EFFECTS OF SOME HEAVY METALS COPPER, ZINC AND LEAD ON CERTAIN TISSUES OF *LIZA PARSIA* (HAMILTON AND BUCHANAN) IN DIFFERENT ENVIRONMENTS

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August, 1993

DECLARATION

I hereby declare that this Thesis entitled **"Effects of some heavy metals copper, zinc and lead on certain tissues of Liza parsia (Hamilton and Buchanan) in different environments"** is based on my own research and has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar titles or recognition.

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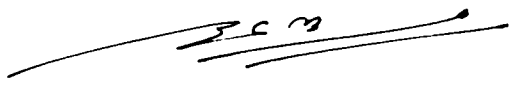

(Bikash Chandra Mohapatra)

CERTIFICATE

This is to certify that the Thesis entitled **"Effects of some heavy metals copper, zinc and lead on certain tissues of** Liza parsia **(Hamilton and Buchanan) in different environments"** is the bonafide record of the work carried out by **Shri. Bikash Chandra Mohapatra** under my guidance and supervision and that no part thereof has been presented for the award of any other Degree or Diploma.

Cochin 682 014

August, 1993

(Dr. K. Rengarajan)
Senior Scientist,
Central Marine Fisheries Research Institute.

DEDICATED TO

MY LOVING PARENTS

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PREFACE

PREFACE

Mother Nature has provided immense natural resources for human beings who worship them as deities. Water is one such natural resource which is a basic requirement for man, without which no life can exist on Earth.

The oceans and seas provide immense amount of both living and nonliving marine wealth. But now, due to scientific, technological and industrial advancements the seas and oceans are regarded as a vast cesspool with an infinite capacity to absorb natural and man-made pollutants. Sufficient reports are available in recent years to understand that there are definite limits to man's abuse of the oceans and in particular many enclosed, semi-enclosed seas and coastal areas. The deleterious effects of pollutants including heavy metals on living resources are much more evident in the estuarine and coastal areas. These ecosystems particularly the estuaries serve as the important feeding and nursery ground and also "passage zone" for a large number of commercially exploitable and cultivable aquatic organisms. We are well aware, knowingly or unknowingly our coastal environments are getting polluted resulting a great concern on our marine wealth. Already many coastal areas have become either unproductive or unharvestable for a variety of finfish, shellfish and other marine living resource due to indiscriminate entry of domestic and industrial pollutants through the dumping of wastes. This needs urgently a thorough knowledge of the pollutants, its entry, source, nature of toxicity on biota, monitoring of the ecosystems, remedial measures and management, legislation

etc. to save our environment . Many countries including India have already promulgated legislations on effluent treatments and pollution control, and established Pollution Control Boards/Agencies by their Government.

The research investigations on pollution, particularly in coastal/ estuarine environments are recent ones and started only in 1970s. Hence the informations available are fragmentary and scattered. They throw some light only on either the concentration of heavy metals in water or in sediment or in organisms. No concerted efforts have been made to consolidate and correlate the results between the environment and biota. Literature on the level of concentration of heavy metals in different tissues of organisms with regard to their availability in the living media, their ratio, their inter-relationship, tolerance limit of organisms, etc. are very few or rather nil.

In view of the importance enumerated above, the candidate has selected the topic "Effects of some heavy metals copper, zinc and lead on certain tissues of Liza parsia (Hamilton and Buchanan) in different environments" for detailed studies and to understand systematically (i) the source of effluents and wastes, (ii) the concentration of heavy metals copper, zinc and lead in water, in sediments and in tissues of the test animal, (iii) their effects, (iv) capacity of tolerance and accumulation in different tissues of the animal, and (v) the "Bioaccumulation Factor", etc.

Importance of the area selected

The estuarine and coastal environments are very important ecosystems as stated earlier, for coastal fishing activities and culture of finfishes and shellfishes As most of the oil refineries, chemical industries, thermal power plants and a large number of small scale industries of different nature are located in the vicinity of estuaries and on the coastal belt discharging their wastes and effluents, these have been selected for the present study.

The heavy metals are (i) normal constituents of living matter, (ii) essential for many metabolic processes at low concentrations, and (iii) regarded as harmful when available in excess either in the environment or in the body of the organisms.

As copper is a toxic heavy metal in aquatic medium (Dehadrai, 1990), lead is a non-essential element to organisms (Qasim et al., 1988) and zinc is essential for biological functions (Walidchuk, 1974), these heavy metals were selected for detailed studies for their effects in the environment and in different tissues of the test animal.

Pollutants in water

The wastes and effluents with toxicants particularly the heavy metals content of water body was analysed in the study along with water quality such as salinity and total hardness.

Pollutants in sediments

On dilution in water, certain amount of heavy metals sink to the bottom and settles along with sediment, which was also felt essential and studied as an important parameter.

Test animal

The fish Liza parsia, a detritus feeder on bottom sediments and brouser on algal and other matters just above the bottom in the water column, was selected as test animal to study the heavy metals in different tissues in its body.

Different centres

To have an overall and comparative informations of heavy metals and their effect on L. parsia, from the west coast of India - two centres (viz. Korapuzha Estuary and Cochin Backwater) and from the east coast of India - four centres (viz. Tuticorin Bay, Mandapam water, Ennore Creek and Rusikulya Estuary) have been selected and studied for a period of two years having the samples regularly collected during different monsoon periods for their seasonal fluctuations.

Monitoring

For comparison and better understanding of these heavy metals in water, in sediment and in tissues of L. parsia, monthly collections were made at Cochin Backwater continuously for a period of one year from June 1991 to May 1992 for monthly fluctuations. It was also aimed

to know whether the results from this monthly monitoring study, support, corroborate and substantiate the finding from the other 5 centres studied in Chapter 1.

Laboratory experiments

In addition to the findings and observations from the environments the candidate conducted laboratory experiments leaving L. parsia to the media with different concentrations of copper, zinc and lead for "bioassay" and followed by "long-term" chronic exposure studies for bioaccumulation of different heavy metals by L. parsia and "Bioaccumulation Factor" in different organs/tissues. This study was aimed at to assess:

i. the 96 hr LC50s of heavy metals copper, zinc and lead, and their combination to L. parsia,

ii. the "Safe-level" of heavy metals in the environments,

iii. the "Toxic unit" for understanding the synergistic effects of heavy metals on L. parsia ,

iv. the specific tissue for accumulation of a specific metal,

v. the relationship between bioaccumulation and "Bioaccumulation Factor", and

vi. the relationship between the findings from the field study and laboratory experiments.

It is very clear from the above, that the research programmes for the present study have been well planned at the fields and also in the laboratory.

Samples of water, sediments and L. parsia were collected from different centres for analysis of water quality, heavy metal concentration and other parameters, at the laboratory. The latest available laboratory techniques, field techniques, methodologies, analytical procedures and statistical methods/formulae were applied.

The data collected have been systematically arranged in Tables, statistically analysed, results presented, discussed elaborately, significant findings and conclusions drawn, and presented in the following three chapters preceded by an "Introduction", "Review of Literature" and "Materials and Methods". The Chapters are:

Chapter 1: Heavy metals copper, zinc and lead in water, sediment and different tissues of Liza parsia and their interrelationships with environmental conditions.

Chapter 2: Monthly and seasonal variation in copper, zinc and lead in water, sediment, and different tissues of Liza parsia in Cochin Backwater.

Chapter 3: "Bioassay" and 'chronic exposure ' studies on Liza parsia with heavy metals copper, zinc and lead.

Some of the salient and significant results from the studies in different chapters are listed below:

Chapter 1

1. The copper, zinc and lead in water of all centres were found below the Environmental Protection Agency's (EPA) safe limits.

2. The Cochin Backwater and Ennore Creek sediments showed higher concentrations of copper and zinc; and the former centre for lead also, in comparison with that of the available "standards".

3. Significant and good relationship was estabilished between zinc in water and sediment.

4. In L. parsia, liver, ovary and gills recorded highest content of copper, zinc and lead respectively. The muscle tissue with the lowest level of all the three metals in the present study, is in agreement with the findings elsewhere.

5. The contents of copper and zinc were seen higher than the "Standard Reference Material (SRM) of IAEA/Monaco" in all the seven tissues.

6. The correlation of "Bioaccumulation Factor" (KB) with the content of metal in tissues, showed in most of the cases significant and good relations between them. In all the cases the relationship was found positive.

Chapter 2

7. Significant monthly variations in heavy metals copper, zinc and lead in water were seen in Cochin Backwater.

8. Monthly variations of lead (only) in sediment was seen at Cochin Backwater.

9. The seasonal variation of heavy metals copper, zinc and lead in environment and tissues of L. parsia; their interrelationships have given interesting and important findings.

Chapter 3

The significant results in Chapter 3 includes the three aspects viz. "Bioassay", "Toxic unit" and "Chronic exposures".

10. Based on 96 hr LC50, zinc was found toxic than copper, lead and its combinations.

11. The reported toxic unit is greater than the "unit" showing the synergistic effect of studied heavy metals on L. parsia.

12. As discussed in Chapter 1 and 2, the accumulation of heavy metals in different tissues was found almost similar in chronic exposures.

13. The relationship was found negative between the "Bioaccumulation" and "Bioaccumulation Factor" for a particular tissue and metal.

14. The results indicated the accumulation of a metal is not proportional to the increase of metal in the medium, even we apply in higher dose.

15. The better correlated, significant relationships from the laboratory experiment for "KB" and accumulation were superimposed to that from field observations to get "composite graphs" in a computer. This type of graphs were obtained for copper in kidney, zinc in liver, and lead in gills and kidney. These graphs elucidated in the Thesis, are self explanatory to give the idea to what level a particular metal will be accumulated in a particular tissue.

The results, significant findings and discussions are briefly summarised at the end in "Summary". This is followed by a list of literature consulted and referred in the Thesis, at the end under "References".

The study carried out by the candidate for the first time from this part of the country, is a regular, continuous, detailed and systematic investigation on the concentrations of copper, zinc and lead in water, in sediments and in different tissues of Liza parsia from six different environments as well as in thelaboratory, and their interrelationships between each other.

It is hoped that the results obtained, the significant findings and the conclusions drawn in this study, would definitely and appreciably enhance our knowledge on the subject.

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ACKNOWLEDGEMENTS

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INTRODUCTION

INTRODUCTION

The seas and oceans with their three dimensional water body, occupy roughly 71% of the earth's surface and they are more populous than land. We not only exploit the resources - both living and nonliving from them, but also pollute the environment very particularly the estuaries and coastal waters. The world's largest and natural "septic tank" is our seas receiving all the wastes from the land. How long it will tolerate this dumping of our wastes! Estuarine and coastal waters are the sites of immediate attack from industrial effluents which are harmful to the biota. The effluents have invariably heavy amount of toxic substances in the form of heavy metals. Hence, it is a paramount duty to save the estuaries and inshore waters by (i) studying and understanding the toxic substances particularly heavy metals, (ii) their concentration in water and sediment, (iii) their effects on biota, and (iv) ultimately the impact on human being who depend on marine wealth.

Metals

The term "metal" designates an element which is a good conductor of electricity and whose electric resistance is directly proportional to the absolute temperature (Wittmann, 1979). In addition to this distinctive characteristic, metals share several other typical physical properties such as high thermal conductivity, high density, malleability and ductility - the ability to be drawn into sheets and wires. Several non-metallic

elements exhibit one or more of these properties, so that the only feature that defines a metal unambiguously is the electric conductivity which decreases with increasing temperature. Within a given period the properties of the elements vary gradually from a high electropositive (metallic) character at the left-hand side of the series to a highly electronegative (non-metallic) character at the end of the series. The "metalloids (or halfmetals)" such as boron, silicon, germanium, arsenic and tellurium situated in the Periodic Table, between metals and non-metals. The "trace metals" for practical purposes used synonymous to the "heavy metals", "trace inorganics", "microelements" and "micronutrients" and its abundance in the lithosphere is less than 0.1% (Wittmann, 1979). According to Qasim et al. (1988) the manganese, copper, iron and zinc are considered as "essential micronutrients"; mercury, cadmium and lead are not required for any important biological function by organisms and are deemed as "non-essential elements".

Viewed from the standpoint of environmental pollution, metals are classified into three categories: (1) noncritical, (2) toxic, but very insoluble or very rare and (3) very toxic and relatively accessible (Wood, 1974). Copper, zinc and lead come under the third category of pollutants (Nammalwar, 1983). According to Piotrowski and Coleman (1980) and Moore and Ramamoorthy (1984) lead, copper and zinc are high, low medium and low toxic to human beings; medium, high and low toxic to aquatic invertebrates, and medium, high and medium toxic to fishes respectively.

The zinc and copper are extremely toxic in marine and freshwater environments (Phillips, 1980), and lead is hepat-and nephrotoxic to fish (Salmeron-Flroes et al., 1990).

With the early use of metals, there was little concern about environmental contamination. The oxides of the metals from corrosion products were hardly sufficient to be a cause for alarm. However, salts of the metals began to find their way into agricultural, commercial and industrial applications. Then it became evident that metallic salts possess certain biocidal properties.

The current alarm of metal pollution in the sea, however, started with the tragedy of "Minimata" and later "Niigata" in Japan. These tragedies resulted in an awareness of the problem of bioaccumulation of mercury by aquatic organisms and generated research in the examination of the levels of metals in aquatic organisms and other foods for human. It is very clear from a scrutiny of literature that a great deal of research still has to be done on the effects of metals on aquatic organisms. National and international legislation is being formulated on the control of all substances entering our waters from an advanced technological society (Waldichuk, 1974). Mercury and cadmium compounds have been banned from dumping into the sea, except in trace concentrations since 1972 (Anon., 1972). Other metals such as zinc, lead and copper require strict control (Waldichuk, 1974). Regulations for the control of pollution by

these substances must be based on a certain degree of scientific knowledge concerning their effects to marine organism.

Heavy metals

. The term "heavy metal" is widely used in scientific literature with reference to several elements beginning with beryllium and going upto actinides (Nair, 1984). The heavy metals are normally regards as the ones having an atomic number of 22 to 92 in all groups from period 3 to 7 in the Periodic Table (Waldichuk, 1974). The Monitoring and Assessment Research Centre (MARC) at Chelsea College, London broadly defines the term "heavy metals" as metals of atomic weight higher than that of sodium and having a specific gravity of more than 5.0. This definition includes over 70 metallic elements, although only a few of these are recognised as potentially damaging (Piotrowski and Coleman, 1980). The term "trace metal" may describe a metal found in trace amounts in an organism (e.g. less than 0.01% of the mass of the organism (Wittmann, 1979)) or may have a further restriction and apply only to those metals required in metabolism (Rainbow, 1988).

Toxic metals

Although at natural concentrations, trace elements either constitute the prosthetic group of enzymes or function as enzyme activators, at elevated concentrations they act as inactivators of enzyme systems and as protein precipitants (Nair, 1984). Metals in their pure state present

little hazard, except those having a high vapour pressure such as mercury and those which may be present in the particulate form in the atmosphere such as vanadium. It is the soluble compounds of the metals which create the problems in the aquatic environments. The different oxidation states of metals, determine certain degree or toxicity in aquatic organisms. The power of the elements to attract and accept electrons in compound formation is called "electronegativity", which has definitely some bearing on its ecological effects, with respect to toxicity to aquatic organisms as there are more electronegativity resulting to more toxicity (Waldichuk, 1974; Wittmann, 1979). This is extensively dealt in detail in Discussion on Chapter III: Acute Toxicity Studies (Bioassay). The more electronegative a metal, the more toxic it is (Nair, 1984). The electronegativity values also known as "Pauling" of the elements such as copper, zinc and lead are 1,8; 1,6 and 1,8 respectively and the "Paulings" method is generally used to estimate electronegativities based on bond energy data (Wittmann, 1979). In a Periodic Table Cu, Zn, and Pb bear atomic numbers 29,30 and 82 and atomic weights 64, 65 and 207 respectively.

The priority list of pollutants complied by the Environmental Protection Agency (EPA) of the United States gives the eight most widespread heavy metals - arsenic, cadmium, chromium, copper, lead, mercury, nickel and zinc (Moore and Ramamoorthy, 1984). The toxicity of different salts of the same metal differs: e.g. nitrates of copper, zinc, cadmium and nickel are more toxic than their sulphates(Metelev et al., 1983).

Speciation of copper, zinc and lead in sea water

The term speciation refers to the particular physical and chemical forms in which an element occurs (Zirino and Yamamoto, 1972; Wittmann, 1979). At the average pH 8.1 the predominant chemical species of copper are $Cu(OH)_2^o$ (90%) and $CuCO_3^o$ (80%); the fractions of the uncomplexed copper ion Cu^{2+} and the ion pair $CuOH^+$ are about 1%. The distribution is mainly dominated by $Cu(OH)_2^o$, $CuCO_3^o$ and Cu^{2+} at low pH values and $Cu(OH)_2^o$ above pH 7.2.

Zinc undergoes less complexation than the other metals. At the average pH 8.1, 17% remains uncomplexed, 62% $Zn(OH)_2^o$, 6.4% $ZnCl^+$ and 5.8% $ZnCO_3^o$. The ion pairs $ZnSO_4$ and $ZnCl_2^o$ are both about 4%. At higher pH nearly all of the total zinc is in the form of $Zn(OH)_2^o$.

Between pH 7 and 9 lead is mainly combined with CO_3^{2-} and to a lesser extent with Cl^-. At pH values near 7 $PbCO_3^o$ and $PbCl^+$ are present in nearly equal amount and there is an appreciable amount of $PbCl_2^o$. However, as pH increases, $PbCO_3^o$ becomes the predominant species.

In sea water, therefore, the uncomplexed heavy metal ion may not be the predominant chemical form (Bruland, 1983), yet there is considerable evidence that this form of heavy metal is the most biologically available (Canterford and Canterford, 1980; Zamuda and Sunda, 1982; Rainbow, 1985). Any physico-chemical change that reduces the hydrophilic complexation of the metal, enhances its bioavailability.

The sources of metal pollution

In general, it is possible to distinguish five different sources from which metal pollution of the environment originates: (1) weathering of rocks (2) industrial processing of ores and metals, (3) the use of metals and metal compounds for example from pharmaceuticals, (4) leaching of metals from garbage and solid waste dumps, sewerage, slaughter house discharge and meat processing centres, and (5) animal and human discharges which contain heavy metals - mainly from "point" areas. Upon attempting to locate the source of metal input of receiving water bodies, a distinction is often made between diffused "nonpoint" and "point" sources. Essentially, rural areas are regarded as "nonpoint" sources, since the metal supply originates from vast areas (Wittmann, 1979). Johnston (1976) has classified three broad categories of marine pollutants : (i) native or natural which are not caused by man, (ii) generated by man, but not created by him and (iii) the synthetic pollutants wholly created by man. Generally heavy metals are broadly put in the first category.

The natural sources of metals in coastal waters are through river run-off. The mechanical and chemical weathering of rocks serve as another major source. In addition, metals washed from the atmosphere through rain fall, wind blown dust and volcanic lava also add to this. All industrial processes involving water are potential sources of metallic contamination in water bodies. The state of marine pollution in the seas around India is summarized by Qasim et al. (1988). According to them the coastal water receives 4.1 Km^3 of domestic sewage and 0.41 Km^3 of industrial

wastes per year. The annual river run-off to the sea is 1645 Km^3 and the sewage and effluents added by the rivers to the sea per is 50 x $10^6 m^3/yr$. The solid waste and garbage generated by coastal population per year is 33 x 10^6 tonnes.

Copper

The major sources of copper to the coastal environment are industrial cooling water discharge, corrosion of pipelines, municipal drainage/ sewerage, combustion of coal, excavation and dumping of ores from mines, silt and sediment dredged from habours, antifouling paints in harbour and vessels (Ramachandran et al., 1991); pulp and paper board mills, fertilizers, petroleum refining, steel works foundaries (Hughes, 1985); fly-ash (Crecelius, 1985) and copper fungicides (Dehadrai, 1990).

Zinc

The sources of zinc to the environment are plating and galvanizing (machine tools and metal products), fly-ash (Smith and Anderson, 1981); dredging and dumping of sediments from harbours (Ramachandran et al., 1991); particulate metals from the atmosphere (Salomons, 1989); pulp and paper board mills, petrochemicals, organic chemicals, alkalies, inorganic chemicals, fertilizers, steel works foundaries and petroleum refining (Hughes, 1985).

Lead

A Swedish study (Taylor, 1982) concludes that most of the lead, cadmium and nickel deposited to the atmosphere is a direct result of

human activities. Coal and oil combustion contributes vanadium and nickel, and the use of leaded petrol in the form of tetra-ethyl lead, has greatly increased the lead content of topsoil in industrial areas. The exhausts of motor vehicles using petrol added with tetra-ethyl lead has been added potential source of air pollution. Most of the lead discharged in atmosphere eventually reaches the lakes, rivers and coastal zones of the sea (Chandra, 1980). The major sources of lead to the water body are pulp and paper board mills, oil refineries, inorganic and organic chemical industries, fertilizer plants, steel industries (Hughes, 1985); fly-ash (Smith and Anderson, 1981; Crecelius, 1985); dredging and dumping of sediments from harbours (Ramachandran et al., 1991); paints, battery plates, lead oxide from defence explosives industry (Ghosh, 1977) and ship breaking industry (Nosal and Wilhelm, 1990). Although, world lead production has remained fairly stable since 1970, there have been significant change in the relative importance of different uses of lead and amount of recycling, and these changes undoubtedly affected the rate of lead emissions to the environment (Laws, 1981).

Process in estuaries and coastal waters

The estuaries, although not acting as major sinks, act as active geochemical areas through which the heavy metals find their way to the environment. In general, concentrations of metals are high in both soluble and particulate fractions of polluted freshwater out-falls (Phillips, 1977). As this freshwater mixes with sea water at the estuary, metals may be lost from the soluble fraction to the sediments by precipitation or to

the phytoplankton by adsorption. By this it is meant the transfer of heavy metals from the particulate to the soluble state and vise versa (Salomons, 1989). The net result is the exchange of metals in soluble form from freshwater to saltwater in particulate form mostly as inorganic nutrients further to mostly organic products as phytoplankton in sea water (Phillips, 1980). If in an estuary a simple mixing of marine and freshwater derived heavy metals would take place, then the relationship between metal concentrations and salinity would be a straight-line. The mixing ratio of river and seawater determines the concentration. The curves showing the relationship between salinity and dissolved metal concentration, are not straight-lines, but show negative deviation in the Rhine Estuary, Netherlands (Duinker and Nolting, 1977) indicating the decrease in metal with increase of salinity. In the Scheldt Estuary, Netherlands the relationship is reverse; a release from the particulate to the dissolved state (Salomons and Forstner, 1984). These differences in geochemical processes are also reflected in metal uptake by organisms. Field observations have shown that the removal of a substantial proportion of the riverine influx of dissolved trace metals is a consistent feature of the very low salinity, high turbidity zone of the Tamar Estuary, Southwest England (Morris, 1986). Comparison of field data with the predictions of a simple sorptive equilibrium model indicates that the removal occurs through rapid uptake onto suspended particles comprising the estuarine turbidily maximum (Morris, 1986 ; Morris et al., 1987). Copper is highly complex by carbonate any hydroxide ions in natural water and this complexity determines the concentration of copper species in solution (Pagenkopf et al., 1974).

Wolfe and Rice (1972) have given a clear picture of cycling of elements in estuaries. The loss of dissolved copper, mercury and lead in association with surface active organic matter in coastal sea water is described by Wallace Jr. (1982). In coastal lagoons, embayments and other sheltered areas where water movement is restricted, pollutants rapidly build up in concentrations and the effects of pollution are more pronounced (Prakash, 1981). A study conducted in North Sea showed the removal of heavy metals taken place through settling and by incorporation of dissolved metals in biological tissues and adsorption on particulate matter (Salomons, 1989).

The processes affecting heavy metals can be classified with a three box model comprising of the surface layers, the remained of the water column and the sediments. The sediment box is devided in two parts. The upper part is the active sediment: e.g. the oxidised layer in which organisms are living and is in active exchange with the water column. The deeper, anoxic part does not play an active role in sediment water exchange processes. In the surface layer dissolved metals are removed by uptake in biological tissues (algae) and by adsorption on particulate matter. The water column beneath the surface layer is subjected to a continuous passing through particulate matter. Part of the biogenic material decomposes in this layer which results the release of dissolved metals to the water column. These decomposition and release processes into the water column continue in the sediment surface. In addition the environmental conditions such as pH, complexing agents cause a remobilisation of heavy metals from the particulate matter (Salomons,

1989). The highly mineralized water containing calcium, potassium, sodium, magnesium and barium salts decreases the solubility of toxic substances, forming insoluble sediments with them and hence reducing their toxicity many times over (Metelev et al., 1983). Phillips, (1977) included stratification of waters, tides and currents and the intermittent flow of industrial effluent as additional factors which may elicit changes of trace metal levels in estuarine or coastal waters. Various authors have discussed the seasonal variation of dissolved metals in inshore waters (Atkins, 1953; Knauer and Martin, 1973; Morris, 1974).

Effect of heavy metals on aquatic life

The implact of pollutants on marine environment is more acute and its deleterious effects on living resources are much more evident in coastal and estuarine areas than the open ocean. Besides being the most fertile part of the marine ecosystem and important feeding, nursing and passage zones for a large number of commercially exploitable aquatic species, coastal and estuarine regions are chief recipients of man-made and natural pollutants. Already many coastal areas have become either unproductive or unharvestable of a variety of fish, shellfish and other marine renewable resources due to indiscriminate entry of domestic and industrial pollutants through their dumping of wastes.

The effect of the pollutants containing heavy metals has been reported in different aquatic lives by different authors. Bryan (1971) has shown the inhibition of growth of Laminaria digitata by zinc, lead and copper. In the case of zooplankton, Qasim et al. (1988) have noted

the high concentration of all metals except mercury. Benthic macrofauna such as polychaete worms, crustaceans bivalves and echinoderms occurring in the sediments rich in heavy metals showed varying degree of accumulation of the metals in their tissues, resulting in high contents even in habitats with low metal concentrations (Everaarts et al., 1989). If the level exceeds the limit, it acts toxic to the animal.

Some heavy metals have high affinities for ligands containing sulphur and nitrogen and hence are bound easily to organic molecules such as proteins (Nieboer and Richardson, 1980). "They, therefore have the capacity to be incorporated into biological molecules to play roles in metabolism and thus be selected in evolution as essential metals. Similarly, however, such high affinities for biological molecules also provide heavy metals with the potential to play toxic roles, for example by substituting for an essential metal of lower affinity in a biologically active molecule or by binding elsewhere onto such a molecule, in either case distorting the geometry of the molecule and thereby inhibiting its function. Thus all heavy metals have the potential to be toxic whether essential or not" (Rainbow, 1988).

The more toxic compounds to fish are the salts of cadmium, copper, mercury, lead, zinc, iron and trivalent chromium (Metelev et al., 1983). "The harmful effect of salts of heavy metals is by the following ways: (a) action of precipitated insoluble hydroxides of metals deposited on the gills and eggs cause mortality of both eggs and fish, (b) some

compounds of heavy metals reduce the pH of the water on hydrolysis, and (c) specific toxic effect "(Metelev et al., 1983). Respiration of fish is disturbed as a result of the direct action of salts of heavy metals on the respiratory epithelium of gills (Metelev et al., 1983). A thick membrance of coagulated mucus on the skin and gills of fish, forms after poisoning with all heavy metals resulting the interference in gaseous exchange between the medium and the gill lamellae. The white coating of the mucus layer is the result of chemical reaction between metal ions and the mucus secretion. The combined effect of copper and zinc has been reported on Cyprinus carpio and Ctenopharyngodon idellus (Wong et al., 1977) and on Ambassis commersoni (Pragatheeswaran, 1987).

Bioaccumulation

In contrast to the non-essential trace metals such as lead, cadmium, mercury, arsenic and others, the essential metals such as copper, zinc, iron and cobalt have important biochemical functions in the organisms. They form either an electron donor system or function as ligands in complex enzymatic compounds. Since essential elements are only used by the organisms in trace amounts and generally as they exist in the environment in small concentrations, their enrichment in the organism does not exceed the level which allows the enzyme system to function without interference. This means that the concentrations of essential trace elements are generally higher in the organisms than in water. If there is excess in the body, the metal content in the organism can be regulated by homeostasis (Bryan and Hummerstone, 1973). However, if the heavy metal concentration at the source of supply (e.g. water, food) is too high, the homeostatic

mechanism ceases to function and the essential neavy metals act in either acutely or chronically toxic manner. Thus in the event of a resulting extended bioaccumulation of heavy metals, the organism may be damaged. The availability of elements to organisms depends on their physicochemical nature which inturn depends on the overall biogeochemical cycle (Boniforti, 1978).

The accumulation of metal in fish, is a function of uptake and excretion. Uptake is considered to be passive and involves diffusion gradients created by adsorption or binding of the metal to the tissue and cell surfaces (Bryan, 1976). The gills of teleosts are likely sites of metal uptake from the ambient water, due to their large surface area and the close proximity of the internal constituent of body and external environment. Within the body, the degree of accumulation in various tissues is dependent on the binding of the metal to specific ligands. There are organs such as liver and kidney, which secrete specific metal binding proteins and there are organs which are the targets of the toxicant action and accumulate metals to significant levels (Stagg and Shuttleworth, 1982). Dallinger et al. (1987) stated that as far as fish is concerned, there are three possible ways by which metals may enter the body: (i) the body surface, (ii) the gills ,and (iii) the alimentary tract. But little is known about the uptake of heavy metals through the skin. It can be assumed that the body surface of fish is more or less impervious to harmful substances in the surrounding water (Dallinger et al., 1987). There are some indications that mucus secretion may prevent heavy metals from entering the body of fish (Varanasi and Markey, 1978; Eddy and

Fraser, 1982). So it may be assumed that the gills and gut are both important path ways for metal uptake in fish (Wills and Sunda, 1984). The work of Honda et al. (1983) on the distribution of heavy metals in organs and tissues showed high concentration of metals in liver and low ones in muscle. However, the concentration of manganese, zinc, copper, lead and nickel were relatively high in ovary and testis and also manganese and zinc were the highest in the skin. The concentration of metals in the organs and tissues characteristically changed with the growth of the fish. The small fish showed faster uptake and excretion rates of metals than the larger ones.

Mechanisms of heavy metal detoxification in invertebrates are well documented (Noel-Lambot, 1976; Viarengo et al., 1980). But very few informations are available on marine vertebrates (Overnell and Coombs, 1979; Kito et al., 1980). Heavy metals are bound to "metal binding proteins" or stored in cellular structures such as in vacuoles and lysosomes (Bouquegneau et al., 1984). Zinc is excreted into the digestive tract and about 45% of it is eliminated with the faeces (Baudin, 1981). Neol-Lambot (1981) found white mucus corpuscules in the intestinal lumen of fishes Anguilla anguilla, Myoxocephalus scorpius, Serranus cabrilla, Moena chryselis and Scorpaena sp. In fish intoicated with $CdCl_2$, $ZnCl_2$ or $CuCl_2$ added to sea water, mucus corpuscules in the intestine contain enormous concentrations of these metals. It seems therefore, to limit the entry of the metals through the intestinal wall to protect the fish against the potentially hazardous concentrations of heavy metals. The heavy metals are eliminated predominantly by the kidney or by the bile (Grahl et al., 1985).

The effect of heavy metals on different aquatic organisms is often complex and difficult to interpret. The role of oxygen, pH, salinity, temperature and hardness in the environment have been demonstrated to be factors that influence the physiology of an organism and the rate of uptake of heavy metals (Wittmann, 1979; Waiwood and Beamish, 1979; Winner, 1985; Bradley and Sprague, 1985 a; Everall, 1987). In general salinity in the marine environment is relatively constant and has little influence on the heavy metal concentrations compared to their role in estuaries. In estuaries, salinity however, plays a dominant role in influencing metal concentrations in free water (Wittmann, 1979). In sea water, the dissolved heavy metal concentrations are generally much lower than in fresh water. Moreover, the high salt content alters the pH of the milieu and consequently the metal solubility. In the case of brackish-water organisms the negative potential difference of the inner body wall increases with lower salinity (Fletcher, 1970); ion transport in to the organisms consequently increases. Bryan and Hummerstone (1973) demonstrated in laboratory experiments on Nereis diversicolor that the absorption of zinc rate per mass unit and time period increases with rising salinity. In the case of copper no direct correlation could be established and seem apparently plays only a secondary role.

The embryological stage of an organism is an important consideration, when examining the effects of heavy metals and concentration of these in the body tissues. It can vary with age or size of the organism (Phillips, 1977; Bennett and Dooley, 1982; Honda et al., 1983; Newman and Mitz, 1988).

The major factors involved in determining the seasonal fluctuation of trace metal levels in aquatic biota, are the extent of pollutant delivery into the aquatic environment, the weight changes occurring in the organisms and the direct effects of salinity, temperature and other water qualities which vary seasonally (Phillips, 1980). It is clear that an increased ambient supply of metal will lead to more rapid uptake of that metal by organisms. Several physiological changes occur in organisms with seasons and may cause fluctuations in the trace metal levels present either in the whole organisms or its component tissues (Phillips, 1980).

Sediment/detritus feeders particularly benthos are exposed to metals both in solution and through ingestion of metal-enriched particulate material (Louma, 1983). It has been shown for instance, that bottom-dwelling fishes accumulate heavy metals, because of their association with metal containing sediments (Ney and Van Hassel, 1983).

Monitoring

Biological monitoring is a mean of assessing water quality or the toxicity of chemicals employing living organisms as the sensors. Relatively recent environmental regulations have lead to the application of bio-monitoring techniques by waste water dischargers and chemical industries. Classical approaches to biomonitoring have included acute bioassay which takes death as the end-point of the test. Recent developments include automated and real time biomonitors which utilise computer technologies for assessing changes in physiological or behavioural parameters to indicate

the presence of toxics (Sivasankaran, 1990). In biomonitoring models the changes measured in the biological response of the test animals used are likely to reflect a meaningful change in the chemical and/or physical conditions of the water concerned. Toxicological hazards measured by bioassay procedures may therefore, be more realistic than those predicted from the results of chemical analyses and the available information on the toxicity of the compounds detected (Koeman et al., 1978; Genjatulin, 1990). Since 1950s, the acute toxicity testing has become the "Workhorse" in monitoring pollution effects (Buikema et al., 1982). Information generated from various toxicity tests can be of use in the management of pollution for the purposes of (a) prediction of environmental effects of a waste, (b) comparison of toxicants or animals or test conditions, and (c) regulation of discharge.

The water quality standards are (1) to first determine the pollutant concentration whether it is acutely toxic to the organism or not and (2) to estimate the pollutant concentration that will have no adverse effect on the organism by multiplying the acutely toxic concentration by a so called "Application Factor". Typically the application factor is a number on the order of 0.01-0.1 (Laws, 1981).

Various groups of aquatic organisms including bacteria, algae, molluscs and fish may serve as indicators for continuous biomonitoring of the media or environment. Cairns et al. (1977) stated that fishes are used as bioassay organisms to test the media or environment. Benoit and Holcombe (1978) have demonstrated that the fragileness of the egg

and poor adhesiveness are due to the more zinc toxicity, when they experimented with Fathead minnow Pimephales promelas. Lesions in organs such as gills, liver and kidney may be indicative for the presence of certain toxic agents (Koeman et al., 1978). Certain changes in the structure of gills such as proliferation of epithelial cells in gill lamellae may indicate that the fish were exposed to toxic sublethal levels of metals (Strik et al., 1975). Early signs of spinal and vertebral aberrations are the indications of compounds such as Zn^{++} and Cd^{++} (Bengtsson, 1975). Saleh (1982) has reported that the fish liver is a place of accumulation of metals. Metallothionein a low molecular weight, heat-stable, metal binding protein was found in liver (Roch et al., 1986; Overnell et al., 1988). The lower "RNA-DNA" ratio in the muscle tissue may predict the pollution interference in fish health and growth (Mohapatra and Noble, 1992).

In the long run, the sublethal concentrations may prove more deleterious than the lethal concentrations, because subtle effects on the fish may alter their behaviour, feeding habits, position in the school, reproductive success, etc. Subtle effects at the organ or cellular level may alter the metabolism of the fish and hence its ability to withstand the stress. If the fish is not directly affected by the pollutants, it liable for infection or toxication through their food which was affected by pollutants.

To evaluate the level of pollution, the "baseline" or "background" has been established for several heavy metals in various near-shore and estuarine environments (Katz and Kaplan, 1981). They can be used as references for monitoring possible future metal pollution. Such values

are available for coastal waters (Anon, 1991a,b), sediments (Flanagan, 1976) and tissues (Anon., 1988,1991c).

From the foregoing pages, it is very clear that there is a global awareness in recent years on pollution and their effects both in the terrestrial and aquatic environments, as these two are meeting the food requirements by way of agriculture, fishing and aquaculture for ever increasing human population. We should not spoil the very useful agriculture lands and aquatic environments for our short-term benefits and we must preserve the environments for our long-term use . We are well aware, knowingly or unknowingly our environments are getting polluted resulting great concern on our livelyhood. This needs an urgent monitoring of the amount of pollutants entering in to the environment, source of pollution, factor contributing the pollution, the pollutant nature, toxicity of the pollutants, mortality of organisms, its effect on food resources, monitoring, remedial measures and legislation.

Bearing all these in mind, an attempt is made here to study the effects of heavy metals particularly copper, zinc and lead in the estuarine environments from both the east and west coasts of India, the concentration of the above mentioned heavy metals in water, sediment and in some tissues of Liza parsia, the test organism. Laboratory studies have also been carried to evaluate the toxic nature of these heavy metals.

The present investigations have brought to light so many interesting results and significant findings which are elaborately presented and discussed in detail in the following three chapters.

REVIEW OF LITERATURE

A RESUME OF LITERATURE ON HEAVY METAL POLLUTION IN AQUATIC ENVIRONMENT

It is a pre-requisite in any type of research to collect all literatures and informations on the field, from all over the world and fully understand.

A thorough scrutiny of literatures on pollution in the marine environment including coastal and estuarine areas was made till the finalisation of this Thesis and every effort has been made to understand and review them.

The elucidation of the comparative trace metal pollution of coastal areas throughout the world must be attempted in order to formulate realistic planning of future industries and to minimise the manmade pollution in the environment. A knowledge of the relative abundance of trace metals in different environments would also help fruitful laboratory studies of metal toxicities on the biota, resulting the prediction of the impacts and effects of metal pollution in the ecosystem and leading useful suggestions to save the environment by relating laboratory results to the environment.

Heavy metals in water

Many authors have reported the results of their studies specifically concerning the concentration of trace metals in water from oceanic areas. Some of these include the pioneering works of Goldberg (1965) for "average" sea water; Topping (1969) for North Indian Ocean and the Arabian Sea; Preston et al. (1972) for Northeast Atlantic; Chester and Stoner (1974) for nearshore and open ocean waters of World Oceans; Duinker et al.,

(1974) for Dutch Wadden Sea; Sengupta et al. (1978) for the Arabian Sea; Sanzgiri and Caroline (1979) for Lakshadweep (Laccadive) Sea; Braganca and Sanzgiri (1980) for coastal and offshore regions of Bay of Bengal; Sanzgiri and Braganca (1981) for the Andaman Sea; Duinker and Nolting (1982) and Nolting (1986) for North Sea and Bethoux et al. (1990) for Mediterranean Sea.

The reports available for heavy metals in near shore, coastal and estuarine areas from various parts of the world, include the Conway Estuary, U.K. (Elderfield et al., 1971); the coast of United Kingdom (Preston et al., 1972); Liverpool Bay, Cardigan Bay and Bristol Channel, U.K. (Abdullah et al., 1972); Seven Estuary, U.K. (Butterworth et al., 1972); Monterrey Bay, California (Knauer and Martin, 1973); Texas Coast, USA (Holmes et al., 1974); Poole Estuary, U.K. (Darracott and Watling, 1975); Rhine Estuary, Netherlands (Duinker and Nolting, 1977); Belgian and Dutch Coasts (Mart et al., 1982); River Tees Estuary (Taylor, 1982); Upper Humber Estuary (Gardiner, 1982); Gota River Estuary, Sweden (Danielsson et al., 1983); South Eastern United States Estuaries (Windom et al., 1983); United Kingdom Shelf waters and the North Atlantic (Jones and Jeffries, 1983); Surface waters of the open Atlantic and European Shelf areas (Kremling, 1985); German Bight (Mart and Nurnberg, 1986); Tamar Estuary, U.K. (Ackroyd et al., 1986); Estuaries of Western Taiwan (Hung Tsu-Chang, 1987); Coastal waters of Southeast Asia (Hungspreugs, 1988) and coastal sea waters of Singapore (Ang et al., 1989).

The study of heavy metals in the coastal environments including estuaries in India started during nineteen seventies. Some of the important reports available are the studies conducted in the inshore and estuarine waters of the Central westcoast of India (Sankaranarayanan and Reddy, 1973); in coastal and estuarine waters around Goa (Zingde et al., 1976); in Bombay Harbour Bay (Matkar et al., 1981); in Pitchavaram Mangroove area of Porto Novo, Tamil Nadu (Subramanian, 1981); in the estuarine waters of Mahanadi Estuary (Ray et al., 1984); in Adyar Estuary, Madras (Nammalwar, 1984); in Auranga River Estuary, Gujarat (Zingde et al., 1985); in Ennore Estuary, Madras (James et al., 1986); in Saurashtra coastal waters (Kesava Rao and Indusekhar, 1986); in the inshore waters of Porto Novo (Shekhar, 1987); in Rusikulya Estuary, Ganjam, Orissa (Sasamal et al., 1987; Gouda and Panigrahi, 1992); in Kodikkarai coastal environment of Southeast coast of India with seasonal variation of heavy metals (Pragatheeswaran et al., 1988); im Ganges Estuary (Subramanian et al., 1988); in Mindhola River Estuary (Zingde et al.,1988); in the Cauvery Estuary (Subramanian et al., 1989); in Kali River Estuary, Karwar (Veer et al.,1990); in the estuaries of southeast coast of India with seasonality of heavy metals (Senthilnathan, 1990) and in Vellar Estuary (Lyla, 1991). The distribution of particulate iron, manganese, copper, zinc in Cochin Backwater has been reported by Sankaranarayan and Stephen (1978). Almost all the studies conducted by various workers mentioned above are somewhat the survey of heavy metal concentrations in the respective study areas.

Heavy metals in sediments

In the studies of pollution, it is of great importance to know the distributioon of metals in sediments, water and biological material and their stresses and impact on the environment and biota by anthropogenic activity. The distribution of heavy metals in particulate matter and in sediments is dominated by mixing processes between river discharge materials and sea-derived (fairly uncontaminated) particulates. The mobilisation of metals from the particulates to the dissolved state is caused by desorption or by dissolution (Wittmann, 1979).

The study of sediments have indicated the areas of heavy metal pollution. Some of them are the studies conducted in the sediments of Tasman Bay, New-Zealand (Brooks and Rumsey, 1965); Seven Estuary, U.K. (Butterworth et al., 1972; Chester and Stoner, 1975); Swan Sea (Bloxam et al., 1972); 27 Estuaries in England (Bryan and Hummerstone, 1973); Cardigan Bay, Wales (Jones, 1973); Hudson River Estuary (Williams et al., 1978); southern coastal areas of Korea (Lee and Han, 1978); southern coast of California (Katz and Kaplan, 1981); Southeastern United States Estuaries (Windom et al., 1983); Norwegian Fjords (Rygg, 1985); Texas marine sediments with seasonality (Holmes, 1986); Nakdon River Estuary, Haengam Bay, Masan Bay, Western part of Jinhae Bay, Chungmu Harbour, Kojae-Hansan Bay (Lee et al., 1986); Sicily Channel Coast (Castagna et al., 1987); Jakarta Bay (Hungspreugs, 1988); Chesapeake Bay (Sinex and David, 1988); Northeast Pacific coastal sediments (Harding and Goyette, 1989) and Humber Estuary, England (Grant and Middleton, 1990).

The reports available from Indian subcontinent include the works of Gogate et al. (1976) and Matkar et al. (1981) for Bombay Harbour sediments; Murty et al. (1978) for Northern half of Western Continental Shelf of India; Murty and Veerayya (1981) for Vembanad Lake, Kerala; George and Sawker (1981) for Mandovi and Zuary Estuary, Goa; Borole et al. (1982) for Narmada and Tapti Estuary and adjacent Arabian Sea; Venugopal et al. (1982) and Nair et al. (1990) for Cochin Backwater with seasonal variation; Pragatheeswaran et al. (1986) for Madras and Visakhapatnam Coasts; Seralathan and Setharamaswamy (1987) and Subramanian et al. (1989) for deltaic sediments of Cauvery River; Sasamal et al. (1987) for mercury distribution in the estuarine and the near shore sediments of the western Bay of Bengal; Nair et al. (1987) for Asthamudi Estuary, Kerala; Ramanathan et al. (1988) for the upper reaches of the Cauvery Estuary; Mohanachandran and Subramanian (1990) for the Southeast Coast of India and Lyla (1991) for Vellar Estuary.

The trace metal concentrations found in sediments varies according to the rate of trace metal deposition, the rate of particle sedimentation, the particle size and nature and the presence or absence of organic material (Phillips, 1977). In general, metal concentrations are found to increase in an approximately linear fashion with increased organic content, measured as total carbon (Halcrow et al., 1973).

Effect of heavy metals on fishes

There are indications of depressed or accelerated enzyme activity in aquatic organisms exposed to low concentrations of metals. Activity

of the enzyme alphaglycerophosphate dehydrogenase found in fish (trout) muscle tissue was found to be inhibited by a number of metals in the following descending order: $Hg^{2+} > Cd^{2+} > Zn^{2+} > Pb^{2+} > Ni^{2+} > Co^{2+}$ (Bargmann and Brown, 1974). Some of the classical works in this regard have been conducted by Jackim et al.(1970) with respect to metal poisoning of a number of liver enzymes in Killifish Fundulus heteroclitus. The influences of lead and other metals on δ-aminolevulinate dehydrase activity in fish has also been studied by Jackim (1973). The study of Bilinski and Jonas (1973) has shown that the oxidation of lactate by gills in rainbow trout Salmo gairdneri is inhibited by over 50%, when the fish is exposed to 0.064 mg Cu/l for 48 hrs.

The effects of copper studied on different activities of fish, are on apetite and growth of Salmo gairdneri (Lett et al., 1976); osmoregulation in marine teleosts (Cardeilhac et al., 1979); the growth of Channa gachua (Marathe and Deshmukh, 1980); chloride transport across the opercular epithelium of sea water-adapted Killifish Fundulus heteroclitus (Crespo and Karnaky, 1983); susceptibility of Japanese eel Anguilla japonica (Mushiake et al., 1984); phagocytosis in the blood of eels (Mushiake et al., 1985); diel activity of marine catfish Arius felis (Steele, 1989); the foraging behaviour of Blue-gill Lepomis macrochirus (Sandheinrich and Atchinson, 1989); degeneration of olfactory receptors in Rainbow-trout Oncorhynchus mykiss (Klima and Applehans, 1990) and chemoreception of Zebrafish Brachydanio rerio (Steele et al., 1990).

The fish organs mainly damaged by exposure to copper are the liver (Gardner and Laroche, 1973; Wong et al., 1977; Leland, 1983; Benedetti et al., 1989); gills (Garner and Laroche, 1973; Wong et al., 1977); kidney (Cardeilhac et al., 1979); stomach (Singh, 1985); mitochondria (Aloj Totaro et al., 1986) and neuron (Enesco et al., 1989).

The effects of zinc studied on different activities of fish are on osmoregulation in Rainbow-trout Salmo gairdneri (Skidmore, 1970) and the mucus production of the same specimen (Eddy and Fraser, 1982); erythrocyte haemolysis (Kodama et al., 1982); the tissue glycogen content of air-breathing Climbing perch Anabas scandens (Natarajan, 1982); lymphoid cells of Carp Cyprinus carpio (Cenini and Turner, 1983); the muscles of whitefish (Wunder et al., 1984); intestinal absorption of some nutrients in Catfish Heteropneustes fossilis (Subhadra and Sastry, 1985); respiration and liver glycogen of Labeo rohita (Benegeri and Patil, 1986a) and tissue melanomacrophage induction (Everall, 1987).

The fish organs damaged by expsure to zinc are gills (Matthiessen and Brafield, 1973; Tuurala and Soivio, 1982; Crespo and Sala, 1986a,b); liver (Leland, 1983) and epidermis of Clupea harengus (Somasundaram, 1985). The fish mortality after acute zinc contamination has been related to desquamation of gill epithelia (Crespo et al. 1981). Matthiessen and Brafield (1973) have described the sloughing of epithelial cells and various cytoplasmic abnormalities in the epithelial cells in the gills of Gasterosteus aculeatus during exposures to sublethal concentrations of zinc.

Lead another poisonous and dangerous metal, enters the human body primarily *via* inhalation of polluted air and through the ingestion of contaminated food and water. Once absorbed into the blood stream, lead is transported to all parts of the body and begins to appear in the liver and kidney within a few hours after absorption and ultimately deposited in bones (Wittmann, 1979). Lead is known to disrupt several enzymes involved in the production of heme, damage to both peripheral and central nervous system and damage of the kidney (Waldron and Stofen, 1974). The effects of lead to aquatic organisms have not been thoroughly studied and the data are particularly wanted or scanty. It is believed that the effect of lead on fishes may be on the similar lines of human beings. The lead is absorbed in fish haemolytically (Metelev *et al.*, 1983). The lead poisoning forms intranuclear inclusions (Choie and Richter, 1972) and exposure to lead nitrate affects the serum calcium and inorganic phosphorus levels in *Channa striatus* (Tewari, 1990). The effect of lead nitrate on the kidney of Tench *Tinca tinca* has been reported by Roncero *et al.* (1988) and on the formation of nuclear inclusions in the oocytes of the Catfish *Clarias batrachus* by Katti and Sathyanesan (1987). The effect of lead has also been reported in the liver of *Puntius arulius*(Bengeri and Patil, 1986b); in the gills of *Puntius arulius* (Bengeri and Patil, 1987); in testis (Srivastava, 1987) and lysosomalmembrane in *Oreochromis hornorum* (Martinez-Tabche *et al.*, 1990).

Bioaccumulation in fishes

Some informations are available regarding the bioaccumulation of heavy metals by fishes in different estuarine and coastal environments.

Some of them have been reviewed by Phillips (1977). After that the valuable works available are for the lower Medway Estuary, Kent (Wharfe and Van Den Broek, 1977); the coastal water of Malayasia (Babji et al., 1979); the Andaman Sea (Kureishy et al., 1981) endorheic saline lake in the tin-silver province of Bolivia(Beveridge et al., 1985); St. Vincent Gulf, South Australia (Maher, 1986); Loire Estuary (Amiard et al., 1987); Kelang Estuary, Malayasia (Law and Singh, 1988); the Gulf of Thailand (Hungspreugs, 1988) and the Arabian Sea (Ashraf and Jaffer, 1990).

The reports available on bioaccumulation in fish from Indian Sub-continent include the works of Zingde et al. (1976) in coastal and estuarine waters around Goa; Matkar et al.(1981) in Bombay Harbour Bay; Nammalwar (1985) in Adyar Estuary, Madras; Ghose et al. (1985) in Hoogly Estuary and Veer et al. (1990) in Kali Estuary, Karwar.

Copper content in various organs of fish after exposures to its salts has been reported for rainbow-trout (Dixon and Sprague, 1981); Fundulus heteroclitus and F. majalis (Bennett and Dooley, 1982); Hetero-pneustes fossilis and Channa punctatus (Rajbanshi and Gupta, 1986); Morone americana (Bunton et al., 1987); Labeo rohita (Radhakrishnaiah, 1988); Clarias anguillaris and Oreochromis niloticus (Daramola and Oladimeji, 1989) and Tilapia nilotica (Eardem and Kargin, 1990; Chait and Kargin, 1990). Uptake and accumulation of zinc for Scyliorhinus canicula (Flos et al., 1979), Salmo gairdneri (Lovegrove and Eddy, 1982) and Labeo rohita (Radhakrishnaiah, 1988) have been reported. The accumulation of lead in liver, kidney and gills of the Carp Cyprinus carpio is given

by Kralj-Klobucar and Spasojevic (1989). The levels of copper, zinc and lead are estimated in gonad, liver, kidney and gills of Barbus grypus and B. belaywin (Latif et al., 1982), in various estuarine and coastal organisms (Amiard et al., 1987) and in liver and kidney of Pampus argenteus and Formio niger (Jaffer and Ashraf, 1988). Concentrations of zinc and copper are reported in dogfish (Sanpera and Vallribera, 1983) and Catostomus commersoni (Young and Harvey, 1989). The values obtained from the field for different metals for different tissues in different fishes have been critically reviewed by Eisler (1981).

Bioassay on fishes

"Standard bioassays on metals in sea waters, where the organism is exposed to the concentration of the metal salts in static set up system, have not been reported widely in the literature. Perhaps, this is not surprising, in as much as sea water facilities are not always readily available to biological laboratories and the use of sea water presents certain problems which are not present with freshwater" (Waldichuk, 1974). The factor that must be considered in terms of toxicity of metals to aquatic organisms is synergism (Waldichuk, 1974). If all the bioassay data had been obtained in one laboratory, where consistency in experimental technique and conditions could have been maintained, perhaps the relationship with position of the metal in the Periodic Table would have been better.

"It is clear that bioassay tests for acute toxicity of heavy metals require special precautions. In addition to the usual care in maintaining

concentrations of the metals in solution, one must be certain of maintaining uniform concentrations of dissolved oxygen, salinity and temperature through out the tests" (Waldichuk, 1974). A number of works had addressed the variables that have an effect upon the toxicity. Of these factors hardness and pH are considered to be of prime importance (Howarth and sprague, 1978; Bradly and Sprague, 1985 b; Hutchinson and Sprague, 1989). Increase in the level of total hardness or calcium ion in a neutralized lake would have a moderating effect on lethality of zinc (Bradly and Sprague, 1985b; Moni and Dhas, 1989) and copper (Howarth and Sprague, 1978), regardless of pH. The effect of pH on the toxicities of different metals is also well documented (Cusimano et al., 1986; Stripp et al., 1990). In effect of calcium concentration on the toxicity of copper, lead and zinc to yolk-sac fry of Brown-trout Salmo trutta is reported by Sayer et al. (1989).

Some of the reports available on toxicity tests in various fishes from India and abroad in 1980s are the toxicity of copper to Rasbora daniconius (Durve et al., 1980) and marine catfish Arius felis (Steele, 1983); copper and zinc to Puntius conchonius (Pant et al.,1980) and Clarias lazera (Hilmy et al., 1987); copper, cadmium and zinc to northern Squawfish Ptychocheilus oregonesis (Andors and Garton, 1980) and to Chinook salmon (Finlayson and Verrue, 1982); lead to Channa punctatus (Saxena and Parashari, 1981), Clarias lazera, Oreochromis niloticus, Chironomus tentans and Benacus sp. (Oladimeji and Offen, 1989); copper and zinc to Nile Tilapia Tilapia nilotica (Somsiri, 1982); zinc to Rainbowtrout Salmo gairdneri (Kodama et al., 1982; Meisner and Hum, 1987), Lebistes reticulatus (Sehagal and Saxena, 1986), Tilapia zilli and Clarias lazera (Hilmy et al., 1987);

copper and mercury to Poecilia reticulata (Khangarot and Ray, 1987) and cadmium, lead, zinc and molybdenum to Nemacheilus botia (Pundir, 1989).

Acute toxity test i.e. bioassay is a first step in monitoring the effects of pollutants. Responses of fish to chronic stress are usually predicted from water quality standards (e.g. LC50 tests and lifecycle toxicity tests). Such approaches is generally acceptable for screening the effects of contaminants on a short-term experiments. These short-term experiment results have limitations and it will not reflect appropriate for providing informations on the real condition of environment as well as the effect on fish.

Hence chronic studies have been carried out to understand the "Bioaccumulation" and "Bioaccumulation Factor" in different tissues of the experimental animal Liza parsia. The efforts were made to correlate the results obtained form the laboratory experiments with that of field samples applying proper and suitable statistical formulae and method, perhaps for the first time in this field.

MATERIALS AND METHODS

MATERIALS AND METHODS

Study Area

It is a gift from Mother Nature to the Indian sub-continent in the tropic, to have (i) a long coastline of about 7000 km (Qasim et al.,1988) with excellent marine wealth from both east and west coasts; (ii) a large number of rivers flowing on both directions into the sea forming reasonably broad estuarine systems and; (iii) enormous exploitable and cultivable fishery resources. The brackishwater area available in our country is estimated to be about 1.4 million hectares (Anon., 1991 a). These estuarine and brackishwater areas now-a-days, are increasingly polluted particularly due to industrial developments. The conservation of our estuarine and brackishwater environments is of paramount importance and their monitoring of pollution is highly essential. Hence, for the present study, six centres, each one is unique in its nature, were selected along the east and west coasts of India. Their location, station positions and major possible sources of pollutants, etc. are given in Table 1. The selected centres are the Korapuzha Estuary, Elathur (Centre Code I); north extension of the Cochin Backwater (Centre Code II), both from Kerala in the west coast of India; the Tuticorin Bay, Tuticorin (Centre Code III); the Gulf of Mannar and the Palk Bay, Mandapam (Centre Code IV); the Ennore Creek, Madras (Centre Code V) - Centres III to V in Tamil Nadu and the Rusikulya Estuary, Ganjam (Centre Code VI) in Orissa. The last 4 centres are from the east coast of India. The period of investigation is from January 1991 to December 1992. The first two centres (I &

TABLE 1. **Location of study area, station code, station numbers, sources of fresh water, type of estuarine condition and major possible source of pollutants**

Name of Estuary/Area	Stations	Place	Coast	Latitute (North)	Longitude (East)	Source of Freshwater	Source of pollution
Korapuzha Estuary (Code I)	1-3	Elathur, Calicut	Southwest	11°21' 11°24'	75°44' 75°46'	Agala Puzha, Pannur Puzha	Coconut husk retting, coir industry, fishing, etc.
Cochin Backwater (Code II)	4-6	Cochin	Southwest	09°58' 10°10'	76°14' 76°20'	River Periyar and Chalakudy at northern side, Meenachil Pamba, Moovattupuzha, Achancoil and Manimala at southern side	Domestic waste, effluents from Petroleum Refinery, Fertilizer Plant, Caprolactam Plant, Cochin Port, Cochin City, Fisheries Harbour, etc.
Tuticorin Bay (Code III)	7-9	Tuticorin	Southeast	08°45' 08°48'	78°09' 78°12'	Uppar Odai Creek and Korampallam Creek	Discharges and Fly-ash from Tuticorin Thermal Power plant, Oil and other effluents from Southern Petrochemical Industries Corporation, Tuticorin Municipal Sewage, paint and debris from craft manufacturing, Port Trust, fishing etc.
Gulf of Mannar and palk Bay (Code IV)	10-12	Mandapam	Southeast	09°15' 09°20'	79°5' 79°13'	-	-
Ennore Creek (Code V)	13-15	Ennore, Madras	Southeast	13°20' 13°23'	79°28' 79°31'	Kotaliar River	Madras Metropolitan sewage, effluents from Kothari Chemicals Ltd., Alkali Chemicals, Madras Fertilizers Ltd., Petrochemicals, Ennore Thermal Power Plant, Madras Refineries, fishing, etc.
Rusikulya Estuary (Code VI)	16-18	Ganjam, Orissa	Northeast	19°22' 19°24'	85°02' 85°06'	Rusikulya River	Effluents from Chloro-Alkali Plant, fishing, etc.

