed higher rate of metal loss in 25 x 10^{-3} salinity in comparison with 35 x 10^{-3} salinity.

4. DISCUSSION

Results of the present study clearly indicate that the ability of different body parts to eliminate metals vary from each other. Comparatively rapid Cu, Hg and Zn loss was found in external tissues like gill and mantle. Because of that gills and mantle showed shorter TB/2 for the above metals (Fig. 5.8). Similar rapid decline of Hg was noticed in gills together with an increase in foot and muscle of bivalves (Fowler *et al.*, 1978; Cunningham & Tripp, 1975b; Smith *et al.*, 1975; Denton and Burdon-Jones, 1981). A rapid Cu elimination was reported from the gills of *M. galloprovincialis* (Viarengo *et al.*,1985).

Comparatively less Cu and Hg were lost from viscera and muscle + foot of *P.viridis*. Similarly highest amount of Hg was found to be retained in the visceral organs of *M.galloprovincialis* and *Tapes*

decussatus (Miettinen *et al.*, 1972). In *Ragia cuneata* large amount of Hg was retained in viscera after eight days of depuration (Dillon and Neff, 1978). These types of high Hg and Cu retention in viscera may be due to slower release of metal or a net internal flow of Hg towards viscera as noticed by Dillon & Neff (1978).

Bryan (1971) proposed three mechanisms for the loss of metals (1) excretion across the body surface or gill (2) excretion via gut and (3) excretion via urea. In the present study external tissues like gills and mantle showed a rapid elimination of metals while, internal tissues like viscera and muscle retained major portion of the metals. Most probabaly the excretion of metals might have occured across the body surface of gill or mantle, since this tissues are always in direct contact with ambient seawater.

In *P.viridis* after 25 days depuration, Cu and Hg in gills showed shorter TB/2. Cu, Hg & Zn showed comparatively longer TB/2 in the viscera. The bioaccumulation study (Chapter-4) showed that after 96 h gills and viscera have highest concentrations of these metals. But TB/2 of these metals in gills and viscera after 25 days of depuration, indicate a rapid metal loss from the former and a slow release from the latter.

Cu showed a short TB/2 (15.24 days) in the gill which is comparable with the value reported in *M.galloprovincialis* (Viarengo *et al.*, 1985). But the TB/2 of Cu reported in the gills of oysters were higher than the present value (Okazaki and Panietz, 1981).

The dosage, duration of exposure to a specific metal, duration of depuration and physiological conditions of the organisms may affect the TB/2 of heavy metals (Unlu *et al.*, 1972; Cunningham and Tripp, 1973). So before comparing the TB/2 values of different studies these factors should be considered. The total mean TB/2 obtained for Hg in the present study (41.04 days) is comparable with values reported for bivalves (Cunningham & Tripp, 1975 b; Denton & Burdon-Jones, 1981; Okazaki & Panietz, 1981; Riisgard *et al.*,1985). Very long TB/2 was reported for organic mercury in comparison with inorganic mercury (Miettinen *et al.*, 1972; Cunningham & Tripp, 1973; Riisgard *et al.*, 1985). In *P.viridis* Zn showed longer TB/2 than Cu as noticed in *Crassostrea gigas* (Okazaki & Panietz 1981).

Cunningham and Tripp (1975a) have recognised following categories of heavy metal release; 1) increase in TB/2 with increase in body burden of the heavy metal. 2) stable TB/2, when an equilibrium is maintained with a proportionate increase in the rate of heavy metal loss as its body burden increase, and 3) decrease in TB/2 with increase in body burden of heavy metals. In the present study release of Cu, Hg and Zn from the viscera apparently followed the first category of metal release. But metal release from gills followed the third category of release. Based upon the results of heavy metal accumulation (Chapter-4) and depuration in *P.viridis* the behaviour of metal can be classified as follow :

1) Cu - showed rapid accumulation and release from the tissues.

2) Hg - showed comparatively slow accumulation and release

3) Zn - showed rapid accumulation and slow release.

Heavy metal depuration may be related to the deposition or binding characteristics of each metal (Okazaki and Panietz, 1981). Hg may be tightly bound or incorporated into areas where depuration may not be possible; and hence it showed high TB/2.

Metallothioneins were found to be playing a fundamental role in accumulation and elimination of heavy metals in mussels (Roesijadi 1982, Viarengo *et al.*, 1985). Elimination of Cu thioneins were observed in gills and digestive glands of mussels in parallel with Cu elimination (Viarengo *et al.*,1985). And they suggested that different TB/2 of metals are related to the different capacity of cells to eliminate the metals bound to thioneins or metalothioneins themselves. So slow depuration of metals like Hg could be related to the chemical and physical difficulty in removing Hg or Hg containing substances such as metalloproteins to the exterior (Dillon & Neff, 1978).

In the present study low salinity enhanced the loss of Cu, Hg and Zn from the tissues. Similiar rapid Hg loss at low salinity, were reported in marsh clams (Dillon & Neff, 1978) and blacklip oysters (Denton & Burdon-Jones, 1981). Based upon the results of chapter-4 and present study it can be summarised that low salinity enhanced the rate of accumulation and rate of depuration of Cu and Zn. While low salinity decreased the rate of accumulation and increased the rate of depuration of Hg.

So the findings of accumulation and depuration studies of Cu, Hg and Zn are significant regarding the future use of *P.viridis* for monitoring, levels of above heavy metals in the tropical marine environment.

CHAPTER - 6

TOXIC EFFECT OF HEAVY METALS ON RESPIRATION AND FILTRATION RATE

1. INTRODUCTION

Wide range of possible techniques has been proposed for biological effect monitoring,(IMCO *et al.*, 1980; Bayne *et al.*, 1985). These include the study of physiological indices like respiration, feeding rate, body condition index and O:N ratio. Many features of aerobic metabolism of invertebrates can be studied indirectly by measurement of the oxygen consumption (Bayne *et al.*, 1976).

Changes in oxygen consumption of animals have been measured to study the response to toxicants (Sprague, 1971). But respiration is not considered suitable as an isolated response because it lacks a clear quantitative relationship with stress and pollution, but it should be measured as a component of balanced energy equation from which "scope for growth" is derived (IMCO *et al.*, 1980; Widdows, 1985a). Even then as Sprague (1971) suggested it seems logical that internal toxic action could change rate of respiration, although if the mode of action is not understood. And it is equally easy to suggest reasons why the rate should increase or decrease.

In bivalves the most commonly used measurement of filtering activity is filtration rate which is defined as that volume of water completely cleared of particles in unit time (Bayne *et al.*, 1976). Mussel feeds and respires by drawing water into its body under the influence of ctenidial cilia. Both mechanical and chemical factors were

found to influence the beat frequency of lateral cilia, contraction of gill musculature and the activity of mantle margin, which determine the rate of water flow through the gill (Badman, 1975; Mane, 1975; Bayne et al,1976). Abel (1976) made use of the filtration rate measurements in assessing the effects of environmental pollutants.

Results of *in vitro* and *in vivo* studies have shown that extremely low concentrations of heavy metals can inhibit oxidative phosphorylation, and mitochondria may be considered one of the most important targets of heavy metals in cells (Akberali & Earnshaw, 1982; Tort *et al,1982; 1984; a; b; Soma*sundaram *et al.*, 1984, Babu & Rao, 1985 Crespo & Sala, 1986; Earnshaw *et al,1986). The rates of filtering* activity and oxygen consumption were used as indicators of physiological effects of heavy metals in bivalve molluscs (Brown & Newell, 1972; Scott & Major, 1972; Mclness & Thrunberg, 1973; Delhaye & Cornet, 1975, Abel, 1976; Capuzzo & Sanser, 1977; Saliba & Vella, 1977; Watling and Watling, 1982; Latha, 1986).

The effect of heavy metals on oxygen consumption and filtration rates of *Perna* was studied by a few workers (Mathew & Menon; 1983; 1984; Prabhudeva & Menon, 1985, 1986; Baby & Menon, 1986). In Chapter - 3 the acute toxicity of Cu, Hg and Zn was quantified in terms of LC50 and EC50. From the LC50 values Cu was found to be more toxic than Hg. At the same time EC50 values obtained based on filtration rate showed that Hg was more toxic to *P.viridis* (Chapter - 3). The main objective of this study is to compare the toxicity of same concentration

of Cu and Hg on *P.viridis* by studying oxygen consumption and filtration rate.

2. MATERIALS AND METHODS

Mussels were collected and acclimated as explained in Chapter - 2. Metal stock solutions were prepared as explained in Chapter - 3. 20 l of filtered natural seawater were taken in each of three clean plastic tubs. Then 0.05 ppm Cu, 1.5 ppm Zn and 0.05 ppm Hg were added to the seawater in each tub respectively. In each tub 40 numbers of cleaned mussels (30-40mm) were exposed. During the experiment mussels were starved. The physio-chemical parameters of the experiments were as given below:

Salinity = 33.5 x 10^{-3}; Temp = 28.5 ± 1.0°C, pH = 7.8±0.05

Dissolved oxygen > 4 ml/l.

Every 24, 48, 72 and 96 h of exposure, eight mussels were removed from each tub to determine the oxygen consumption and filtration rate. Control mussels were used to determine the control values. For the measurement of O_2 consumption and filtration rate filtered seawater having metal concentration similar to their exposure medium were used.

a) Measurement of Oxygen consumption:

Using the respiratory chamber O_2 consumption of eight mussels were determined individually as explained in Chapter - 2.

b) Measurement of filtration rate:

After the measurement of O_2 consumption mussels were transferred individually to conical flask for measuring filtration rate

as explained in Chapter - 2. After the experiment soft tissues were removed and dried at 80°C for 24 h.

3. RESULTS

a) Effect on O_2 consumption:

After 48 h mussels exposed to Cu showed a tendency to close the valves by withdrawing their siphons in comparison with Hg exposed mussels. Zn exposed mussels behaved similarily after 72 h. The effect of Cu, Hg, and Zn on oxygen consumption were given in table - 6.1. Mussels exposed in 0.05 ppm of Cu showed 60% reduction in O_2 consumption after 48 h (from 0.7522 to 0.3011 ml min^{-1}). After that more or less the same trend continued and 40% reduction was observed at the end of 96h exposure. But the effect of Hg (0.05 ppm) on oxygen consumption was less severe when compared to Cu. After 48 h only 8% reduction was observed in the presence of Hg in the medium (0.6360 to 0.5844 ml min^{-1}). After 72 h exposure O_2 consumption value showed a sudden increase (Fig. 6.1 b). At the end of 96 h exposure, only 28% reduction was observed. Mussels exposed for 48 h in 1.5 ppm of Zn showed 29% reduction in O_2 consumption. And at the end of 96 h 39% reduction was observed (0.6331 to 0.3858 ml min^{-1}). These results show that Cu is more toxic than Hg during 96 h exposure (Fig. 6.1).

b) Effect on filtration rate:

After 48h exposure in 0.05 ppm Cu filtration rate decreased by 61% (from 8.3694 to 2.8805 ml min^{-1}). Then the animals slowly recovered from the effect of Cu and after 96 h only 33% reduction was recorded

Table - 6.1. Oxygen consumption and filtration rate calculated for *P. viridis*(1g. dry wt) exposed to Cu, Hg and Zn ($\bar{x}$ ± SD, N=8)

Metal	Concentration (ppm)	Exposure time (h)	O_2 Consumption ml h^{-1}	Filtration rate ml min^{-1}
Cu^{2+}	0.05	0	0.7522±0.0596	8.3694±2.4606
		24	0.6154±0.2217	2.8805±0.6237
		48	0.3011±0.1382	2.3427±1.1780
		72	0.3455±0.1835	4.1642±0.5587
		96	0.4546±0.1446	5.6187±2.4515
Hg^{2+}	0.05	0	0.6360±0.187	9.0864±2.3813
		24	0.5454±0.1942	3.0841±1.3355
		48	0.5844±0.2072	2.6884±1.1099
		72	0.8424±0.3099	2.7816±1.3800
		96	0.4599±0.2499	2.6271±0.7420
Zn^{2+}	1.50	0	0.6331±0.1969	9.0167±1.5275
		24	0.3504±0.1697	7.0014±0.7915
		48	0.4494±0.0584	4.1280±0.6134
		72	0.5710±0.2449	5.5233±1.1320
		96	0.3856±0.1776	4.8114±1.6821

(Table - 6.1 and Fig - 6.1a). After 24 h exposure in 0.05 ppm of Hg filtration rate showed 66% reduction. And after 48 h it reduced by 70%. The effect of Hg on filtration rate was prolonged upto 96 h and showed a maximum reduction of 71% (from 9.0864 to 2.6271 ml min^{-1}). Compared to Cu, effect of Hg on filtration rate was more severe (Fig. 6.1).

4. DISCUSSION

In the present study Cu and Zn were found to be inhibiting O_2 consumption. Cu and Zn were reported to be potent inhibitors of respiration in bivalves (Brown & Newell, 1972; Scott & Major, 1972; McInnes & Thurnberg, 1973; Delhaye & Cornet, 1975; Wilson and McMahon, 1981; Akberali & Earnshaw, 1982; Mathew & Menon, 1983; Akberali *et al*, 1985; Prabhudeva & Menon, 1986; Latha, 1986) and in dogfish (Tort *et al*, 1982; 1984; Crespo & Sala 1986). Mitochondria was reported to be one of the most important targets of heavy metal in cells (Viarengo, 1985). Zaba & Harris (1976; 1978) have suggested the biphasic mode of action of Cu on vertebrate mitochondrial respiration. They have shown that the initial stimulation of mitochondrial respiration is a result of enhanced K^+ influx, which is accompanied by mitochondrial swelling. The ensuring progressive inhibition of mitochondrial respiration appears to be the result of K^+ loss. Further the respiratory inhibition of Cu in bivalves was reported to be connected to uncoupling of respiratory enzyme (Akberali *et al.*, 1985) and inhibition of cytochrome oxidase (Babu & Rao, 1985). Zaba & Harris (1978) reported that Zn was the most potent inhibitor of mitochondrial respiration.

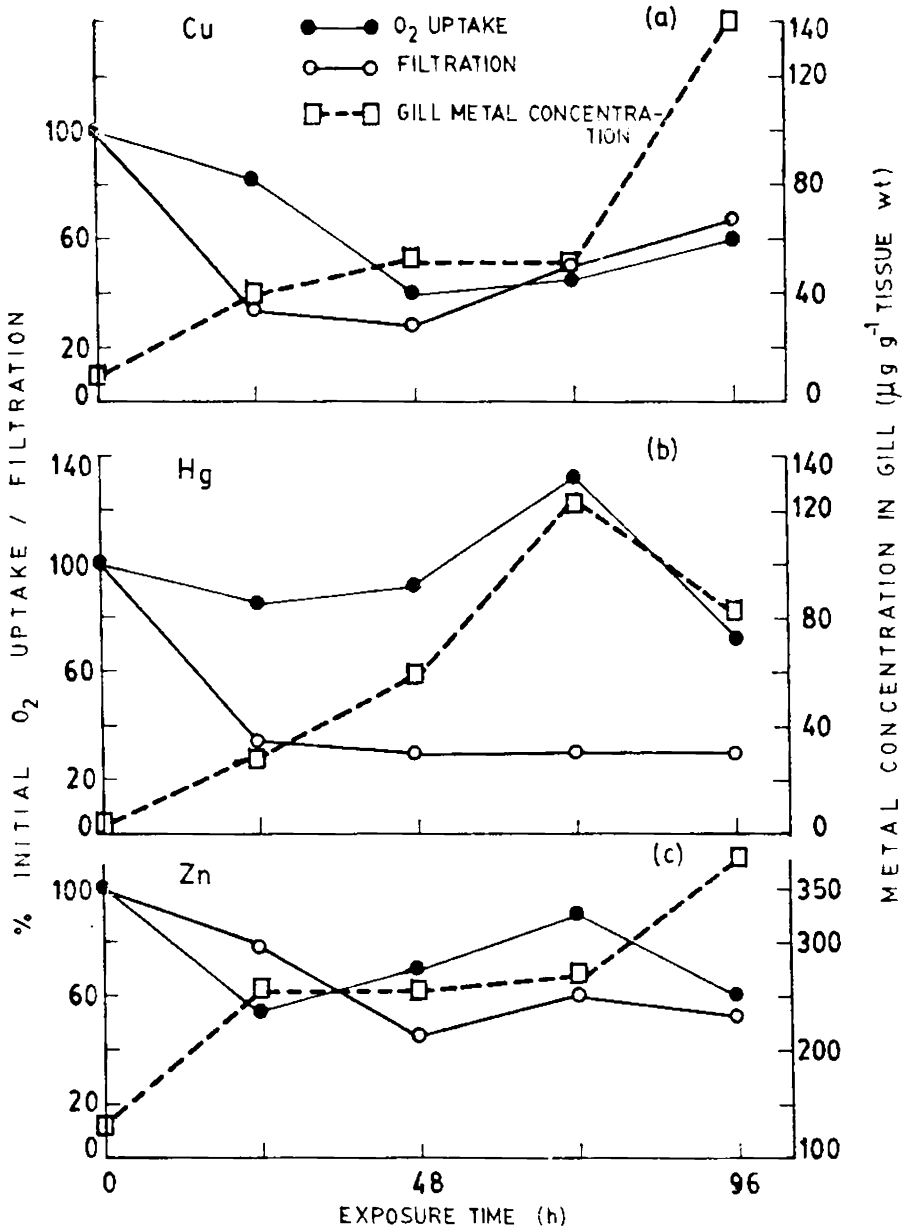


Fig-6.1. Effect of (a) 0.05 ppm Cu (b) 0.05 ppm Hg and 1.5 ppm Zn on Oxygen consumption and filtration rate of Perna viridis. Values of heavy metal concentration taken from chapter-4.

Gill serves a dual function in feeding and respiration, by means of water current drawn into its body under the influence of ctenidial cilia. It was found that metals like Cu, Hg and Zn quickly and preferably accumulated in the gills (Delhaye & Cornet, 1975; Moore *et al*, 1984; Chapter - 4 of this study). From the fig 6.1 it is found that Cu, and Zn inhibited respiration and filtration rate with increase in concentration of metal in the gill. Effect of Hg on filtration rate was more drastic in comparison with Cu and Zn.