II) experience heavy southwest monsoon rain, while Ganjam (VI) has moderate southwest monsoon effects. The rest of the centres (III, IV and V) have northeast monsoon. The total number of stations studied were 18 i.e. three in each centre. A brief description of each centre along with the importance of the stations is given below:

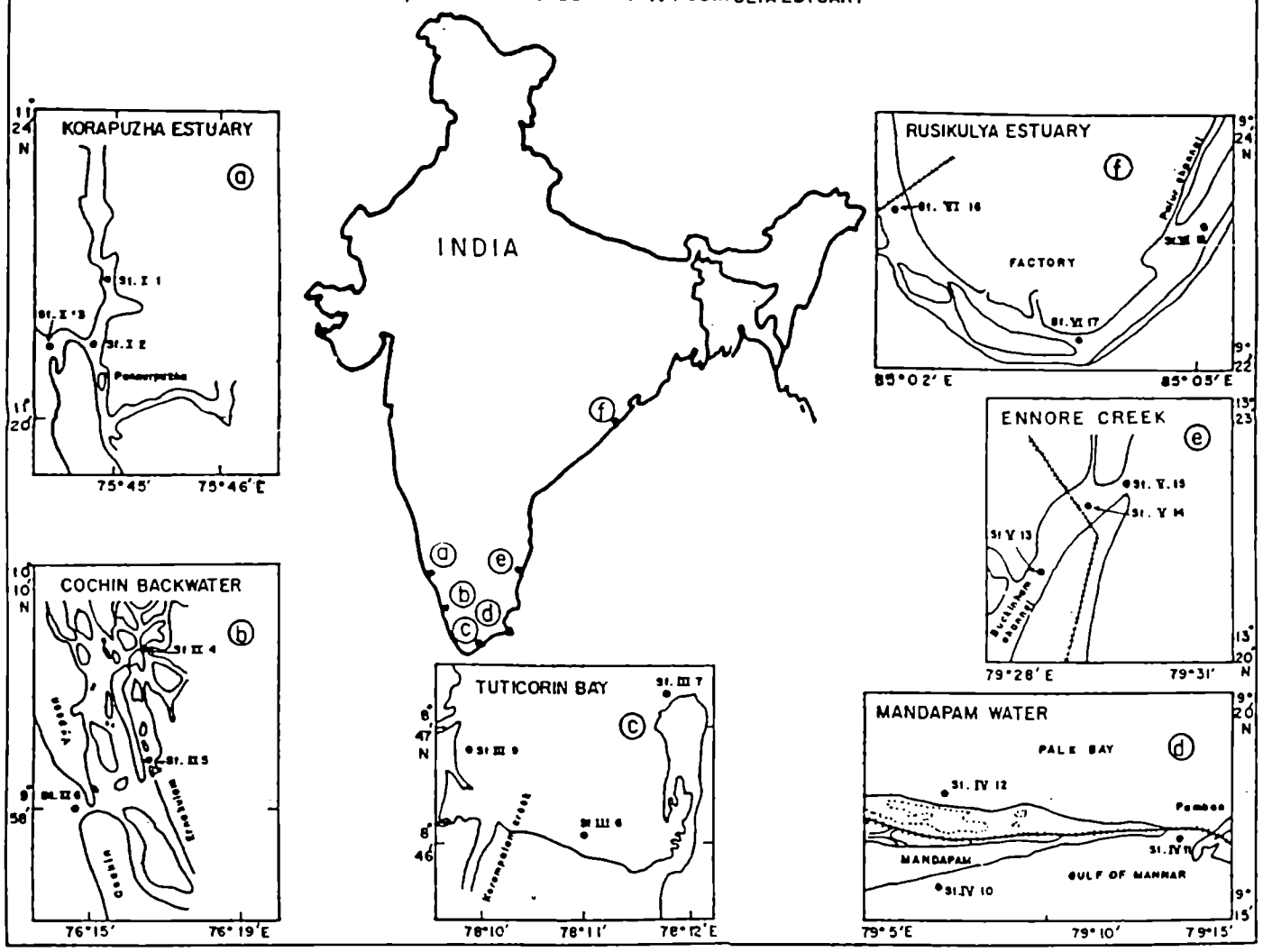
The Korapuzha Estuary (Centre Code I)

This estuary is located at Elathur, Kozhikode (Calicut), along the west coast of India (75° 44' to 75° 46'E and 11° 21' to 11° 24'N). Agala Puzha (River) from northern side and Pannur Puzha (River) from southern side, discharge the freshwater into this estuary which is a perennial one. The major pollutants in this Centre are coconut husk retting waste materials producing H_2S in the area, fish wastes, municipal sewage, agricultural seepage with fertilizer residue, etc. During monsoon the flow of freshwater was recorded 8 - 15 km/hr.

Three stations viz. St. I 1, St. I 2 and St. I 3 were fixed in the estuary and the details of topography with station positions are given in (Fig. 1 a).

St. I 1 (Fig. 1 a): It was fixed at the upper stretch of the estuary i.e. lower end of the Korapuzha River. The water was clear in premonsoon and postmonsoon and turbid in monsoon. The depth recorded at the station was 1.25 to 2.00 m during the collection time. The colour of the bottom soil was dark-ash type. The distance of the station from barmouth was approximately 4 km.

FIG. 1. SAMPLE COLLECTION CENTRES ALONG THE EAST AND WEST COASTS OF INDIA
a. KORAPUZHA ESTUARY, b. COCHIN BACKWATER, c. TUTICORIN BAY,
d. MANDAPAM, e. ENNORE CREEK AND f. RUSIKULYA ESTUARY



St I 2 (Fig. 1 a): This station was fixed at the point where the Pannur Puzha (River) joins to the Korapuzha Estuary. This station location was near to the fish landing centre and the railway bridge with a distance from barmouthapproximately 1.5 km. The colour of water, soil and depth were similar to the St 1 1.

St. 1 3 (Fig. 1 a): The location of this station was at the barmouth of the estuary. The width of the barmouth was found to be 200 to 300 m. The depth at the station at the observation days was 1.25 to 2.50 m. The water was turbid in all the seasons due to the churning process by wave actions. The sediment was clayey with sand. The landward side of the estuary pritched with rocks found with oyster shells attached to it.

The Cochin Backwater (Centre Code II)

It is situated in the central Kerala along the southwest coast of India and its barmouths open to the Arabian Sea at Cochin and Kodungallur. The study area was located between 76°14' and 76°20'E and between 9°58' and 10°10'N. River Periyar and River Chalakudy discharge the freshwater at the northern side of the backwater. The rivers such as Manimala, Meenachil, Pamba, Achancoll and Moovattupuzha discharge the freshwater to the southern backwater which joins to the northern one at Cochin Barmouth. The islands such as Bolghaty, Willingdon, Gundu and Vallarpadam

formed inside the backwater are thickly populated. This system receives major pollutants particularly sewerage from domestic wastes from Greater Cochin urban area; wash outs containing organic fertilizer residues from agricultural lands, oil spillage and other hydrocarbons from Cochin Refinery and Caprolactam Plant and from Cochin Port; chemical wastes from Fertilizer Plant, effluents from other small industries located on the banks; fish spoilages and others from fish landing centres and Fishery Harbour; oil, paints, metal and paint scrabbings from Cochin Shipyard and Port and other Boatyards and Dockyards situated on both the banks of the backwater in and around Cochin particularly on the southern sides; sediments by dredging the Ernakulam Channel for navigational purposes; fish guts, prawn peelings and their wastes from fish processing and canning industries, etc.

Three stations viz. St. II 4, St. II 5 and St. II 6 were fixed for sampling in the backwater.

St. II 4 (Fig. 1 b): It was fixed at the northern part of the backwater near to the Caprolactam Plant and Fertilizer Plant at Cheranellur. The depth at this station was 2.0 - 3.0 m and water was clear in premonsoon and postmonsoon and turbid during monsoon due to influx of freshwater from the surroundings and rivers. During certain collection times, oil-slicks were noticed on water surface and oil-droplets on aquatic weeds such as Eicchornia crassipes. The colour of the sediment was dark black with sand particles.

St. II 5 (Fig. 1 b): The Station was fixed eastern side of the Bolghaty Island. Near to this station found oil terminal, boat jetty, Cochin Port, etc. The depth at this station was 2.0 - 2.2 m and water was clear in premonsoon and postmonsoon, but turbid during monsoon. The sediment was dark and loose humus with biogenic material derived from nearby mangroove areas with sand particles. The station was situated 2 km away from Cochin Barmouth towards the backwater.

St. II 6 (Fig. 1 b): It was located at Cochin Barmouth and the width of mouth and depth of water recorded here were 450 m and 3.0 - 7.0 m respectively. The water colour was same as St. II 5 and the sediment found here was clayey with sand particles.

The Tuticorin Bay (Centre Code III)

It is situated at Tuticorin of Tamil Nadu along the southeast coast of India. The study area in the bay covered from 78°9' to 78°12'E and from 8°45' to 8°48'N. The pollutants to the bay are fly-ash from Tuticorin Thermal Power Plant, industrial and chemical discharge from Southern Petrochemical Industries Corporation, Municipal sewage from Tuticorin Town, fish wastes from fish landing centres, oil spilage and other solid wastes during loading and unloading in Tuticorin Port, grease and other solid wastes from Boatyards and various other small industrial establishments. According to Ramachandran et al. (1991), this area comes under first category of polluted area and the sewage production is estimated to be 11.5×10^4 litres/day.

Three stations were fixed in the bay (Fig. 1 c) for sampling and they were St. III 7, St. III 8 and St. III 9.

St. III 7 (Fig. 1 c): This station was near to the light house at Pandian Tivu (Islet). The depth was between 1.2 and 1.5 m and water was clear with sand particles in suspension due to wave action. The sediment was sandy with dead and broken shells.

St. III 8 (Fig. 1 c): It was fixed close to the out let of the Tuticorin Thermal Power Station adjacent to Korampallam Creek which receives rain water along with salt from the close by salt pan during northeast monsoon. Water was clear and depth was 0.5 - 0.75 m. The sediment was with fly-ash deposits at the bottom of the bay from Thermal Power Plant.

St. III 9 (Fig. 1 c): This station was located near to the Tuticorin Research Centre of CMFRI at Karappad and Uppar Odai Creek. The depth at station was between 1.2 and 1.5 m and the sediment was clayey. Near to the station is the Experimental Oyster Culture Farm of Tuticorin RC of CMFRI. The bottom was also found with gastropods and sea grasses.

The Gulf of Mannar and the Palk Bay (Centre Code IV)

These water bodies are situated along the southeast cost of India in Tamil Nadu and partitioned by the Mandapam Strip of main land leaving Palk Bay on the north and the Gulf of Mannar on the south. The study area was located between 9°15' 9°20'N and between 79°5' - 79°13'E.

This area was selected as control centre and stations presuming a non-polluted area as there are no industries or ports, etc. and area and water are very clear.

The stations St. IV 10, St. IV 11 and St. IV 12 were selected to carry out the sampling (Fig. 1 d).

St. IV 10 (Fig. 1 d): The station was fixed near to Mandapam Regional Centre of CMFRI, Mandapam Camp in the Gulf of Mannar. The water was clear and the depth at the station was between 1.5 - 2.5 m. The sediment was sandy with seaweed fronds both decayed and fresh.

St. IV 11 (Fig. 1 d): It was at the Pamban Bridge where the waters from the Gulf of Mannar and the Palk Bay mix up. According to Ramachandran et al. (1991) this station comes under fourth category of (Polluted) area and sewage production is 0.45×10^4 litres/day. The major activity in this station is fishing by mechanised boats. The water was clear and the depth at Station was 1.5 - 2.5 m. The sediment was sandy with dead shells and broken coral bits.

St. IV 12 (Fig. 1 d): This station was fixed at Mandapam Camp in the Palk Bay. The depth at station was between 2.0 and 3.0 m and water was clear. The sediment was sandy with coral chips. Near the station the fish landing centre is located, where berthing of mechanised and non-mechanised vessels was found.

The Ennore Creek (Centre Code V)

The creek is situated at Ennore, Madras, Capital of Tamil Nadu along the southeast coast of India. The study area was located in the creek from 79°28' to 79°31'E and from 13°20' to 13°23' N. The Barmouth opens to the Bay of Bengal throughout the year. The creek is fed with freshwater derived from Kotaliar River. The major effluents into the creek were domestic and industrial and refinery discharges. The major industrial pollutants include the organic and inorganic chemical wastes from Kothari Chemicals Ltd., Alkali Chemicals, Madras Fertilizers Ltd. and many other private industries; oils, paraffin and other hydrocarbon from Madras Oil Refineries; fly-ash and coolant from Ennore Thermal Power Station; municipal sewage from Madras City, etc. The quantum of domestic sewage output in the Madras Metropolitan area has been estimated to be about 51 million gallons/day and out of that about 0.9 million gallons/day is allowed to flow to Ennore Creek (Nammalwar et al., 1985). It has been estimated that about 4,49,000 litres/day of industrial effluents carrying heavy metals are let out into this system by these industrial establishments (James et al., 1986). In general the water was noticed creamy white in most of the collections and sometime oil patches over the water medium were also observed.

Three stations St. V 13, St. V 14 and St. V 15 were fixed for sample collection (Fig. 1 e).

St. V 13 (Fig. 1 e): This station was fixed in the Buckingham Canal near to the out let of Ennore Thermal Power Plant. The water was dark with smelling hydrogen sulphide. The depth was between 0.25 and 0.75 m The sediment also was found dark.

St. V 14 (Fig. 1 e): It was located near the railway bridge at a distance 1.5 km (approximately) from the Barmouth. The water was found as in station V 13, creamy white probably due to $CaCO_3$, as in this stations oyster and mussel beds are commonly seen. The depth recorded was 1.5 to 2.5 m. The sediment was dark black with broken oyster shells. This station acts-as the dumping place for Ennore Town garbage.

St. V 15 (Fig. 1 e): It was fixed at the Barmouth of the creek. The width of the Barmouth recorded 100 - 150 m and covered with sand at both sides. The depth at the station was 0.5 - 1.0 m. The sediment was ash coloured.

The Rusikulya Estuary (Centre Code VI)

This perennial estuary is situated at Ganjam, Orissa State along the northeast coast of India and its Barmouth opens to the Bay of Bengal. This estuary is fed with the freshwater derived from the upper catchment area through the Rusikulya River. The study area was located between 85°2' and 85°6'E, and between 19°22' and 19°24'N. The major activity in the estuary is fishing with country crafts. It is exposed to contamination from a Chloro-Alkali Plant (i.e. Jayashree Chemicals) since 1967 (Gouda

and Panigrahi, 1992). During monsoons the flow of freshwater was recorded 6-12 km/hr.

For sampling three stations viz. St. VI 16, St. VI 17 and St. VI 18 were fixed in the estuary (Fig. 1 f).

St. VI 16 (Fig. 1 f): It was fixed near to the railway bridge. The water was clear in premonsoon and postmonsoon, but turbid in monsoon. The sediment was clayey with sand. The station was fixed at a distance of 4 km from barmouth. The depth was 1.0 - 1.2 m.

St. VI 17 (Fig. 1 f): It was near the bend of the estuary towards north and after the outlet of the Jayashree Chloro-Alkali Plant. An uninhabited small island was inside the estuary at the right side of the station. The depth was between 1.5 - 2.0 m and the bottom sediment was sandy.

St VI 18 (Fig. 1 f): This was at the Barmouth of the estuary. The width of the mouth was 200 - 300 m. The sides of the barmouth was loaded with sand. In between the St. Vl 17 and St. VI 18 joins the Palur Canal to the estuary. The water was clear with suspension of sand particles in premonsoon and postmonsoon and turbid in monsoon. The depth at the station was at 1.0 - 1.5 m. The sediment was sandy.

Period of study

The samples of water, sediment and Liza parsia were collected from each centre during premonsoon, monsoon and postmonsoon of 1991

and 1992. The classification of different seasons adopted for the present study is that followed by Qasim and Gopinathan (1969), which is as follows: Premonsoon (February to May), Monsoon (June to September) and Post-monsoon (October to January) for SW monsoon area. But for NE monsoon areas the classification followed was : premonsoon (June to September), monsoon (October to January) and Postmonsoon (February to May) (Lyla, 1991). Towards monitoring programme, monthly samples were collected from Cochin Backwater from June 1991 to May 1992 for one year.

Parameters studied

For the present investigation the following physical, chemical and biological parameters were selected, as each one is significant in its nature and has direct bearing on the other.

Physical parameters: To have an idea about the environmental conditions the temperature and pH of water were recorded at the stations with the help of mercury thermometer and pH paper respectively. The depth was measured by graduated rope with weight. The colour and nature of the soil were recorded by visual observations. The organic carbon content of the soil/sediment was also estimated at the laboratory.

Chemical parameters: For detailed study, the salinity and total hardness were selected as the prime supporting parameters. These two have the direct bearing on the estuarine condition as well as on the distribution of the heavy metals. As the heavy metals such as lead, copper and zinc which are toxic pollutants in aquatic environments, they were selected

as the heavy metals for study in the environment i.e.,in water and sediments from each station.

Biological parameters: To study the heavy metal level in Liza parsia, an estuarine mullet and their impact on organism it was selected, as they are available in all centres with good fishery in the area and they are also important in finfish culture. Their length and weight were recorded at the centres. The tissues such as liver, gills, kidney, intestine, ovary, muscle and skin were selected as these organs are the passage or storage for these metals. The "Bioaccumulation" and "Bioaccumulation Factor" for the heavy metals in tissues mentioned above were selected for detailed study.

Method of collections of samples

Surface water: The surface water was collected directly submerging the polyethylene bottles of 500 ml capacity for heavy metal analysis (Mart and Nurnberg, 1986). Extensive cleaning of the bottles prior to the collection, was done by immersing and allowing to remain in the trough filled with diluted HNO_3 until the collection of water samples. Bottles were washed thoroughly with double distilled water before sample collection. For total hardness and salinity the samples were collected separately in pre-cleaned glass bottles of 125 ml capacity.

Bottom water: The water sample from the bottom was collected by sending a water bottle stoppered with two holes one with a short polythene tubing and other with a long one with a closing mechanism controlled

from the surface by the operator (Plate I). The sampling bottle is attached with bamboo pole above 15 cm. from the tip of it. Now the pole with the bottle is sent to the bottom and the closing mechanism at the surface is opened to enable the bottom water to get into the sampling bottle. The sampling bottle with bottom water is taken up for further analysis. Wherever the depth is more and it was not possible to use the pole, the sampling bottle was attached to a nylon rope as in the case of bamboo pole, but with a weight to sink to the bottom. However, the position of the sampling bottle was maintained at about 15 cm above the bottom through out the period of investigation. The collected sample was transferred to the pre-cleaned polyethylene bottles for metal analysis. The water sample was once again collected for salinity and total hardness. The sampler before and after the sampling in a centre was cleaned in 1:1 hydrochloric acid and kept with chromic acid whenever not in use (without stopper). Before using, it was washed profusely by distilled water (Matkar et al., 1981). The stopper and tubings were cleaned with diluted HNO_3 or hydrochloric acid followed by distilled water before use. In between the stations of a centre the water sampler was cleaned with the water of the station.

Sediment: The bottom sediment samples in duplicate from each station were collected with a Van Veen grab and stored in polythene bags as suggested by Matkar et al. (1981), Venugopal et al. (1982), Pavoni et al. (1988) and Zingde et al. (1988).

Liza parsia: Specimens were identified from other mullets by the help of "FAO species identification sheets for fishery purposes" (Anon., 1974).

Plate I. Bottom water sampler.

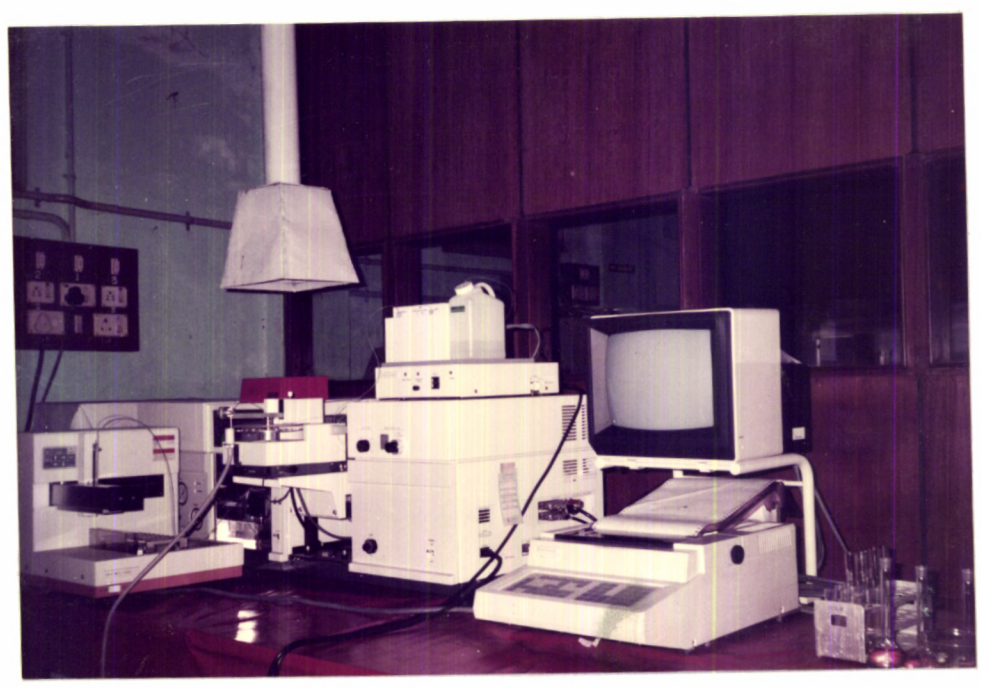


Plate II. Hitachi Polarized Zeeman Atomic Absorption Spectrophotometer.

10 to 15 specimens of size ranging between 7.5 and 14.5 cm, weighing 15.0 and 43.0 g were caught by cast nets in each centre and were immediately cleaned in good water of the sampling station. If specimen was not available in cast netting, purchase of fresh specimens was made at the nearest landing centre (Dybern, 1983). The collected specimens were preserved in an ice box. Difficulty was experienced at certain centres such as Korapuzha Estuary, Cochin Backwater and Rusikulya Estuary during heavy monsoon even in purchasing of L. parsia for analysis from markets as there was no fishing due to rough weather.

Method of preservation of samples

Water samples: The water samples were preserved by adding 2 ml of redistilled concentrated HNO_3 per litre of water (Ekedahl, 1975; Menasveta, 1978) to reduce the pH to 3-4 as the heavy metals will not absorb to container walls.

Sediments: For organic carbon, copper, zinc and lead estimation the sediment samples (station wise) were oven dried at 90°C to constant weight and ground in a ceramic grinder (Holmes, 1986) to pass through a 100 µm screen. The finer sediment particles were kept air tight in plastic bottles for digestion and analysis.

Biological samples: The specimens (L. parsia) on arrival at laboratory in respective centres, were cleaned thoroughly with tap water and later by double distilled water. They were then asceptically dissected using clean stainless steel dissection tools (Harding and Goyette, 1989). The tissues selected for the present study were muscle, skin, gills, liver, kidney,

ovary and intestine. The gills and intestine were rinsed in 5% hydrochloric acid followed by distilled water to remove debris and other adhering particles (Stagg and Shuttleworth, 1982). Each tissue sample analysed was the pooled one of all collected specimens from a centre (Wharfe and Van Den Broek, 1977; Bennett and Dooley, 1982; Dybern, 1983; Amiard et al., 1987). The dissected samples were kept in clean watch glasses (Veer et al., 1990) and dried for three days at 60°C in an oven (Szefer et al., 1990). The dried samples were packed air-tight in clean glass vials till the estimation.

Method of estimation

Temperature: The temperature of the water sample and atmosphere was recorded with a mercury thermometer (0 - 50.0°C).

pH: The pH of water at the collection site was tested with the help of pH paper.

Salinity: The salinity was determined by the classical Mohr titration method (Strickland and Parsons, 1968) and expressed as parts per thousand ($‰_o$).

Total hardness: The concentration of calcium (Ca) plus magnesium (Mg) expressed as equivalent calcium carbonate, is the total hardness. Ca and Mg ions were titrated with the ethylene diamine tetra acetic acid disodium salt (EDTA) to form the stable complexes CaEDTA and MgEDTA.

The end point of the titration was signalled with an indicator called eriochrome black-T. The titration and calculation were done as per the guidelines given in APHA-AWWA-WPCF (1980). The values are expressed as ppm (parts per million) i.e. mg/l as $CaCO_3$.

Heavy Metals: Preconcentration of dissolved copper, zinc and lead from water after filtering through 0.45 µm millipore filters under vacuum was achieved by chelating them with ammonium pyrrolidine dithiocarbamate (APDC) followed by extraction of the metal chelates into an organic solvent (Methyl isobutyl ketone). Again the organic extract was back extracted into inorganic form using concentrated nitric acid. The final extract was diluted to 20 ml and analysed by Hitachi Polarized Zeeman Atomic Absorption Spectrophotometer (Plate II) at the State Pollution Control Board of Kerala at Ernakulam. The method of estimation was followed as per the guide lines of Brooks et al. (1967), Brewer et al. (1969) and Sen Gupta et al. (1978). The values are expressed in ppb (parts per billion). The instrument condition at the time of estimations was as follows:

For copper

Atomization	:	Flame
Lamp current	:	7.50 mA
Wavelength	:	324.80 nm
Slit	:	1.30 nm
Atomizer	:	Standard burner
Oxidant	:	Air
Oxidant pressure	:	1.60 kg/cm^2 (9.50 l/min)

Fuel	:	C_2H_2
Fuel pressure	:	0.30 kg/cm^2 (2.30 l/min)
Burner height	:	7.50 mm
Calculation	:	Integration
Calculation time	:	1.0 sec
Delay time	:	16.0 sec
No. of replicate	:	1
Sample blank	:	Yes
X-scale (time)	:	500 sec.
Time constant	:	1 sec
Calib. curve (Int.)	:	Abs. = F (Conc.) linear.

For zinc

Lamp current	:	10.0 mA
Wave length	:	213.8 nm
Fuel Pressure	:	0.2 Kg/Cm^2 (2.0 l/min)

(rest were same as for copper)

For lead

Wave length	:	283.3 nm
Delay time	:	15.0 sec

(rest were same as for copper)

Sedimentological parameters

Colour of the soil: The soil colour along with its textural property was noted at each station during sampling.

Organic carbon: One gram of ground sediment sample was taken for analysis and was proceeded using Walkley and Black's rapid titration method (Walkley and Black, 1934) for estimation. The values are expressed in percent (%).

Heavy metals: The digestion of the soil samples was done in a mixture of perchloric acid ($HClO_4$) and nitric acid (HNO_3) as recommended by Lithner (1975). Three drops of sodium chloride (30 g/100 ml) added to 1 gm of soil followed by the addition of 8 ml of acid solutions ($HClO_4$: HNO_3 :: 10:2) and digested at 78 - 80°C for 12 hours. After cooling 3 drops of hydroxylamonium chloride (50 g/100 ml) was added to the digested sample and then diluted to 50 ml with double distilled water. Filtration was done with Whatman No.42 filtre paper and final samples were stored in 50 ml plastic bottles. Simultaneously blanks were also prepared. The estimation was done in a Perkin Elmer-2380 model Atomic Absorption Spectrophotometer (Plate III) at CMFRI, Cochin. The values are expressed in ppm (parts per million).

Biological parameters

Length and weight of the fish: Length in cm and weight in gm of L. parsia were recorded immediately on collection.

Moisture in tissues: Samples of each tissue were weighed and then dried in an oven at 60°C for three days (Szefer et al., 1990). After drying, again the final weights were taken. The difference between these two weights is the moisture content expressed in percentage lost during drying

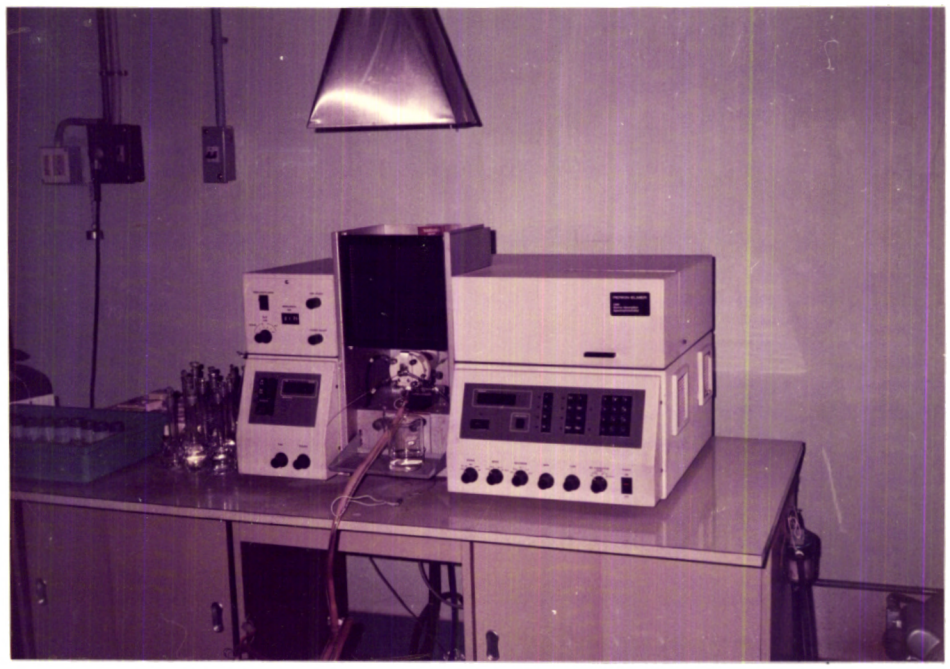


Plate III. Perkin Elmer - 2380 model Atomic Absorption Spectrophotometer.

Plate IV. Arrangement of Experimental tanks for Bioassay.

Heavy metals: The digestion of the tissue samples was carried out as per the methodology given by Dalziel and Baker (1983). The only modification in the methodology effected in the case of the tissue handled is that instead of wet tissue, dry tissue of 1 g (in less available tissues less than 1 g) was taken for digestion. The calculated amount of double distilled water (moisture loss) for each tissue sample was added prior to the addition of acids. The rest was followed as per methodology. A minor modification in the methodology was done after the experience in the laboratory. The original methodology of Dalziel and Baker (1983) is for the wet tissue (fresh tissue) where 10 g of tissue used for digestion. The same generally contains more than 70% moisture and when the concentrated acids (NHO_3 and H_2O_2) are added to this during digestion process it used to be diluted with the moisture content of the tissue. When the same was followed for the digestion of 1 g of dry tissue, fumes generated within few minutes from the digesting material and the tissue seen as black residue in the digesting container. It is assumed that the fuming and black colour of the digestion material may be due to the absence of moisture in the present digestion of dry tissue. To overcome this problem, a specific quantity of DDW is added to the dry tissue powder as calculated from the moisture loss experiment. The values obtained for heavy metals are expressed in ppm (parts per million). The error of estimation for each sample was obtained from the spectrophotometer during estimation. In the text, the accumulation of heavy metals in different tissues are expressed in terms of dry weight, which is more reproduceable than fresh weight (Latif, 1982; Rainbow, 1990).

Bioconcentration Factor: It indicates how many times a fish concentrates a metal above a certain environmental level which is usually (but not always!) that of water (Dallinger et al., 1987). For this purpose in the present study, the metal content in the tissue were back converted to wet weight basis with the help of moisture content value for each specific tissue. The calculation of the Bioconcentration Factor was done by the formula (Buikema et al., 1982 and Nair, 1984):

$$BAF = \frac{\text{Concentration of an element in the tissue}}{\text{Concentration of the element in seawater}}$$

As the unit of metal in tissue is in ppm and that of water in ppb, the units of the tissue were multiplied by 1000 before the calculation to equalise Bioconcentration Factor and it has no unit.

Data analysis

The pH, temperature, salinity and depth of water were used to describe the environmental conditions at each centre. For sediment description the colour, odour, texture and organic carbon content were used as the tool. The length and weight of the fishes were recorded to have the similarity in biological specimen collection at centres. The other parameters including salinity and total hardness were dealt in detail for statistical analysis.

Water: The salinity, total hardness, heavy metals (viz. copper, zinc and lead) of water were tabulated stationwise (indicating surface and bottom water collections) and seasonwise for each centre . Mean values of it were calculated for surface and bottom waters separately within a season

and also the seasonal means with standard deviation and range. The grand mean with standard deviation and range was calculated for each centre from the data for 1991 and 1992, to compare that with that of other similar centres in India and abroad. The average of 3 stations at a particular season in a centre was used for the calculation of Bio-concentration Factor. For statistical analysis "nondetection of metals" was treated as zero.

The ANOVA (analysis of variance) was done in a computer to find out the level of significance as listed below in Table 2.

TABLE 2. **ANOVA for heavy metals in water to find out the level of difference between the factors**

Factor	Degrees of Freedom
Factor A (year)	1
Factor B (Season)	2
Interaction of A and B	2
Factor C (Centre)	5
Interaction of A and C	5
Interaction of B and C	10
Factor D (between Surface and Bottom water)	1
Interaction of A and D	1
Interaction of B and D	2
Interaction of C and D	5

Sediment: The range, mean and standard deviation were calculated seasonwise for each centre. The same was followed to have each centre for all seasons. The ANOVA was done to find out the level of significance as follows (Table 3).

TABLE 3. **ANOVA for heavy metals in sediments to find out the level of difference between the factors**

Factor	Degrees of Freedom
Factor A (year)	1
Factor B (Season)	2
Interaction of A and B	2
Factor C (Centre)	5
Interaction of A and C	5
Interaction of B and C	10
Factor D (Station)	2
Interaction of A and D	2
Interaction of B and D	4
Interaction of C and D	10

The correlation study was made between the metal contents of water and sediment. In the similar way the seasonal average of sediments were correlated with tissues.

Tissue: The metal contents in each tissue were tabulated centrewise and seasonwise with the instrumental estimation error in parenthesis. The two-way ANOVA was done to find the level of significance between the factors as shown in Table 4.

TABLE 4. **ANOVA for heavy metals in tissues to find out the level of significance between the factors**

Factor	Degrees of Freedom
Factor A (year)	1
Factor B (Season)	2
Interaction of A and B	2
Factor C (Centre)	5

Similar study was also done for "Bioaccumulation Factors". The relation between "Bioaccumulation" and "Bioaccumulation Factor" for each tissue for a specific metal was carried out in a computer. The correlation was also studied between the biotic and abiotic factors. The better correlated (+ve or -ve) parameters are graphically presented.

For monitoring programme in the Cochin Backwater, the same methods and procedures were adopted.

Laboratory Study

Collection and transportation of test animals

For acute and chronic exposure studies, live L. parsia of 75-105 mm TL sizes and 15.0 - 30.0 g weight were collected by cast nets from brackishwater canals of Puduvypeen area, as they are easily available in good quantity. The salinity and pH of the collection site were determined to understand the water quality into which the fishes should be transferred on arrival at laboratory. With great care the fishes were

transported to laboratory in plastic bins of 100 litre capacity with the water from the collection site.

Acclimatization of the test animals in the laboratory

The fishes were acclimatized for one week (Bennett and Dooley, 1982) to laboratory condition by maintaining them in plastic pools of 2 tonne capacity with water of salinity 9.8 ± 0.76‰, pH 7.19 ± 0.12, temperature 28.0 ± 1.5°C and total hardness 2956.0 ± 142.2 ppm. To avoid fungal attack on test animals, the water was treated with 11 g/1000 l of malachite green (Mohapatra, 1989). The organisms were fed once in a day, with pellet feed purchased from the local market. The faecal matter and other waste materials were daily siphoned off, to reduce the ammonia content in water the biological filter was used. Once in two days the water in the pools was changed. Electrically operated aerators were used for aeration.

Test reagents/media

Laboratory reagents such as copper sulphate ($CuSO_4$ $5H_2O$), zinc sulphate ($ZnSO_4$ $7H_2O$) and lead nitrate ($Pb(NO_3)_2$) were used for the preparation of stock solutions individually and in combination with a ratio of 1:1:1. The desired concentration of test media were obtained by diluting the stock solution in sea water.

Experimental containers

Fibre glass tanks of 40 litre capacity were used for experiments (Brown et al., 1974). Each of the experimental tank was provided with

facilities for drainage of water from bottom and continuous aeration. Each tank was covered with velon screen netting to prevent the animals from jumping out.

Selection of experimental concentrations

The "range-finding" bioassay for each toxicants and its combination was conducted after APHA-AWWA-WPCF (1976) with experimental organisms exposed to a range of concentrations in logarithmic scale such as 1.0, 10.0, 100.0 and 1000.0 ppm. Ten animals were released to the tanks containing 35 litres of water with individual three toxicants and also a combination of the three in 1:1:1 ratio without feeding. The mortality after 12 hrs in each tank was recorded. In copper sulphate and zinc sulphate 0, 20 and 100% mortality was recorded in 10, 100 and 1000 ppm concentrations respectively. In lead nitrate, the mortality of 0% in 100 ppm and 100% in 1000 ppm was recorded. In 1:1:1 combination of toxicants 0, 10 and 100% mortality was recorded in 10, 100 and 1000 ppm respectively. Based on the Table of APHA-AWWA-WPCF (1976) of the concentrations, between 56 and 180 ppm were selected for copper sulphate and zinc sulphate, and between 75 and 210 ppm for lead nitrate and combination of toxicants.

Bioassay procedure

Static bioassay method (Reish and Oshida, 1987) was used in which the organisms were kept in same experimental medium for the entire experimental period. Each bioassay consisting of a series of five experi-

mental concentrations and a control were used (Plate IV). Each experiment was run in duplicate for combined chemicals. To avoid contamination, the control experiment tanks were maintained away from bioassay experiment tanks. As suggested in the method, the test animals were not fed during the experiment. The percentage of survival at the end of every 12, 24, 48, 72 and 96 hr and in the case or combined toxicants from 6 hr onwards, was accounted and from this the percentage of mortality was calculated. Dead animals were removed from the experimental tanks immediately.

Analysis of Data

The data obtained from the experiments were processed by "Probit analysis" (Reish and Oshida, 1987) for determination of median lethal concentration (LC50). The percentage mortality vs. log concentrations were plotted in probability papers or shortly "Probit Paper" and the "Response curves" were obtained by fitting the best fits (with correlation coefficient 'r') to the points (Mohapatra, 1989). The "Probit Paper" used here is from "AGF Tekniske Papirer" Nr.2107 Normal fordelings blanket. The values for LC16, LC50 and LC84 were obtained from the response curves. The slope function, 95% confidence limit and 95% fiducial limits (upper and lower) were calculated using the following formulae (Reish and Oshida, 1987):

$$\text{Slope (S)} = \frac{\frac{LC84}{LC50} + \frac{LC50}{LC16}}{2}$$

$$95\% \text{ confidence limit } (f_{LC50}) = S^{\frac{2.77}{\sqrt{N}}}$$

Where N = total number of organisms tested at those exposure concentra - tionswhose expected results were between 16% and 84% and 2.77 is a constant.

95% fiducial limits are:

$$\text{Upper} = \text{LC50} \times f_{\text{LC50}}$$

$$\text{Lower} = \frac{\text{LC50}}{f_{\text{LC50}}}$$

The lethal concentrations for each toxicants and its combination were plotted against time in hours in "Nomograph paper" to get the "Toxicity curves" and the corresponding 95% fiducial limits were shown for each LC50 values on graph paper. The nomograph (log-log) paper used here is from "AGF Tekniske Papirer" Nr. 2023.

The level of availability of copper, zinc and lead from its compound from viz. $CuSO_4$ $5H_2O$, $ZnSO_4$ $7H_2O$ and Pb $(NO_3)_2$ when the organisms are died in the experimental tanks were calculated using the formula of Reish and Oshida (1987):

$$\text{Grams of compound containing 1.0 g of element} = \frac{\text{Molecular weight of compound}}{\text{Molecular weight of element}}$$

i.e. 1 g of $CuSO_4$ $5H_2O$, $ZnSO_4 7H_2O$ and $Pb(NO_3)_2$ contain 0.2545, 0.2274 and 0.6256 g of copper, zinc and lead respectively.

The joint toxicity was predicted as per the methodology explained by Brown (1968) and Sprague (1970). They recommended that "acute

toxicity can be described in terms of the incipient LC50 (= lethal threshold concentration), the level of the toxicant which is lethal for 50% of individuals exposed for period sufficiently long that acute lethal action has ceased". "An approximation such as 4-day LC50 (i.e. 96 hr LC50) may be substituted if necessary, and indeed is often equivalent" (Ward and Parish, 1982). For the present study instead of lethal threshold concentration the 96 hr LC50 was taken for calculation. The strength of the toxic material was calculated as follows:

$$\text{Toxic Units} = \frac{\text{actual concentration in solution}}{\text{lethal threshold concentration (LC50)}}$$

For the mixture of metals, the number of toxic units was calculated for each of the component and since the strength of all were expressed in the same units, they were added together (EIFAC, 1980).

Chronic exposure study

This study is otherwise known as "Long-term tests" in which the experiment is "conducted from 7 days to one or more months depending upon the species used and the type of data desired" (Reish and Oshida, 1987). They say "Some long-term tests are simply extensions of the 96 hr test period which generally involve feeding the organisms and may involve renewing the test solution. Death is also used as the criterion for toxicity in this type of long-term tests".

One may ask why do we change the medium during the experiment and will it not affect/disturb the test?

The changing of test medium after every two days, necessitates to (i) remove the wastes from the animal medium, (ii) provide more level of metals in the medium for the uptake by the test animal, (iii) eliminate the effects on the animals from its own discharges such as ammonium in the test medium, and (iv) to make the experiments more realistic.

Two sub-lethal concentrations (1/10th and 1/100th of 96 hr LC50 for combined toxicants) and control experiments in sixtuplates with a total of 18 tanks (containing 10 animal each) were selected for chronic exposure experiments. The animals were fed with pellet feed once a day and the experimental water were kept well aerated as done in the case of acclimation. Half of the water from each experimental tank was replaced in every two days through the drainage pipe provided at the bottom of container. After one week of experimental run, the test organisms were removed from two control tanks and two tanks of each concentrations. All the animals from two control tanks were grouped together and divided into three lots and their tissues except ovary such as gills, muscle , skin, liver, intestine and kidney were dissected out and kept preserved for bioaccumulation and Bioaccumulation Factor study. Similarly for the other concentrations, the same procedures were followed for 2nd and 3rd week. The "Bioaccumulation" and "Bioconcentration Factors" were estimated/calculated in the similar way described earlier under the heading" Method of estimation."

Analysis of Data

The mean values of "Bioaccumulation" and "Bioconcentration Factors" were tabulated with standard deviations. For each concentration the

percentage variation of bioaccumulation from the control was calculated. The "F-test" was carried out for testing the significance between the exposure periods and concentrations for each tissue. The data obtained from the experiments were used for comparision with the results obtained from different enviroments i.e. centres.

BRIEF DEFINITION / EXPLANATION / EXPANSION OF SOME IMPORTANT TERMS USED IN THIS THESIS

(Arranged in alphabatical order)

Accumulation : To go on increasing; the action or process of accumulating.

AF : Application Factor

Bioassay : **Bioassay signifies a test in which a living tissue, organism** or group of organisms is used as a reagent for the determination of the potency of any physiological active substance of unknown activity.

Chronic : Long-term; continued; of long duration.

Confidence interval : An interval which has a specified probability of containing a given parameter or characteristic.

Confidence limit : One of the end points of a confidence interval.

Content : The amount of specified material contained, present, yielded.

Correlation coefficient (r) : The existence of association between pairs of characters where the probability of a individual having a given value of one variate depends on the value it bears of the other variate where the frequency arrays differ by more than such differences as could be caused by random sampling variation.

Electro negativity : The relative ability of an atom to attract electrons to itself.

Fiducial : A line or point established accurately as a basis of reference.

Heavy metals : The metals having specific gravity of 5.0 or above. In the text the copper, zinc and lead together referred as heavy metals.

Highly significant : At 1% F-value

KB : Bioaccumulation Factor

LC50 : The concentration of a substance which is lethal to 50% of the test animals.

Linear-correlation : If the amount of change in one variable tends to bear a constant ratio to the amount of change in the other variable, then the relation is linear.

Log - log paper : Paper ruled with two sets of mutually perpendicular, parallel lines spaced according to the logarithms of consecutive numbers rather than the numbers themselves.

Negative correlation (Reverse relation) : An increase in the value of one variable is followed by the decrease in the value of the other variable.

Positive correlation : The increase in the value of one variable is accompanied by the increases of the other variable likewise the decrease in the value of one variable is followed by the derease in the other variable.

Probit paper (Probability paper) : The scale for the variable in the X-axis is in the ordinary linear scale and that for frequency (%) in Y-axis so arranged that the distribution is "normal" and the cumulative diagram is a straight line.

Response : The value of some measurable quantity after a treatment has been applied.

Significant : At 5% F-value

Standard deviations : The root of the average of the squares of the differences from their mean, $\bar{x}$, of a number, n, of observations, x.

$$SD = \frac{1}{n} \lesssim (x - \bar{x})^2$$

Sublethal	:	Only slightly less than lethal.
Threshold value	:	The minimum input that produces a corrective action in an automatic control system.
Very highly significant	:	At 0.1% F-value

CHAPTER 1

CHAPTER 1

HEAVY METALS COPPER, ZINC AND LEAD IN WATER, SEDIMENT AND DIFFERENT TISSUES OF LIZA PARSIA AND THEIR INTERRELATIONSHIPS WITH ENVIRONMENTAL CONDITIONS

Introduction

Among the various pollutants heavy metals are particularly important due to their toxicity to biosphere. Though the metals are normal constituents of living matter and essential for many life processes at certain required concentrations, their "excess" is regarded as harmful (Wittmann, 1979). In aquatic environments, metals have been termed as "conservative pollutants", because once added to the environment, they prevail for ever (Wittmann, 1979). The metals cannot be broken down to harmless substances by bacterial action. Among the metals manganese, copper, iron and zinc are essential micronutrients, and mercury, cadmium and lead are categorised as non-essential (Qasim et al., 1988). After the catastrophic events of "Minimata" and "Itai-Itai" diseases, much information is available regarding mercury and cadmium poisoning. So the other non-essential metal viz. lead was selected as one of the metal for the present study, as not much information is available at the marine environment and on its effects on the organisms and bioaccumulation by them. From a close scrutiny of literature, the toxic nature of copper is clearly understood (Dehadrai, 1990). Zinc is one of the essential elements for most of the biological functions (Waldichuk, 1974). Along with lead; copper and zinc were also selected for getting comparative informations on their availability in the environment and their effects on biological specimen.

Bewers and Yeats (1977, 1978) defined the analysis of unfiltered water samples as "total" metal, filtered samples as "dissolved" metal and the difference between the two measurements as the "particulate" metal. In the soluble fraction, trace metal ions generally exhibit high biological availability (Phillips, 1980). According to Menasveta (1978) the dissolved form of copper, zinc and lead are the toxic ones. Due to toxic in nature the dissolved copper, zinc and lead were estimated in the estuarine waters, collected from 18 stations in 6 centres for the present study as described under "Materials and Methods".

The coastal zone represents an area of major interphase between seas and land. From either side, a set of boundaries are imposed on the water masses which determine its physical, chemical and biological characteristics as well as the fate and effects of pollutants entering into these zones. In comparison with the offshore waters, concentrations of pollutants in inshore coastal waters tend to be higher and more variable over both time and space (Waldichuk, 1974). Usually pollutant concentrations are more quickly dispersed, diluted and dissipated in Bays and open coastal areas due to water current, wind and wave actions, land run-off, etc. However, in coastal lagoons, embayments and other sheltered areas where water movement is restricted, concentration of pollutants builds up and the effects of pollution are more pronounced. To get the information on the distribution and concentrations of heavy metals in the aquatic environment as well as in the biota particularly on fish, six different coastal environments were selected along the east and west

coast of India. Each environment is unique individually and different from each other.

Among the marine vertebrates, fishes are the first preferred to be exploited and contribute to fishery. Besides contributing to the capture fisheries, mullets form one of the most highly cultivable group of fishes. Infact it is opined that the significant resources lies not so much in the existing capture fisheries, but in their potential as cultivable fishes for extensive and intensive fish farming along the coast. Among the mullets, Liza parsia gained considerable importance as a candidate species for aquaculture owing to its ability of tolerance and resistance to environmental changes, particularly salinity, temperature and pH. L. parsia, a brackishwater species of economic importance inhabiting both the coasts of India (Anon., 1980) spends majority of its life stages in estuaries. During this period, it is subjected to the toxicity of several pollutants discharged into the coastal environment accidentally or directly. On account of this and due to its economic value, L. parsia was selected as the experimental animal for the present study.

To study the content of heavy metals and Bioaccumulation Factor of it, the different tissues and organs viz. liver, kidney, ovary, gills, intestine, muscle and skin of L. parsia were selected. For this purpose the size of the specimen was kept uniform within a range where the largest specimen not more than 50% longer than the shortest as suggested by APHA-AWWA-WPCF (1976). According to Latif (1982), the best tissues to investigate the accumulation of heavy metals are gills, liver and skin

for zinc, copper and lead respectively and kidney for iron and nickel. According to Wittmann (1979) the study of the fish muscle tissue is one of the means for investigating the amount of heavy metals entering the human body through food and it has therefore been investigated more than other organs. Three possible ways of entry of metals viz. through body surface, gills and alimentary tract, have been reported in fishes by Dallinger et al. (1987). Honda et al. (1983) have reported higher concentration of copper, zinc, lead and nickel in ovary and testis of fish. Keeping in mind the reports of various authors, all the seven tissues of L. parsia quoted earlier were selected for the study of accumulation of all the three metals copper, zinc and lead. It is also aimed to:

i. find out the relationship between the biotic and abiotic parameters,

ii. the specific tissue for accumulation of a specific metal, and

iii. the difference in metal concentrations at different centres and in seasons.

Each sampling year was divided into three seasons viz. premonsoon (February - May), monsoon (June - September) and postmonsoon (October - January) in the case of southwest monsoon areas for collection (Qasim and Gopinathan, 1969) and premonsoon (June - September), monsoon (October - January) and postmonson (February - May) in northeast monsoon areas on the east coast (Lyla, 1991).

For statistical analysis of Data, the "two-way ANOVA" was selected to find out the level of significance between the parameters and the

TABLE 5. **Salinity (‰) in the Korapuzha Estuary during premonsoon, monsoon and postmonsoon of 1991 and 1992**

Station		1991			1992		
		Season					
		Premonsoon	Monsoon	Postmonsoon	Premonsoon	Monsoon	Postmonsoon
I 1	Surface	33.00	0.27	22.70	31.10	0	20.70
	Bottom	33.00	0.64	23.50	31.10	0	21.50
I 2	Surface	35.00	0.27	29.20	31.70	0	29.80
	Bottom	34.00	0.36	26.20	32.40	0.50	29.80
I 3	Surface	35.00	0.55	31.50	32.50	1.20	33.70
	Bottom	35.00	0.55	32.40	33.00	1.50	34.00
Mean	Surface	34.33	0.36	27.80	31.77	0.40	28.07
	Bottom	34.00	0.52	27.37	32.17	0.67	28.43
Grand Mean with SD		34.17± 0.98	0.44±0.16	27.58± 4.09	31.97± 7.9	0.53±0.61	28.25± 5.83
Range		(33.00-35.00)	(0.27-0.64)	(22.70-32.40)	(31.10-33.00)	(0 -1.50)	(20.70-34.00)

SD = Standard deviation, Between seasons ($P<0.001$), Mean with SD and range 20.49 ± 14.57 (0-35.0).

TABLE 6. **Salinity (‰) in the Cochin Backwater during premonsoon, monsoon and postmonsoon of 1991 and 1992**

Station		1991			1992		
		Season					
		Premonsoon	Monsoon	Postmonsoon	Premonsoon	Monsoon	Postmonsoon
II 4	Surface	0.50	0.18	0.25	0.50	0	0
	Bottom	0.50	0.27	2.50	0.50	0	0
II 5	Surface	21.00	0.46	8.50	14.10	0	6.50
	Bottom	21.50	8.55	13.20	19.30	0.07	6.50
II 6	Surface	25.00	0.91	9.20	27.00	0.50	29.00
	Bottom	25.00	23.30	25.10	30.50	13.30	29.80
Mean	Surface	15.50	0.52	5.98	13.87	0.17	11.83
	Bottom	15.67	10.71	13.60	16.77	4.46	12.10
Grand Mean with SD		15.58±11.8	5.61±9.25	9.79±8.86	15.31±12.83	2.31±5.39	11.97±13.82
Range		(0.50-25.0)	(0.18-23.30)	(0.25-25.10)	(0.50-30.50)	(0-13.30)	(0-29.80)

SD = Standard deviation, Between seasons ($P < 0.001$), Mean with SD and range 10.10±10.92(0-30.5).

TABLE 6. **Salinity (‰) in the Cochin Backwater during premonsoon, monsoon and postmonsoon of 1991 and 1992**

Station		1991 Season			1992 Season		
		Premonsoon	Monsoon	Postmonsoon	Premonsoon	Monsoon	Postmonsoon
II 4	Surface	0.50	0.18	0.25	0.50	0	0
	Bottom	0.50	0.27	2.50	0.50	0	0
II 5	Surface	21.00	0.46	8.50	14.10	0	6.50
	Bottom	21.50	8.55	13.20	19.30	0.07	6.50
II 6	Surface	25.00	0.91	9.20	27.00	0.50	29.00
	Bottom	25.00	23.30	25.10	30.50	13.30	29.80
Mean	Surface	15.50	0.52	5.98	13.87	0.17	11.83
	Bottom	15.67	10.71	13.60	16.77	4.46	12.10
Grand Mean with SD		15.58±11.8	5.61±9.25	9.79±8.86	15.31±12.83	2.31±5.39	11.97±13.82
Range		(0.50-25.0)	(0.18-23.30)	(0.25-25.10)	(0.50-30.50)	(0-13.30)	(0-29.80)

SD = Standard deviation, Between seasons ($P < 0.001$), Mean with SD and range 10.10±10.92(0-30.5).

TABLE 7. **Salinity (‰) in the Tuticorin Bay during premonsoon, monsoon and postmonsoon of 1991 and 1992**

Station		1991			1992		
		Season					
		Premonsoon	Monsoon	Postmonsoon	Premonsoon	Monsoon	Postmonsoon
III 7	Surface	36.50	31.50	35.00	35.70	33.30	34.50
	Bottom	36.50	33.70	35.00	35.70	33.30	34.50
III 8	Surface	35.60	32.00	35.00	35.00	33.00	35.50
	Bottom	35.60	34.50	35.50	35.00	33.30	35.00
III 9	Surface	36.20	31.10	36.00	35.00	33.70	33.70
	Bottom	36.50	32.00	36.50	35.20	33.70	33.70
Mean	Surface	36.10	31.53	35.33	35.23	33.33	34.57
	Bottom	36.20	33.40	35.67	35.30	33.43	34.40
Grand Mean with SD		36.15 ± 0.44	32.47 ± 1.33	35.50 ± 0.63	35.27 ± 0.34	33.38 ± 0.27	34.48 ± 0.71
Range		(35.60-36.50)	(31.10-34.50)	(35.00-36.50)	(35.00-35.70)	(33.00-33.70)	(33.70-35.50)

SD = Standard deviation, Between seasons ($P < 0.001$), Mean with SD and range 34.54 ± 1.43 (31.1-36.5).

TABLE 8. **Salinity (‰) in the Gulf of Mannar and the Palk Bay during premonsoon, monsoon and postmonsoon of 1991 and 1992**

Station		1991 Season			1992 Season		
		Premonsoon	Monsoon	Postmonsoon	Premonsoon	Monsoon	Postmonsoon
IV 10	Surface	37.00	31.80	31.00	35.00	33.70	30.50
	Bottom	37.30	31.80	31.00	35.00	33.70	30.80
IV 11	Surface	37.00	31.50	30.00	34.20	34.50	29.80
	Bottom	37.00	32.80	31.00	35.00	34.80	30.00
IV 12	Surface	36.50	32.00	30.00	33.70	34.50	27.20
	Bottom	36.90	32.40	30.00	33.70	34.50	27.20
Mean	Surface	36.83	31.77	30.33	34.30	34.23	29.17
	Bottom	36.97	32.23	30.67	34.57	34.33	29.33
Grand Mean with SD		36.95± 0.26	32.05± 0.47	30.50±0.55	34.43 ± 0.65	34.28± 0.47	29.25±1.63
Range		(36.50-37.30)	(31.50-32.80)	(30.00-31.00)	(33.70-35.00)	(33.70-34.80)	(27.20-30.80)

SD = Standard deviation, Between seasons ($P < 0.001$), Mean with SD and range 32.91 ± 2.70 (27.2-37.3).

TABLE 9. **Salinity (‰) in the Ennore Creek during premonsoon, monsoon and postmonsoon of 1991 and 1992**

Station		1991 Season			1992 Season		
		Premonsoon	Monsoon	Postmonsoon	Premonsoon	Monsoon	Postmonsoon
V 13	Surface	21.90	4.37	33.00	29.80	11.50	6.30
	Bottom	23.20	11.60	33.00	33.70	13.00	31.10
V 14	Surface	33.50	9.30	33.50	31.10	14.50	29.80
	Bottom	33.20	24.40	32.00	33.70	19.20	29.80
V 15	Surface	33.90	12.60	33.00	32.40	20.00	31.10
	Bottom	34.40	26.80	33.00	34.00	23.50	32.40
Mean	Surface	29.77	8.76	33.17	31.10	15.33	22.40
	Bottom	30.27	20.93	32.67	33.80	18.57	31.10
Grand Mean with SD		30.02± 5.81	14.85± 8.83	32.92± 0.49	32.45± 1.70	16.95± 4.66	26.75±10.07
Range		(21.90-34.40)	(4.37-26.80)	(32.00-33.50)	(29.80-33.70)	(11.50-23.50)	(6.30-32.40)

SD = Standard deviation, Between seasons ($P < 0.001$), Mean with SD and range 25.65 ± 9.22 (4.37 - 34.4).

TABLE 9. **Salinity (‰) in the Ennore Creek during premonsoon, monsoon and postmonsoon of 1991 and 1992**

Station		1991 Season			1992 Season		
		Premonsoon	Monsoon	Postmonsoon	Premonsoon	Monsoon	Postmonsoon
V 13	Surface	21.90	4.37	33.00	29.80	11.50	6.30
	Bottom	23.20	11.60	33.00	33.70	13.00	31.10
V 14	Surface	33.50	9.30	33.50	31.10	14.50	29.80
	Bottom	33.20	24.40	32.00	33.70	19.20	29.80
V 15	Surface	33.90	12.60	33.00	32.40	20.00	31.10
	Bottom	34.40	26.80	33.00	34.00	23.50	32.40
Mean	Surface	29.77	8.76	33.17	31.10	15.33	22.40
	Bottom	30.27	20.93	32.67	33.80	18.57	31.10
Grand Mean with SD		30.02± 5.81	14.85± 8.83	32.92± 0.49	32.45± 1.70	16.95± 4.66	26.75±10.07
Range		(21.90-34.40)	(4.37-26.80)	(32.00-33.50)	(29.80-33.70)	(11.50-23.50)	(6.30-32.40)

SD = Standard deviation, Between seasons ($P < 0.001$), Mean with SD and range 25.65 ± 9.22 (4.37 - 34.4).

TABLE 10. **Salinity (‰) in the Rusikulya Estuary during premonsoon, monsoon and postmonsoon of 1991 and 1992**

Station		1991 Season			1992 Season		
		Premonsoon	Monsoon	Postmonsoon	Premonsoon	Monsoon	Postmonsoon
VI 16	Surface	9.00	0.18	0.17	31.70	0	0
	Bottom	31.00	0.18	0.50	32.40	0	0.50
VI 17	Surface	8.00	0.09	0.60	32.00	0.09	0.50
	Bottom	32.00	0.27	0.80	33.00	0.18	0.50
VI 18	Surface	28.00	0.27	1.50	33.00	0.18	1.50
	Bottom	33.00	1.82	3.60	33.00	0.18	9.20
Mean	Surface	15.00	0.18	0.76	32.23	0.09	0.67
	Bottom	32.00	0.76	1.63	32.80	0.12	3.40
Grand Mean with SD		(23.50±11.74)	0.47±0.67)	1.20±1.26	32.52±0.57	0.11±0.09	2.03±3.54
Range		(8.00-33.00)	(0.09-1.82)	(0.17-3.60)	(31.70-33.00)	(0-0.18)	(0-9.20)

SD = Standard deviation, Between seasons ($P < 0.001$), Mean with SD and range 25.65 ± 9.22 (4.37 - 34.4).

any significant difference. Very high significant differences were seen between the centres and the values found in descending order were:

Centre	‰	SD	Range
Tuticorin Bay	34.54	1.43	31.10 - 36.50
Mandapam Water	32.91	2.70	27.20 - 37.30
Ennore Creek	25.65	9.22	4.37 - 34.40
Korapuzha Estuary	20.49	14.57	0 - 35.00
Cochin Backwater	10.10	10.92	0 - 30.50
Rusikulya Estuary	9.97	13.82	0 - 33.00

Very high significant difference was found for the interaction of centres with the season. ANOVA showed the significant difference between the values of surface and bottm water at 1% level and surface water found less saline than the bottom. No significant differences were found for the interactions such as centres with year, surface and bottom with year, season and centres.

Total hardness in water

The seasonal values of total hardness in water in 1991 and 1992 for centres are shown in Tables 11 to 16. The ANOVA showed the difference between years at 10% level and in general 1992 showed higher values than 1991. Very high significant differences were found between the

TABLE 11.**Total hardness (ppm) in the Korapuzha Estuary during premonsoon, monsoon and postmonsoon of 1991 and 1992**

Station		1991 Season			1992 Season		
		Premonsoon	Monsoon	Postmonsoon	Premonsoon	Monsoon	Postmonsoon
I 1	Surface	6390.6	73.5	4273.7	6304.3	150.0	5100.0
	Bottom	6520.5	735.6	4860.5	7456.5	170.0	5182.0
I 2	Surface	6701.3	52.5	5036.8	6521.7	158.0	6032.0
	Bottom	6623.4	231.1	5159.2	6721.0	210.0	5890.0
I 3	Surface	6883.1	147.1	6517.6	6820.9	190.0	6760.0
	Bottom	6701.3	105.1	6715.0	6881.0	480.0	6760.0
Mean	Surface	6658.3	91.0	5276.0	6548.9	166.0	5964.0
	Bottom	6615.1	357.3	5578.2	7019.5	286.7	5944.0
Grand Mean with SD		6636.7±169.3	224.2±258.4	5427.1±971.9	6784.2±391.5	226.3±126.2	5954.0±725.9
Range		(6390.6-6883.1)	(52.5-735.6)	(4273.7-6715.0)	(6304.3-6881.0)	(150.0-480.0)	(5100.0-6760.0)

SD = Standard deviation, Between seasons ($P < 0.001$), Mean with SD and range 4292.1 ± 3005.7 (52.5-6883.1).

TABLE 11.**Total hardness (ppm) in the Korapuzha Estuary during premonsoon, monsoon and postmonsoon of 1991 and 1992**

Station		1991 Season			1992 Season		
		Premonsoon	Monsoon	Postmonsoon	Premonsoon	Monsoon	Postmonsoon
I 1	Surface	**6390.6**	73.5	4273.7	6304.3	150.0	5100.0
	Bottom	6520.5	735.6	4860.5	7456.5	170.0	5182.0
I 2	Surface	6701.3	52.5	5036.8	6521.7	158.0	6032.0
	Bottom	6623.4	231.1	5159.2	6721.0	210.0	5890.0
I 3	Surface	6883.1	147.1	6517.6	6820.9	190.0	6760.0
	Bottom	6701.3	105.1	6715.0	6881.0	480.0	6760.0
Mean	Surface	6658.3	91.0	5276.0	6548.9	166.0	5964.0
	Bottom	6615.1	357.3	5578.2	7019.5	286.7	5944.0
Grand Mean with SD		6636.7±169.3	224.2±258.4	5427.1±971.9	6784.2±391.5	226.3±126.2	5954.0±725.9
Range		(6390.6-6883.1)	(52.5-735.6)	(4273.7-6715.0)	(6304.3-6881.0)	(150.0-480.0)	(5100.0-6760.0)

SD = Standard deviation, Between seasons ($P < 0.001$), Mean with SD and range 4292.1 ± 3005.7 (52.5-6883.1).

TABLE 12. **Total hardness (ppm) in the Cochin Backwater during premonsoon, monsoon and postmonsoon of 1991 and 1992**

Station		1991 Season			1992 Season		
		Premonsoon	Monsoon	Postmonsoon	Premonsoon	Monsoon	Postmonsoon
II 4	Surface	41.6	52.5	91.9	500.0	40.0	90.2
	Bottom	42.3	57.6	167.9	434.0	40.0	135.0
II 5	Surface	3922.0	126.0	2740.0	3173.9	40.0	2886.0
	Bottom	4012.0	2210.1	4090.7	3913.0	150.0	3100.0
II 6	Surface	4753.2	134.3	3044.6	4610.0	52.5	4008.0
	Bottom	4753.2	5777.1	4049.7	4804.3	1640.0	4252.0
Mean	Surface	2905.6	104.3	1958.8	2761.3	44.1	2328.1
	Bottom	2935.8	2681.6	2783.1	3050.4	610.0	2495.7
Grand Mean with SD		2920.7±2257.6	1392.9±2309.0	2364.1±1811.8	2905.9±1974.5	327.0±644.6	2411.9±1855.1
Range		(41.6-4753.2)	(52.5-5777.1)	(91.9-4090.7)	(434.0-4804.3)	(40.0-1640.0)	(90.2-4252.0)

SD = Standard deviation, Between seasons ($P < 0.001$), Mean with SD and range 2052.8 ± 1957.8 (40.0-5777.1).

TABLE 12. **Total hardness (ppm) in the Cochin Backwater during premonsoon, monsoon and postmonsoon of 1991 and 1992**

Station		1991 Season			1992 Season		
		Premonsoon	Monsoon	Postmonsoon	Premonsoon	Monsoon	Postmonsoon
II 4	Surface	41.6	52.5	91.9	500.0	40.0	90.2
	Bottom	42.3	57.6	167.9	434.0	40.0	135.0
II 5	Surface	3922.0	126.0	2740.0	3173.9	40.0	2886.0
	Bottom	4012.0	2210.1	4090.7	3913.0	150.0	3100.0
II 6	Surface	4753.2	134.3	3044.6	4610.0	52.5	4008.0
	Bottom	4753.2	5777.1	4049.7	4804.3	1640.0	4252.0
Mean	Surface	2905.6	104.3	1958.8	2761.3	44.1	2328.1
	Bottom	2935.8	2681.6	2783.1	3050.4	610.0	2495.7
Grand Mean with SD		2920.7±2257.6	1392.9±2309.0	2364.1±1811.8	2905.9±1974.5	327.0±644.6	2411.9±1855.1
Range		(41.6-4753.2)	(52.5-5777.1)	(91.9-4090.7)	(434.0-4804.3)	(40.0-1640.0)	(90.2-4252.0)

SD = Standard deviation, Between seasons ($P < 0.001$), Mean with SD and range 2052.8 ± 1957.8 (40.0-5777.1).

TABLE 13. **Total hardness (ppm) in the Tuticorin Bay during premonsoon, monsoon and postmonsoon of 1991 and 1992**

Station		1991 Season			1992 Season		
		Premonsoon	Monsoon	Postmonsoon	Premonsoon	Monsoon	Postmonsoon
III 7	Surface	9453.2	5184.8	6753.2	6852.0	6160.0	7649.0
	Bottom	8192.2	5638.0	6792.5	6860.0	5850.0	7649.0
III 8	Surface	8403.5	5952.9	6467.5	7230.0	3640.0	7826.1
	Bottom	8403.5	7631.5	6623.4	7750.0	3895.0	7739.2
III 9	Surface	7352.5	5730.5	6623.4	7070.0	6882.0	7282.6
	Bottom	7877.7	5958.1	6701.3	7120.0	7904.0	7500.0
Mean	Surface	8403.1	5622.7	6614.7	7050.7	5560.7	7585.9
	Bottom	8157.8	6409.2	6705.7	7243.3	5883.0	7629.4
Grand Mean with SD		8280.4±698.1	6015.9±840.5	6660.2±116.3	7147.0±330.6	5721.8±1672.6	7607.7±192.7
Range		(7352.5-9453.2)	(5184.8-7631.5)	(6467.5-6792.5)	(6852.0-7750.0)	(3640.0-7904.0)	(7292.6-7826.1)

SD = Standard deviation, Between seasons ($P < 0.001$), Mean with SD and range 6905.5±1165.7 (3640.0-9453.2).

TABLE 14. **Total hardness (ppm) in the Gulf of Mannar and the Palk Bay during premonsoon, monsoon and post monsoon of 1991 and 1992**

Station		1991 Season			1992 Season		
		Premonsoon	Monsoon	Postmonsoon	Premonsoon	Monsoon	Postmonsoon
IV 10	Surface	9018.0	5838.2	6182.0	7280.0	6160.0	7391.3
	Bottom	9018.0	5900.8	6182.0	7280.0	5958.0	7750.0
IV 11	Surface	8666.8	6487.2	5688.3	7700.0	6760.0	6630.4
	Bottom	8949.9	6604.0	5792.2	7758.0	6585.0	6782.6
IV 12	Surface	6931.9	6105.0	5792.2	7180.0	8360.0	5760.9
	Bottom	6883.9	6317.3	5760.0	7180.0	8360.0	5760.9
Mean	Surface	8205.6	6143.5	5887.5	7386.7	7093.3	6594.2
	Bottom	8283.9	6273.8	5911.4	7406.0	6967.7	6764.5
Grand Mean with SD		8244.8±1043.7	6208.8±312.6	5899.5±222.1	7396.3±262.2	7030.5±1069.1	6679.4±818.9
Range		(6883.9-9018.0)	(5838.2-6604.0)	(5688.3-6182.0)	(7180.0-7700.0)	(5958.0-8360.0)	(5760.9-7750.0)

SD = Standard deviation, Between seasons ($P < 0.001$), Mean with SD and range 6909.9±1016.4 (5688.3-9018.0).

TABLE 15. **Total hardness (ppm) in the Ennore Creek during premonsoon, monsoon and postmonsoon of 1991 and 1992**

Station		1991			1992		
		Season					
		Premonsoon	Monsoon	Postmonsoon	Premonsoon	Monsoon	Postmonsoon
V 13	Surface	4359.6	687.1	6169.1	5530.0	185.7	4812.5
	Bottom	4980.0	2104.4	6182.0	6470.0	2400.0	6956.5
V 14	Surface	5646.4	1602.7	6545.5	6140.0	2304.0	6782.6
	Bottom	5881.9	4426.9	6182.0	6500.0	3006.0	6739.1
V 15	Surface	8402.8	2289.3	6026.0	6800.0	4384.0	6739.1
	Bottom	7509.3	6181.9	6441.6	6800.0	6550.0	6304.3
Mean	Surface	6136.3	1526.4	6246.9	6156.7	2291.2	6111.4
	Bottom	6123.7	4237.7	6268.5	6590.0	3985.3	6666.6
Grand Mean with SD		6130.0±1537.6	2882.1±2034.0	6257.7±194.8	6383.3±491.8	3416.8±1767.8	6389.0±801.8
Range		(4359.6-8402.8)	(687.1-6181.9)	(6026.0-6545.5)	(5530.0-6860.0)	(1857.0-6550.0)	(4812.5-6956.5)

SD = Standard deviation, Between seasons ($P < 0.001$), Mean with SD and range 5195.1±2018.1 (687.1-8402.8).

TABLE 16. **Total hardness (ppm) in the Rusikulya Estuary during premonsoon, monsoon and postmonsoon of 1991 and 1992**

Station		1991 Season			1992 Season		
		Premonsoon	Monsoon	Postmonsoon	Premonsoon	Monsoon	Postmonsoon
VI 16	Surface	1351.0	84.0	89.0	6347.8	40.0	90.2
	Bottom	5818.2	126.0	100.7	6658.2	40.0	135.0
VI 17	Surface	1558.4	73.5	128.1	6340.2	50.0	135.0
	Bottom	5844.2	158.0	103.8	7717.4	120.0	135.0
VI 18	Surface	1428.6	158.0	167.5	7717.4	210.0	180.5
	Bottom	2389.6	210.0	226.2	7717.4	210.0	486.0
Mean	Surface	1446.0	105.2	128.2	6801.8	100.0	135.2
	Bottom	4864.0	164.7	143.6	7364.3	123.3	252.0
Grand Mean with SD		3065.0±2174.7	134.9±51.3	135.9±52.3	7083.1±704.3	113.3±80.2	193.6±146.1
Range		(1351.0-5844.2)	(84.0-210.0)	(89.0-226.2)	(6340.2-7717.4)	(50.0-210.0)	(90.2-486.0)

SD = Standard deviation, Between seasons ($P < 0.001$), Mean with SD and range 1787.4 ± 2734.5 (50.0-7717.4).

seasons and higher mean values were seen for premonsoon followed by postmonsoon and monsoon. Among the centres very high significant differences were found and the mean values with standard deviation and range were calculated and given below in descending order:

Centre	ppm	SD	Range
Mandapam Water	6909.8	1016.4	5688.3 - 9018.0
Tuticorin Bay	6905.5	1165.7	3640.0 - 9453.2
Ennore Creek	5195.1	2018.1	687.1 - 8402.8
Korapuzha Estuary	4292.1	3005.7	52.5 - 6883.1
Cochin Backwater	2052.8	1957.8	40.0 - 5777.1
Rusikulya Estuary	1787.4	2734.5	50.0 - 7717.4

Between the surface and bottom values the difference was found at 5% level and higher values were recorded for bottom waters. The interaction between centres and seasons was significant at very high level. No significant difference was found for the interactions such as seasons with year, surface and bottom with year, seasn and centre. The interaction of centre with year was significant at 10% level.

The correlation study for pooled data for different seasons showed the positive relatinship between the salinity and total hardness. The coefficients (r) for premonson, monsoon and postmonsoon were found 0.898, 0.967 and 0.937 respectively and were significant at 5% level.

Other parameters in water and sediment

The temperature and pH of water were recorded and organic carbon of the soil were estimated for different stations at different centres to have an idea about the environments.

Centre	Temperature(°C)	pH	Organic carbon (%)
Korapuzha Estuary	28.7 ± 1.3	7.75 ± 0.27	1.90 ± 1.06
Cochin Backwater	29.7 ± 3.1	7.50 ± 0.45	0.86 ± 0.48
Tuticorin Bay	30.7 ± 4.1	8.00 ± 0.13	0.34 ± 0.21
Mandapam Water	29.7 ± 1.3	7.97 ± 0.14	0.16 ± 0.12
Ennore Creek	29.2 ± 1.2	7.08 ± 0.58	0.85 ± 0.83
Rusikulya Estuary	27.6 ± 3.4	7.33 ± 0.41	0.68 ± 0.72

Section 2 : Copper

Copper at a glance (Average of two years)

Centre Code	in water (ppb)	in sediment (ppm dry wt)	in tissue (ppm dry wt)						
			L	G	K	It	O	S	M
I	6.82	16.68	19.2	12.0	35.8	7.3	8.0	5.4	5.2
II	10.38	36.86	15.1	8.9	12.3	8.3	4.5	6.1	4.1
III	5.60	8.65	129.4	4.2	21.7	22.6	7.2	5.8	8.8
IV	6.33	1.64	52.2	4.9	27.2	5.2	4.4	5.4	5.8
V	8.92	45.22	58.1	7.8	16.2	7.6	6.7	5.6	6.3
VI	3.61	8.30	5.7	7.7	12.5	8.5	13.3	3.9	6.3

I = Korapuzha Estuary, II = Cochin Backwater, III = Tuticorin Bay, IV = Mandapam Water, V = Ennore Creek, VI = Rusikulya Estuary, L = Liver, G = Gills, K = Kidney, It = Intestine, O = Ovary, S = Skin and M = Muscle.

TABLE 17. **Copper in water (ppb) and sediment (ppm) in the Korapuzha Estuary during premonsoon, monsoon and postmonsoon of 1991 and 1992**

Sample	Station		1991 Premonsoon	1991 Monsoon	1991 Postmonsoon	1992 Premonsoon	1992 Monsoon	1992 Postmonsoon
			Season					
Water	I 1	Surface	6.5	1.5	18.5	4.5	4.5	3.5
		Bottom	7.0	9.0	6.0	11.5	14.0	2.0
	I 2	Surface	9.0	2.5	3.8	14.0	3.5	4.0
		Bottom	9.5	9.0	3.5	9.5	15.0	3.0
	I 3	Surface	7.5	5.0	1.5	8.0	6.0	3.0
		Bottom	10.5	2.5	3.5	8.0	13.0	3.0
	Mean	Surface	7.67	3.00	7.67	8.83	4.67	3.50
		Bottom	9.00	6.83	4.33	9.67	14.00	2.67
	Grand Mean with SD		8.33 ± 1.57	4.92 ± 3.37	6.00 ± 6.30	9.25 ± 3.27	9.33 ± 5.21	3.08 ± 0.66
	Range		(6.5 - 10.5)	(1.5 - 9.0)	(1.5 - 18.5)	(4.5 - 9.5)	(4.5 - 15.0)	(2.0 - 4.0)
Sediment	I 1		23.25	24.75	21.25	21.25	10.25	16.75
	I 2		24.75	11.75	20.25	11.75	1.75	13.75
	I 3		24.25	1.25	17.75	36.75	1.50	17.25
	Mean with SD		24.08 ± 1.93	12.58 ± 11.08	19.75 ± 2.07	23.25 ± 11.43	4.50 ± 4.62	15.92 ± 2.44
	Range		(21.0 - 26.5)	(ND - 30.0)	(17.0 - 22.0)	(9.5 - 38.0)	(0 - 11.0)	(12.0 - 18.5)

SD = Standard deviation and ND = Not detected.
In water between seasons in a year ($P < 0.01$), between the seasons ($P < 0.001$), Mean with SD and range 6.82 ± 4.87 (1.5 - 18.5).

In sediment between seasons ($P < 0.01$), between seasons within a year ($P < 0.001$), between stations ($P < 0.001$), between the stations within a season ($P < 0.001$), Mean with SD and range 16.68 ± 9.25 (ND - 38.0).

TABLE 18. **Copper in water (ppb) and sediment (ppm) in the Cochin Backwater during premonsoon, monsoon and postmonsoon of 1991 and 1992**

Sample	Station		1991 Premonsoon	1991 Monsoon	1991 Postmonsoon	1992 Premonsoon	1992 Monsoon	1992 Postmonsoon
			Season					
Water	II 4	Surface	6 .0	2 .0	5 .0	6 .0	3 .0	8 .5
		Bottom	8 .0	2 .5	6 .5	10 .0	5 .5	11 .0
	II 5	Surface	7 .5	2 .5	22 .0	8 .5	4 .0	6 .0
		Bottom	9 .0	4 .0	34 .5	14 .5	6 .0	6 .0
	II 6	Surface	5 .0	2 .5	39 .5	6 .0	2 .0	18 .5
		Bottom	7 .0	16 .0	40 .0	9 .5	11 .5	20 .0
	Mean	Surface	6 .33	2 .33	22 .17	6 .83	3 .00	11 .0
		Bottom	8 .00	7 .50	27 .00	11 .33	6 .67	12 .33
	Grand Mean with SD		7.17 ± 1.29	4.29 ± 5.4	24.58 ±15.97	9.08 ± 3.15	4.83 ± 3.55	11.67 ± 6.18
	Range		(5.5 - 9.0)	(2.0 - 16.0)	(5.0 - 40.0)	(6.0 -14.5)	(2.0 - 11.5)	(6.0 - 20.0)
Sediment	II 4		68 .75	52 .25	40.25	80 .25	78 .00	45 .50
	II 5		45 .25	47 .75	41 .00	26 .25	35 .25	30 .00
	II 6		15 .25	15 .00	11 .50	5 .25	11 .50	11 .00
	Mean with SD		43.08 ±24.16	38.00 ±19.61	30.92 ±15.57	37.25 ±34.82	43.58 ±28.58	28.33 ±14.93
	Range		(12.5 -70.5)	(10.5 -63.0)	(11.0 -46.5)	(3.0 -85.5)	(17.5 -87.5)	(11.0 -45.5)

SD = Standard deviation.

In water between seasons in a year ($P < 0.01$), between the seasons ($P < 0.001$), Mean with SD and range 10.38 ± 9.71 (2.0 - 40.0).

In sediment between seasons ($P < 0.01$), between seasons within a year ($P < 0.001$), between stations ($P < 0.001$), between the stations within a season ($P < 0.001$), Mean with SD and range 36.86 ± 22.65 (3.0 - 87.5).

TABLE 19. **Copper in water (ppb) and sediment (ppm) in the Tuticorin Bay during premonsoon, monsoon and postmonsoon of 1991 and 1992**

Sample	Station		1991 Premonsoon	1991 Monsoon	1991 Postmonsoon	1992 Premonsoon	1992 Monsoon	1992 Postmonsoon
			Season					
Water	III 7	Surface	6.0	1.5	7.5	4.0	1.5	8.5
		Bottom	1.5	2.5	9.5	13.0	2.0	11.5
	III 8	Surface	5.0	8.5	6.0	7.5	5.5	3.5
		Bottom	7.0	8.0	6.0	7.5	4.5	3.0
	III 9	Surface	5.0	2.0	4.0	9.5	2.5	4.0
		Bottom	7.0	3.0	5.5	10.5	3.0	4.5
	Mean	Surface	5.33	4.00	5.83	7.17	3.17	5.17
		Bottom	5.17	4.50	7.00	10.33	3.17	6.33
	Grand Mean with SD		5.25 ± 2.05	4.25 ± 3.14	6.42 ± 1.88	8.75 ±2.93	3.17 ±1.54	5.75 ± 3.47
	Range		(1.5 - 7.0)	(1.5 - 8.5)	(4.0 - 9.5)	(4.5 - 13.0)	(1.5 - 5.5)	(3.0 - 11.5)
Sediment	III 7		3.00	1.25	3.25	1.75	2.25	19.25
	III 8		9.00	5.75	6.25	3.25	1.75	36.25
	III 9		4.25	4.25	11.75	5.25	7.00	30.25
	Mean with SD		5.42 ± 3.01	3.75 ± 2.16	7.08 ± 3.89	3.42 ± 1.93	3.67 ± 2.64	28.58 ± 8.11
	Range		(3.0 - 10.0)	(1.0 - 6.5)	(3.0 -12.5)	(1.5 -6.5)	(1.0 - 7.0)	(18.0 - 40.0)

SD = Standard deviation.

In water between seasons in a year ($P < 0.01$), between the seasons ($P < 0.001$), Mean with SD and range 5.60 ± 2.95 (1.5 - 13.0).

In sediment between seasons ($P < 0.01$), between seasons within a year ($P < 0.001$), between stations ($P < 0.001$), between the stations within a season ($P < 0.001$), Mean with SD and range 8.65 ± 9.78 (1.0 - 40.0).

TABLE 20. **Copper in water (ppb) and sediment (ppm) in the Gulf of Mannar and the Palk Bay during premonsoon, monsoon and postmonsoon of 1991 and 1992**

Sample	Station	1991 Premonsoon	1991 Monsoon	1991 Postmonsoon	1992 Premonsoon	1992 Monsoon	1992 Postmonsoon
		Season					
Water	IV 10 Surface	1.0	3.0	4.0	9.5	4.5	2.0
	IV 10 Bottom	1.0	6.5	4.0	11.0	4.5	4.0
	IV 11 Surface	1.0	3.5	6.0	18.0	4.5	3.5
	IV 11 Bottom	1.5	14.5	8.5	24.0	4.5	10.5
	IV 12 Surface	2.0	6.0	3.5	10.5	3.5	3.0
	IV 12 Bottom	10.5	11.5	2.0	13.5	4.0	3.0
	Mean Surface	1.33	4.17	4.50	12.67	4.17	2.83
	Mean Bottom	4.33	10.83	4.83	16.17	4.33	5.83
	Grand Mean with SD	2.83±3.78	7.50±4.57	4.67±2.27	14.42±5.60	4.25±0.42	4.33±3.09
	Range	(1.0-10.5)	(3.0-14.5)	(2.0-8.5)	(9.5-24.0)	(3.5-4.5)	(2.0-10.5)
Sediment	IV 10	0.75	0.50	2.25	1.25	1.75	1.25
	IV 11	4.55	2.75	2.00	ND	5.25	0.75
	IV 12	0.50	0.75	0.75	2.25	1.25	1.00
	Mean with SD	1.93±2.21	1.33±1.25	1.67±0.88	1.17±1.13	2.75±2.02	1.00±1.14
	Range	(ND-5.6)	(ND-3.0)	(ND-2.5)	(ND-3.0)	(1.0-6.0)	(ND-2.5)

SD = Standard deviation and ND = Not detected.
In water between seasons in a year ($P < 0.01$), between the seasons ($P < 0.001$), Mean with SD and range 6.33 ± 5.13 (1.0 - 24.0).

In sediment between seasons ($P < 0.01$), between seasons within a year ($P < 0.001$), between stations ($P < 0.001$), between the stations within a season ($P < 0.001$), Mean with SD and range 1.64 ± 1.51 (ND - 6.0).

TABLE 21. **Copper in water (ppb) and sediment (ppm) in the Ennore Creek during premonsoon, monsoon and postmonsoon of 1991 and 1992**

Sample	Station		1991 Premonsoon	1991 Monsoon	1991 Postmonsoon	1992 Premonsoon	1992 Monsoon	1992 Postmonsoon
			Season					
Water	V 13	Surface	6.5	5.5	5.5	10.5	6.0	9.0
		Bottom	20.5	9.5	8.5	16.0	7.5	11.5
	V 14	Surface	12.0	4.0	4.5	18.0	5.5	2.5
		Bottom	30.0	8.5	9.0	11.0	12.0	14.0
	V 15	Surface	2.5	4.0	9.0	8.5	4.0	2.5
		Bottom	5.0	8.5	10.5	9.5	4.5	4.5
	Mean	Surface	7.00	4.50	6.33	12.33	5.17	4.67
		Bottom	18.50	8.83	9.33	12.17	8.17	10.00
	Grand Mean with SD		12.75 ± 10.61	6.67 ± 2.46	7.83 ± 2.32	12.25 ± 3.83	6.67 ± 3.11	7.33 ± 4.89
	Range		(2.5 - 30.0)	(4.0 - 9.5)	(4.5 - 10.5)	(8.5 - 18.0)	(4.0 - 12.5)	(2.5 - 14.0)
Sediment	V 13		56.00	115.75	24.25	40.25	102.25	57.75
	V 14		45.00	86.75	16.75	31.00	78.00	55.50
	V 15		12.50	21.50	7.25	8.25	33.75	21.50
	Mean with SD		37.83 ± 20.64	74.67 ± 43.34	16.08 ± 8.11	26.50 ± 15.30	71.33 ± 31.30	44.92 ± 19.00
	Range		(11.0 - 61.5)	(21.5 - 121.5)	(7.0 - 28.0)	(7.0 - 46.5)	(32.0 - 108.0)	(20.5 - 65.5)

SD = Standard deviation.

In water between seasons in a year ($P < 0.01$), between the seasons ($P < 0.001$), Mean with SD and range 8.92 ± 5.57 (2.5 - 30.0).

In sediment between seasons ($P < 0.01$), between seasons within a year ($P < 0.001$), between stations ($P < 0.001$), between the stations within a season ($P < 0.001$), Mean with SD and range 45.2 ± 31.86 (7.0 - 121.5).

TABLE 22. **Copper in water (ppb) and sediment (ppm) in the Rusikulya Estuary during premonsoon, monsoon and postmonsoon of 1991 and 1992**

Sample	Station	1991 Premonsoon	1991 Monsoon	1991 Postmonsoon	1992 Premonsoon	1992 Monsoon	1992 Postmonsoon
				Season			
Water	VI 16 Surface	6.0	ND	1.5	2.0	2.0	3.5
	VI 16 Bottom	6.5	3.0	2.5	3.0	2.5	4.0
	VI 17 Surface	6.0	2.0	0.5	6.0	4.0	2.5
	VI 17 Bottom	6.5	ND	5.5	6.0	1.0	4.5
	VI 18 Surface	5.0	2.0	1.5	3.0	2.0	6.0
	VI 18 Bottom	10.0	2.5	5.0	6.5	2.0	3.5
	Mean Surface	5.67	1.33	1.17	3.67	2.67	4.00
	Mean Bottom	7.67	1.83	4.3	5.17	1.83	4.00
	Grand Mean with SD	6.67 ± 1.72	1.58 ± 1.28	2.75 ± 2.04	4.42 ± 1.96	2.25 ± 0.99	4.00 ± 1.18
	Range	(5.0 - 10.0)	(ND - 3.0)	(0.5 - 5.5)	(2.0 - 6.5)	(1.0 - 4.0)	(2.5 - 6.0)
Sedement	VI 16	29.65	2.25	15.50	24.25	1.25	8.75
	VI 17	8.25	4.75	2.25	9.75	0.75	6.25
	VI 18	4.25	4.75	7.25	10.75	1.75	7.00
	Mean with SD	14.05 ± 12.48	3.92 ± 1.72	8.33 ± 6.06	14.92 ± 7.55	1.25 ± 0.69	7.33 ± 1.25
	Range	(2.5 - 33.3)	(1.0 - 6.0)	(1.5 - 16.5)	(7.0 - 26.0)	(ND - 2.0)	(5.5 - 9.0)

SD = Standard deviation and ND = Not detected.
In water between seasons in a year ($P < 0.01$), between the seasons ($P < 0.001$), Mean with SD and range 3.61 ± 2.21 (ND - 10.0).

In sediment between seasons ($P < 0.01$), between seasons within a year ($P < 0.001$), between stations ($P < 0.001$), between the stations within a season ($P < 0.001$), Mean with SD and range 8.30 ± 7.73 (ND - 33.3).

in seawater (Orr and Marshall, 1969). Bryan (1984) reported 0.092 - 0.240 ppb of copper in natural seawater (unpolluted) and 1.80 ppb of the same element in freshwater. From the present investigation the mean values of copper for individual centres were seen higher than that of natural seawater and freshwater.

The World Health Organisation's (WHO) standard for copper in water is 3.0 ppb (Qasim and Sengupta, 1983). The safe level for copper is 25.0 ppb as recommended by Environmental Protection Agency (EPA) of United States of America (Anon., 1991 a). In comparing with WHO standard the copper values in centres were seen polluted (Tables 17 to 22). But in all the centres, the lower ranges given in parentheses were quite below the standard value. In comparing with the safe level of EPA, the copper content in all centres were quite below the permissible safe limit. The copper content was recorded on the higher side in St. II 5 (Surface) and St. II 6 (Surface and Bottom) in postmonsoon of 1991 (Table 18) The probable reason for the same can be attributed to the dredging operations and laying oil pipe line by Dredging Corporation of India and Cochin Oil Refineries respectively in the Ernakulam Channel. The bottom water at St. V 14 of Ennore Creek recorded 30.0 ppb of copper in premonsoon of 1991 (Table 21) was above the EPA safe level. This station was near to the city garbage and waste dumping ground from where the heavy metals might have reached the station. According to Berrow and Webber (1972) "average" sewage sludge and city wastes are the major sources of copper in the aquatic environment.

The ranges of dissolved copper (µg/l or ppb) in the Indian Ocean was determined by various workers. The values reported are:

Value (ppb)	Author(s)
0.5 - 49.1	Topping, 1969
0.2 - 1.2	Chester and Stoner, 1974
01.9 - 19.9	Sanzgiri and Caroline, 1979
20.0 - 37.2	Braganca and Sanzgiri, 1980
01.2 - 17.5	Qasim and Sengupta, 1983
0.22 - 1.2	Fowler et al., 1984

The results and mean values of copper in water obtained from the present study for all the centres (Section : Copper at a glance), were however, well within the range reported by the above authors. The values reported by Chester and Stoner (1974) and Fowler et al. (1984) for copper in the Indian Ocean are 0.2 - 1.2 and 0.22 - 1.2 ppb respectively. Comparing with these values of copper from the Indian Ocean, the centres in the present study recorded on the higher side. This coincides with result of Tkalin (1992) who worked on the coastal zone near Vladivostok and Nakhodka, and reported significantly higher trace metals in the zone than the open Japan Sea. Braganca and Sanzgiri (1980) have shown that the nearshore surface water have higher dissolved and particulate copper than the waters at the stations further away from shore.

The copper concentration obtained in the present study for different centres are well compared with the values (in ppb) reported by several

authors for different coastal and estuarine waters in India and abroad. Some of them from abroad are:

Environment	Value (ppb)	Author(s)
Chao Phraya Estuary, Thailand	5.5	Menasveta, 1978
East Coast of England	2.9	Taylor, 1982
Belgian and Dutch Coast	0.34 - 1.91	Mart et al., 1982
River Tees Estuary	0.5 -10.0	Taylor, 1982
Upper Humber Estuary	8.0	Gardiner, 1982
East Coast of Britain	0.12 - 0.58	Balls, 1985
From India		
Mandovi-Zuari Estuaries	2.5 -69.77	Zingde et al., 1976
Bombay Harbour Bay	2.6 - 7.34	Matkar et al., 1981
Saurashtra Coast	5.73 - 8.0	Kesava Rao and Indusekhar, 1986
Mindhola River Estuary	2.7 -15.9	Zingde et al., 1988
Vellar Estuary	8.0 -26.5	Lyla, 1991
Korapuzha Estuary	1.5 -18.5	Present Study
Cochin Backwater	2.0 -40.0	"
Tuticorin Bay	1.5 -13.0	"
Mandapam Water	1.0 -24.0	"
Ennore Creek	2.5 -30.0	"
Rusikulya Estuary	ND -10.0	"

Though the copper content for each centre in the present investigation was well compared, they differ significantly among themselves.

This may be due to different sources of pollutants to each system as mentioned in Table 1.

The average world concentration of copper in stream water (upper reaches of the river) is 7.0 ppb (Goldberg et al., 1971). The mean values in Cochin Backwater (River Periyar) and Ennore Creek (Kotaliar River) were seen considerably higher than that of average world stream concentration. In all the centres the upper range were seen higher (Tables 17 to 22).

A distinct stratification, resulting from suspension and upwhirling of sediments in the bottom waters was found in the tidal Elbe (Mart and Nurnberg, 1986). For all trace metals such as Cu, Pb and Cd, results from 4-6 m depth samples were generally higher. In the present work a very high significant difference was seen between the bottom and surface collections placing the bottom concentrations to the higher side. At some stations in the open sea area, Mart and Nurnberg (1986) noted that depth samples (4-12 m) yielded slightly enhanced (10-20 %) total trace metal, compared to the surface water. According to Sengupta et al. (1978), higher copper values were encountered frequently near the bottom of the shallow station indicaing that the fraction of this metal concentrated at the surface has been released by the process of decomposition of organic matter at the bottom.

The trace metal distribution in the coastal environment to a great extent is influenced by freshwater inflow (Riley and Chester, 1971).

Both the extractable and organically associated forms of copper decrease with the onset of monsoon by dilution and explained below. The input of copper which is expected to be high in the upper reaches of the river during the monsoon period, might have been affected by the increased load of suspended matter causing adsorption of soluble copper in the fresh-water zone and gradually released in the gradient zone, reaching maximum near the mouth (George and Sawkar, 1981). From statistical analysis, the decreased copper content was seen in monsoon than premonsoon and postmonsoon in the present study. The reason may be attributed to that given by George and Sawkar (1981). Sholkovitz (1978) suggested that as much as 40% of the dissolved copper in some rivers might be removed during mixing, presumably, due to flocculation with humic substances. According to Nair (1984) copper could effectively be removed from solution by adsorption on particles of hydrated ferric hydroxide and manganese oxide, the former being more abundant in coastal waters. According to the reports of Phillips (1980) the tendency of each metal to form che-lates varies widely and the copper is probably the only element to exhibit significant chelate formation with humic materials in freshwater. That may also be a reason for the decreased content of copper in monsoon than other seasons. The premonsoon recorded higher concentrations followed by postmonsoon. Turekian (1971) observed that the absorbed form of metals in streams and rivers are always released to a greater or lesser extent on contact with seawater due to their displacement by major ions like magnesium and calcium in seawater. In the present study premonsoon and postmonsoon recorded higher salinity in the study area indicating

the low run-off. The contribution of calcium and magnesium ions are more in higher saline areas. The above reason supports the increase of copper in premonsoon time. During low run-off the stagnating time of water in the estuarine nearshore zone is longer than during high run-off monsoon period. Release from sediments therefore, would be expected to have a greater dissolution of copper in stagnating water in the estuary during low run-off (Windom et al., 1983).

In the present study, very weak negative relations were obtained between salinity and copper content in premonsoon and postmonsoon as described earlier under results. The same was also seen with total hardness. In the works of Duinker and Nolting (1982) in Southern Bight of the North Sea and Windom et al. (1983) in southeastern United States estuaries; it was seen the similar relations between salinity and copper. A very good and highly significant relationship was obtained between the salinity and copper. A very good and highly significant relationship was also obtained between the salinity and total hardness (Section : Total hardness). The similar relationship of total hardness with copper as seen in the case of salinity is self explanatory.

Copper in sediment

The yearwise, seasonwise and stationwise copper contents in sediments are presented in Tables 17 to 22. The two-way ANOVA from the computer indicated no significant difference between the years. High significant differences were found (at 1% level) between seasons i.e., higher concentrations in monsoon followed by premonsoon and postmonsoon.

Similar significances (at 0.1% level) were obtained for the interactions such as between seasons and year, and between stations and season. Very high significant differences were seen between the centres. Very high significant differences were also seen for the interactions between centre and season, between station and centre, and between the stations. No significant differences were observed for interactions between centre and year and between station and year.

From the correlation study pooling the data for copper in water and sediment, a poor and nonsignificant positive coefficient ($r = 0.255$) was found between them.

Discussion

The concentration of copper was reported with coefficient of variation in parentheses 46.4 (0) ppm for USGS rock standards such as MAG-1, SCO -1 and G-2 (Flanagan, 1976) and by Subramanian et al. (1989) as 45.6 (0.007), 45.0 (0.018) and 46.0 (0.003) ppm for MAG-1, SCO-1 and G-2 standards respectively. In the present invesigation, the mean copper in sediment for respective centres were found below the standard values. None of the stations in Korapuzha Estuary, Tuticorin Bay, Mandapam coastal area and Rusikulya Estuary showed higher concentration than the standards. In Cochin Backwater St. II 4, and Ennore Creek St. V 13 and St. V 14 the sediments showed higher copper concentration than that of standards. The St. II 4 and St. V 13 were at upstream of Cochin Backwater and Ennore Creek respectively. The middle station V 14 of Ennore Creek was near to the city garbage dumping ground. Pregatheeswaran

et al. (1986) observed high concentrations of copper at stations near the industrial (metallurgical) and sewage discharges along Visakhapatnam and Madras Coasts. As the above mentioned three stations were near to the industrial belt and sewage discharges (Table 1), the same might have been the reason for increased copper content. According to Berrow and Webber (1972) the "average" sewage sludge contains 700 ppm of copper. Holmes (1986) reported the oxygen-poor zone in the water column near the sediment-water interface in the harbour at Corpus Christi, Texas. This causes the chalcophilic metals such as copper, zinc, lead, etc. to precipitate from the water resulting to settlement of copper in high concentrations in the sediments particularly near the outlets of the industrial area. The same reason may be attributed to the present increase of copper in St. II 4 (Cochin Backwater), St. V 13 and St. V 14 (Ennore Creek, Madras).

The natural content of copper in the world sediments was given 0.20 ppm by Rankama and Sahama (1960) and reported by Menasveta (1978). Two marine sediment reference materials such as "Miramichi Estuary Standard Sediment (MESS-1)" and "Baie des Chaleurs Standard Sediments (BCSS-1)" were analysed by Ackroyd et al. (1987). The results obtained by them for copper were 29.3 ± 1.6 and 23.4 ± 2.4 ppm dry wt for MESS-1 and BCSS-1 respectively. In comparison with these standards the mean concentration of coppeer in Cochin Backwater and Ennore Creek were found higher. Mandapam area showed very low concentration of copper. "Pollution Peak" value of 782 ppm dry wt copper was reported by Katz and Kaplan (1981) in polluted Southern California surface sediments

In the present study, the centres were seen far below the peak pollution level of copper in its sediments.

For comparison a few results in ppm from abroad are quoted:

Value (ppm)	Environment	Author(s)
Maximum 1400	Rio Tinto Estuary, Spain	Stenner and Nickless, 1975
Maximum 4500	Restronguet Estuary, U.K	Thornton et al., 1975
10,000	Outfall of refineries in Derwent Estuary, Australia	Bloom and Ayling, 1977
2.67-2.58	Chao Phraya River Estuary	Menasveta, 1978
11.8-82.9	Jakarta Bay	Hungspreugs, 1988
4.34-100.0	North Pacific Coastal sediments	Harding and Goyette, 1988.
91.0	Golden Horn Bay, Japan	Tkalin, 1992
ND - 38.0	Korapuzha Estuary	Present study
3.0 - 87.5	Cochin Backwater	"
1.0 - 40.0	Tuticorin Bay	"
ND - 6.0	Mandapam Water	"
7.0 - 121.5	Ennore Creek	"
ND - 33.3	Rusikulya Estuary	"

The values reported from estuaries of Europe showed very high degree of accumulation of copper in sediments than the estuaries in India reported. The periphery of Cochin Backwater and Ennore Creek has

the establishment such as oil refineries. This may be the reason for enrichment of copper in the sediments through its discharges as it contains very high amount of copper. This is supported by report of Bloom and Ayling (1977).

From India the reports for copper in sediment(in ppm)are:

Value(ppm)	Environment	Author(s)
10.0	Northern half of the western continental shelf of India	Murty et al., 1978
162.1-276.3	Bombay Harbour Bay	Markar et al., 1981
9.0 - 63.0	Vembanad Lake, Kerala	Murty and Veerayya,1981
Maximum 70.8	Cochin Backwater	Venugopal et al., 1982
136.0	Narmada Estuary	Borole et al., 1982
128.0	Tapti Estuary	"
24.0-180.0	Off Madras and Visakhapatnam	Pragatheeswaran et al., 1986
73-213	Mindhola River Estuary	Zingde et al., 1988
4.0-53.0	Ganges Estuary	Subramanian, et al.,1988
111.0	Cauvery Estuary	Subramanian et al., 1989
17.0±22.0	Southeast Coast of India	Mohanachandran and Subramanian, 1990
0.16-25.2	Madras Coast	Ramachandran et al.,1991
18.0-92.0	Vellar Estuary	Lyla, 1991

The results of Venugopal et al. (1982) in Cochin Backwater is in agreement with the present report of copper in the same environment. But the upper limit (121.5 ppm) was found slightly on the higher side than that of Venugopal et al. (1982). It may be due to the stations located at different sites and the sampling time. The results obtained by the candidate for Ennore Creek found well within the report of Pragatheeswaran et al. (1986), but higher than that reported by Ramachandran et al. (1991).

The results of Mohanachandran and Subramanian (1990) revealed high concentration of copper at the river mouths compared to the area on the river side from the mouth. The possible reasons attributed by them are:

i. the river carries sediment with iron content which can act as scavenger or carrier of metals, and

ii. of anthropogenic contributions.

In the present work the estuaries such as Cochin Backwater, Ennore Creek and Korapuzha Estuary showed higher copper concentration.

The seasonal difference was noticed from the statistical analysis. The monsoon recorded higher copper content in sediments that the dry seasons. During the monsoon the estuary carries all the washouts from catchment areas which are rich in organic content. The main effluent discharge sites show significant enrichment in copper during the monsoon in Cochin Backwater (Venugopal et al., 1982). The high organic content encourage the mobilisation processes and contribute to the variations of metals (Remani et al., 1980; Arzul and Maguer, 1990). According

to Murty and Veerayya (1981) coppeer bear significant relationship with organic matter of the sediment. The enrichment of the environment with organic washouts might have been the source for higher concentration of copper in monsoon. With a few exceptions, higher concentration of elements are associated with the silt-clay fractions of sediments (Murty and Veerayya, 1981). According to them the estuarine environments are favourable for the formation of colloids of iron and manganese oxides derived with freshwater run-off and their flocculation. It is therefore considered possible that part of these elements might be incorporated into the sediments in association with these hydroxides through adsorption.

Ackroyd et al. (1987) stated that "in general the estuarine chemistry of copper is poorly understood". But they concluded while working in Tamar Estuary that the metals show higher level of copper in the upper reaches which are dependent on the input of new particulate material associated with the seasonal variations in river flow. The increased level of copper in monsoon in the present study may also be due to wastes washed out from industries, sewage dumpings, land drainage and leaching by floods into the environment. As observed here, Kumaraguru (1980) also recorded increased levels of copper during monsoon season in Vellar Estuary and Killai Backwater, Tamil Nadu. In the same Vellar Estuary, Lyla (1991) reported the increased level of copper in sediment in monsoon followed by postmonsoon and premonsoon.

Copper in fish tissues

The copper content in liver, gills, kidney, intestine, ovary, skin and muscle of L. parsia are given for different seasons in 1991 and 1992, and at different centres with grand mean and standard deviation (Tables 23 to 29). Overall concentration of copper (ppm dry wt) in different tissues by pooling the data collected from all centres during different seasons are given below in descending order:

Tissue	Value (ppm dry wt)
Liver	52.95
Kidney	19.62
Intestine	9.47
Gills	7.04
Ovary	6.75
Muscle	5.76
Skin	5.14

Liver: From two-way ANOVA on computer for accumulation of copper (Table 23) in different years, seasons and centres; significant difference (at 10% level) was observed between the centres only. Copper content was in decreasing order:

TABLE 23. **Copper (ppm in dry wt) in liver of L. parsia at different centres during premonsoon, monsoon and post-monsoon of 1991 and 1992**

Centre	Centre Code	1991			1992			Mean with SD
		Season						
		Premonsoon	Monsoon	Postmonsoon	Premonsoon	Monsoon	Postmonsoon	
Korapuzha Estuary	I	10.07 (0.008)	15.18 (0.008)	28.57 (0.007)	18.67 (0.006)	-	23.68 (0.016)	19.23 ± 7.21
Cochin Backwater	II	7.19 (0.006)	12.55 (0.007)	9.90 (0.005)	10.16 (0.004)	18.22 (0.007)	32.26 (0.017)	15.05 ± 9.22
Tuticorin Bay	III	160.60 (0.079)	60.97 (0.016)	119.64 (0.049)	144.00 (0.019)	152.06 (0.081)	139.00 (0.017)	129.38± 6.25
Mandapam	IV	13.00 (0.006)	50.40 (0.007)	56.85 (0.013)	12.55 (0.008)	50.16 (0.022)	130.20 (0.021)	52.19 ± 42.95
Ennore Creek	V	118.91 (0.077)	19.90 (0.077)	126.38 (0.021)	14.12 (0.013)	17.08 (0.007)	52.11 (0.011)	58.08 ± 51.91
Rusikulya Estuary	VI	45.65 (0.009)	18.75 (0.008)	78.50 (0.032)	42.43 (0.001)	-	96.63 (0.031)	56.39 ± 30.96

SD = Standard deviation and coefficient of variation in parentheses.
Between centres($P < 0.1$)

TABLE 24. **Copper (ppm in dry wt) in gills of L. parsia at different centres during premonsoon, monsoon and postmonsoon of 1991 and 1992**

Centre	Centre Code	1991			1992			Means with SD
		Season						
		Premonsoon	Monsoon	Postmonsoon	Premonsoon	Monsoon	Postmonsoon	
Korapuzha Estuary	I	9.98 (0.004)	3.33 (0.008)	6.00 (0.004)	16.84 (0.005)	-	24.03 (0.007)	12.04 ± 8.42
Cochin Backwater	II	3.85 (0.005)	12.73 (0.004)	5.00 (0.006)	4.25 (0.006)	11.00 (0.006)	16.43 (0.007)	8.88 ± 5.26
Tuticorin Bay	III	3.06 (0.006)	3.77 (0.006)	3.40 (0.003)	3.61 (0.004)	8.21 (0.018)	3.41 (0.003)	4.24 ± 1.96
Mandapam	IV	5.07 (0.007)	3.66 (0.006)	3.11 (0.008)	4.74 (0.003)	8.46 (0.016)	4.31 (0.005)	4.89 ± 1.89
Ennore Creek	V	3.14 (0.006)	6.98 (0.007)	3.52 (0.008)	4.61 (0.005)	11.04 (0.005)	17.66 (0.008)	7.83 ± 5.63
Rusikulya Estuary	VI	3.62 (0.010)	2.50 (0.006)	14.56 (0.007)	3.70 (0.007)	-	13.88 (0.021)	7.65 ± 6.10

SD = Standard deviation and coefficient of variation is in parentheses.

Between years ($P < 0.1$).

TABLE 25. **Copper (ppm in dry wt) in kidney of L. parsia at different centres during premonsoon, monsoon and postmonsoon of 1991 and 1992**

Centre	Centre Code	1991			1992			Mean with SD
		Seasons						
		Premonsoon	Monsoon	Postmonsoon	Premonsoon	Monsoon	Postmonsoon	
Korapuzha Estuary	I	7.64 (0.004)	10.42 (0.005)	105.40 (0.007)	8.77 (0.001)	-	46.91 (0.022)	35.83 ± 42.23
Cochin Backwater	II	6.97 (0.007)	5.62 (0.006)	7.27 (0.008)	ND	7.76 (0.003)	46.36 (0.019)	12.33 ± 16.91
Tuticorin Bay	III	6.41 (0.007)	2.40 (0.005)	8.08 (0.008)	19.89 (0.009)	93.14 (0.050)	ND	21.65 ± 35.69
Mandapam	IV	37.88 (0.066)	39.47 (0.008)	12.33 (0.005)	14.53 (0.011)	43.45 (0.009)	15.63 (0.008)	27.22 ± 14.45
Ennore Creek	V	8.85 (0.005)	25.00 (0.005)	12.61 (0.004)	7.49 (0.007)	34.48 (0.013)	8.77 (0.006)	16.20 ± 11.04
Rusikulya Estuary	VI	10.00 (0.005)	6.82 (0.006)	27.60 (0.016)	7.58 (0.007)	-	10.60 (0.058)	12.52 ± 8.58

SD = Standard deviation, coefficient of variation is in parentheses and ND = Not detected.

TABLE 26 **Copper (ppm in dry wt) in intestine of L. parsia at different centres during premonsoon, monsoon and postmonsoon of 1991 and 1992**

Centre	Centre Code	1991			1992			Mean with SD
		Season						
		Premonsoon	Monsoon	Postmonsoon	Premonsoon	Monsoon	Postmonsoon	
Korapuzha Estuary	I	7.00 (0.006)	4.82 (0.008)	6.12 (0.003)	6.09 (0.005)	-	12.49 (0.008)	7.30 ± 3.00
Cochin Backwater	II	8.20 (0.005)	4.48 (0.005)	6.67 (0.008)	12.86 (0.003)	7.50 (0.005)	9.80 (0.003)	8.25 ± 2.86
Tuticorin Bay	III	10.62 (0.014)	45.45 (0.008)	8.04 (0.004)	4.85 (0.005)	44.16 (0.029)	22.60 (0.013)	22.62 ± 18.21
Mandapam	IV	0.73 (0.002)	5.29 (0.008)	6.92 (0.006)	1.55 (0.004)	7.25 (0.006)	9.30 (0.009)	5.17 ± 3.38
Ennore Creek	V	6.83 (0.004)	6.79 (0.005)	6.26 (0.003)	4.24 (0.007)	7.78 (0.006)	13.80 (0.007)	7.62 ± 3.25
Rusikulya Estuary	VI	8.98 (0.005)	4.38 (0.004)	10.74 (0.054)	9.09 (0.004)	-	9.39 (0.088)	8.52 ± 2.42

SD = Standard deviation and coefficient of variation is in parentheses.

Between centres ($P < 0.01$).

TABLE 27 **Copper (ppm in dry wt) in ovary of L. parsia at different centres during premonsoon, monsoon and postmonsoon of 1991 and 1992**

Centre	Centre Code	1991 Premonsoon	1991 Monsoon	1991 Postmonsoon	1992 Premonsoon	1992 Monsoon	1992 Postmonsoon	Mean with SD
		Season						
Korapuzha Estuary	I	4.46 (0.005)	5.43 (0.007)	4.60 (0.005)	2.43 (0.005)	-	23.29 (0.012)	8.04 ± 8.60
Cochin Backwater	II	3.51 (0.006)	4.90 (0.007)	4.47 (0.006)	1.68 (0.003)	6.62 (0.003)	5.52 (0.004)	4.45 ± 1.71
Tuticorin Bay	III	6.33 (0.005)	3.00 (0.005)	2.92 (0.005)	4.66 (0.008)	17.11 (0.041)	9.09 (0.004)	7.19 ± 5.38
Mandapam	IV	3.42 (0.003)	6.10 (0.006)	4.39 (0.006)	3.38 (0.008)	8.95 (0.011)	ND	4.37 ± 3.00
Ennore Creek	V	4.41 (0.006)	6.02 (0.004)	3.95 (0.004)	4.24 (0.003)	8.20 (0.004)	13.59 (0.009)	6.74 ± 3.72
Rusikulya Estuary	VI	5.77 (0.008)	5.96 (0.007)	19.66 (0.007)	10.48 (0.013)	-	24.42 (0.014)	13.26 ± 8.41

SD = Standard deviation, coefficient of variation is in parentheses and ND = Not detected.

Between seasons ($P < 0.1$).

TABLE 28. **Copper (ppm in dry wt) in skin of L. parsia at different centres during premonsoon, monsoon and postmonsoon of 1991 and 1992**

Centre	Centre Code	1991			1992			Mean with SD
		Season						
		Premonsoon	Monsoon	Postmonsoon	Premonsoon	Monsoon	Postmonsoon	
Korapuzha Estuary	I	5.05 (0.009)	4.66 (0.004)	3.74 (0.004)	6.63 (0.004)	-	7.13 (0.009)	5.44 ± 1.41
Cochin Backwater	II	3.90 (0.006)	3.06 (0.004)	3.21 (0.007)	3.56 (0.004)	7.88 (0.009)	15.00 (0.014)	6.10 ± 4.72
Tuticorin Bay	III	2.34 (0.009)	4.60 (0.003)	3.66 (0.005)	3.56 (0.004)	19.16 (0.021)	1.77 (0.006)	5.84 ± 6.60
Mandapam	IV	3.55 (0.006)	4.17 (0.008)	3.17 (0.003)	3.08 (0.002)	9.93 (0.009)	8.60 (0.005)	5.42 ± 3.03
Ennore Creek	V	1.12 (0.005)	4.12 (0.009)	4.09 (0.006)	4.06 (0.005)	5.26 (0.003)	15.19 (0.006)	5.64 ± 4.88
Rusikulya Estuary	VI	4.14 (0.004)	2.22 (0.004)	6.36 (0.008)	3.08 (0.006)	-	3.88 (0.017)	3.94 ± 1.55

SD = Standard deviation, coefficient of variation is in parentheses.

Between years ($P < 0.05$).

TABLE 29. **Copper (ppm in dry wt) in muscle of L. parsia at different centres during premonsoon, monsoon and postmonsoon of 1991 and 1992**

Centre	Centre Code	1991			1992			Mean with SD
		Season						
		Premonsoon	Monsoon	Postmonsoon	Premonsoon	Monsoon	Postmonsoon	
Korapuzha Estuary	I	3.34 (0.008)	2.43 (0.004)	3.00 (0.006)	3.35 (0.005)	-	13.70 (0.009)	5.16 ± 4.79
Cochin Backwater	II	2.12 (0.003)	3.26 (0.006)	3.88 (0.005)	2.95 (0.008)	4.11 (0.002)	8.10 (0.003)	4.07 ± 2.10
Tuticorin Bay	III	2.04 (0.008)	8.49 (0.006)	2.42 (0.006)	10.97 (0.001)	23.48 (0.041)	5.32 (0.006)	8.79 ± 7.99
Mandapam	IV	4.35 (0.005)	5.15 (0.004)	2.12 (0.003)	6.36 (0.006)	12.88 (0.019)	3.87 (0.007)	5.79 ± 3.75
Ennore Creek	V	3.39 (0.007)	3.21 (0.006)	3.03 (0.004)	4.88 (0.008)	6.58 (0.007)	16.67 (0.008)	6.29 ± 5.26
Rusikulya Estuary	VI	3.01 (0.006)	2.07 (0.006)	10.40 (0.007)	5.18 (0.004)	-	11.06 (0.004)	6.34 ± 4.17

SD = Standard deviation, coefficient of variation is in parentheses.

Between years ($P < 0.05$).

Centre	Value (ppm dry wt)
Tuticorin Bay	129.38 ± 36.25
Ennore Creek	58.08 ± 51.91
Rusikulya Estuary	56.39 ± 30.96
Mandapam water	52.19 ± 42.95
Korapuzha Estuary	19.23 ± 7.21
Cochin Backwater	15.05 ± 9.22

From correlation study, very poor positive nonsignificant relationships were obtained for copper content in liver with that of copper in water (r = 0.167) and sediment (r = 0.299).

Gills: ANOVA showed the significant difference (at 10% level) between the years only (Table 24) and higher content was recorded in 1992 (mean 8.677) than 1991 (mean 5.404).

The copper content in gills showed very poor positive nonsignificant relationship with that of water (r = 0.008) and sediment (r = 0.294).

Kidney: Table 25 shows the centrewise, yearly and seasonal copper content in kidney. From ANOVA no signifiant differences were observed between years, centres and seasons.

The correlation study indicated the poor positive nonsignificant relationships between copper in kidney and water (r = 0.17), and kidney and sediment (r = 0.076).

Intestine: Very high significant difference (at 1% level) was observed between the centres only (Table 26). The copper content recorded below in descending order:

Centre	Value (ppm dry wt)
Tuticorin Bay	22.62 ± 18.21
Rusikulya Estuary	8.52 ± 2.42
Cochin Backwater	8.25 ± 2.86
Ennore Creek	7.62 ± 3.38
Korapuzha Estuary	7.30 - 3.00
Mandapam Water	5.17 = 3.38

From correlation study very poor positive nonsignificant relationship was obtained for copper content in intestine with the available concentration of it in water ($r = 0.207$) and sediment ($r = 0.064$). The copper content in intestine showed a significant (at 1% level) positive bearing ($r = 0.41$) with the content in liver.

Ovary: Table 27 shows the variation in copper content in ovary at different centres during different seasons of 1991 and 1992. From ANOVA the significant difference (at 10% level) was obtained for the seasons and maximum content was noticed in postmonsoon (mean 9.658) followed by monsoon (mean 6.024) and premonsoon (mean 4.564).

The copper in ovary showed very poor positive nonsignificant relation with the values in water (r = 0.309) and sediment (r = 0.007). The copper content in ovary showed highly significant (at 1% level) correlation coefficient (r = 0.643) with copper in gills and a significant (at 5% level) coefficient (r = 0.32) with kidney.

Skin: ANOVA indicated significant difference between the years at 5% F-value (Table 28). The higher contents were recorded in different centres and seasons in 1992 (mean 6.543) than 1991 (mean 3.731).

The poor positive nonsignificant correlation coefficients were found out for copper in skin with that of water (r = 0.099) and sediment (r = 0.076). The highly significant (at 1% level) positive correlation coefficients were obtained for copper in skin with that of gills (r = 0.565), kidney (r = 0.509), intestine (r = 0.476) and the significant (at 5% level) correlation coefficient (r = 0.396) with ovary.

Muscle: Table 29 shows the variation in copper in muscle tissue of L. parsia from different centres during different seasons of 1991 and 1992. ANOVA indicated the significant difference (at 5% F-value) between the years only and higher copper content was recorded in 1992 (mean 7.748) than 1991 (mean 3.762).

From correlation study the poor positive nonsignifiant correlation coefficients were obtained for copper in muscle with that in water (r = 0.16) and sediment (r = 0.081). The copper content in muscle showed

highly significant (at 1% level) relation (positive) with the content in gills (r = 0.54), kidney (r = 0.497), intestine (r = 0.585), ovary (r = 0.716, Fig. 2) and skin (r = 0.772, Fig. 3).

Discussion

Marine organisms have the ability to accumulate trace elements from the sea water. In sea water the uncomplexed heavy metal ions may not be predominant (Bruland, 1983), but several reports show that this form of heavy metal is available to organisms which accumulate (Zamunda and Sunda, 1982; Rainbow, 1985). All elements are accumulated by organism to a certain degree except fluorine, bromine, magnesium, sulphur, sodium and chlorine (Nair, 1984). The accumulation can be an active metabolic process in which the ions are transported across the cell membrane as organic molecules or it can be a passive processof adsorption on the surface (Rainbow, 1988). Generally higher accumulation of heavy metals by estuarine and marine finfishes and shellfishes pose a threat to the use of these resources as human food. At present, no standards are available on heavy metals level in different tissues/organs of fish to indicate the acceptable limits for human consumption. According to MPEDA's "TIT-BITS" (Anon, 1991c) the maximum permissible limit of copper for canned shrimp/prawn is 20 ppm. But this cannot be used as a standard for the present study as prawn and fish belong to two different phyla i.e. invertebrata and chordata respectively. Capelli et al. (1987) reported 4.5 ± 0.3 ppm dry wt copper for "Standard Reference Material (SRM)" obtained from the "International Laboratory for Marine Radioactivity (IAEA/Monaco)".

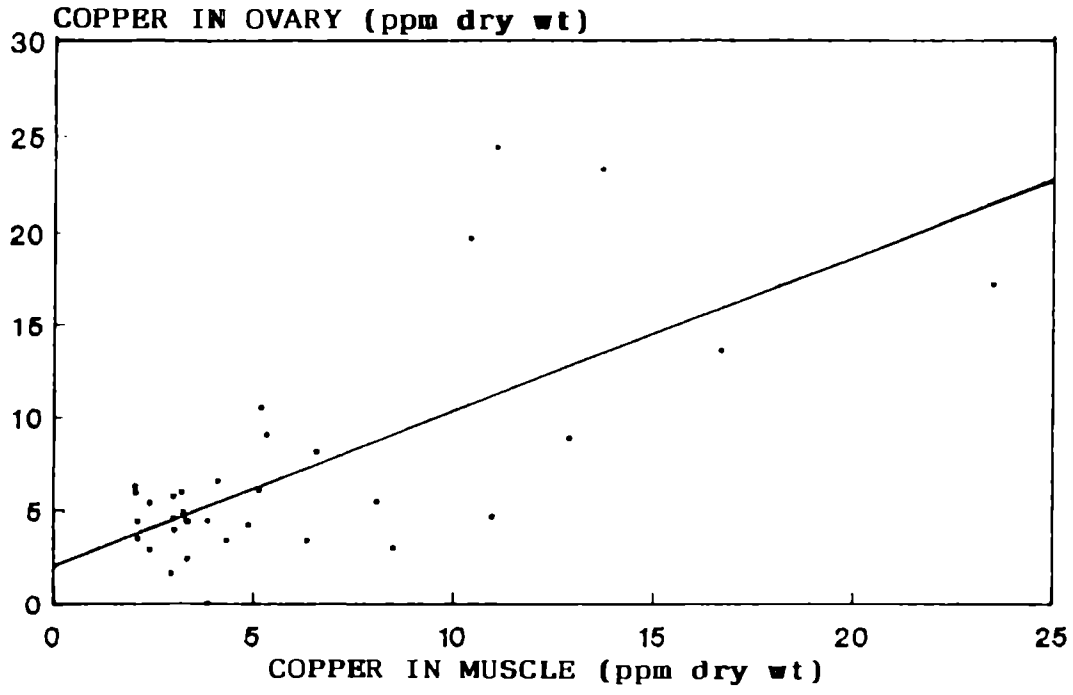


FIG. 2. Relationship between copper in Muscle and copper in ovary of L. parsia.

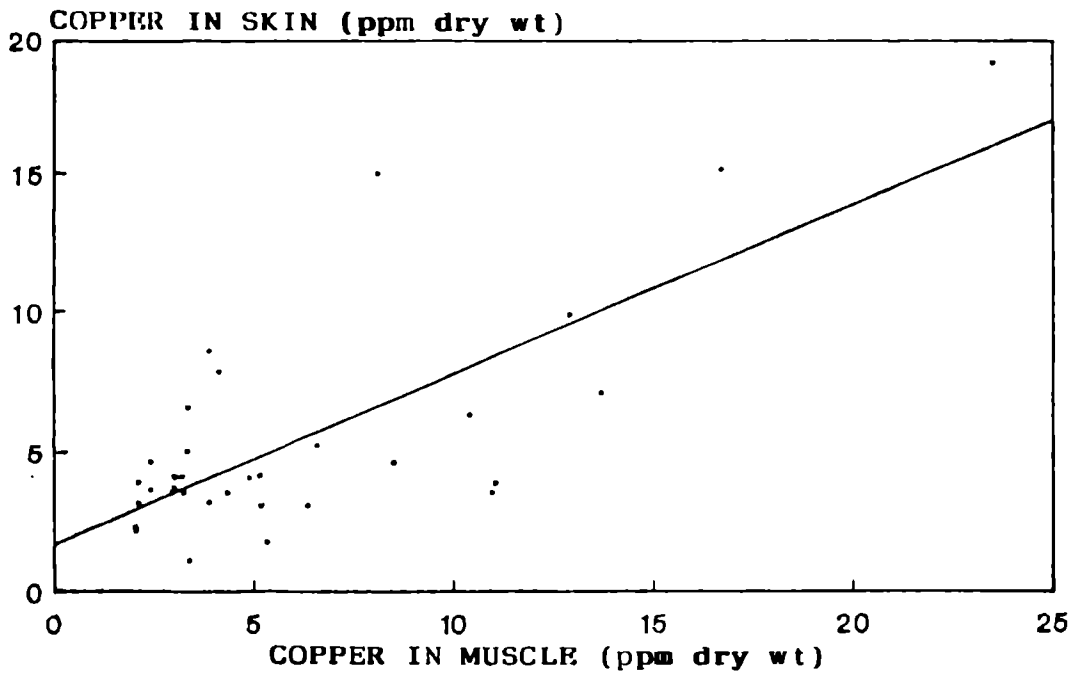


FIG. 3. Relationship between copper in Muscle and copper in Skin of L. parsia.