In the present study same concentrations of Hg and Cu (0.05 ppm) were used. But the fig. 6.1 a & b show the different mode of toxic action of Cu and Hg. Result of filtration rate effect studies and EC50 values (Chapter - 3) have shown that Hg is more toxic than Cu. Abel (1976) and Watling & Watling (1982) also came to similar conclusions based on filtration rate effect studies. Neurotoxic effects of Hg was well established in a variety of animals including fish (Kobayashi *et al.*, 1980; Ram & Sathyanesan, 1985). The pumping of water by mussel is accomplished primarily by the beating of the cilia on the lateral epithelium of the gill filaments (Bayne *et al.*, 1976). Recent studies on the *Mytilid* gill have shown that the epithelial cells are supplied by the brachial nerve, and also that the activity of frontal, latero-frontal and lateral cilia is regulated via the nervous system (Paparo, 1972). So the drastic effect of Hg on filtration rate, without any apparent effect on O_2 consumption, may be due to its neurotoxic effect on the nervous system controlling ciliary activity in P.viridis.

Brown & Newell (1972) concluded that reduction in O_2 consumption due to Cu was caused by suppressed ciliary activity, rather than a direct effect on the respiratory enzymes. But the effect of Hg on both O_2 consumption and filtration rate shows that reduced filtration may not result in low O_2 consumption (fig. 6.1 b). So the reduced O_2 consumption recorded in the presence of Cu and Zn may be due to their inhibitory effect on the respiratory system as shown by several authors (Delhaye & Cornet, 1975; Akberali *et al.*, 1985; Babu & Rao, 1985; Viarengo, 1985).

Cu inhibited both O_2 consumption and filtration rate in comparison with Hg during 96 h exposure. That may be the reason for low 96 h LC50 value obtained for Cu in comparison with Hg. The present study indicates that Cu and Zn are mainly inhibiting the respiratory system, while Hg is damaging the nervous system controlling ciliary activity in *P. viridis*.

CHAPTER - 7

CHRONIC TOXICITY OF COPPER ON PHYSIOLOGY

1. INTRODUCTION

Measurement of physiological responses of bivalve molluscs will be useful in detecting the changes in the environment. These responses can be used in quantifying an organism's condition, its performance and the efficiency with which it functions under conditions of environmental stress and pollution (Widdows 1985a). The physiological indices like scope for growth, growth efficiency, O:N ratio, and body condition index were developed and evaluated by conducting laboratory and field studies using bivalve molluscs. It is found that these indices will make possible a more comprehensive appreciation of the health of environment, than, informations based solely on bioaccumulation factors (Widdows *et al.*, 1980; Martin *et al.*, 1984; Martin, 1985; Lack and Johnson, 1985).

Copper is one of the trace metals that is required by living systems for metabolism but it can have determinal effects at high concentration (See review, INCRA, 1982; Lewis and Care, 1982). Akberali and Black (1980) found that *Scrobicularia plana* (bivalve) could detect Cu at a concentration of 0.01 ppm in sea water. Cu caused respiratory inhibition in *M.edulis* even at low level concentration (Scott and Major, 1972). Delhaye and Cornet, (1975) observed rapid accumulation of Cu in the gills of *M.edulis* and instantaneous inhibition of respiration. Devenport and Manley, (1978) reported valve closure in *M.edulis* as an

avoidance mechanism towards Cu. They reported a threshold Cu concentration of 0.021 ppm in non-acclimated animals. Moore *et al.*, (1984) found that 3 days of exposure in 20 $\mu g\ l^{-1}$ of Cu resulted in reduced scope for growth and clearance efficiency of *M.edulis*.

The physiological indices of natural and transplanted mussels have been used to asses the quality of the coastal environment (Widdows, *et al.*, 1980; Wilson and McMahon, 1981; Martin *et al.*, 1984; Martin, 1985; Lack and Johnson, 1985). Poulson *et al.* (1982); Stickle *et al.* (1984) and Moore *et al.* (1984) investigated the physiological effect of pollutants on molluscs in the laboratory condition.

Before using bivalves for field monitoring programme it is necessary to know their physiological responses to extrinsic factors like pollutants (Widdows, 1985a). While studying the acute and sublethal toxic effects of heavy metals Cu was found to be highly toxic to *P.viridis* (Chapters 3 and 6). So Cu is selected to investigate further its toxic impact on the physiology of *P.viridis*.

The main objective of this study is to find out the chronic toxic effect of Cu on some of the physiological responses like O_2 consumption, ammonia excretion, O:N ratio, filtration rate and body condition index.

2. MATERIALS AND METHODS

Specimens of *P.viridis* were collected from the site, transported and acclimated in the laboratory as explained in Chapter-2, for 2 days. Algal culture (*Synechocytis sp.*) cultured in the heavy metal

free medium was given as food. (Salinity = 33.5 x 10^{-3}; Temp = 27.5±0.5°C; pH = 7.8 ±0.05; D.02 > 90% saturation).

a) Continuous seawater flow system :

The flow through system explained in Chapter-5 was used for the present study. The above system is filled with filtered (Whatman, 42), natural seawater. Copper solution ($CuSO_4.5H_2O$) is prepared as explained in the Chapter-3 and added 7.5 $\mu g\ l^{-1}$ to the system. The lower reservoir is always kept ready with the Cu added sea water for pumping.

Cleaned mussels (30-40 mm size) 20 numbers in each of the experimental tanks were distributed (total 120 nos). Concentrated *Synacocystis* sp.culture was added into the mixing take and well mixed with the stirrer to maintain, a concentration of 3 x 10^3 cells ml^{-1} in the experimental tanks. Sea water was always aerated in the aerating tower and maintained a flow rate of 160 ml min^{-1} through the system. Every 2 days seawater from the outlet was collected for monitoring the physio-chemical parameters. During 20 days of the experimental period the environmental parameters were:

Salinity = 33.5 x 10^{-3}; Temp = 28±1.0°C;pH = 7.8±0.1; D.02 > 4.0 ml l^{-1}

Before starting the experiment 10 mussels were taken from the acclimating system to find out the control values (0' day exposure) of physiological indices and tissue Cu content. The acclimating system was there after kept as control for 20 days for observation. 10 mussels were taken from each of the experimental system on 4th, 8th, 12th and 20th days of exposure to find out the physiological indices.

b) Measurement of physiological indices:

Physiological indices like oxygen consumption, ammonia excretion, O:N ratio, filtration rate and B.C.I. of 10 mussels were measured individually keeping in filtered seawater containing 7.5 $\mu g\ l^{-1}$ Cu as explained in the Chapter-2. The filtration efficiency (FE) or the index of relative efficiency were calculated using the following relationship (Jorgensen, 1975):

$$FE = \frac{1 \text{ of water filtered } h^{-1}}{\text{ml } O_2 \text{ consumed } h^{-1}}$$

Together with the physiological indices measurement, 6 mussels were sacrificed to find out the tissue Cu content by the method explained in Chapter-4. All the physiological indices were calculated for animals of 1g standard body weight (Bayne and Newell, 1983).

3. RESULTS

In the beginning of the experiment mussels remained attached to the sides of the tank near the water surface. But after 12 days of exposure their byssus producing capacity relatively reduced and most of the mussels remained at the bottom. But those in the control system remained healthy by attaching to the sides of the tank. The results of the studies are summarised in the table - 7.1.

After 4 days of exposure, oxygen consumption of the animal showed a 48% reduction (0.8832 to 0.4284 ml h^{-1}) and then increased upto 1.064 ml h^{-1} by 12th day. After that it showed a marked decrease and recorded only 70% of the control value (Fig. 7.1a) Ammonia excretion

Table - 7.1. Physiological responses and copper concentration ($\mu g/g^{-1}$ dry wt) in tissue of *P.viridis* during 20 days exposure to 7.5 ppb of Cu. Calculated for animal of 1 g. standard body weight ($\bar{X}$ ± SD, N = 10)

Exposure days.	Oxygen uptake (ml h^{-1})	Ammonia excretion (μg NH_4N hr^{-1})	O : N	Filtration rate (l h^{-1})	Filtration efficiency	Body condition index	Copper content. (μg g^{-1} dry wt.
Control	0.8832 ± 0.27	36.5 ± 2.96	26.53 ± 7.46	0.9234 ± 0.25	1.05	58.85 ± 12.5	8.34
4	0.4284 ± 0.17	29.78 ± 7.96	21.23 ± 1.47	0.3168 ± 0.06	0.7395	51.06 ± 4.78	23.61
8	0.6748 ± 0.31	25.97 ± 8.96	32.52 ± 11.27	0.6540 ± 0.22	0.9692	49.10 ± 11.84	38.18
12	1.0640 ± 0.17	23.04 ± 5.77	67.56 ± 12.25	0.3816 ± 0.11	0.3586	46.86 ± 10.78	33.00
20	0.6300 ± 0.27	31.35 ± 3.94	28.15 ± 5.15	0.2310 ± 0.074	0.3667	29.32 ± 5.32	196.91

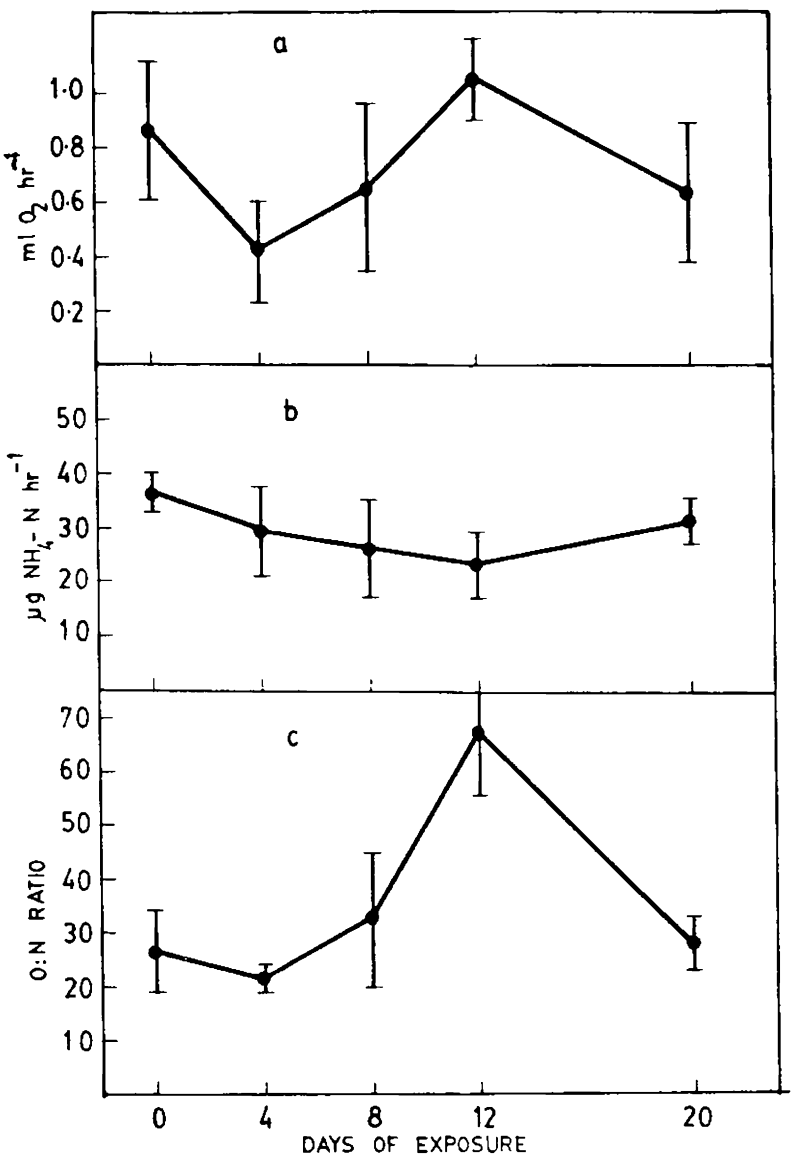


Fig-7.1. Physiological responses of *Perna viridis* exposed to 7.5 ppb of Cu. a) Oxygen consumption. b) Ammonia excretion. c) O:N ratio. Calculated for 1 g body weight. ($\bar{X}$ ± SD, N = 10).

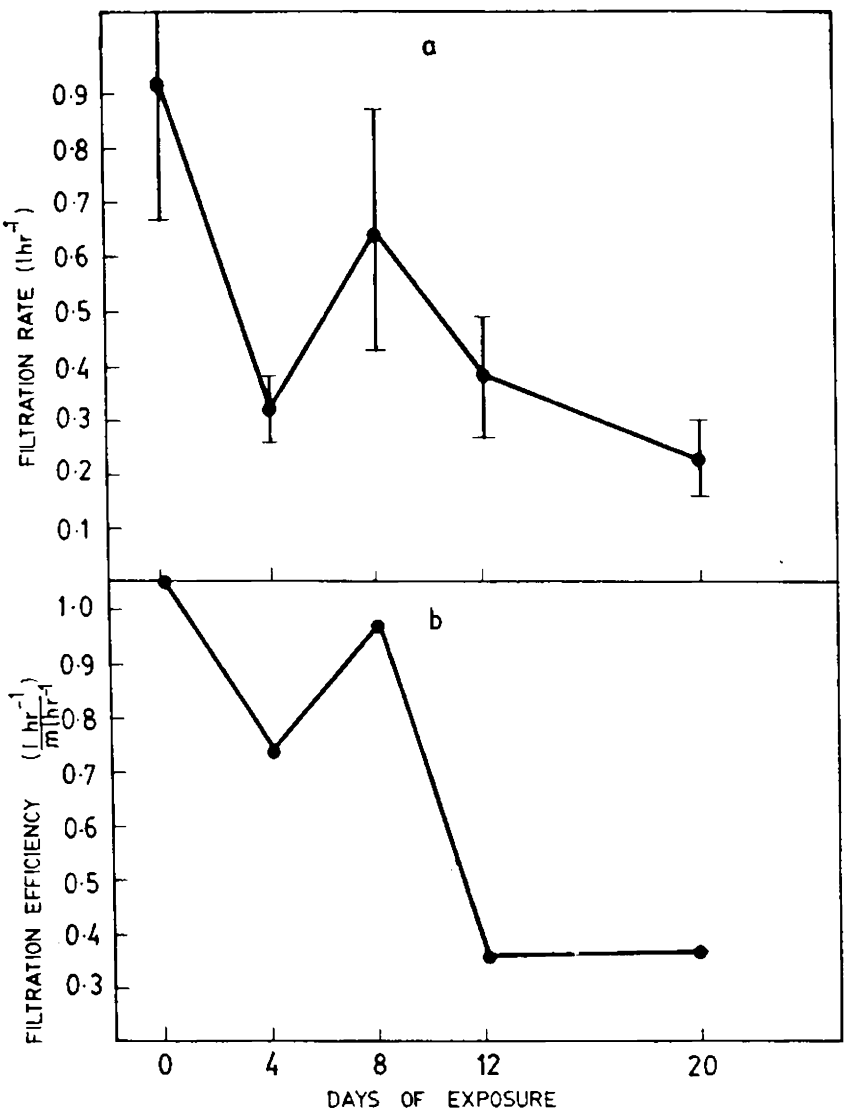


Fig-7.2. Physiological responses of *Perna viridis* exposed to 7.5 ppb of Cu. a) Filtration rate. b) Filtration efficiency. For 1 g body weight. ($\overline{X} \pm$ SD, N = 10).

rate showed slow decrease upto 12th day of exposure from 36.5 to 23.04 $\mu g\ NH_4\text{-}N\ h^{-1}$, then it showed a slight increase by 20th day (Fig. 7.1b). O:N ratio showed a slight decrease from 26.53 to 21.23 by the 4th day. Then it sharply increased to $2\frac{1}{2}$ times of the control value by the 12th day. After that the value decreased to 28.15 which is slightly higher than the control (Fig. 7.1c).

Variation in filtration rate was similar to that of oxygen consumption but the value showed a sharp decrease on 4th day, from 0.9234 l h^{-1} to 0.3168 l h^{-1}. Then the value showed an increase on 8th day and finally reduced to only 25% of the control value (Fig.7.2a). The calculated filtration efficiency followed the pattern of filtration rate upto 12th day of exposure (Fig.7.2b) and then remained more or less constant. Body condition index showed steady decrease due to copper stress upto 12th day. And on 20th day it decreased to nearly half of the control value (Fig - 7.3a).

The Cu uptake pattern of *P.viridis* is shown in the fig-7.3b. From 8.34 $\mu g\ g^{-1}$ dry wt (control value) it increased to 33.00 $\mu g\ g^{-1}$ dry wt by 12th day. After 12th day the tissue copper content showed a significant increase ($P < 0.05$) to 196.91 $\mu g\ g^{-1}$ dry wt (Fig -7.3b).

After the 4th day of exposure all the physiological indices showed a sudden decrease due to Cu stress. After 12th day all the indices except ammonia excretion declined coinciding with significant rise in tissue Cu content (Table-7.1).

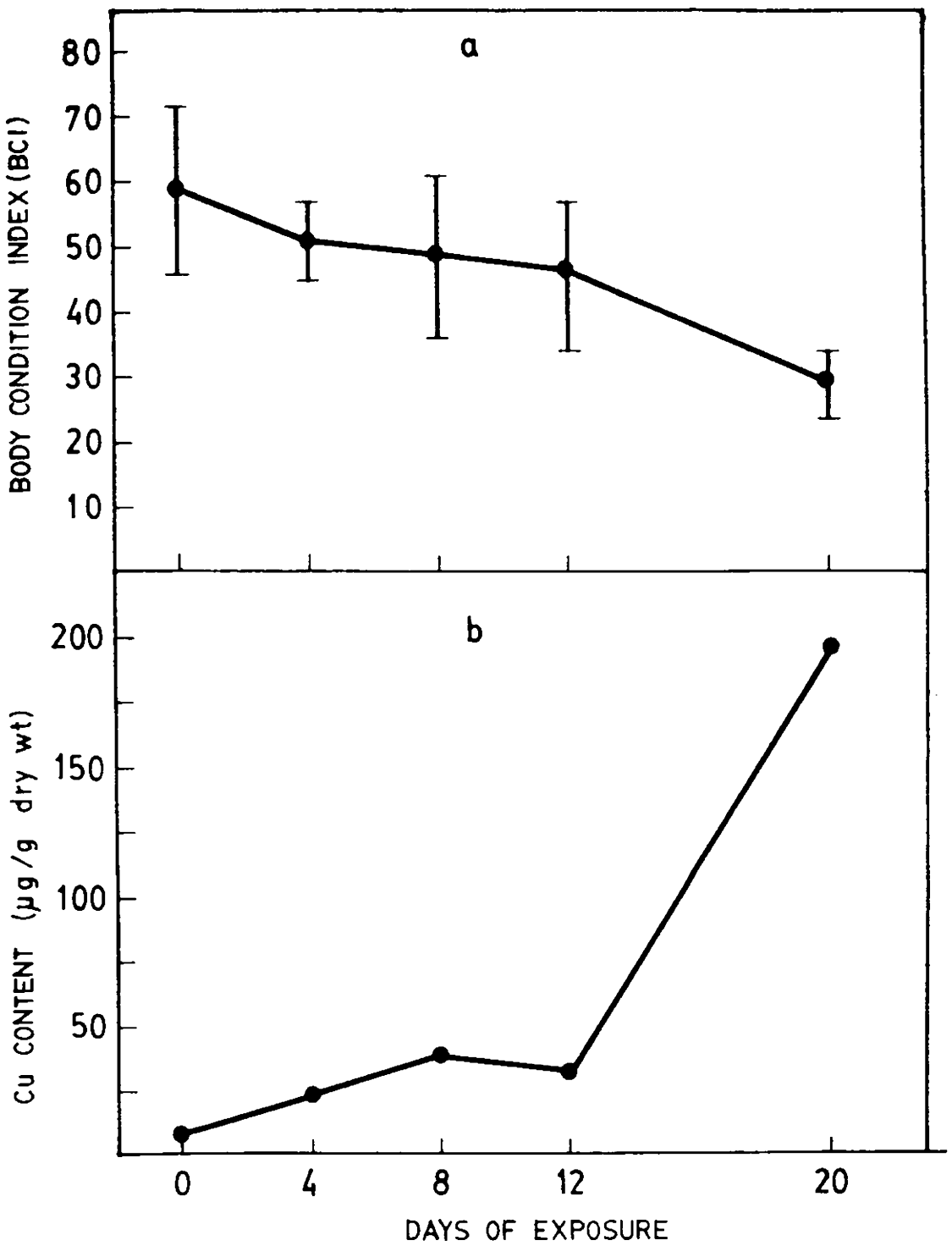


Fig-7.3. Physiological responses of *Perna viridis* exposed to 7.5 ppb of Cu. a) Body condition index ($\bar{X} \pm SD$, N = 10). b) Tissue Cu concentration ($\mu g\ g^{-1}$ dry wt.)

4. DISCUSSION

The LC50 and EC50 values of Cu to *P.viridis* were 86 ppb and 135 ppb respectively (Chapter-3). Sub-lethal concentrations of Cu is found to be inhibiting respiratory mechanism in bivalves (Scott and Major, 1972; Delhaye and Cornet, 1975; Akberali *et al.*, 1985; Viarengo, 1985; Babu and Rao, 1985). In the present study initial oxygen consumption showed fluctuations indicating a sudden change in the metabolic rate. But finally it decreased significantly showing a reduced metabolic rate. Similar observations were reported by Moore *et al.*, (1984) in *M.edulis* after 3 days exposure in 20 ppb of Cu.

From 4th to 12th day of exposure O:N ratio showed a marked increase in the present study (Fig. 7.1c). A similar increase in O:N ratio of marine snails observed after 7 days of exposure in high concentration of water soluble fraction of crude oil (Stickle*et al.*, 1984). This type of 'immediate' increase in O:N ratio reflects a period of rapid utilization of glycogen at a time of excessive oxygen demand (Bayne, 1975). But after 20 days exposure O:N ratio decreased and *P.viridis* showed a value slightly higher than the control. In the case of animals like *P.viridis* where O:N ratio is near its minimum under optimum conditions, (See Chapter-2) physiological stress by Cu may not result in a reduced O:N ratio. Moreover in this type of short term studies O:N ratio may be unreliable if gluconegenesis occurs (Bayne, 1975).

Due to Cu stress, filtration rate and filtration efficiency

showed a clear cut decrease by the 20th day of exposure. From the result of the present study it is clear that body condition index can be taken as a reliable stress index even in short term effect studies (Fig. 7.3a). A similar decrease in tissue weight of *M.edulis* was reported by Wilson and McMahon (1981) with the increase in Cu concentration in a copper laden estuary.

In the present study the general decline in the physiological indices of *P.viridis* may be due to high concentration of Cu accumulated in the tissue from water. Similar fall in the physiological conditions were reported coinciding with an increased body burden of pollutants in natural and transplanted populations of mussels (Widdows, *et al.*, 1980, Martin *et al.*, 1984).

In this study Cu concentration used (7.5 ppb) has relation to the environmental concentration because the reported Cu concentration in the Arabian Sea is 4.7 ppb (Gupta *et al.*, 1978). This type of short term exposures (20 days) to heavy metals in the laboratory may not reveal the more subtle effects on growth, morphology and physiology of organisms that become apparent only after long periods. But these types of short term studies are necessary to plan and execute long term studies which will help us to understand the impact of extrinsic factors on the population.

CHAPTER - 8

CAROTENOID CONCENTRATION AS A BIOCHEMICAL INDEX OF HEAVY METAL STRESS

1. INTRODUCTION

Mussels are known to have considerable tolerance for long periods of anoxia, and bivalves in general have been called 'facultative' anaerobes (Zwaan, 1977 ; Hochachka, 1985). Bivalves have the ability to withstand periods of valve closure and resultant lack of oxygen (Moon and Pritchard, 1970; Coleman and Trueman, 1971; Akberali and Trueman, 1979; 1982; 1985; Widdows *et al.*, 1979). Research carried out by a number of workers have led to the greater appreciation of the metabolic pathway operating in bivalve molluscs during anaerobiosis, and has shown that the pathway differs in some ways from the classical one discussed in vertebrates (Hochachka and Mustafa, 1972; Zwaan and Zandee, 1972; Zurburg and Kluytmans, 1980; Hochachka, 1985).

Under anoxic conditions the breakdown of glucose or glycogen upto phosphoenol pyruvate (PEP) in bivalves is similar to the process in vertebrates. Further, while vertebrates convert PEP to pyruvate and accumulate lactate, the main end products of anaerobic glucose (glycogen) catabolism in intertidal bivalve molluscs are succinate and alanine (Zwaan and Zandee, 1972; Zwaan and Marrewijk, 1973; Hochachka, 1985).

According to Hochachka (1985) the two most critical metabolic problems related to anaerobic tolerance are 1) conservation of

fermentable substances and 2) avoidance of self pollution by accumulation of undesirable end products. But 'good' anaerobs solve this problem by coupling metabolic arrest with channel arrest or the fundamental features of cells, tolerant to hypoxia are: 1) metabolic arrest capacity (reversed Pasteur effect); (2) low permeability membrane; (3) ATP synthesis rate equal to ATP utilization rate; and (4) stable membrane function (Hochachka, 1985). The complex biochemical processes behind anoxic energy producton, and reduced metabolic rates are not yet completely revealed (Widdows *et al.*, 1978; Hochachaka, 1985). The known anoxic energy producing mechanisms, such as glycogenolysis, may contribute energy to a certain extent for the molluscan tolerance to anoxia/hypoxia. But none of this path way can explain all the phenomena of anoxic energy production and survival of molluscs during anoxia/hypoxia (Zs-Nagy, 1977). The possible role of cytosomes (Yellow pigment granules) or 'carotenoxysomes' in the anoxic energy production of molluscan neurons have been reviewed by Zs-Nagy (1977) and Karnaukhov (1979).

The yellow pigment granules present in molluscan tissues have been called 'cytosome' by Zs-Nagy (1977). The size of cytosomes varies from 0.3 to 12.0 μm and they have a membrane, granulated matrix, lipid droplets and a coloured component of carotenoid called lipochrome. The occurence of cytosomes were reported in all basic tissues of molluscs especially in the epithelial tissues of intestine, kidney, statocyst, syphon, gonads, mantle and pedal gland (Zs-Nagy, 1973). So cytosomes are

considered as a cell organells of general occurence in molluscs and they differ from lysosomes in their morphology, high lipid and pigment content (Zs-Nagy, 1977). But Karnaukhov (1971; 1979) suggested the term 'carotenoxysomes' for molluscan granules rich in carotenoids and according to him they can be considered universal energy providing structure of cells, phylogenetically older than mitochondria.

Regarding the role of cytosomes (yellow pigment granules) or carotenoxysomes in the anoxic tolerance of molluscs, two different hypothesis have been put forward independently by Zs-Nagy, (1977) and Karnaukhov (1979).

According to Zs-Nagy and co-workers (Zs-Nagy, 1971 a & b;1973; 1974; 1977; Zs-Nagy and Ermini, 1972a; 1972b; Zs-Nagy and Kerpel-Fronius, 1970) a part of energy production in molluscs during prolonged anoxia is realized by means of mechanism called 'anoxic endogenous oxidation'. This mechanism is localized in the cytosomes and its essential point is that the terminal electron acceptor function of the molecular oxygen is replaced by an internal electron acceptor. According to them the assumed electron acceptor function during anoxia is performed by unsaturated fatty acids, the bio-degradation of which represents an oxidative metabolic step. Zs-Nagy (1977) observed irreversible transformation of cytosomes during prolonged anoxia and increased acid phosphatase activity in it. They reported high survival capacity among molluscs having strong pigmentation in the central nervous system during anoxia (Zs-Nagy 1971a).

But Karnaukhov and co-workers (Karnaukhov, 1971; 1979; Karnaukhov *et al.*, 1977, Karnaukhov and Fedorov, 1977) put forward another hypothesis that carotenoid together with haemoprotein and some respiratory enzymes form a special intracellular organ or 'carotenoxysome' (the former group called cytosome) take part in oxygen metabolism of animal cells. Carotenoxysome can provide an intracellular reserve of oxygen (or its electron acceptor equivalent) and it allows them to provide energy requirement of the cell during anoxia or in the presence of respiratory inhibitors in water.

The electron acceptor and electron donor properties of carotenoid in the lipochrome pigment of cytosome/carotenoxy some were supported by both Zs-Nagy (1977) and Karnaukhov (1979). The electron acceptor - electron donor properties of the conjugated double bond chain of carotenoids allow it to connect an oxygen molecule in place of central unsaturated double bond with the help of haemoprotein (Karnaukhov, 1979). This decreases of double bond in carotenoid leads to the loss of its colour. According to Karnaukhov *et al.* (1977) and Karnaukhov (1979), this colourless oxygenated carotenoid may serve as an electron acceptor equivalent of molecular oxygen and can be considered analogous with the oxidized form of well known cytochrome oxidase ($a+a_3$) of the mitochondrial respiratory chain. Any oxygen deficiency arising within the cell is relieved by oxygen released from the carotenoid intracellular accumulator, and carotenoids once more assume their colour. This leads to an increase in absorption bands of carotenoids in

the characteristic three band absorption spectra.

According to above workers the system of terminal oxidation localized in the carotenoxysomes is similar to mitochondrial substrate - NADH system. The mitochondria are connected with carotenoxysomes by a specific regulatory mechanism and so the carotenoxysomes will not be active during normal mitochondrial activity (Fig. 8.1). At the time of low oxygen availability or if mitochondrial cytochrome system is inhibited, mitochondria lose their activity and the NADH oxidation system localized in carotenoxysomes become activated (Fig. 8.1). But this hypothesis, that carotenoids functioning as oxygen store has been rejected by Zs-Nagy, (1974; 1979) based on the calculations on oxygen storage capacity of carotenoids. According to him carotenoid or other haemopigments cannot store an amount of oxygen sufficient for the high energy yield during prolonged anoxia. But according to Karnaukhov (1979) the above conclusion is not valid on the basis of fact that during anoxic tolerance molluscs can almost arrest their metabolic activity and sustain on small amount of oxygen supply. Eventhough these two groups of workers disagree regarding the functional mechanism of cytosomes/ carotenoxysomes, they reached to a common conclusion that these 'structures' participate in the oxidative metabolism of molluscan tissue during anoxic tolerance (Zs - Nagy 1977; Karnaukhov, 1979). This will be resulted in an increase in cytosome number or carotenoxysomes (and concentration of carotenoid consequently) in the body of molluscs undergoing anaerobiosis or anoxic tolerance.

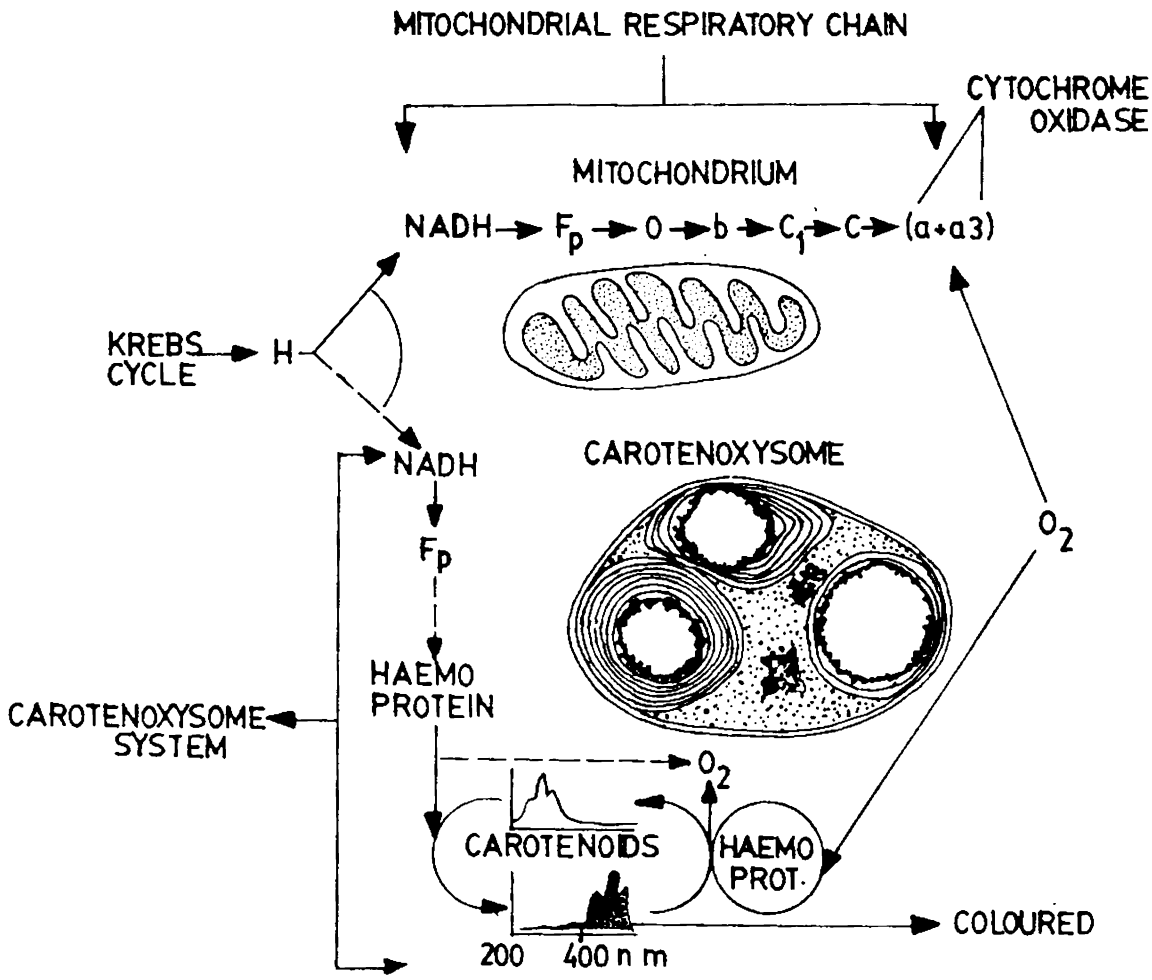


Fig-8.1. Role of carotenoxysomes in the oxidative metabolism of molluscan cells and its interrelation with the respiratory chains of mitochondria. (after Karnaukhov, 1979).

The ability of mussels to protect themselves against toxic substances in the ambient seawater, by closing their valves and ceasing filtration are considered as a great disadvantage as test animals (Cole, 1979). During this period of valve closure they may be depending upon anaerobic respiration to sustain basal metabolism (Akberali & Trueman, 1985).

Most of the heavy metals were reported to damage the physiological and biochemical system of bivalve molluscs (Bayne *et al.*, 1985). Metals may alter the enzyme activities within the mitochondria and damage respiratory mechanism (Viarengo, 1985). Metals were found to damage mitochondrial cytochromes and inhibit oxidative phosphorylation (Zaba & Haris, 1978; Akberali and Earnshaw, 1982; Tort *et al.*, 1982; 1984 a & b; Somasundaram *et al.*, 1984; Babu & Rao , 1985; Crespo & Sala, 1986).

In short, the metal toxicity at higher concentration will lead to two major changes in the overall functioning of the bivalves. They are:

1. Changes due to anaerobic metabolism resulting from prolonged valve closure.
2. Changes due to toxic action of metals on the physiological and biochemical functioning of the animals.

These may be the reasons for the similarity between the reported biochemical effect of heavy metal stress and anoxic/hypoxic stress (Table - 8.1).

Table - 8.1. Reported biochemical effects of anoxia/hypoxia and heavy metal stress and their close resemblance in molluscs.