Comparing with the standard copper of IAEA, the copper content was found higher in all the seven tissues such as liver, gills, kidney, intestine, ovary, skin and muscle of L. parsia in the present study.

Among the very few reports available on copper content in mullets, Windom et al. (1973) reported 1.9 ppm dry wt in muscle tissue of Mugil cephalus collected from the North Atlantic. In the present study, almost a 3 fold increase i.e. 5.76 ppm dry wt of copper in the muscle of L. parsia was recorded, compared to the results of Windom et al. (1973). This increase in copper may be possibly due to the difference in species and collection site. From different fishing areas in and around coastal and estuarine waters of Goa, Zingde et al. (1976) reported 28.6-32.5 ppm dry wt copper in Mugil (= Liza) parsia. From their study, content of copper in specific tissue was not found, and thus the candidate has attempted here to investigate copper content in different tissue of L. parsia.

In species of Liza, the available reports on copper content (ppm dry wt) are:

Species	Liver	Gill	Muscle	Author and Place
L. macrolepis	8.19	1.63	0.60	Nammalwar (1987) - in Adyar Estuary, Madras.
"	6.76	2.83	2.50	Nammalwar (1987)- in Ennore Estuary, Madras.
L. parsia	58.08	7.83	6.29	Present study - in Ennore Creek, Madras.

The higher concentrations obtained here may probably be due to the difference in experimental animals, time and season, and the station position of the collections.

The higher copper content (ppm dry wt) was noticed in liver followed by kidney, intestine, gills, ovary, muscle and skin in this order in L. parsia. Similar reports are available from different parts of the world for different fishes. Some of them (in ppm dry wt) are:

Species	Liver	Gut-wall	Ovary	Muscle	Author(s)
Pleuronectes platessa	3.3	2.4	-	0.8-1.1	Wharfe and Van Den Broek (1977)
Platichthys flesus	12.9-18.3	2.7	2.4	0.3-1.3	"
Merlangus merlangus	1.3- 3.5	0.5-2.5	-	0.2-1.0	"
Gadus morhua	1.2	0.85	0.41	0.24	Szefer et al. (1990)
L. parsia	52.95	9.47	6.75	5.70	Present study

According to Jaffer and Ashraf (1988) the level of copper was found minimum in muscle of Pampus argenteus and Formio niger, while in liver and kidney the levels were higher. Szefer et al. (1990) found the content of copper, 1.50 and 0.91 ppm wet wt in kidney and gills of Gadus morhua respectively. Their findings showed slight deviation than the present investigation. They reported higher level in kidney than liver; but gill showed more than intestine as reported in present study.

The liver was the most active site of copper deposition followed by kidney, gills and muscle in that order in Scardinius erythrophthalmus (Vorob, yev and Zaystev, 1975). Higher content of copper in liver of Ictalurus nebulosus was reported by Benedetti et al. (1981) in Barbus

grypus and B. belaywin (Latif et al., 1987), in Pagothenia borchgrevinki (Honda et al., 1983), in six species from Aquba (Wahbeh and Mahasneh, 1987), in Catostomus commersoni (Young and Harvey, 1989). According to Overnell et al. (1988) the metal binding protein is found in liver and kidney of the fish Scophthalmus maximus. Roch and McCarter (1984) have demonstrated experimentally that elevated levels of copper can induce enhanced levels of hepatic metallothionein in freshwater fish. In the present study the copper present in the aquatic environments might have induced the enhancement of metallothionein in liver and kidney for binding it. These organs also function as the main reservoirs for a number of substances such as trace metals and other pollutants (Romeril and Davis, 1976; Wharfe and Van Den Broek, 1977). The intestine next to liver and kidney shows higher concentrations of metals by Anguilla sp. This above finding is in agreement with the present investigation from Indian waters. An interesting protective mechanism against heavy metal toxicity was described by Noel-Lambot (1981) who stated that corpuscules are present in the intestinal lumen of A. anguilla before elimination with faeces. According to him these corpuscules contain high concentration of copper chloride from water ingested by the eel. The similar reason can also be attributed to the copper accumulation in intestine of L. parsia. Gill metallothionein binds only a very small amount of copper compared to liver (Noel-Lambot et al., 1978). As Phillips (1977) from his review of literature reported that copper tend to concentrate in soft organs such as liver and kidney of fish, gonad contains extremely low concentrations of all elements other than zinc. The results from the present study also confirms his views. Only the axial muscle concentrates very less amount of all heavy metals (Phillips, 1977).

In the present study no good relationships for copper was seen between the tissues and abiotic factors such as copper in water and sediment. Similar findings are available for copper in flesh with water by Wilson et al. (1981). Harding and Goyette (1989) found that tissue metal levels of any species on both raw and log transformed data with sediment and tissue copper did not show relationship with metal concentrations in lake water (Yong and Harvey, 1989). According to Young and Harvey (1989) the absence of relation between concentrations of Fe, Zn and Cu in the liver, kidney and muscle tissues and fish size ($P < 0.05$) implies that these metals were homeostatically controlled. The absence of correlation may reflect either regulation by the fish or limited input from the environment (Milner, 1982).

In the present study no seasonal differences were seen for tissues (except ovary at 10% level) and may be linked with the homeostatic control of copper by the animal with that of environment, as stated by Milner (1982). From the present investigation it is seen annual variations in copper content in ovary, muscle and skin of L. parsia, but no possible reason is seen for their regulation. The centrewise difference in copper content in liver and intestine may be linked with the availability of copper in the environments. The variation in concentration of copper in different centres in water and sediment is already discussed.

Concentrations of trace metal "copper" found in fishes of different centres were variable. Sample standard deviations were often as large as the sample mean itself. Similar results were reported for the fishes in Palestine Lake by Murphy et al. (1978). Giesy and Wiener (1977) found

the trace metal concentration in fishes usually exhibit positive skew and are frequently non-normal. They stated that when dealing with a sample that departs substantially from normality, characterisation of the sample by mean and standard deviation may be misleading. Unfortunately, all data in the literature on trace metal concentration in fish have utilised means and standard deviation.

In marine pollution studies there is little evidence of correlation between measured levels of trace elements (except mercury) in fish tissues and environmental levels (Windom et al., 1973; Eustace, 1974). This has been attributed to the mobility of fish with the localised nature of metal contamination in the sea.

Moisture content in the fish tissue

The moisture content (%) in different tissues of L. parsia was found to be:

Tissue	Value (%)
Liver	73.22 ± 3.98
Kidney	68.33 ± 5.44
Ovary	71.72 ± 5.33
Muscle	75.74 ± 7.19
Skin	64.83 ± 5.19
Gills	81.25 ± 2.18
Intestine	73.57 ± 4.55

These moisture contents were used as tool to convert the metal content in tissues from dry wt to weight wt basis. Subsequently the tissue metal contents in wet wt basis were used for the calculation of "Bioaccumulation Factor" as it is more applicable than calculated in dry wt basis.

Bioaccumulation Factor

The Bioconcentration/Bioaccumulation Factor is usually abbreviated as "KB" which is defined as "$\frac{CB}{CW}$" at equilibrium, where CB is the concentration in the organism and CW is the concentration in surrounding water (Connell and Schuurmenn, 1988). The ability of marine animals to accumulate trace elements from sea water has often been used to estimate the levels of such elements in sea water with the help of accumulation factor. If the factor as well as the bioaccumulation for a particular organism is known, the level of the trace metal in the water medium can be found out (Nair, 1984). Very few reports are available regarding the Bioaccumulation Factor in marine organisms and specifically in fishes. Stray reports are available regarding the Bioaccumulation Factor of elements for different tissues/organs in case of molluscs (Eisler, 1981), but almoost no report on fish tissues. Thus the Bioaccumulation Factor studied in detail in the present investigation for different tissues/organs of L. parsia is very important.

The Bioaccumulation Factor in relation with water in different seasons in 1991 and 1992 at different centres with grand mean and standard deviation are presented in Tables 30 to 36. Pooling the data for specific

TABLE 30. **Bioaccumulation Factor for copper in liver of L. parsia at different centres during premonsoon, monsoon and postmonsoon of 1991 and 1992**

Centre	Centre Code	1991 Season			1992 Season			Mean with SD
		Premonsoon	Monsoon	Postmonsoon	Premonsoon	Monsoon	Postmonsoon	
Korapuzha Estuary	I	322.5	823.2	1270.4	523.8	-	2051.2	998.2 ± 688.3
Cochin Backwater	II	267.5	680.6	107.5	298.5	1006.4	737.5	516.3 ± 3 44.3
Tuticorin Bay	III	8161.5	3827.5	4972.0	4390.8	12798.0	6449.6	6766.6 ± 3344.5
Mandapam	IV	1225.6	1792.9	3247.9	232.2	3148.9	8022.5	2945.0 ± 2740.7
Ennore Creek	V	2488.3	796.0	4306.3	307.5	683.2	1896.7	1746.3 ± 1498.7
Rusikulya Estuary	VI	1826.0	3166.1	7615.9	2561.2	-	6445.2	4322.9 ± 2550.7

SD = Standard deviation, between stations ($P < 0.01$).

TABLE 31. **Bioaccumulation Factor for copper in gills of L. parsia at different centres during premonsoon, monsoon and postmonsoon of 1991 and 1992**

Centre	Centre Code	1991 Season			1992 Season			Mean with SD
		Premonsoon	Monsoon	Postmonsoon	Premonsoon	Monsoon	Postmonsoon	
Korapuzha Estuary	I	224.6	126.9	187.5	341.4	-	1462.8	468.6 ± 561.2
Cochin Backwater	II	100.7	485.1	38.1	87.8	427.0	264.0	233.8 ± 189.1
Tuticorin Bay	III	109.3	166.3	99.3	77.4	485.6	111.2	174.9 ± 155.0
Mandapam	IV	335.9	91.5	124.9	61.6	373.2	186.6	195.6 ± 130.5
Ennore Creek	V	46.2	196.2	84.3	70.6	310.0	451.7	193.2 ± 160.7
Rusikulya Estuary	VI	101.8	296.7	992.7	157.0	-	650.6	439.8 ± 375.8

SD = Standard deviation.

TABLE 32. **Bioaccumulation Factor for copper in kidney of L. parsia at different centres during premonsoon, monsoon and postmonsoon of 1991 and 1992**

Centre	Centre Code	1991			1992			Mean with SD
		Season						
		Premonsoon	Monsoon	Postmonsoon	Premonsoon	Monsoon	Postmonsoon	
Korapuzha Estuary	I	290.5	670.7	5563.4	300.3	-	4823.5	2329.7 ±2631.8
Cochin Backwater	II	307.9	361.8	93.7	ND	508.8	1258.1	421.7 ± 449.2
Tuticorin Bay	III	386.7	178.8	398.6	719.9	9305.2	ND	1831.5 ± 3669.3
Mandapam	IV	4239.1	1666.7	836.2	319.1	3237.8	1143.2	1907.0 ± 1517.8
Ennore Creek	V	219.8	1187.0	510.0	193.6	1637.1	249.3	666.1 ± 605.8
Rusikulya Estuary	VI	474.8	1367.0	3178.5	543.1	-	839.3	1280.5 ± 1117.7

SD = Standard deviation and ND=Not detected.

TABLE 33. **Bioaccumulation Factor for copper in intestine of L. parsia at different centres during premonsoon, monsoon and postmonsoon of 1991 and 1992**

Centre	Centre Code	1991 Season		1992 Season				Mean with SD
		Premonsoon	Monsoon	Premonsoon	Premonsoon	Monsoon	Postmonsoon	
Korapuzha Estuary	I	222.1	258.9	269.6	174.0	-	1071.8	399.3 ± 377.8
Cochin Backwater	II	302.3	240.7	71.7	374.3	410.4	221.9	270.2 ± 121.8
Tuticorin Bay	III	534.6	2826.5	331.0	146.5	3681.9	1038.8	1426.6 ± 1471.8
Mandapam	IV	68.2	186.4	391.6	28.4	450.9	567.7	282.2 ± 220.0
Ennore Creek	V	141.6	269.1	211.3	91.5	308.3	497.6	252.2 ± 143.8
Rusikulya Estuary	VI	355.8	732.7	1032.3	543.5	-	620.4	656.9 ± 250.8

SD = Standard deviation, between centres ($P < 0.01$).

TABLE 34. **Bioaccumulation Factor for copper in ovary of L. parsia at different centres during premonsoon, monsoon and postmonsoon of 1991 and 1992**

Centre	Centre Code	1991			1992			Mean with SD
		Season						
		Premonsoon	Monsoon	Postmonsoon	Premonsoon	Monsoon	Postmonsoon	
Korapuzha Estuary	I	152.4	312.1	216.8	74.3	-	2138.4	578.8 ± 876.2
Cochin Backwater	II	138.4	281.7	51.4	52.3	387.6	133.8	174.2 ± 134.1
Tuticorin Bay	III	341.0	199.6	128.6	150.6	1526.4	447.1	465.6 ± 533.8
Mandapam	IV	341.8	230.0	265.8	66.3	595.5	ND	249.9 ± 212.0
Ennore Creek	V	97.8	255.2	142.7	97.9	347.7	524.3	244.3 ± 168.8
Rusikulya Estuary	VI	244.6	1066.8	2021.8	670.5	-	1726.5	1146.0 ± 733.0

SD = Standard deviation and ND=Not detected.

TABLE 35 **Bioaccumulation Factor for copper in skin of L. parsia at different centres during premonsoon, monsoon and postmonsoon of 1991 and 1992**

Centre	Centre Code	1991			1992			Mean with SD
		Season						
		Premonsoon	Monsoon	Postmonsoon	Premonsoon	Monsoon	Postmonsoon	
Korapuzha Estuary	I	213.2	333.1	219.2	252.1	-	814.2	366.4 ± 254.9
Cochin Backwater	II	191.3	218.7	45.9	137.9	573.8	452.1	270.0 ± 201.0
Tuticorin Bay	III	156.8	380.7	200.5	143.1	2125.7	108.3	519.2 ± 792.8
Mandapam	IV	441.2	195.5	238.7	75.1	821.7	698.5	411.8 ± 297.1
Ennore Creek	V	30.9	217.2	183.7	116.6	277.4	728.8	259.1 ± 245.3
Rusikulya Estuary	VI	218.3	494.2	813.4	245.1	-	341.1	422.4 ± 243.8

SD = Standard deviation.

TABLE 36. **Bioaccumulation Factor for copper in muscle of L. parsia at different centres during premonsoon, monsoon and postmonsoon of 1991 and 1992**

Centre	Centre Code	1991			1992			Mean with SD
		Season						
		Premonsoon	Monsoon	Postmonsoon	Premonsoon	Monsoon	Postmonsoon	
Korapuzha Estuary	I	97.3	119.8	121.3	87.9	-	1079.1	301.1 ± 435.2
Cochin Backwater	II	71.7	160.7	38.3	78.8	206.4	168.4	120.7 ± 66.6
Tuticorin Bay	III	94.3	484.6	91.4	304.2	1796.9	224.5	499.3 ± 652.4
Mandapam	IV	372.9	166.6	110.1	107.0	735.2	216.8	284.8 ± 241.4
Ennore Creek	V	64.5	116.8	95.1	96.6	239.3	551.7	194.0 ± 185.5
Rusikulya Estuary	VI	109.5	317.8	917.5	284.3	-	670.8	460.0 ± 327.0

SD = Standard deviation.

tissues, the grand mean values obtained from computer in descending order are:

Tissue	Value
Liver	2734.75
Kidney	1305.68
Intestine	518.73
Ovary	428.55
Skin	352.89
Muscle	288.84
Gills	259.08

Liver: From ANOVA no significant differences were obtained between the years and between the seasons (Table 30). Highly significant difference (at 1% level) was observed between the centres placing them in descending order as follows:

Centre	Value
Tuticorin Bay	6766.6 ± 3344.5
Rusikulya Estuary	4322.9 ± 2550.7
Mandapam Water	2945.0 ± 2740.7
Ennore Creek	1746.3 ± 1498.7
Korapuzha Estuary	998.2 ± 688.3
Cochin Backwater	576.3 ± 344.3

Highly significant (at 1% level) and poor positive correlation coefficient (r = 0.391) were found for Bioaccumulation Factor in liver with

the copper content in water. Similar result with 'r' value of 0.398 and significant at 5% level was found with sediment. The highly significant (at 1% level) good positive correlation (r = 0.859) was observed between the copper content and Bioaccumulation Factor in liver.

Gills: No significant differences were found in gills between years of collection, seasons and centres (Table 31).

A highly significant (at 1% level) and poor positive relation (r = 0.394) was seen between the Bioaccumulation Factor in gills and the available concentration in water. While correlating copper in gills with the available concentration in sediments, a nonsignificant and very poor coefficient (r = 0.011) was obtained. The accumulation and Bioaccumulation Factor of copper showed a good positive relationship (r = 0.822) with the significance at 1% level.

Kidney: The variation in copper Bioaccumulation Factor in kidney at different centres during different seasons in 1991 and 1992 is presented in Table 32. No significant difference was seen between the years and also between the seasons and centres.

A significant (at 5% level) and weak positive relation (r = 0.352) was seen between the Bioaccumulation Factor and concentration of copper in water. A poor positive nonsignificant coefficient (r = 0.201) of copper in kidney was obtained when correlated with the sediment. The concent and Bioaccumulation Factor of copper in kidney showed a highly significant (at 1% level) and good positive relationship (r = 0.898).

Intestine: From ANOVA, no significant difference was seen between the years and seasons (Table 33). But the centrewise Bioaccumulation Factors were seen significant at 1% F-value. Maximum to minimum values reported are:

Centre	Value
Tuticorin Bay	1426.6 ± 1471.8
Rusikulya Estuary	656.9 ± 250.8
Korapuzha Estuary	399.3 ± 377.8
Mandapam water	282.2 ± 220.0
Cochin Backwater	270.2 ± 121.8
Ennore Creek	253.2 ± 143.8

The correlation study indicated a significant (at 5% level) poor positive relation (r = 0.357) between the Bioaccumulation Factor and the available concentration in water. But the relationship was weak (r = 0.203) and nonsignificant with the concentration in sediment. A very good positive and highly significant (at 1% level) coefficient (r = 0.945) was observed between the content and Bioaccumulation Factor of copper in intestine.

Ovary: No significant differences were observed from ANOVA between years, seasons and centres (Table 34).

From correlation study a highly significant (at 1% level) positive relation (r = 0.456) was obtained between the Bioaccumulation Factor and the available concentration in water. The nonsignificant poor coefficient

(r = 0.171) showed the weak relationship between the Bioaccumulation Factor and the concentration in sediment. Between copper content in ovary and its factor a very good positive relationship (r = 0.928) was seen with higher level (at 1%) of significance.

Skin: The year and centerwise seasonal variation in copper Bioaccumulation Factor in skin is given in Table 35. From ANOVA, no significant difference was seen between the years, seasons and centres.

A significant (at 5% level) and weak positive relation was obtained (r = 0.401) for Bioaccumulation Factor with that of copper in water. But very poor relation was seen (r = 0.16) and significant with the concentration of sediment. The highly significant (at 1% level) and good coefficient (r = 0.818) was obtained for the Bioaccumulation Factor with the copper content in skin.

Muscle: No significant difference was seen between the years, seasons and centres (Table 36).

The correlation study indicated a weak coefficient (r = 0.401) with significance level at 5% between the Bioaccumulation Factor and the available concentration of copper in water. But a significant and weak correlation coefficient (r = 0.219) was seen while correlating Bioaccumulation Factor with copper in sediments. A very good positive correlation (r = 0.893) with 1% level was established between the copper content in muscle and Bioaccumulation Factor.

Discussion

Copper, the essential element found in seawater is readily absorbed by plants in seawater by factors:

Ascophyllum nodosum	6,000 to 20,000	Foster, 1976
Sargassum sp.	2,000	Ishii et al., 1978
Fucus vesiculosus	3,600 to 27,000	Foster, 1976
Other species of seaweeds	4,700 to 200,000	Kim and Won, 1974

Indeed, of all marine organisms examined, algae together with oysters and scallops have the greatest ability to absorb copper from its surrounding environment (Eisler, 1979). High biomagnification from sea water was the rule among the molluscs (Eisler, 1981). The bivalves from Greek water contained 4,100 to 15,000 times more copper than the ambient medium (Papadopoulu, 1973). A review of copper in marine organisms indicates that molluscs, not surprisingly, contained more copper in tissues and soft body than any other group, more than plants, other invertebrates and vertebrates (Eisler, 1979). The tissues of L. parsia reported lesser Accumulation Factors of copper than plants and molluscs. It indicates that the tissues might have been supersaturated with copper in relation to the seawater or might have been regulated by the respective tissues/ organs by homeostasis Among molluscs, highest Accumulation Factors were generally observed in cephalopods and oysters: blood, digestive gland and kidney (Eisler, 1981). In the present study also the liver and kidney recorded higher Bioaccumulation Factors. The levels of copper concentrated

by Crassostrea cuculata and C. gryphoides in coastal and estuarine waters around Goa have been related to the levels in water (Zingde et al., 1976). Around Dona Paula, Goa the average dissolved copper concentration in water was 4.0 ppm. The Accumulation Factor was evaluated in relation to the concentration of the soluble metal in the water in which the oyster inhabits (Zingde et al., 1976). The reports available on factors in different species are:

Species	AF	Place	Author (s)
Crassostrea cuculate and C. gryphoides	15,000	Goa	Zingde et al., 1976
Ascelis indicus (prawn)	2,000	Bombay Harbour Bay	Matkar et al., 1981
Penaeus indicus	139.3-3049.2	Cochin	Anikumari, 1992
Scylla serrata (crab)	4300 - 6200	Bombay Harbour Bay	Matkar et al., 1981
Pleuronectes platessa (plaice)	130 - 660	Pacific	Vink, 1972
Hippoglosus stenolepis (Pacific halibut)	50 - 250	Pacific	Waldichuk, 1974
Arius sp.	160 - 790	Bombay Harbour Bay	Matkar et al., 1981
L. parsia (7 tissues)	259.08-2734.75		Present study

As no reports are available for Bioaccumulation Factor of copper in different organs/tissues of other fishes, the present results of L. parsia cannot be compared.

The present investigation revealed the centrewise differences of Bioaccumulation Factor of copper for liver and intestine only. It may be due to the differences of copper availability in aquatic environment of different centres. In all the tissues, the copper content was seen significantly related with the Bioaccumulation Factor. It indicates the increase in Bioaccumulation Factor with the increase in copper content in the tissue.

Section 3 : Zinc

Zinc at a glance (Average of two years)

Centre Code	in water (ppb)	in sediment (ppm dry wt)	in tissue (ppm dry wt)						
			L	G	K	It	O	S	M
I	20.22	28.54	51.7	54.5	33.7	71.1	223.8	79.9	15.3
II	82.10	409.65	129.2	101.4	32.8	63.6	264.6	102.7	16.5
III	14.14	4.89	185.9	60.4	66.5	91.7	211.4	91.3	34.5
IV	19.39	2.99	89.8	55.3	35.6	69.4	188.6	94.3	17.4
V	38.88	112.75	150.9	121.4	55.3	73.1	333.3	64.7	15.6
VI	19.33	9.68	19.8	53.6	77.9	100.2	355.5	56.4	14.1

I = Korapuzha Estuary, II = Cochin Backwater, III = Tuticorin Bay, IV = Mandapam Water, V = Ennore Creek, VI = Rusikulya Estuary.

L = Liver, G = Gills, K = Kidney, It = Intestine, O = Ovary, S = Skin and M = Muscle.

Zinc in water

The variation in zinc in water during different seasons in 1991 and 1992 for eighteen stations from six centres studied are presented in Tables 37 to 42. From two-way ANOVA, no significant difference between the years of study was noted. The significant difference (at 5% level) was seen between the seasons and higher concentration was found in postmonsoon followed by monsoon and premonsoon. The interaction of seasons with years showed significant difference at 5% level. Very high significant differences (at 0.1% level) were seen between the centres. Significant differences were seen for the interactions e.g. centre with year (at 5% level) and centre with season (at 0.1% level). Very high significant difference (at 0.1% level) was observed between the surface and bottom water, showing higher concentratins in bottom than the surface. No significant differences were found for interactions between surface and bottom values with years and seasons. But 10% level of significnce was seen for the interaction of bottom and surface with centre.

The correlation study indicated the significant (5% level) negative correlation coefficients for zinc with salinity ($r = -0.585$) and hardness ($r = -0.586$) showing the decrease of zinc with increase in salinity and total hardness in premonson. The relationship was found nonsignificant and negative in monsoon (i.e. with salinity $r = -0.061$ and with total hardness $r = -0.027$) and significant (at 5% level) negative in monsoon (i.e. with salinity $r = -0.278$ and with total hardness $r = -0.279$). A significant and poor positive correlation ($r = 0.308$) was noted for copper

TABLE 37. **Zinc in water (ppb) and sediment (ppm) in the Korapuzha Estuary during premonsoon, monsoon and postmonsoon of 1991 and 1992**

Sample	Station		1991 Premonsoon	1991 Monsoon	1991 Postmonsoon	1992 Premonsoon	1992 Monsoon	1992 Postmonsoon
			Season					
Water	I 1	Surface	30.5	5.0	ND	4.5	9.5	7.0
		Bottom	37.5	29.0	43.5	45.0	23.5	18.5
	I 2	Surface	19.0	4.5	24.5	5.5	11.5	12.0
		Bottom	19.0	12.5	13.0	47.0	32.0	20.5
	I 3	Surface	33.5	4.0	36.0	11.5	4.5	11.0
		Bottom	39.0	9.5	17.5	52.5	23.5	11.0
	Mean	Surface	27.67	4.50	20.17	7.17	8.50	10.00
		Bottom	31.83	17.00	24.67	48.17	26.33	16.67
	Grand Mean with SD		29.75 ± 8.85	10.75 ± 9.54	22.42 ± 15.80	27.67 ± 22.72	17.42 ± 10.50	13.33 ± 5.12
	Range		(19.0-39.0)	(4.0-29.0)	(ND-43.5)	(4.5-52.5)	(4.5-23.5)	(7.0-20.5)
Sediment	I 1		41.75	50.25	31.75	27.75	44.25	18.25
	I 2		41.00	28.75	37.25	31.25	22.25	20.25
	I 3		38.75	4.70	24.50	14.00	16.75	20.25
	Mean with SD		40.50±1.97	27.90±20.54	31.17±7.24	24.33±8.62	27.75±13. 37	19.58±1.93
	Range		(37.0-43.0)	(3.4-54.0)	(20.5-40.5)	(11.5-34.5)	(16.0-49.0)	(18.0-22.5)

SD = Standard deviation, ND = Not detected.
In water between seasons ($P < 0.05$), between seasons within a year ($P < 0.05$), between surface and bottom within a centre ($P < 0.1$), Mean with SD and range 20.22± 14.08 (ND- 52.5).

In sediment between years ($P < 0.05$), between seasons ($P < 0.001$), between seasons within a year ($P < 0.001$), between stations ($P < 0.001$), Mean with SD and range 28.54 ± 11.98 (3.4 - 54.0).

TABLE 38. **Zinc in water (ppb) and sediment (ppm) in the Cochin Backwater during premonsoon, monsoon and postmonsoon of 1991 and 1992**

Sample	Station		1991 Premonsoon	1991 Monsoon	1991 Postmonsoon	1992 Premonsoon	1992 Monsoon	1992 Postmonsoon
			Season					
Water	II 4	Surface	66.5	117.5	151.5	68.5	77.5	80.0
		Bottom	123.0	108.5	165.5	94.5	84.0	112.5
	II 5	Surface	25.0	30.0	132.5	86.0	25.0	42.5
		Bottom	42.0	32.0	154.0	97.5	28.5	61.0
	II 6	Surface	38.0	38.5	123.5	42.5	62.5	94.5
		Bottom	38.0	162.0	162.5	51.5	28.5	108.0
	Mean	Surface	43.17	62.00	135.83	65.67	55.00	72.33
		Bottom	67.67	100.83	160.67	81.17	47.00	93.83
	Grand Mean with SD		55.42±35.78	81.42±55.60	148.25±16.76	73.42±23.00	51.0±26.88	83.08±27.43
	Range		(25.0-123.0)	(30.0-162.0)	(123.5-165.5)	(42.5-97.5)	(25.0-84.0)	(42.5-112.5)
Sediment	II 4		758.75	861.00	829.25	665.50	811.50	889.00
	II 5		227.75	607.25	335.25	90.75	205.25	395.25
	II 6		111.00	189.75	120.75	26.75	148.75	100.25
	Mean with SD		365.83±319.63	552.67±67	428.42±329.04	261.00±325.94	388.50±332.86	461.50±362.20
	Range		(110.0-885.0)	(167.0-924.0)	(118.0-910.5)	(25.0-798.0)	(117.5-887.0)	(92.0-990.0)

SD = Standard deviation.
In water between seasons ($P < 0.05$), between seasons within year ($P < 0.05$), between surface and bottom within a centre ($P < 0.1$), Mean with SD and range 82.10 ± 44.12 (25.0 - 165.5).

In sediment between years ($P < 0.05$), between seasons ($P < 0.001$), between seasons within a year ($P < 0.001$), between stations ($P < 0.001$), Mean with SD and range 409.65 ± 314.20 (25.0 - 990.0).

TABLE 39. **Zinc in water (ppb) and sediment (ppm) in the Tuticorin Bay during premonsoon, monsoon and post-monsoon of 1991 and 1992**

Sample	Station		1991 Season Premonsoon	1991 Season Monsoon	1991 Season Postmonsoon	1992 Season Premonsoon	1992 Season Monsoon	1992 Season Postmonsoon
Water	III 7	Surface	6.0	6.0	16.0	2.0	12.5	18.0
		Bottom	ND	38.5	20.0	38.5	12.5	24.5
	III 8	Surface	6.0	8.5	23.0	7.5	13.0	32.5
		Bottom	6.0	25.5	25.5	7.0	21.0	16.0
	III 9	Surface	2.0	2.0	11.0	11.0	13.0	32.5
		Bottom	5.5	22.0	17.0	8.5	13.0	6.0
	Mean	Surface	4.67	5.50	16.67	6.83	12.83	20.83
		Bottom	3.83	28.67	20.83	18.00	15.50	15.50
	Grand Mean with SD		4.25 ± 2.60	17.08 ± 13.97	18.75 ± 5.21	12.42 ± 13.11	14.17 ± 3.36	18.17 ± 9.34
	Range		(ND - 6.0)	(2.0 - 38.5)	(11.0 - 25.5)	(2.0 - 38.5)	(12.5 - 21.0)	(6.0 - 32.5)
Sediment	III 7		3.00	1.75	3.00	5.75	0.75	5.00
	III 8		14.25	3.75	3.75	2.50	3.25	5.25
	III 9		7.25	6.25	8.25	3.50	7.75	3.00
	Mean with SD		8.17 ± 5.55	3.92 ± 2.04	5.00 ± 3.73	3.92 ± 1.63	3.92 ± 3.18	4.42 ± 1.16
	Range		(2.5 - 17.5)	(1.5 - 6.5)	(3.0 - 12.5)	(1.5 - 6.0)	(0.5 - 8.0)	(3.0 - 5.5)

SD = Standard deviation, and ND = Not detected.
In water between seasons ($P < 0.05$), between seasons within a year ($P < 0.05$), between surface and bottom within a centre ($P < 0.1$), Mean with SD and range 14.14 ± 9.69 (ND - 38.5).

In sediment between years ($P < 0.05$), between seasons ($P < 0.001$), between seasons within a year ($P < 0.001$), between stations ($P < 0.001$), Mean with SD and range 4. 89 ± 3.32 (0.5 - 17.5).

TABLE 40. **Zinc in water (ppb) and sediment (ppm) in the Gulf of Mannar and the Palk Bay during premonsoon, monsoon and postmonsoon of 1991 and 1992**

Sample	Station		1991 Season			1992 Season		
			Premonsoon	Monsoon	Postmonsoon	Premonsoon	Monsoon	Postmonsoon
Water	IV 10	Surface	13.5	28.5	7.0	6.5	13.5	8.5
		Bottom	13.5	21.5	8.5	37.0	15.0	7.0
	IV 11	Surface	6.0	16.0	38.5	4.5	15.0	9.0
		Bottom	12.5	52.0	41.5	37.5	42.5	46.5
	IV 12	Surface	5.5	21.5	20.0	3.5	8.0	3.5
		Bottom	9.5	21.5	20.5	33.0	26.5	23.5
	Mean	Surface	8.33	22.00	21.83	4.83	12.17	7.00
		Bottom	11.83	31.67	23.50	35.83	28.00	25.67
	Grand Mean with SD		10.08 ± 3.67	26.83 ± 12.95	22.67 ± 14.58	20.33 ± 17.08	20.08 ± 12.53	16.33 ± 16.30
	Range		(5.5 - 13.5)	(16.0 - 52.0)	(7.0 - 41.5)	(3.5 - 37.5)	(8.0 - 42.5)	(3.5 - 46.5)
Sediment	IV 10		3.00	4.25	2.00	0.75	2.75	1.00
	IV 11		5.85	9.75	0.25	4.50	7.75	ND
	IV 12		2.00	6.25	0.25	0.50	2.50	0.50
	Mean with SD		3.62 ± 2.58	6.75 ± 2.77	0.83 ± 0.93	1.92 ± 2.01	4.33 ± 2.68	0.50 ± 0.84
	Range		(1.5 - 8.7)	(2.5 - 10.0)	(ND - 2.0)	(0.5 - 4.5)	(2.0 - 8.0)	(ND - 2.0)

SD = Standard deviation and ND = Not detected.
In water between seasons ($P < 0.05$), between seasons within a year ($P < 0.05$), between surface and bottom within a centre ($P < 0.1$), Mean with SD and range 19.39 ± 13.65 (3.5 - 52.0).

In sediment between years ($P < 0.05$), between seasons ($P < 0.001$), between seasons within a year ($P < 0.001$), between stations ($P < 0.001$), Mean with SD and range 2.99 ± 2.91 (ND - 10.0).

TABLE 41. **Zinc in water (ppb) and sediment (ppm) in the Ennore Creek during premonsoon, monsoon and postmonsoon of 1991 and 1992**

Sample	Station		1991 Season			1992 Season		
			Premonsoon	Monsoon	Postmonsoon	Premonsoon	Monsoon	Postmonsoon
Water	V 13	Surface	25.00	24.0	19.5	18.0	29.0	34.5
		Bottom	27.5	87.5	31.0	40.5	112.5	29.5
	V 14	Surface	54.0	25.5	38.0	27.0	24.0	13.5
		Bottom	75.0	68.0	29.5	60.0	84.0	127.0
	V 15	Surface	11.0	14.5	12.0	17.0	16.0	9.0
		Bottom	15.0	76.5	39.0	21.0	42.5	21.5
	Mean	Surface	30.0	21.33	23.17	20.67	23.00	19.00
		Bottom	39.33	77.33	33.17	40.50	79.67	59.33
	Grand Mean with SD		34.67 ± 25.03	49.33 ± 31.52	28.17 ± 10.59	30.58 ± 16.80	51.33 ± 38.42	39.17 ± 44.07
	Range		(11.0 - 75.5)	(14.5 - 87.5)	(12.0 - 39.0)	(17.0 - 60.0)	(16.0 - 112.5)	(9.0 - 127.0)
Sediment	V 13		120.25	318.25	67.75	92.00	228.75	115.25
	V 14		103.50	255.75	24.75	97.25	219.75	97.00
	V 15		19.75	33.75	10.75	25.25	27.25	112.50
	Mean with SD		81.17±48.63	202.58±133.87	34.42±35.85	71.50±36.91	178.58±121.24	108.25±21.54
	Range		(19.5-130.5)	(33.0-326.0)	(10.0-105.0)	(20.5-109.5)	(26.5-292.5)	(86.0-144.6)

SD = Standard deviation.

In water between seasons ($P < 0.05$), between seasons within a year ($P < 0.05$), between surface and bottom within a centre ($P < 0.1$), Mean with SD and range 38.88 ± 28.85 (9.0 - 127.0).

In sediment between years ($P < 0.05$), between seasons ($P < 0.001$), between seasons within a year ($P < 0.001$), between stations ($P < 0.001$), Mean with SD and range 112.75 ± 93.99 (10.0 - 326.0).

TABLE 42. **Zinc in water (ppb) and sediment (ppm) in the Rusikulya Estuary during premonsoon, monsoon and postmonsoon of 1991 and 1992**

Sample	Station	1991 Season			1992 Season		
		Premonsoon	Monsoon	Postmonsoon	Premonsoon	Monsoon	Postmonsoon
Water	VI 16 Surface	7.5	9.0	9.0	3.5	33.0	3.5
	VI 16 Bottom	45.5	14.0	23.5	58.0	40.5	22.0
	VI 17 Surface	6.0	1.5	2.0	11.5	18.5	14.0
	VI 17 Bottom	15.0	13.5	34.5	42.5	27.0	22.5
	VI 18 Surface	14.0	2.5	7.5	7.0	20.0	2.0
	VI 18 Bottom	65.0	11.0	33.0	18.5	26.5	11.5
	Mean Surface	9.17	4.33	6.17	7.33	23.83	6.50
	Mean Bottom	41.83	12.83	30.33	39.67	31.33	18.67
	Grand Mean with SD	25.50±24.11	8.58±5.42	18.25±13.96	23.5±21.87	27.58±8.22	12.58±8.77
	Range	(6.0 - 65.0)	(1.5 - 14.0)	(2.0 - 34.5)	(3.5 - 58.0)	(18.5 - 40.5)	(2.0 - 22.5)
Sediment	VI 16	37.50	12.75	3.20	22.00	5.25	10.50
	VI 17	10.75	6.25	6.50	15.00	1.75	6.50
	VI 18	1.75	3.25	7.75	15.25	2.00	6.25
	Mean with SD	16.67 ± 17.01	7.42 ± 4.77	5.82 ± 2.29	17.42 ± 5.01	3.00 ± 1.84	7.75 ± 2.16
	Range	(0.5 - 43.0)	(1.0 - 14.5)	(2.0 - 8.5)	(11.5 - 26.0)	(1.0 - 5.50	(6.0 - 10.5)

SD = Standard deviation.

In water between seasons ($P < 0.05$), between seasons within a year ($P < 0.05$), between surface and bottom within a centre ($P < 0.1$), Mean with SD and range 19.33 ± 15.69 (1.5 - 65.0).

In sediment between years ($P < 0.05$), between seasons ($P < 0.001$), between seasons within a year ($P < 0.001$), between stations ($P < 0.001$), Mean with SD and range 9.68 ± 8.84 (0.5 - 43.0).

with zinc in premonsoon, but nonsignificant relationships were seen for monsoon (r = 0.342) and postmonsoon (r = 0.707).

Discussion

The 10.0 ppb of zinc is shown in seawater by Orr and Marshall (1969). Bryan (1984) reported 0.007-0.640 ppb zinc in natural unpolluted seawater and 10 ppb in freshwater. From the present investigation in all the centres (estuarine and brackishwater) the mean values of zinc were seen higher than that of natural seawater and freshwater of Bryan (1984).

The WHO standard for zinc in water is 5.0 ppb (Qasim and Sengupta, 1983). The safe level recommended by EPA for zinc is 100 ppb (Anon., 1991 a). In comparing zinc in water of study centres with WHO standard, it was found polluted. But in all the centres except Ennore Creek and Cochin Backwater, the lower ranges given in parentheses were quite below WHO standard. The upper range seen for Ennore Creek and Cochin backwater is higher than the EPA safe limit. The higher values recorded were:

Centre	Station	Surface/Bottom	Value (ppb)	Season	Year
Cochin Backwater	II 4	Surface	117.5	Monsoon	1991
"	"	"	151.5	Postmonsoon	"
"	"	Bottom	123.0	Premonsoon	"
"	"	"	108.5	Monsoon	"
"	"	"	165.5	Postmonsoon	"

Cochin Backwater	II4	Bottom	112.5	Postmonsoon	1991
"	II 5	"	154.0	"	1991
"	"	Surface	123.5	"	"
"	II 6	"	123.5	"	"
"	"	Bottom	162.0	Monsoon	"
"	"	"	162.5	Postmonsoon	1991
"	"	"	108.0	"	1992
Ennore Creek	V 13	"	112.5	Monsoon	"
"	V 14	"	127.0	Postmonsoon	"

This may be due to the dumping of city garbage and waste to these systems from which zinc might have leached. This coincides with observations of Berrow and Webber (1972) who found "average" sewage sludge and city wastes are the major sources of zinc in the aquatic environment.

The ranges of dissolved zinc (µg/l or ppb) in the Indian Ocean was determined by various workers. Some of them are:

Value (ppb)	Autor(s)
3.9 - 19.5	Topping, 1969
1.2 - 29.7	Sanzgiri and caroline, 1979
2.4 - 20.0	Braganca and Sanzgiri, 1980
1.9 - 174.0	Qasim and Sengupta, 1983

The mean values for all the centres studied, though estuarine and brackish in nature, were found however, well within the range reported by the above authors. The means for Cochin Backwater and Ennore Creek were in the higher side than other centres. Chester and Stoner (1974) and Fowler et al. (1984) reported the zinc values 0.3-3.0 and 0.002-0.91 ppb from the Indian ocean respectively. But the present investigation showed higher concentrations. Braganca and Sanzgiri (1980) reported the average surface zinc concentration at stations very near to the coast are much higher than those at the stations further away from shore. This almost corroborates the present findings. The same is the case with McGrath and Austin (1979) in Belfast Lough.

The zinc obtained in the present study from six centres are well compared with the values reported by several authors for different coastal and estuarine waters in India and abroad.

Some of them from abroad are(in ppb):

Value	Environment	Author(s)
11.0	Chao Phraya River Estuary	Menasveta, 1978
200.0	Golden Horn Bay, Japan	Tkalin, 1992
From India		
2.8 - 42.3	Mandovi - Zuari Estuary, Goa	Zinde et al., 1976
0.4 - 347.0	near shore waters of Bombay	Sengupta et al., 1978
2.65 - 33.9	Bombay Harbour Bay	Matkar et al., 1981

10.54 - 11.89	Saurashtra Coast	Kesava Rao and Indusekhar, 1986
3.00 - 48.0	Mindhola River Estuary	Zingde et al., 1988
25.5 - 95.4	Vellar Estuary	Lyla, 1991
ND - 52.5	Korapuzha Estuary	Present study
25.0 - 165.5	Cochin Backwater	"
ND - 38.5	Tuticorin Bay	"
3.50 - 52.0	Mandapam water	"
9.00 - 127.0	Ennore Creek	"
1.50 - 65.0	Rusikulya Estuary	"

Though the values for each centre in the present investigation were well compared, they differd significantly among themselves. The reason for this may be the different sources of pollutants entering to each system as mentioned in Table 1, under "Materials and Methods".

Goldberg et al. (1971) have the average world concentration of zinc in stream water to be 20.0 ppb. The mean values for Korapuzha Estuary, Cochin Backwater and Ennore Creek were considerably higher than the observation of Goldberg et al. (1971). Rusikulya Estuary and Mandapam coastal water showed almost same zinc value and Tuticorin Bay showed a value below the average of world stream concentration.

The probable reasons may be:

i. the zinc discharged into system at Mandapam is locked into closed environmental, physical and geographical nature which does not allow the pollutants along with the metals to disperse away from the place of accumulation into the open sea,

ii. the molluscs which accumulate more zinc (Eisler, 1981) in their system is comparatively less densed to that of Tuticorin which is an impor - tant centre of molluscan fishery and particularly the oysters and gastropods, and

iii. Tuticorin Bay is more closer to the open sea with more wave actions near Cape Cormorin area.

A distinct stratification in heavy metals resulting from suspension and churning of sediments in the bottom waters was found in Elbe where the tidal effect is pronounced (Mart and Nurnberg,1986). From the present study, it is observed that the bottom waters from most of the stations recorded significantly higher concentration of zinc than the surface. Similar results for dissolved zinc were found in deeper layers than surface waters from the studies of Braganca and Sanzgiri (1980).

From the present study on seasonal fluctation in zinc concentration in water, higher mean values were found for the postmonsoon followed by premonsoon and monsoon. According to Riley and Chester (1971) the trace metal distribution in the coastal environment is to a great extent influenced by freshwater flow. Zinc effectively removed from solution by adsorption on particles of hydrated ferric hydroxide and manga- nese dioxide, the former being more abundant in coastal water (Nair, 1984).

The reduction in zinc content in monsoon may be due to adsorption of zinc on to the suspended land derived particles in water or the dilution

of it by heavy monsoon water discharge. In postmonsoon the zinc might have been released by dissolution from bound suspended particles due to inclusion of saline water to the estuaries. This is strengthened by Turekian (1971) as observed that the adsorbed form of metals in streams and rivers are always released to a greater or lesser extent on contact with seawater due to their displacement by major ions such as magnesium and calcium in seawater. The other cause of increased zinc in postmonsoon and premonsoon may be due to different sources of pollutants into the estuaries and coastal waters, which generally got diluted in monsoons resulting to low zinc value. During low run-off i.e. postmonsoon and premonsoon the residence time of water in the estuarine-nearshore zone is longeer than high run-off in monsoon. Release from sediments, therefore would be expected to have a greater influence on zinc as seen in the case of copper concentrations during low run-off (Windom et al., 1983).

From the present study the negative relationship was obtained between zinc in water and salinity. It was significant in premonsoon and postmonsoon. The same was also seen with total hardness. It may be due to closer relationship between total hardness and salinity. The negative relationship indicated the decrease in zinc with the increase in salinity in water. These findings are similar to that reported by Duinker and Nolting (1977) in Rhine Estuary, the same authors (1982) and Nolting (1986) in Southern Bight of the North Sea. In the present investigation significant and good correlations were obtained between copper and zinc in water in premonsoon and postmonsoon respectively. The present finding is similar and supports the result obtained during the cruise in autumn 1973 in Southern Bight by Duinker and Nolting (1977).

zinc content and Bioaccumulation Factor a positive relationship (r = 0.595) was seen with high level of significance at 1% level).

Zinc in Sediment

The zinc content in sediments at different stations during different seasons and years are presented in Tables 37 to 42. The two-way ANOVA carried out in a computer indicated the significant difference between the years and higher concentrations were seen in 1991 than 1992. Very high significant differences were obtained between seasons (at 0.1% level) and its interactions with years were significant at 1% level. The monsoon recorded the higher concentratins of zinc followed by postmonsoon and premonsoon. The interaction between season and year was significant at 1% level. Very high significant differences were seen (at 0.1% level) between the centres. Very high significant differences (at 0.1% level) were also seen for the interactions of centre with year, centre with season and stations with centre. Among the stations the level of significance was found at 0.1%, but nonsignificance was seen for interactions such as stations with year and season.

From the correlation study, pooling the data for zinc in water and sediment, a highly significant correlatin coefficient (r = 0.744) was found (Fig. 4).

Discussion

The concentration of zinc (in ppm) was reported (coefficient of variation in parentheses):

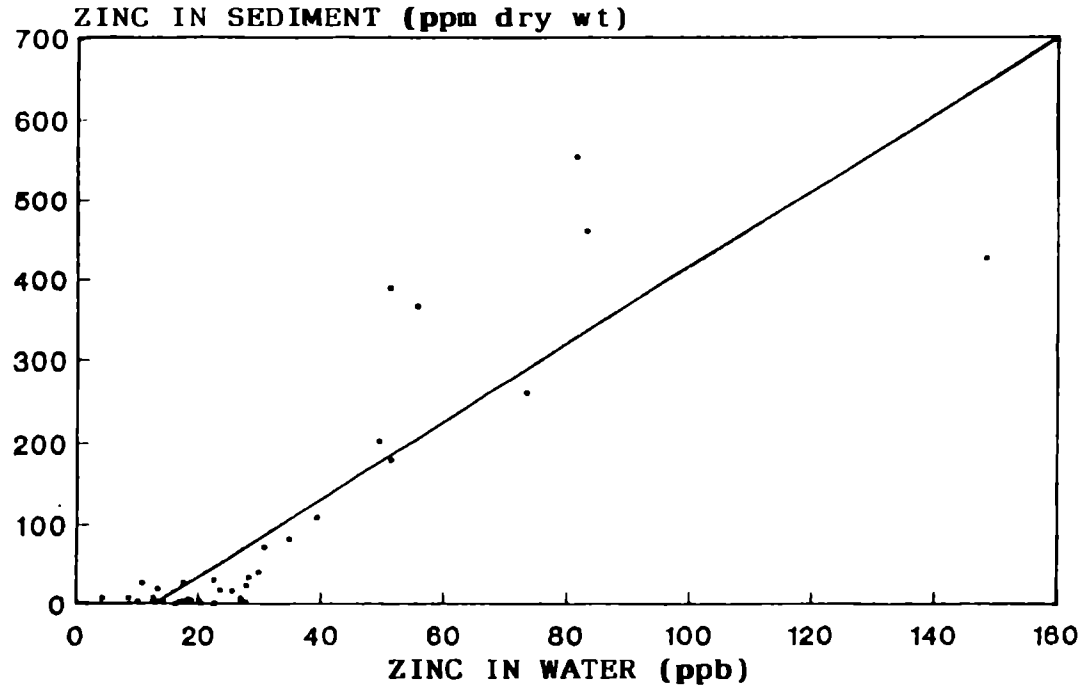


FIG. 4. Relationship between zinc in Water and zinc in Sediment.

USGS Rock Standards	Value (ppm)	Author (s)
MAG-1	102.0(0.057)	Flanagan, 1976
MAG-1	100.0(0.059)	Subramanian et al., 1989
SCO-I	98.8(0.065)	Flanagan,1976
SCO-I	98.0(0.022)	Subramanian et al., 1989
G - 2	68.2(0.055)	Flanagan, 1976
G - 2	66.7(0.006)	Subramanian et al., 1989

In the present investigation the mean zinc values for Cochin Backwater and Ennore Creek were found above the standards and in the other four centres below it. This two centres are situated in the industrial belts of Cochin and Madras respectively. A number of pollutants reach from various sources as already quoted in the "Materials and Methods". All the centres in Cochin Backwater (viz. St. II 4, St. II 5 and St. II 6) and two in Ennore Creek (viz. St. V 13 and St. V 14) showed higher concentration of the zinc in the sediment. These systems act as a major recipients of domestic sewage and industrial wastes (Venugopal et al., 1982; Nammalwar et al., 1985; James et al., 1986). Pragatheeswaran et al. (1988) in their study attributed the increased levels of zinc, to the wastes from chemical industries, sewage , land drainage and leaching by floods into the system. However, there are no literature from Cochin Backwater on the above aspect to compare. The reasons given by the workers from Madras may be more applicable for the present increase

in zinc in sediment of Cochin Backwater also. According to Berrow and Webber (1972) "average" sewage sludge, contains 2600 ppm of zinc. Holmes (1986) reported the zinc to precipitate from the water resulting in high concentrations in sediments near the source. The Cochin Backwater and Ennore Creek contain higher concentrations of zinc in water (Chapter 1, Section : Zinc in water). In the present study also a good relationship was established between zinc in water and sediment (Fig. 4) attributing the increase in zinc in water to that of in sediment.

The zinc concentration in sediments of the stations studied in the present investigation is well within the permissible limit as reported by Rankama and Sahama (1960) who have found the range of zinc in world sediments as 0-1000 ppm. The marine sediment reference materials such as "Miramichi Estuary Standard Sediment (MESS-1)" and "Baie des Chaleurs Standard Sediment (BCSS-1)" were analysed for zinc by Ackroyd et al. (1987). The results obtained by them were 144 ± 40 and 117 ± 7 ppm dry wt for MESS-1 and BCSS-1 respectively. Comparing with this standard values only Cochin Backwater sediments were found polluted. Coastal areas of Mandapam were seen far below the pollution level. "Pollution peak" value of 2096 ppm dry wt zinc was reported by Katz and Kaplan (1981) in polluted South California surface sediments. In the present study all centres have shown the zinc value far below the peak pollution level of zinc in its sediments as given 2096 ppm by Katz and Kaplan (1981). The results in ppm reported by different authors from India and abroad are as follows:

Environment	Value (ppm)	Author(s)
Rio Tinto Estuary, Spain	3100	Stenner and Nickless, 1975
Restronguet Estuary, U.K.	3000	Thornton et al.,1975
Derwent Estuary, Australia	10000	Bloom and Ayling, 1977
Outfall of refineries	104000	"
Corpus Christi Harbour, Texas	235-11000	Holmes,1986
Jakarta Bay	75.8-79.8	Hungspreugs,1988
Northeast Pacific Coastal sediments	33.1-124.0	Harding and Goyette, 1989
Korapuzha Estuary	28.54	Present study
Cochin Backwater	409.65	"
Tuticorin Bay	4.89	"
Mandapam Water	2.99	"
Ennore Creek	112.75	"
Rusikulya Estuary	9.68	"

The zinc values obtained in the present study from different centres are far less than the areas studied by other investigators around the world except Jakarta Bay. The higher zinc concentratin in Cochin Backwater can be very similar to the observation by Bloom and Ayling (1977) at Derwent Estuary as both area are close to oil refineries.

From India the reports for zinc (in ppm) are:

Estuaries	Value(ppm)	Author(s)
Bombay Harbour Bay	80.41±24.5	Matkar et al., 1981
Cochin Backwater	663.5	Venugopal et al., 1982
Narmada Estuary	140.0	Borole et al., 1982
Tapti Estuary	125.0	"
Madras and Visakhapatnam	62.0-106.0	Pragatheeswaran et al.,1986
Ganges Estuary	12.6-611.0	Subramanian et al., 1988
Mindhola River Estuary	75.0-369.0	Zingde et al., 1988
Cauvery Estuary	303.0	Subramanian et al.,1989
Krishna Estuary	1482.0	"
Southeast Coast of India	62.0±34.0	Mohanachandran and Subramanian, 1990
Madras Coast	6.60-50.0	Ramachandran et al.,1991
Vellar Estuary	18.8-202.0	Lyla, 1991

When compared with the results of Venugopal et al. (1982), Cochin Backwater recorded slightly higher concentration of zinc in its sediment, more possibly due to the location of St. II 4 which is near to the industrial area of Cochin. The Ennore Creek where Madras Oil Refinery, Kothari Chemicals, Ennore Thermal Power Station, etc. are situated, recorded

higher concentration of zinc (112.75 ppm dry wt) in sediment than that of Madras Coast (62-106 ppm dry wt) in common.

High concentration of zinc at the river mouths compared to the inter-riverine coastal areas was observed by Mohanachandran and Subramanian (1990). A scrutiny of literature, attributes that the river carries iron bearing sediments which can act as scavenger or carrier of metals by natural or anthropogenic contributions. In the present investigations estuaries dominated by rivers such as Cochin Backwater, Ennore Creek, Korapuzha Estuary and Rusikulya Estuary showed higher zinc concentrations. The seasonal difference was noticed from the statistical analysis. The monsoon recorded higher concentration of zinc than the postmonsoon and premonsoon. The increase in zinc during monsoon in Vellar Estuary and Killai Backwater, similar to the present investigation, was also reported by Kumaraguru (1980). Lyla (1991) has also reported from the same area that increase of zinc in sediment is seen in monsoon followed by postmonsoon and premonsoon as in the case of present study. The rivers during heavy monsoon empties water rich in organic content into the estuary is a probable reason for high concentration. The organic particles have the affinity to trace metals (Wittmann, 1979).

The relatively high organic content encourages the mobilisation processes and contribute to the variations in metals (Remani et al., 1980; Arzul and Maguer, 1990). With a few exceptions, higher concentration of elements are associated with the silt-clay fractions of sediments (Murty and Veerayya, 1981). According to them the estuarine environments

are favourable for the formation of colloids of iron and manganese oxides derived with freshwater run-off and their flocculation. The flocculated material ultimately sinks to bottom enriching sediment with heavy metals. That might be the reason for high metal content in sediment in monsoon.

The estuarine chemistry of zinc, in general, is poorly understood (Ackroyd et al., 1987). The distribution of it over space and time were not found uniform in the sediments in the present study. This variation in the concentration may be due to differences on the sources of heavy metals and complex reactions such as absorption, flocculation, etc. taking place in the sediments. According to Holmes (1986) during the dry seasons when the water is more stagnant, zinc and cadmium which are introduced from the industrial effluent, are concentrated in the oxidizing surface waters. Most of the zinc is probably present in the ionic state (Zn^{2+}) or as ionic pairs with chloride ($ZnCl^{+}$). Ultimately the deposit of zinc to the sediment is reduced. The same may be a probable reason for the reduced level of zinc in sediment in postmonsoon and premonsoon. Similarly, as reported by Holmes (1986), in the present study postmonsoon recorded more zinc in the estuarine water (Chapter 1, Section : Zinc in water).

Zinc in fish tissues

The content of zinc in liver, gills, kidney, intestine, ovary, skin and muscle of L. parsia during different seasons in 1991 and 1992 and at different centres along with grand mean and standard deviation are presented in Tables 43 to 49. Pooling to data over cllections for specific

TABLE 43. Zinc (ppm in dry wt) in liver of L. parsia at different centres during premonsoon, monsoon and postmonsoon of 1991 and 1992

Centre	Centre Code	1991			1992			Mean with SD
		Season						
		Premonsoon	Monsoon	Postmonsoon	Premonsoon	Monsoon	Postmonsoon	
Korapuzha Estuary	I	36.94 (0.018)	60.71 (0.033)	21.40 (0.008)	94.50 (0.019)	-	45.08 (0.021)	51.73 ± 27.81
Cochin Backwater	II	62.62 (0.027)	43.67 (0.018)	108.30 (0.029)	331.00 (0.055)	66.80 (0.113)	162.50 (0.144)	129.15 ± 107.62
Tuticorin Bay	III	211.92 (0.080)	68.30 (0.036)	241.45 (0.313)	170.49 (0.099)	179.12 (0.098)	244.00 (0.115)	185.88 ± 65.19
Mandapam	IV	57.80 (0.023)	85.30 (0.023)	85.76 (0.033)	41.16 (0.056)	95.47 (0.048)	173.50 (0.191)	89.83 ± 45.75
Ennore Creek	V	194.03 (0.152)	47.70 (0.020)	107.24 (0.061)	150.66 (0.147)	58.52 (0.032)	347.10 (0.323)	150.88 ± 110.86
Rusikulya Estuary	VI	82.23 (0.026)	85.88 (0.040)	66.20 (0.084)	91.90 (0.058)	-	72.88 (0.026)	79.82 ± 10.27

SD = Standard deviation, coefficient of variation is in parentheses.

Between seasons ($P < 0.1$).

TABLE 44. **Zinc (ppm in dry wt) in gills of L. parsia at different centres during premonsoon, monsoon and postmonsoon of 1991 and 1992**

Centre	Centre Code	1991			1992			Mean with SD
		Season						
		Premonsoon	Monsoon	Postmonsoon	Premonsoon	Monsoon	Postmonsoon	
Korapuzha Estuary	I	37.55 (0.018)	50.46 (0.023)	53.90 (0.028)	81.10 (0.032)	-	49.59 (0.028)	54.52 ± 16.09
Cochin Backwater	II	65.45 (0.020)	66.82 (0.057)	67.80 (0.030)	272.40 (0.170)	72.58 (0.062)	63.20 (0.083)	101.38 ± 83.84
Tuticorin Bay	III	60.56 (0.054)	60.10 (0.036)	49.56 (0.019)	57.08 (0.058)	59.68 (0.088)	75.20 (0.049)	60.36 ± 8.34
Mandapam	IV	72.24 (0.047)	39.90 (0.021)	39.03 (0.016)	42.63 (0.032)	65.73 (0.072)	72.30 (0.061)	55.31 ± 16.41
Ennore Creek	V	48.95 (0.038)	71.20 (0.032)	42.00 (0.019)	60.30 (0.028)	72.00 (0.038)	433.90 (0.646	121.39 ± 153.56
Rusikulya Estuary	VI	35.08 (0.018)	35.00 (0.008)	57.76 (0.027)	84.90 (0.034)	-	55.23 (0.019)	53.59 ± 20.55

SD = Standard deviation, coefficient of variation is in parentheses.

TABLE 45. Zinc (ppm in dry wt) in kidney of L. parsia at different centres during premonsoon, monsoon and postmonsoon of 1991 and 1992

Centre	Centre Code	1991 Season			1992 Season			Mean with SD
		Premonsoon	Monsoon	Postmonsoon	Premonsoon	Monsoon	Postmonsoon	
Korapuzha Estuary	I	43.31 (0.016)	35.42 (0.010)	37.80 (0.009)	ND	-	51.77 (0.016)	33.66 ± 19.84
Cochin Backwater	II	41.45 (0.021)	30.90 (0.009)	43.00 (00.006)	ND	29.20 (0.038)	51.66 (0.019)	32.80 ± 18.11
Tuticorin Bay	III	87.61 (0.012)	84.70 (0.008)	60.84 (0.012)	65.34 (0.092)	100.49 (0.116)	ND	66.50 ± 35.74
Mandapam	IV	41.67 (0.006)	52.60 (0.009)	44.12 (0.009)	26.26 (0.018)	48.88 (0.073)	ND	35.59 ± 19.64
Ennore Creek	V	49.78 (0.005)	62.70 (0.010)	78.83 (0.018)	25.45 (0.018)	72.55 (0.061)	42.60 (0.036)	55.32 ± 19.94
Rusikulya Estuary	VI	38.46 (0.016)	32.66 (0.024)	82.72 (0.033)	144.00 (0.029)	-	91.77 (0.016)	77.92 ± 45.24

SD = Standard deviation, coefficient of variation is in parentheses and ND = Not detected.