Anoxic/Hypoxic effect	Heavy metal effect
1. Degeneration of cytosomes due to breakage of outer membrane at the end of anoxic tolerance in molluscs (Zs-Nagy, 1977)	Loss of membrane stability of lysosomes and damage to the membrane structure in bivalves. (Viarengo et al., 1980; Moore, 1982; George, 1983b; George and Viarengo, 1984; Moore et al., 1984)
2. Increase in carotenoid concentration in the tissues of *Mytilus galloprovincialis* due to hypoxic tolerance (Karnaukhov et al.,1977)	Increase in carotenoid concentration in the presence of KCN in the nervous tissues of *Lamnaea stongnails (Karnaukhov, 1971).*
3. Increase in CO_2 and calcium in the body fluids of bivalves (Zs-Nagy, 1977).	Increase in pCO_2 and calcium ions in the mantle fluid of bivalves subjected to short term Cu concentration. (Akberali and Black, 1980).
4. Increase in taurine content in the body of molluscs (Zs-Nagy, 1977).	Increase in Taurine; Glycine ratio (Bayne et al, 1985; Viarengo, 1985).
5. Increase in carotenoxysomes number with lipochrome pigment; Increased synthesis of unsaturated fatty acids (Zs-Nagy, 1977; Karunaukhov, 1979).	Increased number of tertiary lysosomes with lipofuschin grannules as a result of heavy metal detoxification in mussels (George et al, 1982; George, 1983b).
6. Activation of certain electron acceptors which can maintain redox potential (Zs-Nagy, 1977).	Increased peroxidase activity with high oxidation potential in snails under Cu stress (Babu and Rao, 1985).
7. Increase in ATP level and Adenylate engery charge (AEC) in molluscs (Wijsman, 1976; Zs-Nagy,1977).	Increase in AEC values of Cd exposed mussels (Haya & Waiwood, 1983; Viarengo, 1985).

The increase in carotenoid concentration in the tissues of molluscs has been observed due to their tolerance to hypoxic condtition or to the mitochondrial inhibitor actions of pollutants (Karnaukhov, 1971, 1979; Karnaukhov *et al.*, 1977). So the first objective of this study is to investigate the relationship between carotenoid concentration in the tissue of *P.viridis* and its heavy metal tolerance. The second one is to investigate the suitability of using carotenoid concentration in the body of *P.viridis* as an index of heavy metal stress.

2. MATERIALS AND METHODS

a) Carotenoid concentration and toxicity tolerance:

Mussels were collected and acclimated as explained in Chapter-2 for one week. Animals were fed with algal culture (*Synechocytis* sp.). Feeding was suspended 48h before and during the experiment. Filtered (42 Whatman), natural seawater was used for the experiment (Salinity = 25 x 10^{-3}; Temp = 26 ± 1.0°C; pH = 7.8 ± 0.2 and dissolved oxygen > 4ml/1).

Mussels (30 - 40) were cleaned and exposed in colourless polythene tubs, containing serial dilutions of Cu and Hg as given in table - 8.2. Each tub contained five 1 of test medium and 10 animals. Hg and Zn solutions were prepared using $HgCl_2$ and $ZnSO_4.7H_2O$ as explained in Chapter - 3. One experimental tub each was kept as control without metal solution. Dead animals were removed at every 12 h intervals and the test medium was renewed once in 24 h. After 96 h all the live

animals were removed for carotenoid extraction.

b) Carotenoid concentration as an index of stress:

i) In different body parts : Mussels exposed to Cu (0.05 ppm), Hg (0.05 ppm), and Zn (1.5 ppm) in the experiments explained in Chapter - 6 were used for the study. Mussels were withdrawn after 0, 24, 48, 72, and 96 h exposure. Animals exposed in Cu and Hg (6 to 8 nos.) were dissected out and gills, viscera, mantle and muscle + foot were separated. Whole soft tissue of mussels (6 to 8 nos) exposed in Zn were collected.

ii) In long term Cu exposed mussels: To find out the carotenoid concentration after long term exposure to Cu (7.5 ppb), mussels from the seawater flow system explained in Chapter - 7 were used. Mussels were withdrawn after 0, 4th and 20th days of exposure. After that whole soft tissues were removed to find out the carotenoid concentration.

iii) Carotenoid concentration and oxygen consumption: In another set of experiment to find out the carotenoid concentration together with the oxygen consumption, mussels were exposed in 1.0 ppm of $HgCl_2$ in a wide mouthed plastic container. Hundred mussels (size 30-40 mm) were exposed in 50 litres of water, and every 24 h water was renewed. Six to eight to 8 mussels were removed every 0, 6, 12, 18, 24, 30, 36, 42 and 48 h of exposure to find out the carotenoid concentration in the whole body. Six mussels were used every 0, 12, 24, 36 and 48 h of exposure to measure the oxygen consumption.

c) Measurement of oxygen consumption :

For oxygen consumption study the methods explained in Chapter - 2 were followed. Experiments were run for 3 h keeping the animals in 1 ppm test solution in the respiratory chamber. At a time 6 to 8 animals were used individually to find out their O_2 consumption with an unpolluted seawater control. After the experiment animals were dissected and the soft tissue removed, blotted and wet weight determined. The results were expressed in ml oxygen consumed g^{-1} wet body weight h^{-1}.

d) Carotenoid extraction and estimation :

The soft tissues collected from the mussels were wiped with filter paper and the wet weight recorded. The carotenoid extraction and estimations were made according to the standard procedures given by Karrer & Jacker (1950); Karnaukhov, *et al.* (1977) and Karnaukhov and Fedorov, (1977). The weighed tissues were ground with chilled acetone in a glass mortar. Then the acetone extract was filtered under reduced pressure through a scintered glass funnel and the solid residues were returned to the mortar for further extraction. The extraction was repeated until the acetone extract was colourless. The volume of acetone extract were measured. Then the absorption spectra and optical density of the extract were recorded for calculation of total carotenoid concentration using a Hitachi spectrophotometer (Model 200-20) at 455nm).

Most of the hydroxylated carotenoids occur in nature in the form of esters and it is necessary to saponify the acetone extract for

further purification and removal of most of non-carotenoid contaminants. So it is saponified for 12 - 14 h at room temperature by the addition of 1 ml 60% KOH solution to each 10 ml extract. Unsaponifiable carotenoids were extracted from the alkali acetone mixture by the addition of 2 - 4 ml of petroleum ether (B.P. = 40-60°C) and 10-15 ml of saturated sodium chloride water solution per each 10 ml of the alkali - acetone mixture in a separating funnel. The petroleum ether solution of unsaponifiable carotenoid was washed several times with distilled water and dried over anhydrous sodium sulphate and then it was kept for 10-12 h in a refrigerator (-4°C) to remove sterols. The volume of the extract was measured and its optical density determined using the spectrophotometer.

The carotenoid concentration in mg $100g^{-1}$ of wet tissue weight was calculated from the equation (Karnaukhov, *et al.*, 1977).

$$\text{Carotenoid mg } 100g^{-1} = \frac{0.4\ DV}{p}$$

where D is the optical density of the extract, V is the total volume of extract in ml and p, the total wet weight in gram of the tissue from which the carotenoid was extracted.

3. RESULTS

a) Carotenoid concentration and toxicity tolerance:

The results of experiments are given in the table - 8.2. Mussels which survived 96 h in 0.8 ppm of Hg have highest carotenoid concentration (1.4278 mg $100g^{-1}$ body wt.) which is nearly five times

higher than the control (0.3055 mg $100g^{-1}$ body wt.). Above 0.2 ppm concentrations of Hg the carotenoid concentration increased sharply. Conversely the percentage survival of the mussels declined above 0.2 ppm (Table - 8.2).

Animals which survived 96 h of exposure in 6.0 ppm of Zn have the highest carotenoid concentration (2.2043 mg $100g^{-1}$ body weight) which is approximately six times higher than that recorded in control animals. Above 1.0 ppm concentration of Zn carotenoid concentration increased sharply. Correspondingly the percentage survival showed a steady decrease. (Table - 8.2). Increase in both total and unsaponifiable carotenoid concentration were found to be highly significant ($p < 0.001$).

b) Carotenoid concentration as an index of stress:

i) In different body parts: The results of the experiments are presented in tables - 8.3 & 8.4. Carotenoid concentrations were found to be high in gills and viscera than in mantle and muscle of animals exposed to metals. In mussels exposed to 0.05 ppm of Cu, total carotenoid concentration in the gills sharply increased to 4.8554 mg $100g^{-1}$ body weight (control value - 0.7682 mg $100g^{-1}$g body wt) after 24 h exposure. Again after 48 h it decreased and remained more or less constant (Fig. 8.2). In viscera, mantle and muscle the sharp increase in total carotenoid was shown only after 72 h. After 96 h carotenoid concentration in all the body parts came down to the normal value (Fig. 8.2). In viscera the maximum value 4.5023 mg $100g^{-1}$ body wt reached

Table - 8.2. Carotenoid concentration ($\bar{X}$ ± SD, N = 6) in *Perna viridis* after 96 h exposure to Hg and Zn and the relationship.

Metal	Metal conc. ppm.	% Survival	Carotenoid concentration mg 100g Wet wt.		Relationship between metal conc. and carotenoid	
			Total	Unsaponifiable	Total	Unsaponifiable
Hg^{2+}	0	100	0.3055±0.0119	0.1139±0.0189		
	0.05	100	0.3800±0.0176	0.3227±0.0177	* b = 1.3763	b = 0.7018
	0.1	100	0.4430±0.0410	0.3795±0.0362	* r = 0.9583	r = 0.9264
	0.2	100	0.4208±0.0608	0.3648±0.0494	p < .001	p < .001
	0.4	70	1.0651±0.0865	0.6381±0.0501		
	0.6	40	0.9756±0.0466	0.5719±0.1055		
	0.8	30	1.4278±0.2080	0.8043±0.1344		
Zn^{2+}	0	100	0.3968±0.0303	0.2380±0.0182		
	1	100	0.5390±0.1189	0.3182±0.0580		
	2	80	1.4692±0.1118	1.0133±0.1134	b = 0.2673	b = 0.2334
	3	70	1.6539±0.3043	1.1870±0.1573	r = 0.8982	r = 0.9370
	4	50	1.5715±0.2481	1.2223±0.1803	p < .001	p < .001
	5	30	1.5181±0.2574	1.2138±0.2292		
	6	10	2.2043±0.5770	1.7499±0.4351		

* = slope of the straight line

** = correlation co-efficient

Table - 8.3. Total (A) and unsaponifiable (B) carotenoid concentration (mg 100 g^{-1} Wet wt.) in different body parts of *P.viridis* exposed to 0.05 ppm of Hg and Cu (each value obtained from pooled sample of 6 animals)

Metal conc.	Period of Exposure (h)		Gills	Viscera	Mantle	Muscle + Foot
Cu^{2+} – 0.05 ppm	0	A	0.7682	1.1688	0.2864	0.1768
		B	0.4609	0.7013	0.1718	0.1031
	24	A	4.8554	1.1223	0.2532	0.1279
		B	2.5248	0.6734	0.1519	0.0767
	48	A	0.6280	1.3712	0.3578	0.1769
		B	0.3768	0.6287	0.2147	0.1061
	72	A	1.1275	4.5023	0.6701	0.5836
		B	0.6765	2.4312	0.4021	0.3502
	96	A	0.7835	1.0993	0.2928	0.1769
		B	0.4701	0.6596	0.1757	0.1061
Hg^{2+} – 0.05 ppm	0	A	0.7562	1.0443	0.2438	0.1679
		B	0.4537	0.6372	0.1463	0.1007
	24	A	0.7655	2.1999	0.4732	0.2913
		B	0.4516	1.2957	0.2839	0.1748
	48	A	0.3030	1.0743	0.1883	0.1758
		B	0.1757	0.6446	0.1130	0.1071
	72	A	0.9287	0.9683	0.2443	0.2491
		B	0.5470	0.4810	0.1466	0.1495
	96	A	0.9423	1.4590	0.2564	0.4538
		B	0.5274	0.8754	0.1538	0.2723

Table - 8.4. Total and unsaponifiable carotenoid concentration in tissue of *P.viridis* exposed to Hg, Zn and Cu, together with oxygen consumption data.

Metal Concentration	Exposure period	Carotenoid in mg $100g^{-1}$ Wet wt		Oxygen consmption ml g^{-1} wet wt.
		Total	Unsaponifiable	
	Control	0.4694 ± 0.021	0.2648 ± 0.049	0.9880 ± 0.237
	6 h	0.5182 ± 0.043	0.3688 ± 0.022	
	12 h	0.5436 ± 0.092	0.5281 ± 0.096	0.5226 ± 0.179
$HgCl_2$	18 h	0.4504 ± 0.038	0.4280 ± 0.043	
1.0 ppm	24 h	0.6348 ± 0.075	0.6111 ± 0.022	0.4911 ± 0.321
	30 h	1.1894 ± 0.206	0.7261 ± 0.081	
	36 h	1.1122 ± 0.192	0.6998 ± 0.034	0.3638 ± 0.299
	42 h	1.0754 ± 0.457	0.6372 ± 0.012	
	48 h	1.5482 ± 0.231	1.1142 ± 0.211	0.3282 ± 0.386
	Control	0.5786 ± 0.039	0.3472 ± 0.034	*0.6331 ± 0.120
$ZnSO_4$	24 h	1.7394 ± 0.363	0.9306 ± 0.059	*0.3504 ± 0.17
$7H_2O$	48 h	0.8088 ± 0.147	0.5015 ± 0.041	*0.4494 ± 0.10
1.5 ppm	72 h	0.6075 ± 0.072	0.3645 ± 0.022	*0.5710 ± 0.245
	96 h	0.6078 ± 0.086	0.2447 ± 0.016	*0.3856 ± 0.178
$CuSO_4$ $5H_2O$	Control	1.7605 ± 0.296	0.9243 ± 0.087	**0.8832 ± 0.27
	4 days	2.5962 ± 0.344	1.3241 ± 0.255	**0.4284 ± 0.17
0.0075 ppm	20 days	5.0571 ± 0.832	2.4780 ± 0.411	**0.6300 ± 0.27

* Data taken from Chapter - 6

** Data taken from Chapter - 7

after 72 h. The oxygen consumption data taken from Chapter - 6 is plotted with the carotenoid concentration (Fig - 8.2) to show the relationship with the change in carotenoid concentration.

Mussels exposed in 0.05 ppm Hg showed less increase in total carotenoid in comparison with Cu exposed mussels (Table - 8.3). In gill the concentration increased from 0.7562 mg $100g^{-1}$ body wt to 0.9423 mg $100g^{-1}$ body wt only after 96 h exposure. But in viscera, mantle and muscle the concentration increased soon after 24 h exposure. But from 72 to 96 h exposure carotenoid concentration increased in all the body parts in relation with the sharp decrease in oxygen consumption rate (Fig. 8.3). Both in Hg and Cu exposed mussels gills and viscera recorded maximum carotenoid concentration in comparison with other body parts.

ii) In long term Cu exposed mussels: Carotenoid concentration in the body of mussels exposed for 20 days in 7.5 ppb Cu is shown in table - 8.4. The oxygen consumption rate is taken from the Chapter - 7. Total carotenoid concentration showed a steady and sharp increase from 1.7605 to 5.0571 mg $100g^{-1}$ body weight after 20 days exposure. The oxygen consumption rate showed a steady decrease just reverse to the change in carotenoid concentration (Table - 8.4).

In all the above experiments unsaponifiable carotenoid also showed variations exactly similar to that of total carotenoid.

iii) In the whole body and oxygen consumption: The results of the studies are tabulated in the table - 8.4. In the first set of experiment 1.0 ppm Hg effect on oxygen consumption and carotenoid

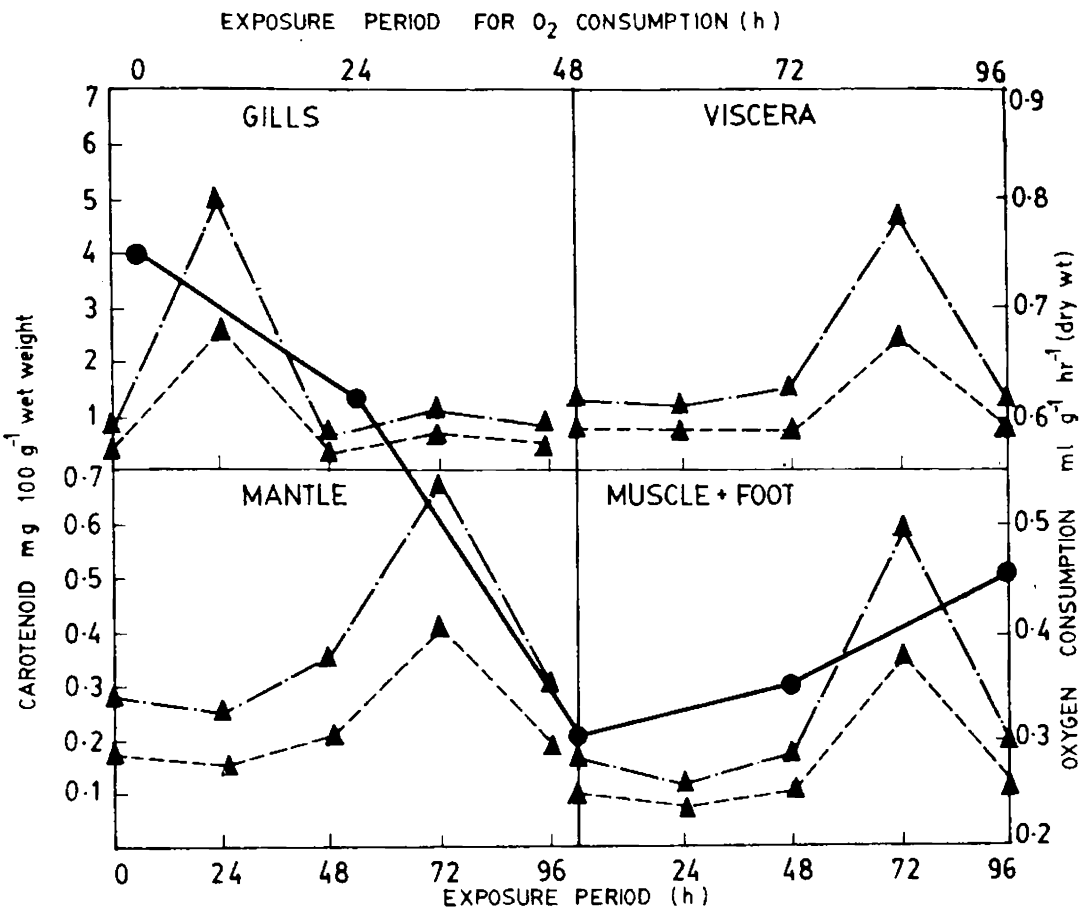


Fig-8.2. Total (▲-▲) and unsaponifiable (▲--▲) carotenoid concentration in different body parts of *Perna viridis* exposed to 0.05 ppm of Cu. O_2 consumption value (●—●) taken from Chapter 6.

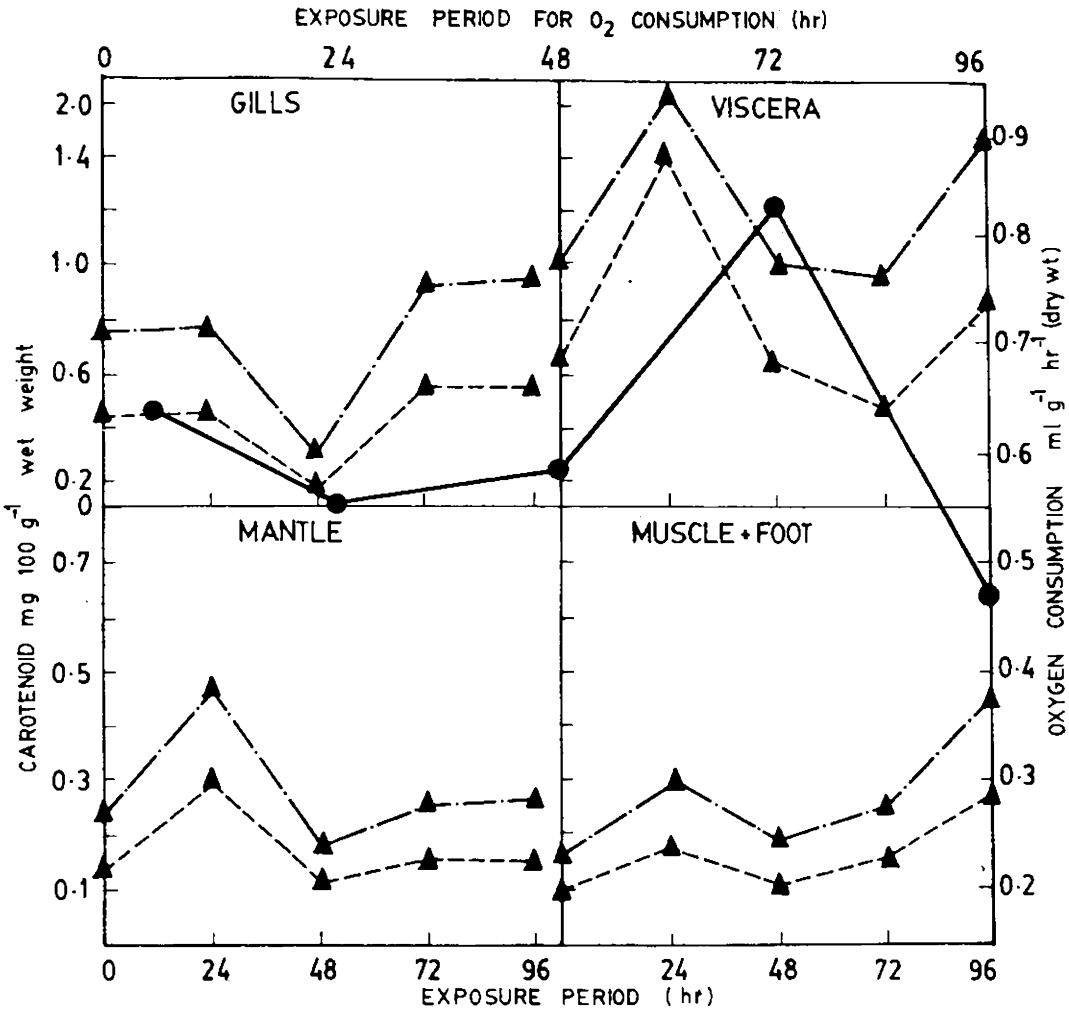


Fig-8.3. Total (▲--▲) and unsaponifiable (▲--▲) carotenoid concentration in different body parts of *Perna viridis* exposed to 0.05 ppm of Hg. O_2 consumption value (●—●) taken from Chapter 6.

concentration were measured every 6 h for 48 h. The total carotenoid concentration sharply increased from 0.4494 mg $100g^{-1}$ body wt to a maximum of 1.5482 mg $100g^{-1}$ body wt after the 48 h exposure. Conversely the oxygen consumption value decreased from 0.98 ml g^{-1} h^{-1} to 0.3282 ml g^{-1} h^{-1} (Table - 8.4). In other words the oxygen consumption rate of the Hg exposed mussels reduced to 1/3 of the control total carotenoid concentration increased 3 times that of the control (Fig. 8.4a).

In mussels exposed to 1.5 ppm of Zn, total carotenoid concentration sharply increased from 0.5786 to 1.7384 mg $100g^{-1}$ after 24 h exposure in the whole body. Then the concentration slowly decreased to 0.6078 mg $100g^{-1}$ body wt. Conversely the oxygen consumption data taken from Chapter - 6 showed a 50% reduction after 24 h exposure. (Fig. 8.4b).

4. DISCUSSION

Animals which survived in acute Hg and Zn concentration were found to be having high carotenoid concentration in their body (Table - 8.3). During their exposure to acute concentration of Cu, Hg and Zn, mussels tightly closed the valves. This type of avoidance mechanism was reported by several investigators in bivalves (See review, Akberali & Trueman, 1985). At the end of this avoidance mechanism animals have to open the valves to release the toxic end products of anaerobic respiration resulted from valve closure. Due to this, soft tissues may come in contact with the heavy metals in the ambient seawater. So physiological and biochemical changes related to metal toxicity and

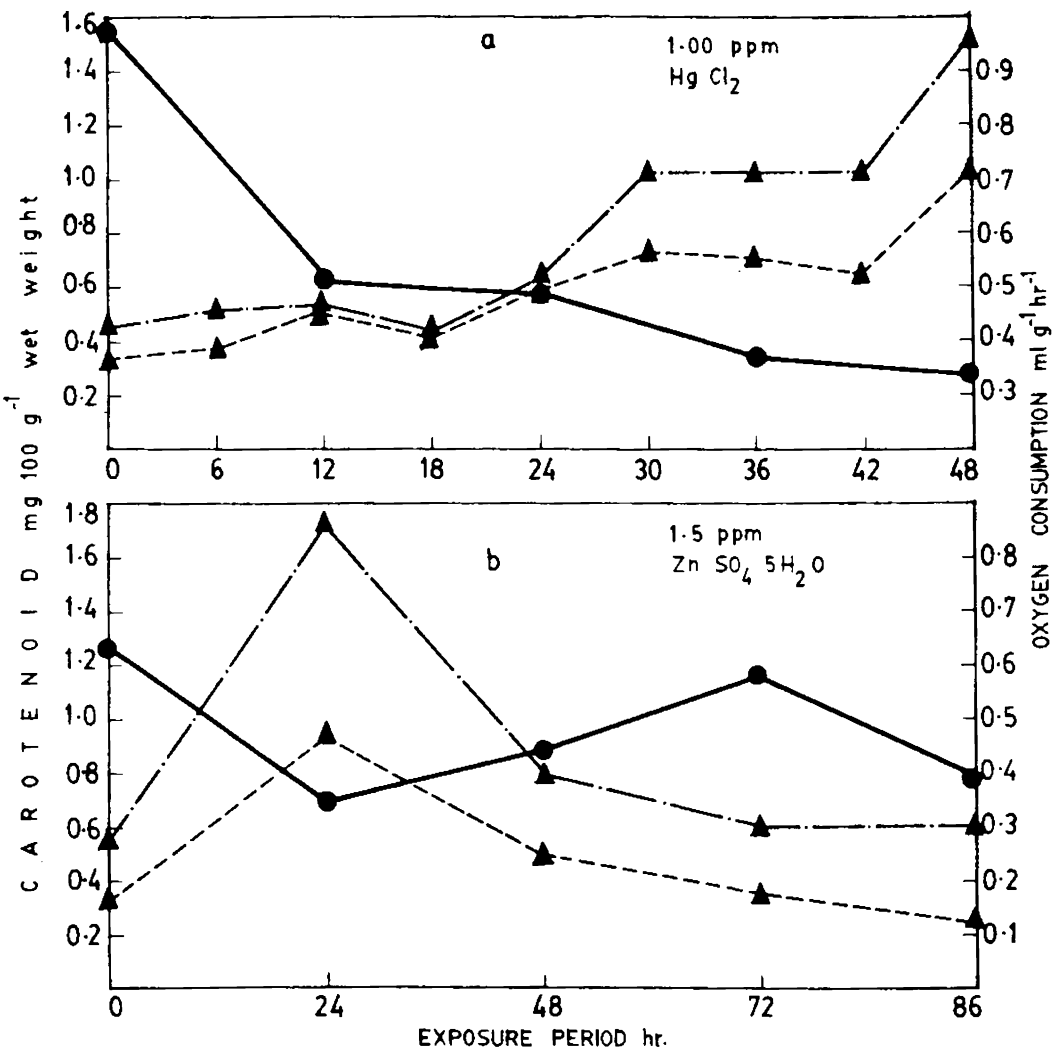


Fig-8.4. Total (▲-▲) and unsaponifiable (▲--▲) carotenoid concentration in the whole body of *Perna viridis* and its O_2 consumption (●—●) exposed to a) 1.00 ppm of Hg and b) 1.5 ppm of Zn.

prolonged valve closure may limit the survival chances of mussels.

Increase in 'carotenoxysome' number (= carotenoid concentration) were reported in molluscs under their adaptation to hypoxic condition or the mitochondrial inhibitor actions of pollutants (Karnaukhov, *et al.*, 1977). Similarly remarkable correlation was reported between the degree of pigmentation of the ganglis and anoxic tolerance in molluscs (Zs-Nagy, 1971a and Schindelmeiser *et al.*, 1979). Karnaukhov *et al.* (1977) observed increase in the population of molluscs having high concentration of carotenoid in the polluted area of Black Sea. At the same time molluscan population with low concentration of carotenoid decreased by pollution. In the present study it is found that animals having high carotenoid concentration in the body survived high toxic concentration of Hg and Zn. This increase in carotenoid concentration was linear with the increase in metal concentration in the ambient medium (Table - 8.2). Mussels which survived 20 days of exposure in 7.5 ppb of Cu also showed very high concentration of carotenoid in comparison with the control. So this study clearly indicates that there is a close correlation between the tolerance of *P.viridis* to heavy metal stress and concentration of carotenoid in their body.

The lethality of heavy metals such as Pb, Cu, Zn and Hg has been ascribed to coagulation of mucus on the gill surface, and damage done to gill tissues leading to respiratory failure (Doudoroff & Katz, 1953). One of the main toxic action of Zn is on the respiratory system by interference with gaseous exchange (Hughes & Flos, 1978; Tort *et*

al., 1982). Skidmore, (1970) reported that some of the effects of Zn toxicity on respiratory parameters were similar to those observed in fishes exposed to anoxic condition. This theory of tissue hypoxia as the main cause of death is supported by several authors (Hughes & Flos, 1978; Viarengo, 1985). Heavy metals were reported as the most potent inhibitor of mitochondrial respiration and oxidative phosphorylation (Zaba & Harris, 1978; Akberali & Earnshaw, 1982; Tort et al., 1984 a & b; Somasundaram *et al.*, 1985; Babu & Rao, 1985; Crespo & Sala, 1986). The glutathione (GSH) reducing system of the cell involved in the maintenance of the ox/redox potential, was found to be inactivated by metals (Viarengo, 1985). This type of effect may reflect in the oxygen consumption rate of metal exposed animals. A steady decrease in respiratory rate of trochid snails were reported under Hg stress (Saliba & Vella, 1977). Cu drastically reduced the endogenous respiration in the digestive gland of snail *Lymnaea luteola* (Babu & Rao, 1985) and resulted in increased peroxidase activity with the subsequent inhibition of cytochrome oxidase and NADH. In the present study also Cu, Hg and Zn were found to be reducing oxygen consumption rate in *P.viridis* with the progress of time (Chapter - 6).

This type of steady decrease in respiratory rate with progress of exposure time may be due to valve closure and toxic action of heavy metals on mitochondria. This may lead to a slow tissue hypoxia within the animal body. When oxygen diffusion in to the tissue is insufficient, withdrawal of oxygen from the saturated carotenoid double bond will take

place (Karnaukhov, 1971; Karnaukhov *et al.*, 1977). This removal of oxygen will be followed by a reconversion of carotenoid to the coloured form with three band characteristic absorption spectra in the visible region as shown in Figs. 8.5 A & B. Such increase in carotenoid concentration in the body of molluscs under the influence of respiratory inhibitors (KCN) and pollutants (mineral oil) were reported by Karnaukhov, (1971) and Karnaukhov *et al.*, (1977).

In the present study mussels exposed in 1.00 ppm Hg showed a marked decrease in oxygen consumption together with an increase in carotenoid concentration as reported by earlier investigators (Karnaukhov *et al.*, 1977). These responses in Hg exposed mussels continued upto 48 h reflecting the acute metal toxic action. These trends in variation of O_2 consumption and carotenoid concentration (Fig. 8.4a) reflect the disability of *P.viridis* to adapt with Hg toxicity. This may be due to the high concentration of Hg used here (1.00 ppm) in comparison with 96 h LC50 and EC50 values reported in Chapter - 3 (0.22 and 0.074 ppm respectively). Mussels exposed in 1.5 ppm of Zn also showed an immediate increase in total carotenoid concentration together with a decrease in respiratory rate within 48 h of exposure. But later carotenoid concentration and oxygen consumption rate remained constant more or less near the control value upto 96 h (Fig. 8.4b). This reflects the ability of mussels to adapt in 1.5 ppm of Zn during short term exposure, since these concentrations is less than its 96 h LC50 (3.9 ppm) value.

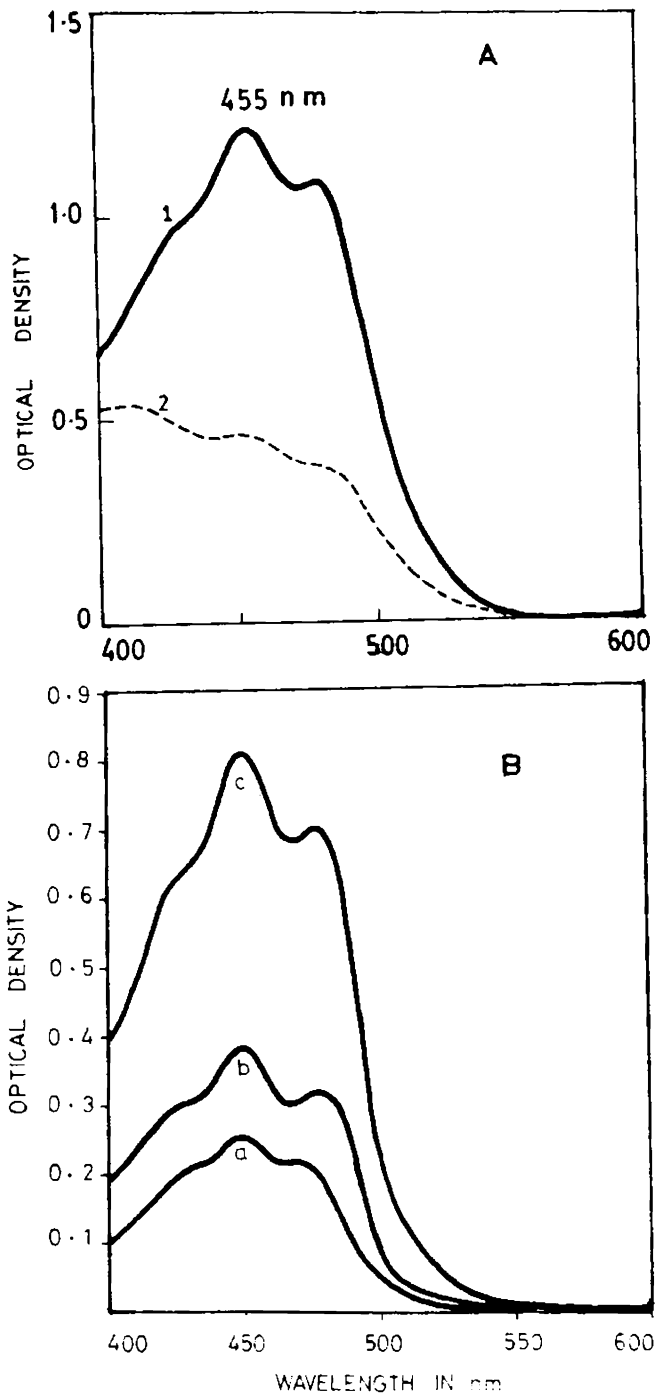


Fig-8.5. The visible light absorption spectra of carotenoid (petroleum ether) from equal amount tissue of *Perna viridis*.
A-1) 20 days exposed in 7.5 ppb of Cu. 2) Control.
B- 96 h exposed to Zn. a) Control. b) 3 ppm. c) 6 ppm.