TABLE 46. **Zinc (ppm in dry wt) in intestine of L. parsia at different centres during premonsoon, monsoon and postmonsoon of 1991 and 1992**

Centre	Centre Code	1991 Season			1992 Season			Mean with SD
		Premonsoon	Monsoon	Postmonsoon	Premonsoon	Monsoon	Postmonsoon	
Korapuzha Estuary	I	57.25 (0.035)	56.80 (0.042)	95.90 (0.016)	87.20 (0.053)	-	58.32 (0.022)	71.09 ± 18.93
Cochin Backwater	II	70.65 (0.048)	46.60 (0.017)	74.40 (0.060)	68.52 (0.044)	39.52 (0.031)	82.21 (0.122)	63.65 ± 16.77
Tuticorin Bay	III	88.70 (0.054)	93.30 (0.028)	49.19 (0.030)	71.20 (0.064)	101.22 (0.114)	146.80 (0.146)	91.73 ± 32.76
Mandapam	IV	93.11 (0.024)	37.00 (0.009)	48.66 (0.024)	96.56 (0.028)	41.52 (0.112)	99.50 (0.059)	69.39 ± 29.88
Ennore Creek	V	46.50 (0.16)	49.20 (0.026)	56.36 (0.027)	50.85 (0.082)	50.61 (0.041)	185.10 (0.134)	73.10 ± 54.96
Rusikulya Estuary	VI	67.49 (0.039)	9.38 (0.009)	128.50 (0.031)	69.80 (0.026)	-	225.81 (0.042)	100.20 ± 81.89

SD = Standard deviation, coefficient of variation is in parentheses.

Between seasons ($P < 0.01$), between seasons within a year ($P < 0.1$).

TABLE 47. **Zinc (ppm in dry wt) in ovary of L. parsia at different centres during premonsoon, monsoon and postmonsoon of 1991 and 1992**

Centre	Centre Code	1991 Season			1992 Season			Mean with SD
		Premonsoon	Monsoon	Postmonsoon	Premonsoon	Monsoon	Postmonsoon	
Korapuzha Estuary	I	229.76 (0.116)	345.11 (0.408)	33.30 (0.164)	452.8 (0.393)	-	58.22 (0.026)	223.84 ± 180.90
Cochin Backwater	II	133.40 (0.064)	192.81 (0.030)	463.20 (0.212)	229.80 (0.037)	212.00 (0.216)	356.61 (0.118)	264.64 ± 121.86
Tuticorin Bay	III	196.20 (0.005)	53.50 (0.020)	334.87 (0.301)	119.57 (0.114)	68.86 (0.077)	495.50 (0.161)	211.42 ± 173.13
Mandapam	IV	52.51 (0.007)	72.00 (0.012)	354.23 (0.311)	411.04 (0.162)	185.71 (0.184)	56.00 (0.026)	188.58 ± 159.14
Ennore Creek	V	857.35 (0.365)	120.50 (0.023)	335.36 (0.142)	283.90 (0.120)	108.0 (0.062)	294.60 (0.122)	333.29 ± 273.72
Rusikulya Estuary	VI	408.65 (0.142)	511.06 (0.163)	388.00 (0.038)	96.00 (0.023)	-	373.87 (0.052)	355.52 ± 154.72

SD = Standard deviation, coefficient of variation is in parentheses.

Between stations ($P < 0.05$).

TABLE 48. **Zinc (ppm in dry wt) in skin of L. parsia at different centres during premonsoon, monsoon and postmonsoon of 1991 and 1992**

Centre	Centre Code	1991			1992			Mean with SD
		Season						
		Premonsoon	Monsoon	Postmonsoon	Premonsoon	Monsoon	Postmonsoon	
Korapuzha Estuary	I	50.48 (0.032)	100.93 (0.027)	48.60 (0.016)	110.20 (0.033)	-	89.36 (0.031)	79.91 ± 28.70
Cochin Backwater	II	135.28 (0.041)	64.29 (0.013)	107.90 (0.029)	131.00 (0.092)	70.06 (0.022)	107.90 (0.038)	102.74 ± 29.86
Tuticorin Bay	III	63.82 (0.025)	76.50 (0.005)	119.39 (0.074)	61.50 (0.027)	153.61 (0.126)	73.10 (0.038)	91.32 ± 37.05
Mandapam	IV	144.80 (0.044)	79.20 (0.015)	106.38 (0.040)	73.85 (0.041)	62.50 (0.092)	98.90 (0.034)	94.27 ± 29.58
Ennore Creek	V	71.86 (0.043)	21.70 (0.010)	98.65 (0.085)	53.57 (0.081)	19.50 (0.012)	122.70 (0.029)	64.66 ± 41.43
Rusikulya Estuary	VI	65.97 (0.032)	40.00 (0.009)	49.90 (0.022)	67.70 (0.040)	-	58.61 (0.028)	56.44 ± 11.57

SD = Standard deviation, coefficient of variation is in parentheses.

Between stations ($P < 0.1$).

TABLE 49. **Zinc (ppm in dry wt) in muscle of L. parsia at different centres during premonsoon, monsoon and postmonsoon of 1991 and 1992**

Centre	Centre Code	1991			1992			Mean with SD
		Season						
		Premonsoon	Monsoon	Postmonsoon	Premonsoon	Monsoon	Postmonsoon	
Korapuzha Estuary	I	13.40 (0.003)	12.56 (0.008)	14.40 (0.011)	22.30 (0.022)	-	13.88 (0.012)	15.31 ± 3.97
Cochin Backwater	II	13.92 (0.013)	8.14 (0.007)	22.30 (0.009)	19.90 (0.019)	11.22 (0.008)	23.32 (0.028)	16.47 ± 6.26
Tuticorin Bay	III	29.97 (0.080)	25.80 (0.013)	16.72 (0.007)	25.86 (0.038)	57.61 (0.082)	50.90 (0.046)	34.47 ± 16.06
Mandapam	IV	15.24 (0.008)	22.90 (0.011)	14.08 (0.006)	11.45 (0.009)	20.65 (0.078)	20.10 (0.017)	17.40 ± 4.45
Ennore Creek	V	19.09 (0.010)	14.10 (0.006)	14.04 (0.006)	18.62 (0.022)	13.00 (0.013)	14.60 (0.020)	15.58 ± 2.60
Rusikulya Estuary	VI	12.62 (0.004)	10.62 (0.007)	8.85 (0.009)	27.60 (0.015)	-	10.60 (0.016)	14.06 ± 7.69

SD = Standard deviation, coefficient of variation is in parentheses.

Between centres ($P < 0.01$).

tissue, the grand mean values obtained from computer in descending order are as follows:

Tissue	Value (ppm dry wt)
Ovary	246.79
Liver	110.89
Skin	77.77
Intestine	73.44
Gills	71.42
Kidney	47.20
Muscle	18.07

Liver: From ANOVA on computer for zinc content in liver, significant difference (at 10% level) was seen between the seasons only. The zinc content was found more in postmonsoon followed by premonsoon and monsoon (Table 43).

From correlation study, very poor and positive nonsignificant relationships were obtained for zinc content in liver with the available concentrations of zinc in water ($r = 0.087$) and sediment ($r = 0.02$).

Gills: ANOVA showed nonsignificant difference between the years, seasons and centres (Table 44).

The zinc values in gills showed very weak positive nonsignificant relationships with that in water ($r = 0.216$) and sediment ($r = 0.179$).

The zinc in gills and liver indicated a very high significant (at 1% level) positive correlation (r = 0.692) among them.

Kidney: Table 45 shows the centrewise yearly and seasonal content of zinc in kidney. No significant differences were observed between the years, centres and seasons as seen from ANOVA.

The correlation study indicated the weak positive nonsignificant relationships between the values of zinc in kidney and water (r = 0.146), and kidney and sediment (r = 0.136).

Intestine: From two-way ANOVA on computer the zinc content in intestine (ppm dry wt) in different years, seasons and centres (Table 46), very high significant difference (at 1% level) was observed between the seasons and significant at 10% level for interactions with year. The higher degree of zinc in intestine was recorded in postmonsoon followed by premonsoon and monsoon.

From correlation study very weak positive nonsignificant relationships were obtained for zinc content in intestine with the available concentration of zinc in water (r = 0.095) and sediment (r = 0.117). The zinc in intestine showed a significant (at 5% level) positive bearing (r = 0.371) with the content in liver and highly significant (at 1% level) positive relationship (r = 0.452) with the content in gills.

Ovary: From ANOVA the significant difference (at 5% level) was obtained for the centres (Table 47) and zinc content in ovary is given below in

the descending order:

Centre	Value (ppm dry wt)
Rusikulya Estuary	355.52 ± 154.72
Ennore Creek	333.29 ± 273.72
Cochin Backwater	264.64 ± 121.86
Korapuzha Estuary	223.84 ± 180.90
Tuticorin Bay	211.42 ± 173.13
Mandapam Water	188.58 ± 159.14

The zinc in ovary showed very weak positive nonsignificant relation with that in water (r = 0.159) and sediment (r = 0.054)

Skin: The two-way ANOVA indicated significant differences between the centres at 10% F-value (Table 48). The content of zinc (ppm dry wt) was estimated and the same in descending order as follows:

Centre	Value (ppm dry wt)
Cochin Backwater	102.74 ± 29.86
Mandapam Water	94.27 ± 29.58
Tuticorin Bay	91.32 ± 37.05
Korapuzha Estuary	79.91 ± 28.70
Ennore Creek	64.66 ± 41.43
Rusikulya Estuary	56.44 ± 11.57

The weak positive nonsignificant correlation coefficients were obtained for zinc content in skin related to zinc concentration in water (r = 0.156) and sediment (r = 0.145). The significant (at 5% level) positive weak coefficients were seen for zinc in skin than in gills (r = 0.391) and intestine (r = 0.330), but a highly significant (at 1% level) relation was obtained with liver (r = 0.496).

Muscle: ANOVA indicated the high significant difference (at 1% F-value) between the centres (Table 49). The content of zinc (ppm dry wt) in descending order were:

Centre	Value (ppm dry wt)
Tuticorin Bay	34.48 ± 16.06
Mandapam Water	17.40 ± 4.45
Cochin Backwater	16.47 ± 6.26
Ennore Creek	15.58 ± 2.60
Korapuzha Estuary	15.31 ± 3.97
Rusikulya Estuary	14.06 ± 7.69

Poor positive and nonsignificant correlation coefficients were obtained for zinc in muscle with that of water (r = 0.067) and sediment (r = 0.127). The zinc content in muscle showed highly significant (at 1% level) positive relation with the content in liver (r = 0.503) and skin (r = 0.438).

Discussion

Zinc is an essential metal, required e.g. in the enzymes carbonic anhydrase and alkaline phosphatase, and a proportion of the absorbed zinc

content in the body must be metabolically available for metabolism (Williams, 1984). Mohapatra (1989) found the alkaline phosphatase in L. parsia in a substantial amount. In general marine vertebrates particularly teleosts have low zinc content, i.e., 6.0 - 400.0 ppm dry wt in tissue when compared to invertebrates (Eisler, 1981). The zinc content in seven tissues of L. parsia of the present study was found within the limits mentioned by Eisler (1981).

Till now no standards have been reported for content and accumulation of heavy metals in different tissues/organs of fish, which would tell us the toxic limits of the same. The maximum permissible limit of zinc for canned shrimp/prawn is 100 ppm (Anon, 1991 c). But this cannot be used as a standard for the present study as the shrimp and fish belong to two entirely different animal groups viz. invertebrates and vertebrates. Capelli et al. (1987) reported 33.0 ± 1.0 ppm dry wt zinc for "Standard Reference Material (SRM)" obtained from he "International Laboratory for Marine Radioactivity (IAEA/Monaco)". Comparing with the standard zinc of IAEA, the present study on zinc content showed higher concentration in all the tissues except muscle.

There are very few reports on zinc content in mullets. Ting (1971) reported 398-1430 and 210-750 ppm ash wt of zinc in viscera and skin of Mugil curema respectively. On muscle tissue the reports are:

Species	Value (ppm)	Author(s)
M. cuerma	98-560 (ash wt)	Ting, 1971
M. cephalus	17.0	Windom et al.,1973
L. parsia (whole body)	47.8 - 63.0	Zingde et al., 1976
L. parsia	18.07	Present study

In the study of Zingde et al. (1976) no specific tissue distribution of zinc was found and thus the present study provides good informations about different metal contents in different tissues. From India the reports available on zinc content (ppm dry wt) in different tissues of mullets are:

Species	Liver	Gill	Muscle	Area	Author
L. macrolepis	76.68	55.2	47.15	Adayar Estuary	Nammalwar,1987
"	86.65	45.2	67.28	Ennore Estuary	"
L. parsia	150.88	121.3	15.58	Ennore Creek	Present study

The probable reason for differential content reported by Nammalwar (1987) and in the present study in liver, muscle and gills in Ennore Creek may be the different species studied.

The higher zinc content (ppm) was recorded in ovary followed by liver, skin, intestine, gills, kidney and muscle in that order in the present study. Simiar reports are available for different fish specimens and are given below:

Species	Liver	Gonad	Kidney	Muscle	Gill	Gut wall	Author(s)
Morone saxatilis	35.90	-	-	3.8	-	-	Windom et al., 1973
Cheiloda-ctylis macropterus	90-1200	52.100	12.20	0.9-7.0	-	-	Brooks and Rumsey, 1974
Latridopsis ciliaris	34-90	70-180	-	1.8-20	26-33	-	"
Merlangus merlangus	28.3	-	-	9.2	-	23.1	"
Gadus morhua	16.7	44.9	-	4.1	-	-	Julshamn and Brekkan, 1975
Polyprion oxygeneios	33-90	11-200	15-29	2.1-12	24-38	-	"
Pleuronectes platessa	38.9	-	-	10-11.9	-	27.5	Wharfe and Van Den Broek, 1977
Platichthys flesus	53.8	146.8	-	12.3	-	36.2	"
Pagothenia borchgrevinki	28.1	32.5	-	5.65	-	-	Honda et al., 1983
L. parsia	110.89	246.79	47.20	18.07	71.42	73.44	Present study

In all the reports muscle tissue recorded the least zinc content as observed in the present investigation too. Most of the above reports indicate that ovary showed more amount of zinc than the other tissue, but the reason for the same is not known.

Among the teleosts, alongwith the gonad, the specific site of zinc concentration is viscera (Eisler, 1981). According to Overnell et al. (1988) the metal binding protein is found in liver and kidney of the fish. Roch and McCarter (1984) have demonstrated experimentally that elevated levels of zinc can induce enhanced levels of hepatic metallothionein in freshwater fish. The zinc present in the aquatic environment might have induced the production of metallothionein in liver and kidney for binding of the metal to it as observed in the present study. These organs also function as the main reservoirs for a number of substances including heavy metals (Romeril and Davis 1976; Wharfe and Van Den Broek, 1977). The kidney plays the key role in excretion of the metals to the outer environment from the body which liver does not. The zinc content was also found more in liver than in kidney in the present study. Skin recorded more zinc next to ovary and liver as seen in the present investigation. The probable reason may be due to direct contact with the medium from which zinc might have been taken through absorption or adsorption. A protective mechanism against heavy metals was described by Noel-Lambot (1981) wherein corpuscules are present in the intestinal lumen of Anguilla anguilla before elimination with faeces. According to him these corpuscules contain high concentration of zinc chloride from water ingested by the eel. The similar reason can be attributed to the zinc accumulation in intestine of L. parsia. Unlike liver metallothionein, gill metallothionein binds only a very small amount of zinc (Noel-Lambot et al., 1978). In most cases axial muscle shows very less of all metals (Phillips, 1977).

In the present study the variation in zinc content in ovary, skin and muscle of L. parsia in different centres may be due to the differences in the concentration in the medium.

From the present investigation no good correlations were found for zinc between the tissues and the abiotic factors such as zinc in water and sediment. Similar reports are available for zinc in flesh with water (Wilson et al., 1981), in eels with natural environment (Brusle, 1989), in tissue levels with sediment (Harding and Goyette, 1989; Young and Harvey, 1989). But in all the cases the poor relationships were positive as seen in the present investigation. Similar positive correlation between the concentration of zinc in fish muscle and in water was found by Jaffer et al. (1988). According to Young and Harvey (1989) the absence of correlation between concentrations of Fe, Zn and Cu in liver, kidney and muscle tissue and fish size ($P < 0.05$) implies that these metals were homeostatically controlled. The absence of correlation, perhaps reflect either regulation by the fish or limited input from the environment (Milner, 1982).

The seasonal variation was seen for zinc content in liver at 10% level and intestine at 1% level in the present study. In these cases, the postmonsoon followed by premonsoon recorded higher content of zinc than the monsoon. The decrease in zinc during monsoon may be due to dilution of the water in the estuaries. As the monsoon subsides, the zinc content increases in liver and intestine. Similar seasonal report is available for zinc in Metapenaeus dobsoni in Cochin Backwater (Sivadasan and Nambisan, 1988).

For statistical analysis the standard deviations were often as large as the sample mean itself, as seen for fishes from Palestine Lake (Murphy et al., 1978). Giesy and Wiener (1977) found the trace metal concentrations in fishes which usually exhibit positive skew are frequently non-normal. They stated that when dealing with a sample that departs substantially from normality, characterisation of the sample by mean may be misleading. Unfortunately, all data in the literature on metal concentrations in fish have utilised means and standard deviation only.

In aquatic pollution studies, only little evidences are available regarding the correlation of trace elements in fish tissues and environments (Windom et al., 1973; Eustace, 1974) and this has been attributed to the mobility of fish with the localised nature of metal contamination in the environment.

Bioaccumulation Factor

The Bioaccumulation Factor in different seasons of 1991 and 1992 at different centres with grand mean and standard deviation for liver, gills, kidney, intestine, ovary, skin and muscle respectively are shown in Tables 50 to 56. Pooling the data over collections for specific tissues the grand mean values obtained from computer were found in decreasing order as given below:

TABLE 50. **Bioaccumulation Factor for zinc in liver of L. parsia at different centres during premonsoon, monsoon and postmonsoon of 1991 and 1992**

Centre	Centre Code	1991			1992			Mean with SD
		Season						
		Premonsoon	Monsoon	Postmonsoon	Premonsoon	Monsoon	Postmonsoon	
Korapuzha Estuary	I	331.3	1506.7	254.7	911.2	-	902.3	781.2 ± 509.3
Cochin Backwater	II	301.5	143.1	194.9	1202.8	349.5	521.8	452.3 ± 390.7
Tuticorin Bay	III	13303.6	1066.9	3435.7	3662.4	3372.6	3582.8	4737.3 ± 4310.4
Mandapam	IV	1529.3	848.2	1009.3	540.2	1268.5	2834.6	1338.5 ± 808.2
Ennore Creek	V	1493.1	258.0	1015.7	1314.5	304.2	2364.2	1125.0 ± 793.0
Rusikulya Estuary	VI	860.4	2670.5	967.8	1043.4	-	1545.7	1417.6 ± 748.2

SD = Standard deviation, between centres ($P < 0.01$).

TABLE 51. **Bioaccumulation Factor for zinc in gills of L. parsia at different centres during premonsoon, monsoon and postmonsoon of 1991 and 1992**

Centre	Centre Code	1991			1992			Mean with SD
		Season						
		Premonsoon	Monsoon	Postmonsoon	Premonsoon	Monsoon	Postmonsoon	
Korapuzha Estuary	I	236.7	880.1	450.8	449.6	-	697.5	542.9 ± 249.3
Cochin Backwater	II	221.4	153.9	85.8	695.7	266.8	142.6	261.0 ± 222.1
Tuticorin Bay	III	2671.8	659.8	495.6	861.7	789.7	776.0	1042.4 ± 808.4
Mandapam	IV	1343.8	278.8	322.8	393.2	613.8	830.1	630.4 ± 405.9
Ennore Creek	V	264.7	270.6	279.6	369.7	263.0	2077.0	587.4 ± 730.9
Rusikulya Estuary	VI	257.9	764.9	593.4	677.4	-	723.2	623.4 ± 222.1

SD = Standard deviation.

TABLE 52. **Bioaccumulation Factor for zinc in kideny of L. parsia at different centres during premonsoon, monsoon and postmonsoon of 1991 and 1992**

Centre	Centre Code	1991 Season			1992 Season			Mean with SD
		Premonsoon	Monsoon	Postmonsoon	Premonsoon	Monsoon	Postmonsoon	
Korapuzha Estuary	I	461.1	1043.5	534.0	ND	-	1230.0	653.7 ± 490.7
Cochin Backwater	II	236.9	120.2	93.2	ND	181.3	196.9	138.1 ± 85.4
Tuticorin Bay	III	6528.5	1570.5	1027.6	1666.1	2246.0	ND	2173.1 ± 2264.0
Mandapam	IV	1309.2	620.9	616.4	409.1	770.9	ND	621.1 ± 430.5
Ennore Creek	V	454.7	402.5	886.2	263.6	447.6	344.4	466.5 ± 217.6
Rusikulya Estuary	VI	477.7	1205.5	1435.5	1940.6	-	2310.3	1473.9 ± 704.1

SD = Standard deviation and ND= Not detected.

Between centres ($P < 0.01$).

TABLE 53. **Bioaccumulation Factor for zinc in intestine of L. parsia at different centres during premonsoon, monsoon and postmonsoon of 1991 and 1992**

Centre	Centre Code	1991 Season			1992 Season			Mean with SD
		Premonsoon	Monsoon	Postmonsoon	Premonsoon	Monsoon	Postmonsoon	
Korapuzha Estuary	I	508.6	1396.5	1130.5	832.9	-	1156.3	1005.0 ± 342.0
Cochin Backwater	II	336.9	151.3	1326.5	246.7	204.8	261.5	421.3 ± 447.7
Tuticorin Bay	III	5516.1	1443.7	693.2	1515.1	1888.0	2135.3	2198.6 ± 1697.6
Mandapam	IV	2441.4	364.5	567.3	1255.3	546.5	1601.5	1130.9 ± 801.2
Ennore Creek	V	354.5	263.6	528.8	439.5	260.6	1249.0	516.0 ± 373.7
Rusikulya Estuary	VI	699.5	288.9	1861.0	785.0	-	4744.2	1675.7 ± 1811.0

SD = Standard deviation, between centres ($P < 0.1$).

TABLE 54. **Bioaccumulation Factor for zinc in ovary of L. parsia at different centres during premonsoon, monsoon and postmonsoon of 1991 and 1992**

Centre	Centre Code	1991 Season			1992 Season			Mean with SD
		Premonsoon	Monsoon	Postmonsoon	Premonsoon	Monsoon	Postmonsoon	
Korapuzha Estuary	I	2184.1	9078.8	420.0	4627.8	-	1235.2	3509.2 ± 3490.8
Cochin Backwater	II	680.7	669.7	883.6	885.1	1175.6	1213.9	918.1 ± 234.2
Tuticorin Bay	III	13055.4	885.8	5050.7	2722.6	1374.3	7704.2	5132.2 ± 4633.8
Mandapam	IV	1473.2	758.9	4418.9	5717.8	2615.5	969.8	2659.0 ± 2016.2
Ennore Creek	V	6993.3	690.8	3366.7	2625.5	595.0	2127.0	2733.1 ± 2352.5
Rusikulya Estuary	VI	4532.0	16844.7	6012.4	1155.3	-	8404.6	7389.8 ± 5902.5

SD = Standard deviation.

TABLE 55. **Bioaccumulation Factor for zinc in skin of L. parsia at different centres during premonsoon, monsoon and postmonsoon of 1991 and 1992**

Centre	Centre Code	1991 Season			1992 Season			Mean with SD
		Premonsoon	Monsoon	Postmonsoon	Premonsoon	Monsoon	Postmonsoon	
Korapuzha Estuary	I	596.8	3302.1	762.4	1709.6	-	2357.7	1745.7 ± 1127.5
Cochin Backwater	II	858.5	277.7	256.0	627.5	483.1	456.8	493.3 ± 226.1
Tuticorin Bay	III	5281.3	1575.2	2239.4	1741.5	3812.6	1414.9	2677.5 ± 1545.7
Mandapam	III	5052.2	1038.2	1650.4	1277.6	1094.7	2130.0	2033.0 ± 1536.1
Ennore Creek	IV	729.0	154.7	1231.6	616.1	133.6	1101.7	661.1 ± 460.6
Rusikulya Estuary	V	909.9	1639.6	961.6	1013.2	-	1638.6	1232.6 ± 372.9

SD = Standard deviation, between centres ($P < 0.01$).

TABLE 56. **Bioaccumulation Factor for zinc in muscle of L. parsia at different centres during premonsoon, monsoon and postmonsoon of 1991 and 1992**

Centre	Centre Code	1991			1992			Mean with SD
		Season						
		Premonsoon	Monsoon	Postmonsoon	Premonsoon	Monsoon	Postmonsoon	
Korapuzha Estuary	I	109.3	283.4	155.8	195.5	-	252.6	199.3 ± 70.6
Cochin Backwater	II	60.9	24.3	36.5	65.8	53.4	68.1	51.5 ± 17.5
Tuticorin Bay	III	1710.8	366.5	216.3	505.1	986.3	679.6	744.1 ± 543.3
Mandapam	IV	366.8	207.1	150.7	136.6	249.5	298.5	234.9 ± 88.5
Ennore Creek	V	133.6	69.3	120.9	147.7	61.4	90.4	103.9 ± 35.4
Rusikulya Estuary	VI	120.1	300.3	117.6	284.9	-	204.4	205.5 ± 87.1

SD = Standard deviation, between centres ($P < 0.01$).

Tissue	Value
Ovary	3420.79
Liver	1580.89
Skin	1392.39
Intestine	1083.44
Kidney	861.91
Gills	582.21
Muscle	245.29

Liver: From ANOVA, no significant differences were observed between the years and seasons (Table 50). Highly significant difference at (1% level) was observed between the centres and in descending order they are:

Centre	Value
Tuticorin Bay	4737.3 ± 4310.4
Rusikulya Estuary	1417.6 ± 748.2
Mandapam Water	1338.5 ± 808.2
Ennore Creek	1125.9 ± 793.0
Korapuzha Estuary	781.2 ± 509.3
Cochin Backwater	452.3 ± 390.7

The 5% significant and weak positive correlation coefficient (r = 0.347) were found out for Bioaccumulation Factor in liver with zinc content in water. The nonsignificant 'r' value of 0.281 was found with sediment. The highly significant (at 1% level) positive relation (r = 0.477) was observed between the zinc content and KB in liver.

Gills: No significant differences were found between years of study, seasons and centres (Table 51).

A significant (at 5% level) and weak positive relation (r = 0.377) was seen between the Bioaccumulation Factor in gills and the available concentration in water. While correlating with the available concentration in sediments, a nonsignificant and weak coefficient (r = 0.309) was obtained. The content and Bioaccumulation Factor of zinc in gills showed a positive relation (r = 0.486) with significance at 1% level.

Kidney: No significant differences were seen between the years and seasons (Table 52). But the centres were differed at 1% F-value. They are:

Centre	Value
Tuticorin	2173.1 ± 2264.0
Rusikulya Estuary	1473.9 ± 704.1
Korapuzha Estuary	653.7 ± 490.7
Mandapam Water	621.1 ± 430.5
Ennore Creek	466.5 ± 217.6
Cochin Backwater	138.1 ± 85.4

A significant (at 5% value) and weak positive relationship (r = 0.404) was seen between the Bioaccumulation Factor and the avilable concentration of zinc in water and sediment (r = 0.322). The content and KB i.e. Bioaccumulation Factor of zinc in kidney showed a highly significant (at 1% level) and positive relationship (r = 0.614).

Intestine: From ANOVA no significant difference was seen between the years and seasons (Table 53). But the centrewise Bioaccumulation Factors were seen significant at 10% F-value as given below in decreasing order:

Centre	Value
Tuticorin Bay	2198.6 ± 1697.6
Rusikulya Estuary	1675.7 ± 1811.0
Mandapam Water	1130.9 ± 801.2
Korapuzha Estuary	1005.0 ± 342.0
Ennore Creek	516.0 ± 373.7
Cochin Backwater	421.3 ± 447.7

A significant (at 5% level) and weak positive relation (r = 0.331) between the Bioaccumulation Factor and the available concentration in water were seen from correlation studies. Similar result was also obtained with sediment (r = 0.318), but not significant. A good positive highly significant (at 1% level) coefficient (r = 0.668) was seen between zinc content and Bioaccumulation Factor in intestine.

Ovary: No significant differences were observed from ANOVA between years, seasons and centres (Table 54).

From correlation study the significant (at 5% level) positive relations were obtained between the Bioaccumulation Factor and the available concentration of zinc in water (r = 0.393) and sediment (r = 0.342). Between

Skin: From ANOVA the highly significant differences (at 1% level) were seen between the centres (Table 55) and in descending order as below:

Centre	Value
Tuticorin Bay	2677.5 ± 1545.7
Mandapam Water	2033.0 ± 1536.1
Korapuzha Estuary	1745.7 ± 1127.5
Rusikulya Estuary	1232.6 ± 372.9
Ennore Creek	661.1 ± 460.6
Cochin Backwater	493.3 ± 226.1

A highly significant (at 1% level) positive relations were obtained from correlation study on computer between the Bioaccumulation Factor and concentration of zinc in water (r = 0.506) and sediment (r = 0.421). The highly significant (at 1% level) and weak positive coefficient (r = 0.478) was obtained for the Bioaccumulation Factor with the zinc content in skin.

Muscle: No significant differences were noticed in ANOVA between years and seasons (Table 56). The centres were seen significant at 1% level. The values recorded are:

Centre	Value
Tuticorin Bay	744.1 ± 543.3
Mandapam Water	234.9 ± 88.5
Rusikulya Estuary	205.5 ± 87.1
Korapuzha Estuary	199.3 ± 70.6
Ennore Creek	103.9 ± 35.4
Cochin Backwater	51.5 ± 17.5

The correlation study indicated weak coefficients (0.409) and (0.341) with significance level at 5% between the Bioaccumulation Factor and the available concentration in water and sediment respectively. The positive coefficient (r = 0.650) with level of significance at 1% was established between zinc content and Bioaccumulation Factor in muscle.

Discussion

Zinc content in algae from the wild and macrophytes from marine environment are always having greater concentration than the corresponding one in the surrounding sea water (Foster, 1976; Ishii et al., 1978). In general uptake of zinc by the algae from the surrounding environment is high than zinc concentrations in seawater, but the relationship is not proportional, in other words it is substantially less than linear.

Oysters and scallops are the most effective accumulators of zinc (Eisler, 1981). Concentration Factors were highest in kidney of scallops with values 1.7 to 4.0 million times those of ambient seawater (Bryan, 1973). In the present study along with ovary, the other soft tissues recordedhigher Bioaccumulation Factors of zinc.

The Concentration Factors of zinc in molluscs collected from field are reported from various parts of the world and some of them are:

Species	Concentration Factor	Author(s)
Ostrea sinuata	1,00,000	Brooks and Rumsby, 1965
Crassostrea virginica	"	Pingle et al., 1968
Surf clam	1525	"
Mercenaria mercenaria	2100	"

Comparing to the Accumulation Factors of zinc in oysters, as stated above the tissues/organs of L. parsia reported lesser Accumulation Factors. It indicates that the tissues of molluscs might have been accumulated the metal to its nigher level as given in the foregoing statements, but in L. parsia zinc might have been regulated by the respective organs/ tissues reducing the zinc content in the body. The Bioaccumulation Factor of 1600-2100 has been reported by Waldichuk (1974) for Pacific Halibut Hippoglosus stenolepis. As no reports are available for Bioaccumulation Factors of zinc in different organs/tissues for other fishes the present results can not be compared.

The present investigation revealed the differences of Bioaccumulation Factor of zinc in liver, skin, intestine, kidney and muscle between different centres. In the centres the differential zinc concentrations in water

is already discussed in "Section : Zinc in Water" in this Chapter. The same might have been the cause for differential zinc Bioaccumulation Factors in different centres for liver, skin, intestine, kidney and muscle. In all the tissues the zinc content was seen positively correlated with the Bioaccumulation Factor. It indicates the increase in zinc content in different tissues ultimately increased the Bioaccumulation Factors for the tissues than the water. The increase in zinc in water also raised the factor in tissues. At the same time the factor i.e. "ratio of zinc in tissues with water" showed the linearity with the content in tissue

Section 4 : Lead

Lead at a glance (Average of two years)

Centre Code	Water (ppb)	Sediment (ppm dry wt)	Tissue (ppm dry wt)						
			L	G	K	It	O	S	M
I	6.67	11.78	15.5	20.2	4.9	4.2	10.7	5.1	7.5
II	6.26	50.91	0.9	5.1	2.4	0.7	0.8	1.3	0.1
III	7.46	8.03	4.4	9.9	6.4	7.5	8.4	2.7	2.0
IV	4.68	4.56	11.7	8.0	5.7	3.7	19.3	5.3	2.6
V	20.74	18.11	4.3	12.3	15.9	34.7	4.0	3.4	1.5
VI	8.46	12.61	0.8	4.8	1.8	1.3	0.5	0.6	0.2

I = Korapuzha Estuary, II = Cochin Backwater, III = Tuticorin Bay, IV = Mandapam Water, V = Ennore Creek, VI = Rusikulya Estuary.

L = Liver, K = Kidney, G = Gills, It = Intestine, O = Ovary, S = skin and M = Muscle.

TABLE 57. **Lead in water (ppb) and sediment (ppm) in the Korapuzha Estuary during premonsoon, monsoon and postmonsoon of 1991 and 1992**

Sample	Station		1991 Season Premonsoon	1991 Season Monsoon	1991 Season Postmonsoon	1992 Season Premonsoon	1992 Season Monsoon	1992 Season Postmonsoon
Water	I 1	Surface	2.0	0.5	46.5	5.5	4.5	14.5
		Bottom	7.5	2.5	0.5	6.5	3.0	5.5
	I 2	Surface	8.5	ND	ND	4.5	3.0	8.0
		Bottom	9.0	1.0	0.5	13.5	2.5	7.5
	I 3	Surface	11.0	ND	ND	6.5	3.5	7.5
		Bottom	26.5	ND	ND	18.5	2.0	7.5
	Mean	Surface	7.17	0.17	15.50	5.50	3.67	10.00
		Bottom	14.33	1.17	0.33	12.83	2.50	6.83
	Grand Mean with SD		10.75 ± 8.29	0.67 ± 0.98	7.92 ± 18.90	9.17 ± 5.57	3.08 ± 0.86	8.42 ± 3.11
	Range		(2.0 - 26.5)	(ND - 2.5)	(ND - 46.5)	(5.5 - 18.5)	(2.0 - 4.5)	(5.5 - 14.5)
Sediment	I 1		5.75	36.75	21.25	1.25	44.50	7.25
	I 2		7.25	18.25	20.75	7.00	5.75	5.50
	I 3		11.75	ND	15.25	1.75	1.00	1.00
	Mean with SD		8.25 ± 3.53	18.33 ± 16.67	19.08 ± 3.43	3.33 ± 2.99	17.08 ± 21.41	4.58 ± 3.06
	Range		(3.5 - 14.0)	(ND - 38.0)	(14.0 - 21.5)	(ND - 7.0)	(ND - 47.0)	(1.0 - 8.5)

SD = Standard deviation and ND = Not detected. In water between years ($P < 0.01$) and Mean with SD and range 6.67 ± 8.82 (ND - 46.5).

In sediment between years ($P < 0.001$),between seasons ($P < 0.001$), between stations ($P < 0.001$), between stations within a season ($P < 0.001$), between the stations within a centre ($P < 0.001$), and Mean with SD range 11.78 ± 12.31 (ND - 47.00).

TABLE 58. **Lead in water (ppb) and sediment (ppm) in the Cochin Backwater during premonsoon, monsoon and postmonsoon of 1991 and 1992.**

Sample.	Station		1991 Premonsoon	1991 Monsoon	1991 Postmonsoon	1992 Premonsoon	1992 Monsoon	1992 Postmonsoon
			Season					
Water	II 4	Surface	5.0	ND	4.0	6.0	4.5	60.5
		Bottom	11.5	1.0	4.5	9.5	2.5	4.5
	II 5	Surface	3.5	1.5	5.5	6.5	2.0	7.0
		Bottom	10.0	5.5	5.0	8.5	ND	7.5
	II 6	Surface	3.0	1.5	7.0	3.5	1.5	6.5
		Bottom	3.5	3.5	7.0	6.0	ND	4.5
	Mean	Surface	3.83	1.0	5.50	5.33	2.67	24.67
		Bottom	9.00	3.33	5.50	8.00	0.83	5.50
	Grand Mean with SD		6.42 ± 3.51	2.17 ± 1.99	5.50 ± 1.26	6.67 ± 2.11	1.75 ± 1.70	15.08 ± 22.29
	Range		(3.0 - 11.5)	(ND - 5.5)	(4.0 - 7.0)	(3.5 - 9.5)	(ND - 4.5)	(4.5 - 60.5)
Sediment	II 4		116.75	139.85	66.00	128.75	116.00	67.00
	II 5		22.65	58.00	27.25	15.45	41.25	21.50
	II 6		12.00	23.75	13.25	16.25	18.25	12.50
	Mean with SD		50.47 ± 55.69	73.86 ± 54.71	35.50 ± 24.57	53.48 ± 59.63	58.50 ± 48.74	33.67 ± 26.19
	Range		(11.5 - 150.0)	(23.5 - 158.5)	(12.5 - 69.5)	(14.4 - 148.5)	(16.0 - 142.5)	(11.5 - 69.5)

SD = Standard deviation and ND = Not detected. In water between years ($P < 0.01$), Mean with SD and range 6.26 ± 9.60 (ND - 60.5).

In sediment between years ($P < 0.001$), between stations, ($P < 0.001$), between stations within a season ($P < 0.001$), between the stations within a centre ($P < 0.001$), Mean with SD and range 50.91 ± 45.14 (11.5 - 158.5).

TABLE 59. **Lead in water (ppb) and sediment (ppm) in the Tuticorin Bay during premonsoon, monsoon and postmonsoon of 1991 and 1992**

Sample	Station		1991 Premonsoon	1991 Monsoon	1991 Postmonsoon	1992 Premonsoon	1992 Monsoon	1992 Postmonsoon
			Season					
Water	III 7	Surface	ND	1.0	11.0	2.0	3.0	4.0
		Bottom	1.5	ND	13.0	1.5	ND	3.5
	III 8	Surface	0.5	31.0	8.5	28.0	326.0*	16.0
		Bottom	1.0	11.5	12.5	89.5	9.5	14.0
	III 9	Surface	6.0	ND	4.0	60.0	16.5	19.5
		Bottom	4.5	ND	4.5	134.5	8.0	12.0
	Mean	Surface	2.17	10.67	7.83	30.00	9.75	13.17
		Bottom	2.33	3.83	10.00	75.17	5.83	9.83
	Grand Mean with SD		2.25 ± 2.42	7.25 ± 12.48	8.92 ± 3.94	52.58 ± 52.73*	7.40 ± 6.36	11.50 ± 6.50
	Range		(ND - 4.5)	(ND - 31.0)	(4.0 - 12.5)	(1.5 - 134.5)	(ND - 326.0)	(3.5 - 19.5)
Sediment	III 7		23.75	6.75	7.25	3.00	0.25	6.00
	III 8		26.26	3.25	1.25	ND	0.50	5.75
	III 9		10.75	12.50	17.00	3.50	10.00	6.75
	Mean with SD		20.25 ± 7.98	7.50 ± 4.23	8.50 ± 7.33	2.17 ± 1.75	3.58 ± 4.98	6.17 ± 0.68
	Range		(9.5 - 28.5)	(3.0 - 13.5)	(ND - 19.5)	(ND - 4.0)	(ND - 10.5)	(5.5 - 7.5)

SD = Standard deviation and ND = Not detected. (*) = Not used for calculation.
In water between years ($P < 0.01$), Mean with SD and range 7.46 ± 7.40 (ND-31.0).

In sediment between years ($P < 0.001$), between seasons ($P < 0.001$), between stations ($P < 0.001$), between stations within a season ($P < 0.001$), between the stations within a centre ($P < 0.001$), Mean with SD and range 8.03 ± 7.57 (ND - 28.5).

TABLE 60. **Lead in water (ppb) and sediment (ppm) in the Gulf of Mannar and the Palk Bay during premonsoon, monsoon and postmonsoon of 1991 and 1992**

Sample	Station	1991 Premonsoon	1991 Monsoon	1991 Postmonsoon	1992 Premonsoon	1992 Monsoon	1992 Postmonsoon
		Season					
Water	IV 10 Surface	ND	ND	7.0	ND	10.5	19.0
	IV 10 Bottom	1.0	ND	7.0	1.0	10.5	2.5
	IV 11 Surface	1.0	0.5	9.5	2.0	17.0	8.5
	IV 11 Bottom	1.0	ND	11.0	2.0	13.5	11.0
	IV 12 Surface	ND	ND	5.5	4.0	4.0	ND
	IV 12 Bottom	0.5	2.0	6.0	ND	3.0	8.5
	Mean Surface	0.33	0.17	7.33	2.00	10.17	9.17
	Mean Bottom	0.83	0.67	8.00	1.00	9.00	7.17
	Grand Mean with SD	0.58 ± 0.49	0.42 ± 0.80	7.67 ± 2.14	1.50 ± 1.52	9.75 ± 5.41	8.17 ± 6.79
	Range	(ND - 1.0)	(ND - 2.0)	(5.5 - 11.0)	(ND - 4.0)	(3.0 - 17.0)	(ND - 19.0)
Sediment	IV 10	1.50	1.75	1.25	1.25	2.00	5.25
	IV 11	12.00	8.25	6.00	0.40	10.25	8.00
	IV 12	0.75	9.75	0.75	ND	8.25	4.75
	Mean with SD	4.75 ± 5.72	6.58 ± 4.14	2.67 ± 2.71	0.55 ± 0.98	6.83 ± 3.91	5.17 ± 2.18
	Range	(ND - 12.0)	(ND - 11.5)	(ND - 6.0)	(ND - 2.5)	(2.0 - 11.0)	(2.0 - 7.5)

SD = Standard deviation and ND = Not detected. In water between years ($P < 0.01$), Mean with SD and range 4.68 ± 5.18 (ND - 19.0).

In sediment between years ($P < 0.001$), between stations, ($P < 0.001$), between stations within a season ($P < 0.001$), between the stations within a centre ($P < 0.001$), Mean with SD and range 4.56 ± 4.02 (ND - 12.0).

TABLE 61. **Lead in water (ppb) and sediment (ppm) in the Ennore Creek during premonsoon, monsoon and post-monsoon of 1991 and 1992**

Sample	Station		1991 Premonsoon	1991 Monsoon	1991 Postmonsoon	1992 Premonsoon	1992 Monsoon	1992 Postmonsoon
			Season					
Water	V 13	Surface	1.5	3.5	4.0	60.5	22.5	10.0
		Bottom	3.5	2.5	12.0	65.5	18.0	7.0
	V 14	Surface	4.5	0.5	33.0	41.5	12.0	8.5
		Bottom	9.5	11.0	13.0	26.5	8.5	25.5
	V 15	Surface	0.5	ND	5.0	132.5	13.0	6.0
		Bottom	2.0	9.5	12.5	53.5	2.0	4.5
	Mean	Surface	2.17	1.33	14.00	111.50	15.83	8.17
		Bottom	5.00	7.67	12.50	48.50	9.50	12.33
	Grand Mean with SD		3.58 ± 3.23	4.50 ± 4.66	13.25 ± 10.45	80.00 ± 46.25	12.67 ± 7.17	10.25 ± 7.71
	Range		(0.5 - 9.5)	(ND - 11.0)	(4.0 - 33.0)	(26.5 - 141.5)	(2.0 - 22.5)	(4.5 - 25.5)
Sediment	V 13		25.25	65.75	5.00	8.25	59.75	12.15
	V 14		26.50	42.75	3.75	10.00	32.50	19.25
	V 15		10.75	0.5	1.5	1.5	ND	0.75
	Mean with SD		20.83 ± 8.58	36.33 ± 29.78	3.42 ± 1.91	6.58 ± 4.24	30.75 ± 26.78	10.72 ± 8.41
	Range		(9.0 - 30.5)	(ND - 70.5)	(ND - 5.0)	(ND - 11.5)	(ND - 61.5)	(ND - 20.0)

SD = Standard deviation and ND = Not detected. In water between years ($P < 0.01$), Mean with SD and range 20.74 ± 32.42 (ND - 141.5).

In sediment between years ($P < 0.001$), between stations ($P < 0.001$), between stations within a season ($P < 0.001$), between the stations within a centre ($P < 0.001$), Mean with SD and range 18.11 ± 19.90 (ND - 70.5).

TABLE 62. **Lead in water (ppb) and sediment (ppm) in the Rusikulya Estuary during premonsoon, monsoon and postmonsoon of 1991 and 1992**

Sample	Station		1991 Premonsoon	1991 Monsoon	1991 Postmonsoon	1992 Premonsoon	1992 Monsoon	1992 Postmonsoon
			Season					
Water	VI 16	Surface	9.0	4.5	6.0	6.0	7.5	4.5
		Bottom	10.5	4.0	39.5	5.0	3.5	5.5
	VI 17	Surface	3.0	1.0	8.0	2.5	16.0	13.3
		Bottom	15.0	4.5	4.5	7.5	4.0	7.5
	VI 18	Surface	10.5	2.0	24.5	8.5	7.5	22.0
		Bottom	7.5	2.5	3.5	12.5	8.5	7.5
	Mean	Surface	7.50	2.50	12.83	5.67	10.33	13.05
		Bottom	11.00	3.67	15.83	8.33	5.33	6.83
	Grand Mean with SD		9.25 ± 3.96	3.08 ± 1.46	14.33 ± 14.56	7.00 ± 3.41	7.83 ± 4.49	9.40 ± 7.16
	Range		(3.0 - 15.0)	(1.0 - 4.5)	(3.5 - 39.5)	(2.5 - 12.5)	(3.5 - 16.0)	(4.5 - 22.0)
Sediment	VI 16		15.50	33.00	45.25	12.75	21.75	7.25
	VI 17		3.25	7.25	20.25	7.25	10.50	2.25
	VI 18		2.75	6.75	11.25	6.15	9.50	4.25
	Mean with SD		7.17 ± 6.74	15.67 ± 13.46	25.58 ± 15.93	8.72 ± 3.38	13.92 ± 7.12	4.58 ± 2.27
	Range		(2.5 - 18.5)	(6.0 - 34.0)	(10.0 - 48.5)	(5.5 - 14.0)	(8.5 - 27.5)	(2.0 - 7.5)

SD = Standard deviation. In water between years ($P < 0.01$), Mean with SD and range 8.46 ± 7.46 (1.0 - 39.5).

In sediment between years ($P < 0.001$), between seasons ($P < 0.001$), between stations ($P < 0.001$), between stations within a season ($P < 0.001$), between stations within a centre ($P < 0.001$), Mean with SD and range 12.61 ± 11.14 (2.0 - 48.5).

higher in estuarine and brackishwater during the present study than that of seawater, freshwater and WHO standard. The safe level of lead recommended by EPA is 100 ppb (Anon., 1991 a). Lohani (1981) reported the critical upper limit of lead is 0.1 ppm. The lead in all centres were quite below the limit to that of EPA standards. Higher values were recorded at St. V 14 and St. V 15 for surface waters during premonsoon of 1992, St. III 9 for bottom water during premonsoon of 1992 and St. III 8 in surface water during monsoon of 1992 (Tables 59 and 61). In station V 14 and V 15 of Ennore Creek in premonsoon 1992 a thin coating of oil was noticed over the water surface and fishes jumping to air was also noticed. This may be due to asphyxiation in fishes as the oxygen level in the water column in the morning (collection time) might have been reduced along with a oil partition between the air and water interphase which might have reduced the oxygen diffusion to water in that layer. The oil is one of the major sources of lead into the aquatic medium (Wittmann, 1979; Laws, 1981). In that two stations the increase of lead content in water might have been due to the oil coating in the water surface. But in St. III 8 and 9 the probable reason for the increase in lead in water is exactly not known, but can be linked to industrial and municipal waste water.

The ranges of dissolved lead in the Indian Ocean was determined by various workers. The values reported are:

Value (ppb)	Author(s)
0.25 - 7.5	Sanzgiri and Braganca, 1981
0.5 - 21.8	Qasim and Sengupta, 1983
0.05 - 0.12	Fowler et. al., 1984

The mean values for all the centres, though estuarine and coastal in nature were, however, well within the range reported by Qasim and Sengupta (1983), but found above the values of Fowler et al. (1984). The mean values of Korapuzha Estuary, Cochin Backwater, Tuticorin Bay and coastal waters of Mandapam were seen within the range reported by Sanzgiri and Braganca (1981), but Rusikulya Estuary and Ennore Creek recorded above the range.

The lead values obtained in the present study from different centres are well compared with the values reported by several authors for different coastal and estuarine waters in India and abroad. Some of them are:

Estuary	Value(ppb)	Author(s)
Chao Phraya River Estuary	5.2	Menasveta, 1978
River Tees Estuary	0.6-3.7	Taylor, 1982
Humer Estuary	0.07-2.4	Balls, 1985
Golden Horn Bay	1.8	Tkalin, 1992

Mindhola River Estuary	2.3-68.0	Zingde et al., 1988
Waters off Tuticorin	2.0-7.8	Ramachandran et al., 1991
Korapuzha Estuary	ND-8.82	Present study
Cochin Backwater	ND-60.5	"
Tuticorin Bay	ND-31.0	"
Mandapam Water	ND-19.0	"
EnnoreCreek	ND-141.5	"
Rusikulya Estuary	1.0-39.5	"

Tuticorin Bay recorded 7.46 ppb of lead as observed by the candidate which is well compared to that reported by Ramachandran et al. (1991) for the same area. But no significant difference was seen among the centres in the present study.

The average world concentration of lead for stream water is 3.0ppb (Goldberg et al., 1971). The mean values for all the centres in the present study were seen considerably higher than that of average world stream concentration.

The Guanabara Bay (Rio de Janeiro) which serves as an estuary for several rivers and channels, showed lead concentrations in surface waters (Rebello and Arras, 1983) with an anthropogenic impact in the area. Dissolved lead levels are generally low, even in highly polluted areas. Apart from few sampling stations in waters affected by waste plumes at German Bight dissolved lead levels are generally below 0.4 ppb even in polluted tidal river areas (Mart and Nurnberg, 1986). The enhanced levels in some samples at German Bight were due to substantial

upwhirling of particulate matter (Mart and Nurnberg, 1986). Nair (1984) found that lead is effectively removed from solution (waters) by absortion on particles of hydrated ferric hydroxide and manganese dioxide.

In the present study the yearly variation in lead was noticed in water for which no clear reason can be attributed, as this metal is more influenced by anthropogenic activity.

Surface waters at westren Brittany (France) are enriched with lead, relative to deep water at some locations by as much as an order of magnitude (Riso et al., 1990) and also obtained in the present study.

Balls (1985) reported no relationship of lead in water with salinity while working in coastal waters of the western North Sea. No good relationship was also reported between salinity and lead in Rhine Estuary and the Southern Bight (Duinker and Nolting, 1977). Similarly no good relationship of lead with salinity and hardness was seen in the present investigation.

Lead in sediment

The two-way ANOVA in computer indicated very high significant difference (at 0.1% level) between years and higher concentrations were seen in 1991 than 1992 (Table 57 to 62). Very high significant differences were seen between seasons (at 0.1% level) and its interactions with year were seen nonsignificant. The monsoon recorded the higher concentration of lead followed by premonsoon and postmonsoon. Very high significant

differences (at 0.1% level) were seen for the interactions of centres with season and no significance was seen between the interactions of centres and year. Very high significant differences were noticed (at 0.1% level) for stations, interaction of stations with season, and stations with centre. No significant difference was seen for the interaction of stations with year.

A nonsignificant poor correlation coefficient (r = 0.214) was found between lead in water and sediment.

Discussion

The concentration of lead with coefficient of variation in parentheses for USGS rock standard such as "G-2" was given 31.3 (0.110) ppm by Flanagan (1976) and as reported by Subramanian et al. (1989) as 29.8 (0.011) ppm. The mean value of lead in sediment of Cochin Backwater was found above the standards (USGS rock standard) in the present investigation. For two marine sediment reference materials i.e. "Miramichi Estuary Standard Sediment (MESS-1)" and "Baie des Chaleurs Standard Sediment (BCSS-1)" the estimated lead given by Ackroyd et al. (1987) was 42.9 ± 1.8&19.7 ± 7.1 ppm dry wt respectively. Similarly, as mentioned earlier, the mean values of lead for Cochin Backwater sediment was found above the standards. The mean lead values were seen below the standards for all other centres. Above the "MESS-1" standard lead, values were reported for:

Centre	Station	Season	Year
Korapuzha Estuary	II 1	Monsoon	1992
Cochin Backwater	II 4	All Seasons	1991 and 1992
Ennore Creek	V 13	Monsoon	"
"	V 14	"	1991
Rusikulya Estuary	VI 16	Postmonsoon	"

In all the above mentioned stations the monsoon recorded higher concentration of lead and is discussed in the subsequent paragraph for seasonal variation. The position of most of the stations studied here, were towards the freshwater side of the estuaries except St. II 5 and St. V 14 which are in Cochin Backwater and Ennore Creek respectively. The increased lead in St. I 1 and St. VI 16 can not be exactly attributed to a definite source as all the stations in Korapuzha Estuary and Rusikulya Estuary assumed to be similar for the respective centres. In case of Rusikulya Estuary the Jayashree Chloro-Alkali Plant discharges its effluents somewhere in the estuary after St. VI 16 to the system. St. II 4 receives the industrial and oily wastes from Caprolactam Plant, industrial complexes and sewages of the area. The St. II 5 receives organic debris and water from nearby mangroove areas and oils leaked from nearby oil terminals and let outs from the ships harboured in the backwater. St. V 13 and 14 receives the Madras Metropolitan City wastes and the industrial wastes in and around Ennore. According to Wittman (1979), these wastes might have contributed the substantial lead content in the sediment at respective stations. The oil and other hydrocarbons are major sources of lead to the environment. The high value of 175 ppm level at Cl 10% for the

bed sediment is reported from Haldia where the petroleum refinery discharges waste into the channel (Subramanian et al., 1988). Bloom and Ayling (1977) reported 11000 ppm of lead at the outfall of the refineries to Derwent Estuary, Australia. The source of lead to the water and sediment near the city area is from the atmosphere. The source of lead to the atmosphere is from the combustion of lead-blended gasoline (Rebello et al., 1986). The more the number of the vehicles in a city the more the contribution of lead by combustion of "tetra-ethyl lead" to the air, which ultimately settles down to the nearby aquatic environment or open land, further from the open space the lead is washed out to the nearby aquatic medium. The incrase in lead in Cochin Backwater may be linked to its location near to the Greater Cohin area which strengthens the views of Rebello et al. (1986). According to Berrow and Webber (1972) the "average" sewage sludge contains 450 ppm of lead. The addition of municipal wastes and sewage from Greater Cochin to the backwater and from Madras City to Ennore Creek might have been the cause for the increase of lead in the sediments.

The range of natural content of lead reported 0-20 ppm in the world sediments by Rankama and Sahama in 1960 (Menasveta, 1978). In comparing with this value all the mean values for different centres except Cochin Backwater in the present study were found within the range. "Pollution peak" value of lead 537 ppm dry wt was reported by Katz and Kaplan (1981) in polluted south California surfac sediments. From the present study, the centres were seen far below the peak pollution level of lead in its sediments as reported by Katz and Kaplan (1981).

For comparison of the present results in various centres, from similar environment from India and abroad are given here:

Environment	Value (ppm)	Author(s)
Rio Tinto Estuary, Spain	Maximum 1600	Stenner and Nickless, 1975
Restronguet Estuary, U.K.	Maximum 1620	Thornton et al., 1975
Derwent Estuary, Australia	Maximum 1000	Bloom and Ayling, 1977
East Coast of England	17.1-238	Taylor, 1982
Jakarta Bay	9.0-438	Hungspreugs, 1988
Northeast Pacific Coast	7.0-11.8	Harding and Goyette, 1989
Golden Horn Bay, Japan	300	Tkalin, 1992
Mindhola River Estuary	4-67	Zingde et al., 1988
Ganges Estuary	16-115	Subramanian et al., 1988
Cauvery Estuary	Average 46.0	Subramanian et al., 1989
Southeast Coast of India	Average 53±30	Mohamachandran and Subramanian, 1990
Madras Coast	0.09-80.7	Ramachandran et al., 1991
Korapuzha Estuary	ND-47.0	Present study
Cochin Backwater	11.5-158.5	"
Tuticorin Bay	ND-28.5	"
Mandapam Water	ND-12.0	"
Ennore Creek	ND-70.5	"
Rusikulya Estuary	2.0-48.5	"

Except Northeast Pacific coastal sediments, in all other centres higher concentration of lead were found than the standards and the values obtained in the present study for different centres.

The mean value and the range for lead in sediment in Ennore Creek were found well compared to the reports of Mohanachandran and Subramanian (1990) and Ramachandran et al. (1991) for Madras Coast. The Mandapam coastal sediment and Tuticorin Bay in present study recorded lower concentration of lead than that reported for southeast coast.

In the present study the variation in lead concentration was found between years and seasons. The variation of lead in space and time may be due to the differences in the sources of the heavy metals and the complex reactions such as absorption, flocculation, etc. taking place in the sediments. According to Balls (1985) the lead distribution is dominated by its association with particulate material. As seen in case of copper and zinc, the lead also showed higher concentrations in monsoon followed by dry seasons. The reasons attributed for the increase of copper and zinc as discussd in previous sections (copper and zinc in sediment) may also be ascribed to the increase in monsoonal heavy river discharge into concerned estuaries. According to Mart and Nurnberg (1986) the lead, being transported mainly with the suspended particulate phase (upto 99.5%) is better settled at the bottom as sediment in the estuaries. According to Menasveta (1978) who worked on Chao Phraya River Estuary, stated"especially in the channel at the outer bar of the river, where the down river freshwater with sediment loads meets the saline water, causing

both chemical and physical changes which result in the sedimentation of the suspended particles". The lead is incorporated in the stern layer of the iron hydroxide structures and hydrogen ions are exchanged (Hilderbrand and Blum, 1974) so that lead exhibits a strong affinity for the hydroxyl group of the Fe(OH) crystal. This complex settles to the bottom sediment in the aquatic environment. The same might have been the reason for the increase in lead in sediment due to iron oxide and lead complexes to the sediment layer. Generally the river carries iron coated sediments which can act as scavenger or carrier of metals (Mohanachandran and Subramanian, 1990). Similarly, in the present study, all the river dominated estuarine systems such as Cochin Backwater, Ennore Creek, Rusikulya Estuary and Korapuzha Estuary recorded higher concentration of lead in its sediments. In monsoon, the rivers carry all the wash out including organic matters from the catchment areas along their flow, through the hills and terrain which are rich in lead.

Lead in tissues

The lead content in liver, gills, kidney, intestine, ovary, skin and muscle during different seasons in 1991 and 1992 and at different centres are presented in Tables 63 to 69. Pooling the data over collections for specific tissues, the grand mean values obtained in computer were found in descending order as below:

TABLE 63. **Lead (ppm dry wt) in liver of L. parsia at different centres during Premonsoon, monsoon and postmonsoon of 1991 and 1992**

Centre	Centre Code	1991			1992		
		Season					
		Premonsoon	Monsoon	Postmonsoon	Premonsoon	Monsoon	Postmonsoon
Korapuzha Estuary	I	ND	ND	5.95 (0.049)	68.29 (0.133)	-	3.19 (0.008)
Cochin Backwater	II	ND	ND	5.21 (0.061)	ND	ND	ND
Tuticorin Bay	III	6.07 (0.65)	0.92 (0.059)	ND	19.34 (0.132)	ND	ND
Mandapam	IV	59.10 (0.103)	2.99 (0.044)	ND	6.53 (0.056)	1.62 (0.032)	ND
Ennore Creek	V	3.23 (0.058)	3.98 (0.051)	ND	8.24 (0.016)	4.90 (0.061)	5.27 (0.076)
Rusikulya Estuary	VI	ND	ND	ND	3.05 (0.091)	-	0.89 (0.018)

Coefficient of variation is in parentheses and ND = Not detected.

Between seasons ($P < 0.1$).

TABLE 64. **Lead (ppm dry wt)in liver of L. parsia at different centres during premonsoon, monsoon and postmonsoon of 1991 and 1992**

Centre	Centre Code	1991			1992			Means with SD
		Season						
		Premonsoon	Monsoon	Postmonsoon	Premonsoon	Monsoon	Postmonsoon	
Korapuzha Estuary	I	6.57 (0.089)	2.82 (0.069)	22.61 (0.070)	47.00 (0.047)	-	21.98 (0.092)	20.20 ± 17.43
Cochin Backwater	II	ND	4.09 (0.051)	13.20 (0.044)	9.27 (0.054)	ND	3.93 (0.028)	5.08 ± 5.24
Tuticorin Bay	III	4.79 (0.080)	16.26 (0.067)	ND	22.40 (0.182)	2.35 (0.069)	13.34 (0.095)	9.86 ± 8.83
Mandapam	III	2.53 (0.063)	13.12 (0.068)	ND	12.63 (0.072)	8.12 (0.028)	11.33 (0.064)	7.96 ± 5.33
Ennore Creek	V	2.09 (0.047)	13.97 (0.054)	ND	18.43 (0.074)	34.48 (0.102)	4.54 (0.074)	12.25 ± 13.02
Rusikulya Estuary	VI	ND	4.17 (0.078)	8.63 (0.084)	8.11 (0.045)	-	2.91 (0.038)	4.76 ± 3.63

SD = Standard deviation,coefficient of variation is in parentheses and ND = Not detected.