Carotenoid concentrations in gills and viscera of *P.viridis* were found to be high in comparison with mantle and muscle. Zs-Nagy (1977) reported that cytosomes are generally found in all basic tissues of molluscs and their number may vary from organ to organ. In the present study (0.05 ppm) Cu reduced oxygen consumption rate of the animals. The gills showed an immediate increase in carotenoid concentration while the other body parts showed at the end of the exposure period (Fig. 8.2). These may be due to the easy accessibility of gills for the toxic action of Cu, and rapid metal uptake by it. Hg exposed (0.05 ppm) mussels also showed more or less similar variations in oxygen consumption and carotenoid concentration (Fig. 8.3). In both Cu and Hg exposed mussels, the recorded high carotenoid concentration in gills and viscera (Figs. 8.2 & 8.3) indicates the increase of toxic action by Cu and Hg to those body parts. This is in agreement with the high Cu and Hg content of gills and viscera after 96 h exposure in 0.04 ppm and 0.0375 ppm of Cu and Hg respectively (See Chapter-4). In the present study body parts of Cu exposed mussels recorded high carotenoid concentration in comparison with Hg exposed mussels. This may be taken as an indication that Cu is more toxic than Hg to *P.viridis*. From the LC50 values and oxygen consumption and filtration rate effect studies (Chapters - 3, 6 & 7) Cu was found to be more toxic than Hg, and the present findings also substantiate the result.

The high carotenoid concentration recorded in the whole body of mussels which survived 20 days in 7.5 ppb of Cu reflects the

deterioration in the physiological conditions of the mussels. This is in good agreement with the recorded reduction in oxygen consumption, filtration rate and body condition index of mussels (Chapter - 7).

The duration and degree of valve closure in bivalves were reported to be related to the external metal concentration (Delhaye & Cornet, 1975; Akberali & Trueman, 1985). So valve closure can be considered as a stress or toxic effect rather than as an avoidance mechanism. The anaerobic metabolism during valve closure may limit the utility of some of the physiological and biochemical indices of stress to assess the health of the animals (Bayne, 1975; Viarengo, 1985). Hence the measurement of carotenoid concentration may be used as a useful tool in such studies.

The main inferences of this study can be summarised as follows:

1. The increase in carotenoids concentration with the decrease in oxygen consumption under the heavy metal stress indicates the possible role of carotenoids in the oxidative metabolism of *P. viridis*.

2. Mussels having high carotenoid concentration in their body were found to be able to withstand acute Hg and Zn toxicity.

3. Carotenoid concentration in the whole body and different body parts behaved as an index of stress to Cu, Hg and Zn with progress of exposure time. This is in good agreement with the results of acute toxicity, bioaccumulation and physiological effect studies.

4. Gills and viscera of *P.viridis* recorded high carotenoid

concentration indicating that they are the first and main targets of toxic action of Cu & Hg.

Based upon the above results carotenoid concentration in the tissue of *P.viridis* can be proposed as an useful and general biochemical index of heavy metal stress. In order to understand the specificity of this index and use it in regular biological effect monitoring programmes, more detailed laboratory and field studies are essential.

SUMMARY AND CONCLUSION

Concern over heavy metal pollution has received greater attention as most of the heavy metals have been proved to be harmful to most organisms at some level of exposure and absorption. The capacity of bivalve molluscs to accumulate toxic heavy metals in their tissues, far in excess of environmental level is well known and has become the focus of an increasing number of studies. People concerned with environmental management increasingly recognise the need for monitoring physiological and biochemical effects of these organisms due to heavy metal contamination in natural systems for detecting changes in the environmental quality. Mussels are now receiving considerable attention as a subject of such pollution studies and have been proposed as suitable indicators for monitoring heavy metal pollution in the marine environment both locally and globally as in 'Mussel Watch' programme.

The green mussel *Perna viridis* is ecologically and economically important with outstanding potential as sentinel organism. Three toxic heavy metals namely mercury, copper and zinc were selected for the present study, since they are widely recognised as most common pollutants in the marine environment.

Base line information on some of the physiological indices like oxygen consumption, ammonia excretion, O:N ratio, filtration rate and body condition index were collected. And the effect of body size on these indices were studied. The weight specific oxygen consumption obtained in *P.viridis* was found to be higher than the mean value calculated for temperate bivalves. The low O:N ratio obtained for *P.*

viridis indicates a heavy dependence on protein for its energy production. Oxygen consumption, ammonia excretion, and filtration rate were found to be positively correlated with increase in body weight. But body condition index showed a negative correlation with the increase in body weight.

The acute toxicity of Cu, Hg and Zn were studied by means of static bioassays, and LC50 and EC50 values were calculated and compared using toxicity curves. Based on LC50 values the order of toxicity of the metals is Cu > Hg > Zn. Animals of smaller size group were found to be more sensitive to the above metals. EC50 values were determined based on filtration effect study and the order of toxicity of metals was found to be Hg > Cu > Zn. The comparatively low EC50 value obtained for Hg indicates its probable neurotoxic effect on the filtration rate of the animal. In the present study the threshold concentrations obtained in larger animals for Cu, and Hg were 0.086 and 0.155 ppm respectively.

Bioaccumulation of Cu, Hg and Zn in gill, viscera, mantle and muscle was determined. Highest concentrations of Cu and Hg were found in the gill followed by viscera, while maximum Zn accumulation was observed in viscera followed by gill. Cu and Zn have shown higher rates of accumulation in comparison with Hg in all the body parts. Cu exposed animals showed highest bioconcentration factor followed by Hg and Zn. Cu and Zn were found to be accumulated more at low salinity than at higher salinity. But the reverse was found in the case of Hg. Animals of smaller size group were found to accumulate high concentration of metals

compared to larger size group. Rate of accumulation of Cu, Hg and Zn were found to be higher in smaller animals (15-20 mm) than in larger animals (30-40 mm). This may be due to the high weight specific metabolic rate of smaller animals in comparison with that of larger animals. So while using *P.viridis* as bioindicator of heavy metal pollution monitoring size group of the animal and habitat salinity should be taken into account.

Depuration of the above heavy metals were studied in gill, viscera, mantle and muscle in higher and lower salinities for 25 days. Cu, Hg and Zn loss from the external tissues like gill and mantle were faster than from the internal tissues like viscera and muscle. After 25 days of depuration Cu and Hg in gills showed shorter biological half lives. But in viscera Cu, Hg and Zn showed comparatively longer half lives. Low salinity enhanced the loss of Cu, Hg and Zn from the tissues, in comparison with high salinity. In *P.viridis* based on accumulation and depuration study, metals can be classified as follows:

a) Cu - showed rapid accumulation and release from the tissues.

b) Hg - showed slow accumulation and release.

c) Zn - showed rapid accumulation and slow release.

Effects of Cu, Hg and Zn on oxygen consumption and filtration rate were studied during 96 h exposure. Same concentration of Cu and Hg were used to understand their mode of toxic action on oxygen consumption and filtration rate. Effects of Cu and Zn on oxygen consumption were more severe than the effect of Hg. But the effect of Hg on filtration

rate was more drastic than the effect of Cu. This shows that the mode of toxic action of Hg is different from that of Cu. These observations are in agreement with the results of LC50 and EC50 experiments. Results of this study show that respiratory system in *P.viridis* is the main target of the toxic action of Cu. As reported by earlier workers Hg is neurotoxic in nature and filtration rate is found to be severely affected by its toxic action.

Chronic toxic effects of Cu on the physiology of mussels were studied in a continuous seawater flow system. During 20 days exposure to 7.5 ppb of Cu a general decline in the physiological indices like O_2 consumption, filtration rate and body condition index were observed coinciding with, an increase in body burden of Cu in the tissues of *P.viridis*.

Carotenoid concentration in the tissues of mussels were studied as a general biochemical index of Cu, Hg and Zn stress. Mussels having high carotenoid concentration in their body were found to be able to withstand acute Hg, Zn and Cu toxicity. The increase in carotenoid concentration with decrease in oxygen consumption of the animal indicates the possible role of carotenoids in the oxidative metabolism. Carotenoid concentration in the whole body and different body parts behaved as a general index of stress to Cu, Hg and Zn with progress of exposure time. Gills and viscera of the mussels recorded high carotenoid concentration indicating that they are the first and main targets of the toxic action of Cu and Hg. This study shows the usefulness of the

measurement of carotenoid concentration in the tissues of *P.viridis* as a general biochemical index of Cu, Hg and Zn stress.

The results of the above physiological effect studies using Cu, Hg and Zn show that *P.viridis* is a sensitive and suitable biological indicator of heavy metal stress. So it can be used as a suitable sentinel organism in programmes like "Mussel Watch" for heavy metal pollution monitoring along the coastal waters of India.

REFERENCES

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

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

Ahrland, S. (1975). Metal complexes present in seawater. In : The nature of seawater. Dahlem Konferenzen, Berlin, 219 - 244.

Ajithkumar, B.S. (1984). Reproductive physiology of Indian species of the Genus *Perna* (family) *Mytilidae*. Ph.D.thesis. Cochin University of Science and Technology.

Akberali, H.B. & Trueman, E R. (1979). pO_2 and pCO_2 changes in the mantle cavity of *Scrobicularia plana* (Bivalvia) under normal and stress conditions. Estuarine and coastal Marine Sciences 9, 499 - 507.

Akberali, H.B. & Black, J.E. (1980). Behavioural responses of the bivalve *Scrobicularia plana* to short term copper concentrations. Mar. Environ. Res. 4, 97 - 107.

Akberali, H.B. & Earnshaw M.J. (1982). Studies of the effects of Zinc on the respiration of mitochondria from different tissues in the bivalve mollusc *M. edulis* L. Comp. Biochem. Physiol. 72 C, 149 - 152.

Akberali, H.B. & Earnshaw, M.J. (1985). Interactions of heavy metals at tissue and cellular level in aquatic organisms. Symposia Biologica Hungarica. 29, 343 - 355.

Akberali, H.B. & Trueman, E.K. (1985). Review : Effects of Environmental Stress on marine bivalve molluscs. Advances in Marine Biology. 22, 101 - 198.

Akberali, H.B., Earnshaw, M.J. & Marriott, K.R.M. (1984). The action of heavy metals on the gametes of the marine mussel *M. edulis* (L) - I. Copper induced uncoupling of respiration in unfertilized egg. Comp. Biochem. Physiol. 77 C. 289 - 294.

Akberali, H.B., Earnshaw. M.J. & Marriott, K.R.M. (1985). The action of heavy metals on the Marine Mussel *Mytilus edulis* II. Uptake of Cu and Zn and their effect on Respiration in the Sperm and

unfertilized egg. Mar. Environ. Res. 16, 36 - 60.

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

APHA, AWWA & WPCF.(1981). Standard Methods for the examination of Water and Wastewater. 15th Edition. American Public Health Association, American Water Works Association and Water Pollution Control Federation. Washington, D.C.

Babu, K. & Rao, V. (1985) Effect of copper sulphate on respiration, electron transport and redox potential in the digestive glands of the snail host *Lymnaea lutola*. Bull. Environ. Contam. Toxicol. 34, 396 - 402.

Baby, K.V. & Menon, N.R. (1986). Oxygen uptake in the brown mussel, *Perna indica* (Kuriakose & Nair) under sublethal stress of Hg, Cd & Zn. Indian J. Mar. Sci. 15, 127 - 128.

Badman, D.G. (1975). Filtration of Neutral Red by freshwater clams in aerobic and hypoxic conditions. Comp. Biochem. Physiol. 51A, 741 - 774.

Bayne, B.L. (1975). Aspects of physiological condition in *Mytilus edulis* (L.) with special reference to the effects of oxygen tension and salinity. In : Proceedings of the 9th European marine biology Symposium, (Ed.) H. Barnes, University Press, Aberdeen, 213 - 238.

Bayne, B.L. (1985a). General Introduction, In : The effect of stress and pollution on marine animals (Eds.) Bayne et al., Praeger Special Studies, Praeger Scientific, New York.

Bayne, B.L. (1985b). Cellular and Physiological Measures of Pollution Effect. Mar. Pollut. Bull. 16, 127 - 129.

Bayne, B.L. & Scullard, C. (1977). Rates of nitrogen excretion by species of *Mytilus* (Bivalvia ; Mollusca) J.Mar.Biol. Ass. U.K. 57, 355 - 364.

Bayne, B.L. & Newell, R.C. (1983). Physiological energetics of marine molluscs. In : The Mollusca, Vol. 4, Physiology, Part, I (Eds.)

Saleuddin, A.S.M. & Wilbur, M.K. Academic Press, New York. pp 407 - 515.

Bayne, B.L., Thompson, R.J. & Widdows, J.(1976). Physiology . I. In : Marine mussels : Their ecology and physiology (Ed.) Bayne, B.L. Cambridge University Press, Cambridege, pp. 121 - 206.

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

Bayne, B.L., Brown, D.A., Burns, K., Dixon, D.R., Ivanovici, A., Livingstone, D.R., Lowe, D.M., Moore, M.N., Stebbing, A.R.D. and Widdows, J. (1985). The effects of stress and pollution on marine animals. Praeger Special Studies. Praeger Scientific Publishers.

Blackstock, J. (1984). Biochemical Metabolic Regulatory Responses of Marine Invertebrates to natural environmental change and marine pollution. Oceanography and Marine Biology. An Annual Review . 22, 263 - 314.

Boyden, C.R. (1977). Effects of size upon metal content of shell fish. J. Mar. Biol. Ass. U.K.57, 675 - 714.

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

Bryan, G.W. (1971). The effects of heavy metals (other than mercury) on marine and estuarine organisms. Proc. of the Royal Society, London, Ser. B, 177, 389 - 410.

Bryan, G.W. (1973). The occurrence and seasonal variation of tracemetals in the scallops *Pecten maximus* (L.) and *Chlamys opercularis* (L.) J. Mar. Biol. Ass. U.K. 53, 145 - 166.

Bryan, G.W. (1976). Some aspects of heavy metal tolerance in aquatic oganisms. In :Effects of pollutants on Aquatic organisms. (Ed.) A.P.M. Lockwood, Cambridge University Press, pp. 7 - 34.

Bryan, G.W., (1979). Bioaccumulaton of Marine pollutants. Phil. Trans. R. Soc. Land. B. 286, 483 - 505.

Bryan, G.W., (1984). Pollution due to heavy metals and their compounds. In : Marine Ecology Vol.V Part - 3 Ed. O. Kinne. John Willey & Sons, New York, pp. 1229 - 1431.

Bryan, G.W. & Hummerstone, (1971). Adaptation of the Polychaete *Nereis diversicolor* to estuarine sediments containing high concentrations of heavy metals. 1. General observations on adaptation to copper. J. Mar. Biol. Ass. U.K. 51, 845 - 863.

Bryan, G.W. & Hummerstone, L.G. (1973). Adaptation of the Polychaet *Nereis diversicolor* to estuarine sediments containing high concentrations of Zn and Cd. J. Mar. Biol. Ass. U.K. 53, 839 - 857.

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

The Bureau International Technique du chlor (B.I.T.C.), (1976). Standardisation of methods for the determination of Traces of mercury Part II. Determination of total mercury in materials containing organic matter. Analytica Chimica Acta. 84, 231 - 257.

Capuzzo, J.M. & Sanser, J.J. Jr. (1977). The effect of chromium on filtration rates and metabolic activity of *Mytilus edulis* L. and Mya arenaria L. In : Physiological responses of Marine biota to pollutants. (Eds.) Vernberg, et.al.. Academic Press, INC, Newyork, pp. 225 - 237.

Carpene, E & George, S.G., (1981). Absorption of Cadmium by gills of *Mytilus edulis* (L) Mol. Physiol. 1, 23 - 24.

Chaudhuri, N. (1980). Increasing danger of mercury pollution. Ind. Exp. XLVIII, P. 9.

Cherry, D.S. & Cairns, J. Jr. (1982). Biological monitoring Part V - Preference and Avoidance studies. Water Res. 16, 263.

Chung, K.S. (1980). Acute toxicity of selected heavy metals to mangrove oyster *Crassostrea rhizophorae*. Bull. Jap. Soc. Sci.Fish. 46, 777 - 780.

Cole, H.A., (1979). Summing - up : deficiencies and future needs. Phil.

Trans. R. Soc. Land. B. 286, 625 - 633.

Cole, H.A & Hepper, B.T (1954). Use of neutral red solution for the comparitive study of filtration rates of lamellibranchs. Journal Cons. 20, 197 - 203.

Coleman, N & Trueman, E.R.(1971). The effect of aerial exposure on the activity of the mussels *Mytilus edulis L. and Modiolus modiolus* (L). J. Exp. Mar. Biol. Ecol. 7, 295 - 304.

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

Coombs, T.L & George, S.G. (1978). Mechanisms of immobilization and detoxification of metals in marine organisms. In : Physiology and Behaviour of Marine Organisms (Eds.) McLusky. D.J. and Berry. A.J. Pergamon Press, Oxford and Newyork, pp. 179 - 187.

Cossa, D & Rondean, J.G. (1985). Seasonal, geographical and size induced variability in Hg content of *Mytilus edulis* (L) in an estuarine environment : a reassessment of Hg pollution level in the Estuary and Gulf of St. Lawrence. Mar. Biol. 88, 43 - 49.

Cossa, D., Bourget, E. & Piuze, J. (1979). Sexual maturation as a source of variation in the relationship between Cd concentration and body weight of *Mytilus edulis* (L). Mar. Pollut. Bull. 10, 174 - 176.

Cossa, D., Bourget, E., Pouliot, J., Piuze. J. & Charut. (1980). Geographical and seasonal variations in the relationship between trace metal content and body weight in *M. edulis* (L). Mar. Biol. 58, 7 - 14 .

Cotter, A.J.R. Phillip, D.J.H. & Ahsanullah, M., (1982). The significance of temperature, salinity and Zinc as Lethal factors for the Mussel *Mytilus edulis* (L) in Polluted Estuary. Mar. Biol. 68, 135 - 141.

Crespo, S. & Sala, R. (1986). Chloride cell mitochondria are target organells in acute Zinc contamination. Mar. Pollut. Bull.17, 319- 331.

Cunningham, P.A. & Tripp, M.R. (1973). Accumulation and depuration of Hg in the American oyster *Crassostrea virginica*. Mar. Biol.

20, 14-19.

Cunningham, P.A. & Tripp, M.R. (1975a). Factors affecting the accumulation and removal of mercury from tissues of the American Oyster *Crassostrea virginica*. Mar. Biol. 31, 311 - 319.

Cunningham, P.A. & Tripp, M.R. (1975b). Accumulation tissue distribution and elimination of $^{203}HgCl_2$ and $CH_3{}^{203}HgCl_2$ in the tissues of the American Oyster *Crassostrea virginica*. Mar. Biol. 31, 321-324.

Dasilva, J.J.R.F., (1978). Interactions of the chemical elements with biological systems. In: New trends in bioinorganic chemistry. (Eds.) Williams R.J. & Dasilva, J.J.R.F. Academic Press, London, 449 - 484.

Davenport, J., (1983). A comparison of some aspects of the behaviour and physiology of the Indian mussel *Perna (=Mytilus) viridis* and the common mussel M. edulis (L). Journal of Molluscan studies. 49, 21 - 26.

Davenport, J. & Manley, A., (1978). The detection of heightened seawater copper concentration by the mussel *Mytilus edulis* (L). J. Mar.Biol. Ass. U.K. 58, 843 - 850.

Davies, I.M. and Pirie, J.M. (1980). Evaluation of a 'mussel watch' project for heavy metals in Scottish coastal waters. Mar. Biol. 57, 87 - 93.

Delhaye , W. & Cornet D, (1975). Contribution the study of the effect of copper on *M.edulis* (L) during reproductive period. Comp. Biochem. Physiol. 50A, 511 - 518.

Denton, G.R.W & Burdon - Jones, C., (1981). Influence of temperature and salinity on the uptake, distribution and depuration of Hg, Cd and Pb by the black lip oyster Saccostrea echinata. Mar. Biol. 64, 317 - 326.

Dillon, T.M., (1977). Mercury and the estuarine marsh clam *Rangia cuneata*, Gray. I. Acute Toxicity. Arch. Environm. Contam. Toxicol. 6, 249 - 255.

Dillon, T.M. & Neff, J.M., (1978). Mercury and the estuarine marsh clam,

Rangia cuneata Gray, 11. Uptake, tissue distribution and depuration. Mar. Environ. Res. 1, 67 - 76.

Doudoroff, P. & Katz, M. (1953). Critical review of literature on toxicity of industrial wastes and their components to fish II. The metals as salts. Sewage Ind. Wastes 25, 802 - 839.

D'Silva, C. & Kureishy, T.W. (1978). Experimental studies on the bioaccumulation of Copper and Zinc in the green mussel. Mar. Pollut. Bull. 9, 187 - 190.

D'Silva, C & Qasim, S.Z. (1979). Bioaccumulation and elimination of Cu in the rock oyster *Crassostrea cucullata*, Mar.Biol. 52, 343 - 346.

Earnshaw, M.J., Wilson, S. Akberali, H.B, Butler, R.D., Marriott, K.R.M. (1986). The action of heavy metals on the gametes of the marine mussel, *Mytilus edilis (L) - 111. The effect of applied* copper and zinc on sperm mortality in relation to ultrastructural damage and intracellular metal localisation. Mar. Environ. Res. 20, 261 - 278.

Eganhouse, R.P. & Young, D.R. (1978). In situ uptake of mercury by the intertidal mussel Mytilus californianus. Mar.Pollut.Bull. 9, 214 - 217.

Ekanath, & Menon, N.R. (1983). Mercury toxcity in two intertidal tropical marine molluscs. Fishery Technology. 20, 84 - 89.

F.A.O. (1975). Mannual of methods in aquatic environmental research. Part I. Methods for detection measurement and monitoring of water pollution. FAO, Fish. Tech. Pap. 137, 238 PP.

Fowler, S.W., Heyrand & La Rosa, J. (1978). Factors affecting methyl and inorganic mercury dynamics in mussels and shrimps. Mar. Bio. 46, 267 - 276.

Gelber, R.D., Lavin, P.F., Mehta, C.R., & Schoenfeld, D.A (1985). Statistical Analysis. In : Fundamentals of Aqatic Toxicology (Eds.) Rang, G.M & Petrocelli. S.R Hemisphere Publishing Corporation, Washington. PP. 110 - 123.

George, S.G (1983a). Heavy metals detoxification in the mussel *Mytilus edulis* - Composition of Cd - containing kidney granules

(tertiary lysosome). Comp. Biochem. Physiol. 76C, 53 - 57.

George, S.G., (1983b). Heavy metal detoxification in *Mytilus Kidney - An* in vitro study of Cd and Zn binding to isolate tertiary lysosomes. Camp. Biochem. Physiol. 76C, 59 - 65.

George,S.G., Pirie, B.J.S., Cheyne, A.R., Coombs. T.L. & Grant, P.T. (1978). Detoxification of metals by marine bivalves: an ultrastructural study of the compartmentation of Cu and Zn in the oyster Ostrea edulis. Mar. Biol. 45, 147 - 156.

George,S.G., Coombs, T.L. & Pirie, B.J.S. (1982). Characterization of metal - containing granules from the kidney of the common mussel *M.edulis*. Biochemica Biophysica Acta 716, 61 - 71.

George,S.G., & Viarengo, A (1984). An integration of current knowledge of the uptake, metabolism and intracellular control of heavy metals in mussels. In : Sixth Symposium on pollution and physiology of Marine organisms.Mystic.Conn.Nov. 1983. PP 1-3.

Goldberg, E.D. (1975). The Mussel Watch a first step in global marine pollution monitoring. Environ. Pollution Bull. 6, 111.

Goldberg, E .D., (1976). In : The health of the Oceans UNESCO press. P.172.

Goldberg, E.D., Bowen, V.T., Farrington, J.W., Harvey, G., Martin, J.H., Parker., Risebrough, R.W. Robertson, W., Schneider, E. & Gamble, E. (1978). The mussel watch. Environ. Conserv. 5, 101 - 125.

Hawkins, A.J.S., Bayne, B.L., Menon, N.R. and Damodaran, R. (1986). The mussels *Perna viridis* and *Perna indica* as transplantable indicators of pollution: Comparison of their metabolic response to reduction of both oxygen tension and salinity. In : Proceedings of the National Seminar on Mussel watch. Vol. I Cochin University of Science and Technology. 51 - 64.

Haya, K. and Waiwood, B.A. (1983). Adenylate energy charge and ATPase activity: Potential biochemical indicator of sublethal effects caused by pollutants in aquatic animals. In : Aquatic Toxicology (Ed.) Nriague, O.J. John Wiley & Sons, New York,pp. 308 - 328.

Herricks, E.E. & Carins, J. Jr. (1982). Biological Monitoring. Part III. Receiving System Methodology Based on Community structure. Water Res. 16, 141 - 149.

Hochachka, P. W (1985) Theorems of environmental adaptations. C. M. F. R. I., Special Publication No. 26, Cochin, India. pp. 153.

Hochachka, P.W. and Mustafa, (1972). Invertebrate facultative anaerobiosis. Science 178, 1056 - 1060.

Hubschman, H.J. (1967). Effect of Copper on the Cray fish Orionectes rusticus. II. Mode of toxic action. Crustaceana. 12 (2), 141 - 150.

Hughes, J.M. and Flos, R. (1978). Zinc content of the gill of rainbow trout (*S. gairdneri*) after treatment with zinc solution under normoxic and hypoxic conditions. J. Fish. Biol. 13, 717 - 728.

Hughes, J.M., Champman, H.F. and Kitching, R.L. (1987). Effects of sublethal concentrations of copper and freshwater on behaviour in an estuarine gastropod Polynices sordidus. Mar. Pollut. Bull. 18, 127 - 131.

Hvilson, M.M. (1983). Copper-induced differential mortality in the mussel *Mytilus edulis*. Mar. Biol. 76, 291 - 296.

IMCO/FAO/UNESCO/WMO/WHO/IAEA/UN/UNEP/(1976). Joint group of Experts on the Scientific Aspects of Marine Pollution (GESAMP). Review of Harmful Substances. Rep. and Stud. GESAMP. No. 2 pp. 80.

IMCO/FAO/UNESCO/WMO/WHO/IAEA/UN/UNEP/ (1980). Joint Group of Experts on the Scientific Aspects of Marine Pollution (GESAMP). Monitoring Biological Variables to marine Pollution. Rep. Stud. GESAMP. 12, pp 20.

IMCO/FAO/UNESCO/WMO/WHO/IAEA/UN/UNEP/ (1982). Joint group of Experts on the Scientific Aspects of Marine Pollution (GESAMP). The review of the health of the Oceans. Rep. Stud. GESAMP, 15, pp. 108.

International Copper Research Association (INCRA)(1982). The biological importance of copper in the Sea - A literature Review. In : INCRA Project (No. 223) Final Report. pp 52.

Indian Standards (IS): 7968. (1976). Tolerance limits for industrial

effluents discharged in to marine coastal areas. Indian Standards Institution, pp. 8.

Jones, S. (1968). Proceedings of the Sympsosium on mullusca, Vol.2 (Marine Biological Association of India, Cochin), 906.

Jorgensen, C.B. (1975). On gill function in the mussel, *Mytilus edulis* L. Ophelia.13, 187 - 232.

Karnaukhov, V.N. (1971). Carotenoids in oxidative metabolism of molluscoid neurons. Exp. Cell. Res. 64, 301 - 306.

Karnaukhov, V.N. (1979). The Role of Filtrator Molluscs Rich in Carotenoid in the Self Cleaning of fresh waters. Symp. Biol. Hung, 19, 151 - 167.

Karnaukhov, V.N. and Fedorov. (1977). The role of Carotenoids and Vitamin A in animals adaptation to high altitude. Comp. Biochem. Physiol. 57 A, 333 - 381.

Karnaukhov, V.N. Milovidova, N.Y.and Kargopolova. (1977). On a role of Carotenoids in tolerance of Sea Molluscs to environment pollution. Comp. Biochem. Physiol. 56 A, 189 - 193.

Karrer, P. & Jucker, E. (1950). The isolation of Carotenoids In : Carotenoids. Elsevier Publishing Company, Inc. New York. pp. 20 - 28.

King, D.G & Davies, M. (1987). Laboratory and field studies of the Accumulation of Inorganic Mercury by the Mussel *Mytilus edulis* (L). Mar. Pollut. Bull. 18, 40 - 45.

Kinne, O. (1984). Pollution and protection of the seas. Radioactive heavy metals and oil. In : Marine Ecology Vol. V, Part. 3, (Ed.). O. Kinne. John Willey & Sons, New York. pp. 1091 - 1109.

Kobayashi, H., Yuyama, A., Matsusaka, N., Takeno, K. and Yanajiya, I. (1980). Effect of methyl mercury on brain acetylcholine concentration and turnover in mice. Toxicol. Appl. Pharmacol. 54, 1 - 8.

Krishnakumar, P.K. & Damodaran, R. (1986). Effect of body size on the physiology of green mussel *Perna viridis* (Linnaeus). In : Proceedings of the National Seminar on Mussel Watch Vol. I, Cochin University of Science and Technology, pp. 77 - 85.

Kumaraguru, A.K. & Ramamoorthi, K. (1978). Toxicity of copper to three estuarine bivalves. Mar. Environ. Res. 1, 43 - 48.

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

Lack, T.J. & Johnson, D. (1985). Assessment of the biological effect of sewage sludge at a Licensed site off plymonth. Mar. Pollut. Bull. 16, 147 - 152.

Lakshmanan, P.T. (1982). Investigations on the chemical constitutents and trace metal interactions in some bivalve molluscs of the Cochin back waters. Ph.D thesis, Cochin University of Science and Technology.

Lakshmanan, P.T. & Nambisan, P.N.K. (1977) Toxicity of copper on the bivalve, *Villorita cyprinoides* var. cochinensis. Ind. J. Mar Sci. 6, 83 - 85.

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

Lakshmanan, P.T. & Nambisan, P.N.K. (1983). Seasonal variation in trace metal content in bivalve molluscs *Villorita cyprinoides* var. cochinensis (Hanely), *Meretrix casta* (Chemnitz) and *Perna viridis* (Linnaeus). Indian J. Mar. Sci. 12, 100 - 103.

Lakshmanan, P.T. & Nambisan, P.N.K. (1986). Bioaccumulation and depuration of some trace metals by the mussel, *Perna viridis*. In Proceedings of the National Seminar on mussel watch. Vol. I, Cochin University of Science and Technology. 35 - 46.

Latha, T. (1986). Physiological effect of copper on *Sunetta scripta*, L. Ph.D thesis, Cochin University of Science and Technology.

Lewis, A.G. & Care, W.R. (1982). The Biological Importance of Copper in Oceans and estuarine. Oceanography and Marine Biology. An Annual Review Vol. 20, 471.

Litchfield, J.T. & Wilcoxon, F. (1949). A simplified method of evaluating dose-effect experiments. J. pharmae. exp. Ther. 96, 99 - 113.

Livingstone, D.R. (1985a). Biochemical measurements. In : The effect of

stress and pollution on marine animals. (Eds.) Bayne et al, Praeger Special Studies. Praeger Scientific. pp. 81 - 132.

Livingstone, D.R. (1985b). Responses of the Detoxification/Toxication Enzyme systems of Molluscs to Organic Pollutants and Xenobiotics. Mar. Pollut. Bull. 16, 158 - 164.

Lobel, P.B. (1986). Role of kidney in determining the whole soft tissue Zn concentration of individual mussels (*M.edulis*). Mar. Biol. 92, 355 - 359.

Luoma, S.N. (1977a). The Dynamics of biologically available mercury in a small estuary. Estuarine and Coastal Mar. Sci. 51, 643 - 652.

Luoma, S.N. (1977b). Physiological characteristics of Mercury uptake by two estaurine species. Mar. Biol. 41, 269 - 273.

Mace, A.M. & Ansell, A.D. (1982). Respiration and Nitrogen excretion of *Polinices alderi* (Forbes) and *P. catena* (dacosta) (Gastropoda : Naticidae). J. exp. Mar. Biol. Ecol. 60, 275 - 292.

McInnes, J. & Thurnberg, F. (1973). Effect of metals on the behaviour and oxygen consumption of the mud snail. Mar. Pollut. Bull. 4, 185 - 186.

Mane, U.K. (1975). Study of the rate of water transport of the clam *Katalysia opima* in relation to environmental conditions. Hydrobiologia, 47, 439 - 451.

Martin, J.L.M. (1979). Schema of lethal action of Copper on mussels. Bull. Environ. Contam. toxicol. 21, 808 - 814.

Martin, M. (1985). State Mussel Watch : Toxic Surveillance in California. Mar. Pollut. Bull. 16, 140 - 144.

Martin, M., Ichikawa, G., Goetzl, J., Reges, M.D.L. & Stephenson, M.D. (1984). Relationship between physiological stress and trace toxic substances in the Bay Mussel *Mytilus edulis* from San Fransisco Bay, California. Mar. Environ. Res. 11, 91 - 110.

Martincic, D., Nuernberg, H.W., Stoeppler, M. & Bramica, M. (1984). Bioaccumulation of heavy metals by bivalves from Lim Fjord (North Adriatic Seas). Mar. Biol. 81, 177 - 188.

Mathew, R. & Menon, N.R. (1983). Oxygen consumption in tropical bivalves *Perna viridis* and *Meretrix casta* exposed to heavy metals.

Indian J. Mar. Sci. 12, 57 - 59.

Mathew, R. & Menon, N.R. (1984). Filtration in *Perna viridis* and *Meretrix casta* under heavy metal stress. Mahasagar, 17, 183 - 186.

Matthews, R.A., Buikema Jr. A.L., Cairns, J. & Rodgers J.H.J. (1982). Biological monitoring Part II A - Receiving systems functional relationship and indices. Water Res. 16, 129 - 139.

McIntyre, A.D. (1984). What Happened to Biological Effects monitoring? Mar. Pollut. Bull. 15, 391 - 392.

Merafante, E (1976). Binding of Mercury and Zinc to Cadmium binding protein in liver and kidney of goldfish (*Carassius auratus*). Experientia. 32, 149 - 150.

Miettinen, J.K., Heyrand, M. & Keckes, S. (1972). Mercury as a hydrospheric pollutant. 11. Biological half time of methyl mercury in four Mediterranean species. A fish, a crab and two molluscs. In : Marine Pollution and Sea life (Ed.) Ruivo, M. Fishing News (Books) Ltd., London, pp. 295 - 298.

Mohan, M.V. & Cheriyan, P.V. (1980).Oxygen consumption of the green mussel *Perna viridis* Linnaeus in relation to body weight and declining oxygen tension. Bull. Dept. Mar. Sci. Univ. Cochin. XI, 75 - 84.

Moon, T.W. & Pritchard, A.W. (1970). Metabolic adaptation in vertically separated populations of *Mytilus californianus* Conard. J. Exp. Mar. Biol. Ecol. 5, 35 - 46.

Moore, M.N. (1982). Lysosomes and environmental stress. Mar. Pollut. Bull. 13, 42 - 43.

Moore, M.N. (1985). Cellular Responses to Pollutants. Mar. Pollut. Bull. 16, 134 - 139.

Moore, M.N., Widdows, J., Clearly, J.J., Pipe, R.K., Salkeld, P.N., Donkin, P., Farrar, S.V., Evans, S.V. & Thompson, P.E. (1984). Responses of the mussel *Mytilus edulis* to copper and Phenanthrene : Interactive effects. Marine.Environ. Res. 14, 167 - 183.

Murphy, C.B. & Spiegel, S.J. (1983). Bioaccumulation and toxcity of

heavy metals and related trace elements. J. Wat. Pollut. control. Fed. 55, 816 - 822.

Nair, A., Ansari, Z.A., Harkantra, S.N. & Parulekar, A.H. (1977). Uptake of copper by the mussel *Mytilus viridis* L. Indian J. Mar. Sci. 6, 90 - 91.

Nambisan, P.N.K. & Lakshmanan, P.T. (1986). Toxicities of selected heavy metals to the mussel *P. viridis In : Proceeding of the National Seminar on Mussel Watch*, Vol. I Cochin University of Science and Technology. pp. 132 - 141.

Nambisan, P.N.K. Lakshmanan, P.T. & Saliah, K.Y.M. (1977). On the uptake of Copper (ii) by Meretrix casta (Chemnitz), an indicator species of metal pollution. Curr. Sci, 46, 437 - 440.