Between years ($P < 0.1$).

TABLE 65. **Lead (ppm dry wt)in kidny of L. parsia at different centres during premonsoon, monsoon and postmonsoon of 1991 and 1992**

Centre	Centre Code	1991			1992		
		Season					
		Premonsoon	Monsoon	Postmonsoon	Premonsoon	Monsoon	Postmonsoon
Korapuzha Estuary	I	ND	ND	14.86 (0.073)	ND	-	9.82 (0.068)
Cochin Backwater	II	ND	ND	ND	ND	ND	14.54 (0.062)
Tuticorin Bay	III	ND	8.26 (0.062)	ND	15.57 (0.095)	14.71 (0.092)	ND
Mandapam	III	ND	19.73 (0.066)	ND	14.53 (0.088)	ND	ND
Ennore Creek	V	5.53 (0.065)	18.75 (0.077)	ND	49.90 (0.089)	21.28 (0.082)	ND
Rusikulya Estuary	VI	ND	ND	2.56 (0.038)	ND	-	6.33 (0.078)

Coefficient of variation is in parentheses and ND = Not detected.

TABLE 66. **Lead (ppm drywt) in intestine of L. parsia at different centres during premonsoon, monsoon and postmonsoon of 1991 and 1992**

Centre	Centre Code	1991			1992		
		Season					
		Premonsoon	Monsoon	Postmonsoon	Premonsoon	Monsoon	Postmonsoon
Korapuzha Estuary	I	ND	ND	19.39 (0.056)	ND	-	1.36 (0.009)
Cochin Backwater	II	ND	ND	4.11 (0.350)	ND	ND	ND
Tuticorin Bay	III	ND	3.20 (0.049)	9.32 (0.108)	24.27 (0.214)	5.43 (0.081)	2.70 (0.068)
Mandapam	III	ND	3.78 (0.072)	5.77 (0.100)	ND	8.67 (0.107)	3.79 (0.040)
Ennore Creek	V	3.41 (0.073)	ND	ND	194.92 (0.145)	6.62 (0.096)	3.45 (0.043)
Rusikulya Estuary	VI	ND	ND	4.32 (0.066)	ND	-	2.26 (0.038)

Coefficient of variation is in parentheses and ND = Not detected.

TABLE 67. **Lead (ppm dry wt)in ovary of L. parsia at different centres during premonsoon, monsoon and postmonsoon of 1991 and 1992**

Centre	Centre Code	1991			1992		
		Season					
		Premonsoon	Monsoon	Postmonsoon	Premonsoon	Monsoon	Postmonsoon
Korapuzha Estuary	I	ND	ND	41.44 (0.070)	5.84 (0.034)	-	6.16 (0.092)
Cochin Backwater	II	ND	ND	1.49 (0.062)	ND	ND	3.42 (0.022
Tuticorin Bay	III	25.32 (0.043)	1.80 (0.066)	ND	4.66 (0.068)	18.86 (0.112)	ND
Mandapam	III	9.13 (0.053)	31.71 (0.048)	2.39 (0.067)	30.41 (0.099)	11.90 (0.085)	30.17 (0.056)
Ennore Creek	V	5.14 (0.075)	1.00 (0.013)	ND	18.10 (0.042)	ND	ND
Rusikulya Estuary	VI	ND	ND	ND	ND	-	2.25 (0.081)

Coefficient of variation is in parentheses and ND = Not detected.

TABLE 68. **Lead (ppm dry wt) in skin of L. parsia at different centres during premonsoon, monsoon and postmonsoon of 1991 and 1992**

Centre	Centre Code	1991			1992		
		Season					
		Premonsoon	Monsoon	Postmonsoon	Premonsoon	Monsoon	Postmonsoon
Korapuzha Estuary	I	ND	ND	2.14 (0.044)	21.19 (0.330)	-	2.13 (0.024)
Cochin Backwater	II	ND	ND	1.07 (0.099)	ND	ND	6.77 (0.082)
Tuticorin Bay	III	ND	4.59 (0.052)	ND	2.63 (0.018)	2.00 (0.022)	7.08 (0.035)
Mandapam	III	ND	ND	ND	22.31 (0.084)	ND	9.31 (0.042)
Ennore Creek	V	4.49 (0.045)	3.61 (0.047)	ND	7.49 (0.008)	5.02 (0.066)	ND
Rusikulya Estuary	VI	ND	ND	ND	ND	-	3.07 (0.026)

Coefficient of variation is in parentheses and ND = Not detected.

Between years ($P < 0.05$).

TABLE 69. **Lead (ppm dry wt) in muscle of L. parsia at different centres during premonsoon, monsoon and postmonsoon of 1991 and 1992**

Centre	Centre Code	1991			1992		
		Season					
		Premonsoon	Monsoon	Postmonsoon	Premonsoon	Monsoon	Postmonsoon
Korapuzha Estuary	I	ND	ND	1.63 (0.083)	31.25 (0.060)	-	4.67 (0.022)
Cochin Backwater	II	ND	ND	ND	ND	ND	0.57 (0.012)
Tuticorin Bay	III	ND	ND	7.99 (0.091)	3.13 (0.042)	1.09 (0.018)	ND
Mandapam	III	1.81 (0.043)	6.29 (0.059)	ND	3.99 (0.028)	3.35 (0.042)	ND
Ennore Creek	V	ND	0.64 (0.020)	ND	5.27 (0.099)	3.28 (0.073)	ND
Rusikulya Estuary	VI	ND	ND	ND	ND	-	1.06 (0.008)

Coefficient of variation is in parentheses and ND = Not detected.

Tissue	Value (ppm dry wt)
Gills	9.32
Intestine	8.52
Ovary	6.98
Kidney	6.01
Liver	5.80
Skin	2.91
Muscle	2.11

Liver: From ANOVA, the significant difference (at 10% level) was seen between the seasons only (Table 63). The lead content was found more during premonsoon followed by postmonsoon and monsoon.

From correlation study, very poor positive nonsignificant relationships were obtained for lead in liver with that of water (r = 0.067) and sediment (r = 0.230).

Gills: The ANOVA showed the significant difference between years at 10% level (Table 64). The higher lead content was estimated in 1992 than 1991. No significant difference was seen between the seasons and centres.

The lead content in gills showed very weak positive nonsignificant relationships compared to water (r = 0.285) and sediment (r = 0.151). The lead in gills and liver indicated a very high significant (at 1% level) positive relation (r = 0.505) among them.

Kidney: From ANOVA, no significant differences were observed between years, centres and seasons (Table 65).

The correlation study indicated the positive highly significant (at 1% level) relation between lead in kidney and water (r = 0.691) and a nonsignificant relationship with sediment (r = 0.129). The lead in kidney and gills showed significant (at 5% level) positive relationship (r = 0.402) between them.

Intestine: From two-way ANOVA lead content in different years, seasons and centres (Table 66), no significant differences were observed between them.

Correlation study showed a good positive and highly significant (at 1% level) coefficient (r = 0.867) for lead in intestine with that of water (Fig. 5). A very weak nonsignificant positive relation was seen with sediment (r = 0.140). The lead in intestine has shown a direct bearing on the same in kidney (r = 0.763) and was significant at 1% level (Fig. 6).

Ovary: Table 67 shows the variation in lead content in ovary at different centres during premonsoon, monsoon and postmonsoon of 1991 and 1992. From ANOVA, no significant difference was observed between years and also between the seasons and centres.

The lead in ovary showed very weak positive nonsignificant relation compared to water (r = 0.051) and sediment (r = 0.284). The lead in ovary and kidney was correlated with a significant (at 5% level) coefficient (r = 0.394).

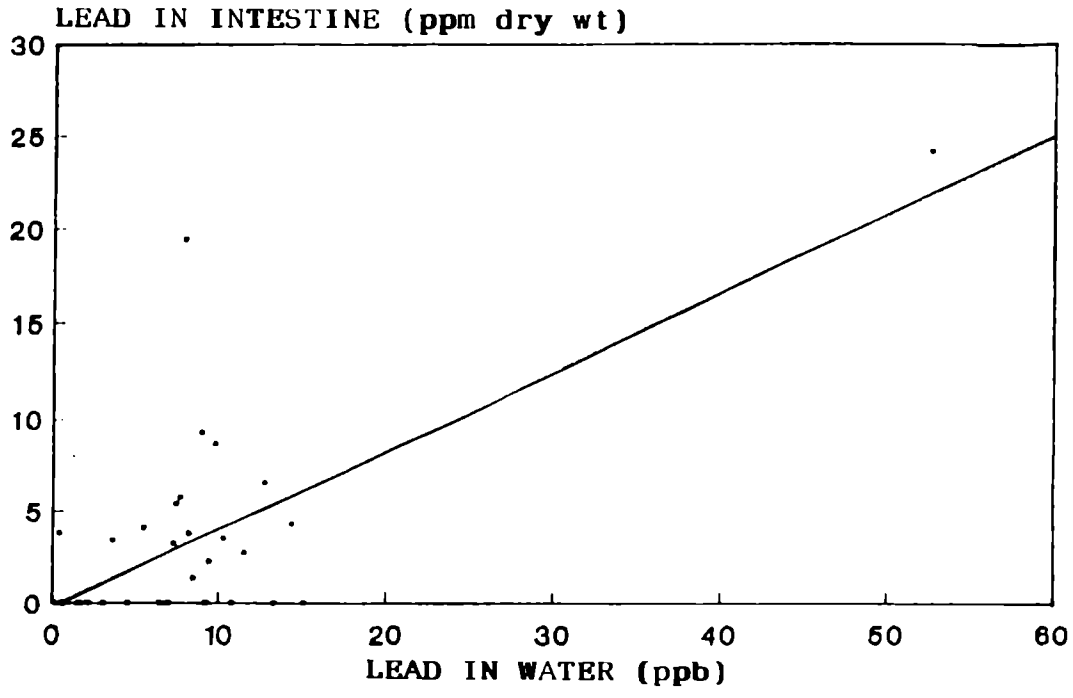


FIG. 5. Relationship between lead in Water and lead in intestine of L. parsia

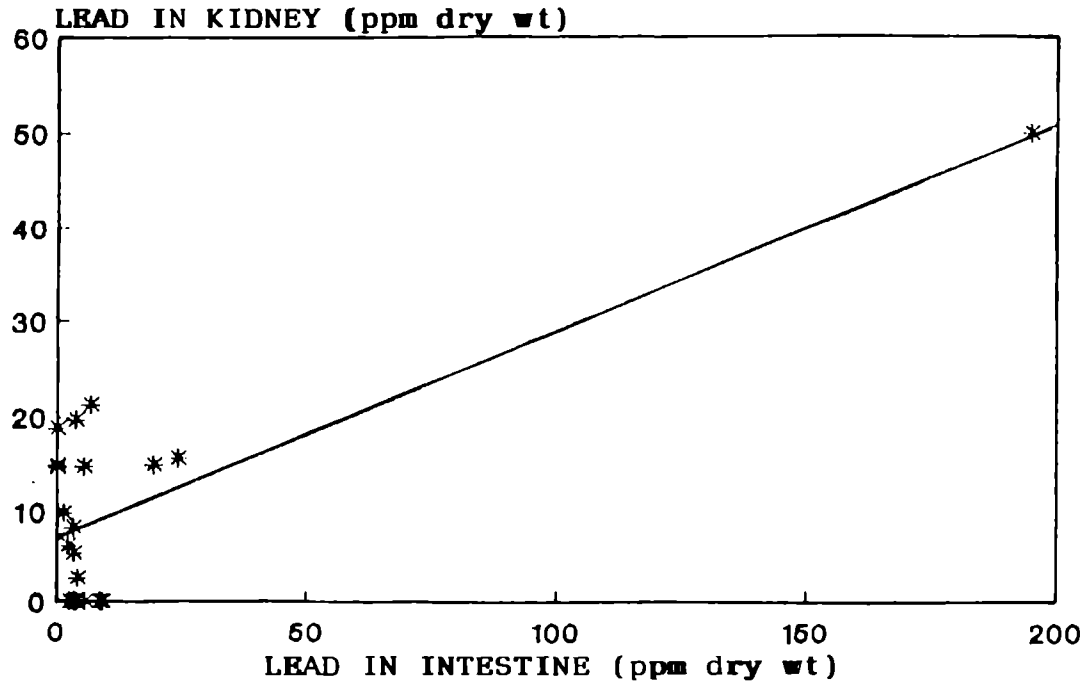


FIG. 6. Relationship between lead in Intestine and lead in Kidney of L. parsia.

Skin: The ANOVA showed significant difference between the years at 5% F-value and higher content was seen in 1992 than 1991 (Table 68). No significant differences were observed among the centres and seasons.

The weak positive nonsignificant correlation coefficients were obtained for lead content in skin with that of lead in water (r = 0.130) and sediment (r = 0.259). The highly significant (at 1% level) positive coefficients were obtained for lead in skin with that of liver (r = 0.43) and gills (r = 0.592). But with ovary (r = 0.334) it was significant at 5% level.

Muscle: No significant differences were seen between years, seasons and centres (Table 69).

From correlation study the weak positive and nonsignificant coefficients were obtained for lead in muscle with the concentrations in water (r = 0.117) and sediment (r = 0.248). The lead in muscle showed highly significant (at 1% level) positive relations with that in liver (r = 0.714, Fig. 7) gills (r = 0.686) and skin (r = 0.614).

Discussion

Lead is a non-essential metal (Rainbow, 1988). The higher the concentration of lead to which Crangon crangon in exposed, the more it accumulates, showing no evidence of regulation (Amiard et al., 1985). Lead will bind to metallothionein, but also has an affinity (probaly higher) for other metabolic ligands, often associating with deposited inorganic

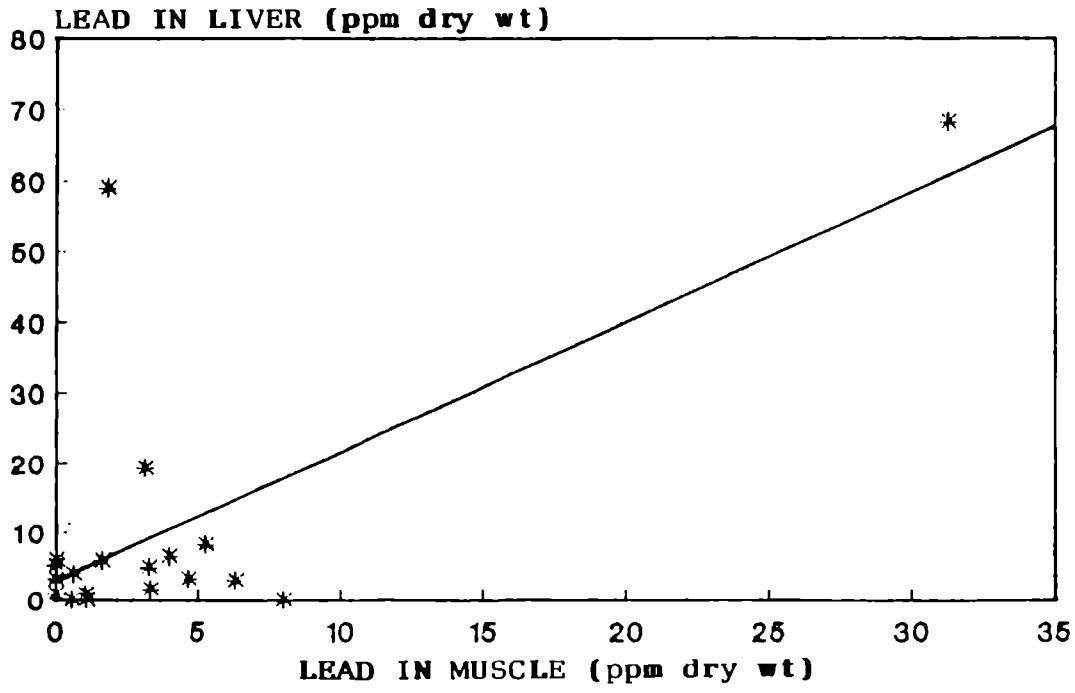


FIG. 7. Relationship between lead in Muscle and lead in Liver of L. pursia.

granules with high concentrations of calcium (Brown, 1982). A regulation of internal level of some essential metals has been observed in some species whereas the bioaccumulation of several non-essential metals paralleled their overloads in water (Bryan, 1971). Being a non-essential element the lead has been studied in different tissues of L. parsia.

To the present date, no standards have been reported for lead content in different tissues/organs of a fish from which we could evaluate the toxic limits for the same. The maximum limit of lead is 2.0 ppm for canned shrimp or prawn (Anon., 1991 b). But this can not be used as a standard for the present study as prawn and fishes belong to invertebrata and vertebrata respectively. Earlier to this the legal admissible limit of 5.0 ppm dry wt lead was reported for sea foods to be exportd to U.K. (Anon., 1988). Comparing to this standard of Anon. (1988) except muscle and skin all other tissues in L. parsia reported more lead.

Few reports are available regarding the lead content in mullets. Bebbington et al. (1977) reported 0.71 (0.2-4.1)ppm wet wt lead in muscle tissue of Mugil cephalus. In the present study muscle of L. parsia recorded 2.1 ppm dry wt (mean) of lead and found well compared with that of M. cephalus.

The content of lead in different tissues of genus Liza reported from Indian waters are:

Species	Gill	Liver	Muscle	Author Place
L. macrolepis	2.83	1.51	1.25	Nammalwar, 1987 - Adyar Estuary
"	2.90	1.62	1.34	Nammalwar, 1987 - Ennore Estuary
L. parsia	12.25	4.27	1.53	Present study Ennore Creek

The differential uptake of lead by various organs/tissues of Liza may be due to the difference in experimental animal.

Higher lead content was recorded in gills followed by intestine, ovary, kidney, liver, skin and muscle in that order in L. parsia in the present investigation. According to Eisler (1981) no pattern was observed for mean lead concentrations in whole fish although these tend to exhibit higher content than muscle and liver, possibly due to lead uptake in hard tissues such as bone which is supposed to be the place of lead accumulation. Distinct tissue specific accumulation rates were found in Gillichthys mirabilis - gill, intestine accumulated the highest amount of lead while liver and muscle accumulated the least lead (Somero et al., 1977). Similar results were also found in L. parsia tissues in the present study. Brooks and Rumsey (1974) found that in general, zinc, cadmium, copper and iron are concentrated in soft organs of teleosts such as liver, kidney, spleen, heart, gonads, whereas lead and manganese are concentrated in bony organs such as gills, backbone and tail. In case of L. parsia gills accumulated more lead than other organs as it contain calcarious bony gill arches which

have the affinity to lead. The other reports available for lead content (in ppm) in different fishes have been cited below:

Species	Gills	Gut wall	Liver	Muscle	Ovary	Skin	Author(s)
Pleuronectes platessa	-	1.33	1.34	1.15	-	-	Wharfe and Van Den Broek, 1977
Pagothenia berchgrevinki	-	-	0.29	0.12	0.76	0.13	Honda et al., 1983
Gadus morhua	4.0	0.5	0.4	0.2	0.9	-	Szefer et al., 1990
L. parsia	9.32	8.52	6.01	2.11	6.98	2.9	Present study

The same order as reported by Honda et al. (1983) was seen for the lead in different tissues in present study. Wahbeh and Mahasneh (1987) reported highest lead in the gills of Abudefduf saxatilis and similarly higher lead content in gills was seen in the present study.

The turn-over of lead in the mucus covered tissues such as gills and intestine is a result of lead complexation with mucus (Somero et al., 1977). The same reason can be attributed to the higher content of lead in gills and intestine of L. parsia. According to Overnell et al. (1988) the metal binding protein is found in liver and kidney of the fish and has more affinity to copper and zinc than lead. The similar reason can be attributed for lower content of lead in liver and kidney of L. parsia. The only tissue with a lower level of metal (except mercury) is the axial muscle, in which metals appear to be strictly regulated (Phillips, 1977). Similarly the muscle of L. parsia reported less content of lead than other tissues studied.

From the correlation study only the interrelationship was found for intestine and kidney with the water. No relations were found for lead in the tissues with water. No relation was also seen for lead in tissue with that in sediments. Harding and Goyette (1989) reported no correlation between sediment and tissue metal levels of any species on both raw and log transformed data. In liver of Baltic herring and cod the spatial differences roughly in accordance with the concentration of lead in sea water were reported by Perttila et al. (1982). Jaffer et al. (1988) did not find relationship of lead in edible muscle tissue of fish with that in water. The absence of correlations implies that this metal either controlled by homeostasis or the tissue concentration is independent to that in environment. Milner (1982) stated that the absence of correlation reflects either the metal is regulated by fish or its limited availability to the tissue from the environment.

In the present study the lead variation was not seen significant in different tissues at different centres. The lead content was found independent in different tissues with whatever concentration was available in the environment. The only seasonal difference was recorded in liver at 10% F-value. Almost all the tissues exhibited no difference in lead content in different seasons.

Statistical analysis on the standard deviations often found as large as the sample mean itself. But its use in analysis discussed earlier in "Section: Zinc in tissue". In aquatic pollution studies very little evidence is available regarding the correlation of trace elements in fish tissues and environments (Windom et al., 1973; Eustace, 1974) and this may be

attributed to the migration of fish from the normal place of metal contamination to other or neighbouring suitable environment.

Bioaccumulation Factor

The Bioaccumulation Factor for liver, gills kidney, intestine, ovary, skin, and muscle are given in Tables 70 to 76. Pooling of data over collections for specific tissues, the grand mean values obtained from computer were found in decreasing order:

Tissue	Value
Ovary	1086.77
Liver	945.92
Kidney	641.84
Gills	439.42
Skin	233.81
Intestine	163.59
Muscle	86.48

Liver: From ANOVA, no significant differences were observed between the years, seasons and centres (Table 70).

From the correlation study the nonsignificant and very weak positive coefficients were seen for Bioaccumulation Factor in liver with the lead content in water (r = 0.127) and sediment (r = 0.145). The highly signifi - cant(1% level) positive relation (r = 0.67) was oserved between the lead content in liver and Bioaccumulation Factor.

TABLE 70. **Bioaccumulation Factor for lead in liver of L. parsia at different centres during premonsoon, monsoon and postmonsoon of 1991 and 1992**

Centre	1991			1992		
	Season					
	Premonsoon	Monsoon	Postmonsoon	Premonsoon	Monsoon	Postmonsoon
Korapuzha Estuary	ND	ND	200.4	1986.9	-	101.1
Cochin Backwater	ND	ND	252.7	ND	ND	ND
Tuticorin Bay	202.9	33.8	ND	98.4	ND	ND
Mandapam	7186.0	1899.4	ND	1165.5	44.3	ND
Ennore Creek	204.7	236.0	ND	27.5	103.2	137.2
Rusikulya Estuary	ND	ND	ND	116.2	-	25.3

ND = Not detected.

TABLE 71. **Bioaccumulation Factor for lead in gills of L. parsia at different centres during premonsoon, monsoon and postmonsoon of 1991 and 1992**

Centre	Centre Code	1991			1992		
		Season					
		Premonsoon	Monsoon	Postmonsoon	Premonsoon	Monsoon	Postmonsoon
Korapuzha Estuary	I	114.6	789.2	535.3	961.0	-	489.5
Cochin Backwater	II	ND	353.4	450.0	260.6	ND	48.9
Tuticorin Bay	III	399.2	420.5	ND	79.9	59.5	217.5
Mandapam	IV	817.9	5857.1	ND	1578.8	156.2	260.0
Ennore Creek	V	109.5	582.1	ND	43.2	510.3	83.0
Rusikulya Estuary	VI	ND	253.8	112.9	217.2	-	58.0

SD = Standard deviation and ND=not detected.

TABLE 72. **Bioaccumulation Factor for lead in kidney of L. parsia at different centres during premonsoon, monsoon and postmonsoon of 1991 and 1992**

Centre	Centre Code	1991			1992		
		Season					
		Premonsoon	Monsoon	Postmonsoon	Premonsoon	Monsoon	Postmonsoon
Korapuzha Estuary	I	ND	ND	594.2	ND	-	369.4
Cochin Backwater	II	ND	ND	ND	ND	ND	305.4
Tuticorin Bay	III	ND	360.8	ND	93.8	629.5	ND
Mandapam	IV	ND	14877.4	ND	3067.2	ND	ND
Ennore Creek	V	489.2	1319.6	ND	197.5	531.9	ND
Rusikulya Estuary	VI	ND	ND	56.6	ND	-	213.3

ND = Not detected.

TABLE 73. **Bioaccumulation Factor for lead in intestine of L. parsia at different centres during premonsoon, monsoon and postmonsoon of 1991 and 1992**

Centre	Centre Code	1991 Season			1992 Season		
		Premonsoon	Monsoon	Postmonsoon	Premonsoon	Monsoon	Postmonsoon
Korapuzha Estuary	I	ND	ND	647.1	ND	-	42.7
Cochin Backwater	II	ND	ND	197.5	ND	ND	ND
Tuticorin Bay	III	ND	116.7	276.2	122.0	193.9	62.1
Mandapam	IV	ND	2378.7	228.6	ND	235.0	122.6
Ennore Creek	V	251.7	ND	ND	644.0	138.1	89.0
Rusikulya Estuary	VI	ND	ND	79.7	ND	-	63.5

ND = Not detected.

TABLE 74. **Bioaccumulation Factor for lead in ovary of L. parsia at different centres during premonsoon, monsoon and postmonsoon of 1991 and 1992**

Centre	Centre Code	1991			1992		
		Season					
		Premonsoon	Monsoon	Postmonsoon	Premonsoon	Monsoon	Postmonsoon
Korapuzha Estuary	I	ND	ND	1479.7	180.1	-	206.9
Cochin Backwater	II	ND	ND	76.6	ND	ND	64.1
Tuticorin Bay	III	3182.4	70.2	ND	147.7	101.4	ND
Mandapam	IV	4451.7	21351.4	88.1	5733.3	345.2	1044.3
Ennore Creek	V	406.0	62.8	ND	64.0	ND	ND
Rusikulya Estuary	VI	ND	ND	ND	ND	-	67.7

ND = Not detected.

TABLE 75. **Bioaccumulation Factor for lead in skin of L. parsia at different centres during premonsoon, monsoon and postmonsoon of 1991 and 1992**

Centre	Centre Code	1991			1992		
		Season					
		Premonsoon	Monsoon	Postmonsoon	Premonsoon	Monsoon	Postmonsoon
Korapuzha Estuary	I	ND	ND	95.0	812.7	-	89.0
Cochin Backwater	II	ND	ND	68.4	ND	ND	157.9
Tuticorin Bay	III	ND	222.7	ND	17.6	95.1	216.5
Mandapam	IV	ND	ND	ND	5231.0	ND	400.8
Ennore Creek	V	441.1	282.1	ND	32.9	139.3	ND
Rusikulya Estuary	VI	ND	ND	ND	ND	-	114.9

ND = Not detected.

TABLE 76. **Bioaccumulation Factor for lead in muscle of L. parsia at different centres during premonsoon, monsoon and postmonsoon of 1991 and 1992**

Centre	Centre Code	1991			1992		
		Season					
		Premonsoon	Monsoon	Postmonsoon	Premonsoon	Monsoon	Postmonsoon
Korapuzha Estuary	I	ND	ND	49.9	826.7	-	134.6
Cochin Backwater	II	ND	ND	ND	ND	ND	9.17
Tuticorin Bay	III	ND	ND	217.3	14.4	35.7	ND
Mandapam	IV	757.1	199.0	ND	645.3	83.4	ND
Ennore Creek	V	ND	34.5	ND	16.0	62.8	ND
Rusikulya Estuary	VI	ND	ND	ND	ND	-	27.4

ND = Not detected, between centres ($P < 0.01$).

Gills: No significant differences were found between years of collection, seasons and centres (Table 71).

The nonsignificant and very weak positive relationship were observed between the Bioaccumulation Factor in gills and the available concentrations in water (r = 0.196) and sediment (r = 0.132). Similar result was also obtained between the lead content and its Bioaccumulation Factor in gills (r = 0.224).

Kidney: From ANOVA no significant differences were seen between the years and also between the seasons and centres (Table 72).

The weak positive and nonsignificant correlation coefficients were obtained between the Bioaccumulation Factor to that of lead concentration in water (r = 0.131) and sediment (r = 0.127). Similar result (r = 0.309) was also seen between the lead content and the Bioaccumulation Factor in kidney.

Intestine: The year, season and centrewise Bioaccumulation Factor of lead in intestine is presented in Table 73. From ANOVA no significant differences were seen between 1991 and 1992 and also between the seasons and centres.

The correlation study indicated the nonsignificant and weak positive coefficients between the Bioaccumulation Factor of lead in intestine and the available concentration in water (r = 0.098) and sediment (r = 0.170).

Similar result was also seen (r = 0.238) between lead content and Bio-accumulation Factor in intestine.

Ovary: No significant differences were observed from ANOVA for years, seasons and centres (Table 74).

From correlation study the nonsignificant positive relations were obtained between the Bioaccumulation Factor and the available concentration of lead in water (r = 0.173) and sediment (r = 0.173). Between the lead content and its factor, a highly significant (at 1% level) and positive relationship (r = 0.548) was seen.

Skin: Table 75 indicates the variation in lead Bioaccumulation Factor in skin in year, centre and seasons. From two-way ANOVA no significant differences were obtained for seasons, years and centres.

The nonsignificant positive relationships were obtained from correlation study on computer between the Bioaccumulation Factor and the available concentration of lead in water (r = 0.111) and sediment (r = 0.187). The highly significant (at 1% level) good positive coefficient (r = 0.749) was obtained for the Bioaccumulation Factor with the lead content in skin.

Muscle: No significant differences were noticed in ANOVA for years and seasons (Table 76). The centres were seen significant at 10% F - value. The mean values calculated are given in descending order:

Centre	Value
Mandapam Water	280.8
Korapuzha Estuary	202.2
Tuticorin Bay	44.6
Ennore Creek	18.9
Rusikulya Estuary	5.5
Cochin Backwater	1.5

The correlation study indicated the weak nonsignificant positive coefficients (0.140 and 0.296) between the Bioaccumulation Factors and the available concentration in water and sediment respectively. The positive coefficient (r = 0.698) with level of significance at 1% was established between the content and its factor for lead in muscle.

Discussion

High intake of lead from ambient seawater by marine flora is documented by several investigators (Eisler, 1981). Concentration Factors (CF) reported for different marine plants and organisms are listed below:

Species	Value	Author(s)
Benthic algae of Raritan Bay, New Jersey	13,000 - 18,000	Seeliger and Edwards, 1977
Algae from sorfjorden, Norway	1,000 - 26,000	Melhuus et al., 1978
Ulva reticulata (in Laboratory)	124	Sivalingam, 1978
Crustaceans (In marine food chain)	100	Heyraud and Cherry, 1979

Sergestes spp. (Hepatopancreas)	10 - 1,000,000	Heyraud and Cherry, 1979
Penaeus indicus	0 - 3475.5	Anikumari, 1992
L. parsia (Seven tissues)	86 - 1086.77	Present study

Most of the lead in crustaceans was localised in the exoskeleton with comparatively low residues in other tissues (Eisler, 1981). Second to ovary, the liver of L. parsia showed higher Bioaccumulation Factor of lead in the present study as equivalent to the hepatopancreas of prawn/shrimp as observed by Eisler (1981). The muscle tissue of L. parsia showed lowest Bioaccumulation Factor of lead as equivalent to the muscle of shrimp.

In fishes, very few reports are available regarding the Bioaccumulation Factors in whole organisms or in different tissues. The ability of tuna to accumulate higher amount of lead from seawater is reported by Heyraud and Cherry (1979). Concentration Factors for whole tuna were approximately 100, being highest in liver and lowest in muscle. In the present study liver has shown the highest and muscle the lowest Bioaccumulation Factors. The value for other tissues/organs were found in between. Waldichuk (1974) reported the Concentration Factor of 6,000 - 10,000 for Pacific Halibut Hippoglosus stenolepis. As no reports are available regarding Bioaccumulation Factors of lead in different tissues of fish, the present result can not be compared and this is the first report from India. In the present study, no centrewise or seasonwise differences were seen in lead Bioaccumulation Factor. The poor relations obtained in the

study concluded that the Bioaccumulation Factor seemed independent of the available concentration in medium. It may also be concluded that, being a non-essential element, the lead maintained in the tissues upto a certain limit irrespective of its availability in the medium; and no regulation of lead found in the body of the experimental animal.

GENERAL DISCUSSION

In the present study all the tissues (with certain exceptions) recorded more zinc followed by copper and lead. The exceptions were the ovary for which the concentration of lead and copper found similar (with lead on higher side) and the gills for which lead was found higher than that of copper. Similar reports are available from the literature to support the present findings. Some of them are:

Species	Tissue	Order of accumulation	Author(s)
M. parsia	—	zinc, copper	Zingde et al.,1976
Pagothenia borchgrevinki	muscle, testis, skin	zinc, copper, lead	Honda et al., 1983
L. macrolepis	liver, muscle	zinc, copper, lead	Nammalwar, 1987
"	gills	zinc, lead, copper	"
Gadus morhua	liver, kidney muscle, intestine	zinc, copper, lead	Szefer et al., 1990
"	gonad, gills	zinc, lead, copper	"
L. parsia	liver, kidney muscle, intestine, skin	zinc, copper, lead	Present study
"	gills, ovary	zinc, lead, copper	"

The Bioaccumulation Factor for zinc found more than copper in prawn Ascelis indicus, crab Scylla serrata and fish Arius sp. as whole organism (Matkar et al., 1981). No other reports particularly from India are available regarding the comparison of Bioaccumulation Factors of different elements in different tissues of fish. From the present study in following order the Bioaccumulation Factor for copper, zinc and lead were found for different tissues:

Tissue	Factor in order
Ovary	Zn, Pb, Cu
Liver	Cu, Zn, Pb
Skin	Zn, Cu, Pb
Gills	Zn, Pb, Cu
Muscle	Cu, Zn, Pb
Kidney	Cu, Pb, Zn
Intestine	Zn, Cu, Pb

CHAPTER 2

CHAPTER 2

MONTHLY AND SEASONAL VARIATIONS IN COPPER, ZINC AND LEAD IN WATER, SEDIMENT AND DIFFERENT TISSUES OF LIZA PARSIA IN COCHIN BACKWATER

Introduction

The concentration of copper, zinc and lead at 6 centres in 18 stations spread on both east and west coasts of India during 3 seasons viz. Premonsoon, Monsoon and Postmonsoon in 1991 and 1992, their relation ship with environment and content of heavy metals in L. parsia and Bio-accumulation Factor have been studied in Chapter 1 and very significant results were found. It was felt essential and very proper to study, with closer observations to substantiate the results by intensively studying in a regular manner monthwise at Cochin Backwater between June 1991 and May 1992 on the same pattern as described in Chapter 1.

The observations and the results obtained for one year at Cochin Backwater are very similar and comparable to the results obtained and the distribution pattern of heavy metals at 6 centres over a period of 2 years covering 6 season is a remarkable finding. A few exceptions and small minor deviations have been discussed with proper explanation and justification at appropriate places. Hence, the candidate straight away proceeded with results without re-explaining the methodology which is the same as explained in Chapter 1.

For this type of monitoring, the samples i.e. water, sediments and tissues of L. parsia was collected during low tide in morning hours

of a day in second week of each month from June 1991 to May 1992. The collections were undertaken at St. II 4, St. II 5 and St. II 6 of Cochin Backwater. To find out the level of significance between the seasons (viz. Premonsoon: February-May; Monsoon: June-September; Postmonsoon: October-January) for a particular parameter, the one-way ANOVA was applied to the data (Snedecor and Cocnran, 1967). In the similar line of Chapter 1, this study also aimed to find out the interrelationship between the biotic and abiotic parameters, the specific tissue for the content of a specific metal and the seasonal differences in metal concentration in water, sediment and tissue. In the begining of each section hereunder a comparative picture of each parameter is given for the Cochin Backwater as obtained from Chapter 1 (over 6 seasonal collectionsons of 1991 and 1992) and at Cochin Backwater (over 12 months collections) along with the percentage deviation between the two results.

The Cochin Backwater was selected, because

(i) it is a perennial estuary,

(ii) it is a key industrial centre in central Kerala, clustered with all types of industries along the estuarine area and both the banks

As analysed

From ANOVA the significant difference (at 5% level) was obtained between the seasons only. The higher amount of copper was found in monsoon followed by premonsoon and postmonsoon. This coincides with the results obtained for two year period in other centres and cited in "Chapter 1, Section: Copper in sediment". Very high significant difference (at 0.1% level) was observed from analysis of variance for stations placing St. II 4 in the higher side followed by St. II 5 and St. II 6. The stations within a season showed marked difference at 1% F-value.

From the correlation study pooling the data for copper in water and sediment, a poor and nonsignificant positive coefficient (r = 0.346) was found between them as reported earlier for other centres in "Chapter 1, Section : Copper in sediment".

Discussion

The mean value (35.12 ppm dry wt) of copper obtained in sediments of Cochin Backwater ranged between 2.5 and 106.2 with the standard deviation 23.51 (Table 78). As discussed in "Chapter 1, Section: Copper in Sediment", the monsoon showed higher concentration of copper than the dry seasons. The three stations viz. St. II 4, St. II 5 and St. II 6 differed significantly with the means 48.54, 43.43 and 13.40 respectively. Comparing with the USGS rock standards of 46.4 ppm (Flangagan, 1976), the St. II 4 showed higher copper concentrations and discussed under "Chapter 1, Section : Copper in sediment". Ackroyd et al. (1987) reported

18.5 ± 2.7 and 25.1 ± 3.8 ppm dry wt of copper for "Miramichi Estuary Standard Sediment (MESS-1)" and "Baie des Chaleurs Standard Sediment (BCSS-1)" respectively. Comparing with these standards the St. II 4 and St. II 5 recorded higher concentrations. The differences among the stations can be attributed to their locations which are discussed in "Materials and Methods". Very few reports are available regarding the copper content in Cochin Backwater. Murty and Veerayya (1981) reported 9-63 ppm of copper for Vembanad Lake, Kerala which is a part of Backwater system. Venugopal et al. (1982) reported maximum of 70.8 ppm dry wt copper for northern side of Cochin Backwater. For all the three stations, the results obtained in present study are well comparable with that of Murty and Veerayya (1981) and Venugopal et. al. (1982).

Working at Tamar Estuary, Ackroyd et al. (1987) concluded that the distribution of metals are dependant on the input of new particulate material derived with river flow. The St. II 4 location was towards the river side of the Backwater and St. II 6 towards the sea side in the Backwater. It was found that freshwater generally contains more iron and manganese oxides favourable for the formation of colloids with heavy metals and their flocculation (Murty and Veerayya, 1981). Location of St. II 4 towards the freshwater side might have favoured for the flocculation of copper with iron and manganese oxides and latter sinking to the bottom as the ultimate reservoir. Katz and Kaplan (1981) stated that majority of heavy metals enter the coastal waters of Southern California are associated with particulate material via waste outfalls. The coarse-grained particles, along with their metal loads are deposited as such near the outfall

and are removed altogether from the system. The St. II 4 location was near to the industrial belt of Cochin area. The industrial waste along with the sewage might have contributed substantial amount of copper to the sediment. According to Berrow and Webber (1972) sewage sludge is the major source of copper to the environment. Station II 4 water was noticed with oil slicks in some collections and sediment with oil coatings. The source of hydrocarbons (oil and paraffin) to the station can be linked with the establishment of Caprolactam Plant near to the station. Report of very high concentration of copper at outfall of refineries (10000 ppm) near Derwent Estuary in Australia are available (Bloom and Ayling, 1977). Organic coatings on particles, which suppress heavy metal leaching in the laboratory , are probably abundant in the reducing environment of the outfalls (Katz and Kaplan, 1981). Smaller particles which are transported through oxic waters towards the 'cleaner' reaches of the ocean loose their organic shield (along with small fraction of metals in it). Thus metal became leachable to the water medium and diluted (Katz and Kaplan, 1981). The same reason is more applicable here for the gradual decrease of copper from St. II 4 to St. II 6 in the seaward direction.

The copper content in the sediment of Cochin Backater is also well comparable with that of similar environments in India as observed by the candidate at six centres and also others elsewhere, but less than the Bombay Harbour Bay as reported for Bombay Harbour sediment is between 162.1-276.3 ppm dry wt (Matkar et al., 1981).

Copper in tissues

As observed

The copper content in liver, gills, kidney, intestine, ovary, skin and muscle of L. parsia in different months from June 1991 to May 1992 in Cochin Backater is given in Table 79.

As analysed

Mean monthly values of copper in different tissues of L. parsia with standard deviation and range are given below in descending order:

Tissue	Value in ppm dry wt		
	Mean	SD	Range
Liver	10.73	3.11	6.34 - 16.56
Intestine	6.53	3.01	2.52 - 12.86
Kidney	5.61	3.12	ND - 14.02
Gills	4.80	3.12	1.33 - 12.73
Ovary	4.35	1.48	1.68 - 6.35
Skin	3.37	1.35	1.05 - 5.28
Muscle	3.07	0.84	1.28 - 3.96

The correlation coefficients (r), level of significance with grand mean values (ppm dry wt) of copper content in L. parsia in premonsoon (PrM), monsoon (M) and Postmonsoon (PtM) (June 1991 to May 1992) are presented below:

TABLE 79. Copper (ppm dry wt) in different tissues of L. parsia in Cochin Backwater from June 1991 to May 1992

Tissues	Monsoon			Postmonsoon						Premonsoon			Mean with SD
	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	
Skin	3.06 (0.004)	1.64 (0.007)	1.53 (0.006)	1.05 (0.006)	5.28 (0.005)	3.21 (0.007)	3.56 (0.004)	3.92 (0.003)	3.78 (0.002)	4.24 (0.008)	4.18 (0.004)	5.00 (0.002)	3.37± 1.35
Liver	12.55 (0.007)	13.33 (0.011)	14.77 (0.012)	8.04 (0.005)	16.56 (0.008)	9.9 (0.005)	12.02 (0.008)	8.77 (0.004)	10.16 (0.004)	7.99 (0.005)	6.34 (0.006)	8.34 (0.006)	10.73± 3.11
Gills	12.73 (0.004)	1.55 (0.006)	1.33 (0.006)	2.89 (0.008)	6.42 (0.002)	5.00 (0.006)	4.77 (0.008)	3.26 (0.008)	4.25 (0.006)	2.35 (0.006)	5.88 (0.003)	7.19 (0.005)	4.80± 4.80
Kidney	5.62 (0.006)	ND	ND	ND	4.30 (0.003)	7.27 (0.008)	7.22 (0.006)	7.25 (0.006)	ND	8.45 (0.004)	14.02 (0.005)	13.24 (0.007)	5.61± 4.98
Intestine	4.48 (0.005)	2.52 (0.006)	3.36 (0.006)	4.77 (0.007)	9.12 (0.003)	6.67 (0.008)	9.30 (0.009)	8.36 (0.007)	12.86 (0.003)	3.70 (0.007)	6.26 (0.005)	6.94 (0.005)	6.53± 3.01
Ovary	4.90 (0.007)	6.35 (0.006)	5.78 (0.005)	2.68 (0.005)	4.26 (0.007)	4.47 (0.007)	3.00 (0.004)	2.88 (0.003)	1.68 (0.003)	4.69 (0.005)	5.70 (0.004)	5.78 (0.006)	4.35± 1.48
Muscle	3.26 (0.006)	3.51 (0005)	2.24 (0.008)	1.28 (0.008)	2.82 (0.007)	3.88 (0.005)	3.96 (0.002)	2.74 (0.002)	2.95 (0.008)	4.13 (0.006)	3.68 (0.004)	2.39 (0.003)	3.07± 0.84

SD = Standard deviation and coefficient of variation is in parentheses.
In kidney between seasons ($P < 0.1$), in intestine between seasons ($P < 0.1$), in skin between seasons ($P < 0.01$).

Tissue	Significance between seasons at	Correlations	
		With water	With sediment
Liver	NS	r = 0.335	r = 0.455
Gills	NS	r = 0.389	r = 0.404
Kidney	10% F-value; PrM 8.93 PtM 6.51 M 1.41	r = 0.366	r = 0.593**
Intestine	10% F-value; PtM 8.36 PrM 7.44 M 3.78	r = 0.188	r = 0.467
Ovary	NS	r = 0.071	r = 0.338
Skin	1% F-value; PrM 4.30 PtM 3.99 M 1.82	r = 0.502	r = 0.522
Muscle	NS	r = 0.177	r = 0.033

** 1% level, NS = Nonsignificant.

The results obtained for liver, gills, intestine, ovary and muscle were seen similar to that obtained in Chapter 1.

Discussion

In the present investigation, the higher copper content was noticed here also in liver as observed in "Chapter 1, Section : Copper in tissues" followed by intestine, kidney, gills, ovary, skin and muscle. In this investigation intestine showed higner content of copper than kidney and skin than muscle. Zingde et al. (1976) reported higher levels of metals such as As, Cu, Zn and Mn in Mugil (Liza) parsia in coastal and estuarine

waters around Goa. This higher levels of metals probably are attributable to the feeding habits as suggested by Zingde et al. (1976). L. parsia is detritus feeder and feed either by sucking up the surface layer of the mud or grazing on the rock surfaces leading to the transfer of mineral particles into the body along with food (Zingde et al., 1976). The sediments are usually enriched with trace metals and as a result,detritus feeders are exposed to more quantities of metals than fishes above water column (pelagic) habitat. From the present investigation the sediments showed higher concentration of copper in the Cochin Backwater than other centres (Chapter 1, Section: Copper in sediment). Being a detritus feeder and as observed above, it substantiates the finding that in intestine of L. parsia more copper is found from the ingested food along with sediments which contains more copper. Noel-Lambot (1981) has stated that the intestinal lumen contains the corpuscules which bind the copper in eel Anguilla anguilla. The finding of Noel-Lambot (1981) strengthens the results of the present study in Cochin Backwater for L. parsia. According to Phillips (1977) the only tissue with a lower level of metal is axial muscle, inwhich metals appear to be strictly regulated. Similar lower copper in muscle of L. parsia was recorded from the present study.

As found and discussed in "Chapter 1, Section: Copper in tissue", no "good correlations" were obtained (except kidney) for copper in tissues with that in water and sediment. Here also it is reflected with no "good correlation" as similar observations were made by Milner (1982). The kidney serves as the excretory organ in the fish. When sediments contained more copper and served as the food for L. parsia, the enrichment of the

metal might have taken place in different tissues. At the same time the elimination of the metal from kidney might have ceased leading to the higher content in kidney. Thus increase of copper in kidney was found positively related with that of sediment.

In the present study the seasonal difference was reported for copper in kidney (at 10% level), intestine (at 10% level) and skin (at 1% level). In all cases, the summer season recorded higher content of copper than the rainy seasoon i.e. monsoon. The decrease in the metal content in the tissues in monsoon may be due to higher dilution of the water. As monsoon subsided the copper content increased in intestine. The sudden spike during premonsoon in kidney and skin may be due to the premonsoon showers which mobilise copper from industrial and domestic source to the receiving backwater Similar seasonal reports are available for Metapenaeus dobsoni in Cochin Backwater (Sivadasan and Nambisan, 1988). The increase in trace metal level found in aquatic organisms may contribute to the seasonality of elments in biota. Phillips (1980) states "in a hypothetical situation in temperate waters where the amount of available metal does not differ through out the year, an organism would exhibit cyclical annual maxima and minima in metal concentration because of the changes in the growth rates with season". But that may not be a case in tropical climate. In tropical estuaries where well-defined wet and dry seasons exist, biota will be exposed to severe seasonal variations in hydrological parameters particularly salinity and the direct effect of salinity on metal uptake could evince marked changes in metal abundance

in such biota (Phillips, 1980). But its exact effect on different organs is still unknown, because of the tissue specific dynamics in metal uptake and elimination which results the metal storage in tissue.

Bioaccumulation Factor

As observed

The Bioaccumulation Factor in relation with water in different months in different tissues are presented in Table 80.

As analysed

Range of "Bioaccumulation Factor" obtained for 12 months for different tissues with grand mean values and standard deviation for copper in descending order are in brief:

Tissue	Value		
	Mean	SD	Range
Liver	256.4	191.1	35.8 - 680.6
Kidney	227.3	262.2	ND - 719.6
Intestine	167.9	127.6	7.4 - 374.3
Skin	131.4	105.4	6.4 - 267.2
Ovary	120.6	104.5	9.5 - 281.7
Gills	107.9	135.2	3.2 - 485.1
Muscle	73.7	61.0	8.9 - 179.6

TABLE 80. Bioaccumulation Factor of copper in different tissues of L. parsia in Cochin Backwater from June 1991 to May 1992.

Tissues	Monsoon			Postmonsoon				Premonsoon				Mean with SD
	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	
Liver	680.6	39.5	126.4	61.6	401.7	107.5	35.8	315.3	298.5	382.0	303.0	325.0	256.4± 191.1
Gills	485.1	3.2	8.0	15.6	109.4	28.1	10.0	82.4	87.8	79.0	178.7	197.4	107.9± 135.2
Kidney	361.8	ND	ND	ND	123.8	93.7	25.5	309.4	ND	479.6	719.8	613.9	227.3± 262.2
Intestine	240.7	7.4	28.5	36.2	219.1	71.7	27.5	297.8	374.3	175.3	268.2	268.6	167.9± 127.6
Ovary	281.7	19.9	52.4	21.8	109.5	51.4	9.5	109.8	52.3	237.7	261.3	239.3	120.6± 104.5
Skin	218.7	6.4	17.3	10.6	168.8	46.1	14.0	185.8	146.4	267.2	238.3	257.5	131.4± 105.4
Muscle	160.7	9.5	17.4	8.9	62.2	38.3	10.7	89.6	78.8	179.6	143.9	84.9	73.7± 61.0

SD = Standard deviation , ND = Not detected.

In kidney between seasons ($P < 0.1$), in skin between seasons ($P < 0.1$).

The correlation coefficients (r), level of significance with grand mean values of copper Bioaccumulation Factor in L. parsia in premonsoon (PrM), monsoon (M), and postmonsoon (PtM) (June 1991 to May 1992) are presented below:

Tissue	Significance between seasons at	Correlations		
		With water	With sediment	With Copper content in tissue
Liver	NS	r = 0.729	r = 0.150	r = 0.005
Gills	NS	r = 0.514	r = 0.179	r = 0.916**
Kidney	10% F-value; PrM 453.13 PtM 138.10 M 90.40	r = 0.574*	r = 0.405*	r = 0.880**
Intestine	10% F-value; PrM 271.50 PtM 154.03 M 78.20	r = 0.758**	r = 0.317	r = 0.577
Ovary	NS	r = 0.668**	r = 0.274	r = 0.457
Skin	10% F-value; PrM 227.35 PtM 103.68 M 63.25			
Muscle	NS	r = 0.703**	r = 0.205	r = 0.374

** 1% level, * 5% level, NS = Nonsignificant.

The results obtained for liver, gills, intestine, ovary, skin and muscle are very well coincides with that obtained in Chapter 1.

Discussion

The Bioaccumulation Factor for copper was found more in liver, kidney and intestine in that order and are similar as observed and discussed in Chapter 1, but muscle recorded the least and ovary, skin, gills with slight deviations. The lower Accumulation Factor in muscle can be attributed to lower level of copper in that tissue in Cochin Backwater (Chapter 2, Section: Copper in tissue). The reports available for other organisms have already been discussed in Chapter 1 elaborately. The differences were seen in Bioaccumulation Factors at 10% F-value in kidney, intestine and skin in different seasons. The dry seasons recorded higher value than the wet season. Similar copper contents were also seen for the same tissue in Cochin Backwater. As discussed in Chapter 1, the good positive correlations were obtained for Bioaccumulation Factor of copper in water and tissue, but not with sediment. From all the results it has been concluded that as the concentration of copper increased in water, it resulted in increase in different tissues and the Bioaccumulation Factor. Lenearity is maintained in all the tissues between copper content and its factor which is a significant finding. When the copper level increased in water, the copper in tissues increased more than proportionately. Thus the factor (copper in tissue devided by copper in water) was seen increasing in a linear fashion.

Section 3: Zinc

At a glance

Parameter	Average value obtained (Range in parentheses)		% Variation (%)
	during 2 years period	monthwise in 1991-92	
Water (ppb)	82.10 (25.0-165.5)	75.23 (15.5-180.0)	-6.1
Sediment (ppm)	406.65 (25.0-990.0)	346.45 (25.0-1044.5)	-3.0
Zinc in tissue (ppm dry wt)			
Liver	129.15 (43.67-331.0)	94.84 (42.84-331.0)	-26.0
Gills	101.38 (63.20-272.40)	108.43 (55.72-272.4)	7.0
Kidney	32.80 (ND -52.66)	36.98 (ND -92.76)	12.7
Intestine	63.65 (39.52-82.21)	72.29 (31.65-124.75)	13.6
Ovary	264.74 (133.4-463.2)	288.79 (130.5-629.1)	9.1
Skin	102.74 (64.29-135.28)	110.53 (62.29-161.0)	7.6
Muscle	16.47 (8.14-23.32)	23.48 (8.14-52.10)	42.6
Bioaccumulation Factor			
Liver	452.3 (143.1-1202.8)	257.3 (91.1-845.3)	-43.1
Gills	261.0 (85.8-695.7)	318.2 (85.8-821.9)	21.0
Kidney	138.1 (ND -236.9)	173.5 (ND -393.1)	25.6
Intstine	421.3 (151.3-1326.5)	296.7 (85.6-794.8)	-29.6
Ovary	918.1 (669.7-1213.9)	1135.5 (554.3-2779.8)	27.7
Skin	493.3 (256.0-058.5)	592.0 (269.1-1232.3)	20.0
Muscle	51.5 (24.3-68.1)	78.6 (23.4-197.5?	52.6

ND = Not detected.

Zinc in Water

As observed

Abstract of Table 38 and 81 is given below for comparison (range is in parentheses):

Months	Mean in (ppb)	Average in seasons during			Variation (%)
			1991-92	2 years period	
June	84 .42 (30.0 -162.00)	Monsoon	74 .67 (19.0-180.0)	62 .21 (25.0 -162.0	-11.3
July	97 .75 (48.0 -126.5)				
August	62 .17 (23.5 -103.5)				
September	54 .33 (19.0 -180.0)				
October	88 .17 (39.5 -126.5)				
November	148 .25 (123,5-165.5)				
December	66 .92 (24.0 -118.5)	Post-monsoon	92 .48 (22.5-165.5)	112 .67 (42.5 -165.5)	25.1
January	66 .58 (22.5 -128.0)				
Febryary	73 .42 (42.5 -97.5)				
March	64 .00 (24.0 -96.0)	Pre-monsoon	52 .04 (15.5-132.0)	64 .42 (25.0 -1230)	11.0
April	63 .00 (18.0 -132.0)				
May	31 .75 (15.5 -79.0)				

TABLE 81. **Zinc in water (ppb) and sediment (ppm) of Cochin Backwater from June 1991 to May 1992**

	Stations		Monsoon June	July	Aug.	Sept.	Postmonsoon Oct.	Nov.	Dec.	Jan.	Premonsoon Feb.	Mar.	Apr.	May
Water	II 4	Surface	117.5	76.5	23.5	35.0	76.0	151.5	86.0	102.0	68.5	96.0	18.0	18.0
		Bottom	108.5	76.5	81.5	35.5	112.5	165.5	112.5	128.0	94.5	77.0	132.0	79.0
	II 5	Surface	30.0	102.5	29.5	19.0	39.5	132,5	28.0	42.0	86.0	24.0	58.0	15.5
		Bottom	32.0	126.5	102.5	31.0	126.5	154.0	118.5	56.0	97.5	86.5	96.0	51.5
	II 6	Surface	38.5	48.0	32.5	25.5	66.5	123.5	24.0	22.5	42.5	32.5	20.5	24.0
		Bottom	162.0	156.5	103.5	180.0	108.5	162.5	32.5	49.0	57.5	68.0	53.5	32.5
	Mean	Surface	62.00	75.67	28.50	26.50	60.67	135.83	46.00	55.50	65.67	50.83	32.17	19.17
		Bottom	100.83	119.83	95.83	82.17	115.67	160.67	87.83	77.67	81.17	77.17	93.83	44.33
	Grand Mean with SD		84,42± 55.60	97.75± 39.19	62.17± 37.82	54.33± 61.88	88.17± 32.98	148.25± 16.76	66.92± 43.92	66.58± 39.99	73.42± 23.00	64.00± 29.35	63.00± 44.26	31.75± 28.25
	Range		(30.0- 162.0)	(48.0- 126.5)	(23.5- 103.5)	(19.0- 180.0)	(39.5- 126.5)	(123.5- 165.5)	(24.0- 118.5)	(22.5- 128.0)	(42.5- 97.5)	(24.0- 96.0)	(18.0- 132.0)	(15.5- 79.0)
Sediment	II 4		861.00	802.00	663.75	781.75	688.75	829.25	914.25	771.50	665.50	1006.00	550.00	411.50
	II 5		607.25	413.75	390.75	403.75	708.50	335.25	208.50	301.25	90.75	153.25	344.00	300.75
	II 6		189.75	128.75	119.25	196.50	108.00	120.75	83.75	57.50	26.75	210.50	126.00	89.25
	Mean with S.D		552.67± 307.95	448.17± 305.30	391.25± 243.93	460.67± 265.54	383.67± 261.20	428.42± 329.04	402.17± 401.17	376.75± 324.74	261.00± 325.94	421.50± 453.94	340.00± 215.02	26717± 146.53
	Range		(167.0- 924.0)	(122.0- 868.0)	(112.5- 685.5)	(188.0- 786.00	(108.0- 712.5)	(118.0- 910.5)	(81.0- 949.0)	(52.5- 772.5)	(25.0- 798.0)	(98.0- 1044.5)	(126.0- 710.0)	(85.5- 416.5)

SD = Standard deviation.
In water between seasons ($P < 0.05$), between months ($P < 0.05$), between surface and bottom collections ($P < 0.001$) Mean with SD and range 75.23 ± 44.87 (15.5-180.0).
In sediment between seasons ($P < 0.001$), between stations ($P < 0.001$), mean with SD and range 394.45 ± 291.87 (25.0-1044.5).

As analysed

ANOVA showed the differences between the seasons and months at 5% F-value. The higher concentration of zinc was found in postmonsoon followed by monsoon and premonsoon. Between the surface and bottom water collections the latter was found significantly higher at 0.1% level than the former one.

In correlating the zinc with salinity in different seasons, the significant (at 1% level) negative coefficients were obtained -8.491, -0.363 and -0.246 for premonsoon, monsoon and postmonsoon respectively. Similarly significant (at 5% level) coefficients were seen with total hardness e.g., -0.279 and -0.259 for premonsoon and monsoon respectively, but the coefficient was -0.4 for postmonsoon and significant at 1% level.

Discussion

The monthly average from June 1991 to May 1992 of zinc in Cochin Backwater was calculated 75.23±44.87 (15.5-180.00) ppb and it was well complied with the result that obtained for the same centre in comparative study in Chapter 1 (Table 39). The mean value was seen below the EPA safe limit of 100 ppb (Anon., 1191 a).

As discussed in "Chapter 1, Section: Zinc in water," the significant seasonal differences were seen for zinc in Cochin Backwater. Similar

to the above observation, postmonsoon recorded higher concentration of zinc followed by monsoon and postmonsoon. In monsoon and postmonsoon of 1991 there was dredging in Backwater near to St. II 5 which resulted the suspension of bottom mud in water column increasing the zinc in postmonsoon followed by monsoon which dilutes estuarine water. But in all the stations in most of the collections, higher zinc concentrations than EPA safe limit of 100 ppb were found (Table 81) for which no specific reason is attributable other than the general industrial discharges.

As discussed in "Chapter 1, Section: Zinc in water", significantly higher zinc level was found in bottom water than surface. Better correlation was also found between zinc in water and salinity, but was significantly negative between them. Similar correlation was also established with total hardness.

Zinc in sediment

As observed

The station, month and seasonwise zinc content in sediments of Cochin Backwater is given in Table 81. Abstract of Table 38 and 81 is given below for comparison (range is in parentheses):

Months	Mean in ppm	Average in season	1991-92	2 years period	Variation (%)
June	552 .67 (267.0 -924.0)				
July	448 .17 (122.0 -868.0)				
August	391 .25 (112.5 -685.5)	Monsoon	463.19 (112.5-924.0)	470.59 (117.5-924.0)	1.6
September	460 .67 (118.0 -786.0)				
October	383 .67 (108.0 -910.5)				
November	428 .42 (118.0 -910.5)	Post-monsoon	397 .75 (52.5 -949.0)	444.96 (92.0-990.0)	11.9
December	402 .17 (81.0 -949.0)				
January	376 .75 (52.5 -772.5)				
February	261 .0 (25.0 -798.0)	Pre-monsoon	322 .4 (25.0-1044.5)	313.42 (25.0-885.0)	-2.8
March	421 .5 (98.0 -1044.5)				
April	340 .0 (126.0 -710.0)				
May	267 .17 (85.5 -416.5)				

As analysed

From analysis of variance highly significant differences (at 0.1% level) were seen between the seasons and stations. The maximum value was seen in monsoon followed by postmonsoon and premonsoon. St. II 4 recorded higher value (mean 745.42) followed by St. II 5 (mean 325.29) and St. II 6 (mean 112.63 ppm dry wt).