Narasimha, K.A. (1983). Fishery and biology of the green mussel *P. viridis* (Linnaeus) C.M.F.R.I., Cochin, India. Bull. 29, 10 - 14.

Nelson, A. & Donkin, P. (1985). Process of Bioaccumulation, the importance of chemical speciation. Mar. Pollut. Bull. 16, 164 - 169.

Nelson, D.A., Calabrese, A. & McInnes, J.R. (1977). Mercury stress on juvenile Bay scallops *Argopecten irradians* under various salinity - Temperature regimes. Mar. Biol. 43, 293 - 297.

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

Olafson, R.W., Sim, R.G. Kearns, A. (1979). Physiological and chemical characterization of invertebrates metallothionein like protein. In : Metallothionein, (Eds.) Kagi, J.H.R., Nordberg. M. Birkhauser verlg, Boston. 197 - 204.

Olson, K. & Harrel, R. (1973). Effect of salinity on acute toxicity of copper, mercury and chromium for *Rangia cuneata (Pelecypoda* matridae). Contr. Mar. Sci. 17, 9 - 13.

Paparo, A. (1972). Innervation of the lateral cilia in the mussel, *Mytilus edulis*, L. Biol. Bull. 143, 592 - 604.

Pentreath, J.R. (1976). The accumulation of inorganic mercury from

seawater by the plaice, *Pleuronectes platessa* L. J. Exp. Mar. Biol. Ecol. 24, 103 - 119.

Phillips, D.J.H. (1976a). The common mussel *Mytilus edulis* as a indicator of pollution by Zn, Cd, Pb, and Cu I. Effects of environmental variables on uptake of metals. Mar. Biol. 38, 59 - 69.

Phillips, D.J.H. (1976 b). Common mytilus *Mytilus edulis* as an indicater of pollution by Zn, Cd, Pb, and Ca II. Relationship of metals in the muscles of those discharged by the industry. Mar. Biol. 38, 71 - 80.

Phillips, D.J.H. (1977a). Effects of Salinity on the net uptake of Zinc by the common mussel Mytilus edulis. Mar. Biol. 41, 79 - 88.

Phillips, D.J.H. (1977b). The use of biological indicator organisms to monitor tracemetal pollution in marine and estuarine environments - A review. Environ. Pollut. 13, 281 - 317.

Phillips, D.J.H. (1980). Quantitative Aquatic Biological indicators. Applied Science Publishers Ltd. London.

Phillips, D.J.H. & Segar, D.A (1986). Use of bio-indicators in monitoring conservative contaminants : Programme Design Imperatives. Mar. Pollut. Bull, 17, 10 - 17.

Pillai, V.K. Ponniah, A.G., Valsala K.K. & Nair, P.V.R. (1986). Heavy metal load in a few bivalves from the coastal waters of South India. In: Proceedings of the National Seminar on Mussel Watch. Vol.I Cochin University of Science and Technology, pp. 93 - 104.

Pirie, B.J.S., George, S.G., Lytton, D.G. & Thomson, J.D. (1984). Metal containing blood cells of oysters : ultrastructure, histochemistry and X-Ray micro analysis. J. Mar. Biol. Ass. U.K. 64. 115 - 123.

Poulson, E., Riisgard, H.U.and Mohlenberg, F. (1982). Accumulation of Cadmium and bioenergetics in the mussel *Mytilus edulis*. Mar. Biol. 68, 25 - 69.

Prabhudeva, K.N. & Menon, N.R. (1985). Rate of filtration by *Perna viridis* pre-exposed to heavy metals. Fishery Technology. 22, 102 - 104.

Prabhudeva, K.N. & Menon. N.R. (1986). Oxygen consumption under copper and Zinc stress in *Perna viridis*. Fishery Technology, 23, 24 - 26.

Qasim, S.Z., Parulekar, A.H., Harkantra, S.N., Ansari, Z.A., & Nair, A. (1977). Aquaculture of green mussel *P. viridis* L : cultivation on ropes from floating rafts. Indian J. Mar. Sci. 6, 15 - 25.

Ram, N.R. & Sathyanesan, A.G. (1985). Mercurial induced brain monoamine oxidase inhibition in the Teleost *Channa punctatus* (Bloch). Bull. Environ. contam. Toxicol. 35, 620 - 626.

Rao, K.V., Krishnakumari, L. & Qasim, S.Z. (1977). Aquaculture of green mussel *Mytilus viridis* L. Spawning, fertilization and Larval development. Indian J. Mar. Sci. 5, 113 - 116.

Renfro, W.C. (1973). Transfer of ^{65}Zn from sediments by marine Polychaete worms. Mar. Biol. 21, 305 - 316.

Riisgard, H.U., Kioerboe, T., Moehlenberg, F., Draback, I. & Madsen, P.P. (1985). Accumulation, elimination and chemical speciation of Hg in the bivalves *Mytilus edulis* and *Macoma balthica*. Mar. Biol. 86, 55 - 62.

Roesijadi, G. (1980). Influence of Copper on the clam *Protothaea staminea*; Effects on gills and occurrence of Copper-binding proteins. Biol. Bull, 158, 233 - 247.

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

Saliba, L.J. & Vella, M.G. (1977). Effects of mercury on the behaviour and Oxygen consumption of *Monodonta articulata*. Mar. Biol. 43, 277 - 282.

Schulz - Baldes, M. (1974). Lead uptake from sea water and food and lead loss in the common mussel *Mytilus edulis*. Mar. Biol.25, 177 - 193.

Schindelmeiser, I., Kuhlmann, D. & Nolte, A (1979). Localization and characterization of hemoproteins in the Central nervous tissue of some gastropods. Comp. Biochem. Physiol. 64 B, 149 - 154.

Scott, D. & Major, C. (1972). The Effect of copper (II) on survival

respiration and heart rate in the common blue mussel, *Mytilus edulis*. Biol. Bull. 143, 679 - 688.

Segar, D.A. & Stamman, E. (1986). Fundamentals of Marine pollution monitoring programme design. Mar. Pollut. Bull. 17, 194 - 200.

Sengupta, R., Singbal, S.Y.S, & Sanzgiri S. (1978). Atomic - absorption Analyses of a few trace metals in Arabian sea waters. Indian J. Mar. Sci. 7, 295 - 299.

Shafee, M.S. (1976). Effect of salinity & Time of exposure to air on the metabolism of green mussel, *Mytilus viridis* L. Indian J. Mar. Sci. 5, 130 - 132.

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

Shirley, T.C. & Stickle, W.B. (1982). Responses of *Leptasterias hexactis* (Echinodermata : Asteroidea) to low salinity II. Nitrogen metabolism, Respiration and Energy budget. Mar. Biol. 69, 155 - 163.

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

Simkiss, K. & Mason, A.Z. (1985). Metal Ions : Metabolic and toxic effects. In : The Mollusca vol-8, part I (Ed.) Wilbur. K.M. Academic press. Inc. Orlando.

Simkiss, K., Tayler, M., & Mason, A.Z. (1982). Metal Detoxification and bioaccumulation in molluscs. Mar. Biol. Letters. 3, 187 - 201.

Simpson, R.D. (1979). Uptake and loss of Zinc and lead by mussels (*Mytilus edulis*) and relationship with body weight and reproductive cycle. Mar. Pollut. Bull. 10, 74 - 78.

Singbal, S.Y.S., Sanzgiri, S. & Sengupta, R. (1978). Total mercury concentration in the Arabian Seawater off Indian coast. Indian J. Mar. Sci. 7, 124 - 126.

Skidmore, J.F. (1970). Respiration and osmoregulation in rainbow trout with gills damaged by zinc sulphate. J. Exp. Biol. 52, 484 - 494.

Smith, A.L., Green, R.H. & Lutz, A. (1975). Uptake of mercury by fresh

water clams (family unionidae). J. Fresh. Wat. Res. Bd. Can. 32, 1296 - 1303.

Snedecor, G.W. & Cochran, W.G. (1968). Statistical Methods.Oxford and IBH publishing Co. New Delhi, 6th edn.

Solorzano, L. (1969). Determination of ammonia in natural water by the phenolhypochlorite method. Limnol. Oceanogr. 14, 799 - 801.

Somasundram, B., King, P.E. & Shackley, S.E. (1984). The effect of Zn on the structure of the trunk muscle of the larvae of *Culpea harengus* L. Comp. Biochem. Physiol. 79C, 311 - 315.

Spotte, H. stepen. (1970). Water management in the closed systems. In : Fish and invertebrate cultures. John wiley and sons. 3 - 59.

Sprague, J.B. (1969). Measurement of pollutant toxicity to fish - 1. Bioassay methods for acute toxicity. Water., Res. 3, 793 - 821.

Sprague, J.B. (1970). Measurement of pollutant toxicity to fish - 11. Utilizing and applying bioassay results. Water. Res. 4, 3 - 32.

Sprague, J.B. (1971). Measurement of pollutant toxicity to fish - 111. sublethal effects and 'safe' concentrations. Water. Res. 5, 245 - 266.

Sprague, J.B. (1985). Factors that modifying toxicity In : Fundamentals of Aquatic toxicology. (Eds.). Rang. G.M. and Petrocelli S. R. Hemisphere Publishing Corporation, Washington. pp. 124 - 163.

Stebbing, A.R.D. (1985). Bioassay. In : The effects of stress and pollution on marine animals (Eds.) - Bayne B.L. et al, Praeger special studies - Praeger scientific. New York. pp. 133 - 140.

Stickle, W.B. & Bayne, B.L. (1982). Effect of temperature and salinity on oxygen consumption and nitrogen exerection in *Thais* (Nucella) *lappillus* (L). J. Exp. Mar. Biol. Ecol. 58, 1 - 17.

Stickle, W.B., Rice, S.d. Moles, A. (1984). Bio-energetics and survival of the marine snail *Thais lima* during long term oil exposure. Mar. Biol. 80, 281 - 287.

Strickland, J.D.H.and Parson, T.R. (1968). A manual of sea water analysis. Bull. Fish. Res. Bd. Can. 167, 11 - 44.

Stromgren, T. (1982). Effect of heavy metals (Zn, Hg, Cu, Cd, Pb, Ni,) on the length growth of *M. edulis*. Mar. Biol. 72, 69 - 78.

Srna, R.F. & Baggaley, A. (1976). Rate of excretion of Ammonia by the Hard clam *Mercenaria mercenaria* and the American oyster *Crassostrea virginica*. Mar. Biol. 36, 251 - 258.

Sunila, I. (1981). Toxicity of copper and cadmium to *Mytilus edulis*.(L) (Bivalvia). Ann. Zool. Fennici. 18, 213 - 223.

Taylor, D. (1979). A review of the lethal and Sub-lethal effects of mercury on aquatic life. Residue Reviews, 72, 1-65.

Topping, G. (1983). Guidelines for the use of biological material in first order pollution Assessment and trend monitoring. Scottish, Fisheries, Research Report No. 28, ISSN 0308 8022 pp. 1-29.

Tort, L., Crespo, S. & Balasch, J. (1982). Oxygen consumption of the Dog fish gill tissue following Zinc treatment. Comp. Biochem. Physiol. 72 C, 145 - 148.

Tort, L., Flos, R. & Balasch, J. (1984a). Dogfish liver and kidney tissue respiration after Zn treatment. Comp. Biochem. Physiol. 77 C, 381 - 384.

Tort, L., Rosell, M. & Flos, R. (1984b). Oxygen consumption of dig fish rectal gland after *in vitro* and *in vivo* zinc treatment. Mar. Pollut. Bull. 15, 253 - 255.

Unlu, M.Y. Heyrand, M & Keches, S. (1972). Mercury as a Hydrospheric Pollutant. 1. Accumulation and Excretion of $HgCl_2$ in *Tapes decussatus*. L. In : Marine Pollution and Sea life. (Ed.) Ruivo. M, Fishing News (Books) Ltd London. pp. 295 - 298.

Vallee, B.L. (1978). Zinc biochemistry and Physiology and their dearrangements. In: New trends in Bioinorganic Chemistry (Eds.) Williams, R.J.P. & Dasilva J.R.R.F. Academic Press, London. pp 11 - 57.

Viarengo, A. (1985). Biochemical Effects of Trace Metals. Mar. Pollut. Bull. 16, 153 - 158.

Viarengo, A., Pertica M, Mancinelli. G., Zanicchi, G. & Orunesu, M. (1980). Rapid induction of copper binding proteins in the gills of metal exposed mussels. Comp. Biochem. Physiol. 67 C, 215 - 218.

Viarengo, A. Palmero, S., Zanicchi G., Capelli R., Vaissiere, R & Orunesu. M. (1985). Role of metallothioneins in Cu and Cd accumulation and elimination in the gill and digestive gland cells of *Mytilus galloprovincialis*. Mar. Environ. Res. 16, 23 - 36.

Ward, G.S. & Parrish P.R. (1982). Manual of Methods in Aquatic Environment Research. Part 6. Toxicity Tests. FAO Fisheries Technical Paper No. 185.

Watling, H.R. (1981). The effects of metals on mollusc filtering rates. Trans. Roy. Soc. S. Afr. 44, 441 - 451.

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

Widdows, J. (1978a). Combined effects of body size, food concentration and season on the physiology of *Mytilus edulis*. J. Mar. Biol. Ass, U.K. 58, 109 - 124.

Widdows, J. (1978b) Physiological indices of stress in *Mytilus edulis*. J. Mar. Biol. Ass. U.K. 58, 125 - 142.

Widdows, J. (1985a). Physiological measurements. In : The effect of stress and Pollution on marine animals. (Ed.) Bayne et al, Praeger Special Studies. Praeger Scientific, New York. pp. 3 - 45.

Widdows, J. (1985b). Physiological Procedures. In : The effect of stress and pollution on marine animals (Eds.) Bayne et al, Praeger Special Studies. Praeger Scientific, New York. pp. 161 - 178.

Widdows, J. (1985c). Physiological responses to Pollution. Mar. Pollut. Bull. 16, 129 - 134.

Widdows, J., Bayne. B.L., Livingstone, D.R., Newell, R.I.E. & Donkin P. (1979). Physiological and biochemical responses of bivalve molluscs to exposure to air. Comp. Biochem. Physiol. 62 A, 301 - 308.

Widdows, J., Phelps, D.K. & Gaallowy, W. (1980). Measurement of Physiological condition of mussels transplanted along a pollution gradient in Narragansett Bay. Mar. Environ. Res. 4,

181 - 194.

Wijsman, T.C.M. (1976). ATP content and energy change in different tissues of *Mytilus edulis* L. under aerobic and anaerobic conditions. J. Comp. Physiol. 107, 129 - 140.

Wilson, T.G., McMahon. R.F. (1981) Effects of high environmental copper concentration on the oxygen consumption, condition and shell morphology of natural populations of *Mytilus edulis* L and *Littorina nidis* Maton. Comp. Biochem. Physiol. 70C, 139 - 147.

Winter, J.E. (1973). The filtration rate of *Mytilus edulis* and its dependence on algal concentration, measured by continuous automatic recording apparatus. Mar. Biol. 22, 317 - 328.

Worrall, C.M., Widdows, J & Lowe D.M. (1983). Physiological ecology of three populations of the bivalve *Scrobicularia plana*. Mar. Ecol. Prog. Ser. 12, 267 - 279.

Zaba, B.N. Harris, E.J. (1976). Uptake and effects of copper in rat liver mitochondria. Biochem. Journal. 160, 707 - 714.

Zaba, B.N. Harris, E.J. (1978). Accumulation and effects of trace metal ions in fish liver mitochondria. Comp. Biochem. Physiol. 61C, 89 - 93.

Zs-Nagy, I (1971a). Pigmentation and energy dependent Sr^{2} accumulation of molluscan neurons under anaerobic conditions. Annal. Biol. Tihany. 38, 117 - 125.

Zs-Nagy, I. (1971b). The lipochrome Pigment of molluscan neurons, a specific electron acceptor. Comp. Biochem. Physiol. 40 A, 595 - 602.

Zs-Nagy, I. (1973). Carbohydrate consumption and ATP production in the tissues of *Anodonta cynea* L. (Mollusca, Pelecypoda) under normal and anoxic conditions. Acta. biochem. biophys. Hung. 8, 143.

Zs-Nagy, I. (1974). Some quantitative aspects of oxygen consumption and anaerobic metabolism of molluscan tissues. A review. Comp. Biochem. Physiol. 49 A, 399 - 405.

Zs-Nagy, I (1977). Cytosomes (Yellow Pigment Granuels) of Molluscs as cell organells of anoxic energy production. Intl. Rev. Cytol.

49, 331 - 377.

Zs-Nagy, I & Kerpel-Fronius, S. (1970). The ultrastructural localization of succinic dehydrogenase activity in the nervous system of *Anodonta cynea* L. (Mollusca, Pelecypoda). Acta Biol. Acad. Sci. Hung. 21, 105 - 113.

Zs-Nagy, I & Ermini, M (1972a). Oxidation of $NADH_2$ by the lipochrome Pigment of the tissue of the bivalve *Mytilus galloprovincialis* (Mollusca, Pelecypoda). Comp. Biochem. Physiol. 43 B, 39 - 46.

Zs-Nagy, I & Ermini, M. (1972b). ATP production in the tissue of the bivalve *Mytilus galloprovincialis* (Pelecypoda) under normal and anoxic conditions. Comp. Biochem. Physiol. 43B, 593 - 600.

Zwaan, A De., (1977). Anaerobic energy metabolism in bivalve molluscs. Oceanogr. Mar. Biol. Ann. Rev. 15, 103 - 187.

Zwaan, A De & Zandee, (1972). The utilization of glycogen and accumulation of some intermediates during anaerobiosis in *Mytilus edulis*. Comp. Biochem. Physiol. 43B, 47 - 54.

Zwaan, A. De & Marrewijk, W.J.A. (1973). Anaerobic glucose degradation in the Sea mussel *Mytilus edulis* L. Comp. Biochem. Physiol. 44 B, 429 - 431.

Zurburg, W, & Kluytmans. J.H. (1980). Organ specific changes in energy metabolism due to anaerobiosis in the Sea Mussel *Mytilus edulis* (L). Comp. Biochem. Physiol. 76B, 317 - 322.