A nonsignificant correlation coefficient was found between sediment and water (r = 0.389).

Discussion

The monthly mean of 394.45 ppm dry wt of zinc was obtained in sediments of Cochin Backwater and ranged between 25.0 and 1044.5 ppm dry wt with the standard deviation 291.87 (Table 81). As discussed in "Chapter 1, Section : Zinc in sediment" here in Cochin Backwater also zinc was found above the standard values. It is also noticed from statistical analysis, a higher concentration of zinc was found in sediments in monsoon followed by postmonsoon and premonsoon. The reasons explained in Chapter 1 stand good here also.

In the present study, the stations differed among themselves significantly. Working in Tamar Estuary Ackroyd et al. (1987) concluded that the distribution of metals are dependent on the input of the particulate material derived with river flow. The St. II 4 location is towards the river side of the Backwater and St. II 6 towards the sea. The St. II 4 is very close to the industrial areas of Cochin discharging their wastes contributing considerable amount of zinc to the sediments through their effluents. Katz and Kaplan (1981) indicated that the majority of heavy metals enter the coastal waters of Southern California are associated with particulate material via. waste outfalls. The coarse-grained particles, along with their metal loads settle as such near the outfall and their concentrations reduced from aquatic medium increasing the zinc in sediment. Similarly at St. II 4 the industrial wastes along with the domestic sewage is a major contributor of zinc to the sediment underneath the water column. According to Berrow and Webber (1972) sewage sludge is one of the major source of zinc to the aquatic environment. The sediments of the St. II 4 were noticed in most of the collections with oil coatings. Bloom and Ayling (1977) reported very high concentration of zinc (104000 ppm) at outfall of refineries and at the same time the contribution of it to the increased level of zinc in sediment in the Derwent Estuary,

Australia is also available. Katz and Kaplan (1981) have also found that organic coatings on particles, which reduces heavy metal leaching is abun - dant in the reducing environment of the outfalls. Smaller particles which are transported through oxic waters towards the "Cleaner" reaches of the ocean loose their organic shield (along with small faction of metals in it). Thus metal became leachable to the aquatic medium (Katz and Kaplan, 1981). The similar reason corroborates the present decrease of zinc in sediment from St. II 4 to St. II 6. Report of Morris et al. (1986 b) showed the higher concentration of zinc in sediment in the upper stream of the Tamar Estuary with concentrations decreasing gradually seawards substantiates the candidate's observations in Cochin Backwater. In general, the zinc content in Cochin Backwater which is below the world estuaries and comparable to that of India, as observed by the candidate is very much agreement with the results of Hungspreugs (1988) in Jakarta Bay, Harding and Goyette(1989) in North Pacific coastal sediments and from India Venugopal et al. (1982) in Cochin Backwater, Subramanian et al. (1988) in Ganges Estuary, Subramanian et al. (1989) in Cauvery Estuary and Lyla (1991) in Vellar Estuary.

Zinc in tissues

As observed

The zinc content in different tissues during 12 months in 1991-1992 at Cochin Backwater was studied for biomonitoring (Table 62).

TABLE 82. **Zinc (ppm dry wt) in different tissues of L. parsia in Cochin Backwater from June 1991 to May 1992**

	Monsoon				Postmonsoon				Premonsoon				
Tissues	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr	May	Mean with SD
Liver	43.67 (0.018)	50.00 (0.021)	50.82 (0.028)	54.46 (0.021)	42.84 (0.086)	108.30 (0.029)	201.63 (0.124)	63.25 (0.028)	331.00 (0.055)	71.40 (0.040)	63.22 (0.088)	57.48 (0.051)	94.84± 86.45
Gills	66.82 (0.057)	55.72 (0.012)	68.65 (0.016)	72.97 (0.034)	149.64 (0.108)	67.80 (0.030)	72.55 (0.053)	78.45 (0.020)	272.40 (0.170)	123.40 (0.065)	133.52 (0.098)	139.18 (0.062)	108.43± 61.47
Kidney	30.90 (0.009)	33.87 (0.005)	41.08 (0.036)	44.38 (0.006)	92.73 (0.098)	43.60 (0.006)	38.86 (0.041)	40.90 (0.036)	ND	11.80 (0.015)	26.26 (0.018)	39.41 (0.048)	36.98± 22.18
Intestine	46.60 (0.017)	31.65 (0.015)	48.66 (0.032)	51.13 (0.013)	124.75 (0.241)	74.40 (0.060)	70.62 (0.009)	65.66 (0.052)	78.55 (0.064)	89.60 (0.031)	90.12 (0.091)	95.49 (0.098)	72.29± 25.80
Ovary	192.81 (0.030)	370.76 (0.012)	220.06 (0.032)	169.35 (0.025)	367.92 (0.312)	463.20 (0.212)	212.50 (0.122)	130.50 (0.026)	229.50 (0.037)	629.10 (0.119)	323.00 (0.212)	156.50 (0.066)	288.79± 147.84
Skin	62.29 (0.013)	74.80 (0.045)	76.06 (0.052)	81.35 (0.022)	119.27 (0.017)	107.90 (0.02)	118.86 (0.029)	135.28 (0.114)	155.66 (0.096)	161.00 (0.038)	122.60 (0.142)	111.25 (0.123)	110.53± 31.82
Muscle	8.14 (0.007)	25.59 (0.012)	21.40 (0.018)	21.37 (0.013)	37.84 (0.092)	22.30 (0.009)	19.90 (0.01)	11.90 (0.012)	19.90 (0.019)	52.10 (0.042)	24.26 (0.018)	16.11 (0.018)	23.48± 12.31

SD = Standard deviation and coefficient of variation is in parentheses.

In gills between seasons ($P < 0.05$), in kidney between seasons ($P < 0.01$), in intestine between seasons ($P < 0.05$), in skin between seasons ($P < 0.01$).

An analysed

The grand mean values in ppm dry wt with standard deviation were found out statistically from the data for specific tissue. In decreasing order they are (range in parentheses):

Tissue	Value (in ppm dry wt)		
	Mean	SD	Range
Ovary	288.79	147.84	130.50 - 629.10
Skin	110.53	31.82	62.29 - 161.00
Gills	108.43	61.47	55.72 - 272.40
Liver	94.84	86.45	42.84 - 331.00
Intestine	72.29	25.80	31.65 - 124.75
Kidney	36.98	22.18	ND - 92.73
Muscle	23.48	12.31	8.14 - 52.10

The correlation coefficient (r), level of significance with grand mean values of zinc content in L. parsia in in premonsoon (PrM), monsoon (M) and postmonsoon (PtM) are presented below:

Tissue	Significance between seasons at	Correlations	
		With water	With sediment
Liver	NS	r = 0.054	r = 0.473
Gills	5% F-Value; PrM 167.13 PtM 92.11 M 66.04	r = 0.222	r = 0.732
Kidney	10% F-Value; PtM 54.03 M 37.56 PrM 19.37	r = 0.171	r = 0.175
Intestine	5% F-value; PrM 88.52 PtM 83.86 M 44.51	r = 0.114	r = 0.537
Skin	1% F-Value; PrM 137.63 PtM 120.33 M 73.63	r = 0.137	r = 0.614
Ovary	NS	r = 0.467	r = 0.159
Muscle	NS	r = 0.065	r = 0.040

* 5% level ** 1% level and NS = Nonsignificant.

The results obtained for kidney and muscle were see, similar to that obtained in Chapter 1, while studying the centrewise during 1991-1992.

Discussion

In the present investigation the higher contentof zinc was recorded in ovary followed by skin, gills, liver, intestine, kidney and muscle. The

discussion on zinc content in different tissues of fish has already been elaborated in "Chapter 1" Section: Zinc in tissue. The ovary and muscle recorded highest and lowest zinc content respectively as observed in Chapter 1. The rest of other tissues showed variation in zinc within them. The zinc is more dynamic in nature as well as in tissues and it serves as an essential metal for metabolism and enzymatic action (Williams, 1984). His statement is substantiated by the present study of the candidate showing venue for further greater detailed investigation. As discussed in Chapter 1, no good correlation was noticed for zinc in tissue and in environment such as water and sediment (except skin, intestine and ovary with sediment). According to Overnell et al. (1988), the liver zinc concentrations responed to both an increase and a decrease of zinc in water and was judged to the most reliable indicator of the water zinc level. Jaffer et al. (1988) reported positive correlation between the concentration of zinc in fish muscle and in water. In the present study in L. parsia, all the tissues showed positive relationship with that of environment. But relationship with water was poor than that with sediment. Harding and Goyette (1989) found no correlation between sediment and shrimp/prawn tissue, zinc level along with copper of any species on both raw and log transformed data. But Young and Harvey (1989) reported that only zinc in liver and muscle were correlatd with zinc in the sediment ($r = 0.83$ and $P < 0.05$). In the present study instead of liver and muscle; the skin, intestine and ovary showed good relationship with that available in sediment. The gonadal availability may be linked with the seasonality of metal in the environment. In the present study the gonad showed the seasonal fluctuation

in zinc and at the same time sediment also. The two thus might have been linked with each other. But, sediment peak for zinc was recorded in monsoon, while in ovary in pre-and postmonsoons. So the availability of zinc in ovary might have been controlled by some other metabolic activities and the availability from the sediments. The metals used to enter the body via. intestinal wall and or skin or any other contact surface of the animal with the environment (Dallinger et al., 1987). The increase in zinc content in tissue such as intestine, skin and ovary of L. parsia which is a bottom detritus feeder and feed either by sucking up the surface layer of the mud or grazing on the hard substratum leading to transfer of mineral particles into the body along with food (Zingade et al., 1976). The sediments in Cochin Backwater were rich in zinc content as observed by the candidate and the same is ingested along with the food. This is also widened by observing L. parsia with its gills coated with sediment and mud particles.

Bioaccumulation Factor

As observed

The Bioaccumulation Factor of zinc in different tissues in different months of collection at Cochin Backwater is given in Table 83.

Analysed

Pooling the data over collections for specific tissue, the grand mean values with standard deviation calculated and (in descending order) they are:

TABLE 83. **Bioaccumulation Factor of zinc in different tissues of L. parsia in Cochin Backwater from June 1991 to May 1992**

Tissues	Monsoon				Postmonsoon				Premonsoon				
	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	Mean with SD
Liver	97.00	95.9	153.3	187.9	91.1	1378.0	564.9	178.1	845.3	209.2	188.2	339.2	257.3± 227.6
Gills	148.4	106.9	207.0	251.8	318.2	85.8	203.3	220.9	695.7	361.5	397.4	821.9	318.2± 227.6
Kidney	115.9	109.7	209.3	258.7	333.1	93.1	183.9	194.5	ND	58.4	132.0	393.1	173.5± 113.5
Intestine	145.9	85.6	206.9	248.7	374.0	132.6	278.9	260.6	283.8	370.0	378.1	794.8	296.7± 184.0
Ovary	645.9	1072.6	1001.0	881.5	1180.1	883.6	898.0	554.3	885.1	2779.9	1449.9	1394.0	1135.5± 581.4
Skin	259.5	269.1	430.3	526.6	475.8	256.0	624.7	714.6	745.6	884.7	684.4	1232.3	592.02 228.5
Muscle	23.4	61.0	83.5	95.4	104.1	36.5	43.1	43.4	39.3	197.5	93.4	123.1	78.6± 49.1

SD = Standard deviation.

In gills between seasons ($P < 0.01$), in intestine between seasons ($P < 0.1$), in skin between seasons ($P < 0.05$).

Tissue	Value		
	Mean	SD	Range
Ovary	1 1135.5	581.4	554.3 - 2779.8
Skin	592.0	288.5	256.0 - 1232.3
Gills	318.2	227.9	85.8 - 821.9
Intestine	296.7	184.0	85.6 - 794.8
Liver	257.3	227.6	91.1 - 845.3
Kidney	173.5	113.5	ND - 393.1
Muscle	78.6	49.1	23.4 - 197.5

ND = Not detected.

The correlation coefficients (r), level of significance with grand mean values of zinc Bioaccumulation Factor in L. parsia in premonsoon (PrM), monsoon (M) and postmonsoon (PtM) (June 1991 to May 1992) are presented below:

Tissue	Significance between seasons at	Correlations		
		with water	With sediment	with zinc content in tissue
Liver	NS	r = 0.256	r = 0.578*	r = 0.944**
Gills	1% F-Value; PrM 569.13 PtM 207.05 M 178.53	r = 0.595*	r = 0. 825* (Fig.8)	r = 0.788**
Intestine	10% F-Value; PrM 456.68 PtM 261.53 M 171.78	r = 0.655*	r = 0.662**	r = 0.647*

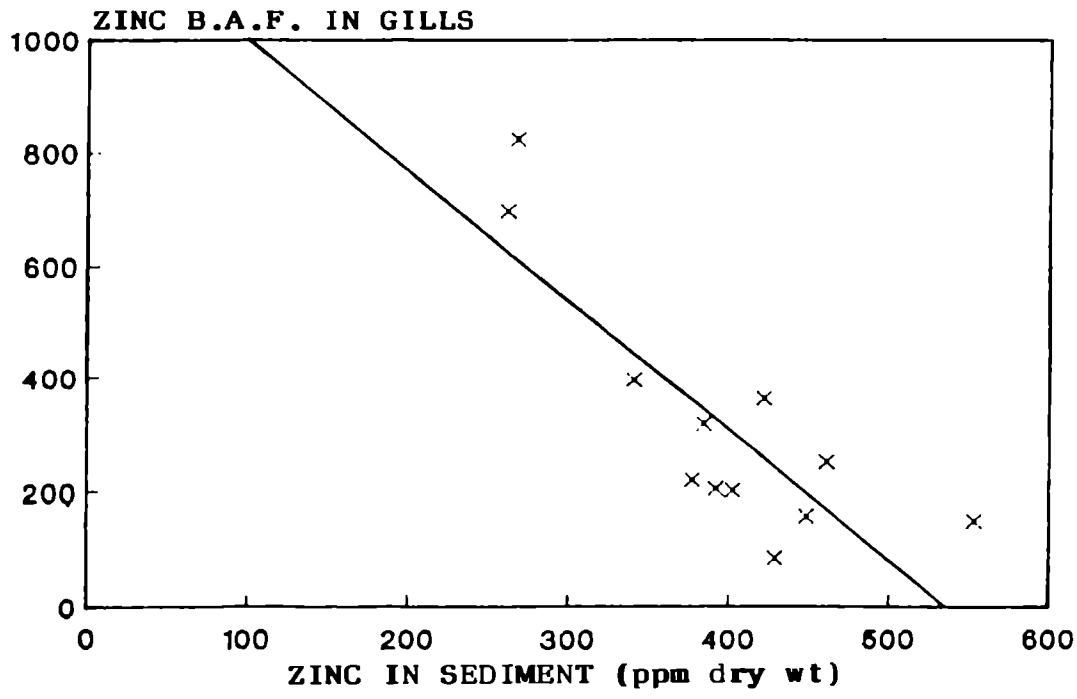


FIG. 8. Relationship between zinc in Sediment and Bioaccumulation Factor(B.A.F.) of zinc in Gills of L. parsia.

Ovary	NS	r = 0.234	r = 0.135	r = 0.742**
Skin	5% F-Value; PrM 886.75 PtM 517.78 M 371.38	r = 0.735**	r = 0.722** (Fig. 9)	r = 0.609*
Muscle	NS	r = 0.443	r = 0.18	r = 0.806**
Kidney	NS	r = 0.440	r = 0.173	r = 0.725**

* = 5% level, ** = 1% level and NS = Nonsignificant.

The results obtained for liver, kidney, intestine, ovary and muscle were seen similar to that obtained in other centres.

Discussion

As discussed in "Chapter 1, Section: Bioaccumulation Factor of zinc" the ovary and muscle recorded the highest and lowest factors respectively in the present study. The Bioconcentration Factor on other tissues were found in between gonad and muscle. As discussed in Chapter 1, the factors for tissues/organs of L. parsia were seen lower than that of algae and molluscs. The seasonal differences were seen for Bioaccumulation Factor in skin, intestine and gills. The summer seasons recorded higher values than the monsoon season. Similar observations on zinc were also seen for the same tissues in Cochin Backwater as observed in "Zinc in tissue". As also seen in Chapter 1, the good correlations were obtained for Bioaccumulation Factor with the available concentrations of zinc in water and tissue, but not in sediment (except gills). The linearity of zinc content with Bioaccumulation Factor is explained in "Chapter 2, Section: Bioaccumulation Factor" in copper.

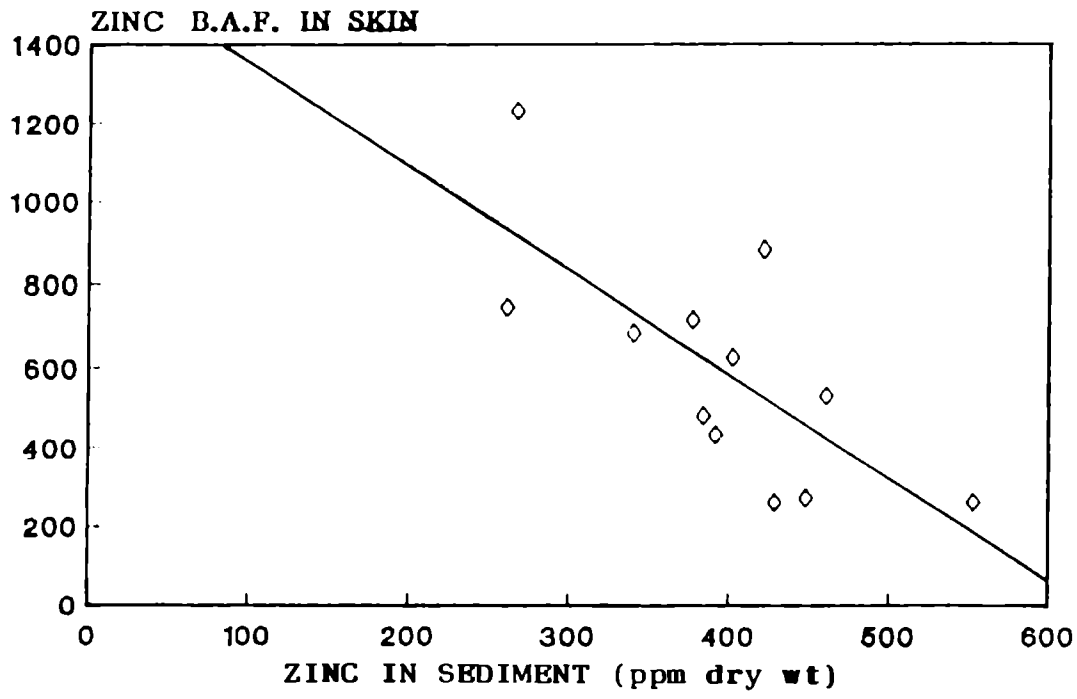


Fig. 9. Relationship between zinc in Sediment and Bioaccumulation Factor (B.A.F) of zinc in Skin of L. parsia.

Section 4: Lead

At a glance

Parameter	Average value obtained (range in parentheses)		Variation (%)
	Dduring 2 years period	Monthwise in 1991-92	
Water (ppb)	6.26 (ND - 60.5)	9.17 (ND - 30.5)	46.5
Sediment (ppm dry wt)	50.91 (11.5 - 158.5)	50.81 (6.0 - 208.0)	- 0.2
Lead in tissue (ppm dry wt)			
Liver	0.87 (ND - 5.21)	1.42 (ND - 8.31)	63.2
Gills	5.08 (ND - 13.2)	5.15 (ND - 13.2)	1.4
Kidney	2.42 (ND - 14.54)	0.74 (ND - 4.26)	-69.4
Intestine	0.69 (ND - 4.11)	1.05 (ND - 4.11)	52.2
Ovary	0.82 (ND - 3.42)	3.38 (ND - 16.95)	312.2
Skin	1.31 (ND - 6.77)	1.33 (ND - 5.76)	1.5
Muscle	0.10 (ND - 0.57)	0.93 (ND - 3.16)	830.0
Bioaccumulation Factor			
Liver	42.1 (ND - 252.7)	54.2 (ND - 260.8)	28.7
Gills	18.55 (ND - 450.0)	139.3 (ND - 450.0)	-24.9
Kidney	50.9	25.2	-49.5

Kidney	50.9 (ND - 305.4)	25.2 (ND - 139.5)	-49.5
Intestine	32.9 (ND - 197.5)	37.8 (ND - 197.5)	14.9
Ovary	23.5 (ND - 76.6)	54.3 (ND - 184.7)	131.1
Skin	37.7 (ND - 157.9)	48.4 (ND - 237.9)	28.4
Muscle	1.5 (ND - 9.17)	18.2 (ND - 101.3)	1113.3

ND = Not detected.

Lead in water

As observed

The monthly fluctuation of lead in Cochin backwater from June 1991 to 1992 as observed, is presented in Table 84.

As analysed

Abstract of Table 58 and 84 is given below for comparison (range is in parentheses):

Months	Mean in (ppb)		Average in seasons during		Variation (%)
			1991-92	2 years period	
June	2.17 (ND - -5.5)	Monsoon	12.5 (ND-30.5)	1.96 (ND-5.5)	-84.3
*July	155.75 (6.5 - 290.0)				

TABLE 84. **Lead in water (ppb) and sediment (ppm) of Cochin Backwater from June 1991 to May 1992**

	Stations		Monsoon				Postmonsoon				Premonsoon			
			June	July *	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	
Water	II 4	Surface	ND	177.0	17.0	14.0	8.5	4.0	6.5	11.5	6.0	3.0	13.5	26.0
		Bottom	1.0	130.0	13.0	16.5	6.0	4.5	8.0	8.0	9.5	5.5	6.0	7.5
	II 5	Surface	1.5	290.0	17.0	10.0	12.0	5.5	18.5	11.0	6.5	3.0	11.0	5.5
		Bottom	5.5	216.5	40.0	8.5	6.5	5.0	6.5	6.0	8.5	7.5	5.	3.0
	II 6	Surface	1.5	6.5	17.5	21.0	18.5	7.0	12.5	8.5	3.5	18.0	7.5	7.0
		Bottom	3.5	111.5	30.5	7.0	6.5	7.0	6.0	3.5	6.0	4.5	7.0	2.0
	Mean	Surface	1.00	157.83	17.17	15.00	13.00	5.60	12.50	10.33	5.33	8.00	10.67	12.83
		Bottom	3.33	152.67	27.83	10.67	6.33	5.50	6.83	5.83	8.00	5.83	6.17	4.17
	Grand Mean with SD		2.17± 1.99	155.25± 97.02	22.50± 10.45	12.83± 5.34	9.67± 4.86	5.50± 1.26	9.67± 4.95	8.08± 3.02	6.67± 2.11	6.92± 5.69	8.42± 3.15	8.50± 8.84
	Range		(ND- 5.5)	(6.5 290.0)	(13.0- 30.5)	(7.0- 21.0)	(6.0- 18.5)	(4.0- 7.0)	(6.0- 18.5)	(3.5- 11.5)	(3.5- 9.5)	(3.0- 18.0)	(5.5- 13.5)	(2.0- 26.0)
Sediment	II 4		139.85	76.90	67.50	59.75	86.50	66.00	127.00	124.25	128.75	175.75	56.00	108.75
	II 5		58.00	36.50	43.75	29.75	30.50	27.25	23.50	18.00	10.45	25.75	34.50	53.25
	II 6		23.75	33.25	10.75	20.00	20.00	13.25	7.25	17.75	16.25	10.50	22.75	25.50
	Mean with SD		73.86± 54.71	48.88± 21.84	40.67± 25.59	36.50± 18.55	47.33± 30.72	35.50± 24.57	52.58± 58.52	53.33± 54.95	53.48± 59.63	70.67± 84.20	37.75± 15.96	62.50± 38.06
	Range		(23.5 158.5)	(32.5- 80.0)	(10.0- 70.5)	(20.0- 61.0)	(18.5- 86.5)	(12.0- 69.5)	(6.0- 138.0)	(17.0- 126.5)	(14.4 148.5)	(10.5- 208.0)	(22.5- 64.0)	(22.5- 112.5)

SD = Standard deviation, * = Not used for calculation of mean, range and SD in Cochin backwater.
In water between seasons ($P < 0.01$), between months ($P < 0.001$) and mean with SD and range 9.17 ± 7.0 (ND - 30.5).
In sediment between stations ($P < 0.001$), between months ($P < 0.05$) and mean with SD and range 50.81 ± 43.49 (6.0 - 208.0).

August	22.59 (13.0-30.5)				
September	12.83 (7.0-21.0)				
October	9.67 (6.0-18.5)				
November	5.50 (4.0-7.0)				
December	9.67 (6.0-18.5)	Post-monsoon	8.23 (3.5-18.5)	10.29 (4.0-60.5)	25.0
January	8.08 (3.5-11.5)				
February	6.67 (3.5-9.5)				
March	6.92 (3.0-18.0)				
April	8.50 (5.5-13.5)	Pre-monsoon	7.63 (2.0-26.0)	6.55 (3.0-11.5)	-14.2
May	8.50 (2.0-36.0)				

*Not used for calculation, ND = Not detected.

The seasonal values pooled from the monthly ones were used for statistical analysis. From one-way ANOVA the significant difference at 1% level was seen between the seasonal collections and at 0.1% between the months. The monsoon showed the higher concentrations than the postmonsoon and premonsoon.

From correlation study nonsignificant and weak coefficients (0.178, 0.042, and 0.214 for premonsoon, monsoon and postmonsoon respectively) were obtained for lead in water compared to salinity. The relationships found significant (at 1% level in premonsoon and, at 5% level in monsoon and postmonsoon) with total hardness, but were weak (0.323, 0.245 and 0.245 for premonsoon, monsoon and postmonsoon respectively).

Discussion

The Environmental Protection Agency (EPA) has recommended 100 ppb as "safe level" of lead (Anon., 1991 a). The same value was also recommended by Lohani (1981) as the critical upper limit of lead. In the present study the mean value obtained from 12 months of collection from June 1991 to May 1992 in Cochin Backwater was 9.17 ± 7.0 (ND - 30.5) ppb. As the values were exhorbitantly high in all the stations i.e. II 4, II 5 and II 6 in July 1991 theywere not taken for statistical analysis i.e. for finding the mean value. In comparative study, for the same centre (Chapter 1, Section : Lead in water) the value for lead obtained was 6.26 ± 9.60 (ND - 60.5) ppb. The higher limits (by adding standard deviation values to respective means) obtained in both reports for the same centre were similar.

In the present investigation as discussed in "Chapter 1, Section : Lead in water" the bottom and surface waters did not differ significantly and also no good relation was seen for lead with salinity and lead with total hardness. But monsoon recorded higher lead in water than other seasons. According to Riley and Chester (1971) the trace metal distribution

in the coastal environment is, to a great extent, influenced by freshwater inflow. The Cochin Backwater generally receives a higher run-off in monsoon from its upper and surrounding catchment areas along with the domestic and industrial wash outs. Rebellow and Arras (1983) attributed the higher lead content in Guanabara Bay (Rio de Janeiro) to the untreated domestic and industrial sewage from a densely populated and industrialised area. The same may be the reason for the increase of lead in Cochin backwater also. Waldron and Stofen (1974) reported that the river run-off alone contributes the major share of lead to the oceans in each year. The tetraethyl lead which is used as an antiknock for gasoline in motor vehicles finds the way to air medium after combustion and also found more in the air above the city where number of motor vehicles are more (Laws, 1981). The same might have been washed from air medium to the aquatic medium by rain water causing the increase of lead in water. According to Mart and Nurnberg (1986) the enhanced level of lead in water are due to substantial churning of particulate matter in the medium. The same reason also can be linked to the increase of lead in monsoon as all the particulate substances and mud found in suspension.

Lead in sediment

As observed

The stationwise monthly values of lead in sediments of Cochin Backwater is given in Table 84.

As analysed

Abstract of Table58 and 84 is given below for comparison (range in parentheses):

Months	Mean in ppm		Average in seasons during 1991-92	Average in seasons during 2 years period	Variation (%)
June	73.86 (23.5-158.5)	Monsoon	50.00 (10.0-158.5)	66.18 (16.0-158.5)	32.4
July	48.88 (32.5-80.0)				
August	40.67 (10.0-70.5)				
September	36.50 (20.0-61.0)				
October	47.33 (18.5-86.5)	Post-Monsoon	47.19 (6.0-138.0)	34.59 (11.5-69.5)	26.7
November	35.50 (12.0-69.5)				
December	52.58 (6.0-138.0)				
January	53.33 (17.0-126.5)				
February	53.48 (14.4-148.5)	Pre - monsoon	56.10 (10.5-208.0)	51.98 (11.5-150.0)	- 7.3
March	70.67 (10.5-208.0)				
April	37.75 (22.5-64.0)				
May	62.50 (22.5-112.5)				

No significant differences were seen between the seasons. The stations differed among themselves at 0.1% F-value and months at 5% F-value. The St. II 4 recorded higher concentration of lead (mean 101.43 ppm) followed by St. II 5 (mean 32.60) and St. II 6 (mean 18.42).

From the correlation study lead in water with sediment, a nonsignificant week coefficient (0.112) was found between them as also observed in Chapter 1 at other centres.

Discussion

The monthly mean value 50.81 ppm dry wt of lead was obtained in sediments of Cochin Backwater and ranging between 6.0-208.0 with the standard deviation 43.49 (Table 84). The monthly collections of one year from June 1991 to May 1992 in Cochin Backwater was seen similar to that obtained in the same environment in Chapter 1. As discussed in "Chapter 1, Section : Lead in sediment" the lead in the sediments of Cochin Backwater was found above the "G-2", "MESS-1" and "BCSS-1" standards. Similarly the St. II 4 showed higher concentration of lead in sediment followed by St. II 5 and St. II 6. The reasons and discussions given in Chapter 1 stands good here also. Balls (1985) reported the seaward attenuation in total lead concentration and suggested that the lead is transported only a short distance from the coast/source. This restricted dispersal of lead to very short distance from its place of entry, is most probably due to its heaviness. Similarly in the present study the respective stations might have received the lead from nearby catchment area showing the individuality among themselves. Mohanachandran and Subramanian

(1990) from Ashtamudi Lake, Kerala have reported the decrease of lead towards the coast from the land as observed in the present study. But based on the one year data (12 collections per station) no statistically significant seasonal differences were found out. This indicated the continuous supply of lead from various sources to the environment from June 91 to May 92.

Lead in tissues

As observed

The lead content in different tissues in different months at Cochin Backwater was studied (Table 85).

As analysed

Data over months for specific tissues, the grand mean with standard deviation found in decreasing order are:

Tissue	Value (ppm dry wt)		
	Mean	SD	Range
Gills	5.15	3.77	ND - 13.20
Ovary	3.38	5.14	ND - 16.95
Liver	1.42	2.77	ND - 8.31
Skin	1.33	2.17	ND - 5.76
Intestine	1.05	1.58	ND - 4.11
Muscle	0.93	1.27	ND - 3.16
Kidney	0.74	1.34	ND - 4.26

TABLE 85. Lead (ppm dry wt) in different tissues of L. parsia in Cochin Backwater from June 1991 to May 1992

	Monsoon				Postmonsoon				Premonsoon			
Tissues	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May
Skin	ND	1.97 (0.068)	ND	ND	5.76 (0.083)	1.07 (0.099)	ND	ND	1.36 (0.022)	ND	ND	5.75 (0.094)
Liver	ND	ND	ND	ND	ND	5.21 (0.061)	ND	ND	ND	3.55 (0.087)	ND	8.31 (0.082)
Kidney	ND	ND	ND	ND	4.26 (0.031)	ND	2.36 (0.081)	ND	ND	ND	0.89 (0.012)	1.41 (0.028)
Gills	4.09 (0.051)	6.18 (0.062)	2.02 (0.058)	ND	3.26 (0.034)	13.20 (0.044)	5.76 (0.098)	2.86 (0.052)	9.27 (0.054)	1.41 (0.053)	5.00 (0.076)	8.70 (0.063)
Intestine	ND	ND	ND	ND	2.89 (0.048)	4.11 (0.350)	ND	2.66 (0.087)	ND	ND	ND	2.89 (0.770)
Muscle	ND	3.16 (0.064)	1.60 (0.042)	ND	0.98 (0.022)	ND	ND	ND	ND	2.89 (0.057)	ND	2.48 (0.034)
Ovary	ND	16.95 (0.096)	8.72 (0.084)	ND	4.62 (0.081)	1.49 (0.062)	ND	3.26 (0.098)	ND	ND	5.50 (0.082)	ND

SD = Standard deviation and coefficient of variation is in parentheses.

In intestine between seasons ($P < 0.1$).

The correlation coefficients (r), level of significance with grand mean values (ppm dry wt) of lead in different tissues of L. parsia in premonsoon (PrM), monsoon (M) and postmonsoon (PtM) are presented below:

Tissue	Significance between seasons at	Correlations With water	With sediment
Liver	NS	r = 0.185	r = 0.210
Gills	NS	r = 0.037	r = 0.117
Kidney	NS	r = 0.170	r = 0.044
Intestine	10% F-value; PtM 2.42 PrM 0.73 M ND	r = 0.2323	r = 0.161
Ovary	NS	r = 0.869 **	r = 0.324
Skin	NS	r = -0.084 **	r = 0.085
Muscle	NS	r = 0.869	r = 0.324

** 1% level, ND = Not detected and NS = Nonsignificant.

The results obtained for liver, gills, kidney, ovary, skin and muscle corroborates the results obtained in Chapter 1.

Discussion

In the present study for statistical analysis the "Not detected" (ND) limit was treated as zero. Thus the standard deviation was found more in the case of most tissues. The lead content in different tissues

has been discussed in "Chapter 1, Section : Lead in tissue". As explained in Chapter 1, the gills contained more lead than other tissues in L. parsia. As seen in the present study Szefer et al. (1990) reported the least amount of lead in kidney of Gadus morhua. As the lead is non-essential for animal, its distribution over tissues is difficult to discuss.

From correlations study, "good relationships" were found for lead in ovary and muscle with that of water. For other tissues with water and for all tissues with sediment "no relationships" were obtained. No seasonal fluctuation was seen for none of the tissues in L. parsia. It is discussed in earlier chapter.

In the present investigation all the tissues (except gills) contained more zinc followed by copper and lead. For gills the content was found more for zinc followed by lead and copper. It is discussed with supporting evidences of other species in Chapter 1.

Bioaccumulation Factor

As observed and analysed (Table 86)

The Bioaccumulation Factor of lead in different tissues of L. parsia in Cochin Backwater from June 1991 to May 1992 shows the grand means with standard deviation and range for each tissue as follows:

TABLE 86. **Bioaccumulation Factor of lead in different tissues of L. parsia in Cochin Backwater from June 1991 to May 1992**

Tissue	Monsoon				Postmonson				Premonsoon			
	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	April	May
Skin	ND	4.46	ND	ND	198.6	68.4	ND	ND	71.7	ND	NF	237.9
Liver	ND	ND	ND	ND	ND	262.7	ND	ND	ND	136.9	ND	260.8
Kidney	ND	ND	ND	ND	139.5	ND	77.4	ND	ND	ND	33.5	52.5
Gills	353.4	7.5	16.8	ND	63.2	450.0	11.7	66.4	260.6	38.2	111.3	191.9
Intestine	ND	ND	ND	ND	79.0	197.5	ND	87.0	ND	ND	ND	89.9
Muscle	ND	4.9	17.3	ND	24.6	ND	ND	ND	ND	101.3	ND	70.8
Ovary	ND	31.5	109.6	ND	135.1	76.6	ND	ND	ND	ND	184.7	ND

SD = Standard deviation.

In intestine between seasons ($P < 0.1$).

Tissue	Value		
	Mean	SD	Range
Gills	139.3	146.0	ND - 450.0
Ovary	54.3	66.7	ND - 184.7
Liver	54.2	102.4	ND - 260.8
Skin	48.4	84.1	ND - 237.9
Intestine	37.8	63.1	ND - 197.5
Kidney	25.2	44.4	ND - 139.5
Muscle	18.2	33.3	ND - 70.8

ND = Not detected.

The correlation coefficients (r), level of significance with grand mean values of lead "Bioaccumulation Factor" in L. parsia in premonsoon (PrM), monsoon (M) and postmonsoon (PtM) (June 1991 to May 1992) are presented below:

Tissue	Significance between seasons at	Correlations with		
		Water	Sediment	lead content in tissue
Liver	NS	r=0.195	r=0.119	r=0.975**
Gills	NS	r=0.146	r=0.353	r=0.761**
Kidney	NS	r=0.176	r=0.041	r=0.998**
Intestine	10% F-value; PtM 09.88 PrM 22.48 M ND	r=0.216	r=0.243	r=0.963**
Ovary	NS	r=0.075	r=0.545*	r=0.375

Muscle	NS	r=0.176	r=0.090	r=0.955**
Skin	NS	r=0.127	r=0.534	r=0.208

* = 5% level, ** = 1% level and NS = Nonsignificant

The results obtained for all the tissues were seen similar to that obtained in all other centres as given in Chapter 1.

Discussion

The reports regarding the "Bioaccumulation Factor" of lead in various species have already been discussed in "Chapter 1, Section : Bio-accumulation Factors" for lead. As seen in other centres (Chapter 1), here in Cochin Backwater also the factor did not vary during different seasons. the factor also showed "positive relationship" with lead in water and tissues of L. parsia.

CHAPTER 3

CHAPTER 3

BIOASSAY AND CHRONIC EXPOSURE STUDIES ON L. PARSIA WITH HEAVY METALS COPPER, ZINC AND LEAD

Introduction

India is a developing country and it has tremendously advanced towards the industrial developments which results to certain extent, in polluting the environments, both atmosphere and hydrosphere, the latter has been blessed by Mother Nature providing 7000 km length of coastal belt with full of marine wealth.

In view of the above, an attempt has been made in earlier two chapters to study the extent of heavy metal pollution of six estuaries along the east and west coast of India. Most of these estuaries are heavily surrounded and clustered with industries as stated in Table 1 earlier. To substantiate the actual findings from the environments studied in Chapter 1, the candidate attempted in Chapter 2 by regular monitoring and observing for a period of 12 months continuously at Cochin Backwater which gave good findings supporting the observations made at other five centres. In addition to the above field observations, an attempt was made in the laboratory exposing the test animal L. parsia to media with different concentration of copper, zinc and lead for bioassay and accumulating capabilities of the animal of the heavy metal and its tolerance limit of the heavy metals. This has given very interesting, significant and important findings through the experiment. The results are enumerated and discussed in this chapter in following paragraphs.

This is essential to fully understand the effects of heavy metals on our coastal exploitable fishery resources as also cultivable fishes and prawns for better management and formulation of policies.

Heavy metals (Mn, Ni, Cr, Zn, As, Cd, Pb, Fe, Cu) and their salts constitute the widely distributed group of highly toxic and long-retained substances (Metelev et al., 1983). To obtain a measure of acute toxicity the standard bioassays are normally conducted for 48-hr or 96-hr period (Waldichuk, 1974). But, most of heavy metals produce a toxic effect on fish through constant accumulation (Metelev et al., 1983). The toxicity of heavy metals which is stored in the body of the organisms, is not such that an LC50 with peak bioaccumulation can be clearly obtained in a short period viz. 96 hrs. Because of the uptake of metal by the organism being tested, the toxicity continues over a prolonged period and there is no so-called toxic threshold. Thus, for practical purposes it is necessary to conduct long-term experiments for a month or longer to obtain a better understanding and reliable results on toxicity level in the animal in the laboratory (Waldichuk, 1974; Reish and Oshida, 1987).

The present study aimed to find out the acute toxicities of copper, zinc, lead and their combination on L. parsia through bioassays and their bioaccumulations with "Bioaccumulation Factors" in different tissues in chronic experiments for 21 days. One may wonder why the candidate has concluded 21 days experiment while he has suggested earlier to carry out experiments for about a month. This bioassay studies and experiments

have yielded required and comparable results in 21 days in the laboratory to compare with that of the results obtained from the field.

For the tests, the L. parsia, an economically important backishwater fish and a native of the study area was selected for the experiments. According to Buikema et al. (1982), fish has been the most popular test organism, because they are presumed to be the best understood organism in the aquatic environment and perceived as most valuable by the majority of the scientists.

Range finding bioassay

The range finding bioassay of the heavy metals and its combinations for the main experiments with the animal L. parsia was conducted as per the guide lines given by APHA-AWWA-WPCF (1976) and Reish and Oshida (1987). It has been already discussed under "Materials and Methods".

Acute toxicity test (Bioassay)

The acute toxicity test "the bioassay" was conducted by placing 10 L. parsia of size from 7.5 to 10.5 cm in each different concentrations of metals. The control tests were typically conducted in "dilution water" of $10\%_0$ from the collection site and freshwater with no addition of heavy metals. As a rule of thumb, a toxicity test is valid if control mortality is less than 10% (Buikema et al., 1982). The objective of the toxicity test was to define the concentrations of heavy metals at which level they initiate some "selected" response, usually "deleterious" or "Quantal response" in a population in experimental conditions of exposure. As suggested by Ward and Parish (1982) the "Quantal response" i.e. death,

was taken as the unit for the measurement of the response from which the relation between concentration and percentage effect could be defined. "Quantal data" allows statistical definition of the position and slope of the concentration-mortality curve i.e. "Response curve" for each selected period of exposure (Ward and Parish, 1982). Raw data from the bioassay experiments were plotted as the percentage of deaths verses the exposure concentration in log at each observation period to obtain the "Response curve" on Probit paper (Reish and Oshida, 1987; Mohapatra, 1989). A dose-response curve on "Coordinate paper" typically shows that the percentage mortality rapidly increases and then levels out (asymptotic) as the concentration increases (Buikema et al., 1982). But, by transforming the response data to a probit scale, the dose-response curve becomes a straight line as observed in the present study. Like any line, the "dose-response line" can be described with two factors: an "Intercept" in the case of acute toxicity data, i.e. the LC50 and the "Slope" (Buikema et al., 1982). In the present study the "Probit analysis" was done to get the LC50 values at different exposures to different heavy metals and combinations. Often, the fiducial limits for the LC50 were calculated and reported. "Fiducial limits" mean a 19 of 20 chance i.e. LC50 value falls within the specified limits (P = 0.05). Fiducial limits and "Confidence limits" are different even though many times the limits derived are similar(Buikema et al., 1982). The LC50 with fiducial limits provides information about the median lethal concentrations. The estimate of the slope with fiducial limits provides information about the standard deviation of the log tolerance distribution (Buikema et al., 1982). The information on LC50s with fiducial limits permitted the construction of a "Toxicity curve" for each heavy metals and combinations.

"Long-term "or chronic toxicity tests are most commonly designed to provide informations on the effect of various concentrations of toxicant on the survival, growth and reproductive success of an organism (Buikema et al., 1982). The tendency to be bioaccumulated, is perhaps one of the most important biological properties of metals (Waldichuk, 1974). The bioaccumulation is a continuous process over time and a balance between the uptake and excretion by the animal. This present investigation aimed to estimate the accumulation of metals in tissues of L. parsia in anticipation to obtain the maximum limit for the same in a tissue. For carrying out the long-term experiments, the 96 hr LC50 of copper sulphate : zinc sulphate : lead nitrate :: 1:1:1 was devided into two fractions such as 0.1 and 0.01. The experiments were conducted for 21 days as described in "Materials and Methods". The data obtained from the experiments were processed applying two-way ANOVA in a computer (Snedecor and Cochran, 1967). This was done to obtain the level of significance between the treatments and exposure periods. The correlation study was carried out to find out the relationship between the " Bioaccumulation" and the "Bioaccumulation Factor". The present study was also aimed to find out the specific tissue which accumulates the specific metal and to study its similarity with that of results obtained in different environment i.e. Chapter 1 and 2. Based on bioaccumulation of heavy metals, this investigation also aimed to find out whether 0.01 fraction of 96 hr LC50 can be suggested as the "Safe level" for the environments.

Section 1: Acute toxicity studies (Bioassay)

Copper sulphate

The mortality rate (in percentage) of L. parsia in different concentrations of copper sulphate such as 56, 76, 100, 135 and 180 ppm and in different exposure periods of 12, 24, 48, 72 and 96 hours is given in Table 87. The results of bioassay expressed in terms of LC50 values for 12, 24, 48, 72 and 96 hours obtained in Probit analysis on Probit papers (Fig. 10) are given in Table 88. The LC50 values were found:

Hours	ppm
12	141.2
24	137.0
48	109.9
72	86.5
96	85.6

showing gradual decrease in concentration with increase of time. The 95% "fiducial limits", LC16, LC84, slope function and 95% confidence limit of each response curve for different exposure periods in hours are also given in Table 88. The 12, 24, 48, 72 and 96 hours LC50 values with its 95% fiducial limits were plotted on log-log paper to get the "toxicity curve" (Fig. 14). All the results were obtained at salinity 9.8 ± 0.76‰, temperature 27.5 ± 1.5°C, pH 7.19 ± 0.12 and total hardness 2956.0 ± 142.2 ppm.

TABLE 87. **Mortality rate of L. parsia in different concentrations of copper sulphate during acute toxicity studies**
(Animals exposed in each concentration are 10)

Exposure period (hrs)	Concentration (ppm)									
	56		75		100		155		180	
	Nos	%	Nos	%	Nos	%	Nos	%	Nos	%
12	-	-	-	10	3	30	4	40	8	80
24	2	20	2	20	3	30	6	60	10	100
48	2	20	3	30	4	40	7	70	10	100
72	3	30	4	40	5	50	8	80	10	100
96	3	30	4	40	6	60	8	80	10	100

TABLE 88. **Acute toxicity of copper sulphae on L. parsia**

Exposure period (hrs)	LC50 (ppm)	95% Fiducial limits (ppm)		LC16 (ppm)	LC84 (ppm)	Slope fun-ction	95% confi-dence limit
		Upper	Lower				
12	141.2	185.6	107.4	82.3	242.7	1.718	1.315
24	137.0	199.7	94.0	59.1	330.3	2.365	1.458
48	109.9	150.3	80.4	53.0	221.0	2.044	1.368
72	86.5	118.6	63.1	42.5	179.5	2.055	1.371
96	85.6	116.6	62.8	42.9	175.9	2.025	1.362

Conversion of log value (ppm) to observed value (ppm)

Hours	LC	Log value	Observed value
12	LC84	5.492	242.7
	LC50	4.950	141.2
	LC16	4.410	82.3
24	LC84	5.800	330.3
	LC50	4.920	137.0
	LC16	4.080	59.1
48	LC84	5.400	221.0
	LC50	4.700	109.9
	LC16	3.970	53.0
72	LC84	5.190	179.5
	LC50	4.460	86.5
	LC16	3.750	42.5
96	LC84	5.170	175.9
	LC50	4.450	85.6
	LC16	3.760	42.9

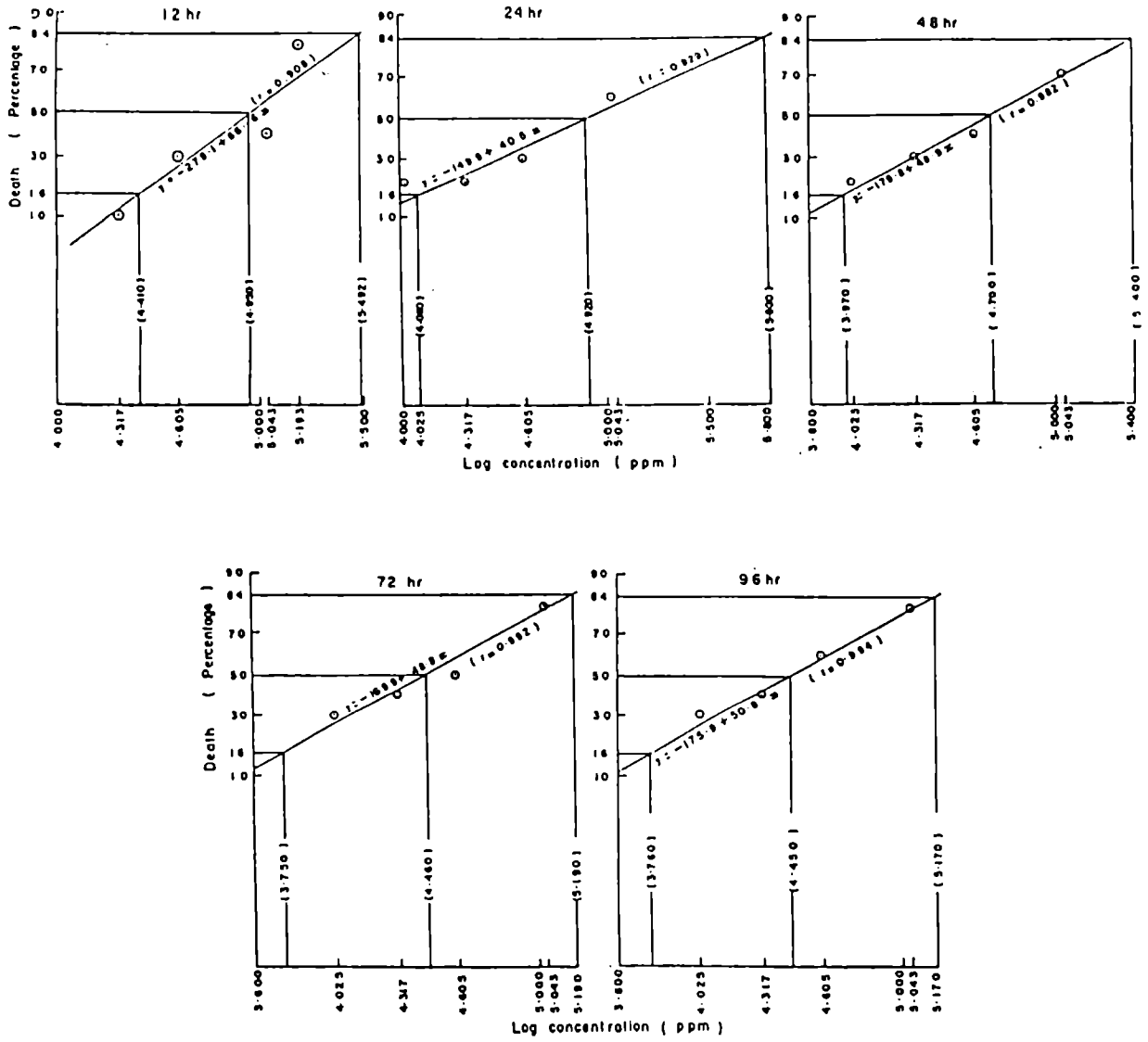


FIG. 10. Response curve of copper sulphate at 12, 24, 48, 72 and 96 hours

Zinc sulphate

The mortality rate of L. parsia at different hours in different concentrations such as 56, 75, 100, 155 and 180 ppm of zinc sulphate is given in Table 89. The results of bioassay expressed in terms of LC50 values for 12, 24, 48, 72 and 96 hours obtained in Profit analysis on Profit papers (Fig. 11) along with 95% fiducial limits, LC16,LC84, slope function and 95% confidence limit are given in Table 90. The LC50 were found to be:

Hours	ppm
12	180.0
24	127.7
48	98.5
72	75.2
96	60.3

The LC50 values for different exposure periods along with its 95% fiducial limits were plotted on log-log paper to get the toxicity curve (Fig. 14). All the experiments were conducted in the similar laboratory conditions as mentioned for $CuSO_4 5H_2 0$.

Lead nitrate

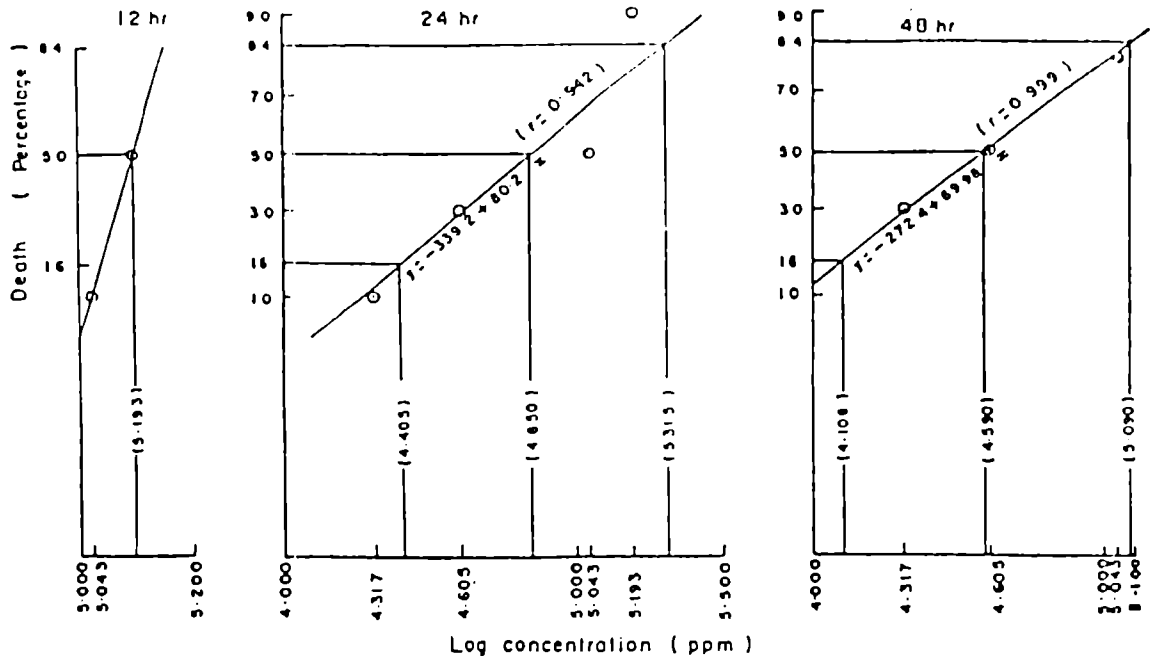
Table 91 presents the mortality rate of L. parsia in different concentrations of lead nitrare such as 75,100, 155, 180 and 210 ppm and exposure periods such as 12, 24, 48, 72 and 96 hours. The results obtained in terms of LC50 values for different periods such as 12, 24,48, 72 and 96 hours from Probit analysis (Fig. 12) are given in Table 91. The LC50

TABLE 89. **Mortality rate of L. parsia in different concentrations of zinc sulphate during acute toxicity studies**
(Animals exposed in each concentration are 10)

Exposure period (hrs)	Concentration (ppm)									
	56		75		100		155		180	
	Nos	%	Nos	%	Nos	%	Nos	%	Nos	%
12	-	-	-	-	-	-	1	10	5	50
24	-	-	1	10	3	30	5	50	9	90
48	-	-	3	30	5	50	8	80	10	100
72	1	10	6	60	8	80	10	100	10	100
96	1	10	7	70	8	80	10	100	10	100

TABLE 90. **Acute toxicity of zinc sulphate on L. parsia**

Exposure period (hrs)	LC50 (ppm)	95% Fiducial limits (ppm)		LC16 (ppm)	LC84 (ppm)	Slope function	95% confidence limit
		Upper	Lower				
12	180.0	-	-	-	-	-	-
24	127.7	169.3	96.3	81.9	203.4	1.576	1.325
48	98.5	126.3	76.8	60.8	162.4	1.634	1.282
72	75.2	90.7	62.4	55.7	102.0	1.353	1.206
96	60.3	72.6	50.1	55.7	97.5	2.350	1.204



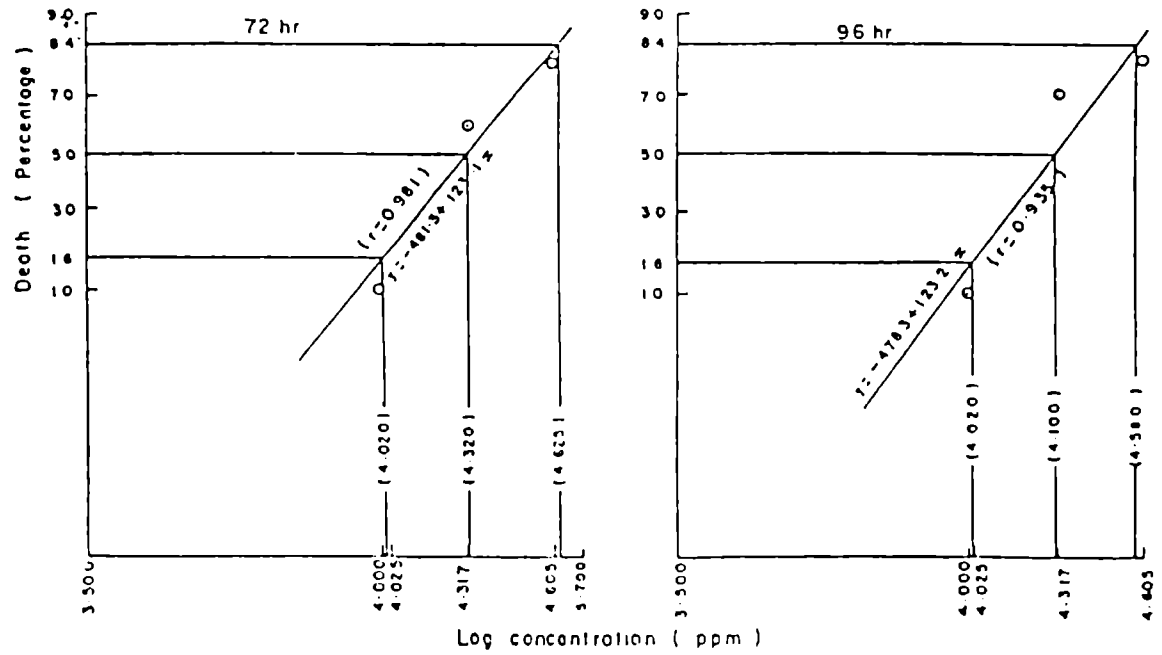


FIG. 11, Response curve of zinc sulfate at 12, 24, 48, 72 and 96 hours

TABLE 91. **Mortality rate of L. parsia in different concentrations of lead nitrate during acute toxicity studies**
(Animals exposed in each concentration are 10)

Exposure period (hrs)	Concentration (ppm)									
	56		75		100		155		180	
	Nos	%	Nos	%	Nos	%	Nos	%	Nos	%
12	-	-	-	-	1	10	2	20	5	50
24	-	-	1	10	3	30	5	50	8	80
48	1	10	2	20	8	80	9	90	10	100
72	2	20	3	30	8	80	9	90	10	100
96	2	20	4	40	9	90	10	100	10	100

TABLE 92. **Acute toxicity of lead nitrate on L. parsia**

Exposure period (hrs)	LC50 (ppm)	95% Fiducial limits (ppm)		LC16 (ppm)	LC84 (ppm)	Slope function	95% confidence limit
		Upper	Lower				
12	217.0	255.3	184.5	163.7	282.9	1.300	1.176
24	170.7	211.5	137.8	112.4	262.4	1.528	1.239
48	121.5	153.8	96.0	83.9	179.5	1.462	1.266
72	112.4	138.5	91.2	75.2	171.6	1.511	1.232
96	103.5	126.9	84.4	73.4	142.3	1.390	1.226

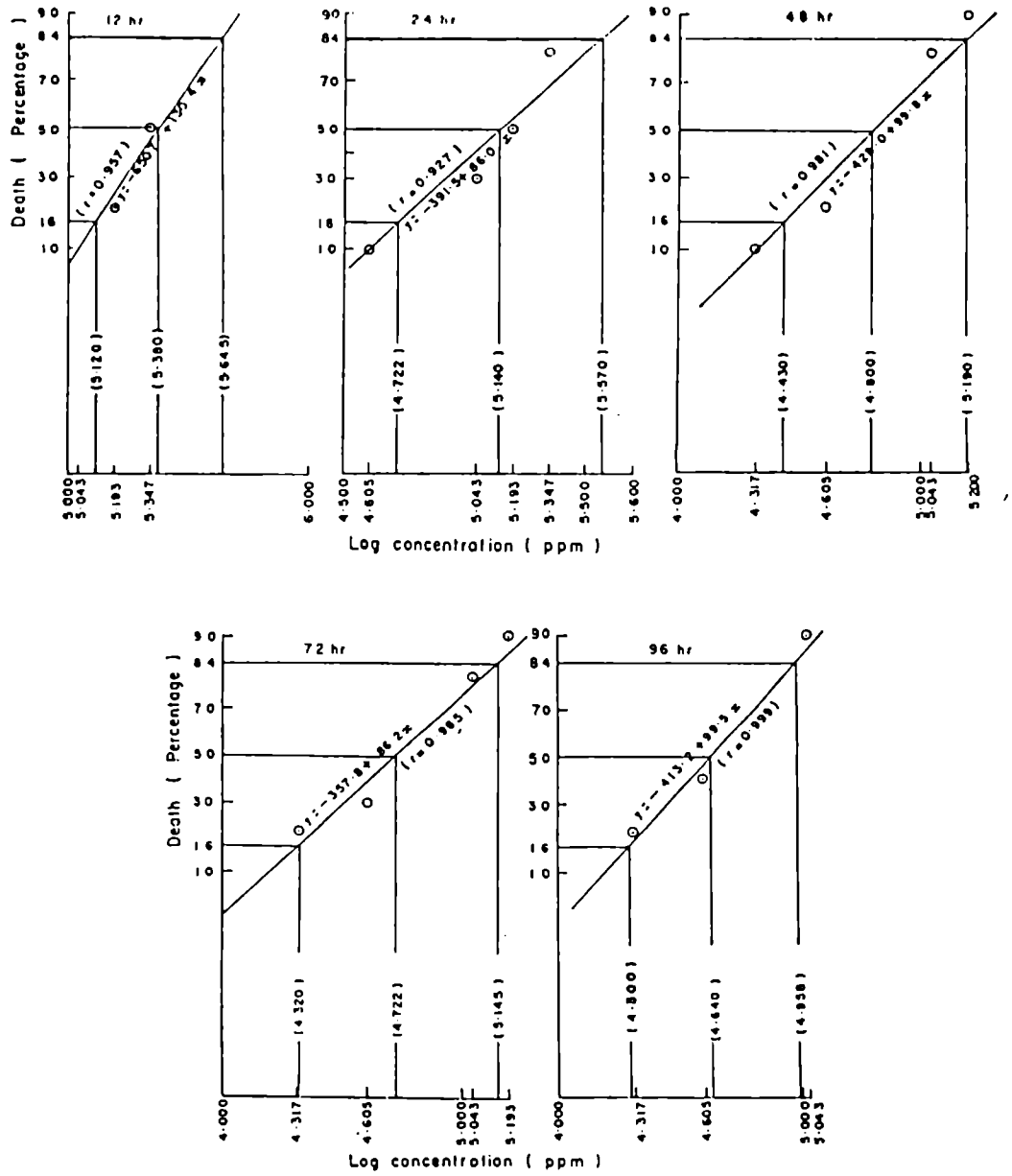


FIG. 12. Response curve of lead nitrate at 12, 24, 48, 72 and 96 hours

values were found:

Hours	ppm
12	217.0
24	170.7
48	121.5
72	112.4
96	103.5

showing the gradual decrease in concentration with increase of time. The 95% fiducial limits, LC16, LC84, slope function and 95% confidence limit of each response curve for different exposure periods in hours are also given in Table 92. The 12, 24, 48, 72 and 96 hours LC50 values with its 95% fiducial limits were plotted on log-log paper to get the toxicity curve (Fig. 14).

Combined Toxicant - $CuSO_4 5H_2O$: $ZnSO_4 7H_2O$: $Pb(NO_3)_2$

The mortality rate in percentage of L. parsia exposed to different concentrations such as 75, 100, 115, 135 and 210 ppm of copper sulphate, zinc sulphate and lead nitrate at a ratio of 1:1:1 in different exposure periods such as 6, 12, 24, 48, 72, and 96 hours, is given in Table 93. The results of acute toxicity studies expressed in terms of LC50 values for 6, 12, 24, 48, 72 and 96 hours obtained in Probit analysis on Probit papers (Fig. 13) are given in Table 94. The LC50 values were found:

TABLE 93. **Mortality rate of L. parsia in different concentrations of copper sulphate : zinc sulphate :lead nitrate:: 1:1:1 during acute toxicity studies**
(Animals exposed in each concentration are 20)

Exposure period (hrs)	Concentration (ppm)									
	56		75		100		155		180	
	Nos	%	Nos	%	Nos	%	Nos	%	Nos	%
6	-	-	-	-	-	-	4	20	6	30
12	-	-	-	-	1	5	6	30	16	80
24	1	5	2	10	4	20	10	50	20	100
48	1	5	2	10	5	25	12	60	20	100
72	1	5	4	20	7	35	16	80	20	100
96	4	20	6	30	11	55	17	85	20	100

TABLE 94. **Acute toxicity values of copper sulphate : zinc sulphate : lead nitrate:: 1:1:1 in L. parsia**

Exposure period (hrs)	LC50 (ppm)	95% Fiducial limits (ppm)		LC16 (ppm)	LC84 (ppm)	Slope function	95% confidence limit
		Upper	Lower				
6	445.8	-	-	-	-	-	-
12	160.5	180.6	142.6	122.7	210.6	1.310	1.126
24	152.2	188.4	122.9	93.2	247.2	1.628	1.238
48	135.6	160.0	114.9	92.8	197.4	1.458	1.180
72	117.1	128.8	106.5	89.6	152.6	1.305	1.100
96	106.7	118.6	95.9	79.4	143.7	1.345	1.112

Conversion of log value (ppm) to observed value (ppm)

Hours	LC	Log value	Observed value
6	LC50	6.100	445.8
12	LC84	5.350	210.6
	LC50	5.078	160.5
	LC16	4.810	122.7
24	LC84	5.570	247.2
	LC50	5.025	152.2
	LC16	4.535	93.2
48	LC84	5.285	197.4
	LC50	4.910	135.6
	LC16	4.530	92.8
72	LC84	5.028	152.6
	LC50	4.763	117.1
	LC16	4.495	89.6
96	LC84	4.968	143.7
	LC50	4.670	106.7
	LC16	4.375	79.4

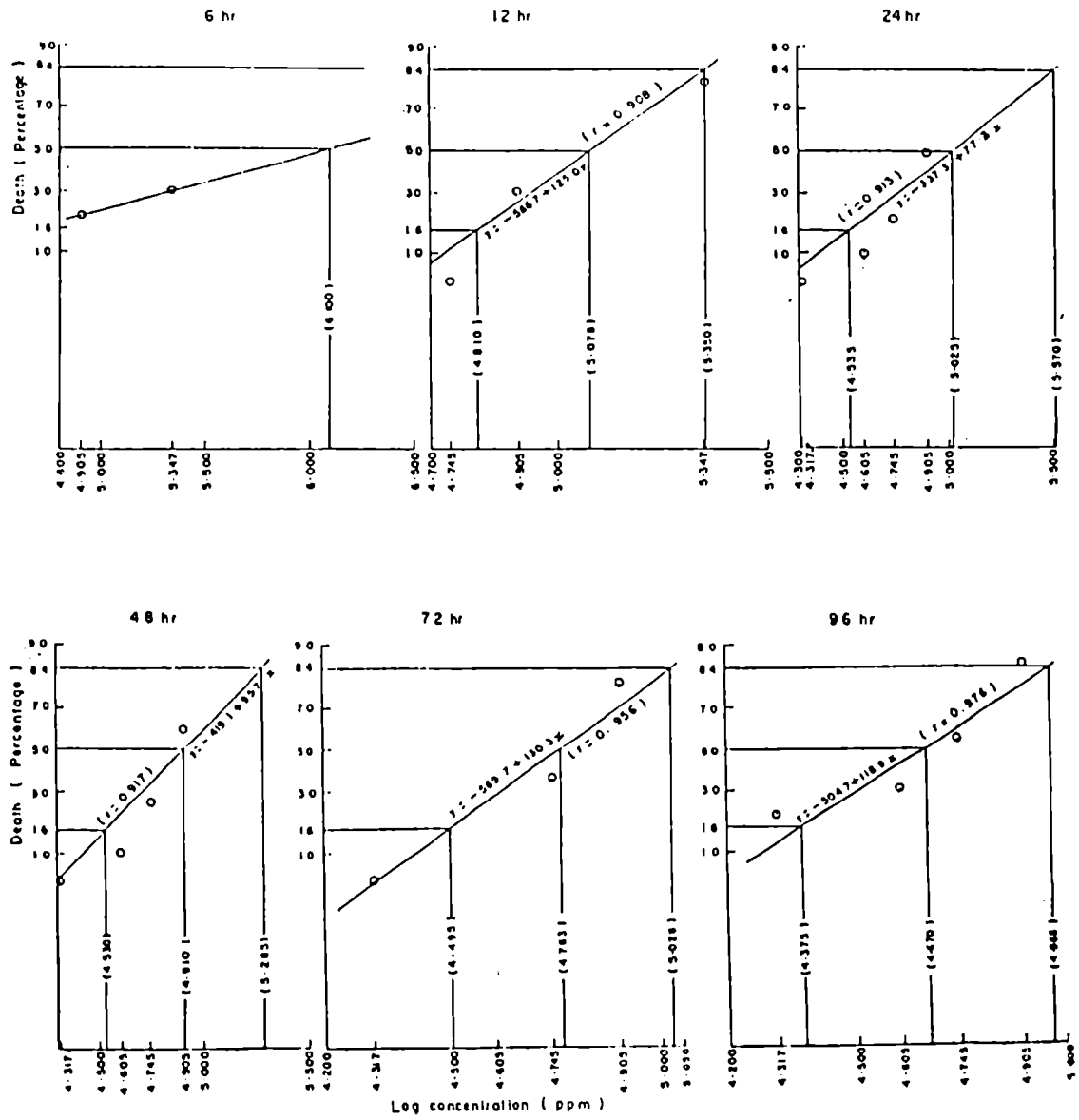


FIG. 13. Response curve of copper sulphate : zinc sulphate : lead nitrate :: 1:1:1 at 6, 12, 24, 48, 72 and 96 hours

Hours	ppm
6	445.8
12	160.5
24	152.2
48	135.6
72	117.1
96	106.7

The LC50 values showed gradual decrease with increase of time. The 95% fiducial limits, LC16, LC84, slope function and 95% confidence limit of each response curve for different exposure periods in hours are also given in Table 94. The LC50 values along with its 95% fiducial values were plotted on the log-log paper to get the toxicity curve (Fig. 14).

Toxic unit

The joint toxicity was predicted following the method of Brown (1968) and Sprague (1970) which was explained under "Materials and Methods".

The 96 hr LC50 of 1:1:1 combination = 106.7 ppm

The individual contribution $= \frac{106.7}{3}$ ppm = 35.67 ppm

The 96 hr LC50 of copper sulphate = 85.6 ppm

The 96 hr LC50 of zinc sulphate = 60.3 ppm

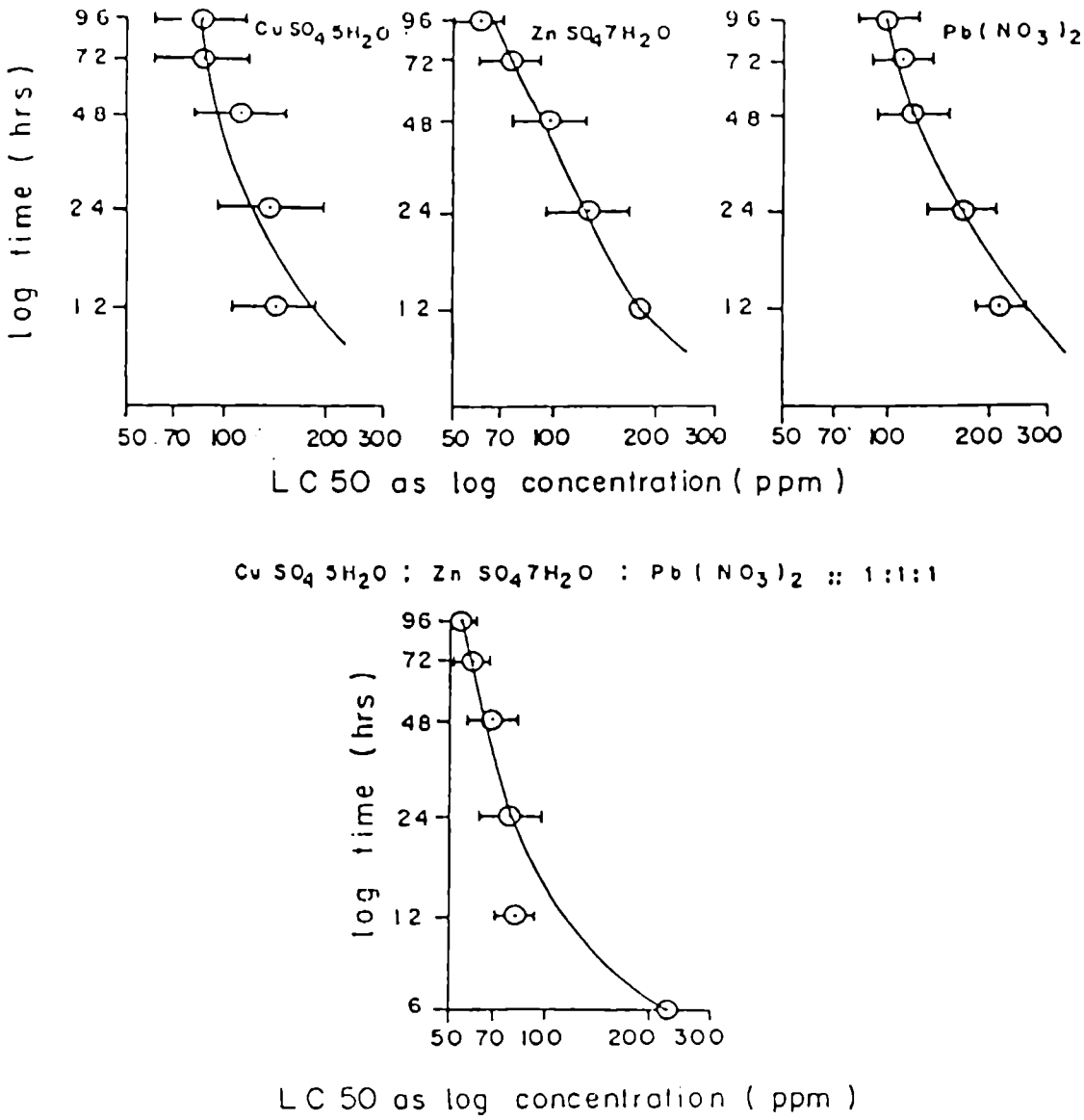


FIG. 14.Acute toxicity curve of copper sulphate, zinc sulphate, lead nitrate and combination 1:1:1

The 96 hr LC50 of lead nitrate = 103.5 ppm

The toxic unit of copper sulphate = $\frac{35.57}{85.6}$ = 0.4155

The toxic unit of zinc sulphate = $\frac{35.57}{60.3}$ = 0.5898

The toxic unit of lead nitrate = $\frac{35.57}{103.5}$ = 0.3436

The toxic units are 0.4155 + 0.5898 + 0.3436 = 1.3489 and was found greater than unit (i.e. 1).

Toxicity of Elementary metals

The heavy metals such as copper, zinc and lead in their metallic forms are not soluble in water. The bioassays were carried out in dissolving their compound forms such as copper sulphate, zinc sulphate and lead nitrate. But for the comparison of the metal toxicity, the compounds are not having much use as the elementary forms have. For the purpose, the elementary forms were calculated based on the formulae given by Reish and Oshida (1987) and elucidated under "Materials and Methods".

The results of acute toxicity studies expressed in terms of LC50 values for copper, zinc, lead and its combinations in a ratio of 23:20:57 (calculated from the acute toxicity results for compound forms) for 6, 12, 24, 48, 72 and 96 hours are given in Table 95. Based on the 95 hr LC50 values zinc found more toxic (13.7 ppm) than copper (21.8 ppm), combinations (39.5 ppm) and lead (64.7 ppm).

Discussion

Copper

Copper poisoning, though not very common, is still a great hazard since it leads mortality of marine organisms including fishes. Shashi

TABLE 95. **Acute toxicity of Cu, Zn, Pb and Cu: Zn: Pb:: 20:23:57 on L. parsia**

Exposure period (hrs)	LC50 (ppm)			
	Cu	Zn	Pb	Cu:Zn:Pb:: 23:20:57
6	-	-	-	164.6
12	35.9	40.9	135.8	59.3
24	34.9	29.0	106.8	56.1
48	28.0	22.4	76.0	50.1
72	22.0	17.1	70.3	43.2
96	21.8	13.7	64.7	39.5

Kant (1990) states "Copper concentration of more than 1-3 µ/l is responsible for acute toxicity and the crystals of copper sulphate have been discovered from the aquatic environments". The statement of Sashikant (1990) is contradictory to all available information and not acceptable.

The results obtained for different species of fishes from India and abroad through bioassay to copper are given below (in ppm):

Species	24 hr LC50	Author(s)
Mystus bleekeri	4.17	Gupta and Rajbanshi, 1981
Tilapia nilotica	73.4	Somsiri, 1982
L. parsia	34.9	Present study
	48 hr LC50	
Pleuronectes flesus	1.0-3.3	Jackim et al., 1970
Fundulus heteroclitus	3.2	"
Mystys bleekeri	1.85	Gupta and Rajbanshi, 1981
Tilapia nilotica	63.92	Somsiri, 1982
L. parsia	28.0	Present study

From these results it can be concluded that L. parsia is more resistant to copper than P. flesus and F. heteroclitus. According to Wittmann (1979) the tolerance level of $CuSO_4$ is 0.03 - 0.8 ppm for trout. Based on the results otained for $CuSO_4$ (Table 88) the L. parsia found to be more resistant to the metal than the trout. The differential toxicities to different species of fishes have been reported by various authors. Some of them are (in ppm):

	for 96 hr LC50	
Agosia chrysogaster (Longfin dace)	0.86	Lewis, 1978
Ptychocheilus oregonesis (Northern squawfish)	18.00	Andros and Garton, 1980
Rasbora daniconicus	0.203	Durve et al., 1980
Mystus bleekeri	0.85	Gupta and Rajbanshi, 1981
L. parsia	21.80	Present study (Tale 95)
	for 72 hr LC50	
Mystus bleekeri	0.95	Gupta and Rajbanshi 1981
Tilapia nilotica	58.30	Somsiri (1982)
L. parsia	22.00	Present study (Table 95)

L. parsia was found more resistant to copper than M.bleekeri and less resistant than T nilotica based on 24 and 48 hr LC50s.

Zinc

Zinc compounds damage the gills of fish very severely and cause to increase the mucus discharge. Zinc intoxicated fish first undergoes a phase of excitation with an increase in gill ventillatory rhythm with damage in respiratory epithelium of the gills leading to asphyxia and death (Metelev et al., 1983). According to them, concentration of 15 ppm is toxic to all fish within 8 hours. But from the present study it was noticed that 40.9 ppm zinc (Table 95) needed to kill 50% population of L. parsia in 12 hours. Bradly and sprague (1985b) reported 4.53 (3.07-6.69) ppm zinc as the "incipient" LC50 for Salmo gairdneri. In the present

study L. parsia was found more resistant than S. gairdneri with the 96 hr LC50 (= incipient LC50) of 13.7 ppm (Table 95). Very few reports are available regarding LC50s to differeent species of fish with zinc intoxication. Some of them are (in ppm):

Species	96 hr LC50	Author(s)
Agosia chrysogaster (Logfin dace)	0.79	Lewis, 1978
Ptychocheilus oregonesis (Northern squawfish)	3.69	Andros and Garton, 1980
Nemacheilus botia	25.00	Pundir, 1989
L. parsia	13.70	Present study (Table 95)
	72 hr LC50	
Salmo gairdneri	2.00	Lovegrove and Eddy, 1982
Tilapia nilotica	65.55	Somsiri, 1982
L. parsia	17.10	Prsent study (Table 95)

Wittmann (1979) reported 5 ppm of $ZnSO_4$ as the toxicological tolerance limit to trout. Similarly, in the present study the lethal concentrations for L. parsia were found after 5 ppm towards higher side. The finding of Wittmann (1979) supports the results of the present study. According to Portman (1972) the 48 hr LC50 of zinc to Salmo gairdneri was found to be 3.3 ppm. In present case the same for L. pasia was 22.4 ppm. The difference between these two species may be that L. parsia is a brackishwater inhabitant compared to Salmo gairdneri and the total hardness of water may also play an important role.

Lead

According to Wittmann (1979) the tolerance limit of $Pb(NO_3)_2$ to trout was 0.33 - 200 ppm. But in the present investigation also the same limits were found to be lethal for L. parsia (Table 92). Comparatively very few reports are available regarding the LC50s of lead to fish. Pundir (1989) found 25 ppm lead as the 96 hr LC50 to a freshwater fish Nemacheilus botia. From the present study it is seen that 64.7 ppm of lead as 96 hr LC50 to L. parsia and found more resistant to lead than N. botia. The 48 hr LC50 of lead to Killifish Fundulus heteroclitus was reported to be 188 ppm and to sticklebacks Eucatia inconstans and Coho-salmon Oncorhynchus kisutch to be 0.34 ppm (Jackim et al., 1970). The same for goldfish was found 117 ppm (Laws, 1981). For L. parsia the 48 hr LC50 was found to be 76 ppm in the present study (Table 95).

L. parsia is seen less reistant to lead than F. heteroclitus and gold fish. However, the comparisons cannot be accurate, as the toxicity varies with the variation of total hardness and other parameters.

In the present investigation based on 12 hr LC50, the copper was found more toxic than zinc, combination and lead (Table 95). It shows the quickest action of copper on L. parsia than other metals. Based on the toxicity data Cu^{2+} was found more toxic than Zn^{2+} and Pb^{2+} to fish (Waldichuk, 1974). Conducting bioassays for 12, 24 and 72 hrs with Tilapia nilotica, Somsiri (1982) reported that the copper is more toxic than zinc. Based on 24, 48, 72 and 96 hr LC50s the zinc was found toxic to L. parsia than copper, combination and lead in the present

study (Table 95). The toxicities of heavy metals can be linked with the electronegativity of the same. Based on "Periodic Table" the copper is more electronegative than zinc and lead (Wittmann, 1979). But copper and zinc display similar characters and also found in Periodic Table side to each other with atomic numbers and atomic weights 29, 64 and 30, 65 respectively. Based on these properties copper could have exhibited slightely more toxic than zinc. But in the present study in prolonged exposure (i.e after 12 hrs), the zinc became more toxic than copper and lead. Though cause for the same is not exactly traced out, it is possibly assumed that toxicity is controlled by various physico-chemical and biological parameters. Similar type of results were also reported for other fishes by various authors. Based on 96 hr LC50s of copper and zinc on Longfin dace Agosia chrysogaster Lewis (1978) reported higher toxic nature of zinc than copper. According to Metelev et al. (1983) rainbow trout can live upto 133 min at 18°C in distilled water containing 25 ppm zinc. After that the symptoms of death have appeared. But 143 ppm of copper sulphate did not cause death in trout. It proved the toxicity of zinc over copper. The copper toxicity is generally reduced in the presence of the synthetic chelating agents, sewage effluents and purified humic acid (Winner, 1985). The lead with atomic number 82 and atomic weight 207 found least toxic in the present study. Pundir (1989) reported that the lead is less toxic than zinc in Nemacheilus botia.

Toxic Unit

The "synergism" of copper and zinc to aquatic organisms is explained by Waldichuk (1974). The effect also explained by other authors working

on different species of fish such as Puntius conchonius (Pant et al., 1980) and Clarias lazera (Hilmy et al., 1987). The most exciting and potentially useful recent development in pollution biology has been a method of predicting toxicity of mixtures of pollutants (Sprague, 1970). In the present study the mixture of copper, zinc and lead was experimented on L. parsia to find out the joint toxicity. As reported earlier the addition of "toxic units" for copper sulphate (Cu), zinc sulphate (Zn) and lead nitrate (Pb) was found to be more than 1.0 (i.e. 1.3489). This present study and results proved that the toxicities of the three components such as Cu, Zn and Pb are indeed strictly "additive" as suggested by Laws (1981). For more details, again the results in the present study were compared with the "Gaddum's diagram" explained and given by Sprague (1970). From the same it is concluded that the "Joint action" of copper, zinc and lead was "less-than-additive". To get the 96 hr LC50 the amount of toxicants such as copper and zinc individually were needed less than the combinations. But to get the similar response as obtained in the case of combination of metals, more amount of lead is needed. Thus the synergistic effect of zinc and copper somehow balanced with the addition of lead in the combinations.

Section 2: Chronic Exposure Studies

The 96 hr LC50 (i.e. 106.7 ppm) of copper sulphate, zinc sulphate and lead nitrate in the ratio of 1:1:1 was divided by 10 and 100 to get two different concentrations i.e. $\frac{1}{10}$th 96 hr LC50 and $\frac{1}{100}$ th 96 hr LC50 which is supposed to be the threshold level for carrying out the

laboratory experiments as suggested and followed by Ward and Parish (1982). To these concentrations fish L. parsia was exposed for three weeks. Simultaneously controls were maintained. The accumulation of copper, zinc and lead in different tissues such as liver, gills, kidney, intestine, skin and muscle were estimated after first, second and third weeks of the experiments.

Copper

Accumulation in tissue

The accumulation of copper in control and in different concentra - tions with percentage variation from control in different tissues are presented in Tables 96-101 for liver, gills, kidney, intestine, skin and muscle respectively. Analyses of data of the experiments for specific tissues, the grand mean values obtained were found in decreasing order:

Tissue	Value (ppm dry wt)
Liver	638.66
Intestine	224.21
Kidney	73.91
Gills	18.76
Skin	4.29
Muscle	3.25

Liver: From two-way ANOVA, very high significant differences (at 0.1% level) were seen among the treatments, exposure periods and their inter-

TABLE 96. **Accumulation of copper in liver of L. parsia exposed to 1:1:1 ratio of lead nitrate: copper sulphate: zinc sulphate at different sub-lethal levels in different exposure periods**

Exposure period (weeks)	Copper (ppm dry wt) in control tissue	Sub-lethal concentrations			
		$\frac{1}{100}$ th 96 hr LC50		$\frac{1}{10}$ th 96 hr LC50	
		Tissue content (ppm dry wt)	% variation from control	Tissue content (ppm dry wt)	% variation from control
0	11.15±2.08	11.15± 2.08	0	11.15± 2.08	0
1	8.21±1.07	381.07±10.68	4541.5	932.86±46.96	11262.5
2	8.30±0.04	484.93±12.73	5742.5	1123.27±42.62	13433.4
3	8.00±0.14	901.80±18.88	11172.5	1899.53± 9.10	23644.1

± Standard deviation.

Between treatments ($P < 0.001$) and between periods ($P < 0.001$).

TABLE 97. **Accumulation of copper in gills of L. parsia exposed to 1:1:1 ratio of lead nitrate: copper sulphate: zinc sulphate at different sub-lethal levels in different exposure periods**

Exposure period (weeks)	Copper (ppm dry wt) in control tissue	Sub-lethal concentrations			
		$\frac{1}{100}$ th 96 hr LC50		$\frac{1}{10}$ th 96 hr LC50	
		Tissue content (ppm dry wt)	% variation from control	Tissue content (ppm dry wt)	% variation from control
0	5.11±0.77	5.11± 0.77	0	5.11± 0.77	0
1	6.03±1.02	8.63± 0.60	43.1	27.65± 1.63	358.5
2	4.87±1.14	13.73± 1.06	181.9	46.20± 3.63	848.7
3	4.87±0.39	13.12± 1.39	169.4	43.76± 5.07	798.6

± Standard deviation.

Between treatments ($P < 0.001$) and between periods ($P < 0.001$).

TABLE 98. **Accumulation of copper in kidney of L. parsia exposed to 1:1:1 ratio of lead nitrate: copper sulphate: zinc sulphate at different sub-lethal levels in different exposure periods**

Exposure period (weeks)	Copper (ppm dry wt) in control tissue	Sub-lethal concentrations			
		$\frac{1}{100}$ th 96 hr LC50		$\frac{1}{10}$ th 96 hr LC50	
		Tissue content (ppm dry wt)	% variation from control	Tissue content (ppm dry wt)	% variation from control
0	10.67±4.86	10.67± 4.86	0	10.67± 4.86	0
1	9.32±3.42	36.41± 2.09	290.7	119.87±12.76	1186.2
2	7.52±0.56	51.00± 3.80	578.2	166.82± 7.41	2118.4
3	7.54±0.32	61.12± 4.50	710.6	206.63± 6.63	2627.2

± Standard deviation.

Between treatments($P < 0.001$) and between periods ($P < 0.001$).

TABLE 99. **Accumulation of copper in intestine of L. parsia exposed to 1:1:1 of lead nitrate: copper sulphate: zinc sulphate at different sub-lethal levels in different exposure periods**

Exposure period (weeks)	Copper (ppm dry wt) in control tissue	Sub-lethal concentrations			
		$\frac{1}{100}$ th 96 hr LC50		$\frac{1}{10}$ th 96 hr LC50	
		Tissue content (ppm dry wt)	% variation from control	Tissue content (ppm dry wt)	% variation from control
0	6.90±1.29	6.90± 1.29	0	6.90± 1.29	0
1	6.73±0.19	181.17± 2.41	2592.0	70.05± 4.15	940.9
2	7.23±0.14	382.60±116.04	5191.8	585.75± 8.03	8001.7
3	7.35±0.12	259.80± 8.05	3434.7	517.18± 5.14	6936.5

± Standard deviation.

Between treatments($P < 0.001$) and between periods ($P < 0.001$).

TABLE 100. **Accumulation of copper in skin of L. parsia exposed to 1:1:1 ratio of lead nitrate: copper sulphate: zinc sulphate at different sub-lethal levels in different exposure periods**

Exposure period (weeks)	Copper (ppm dry wt) in control tissue	Sub-lethal concentrations			
		$\frac{1}{100}$ th 96 hr LC50		$\frac{1}{10}$ th 96 hr LC50	
		Tissue content (ppm dry wt)	% variation from control	Tissue content (ppm dry wt)	% variation from control
0	3.61±0.74	3.61±0.74	0	3.61±0.74	0
1	3.06±0.06	3.44±0.31	12.4	7.90±0.92	158.2
2	2.97±0.05	4.72±0.17	58.9	3.85±0.12	29.6
3	2.74±0.07	5.45±0.43	98.9	4.48±0.43	63.5

± Standard deviation.

Between treatments ($P < 0.001$) and between periods ($P < 0.001$).

TABLE 101. **Accumulation of copper in muscle of L. parsia exposed to 1:1:1 ratio of lead nitrate: copper sulphate: zinc sulphate at different sub-lethal levels in different exposure periods**

Exposure period (weeks)	Copper (ppm dry wt) in control tissue	Sub-lethal concentrations			
		$\frac{1}{100}$ th 96 hr LC50		$\frac{1}{10}$ th 96 hr LC50	
		Tissue content (ppm dry wt)	% variation from control	Tissue content (ppm dry wt)	% variation from control
0	2.31±0.44	2.31±0.44	0	2.31±0.44	0
1	2.48±0.43	2.62±0.20	5.6	3.72±0.21	50.0
2	2.76±0.09	3.61±0.52	30.8	2.82±0.11	2.2
3	2.84±0.20	4.99±0.25	75.7	3.42±0.26	20.4

± Standard deviation.

Between treatments ($P < 0.001$) and between periods ($P < 0.001$).

actions. The mean bioaccumulation was seen high from computer analysis in $\frac{1}{10}$ th 96 hr LC50 followed by $\frac{1}{100}$ th 96 hr LC50 and control. Similarly it was seen higher in the liver of the fishes exposed for 3 weeks followed by 2 weeks and 1 week (Table 96).

Gills: Very high significant differences (at 0.1% level) were observed among the treatments, exposure periods and their interactions here also as seen in liver. The higher mean bioaccumulation was noticed in the gills, exposed to $\frac{1}{10}$ th 96 hr LC50 followed by $\frac{1}{100}$ th 96 hr LC50 and control. Among exposure periods the higher mean values were recorded in 2nd week of exposure followed by 3rd week and 1st week (Table 97) which is unlike as observed in liver.

Kidney: The analysis of variance obtained from computer analysis indicated the very high significant differences (at 0.1% F-value) among the treatments, exposure periods and their interactions (Table 98). The gradual increase in bioaccumulation was noticed from 1st week to 3rd week and from control to $\frac{1}{10}$ th 96 hr LC50. The maximum bioconcentration was seen at higher concentration of heavy metals at longer exposure period, which followed a normal trend.

Intestine: The higher mean bioconcentration of copper in intestine was seen in $\frac{1}{10}$ th 96 hr LC50 followed by $\frac{1}{100}$ th 96 hr LC50 and control and very high significant differences among the treatments, exposure periods and their interactions at 0.1% F-value through computer ANOVA. Among exposure periods the higher mean bioaccumulation was recorded

in 2nd week followed by 3rd week and 1st week (Tale 99) as seen in the case of gills.

Skin: Very high significant differences (at 0.1% level) were seen from two-way ANOVA among the treatments, exposure periods and their interactions (Tale 100). The higher mean bioaccumulations were recorded for $\frac{1}{10}$ th 96 hr LC50 than $\frac{1}{100}$ th 96 hr LC50 and control. In $\frac{1}{10}$ th 96 hr LC50 the 1st week recorded higher bioaccumulation (7.90 ± 0.92 ppm in dry wt) followed by 3rd week (4.48 ± 0.43 ppm dry wt) and 2nd week (3.85 ± 0.12 ppm dry wt). But in $\frac{1}{100}$ th 96 hr LC50 the gradual increase in bioaccumulation was noticed from the 1st week to 3rd week.

Muscle: The very high significant differences (at 0.1% level) among the treatments, exposure periods and their interactions were seen from two-way ANOVA (Table 101). The higher percentage variation in bioaccumulation from control was recorded in 1st week followed by 3rd week and 2nd week in $\frac{1}{10}$ th 96 hr LC50. But the gradual increase in copper was seen from 1st week to 3rd week in $\frac{1}{100}$ th 96 hr LC50. The higher accumulation was recorded in $\frac{1}{100}$ th 96 hr LC50 at 3 weeks of exposure and the recorded value was 4.99 ± 0.26 ppm dry wt.

Discussion

Copper was seen to accumulate in the gills, liver, kidney and plasma of seawater-adapted and freshwater-adapted Platichthys flesus exposed to higher concentrations of the metal (Stagg and Shuttleworth, 1982). According to them the copper content was significantly greater in fish

exposed to elevated copper than the controls. Similar result was reported by Cahit and Kargin (1990), who studied the accumulation of copper in spleen, liver, intestine, stomach, gills and muscle of Tilapia nilotica exposed to the water containing 0.1, 1.0, and 10 ppm of copper. In the present experimental investigation all the six tissues showed higher accumulation of copper in L. parsia proportional to copper concentration in medium. In all the tissues higher accumulations of copper were seen in $\frac{1}{10}$ th 96 hr LC50 followed by $\frac{1}{100}$ th 96 hr LC50 and control. According to Drbal and Svobodova (1980) the different degree of copper accumulation in fish was associated with the formation of complex and barely soluble compounds in pond water. Cahit and Kargin (1990) reported the accumulation of copper in different tissues and organs of T. nilotica was a function of exposure period. In the present study the accumulation of copper in gills and kidney of L. parsia was seen as a function of exposure period, but in other tissues the differential accumulation of copper was noticed over time. It is discussed below elsewhere.

As seen in field observations (Chapter 1 and 2), the liver showed maximum amount of copper whereas the skin and muscle with less than 5 ppm in dry wt in laboratory experiments. Many fold increase in copper over the same reported from different centres was recorded for liver, intestine, kidney and gills only. Copper has been shown to accumulate in the gills, liver and kidney of Ictalurus nebulosus by Brungs et al. (1993) following exposure to copper concentrations between 27 and 152 ppb. Yamamoto et al. (1977) recorded statistically significant increase in copper content of the gills, liver, intestine, kidney and serum of cyprinus carpio,

the highest concentrations being in the liver. The above results are very well in agreement with the present study for different tissues of L. parsia. This indicates that in these experimental conditions, the rate of uptake is greater than the rate of excretion of copper. The higher bioaccumulation of copper in liver followed by intestine, gills and muscle as seen from the present investigation was reported by Cahit and Kargin (1990) in T. nilotica. At lower (i.e. 0.01 and 1.0 ppm) concentrations of copper in water Romanenko and Yevtushenko (1985) observed gradual increase in its accumulation in the liver of fish. Halcrow et al. (1973) found 16 times more copper in the dumpsite of firth of Clyde sediments compared to control sites and copper in Crangon sp. to be raised by a factor of two. Copper in the liver of Dab Limanda limanda feeding exclusively on macrobenthos at the dumpsite was 17 times higher than that in cod (and also much higher than in all the other species of fish measured). Segner (1987) reported higher bioaccumulation of copper in liver tissue of starved specimens of Roach Rutilus rutilus exposed to sulethal copper contamination of 80 ppb for 7 days.

There is evidence that the low molecular weight proteins (metallothioneins) in liver and kidney bind heavy metals and thereby reduce their toxicity (Stagg and Shuttleworth, 1982). Metallothionein-like proteins capable of binding copper have been described in the liver of Cyprinus carpio (Yamamoto et al., 1978), Anguilla anguilla (Noel-Lambot et al.,1978) and Dab Limanda limanda (Overnell and McIntosh, 1988). The accumulation of copper in liver and kidney of L. parsia as observed here is likely by binding of copper to metallothionein-like protein as part of a detoxification function.

Bivalent ions such as copper and zinc are excreted *via* the kidney. However, it is not unlikely that, when the kidney is overloaded, as after exposure to heavy metals, the gill play an important role as an external route for detoxification (Crespo *et al.*, 1981). In the present study the continuous overloading of copper was seen in kidney of *L. parsia* due to over exposure with fluctuations in copper in gills. The reason may be attributed to that of Crespo *et al.* (1981) as explained above. In conditions of acute copper stress, the gill tissue secretes large quantities of mucus as an excretory mchanism (Bryan, 1971). Mucus cells are dilated (Cardeilhac *et al.*, 1979) and are more abundant (Baker, 1969; Bryan, 1971) in copper-exposed fish suggesting an increased activity which may be associated with the excretion of the metal bound to mucus. The same may be the cause for decreased content of copper in gills of *L. parsia* after 3 weeks exposure. Fluctuations in copper accumulation have been shown to occur in the organs concerned with osmotic and ionic regulation in teleosts exposed to higher copper concentrations in the water. These include the gills and kidney of *Ictalurus nebulosus* (Brungs *et al.*, 1973), *Platichthys flesus* (Stagg and Shuttleworth, 1982) and the gills, kidney and intestine of *C. carpio* (Yamamoto *et al.*, 1977). In the present study the intestine absorbed lower concentration of copper, exposed to copper for 3 weeks than for 2 weeks. In fish intoxicated with $CdCl_2$, $ZnCl_2$, $CuCl_2$ added to seawater, the corpuscule attached to the lumen of intestine contains enormous concentrations of these metals (Noel-Lambot, 1981). Intestinal corpuscules seem therefore to limit the entry of metals through the intestinal wall and the corpuscules themselves excreted with heavy metal to the exterior through the faeces. This reason can also be attributed to

the present decrease in copper in intestine of L. parsia in 3rd week of exposure. This is to be checked up in further studies for verification.

Somatic tissue of fish has been demonstrated to have low concentration of copper, even in systems containing high copper levels (Bennett and Dooley, 1982). It has been shown that copper content in muscle tissue of Fundulus heteroclitus has little relationship to osmoregulatory stress. Although copper content was high in the muscle of F. heteroclitus, its percentage increase above controls was low. The results of the present study obtained for skin and muscle of L. parsia corroborate the observations of Bennett and Dooley (1982). Skin is essentially impermeable to water and solute molecules (Bartholomew et al., 1972). It is probale that the increase in copper content is related to an increase in slime secretions by the skin (Bryan, 1971) as a mode of heavy metals excretion. According to Bryan (1971) heavy metals can be absorbed via digestive tract either from water being swallowed for osmoregulation or from food .

Bioaccumulation Factor

The "Bioaccumulation Factor" in relation with water in different sublethal concentrations of heavy metals (copper sulphate : zinc sulphate : lead nitrate ::: 1:1:1) and exposure periods for different tissues such as liver, gills, kidney, intestine, skin and muscle are presented in Tales 102-107. Analysis of data for specific tissues, the grand mean values otained were in descending order:

TABLE 102. **Bioaccumulation Factor of copper in liver of L. parsia exposed to 1:1:1 ratio of lead nitrate: copper sulphate: zinc sulphate at different sub-lethal concentrations in different exposure periods**

Exposure period (weeks)	Control tissue factor	Sub-lethal concentrations			
		$\frac{1}{100}$ th 96 hr LC50		$\frac{1}{10}$ th 96 hr LC50	
		Tissue factor	% variation from control	Tissue factor	% variation from control
0	991.3±184.9	991.3±184.9	0	991.3±184.9	0
1	730.4± 95.0	1119.1± 31.4	53.2	274.0± 13.8	- 62.5
2	738.2± 3.2	1424.1± 37.4	92.9	329.9± 12.5	- 55.3
3	711.2± 12.3	2648.3± 55.4	272.0	557.8± 2.7	- 21.6

± Standard deviation.

Between treatments($P < 0.001$) and between periods ($P < 0.001$).

TABLE 103. **Bioaccumulation Factor of copper in gills of L. parsia exposed to 1:1:1 ratio of lead nitrate: copper sulphate: zinc sulphate at different sub-lethal concentrations in different exposure periods**

Exposure period (weeks)	Control tissue factor	Sub-lethal concentrations			
		$\frac{1}{100}$ th 96 hr LC50		$\frac{1}{10}$ th 96 hr LC50	
		Tissue factor	% variation from control	Tissue factor	% variation from control
0	319.6± 48.3	319.6± 48.3	0	319.6± 48.3	0
1	376.9± 63.6	17.8± 1.3	- 95.3	5.7± 0.3	- 98.5
2	304.2± 71.1	28.3± 2.2	- 90.7	9.5± 0.8	- 96.9
3	304.2± 24.2	27.1± 2.9	- 91.1	9.0± 1.1	- 97.0

± Standard deviation.

Between treatments ($P < 0.001$).

TABLE 104. **Bioaccumulation Factor of copper in kidney of L. parsia exposed to 1:1:1 ratio of lead nitrate: copper sulphate: zinc sulphate at different sub-lethal concentrations in different exposure periods**

Exposure period (weeks)	Control tissue factor	Sub-lethal concentrations			
		$\frac{1}{100}$ th 96 hr LC50		$\frac{1}{10}$ th 96 hr LC50	
		Tissue factor	% variation from control	Tissue factor	% variation from control
0	1125.7±513.4	1126.7±513.4	0	1126.7±513.4	0
1	1089.4±291.1	126.9± 7.3	-88.3	42.8± 4.5	-96.2
2	794.2± 48.8	177.8± 13.2	-77.6	58.1± 2.6	-92.7
3	795.6± 34.1	213.0± 15.7	-73.2	71.7± 2.3	-91.0

± Standard deviation.

Between treatments($P < 0.001$).

TABLE 105. **Bioaccumulation Factor of copper in intestine of L. parsia exposed to 1:1:1 ratio of lead nitrate: copper sulphate: zinc sulphate at different sub-lethal concentrations in different exposure periods**

Exposure period (weeks)	Control tissue factor	Sub-lethal concentrations			
		$\frac{1}{100}$ th 96 hr LC50		$\frac{1}{10}$ th 96 hr LC50	
		Tissue factor	% variation from control	Tissue factor	% variation from control
0	608.4±113.8	608.4±113.8	0	608.4±113.8	0
1	592.9± 16.5	527.0± 7.0	-11.1	20.4± 1.2	-96.6
2	663.7± 12.5	1113.0±337.6	74.8	170.4± 2.3	-73.2
3	647.5± 10.4	755.8±88.6	16.7	150.5± 1.5	-76.8

± Standard deviation.

Between treatments($P < 0.001$) and between periods ($P < 0.001$).

TABLE 106. **Bioaccumulation Factor of copper in skin of L. parsia exposed to 1:1:1 ratio of lead nitrate: copper sulphate: zinc sulphate at different sub-lethal concentrations in different exposure periods**

Exposure period (weeks)	Control tissue factor	Sub-lethal concentrations			
		$\frac{1}{100}$ th 96 hr LC50		$\frac{1}{10}$ th 96 hr LC50	
		Tissue factor	% variation from control	Tissue factor	% variation from control
0	187.9±35.1	187.9±35.1	0	187.9±35.1	0
1	358.3± 6.5	13.3± 1.2	-96.3	3.1± 0.4	-99.1
2	348.2± 5.4	18.3± 0.6	-94.8	1.5± 0.1	-99.6
3	321.2± 8.1	21.1± 1.7	-93.4	1.7± 0.2	-99.5

± Standard deviation.

Between treatments ($P < 0.001$) and between periods ($P < 0.001$).

TABLE 107. **Bioaccumulation Factor of copper in muscle of L. parsia exposed to 1:1:1 ratio of lead nitrate: copper sulphate: zinc sulphate at different sub-lethal concentrations in different exposure periods**

Exposure period (weeks)	Control tissue factor	Sub-lethal concentrations			
		$\frac{1}{100}$ th 96 hr LC50		$\frac{1}{10}$ th 96 hr LC50	
		Tissue factor	% variation from control	Tissue factor	% variation from control
0	187.9±35.1	187.9±35.1	0	187.9±35.1	0
1	200.6±34.9	7.0± 0.5	-96.5	1.0± 0.04	-99.5
2	223.2± 7.0	9.6± 1.4	-95.7	0.8± 0.02	-99.7
3	229.7±16.4	13.3± 0.7	-94.2	09.± 0.07	-99.6

± Standard deviation.

Between treatments ($P < 0.001$).

Tissue	Value
Liver	3550.65
Intestine	1939.82
Kidney	1181.86
Gills	641.60
Skin	343.22
Muscle	314.19

The highest and lowest Bioaccumulation Factors were seen for liver and muscle respectively. Similar results of Bioaccumulation Factor were also obtained in Chapter 1 for different centres.

Liver: Bioaccumulation Factor shows very high signficant differences (at 0.1% level) among the treatments, exposure periods and their interactions (Table 102). The higher mean Bioaccumulation Factors were calculated for $\frac{1}{100}$ th 96 hr LC50 followed by control and $\frac{1}{10}$ th 96 hr LC50. The gradual increase was noticed in both the sublethal concentrations from 1st week to the 3rd week. In correlating the "Bioaccumulation" of copper with "its factor" in liver of L. parsia, a very poor and nonsignificant coefficient (r = 0.064) was obtained between them.

Gills: Bioaccumulation Factor for gills was very highly significant (at 0.1% level) between the treatments. Higher mean Bioaccumulation Factor was calculated for control followed by $\frac{1}{100}$ th 96 hr LC50 and $\frac{1}{10}$ th 96 hr LC50 (Tale 103). No significant differences were seen among the exposure periods and the interactions of concentrations with exposure

periods. The correlation study indicated a negative relation (-0.616) between the "Bioaccumulation" of copper and "Bioaccumulation Factor" in gills.

Kidney: ANOVA showed very high significant differences between the treatments at 1% F-value. The higher mean factor was found for control followed by $\frac{1}{100}$ th 96 hr LC50 and $\frac{1}{10}$ th 96 hr LC50 (Tale 104). This indicated the decrease in Bioaccumulation Factor with increase in concentration. The nonsignificant differences were found among the periods, but significant differences were found among the periods, but was significant (at 5% level) for the interactions of periods with treatments. The relationship between "Bioaccumulation" and "its factor" for copper in kidney was found negative and good (r = -0.702). The bioaccumulation in animal tissue on six different field centres and experimental animal along with its factors were plotted graphically on computer (Fig. 15) to get the break-up concentration, where the relationship begins to be negative and the same concentration was found to be 10 ppm dry wt.

Intestine: Very high significant differences were seen among the treatments and periods at 0.1% F-value. The higher mean Bioaccumulation Factor was calculated for $\frac{1}{100}$ th 96 hr LC50 followed by control and $\frac{1}{10}$ th 96 hr LC50 (Table 105). Similarly higher mean factor was obtained for 2nd week followed by 3rd and 1st week. No significant differences were seen for the interactions between treatment and periods. A very poor and nonsignificant negative relationship was obtained in correlating the bioaccumulation of copper with Bioaccumulation Factor in intestine (r = -0.150).

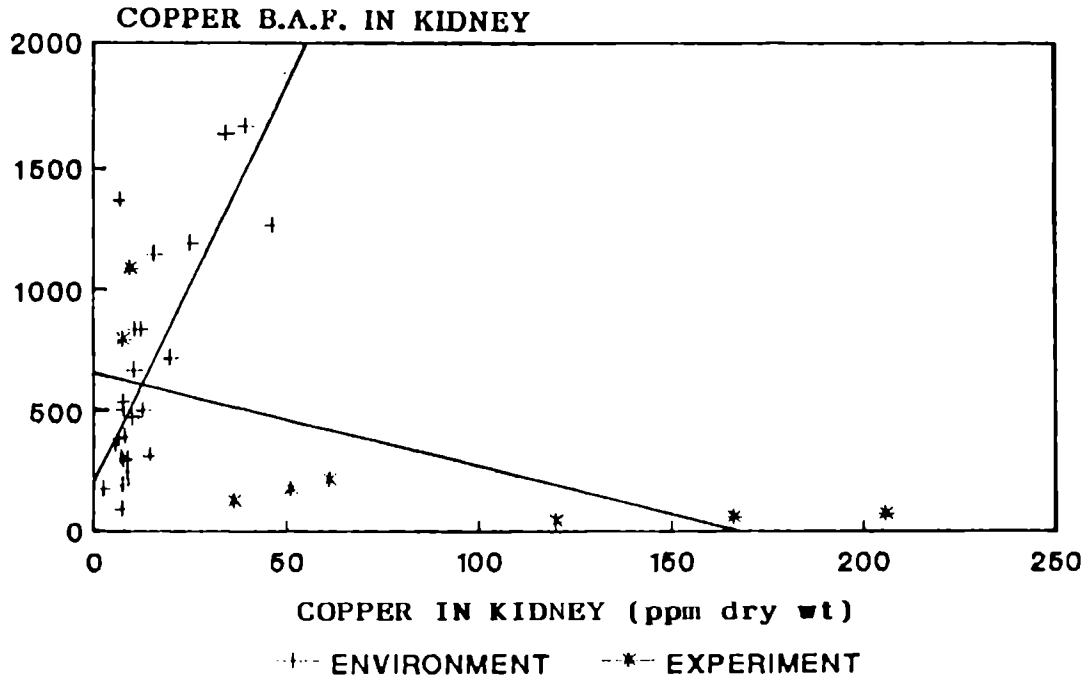


FIG. 15. Relationship between Bioaccumulation and Bioaccumulation Factor (B.A.F.) of copper in Kidney of L. parsia.

Skin: From two-way ANOVA in computer, very high significant differences (at 0.1% F-value) were seen among the treatments, periods and interactions between them (Table 106). The higher mean Bioaccumulation Factor was calculated for control followed by $\frac{1}{100}$th 96 hr LC50 and $\frac{1}{10}$th 96 hr LC50. The highest mean factor was calculated in 1st week followed by 2nd and 3rd weeks with lowest. From the correlation study the negative relationship ($r = -0.616$) was obtained between the bioaccumulation of copper and its factor in skin.

Muscle: Very high significant difference (at 0.1% level) was seen between the treatments placing the control at higher side followed by $\frac{1}{10}$ th 96 hr LC50, $\frac{1}{100}$ th 96 hr LC50 (Table 107). But no significant differences were seen between the periods of exposure and the interactions of periods with treatments. The correlation study for bioaccumulation of copper with its factor in muscle produced a weak negative relationship ($r = -0.456$). The discusssion is given at the end of this Chapter 3.

Zinc

Accmulation in tissue

The bioaccumulation of zinc in different tissues studied in L. parsia exposed to 1:1:1 ratio of copper sulphate, zinc sulphate and lead nitrate at different concentrations in different exposure periods are presented in Tables 108-113. The grand mean values for specific tissues from the experiments were found from computer and in decreasing order, they are:

TABLE 108. **Accumulation of zinc in liver of L. parsia exposed to 1:1:1 ratio of lead nitrate: copper sulphate: zinc sulphate at different sub-lethal levels in different exposure periods**

Exposure period (weeks)	Zinc (ppm dry wt) in control tissue	Sub-lethal concentrations			
		$\frac{1}{100}$ th 96 hr LC50		$\frac{1}{10}$ th 96 hr LC50	
		Tissue content (ppm dry wt)	% variation from control	Tissue content (ppm dry wt)	% variation from control
0	54.67±16.05	54.67±16.05	0	54.67±16.05	0
1	56.26± 3.33	121.70± 7.93	116.3	203.83± 7.60	262.3
2	51.05± 4.25	181.74±13.07	256.0	284.30± 8.67	456.3
3	53.95± 2.85	206.90±12.63	283.5	261.60±10.69	383.8

± Standard deviation.

Between treatments($P < 0.001$) and between periods ($P < 0.001$).

TABLE 109. **Accumulation of zinc in gills of L. parsia exposed to 1:1:1 ratio of lead nitrate: copper sulphate: zinc sulphate at different sub-lethal levels in different exposure periods**

Exposure period (weeks)	Zinc (ppm dry wt) in control tissue	Sub-lethal concentrations			
		$\frac{1}{100}$ th 96 hr LC50		$\frac{1}{10}$ th 96 hr LC50	
		Tissue content (ppm dry wt)	% variation from control	Tissue content (ppm dry wt)	% variation from control
0	35.70± 6.45	35.70± 6.45	0	35.70± 6.45	0
1	39.57± 3.53	46.89± 3.67	18.5	49.93± 0.65	26.2
2	40.34± 3.19	59.95± 3.73	48.6	65.64± 5.41	62.7
3	42.81± 3.39	65.52± 4.30	53.0	67.51± 3.84	57.7

± Standard deviation.

Between treatments($P < 0.001$) and between periods ($P < 0.001$).

TABLE 110. **Accumulation of zinc in kidney of L. parsia exposed to 1:1:1 ratio of lead nitrate: copper sulphate: zinc sulphate at different sub-lethal levels in different exposure periods**

Exposure period (weeks)	Zinc (ppm dry wt) in control tissue	Sub-lethal concentrations			
		$\frac{1}{100}$ th 96 hr LC50		$\frac{1}{10}$ th 96 hr LC50	
		Tissue content (ppm dry wt)	% variation from control	Tissue content (ppm dry wt)	% variation from control
0	40.78±6.49	40.78± 6.49	0	40.78± 6.49	0
1	39.70±3.60	75.64± 2.90	90.5	56.30± 5.75	41.8
2	33.94±2.06	99.51± 6.39	193.2	132.46± 5.93	290.3
3	33.97±2.23	101.02±15.08	197.4	107.51± 4.31	216.5

± Standard deviation.

Between treatments($P < 0.001$) and between periods ($P < 0.001$).

TABLE 111. **Accumulation of zinc in intestine of L. parsia exposed to 1:1:1 ratio of lead nitrate: copper sulphate: zinc sulphate at different sub-lethal levels in different exposure periods**

Exposure period (weeks)	Zinc (ppm dry wt) in control tissue	Sub-lethal concentrations			
		$\frac{1}{100}$ th 96 hr LC50		$\frac{1}{10}$ th 96 hr LC50	
		Tissue content (ppm dry wt)	% variation from control	Tissue content (ppm dry wt)	% variation from control
0	45.33±6.21	45.33± 6.21	0	45.33± 6.21	0
1	50.17±2.73	76.93± 4.40	53.3	59.74± 3.23	19.1
2	58.35±4.78	111.15± 2.54	90.5	303.41±12.78	420.0
3	60.47±3.70	110.35± 4.61	82.5	213.48±20.81	253.0

± Standard deviation.

Between treatments($P < 0.001$) and between periods ($P < 0.001$).

TABLE 112. **Accumulation of zinc in skin of L. parsia exposed to 1:1:1 ratio of lead nitrate: copper sulphate: zinc sulphate at different sub-lethal levels in different exposure periods**

Exposure period (weeks)	Zinc (ppm dry wt) in control tissue	Sub-lethal concentrations			
		$\frac{1}{100}$ th 96 hr LC50		$\frac{1}{10}$ th 96 hr LC50	
		Tissue content (ppm dry wt)	% variation from control	Tissue content (ppm dry wt)	% variation from control
0	50.85±6.02	50.85±6.02	0	50.85±6.02	0
1	57.26±3.17	61.03±3.79	6.6	69.87±3.22	22.0
2	55.48±3.55	63.55±4.48	14.5	101.27±7.70	82.5
3	54.76±3.33	81.14±2.14	48.2	103.99±4.38	89.9

± Standard deviation.

Between treatments($P < 0.001$) and between periods ($P < 0.001$).

TABLE 113. **Accumulation of zinc in muscle of L. parsia exposed to 1:1:1 ratio of lead nitrate: copper sulphate: zinc sulphate at different sub-lethal levels in different exposure periods**

Exposure period (weeks)	Zinc (ppm dry wt) in control tissue	Sub-lethal concentrations			
		$\frac{1}{100}$ th 96 hr LC50		$\frac{1}{10}$ th 96 hr LC50	
		Tissue content (ppm dry wt)	% variation from control	Tissue content (ppm dry wt)	% variation from control
0	15.77±0.81	15.77±0.81	0	15.77±0.81	0
1	16.11±1.11	17.27±0.53	7.2	21.72±2.81	34.8
2	14.80±1.07	37.82±3.15	155.5	45.75±3.15	209.1
3	15.19±1.36	33.41±1.02	119.9	31.92±4.29	110.1

± Standard deviation.

Between treatments ($P < 0.001$) and between periods ($P < 0.001$).

Tissue	Value (ppm dry wt)
Liver	157.74
Intestine	116.01
Kidney	75.56
Skin	72.04
Gills	53.13
Muscle	26.00

The sufficient and required quantity of ovary was not available for estimation of heavy metals for the present study, as the size selection of L. parsia was such from smaller animals it was difficult to get it (APHA-AWWA-WPCF, 1976). The zinc accumulation in muscle was found less both in experiments as well as at field centres.

Liver: From analysis of variance, very high significant differences (at 0.1% level) were observed within treatments, between exposure periods and within their interactions. The higher mean bioaccumulation was recorded for $\frac{1}{10}$ th 96 hr LC50 followed by $\frac{1}{100}$ th 96 hr LC50 and control in 3rd week followed by 2nd and 1st week. The gradual increase in bioaccumulation over the time was seen for $\frac{1}{100}$th 96 hr LC50 (Table 108). In the case of $\frac{1}{10}$ th 96 hr LC50 the higher bioaccumulation was recorded in 2nd week.

Gills: The ANOVA indicated very high significant differences (at 0.1% level) among the treatments and the periods (Table 109). But their interactions were found significant at 1% F-value. The mean bioaccumulation seen higher for $\frac{1}{10}$ th 96 hr LC50 followed by $\frac{1}{100}$ th 96 hr LC50 and

control. The highest mean values were recorded in the 3rd week followed by 2nd and the lowest in 1st week. The gradual increase in bioaccumulation was noticed in $\frac{1}{100}$ the 96 hr LC50 from 1st week to 3rd week. But in $\frac{1}{10}$ th 96 hr LC50 higher value was obtained in the 2nd week.

Kidney: From two-way ANOVA on the accumulation of zinc by kidney of L. parsia exposed to different concentrations of heavy metals in different exposure periods (Tale 110), very high significant differences were seen among them at 0.1% F-value. The interactions of periods with the tratment were also seen significant at 0.1% level. The mean bioaccumulation was seen highest for $\frac{1}{10}$ th 96 hr LC50 followed by $\frac{1}{100}$ th 96 hr LC50 and control. Similarly highest mean was seen in 2nd week followed by 3rd and 1st one. The gradual increase in bioaccumulation from 1st week to 3rd week was noticed in $\frac{1}{100}$ th 96 hr LC50. But higher bioaccumulation was observed in 2nd week in case of exposure to $\frac{1}{10}$ th 96 hr LC50.

Intestine: Among the exposure periods, treatments and their interactions, the differences were seen at 0.1% F-value (Table 111). The higher mean bioaccumulation of zinc was recorded in intestine of L. parsia exposed to $\frac{1}{10}$ th 96 hr LC50 followed by $\frac{1}{100}$ th 96 hr LC50 and control. Among exposure periods the 2nd week recorded higher mean bioaccumulation than 3rd and 1st week.

Skin: The ANOVA indicated very high significant differences (at 0.1% level) among the exposure periods, treatments and their interactions (Table 112). The skin of L. parsia in $\frac{1}{10}$ th 96 hr LC50 showed higher mean

bioaccumulation of zinc than $\frac{1}{10}$ th 96 hr LC50 and control. The mean accumulation was maximum in 3rd week followed by 2nd and minimum in the 1st week. The percentage variation in bioaccumulation from control calculated for sublethal concentrations (Table 112) indicated the gradual increase in it from 1st week to 3rd week.

Muscle: Very high significant differences were noticed from the analysis of variance among the treatments, exposure periods and their interactions. The mean bioaccumulation was high in $\frac{1}{10}$ th 96 hr LC50 followed by $\frac{1}{100}$ th 96 hr LC50 and control (Table 113). In sublethal concentrations the high mean bioaccumulation was recorded in the 2nd week followed by 3rd and least in the 1st week.

Discussion

Early interest in the environmental behaviour of zinc was stimulated by the observation of Japanese scientists that radio-zinc-65 from fall out was concentrated strongly in tunas. Subsequently, the nuclide was reported in large amounts in many species of marine organisms when compared to ambient sea water (Eisler, 1981). This lead to the study of zinc in marine organisms in subsequent years. Concentrations of zinc in different tissues of fish from the field worldover are already discussed in "Chapters 1 and 2; Section : Zinc in tissue".

According to Pentreath (1976), the direct accumulation of zinc from seawater plays only a minor role relative to food in the metabolism by the Flatfish Pleuronectes platessa. In the present study the differential

accumulations were seen in different tissues over time. But the accumulation of zinc in tissues was seen a function of concentration in the water. The accumulation was high in tissues of L. parsia exposed to high concentration of zinc in water. The accumulation in experiments was seen slightly higher or similar in the tissues of L. parsia with that obtained from different observation centres.

As observed from other field centres (Chapter 1, Section : Zinc in tissue), the liver accumulated maximum amount of zinc and the muscle minimum in the experiments. The zinc in ovary could not be estimated in the experiments due to nonavailability of sufficient quantity of ovary. Evtushenko et al. (1984) have presented the results on the effect of different concentrations of zinc in solution on its accumulation in the organs and tissues of carp Cyprinus carpio and the incorporation rate of radio labelled C^{14} from sodium acetate into the liver proteins. The extent of zinc accumulation in the organs and tissues and the rate of incorporation of radioactive carbon into liver proteins were determined by the concentration in water and duration of exposure. Similar results were also obtained in the present study, but in most of the tissues the "Saturation level" of zinc was reached within 2 weeks of exposure to $\frac{1}{10}$th 96 hr LC50.

The interrelationship of zinc and copper in kidney of Limanda limanda has been explained by Overnell and McIntosh (1988) stating that the reduction in one would increase in accumulation of the other. Similarly in the present study the variations in zinc accumulation in different tissues of L. parsia may be linked with the copper accumulation in same tissues.

Both the metals possess the similar properties in Periodic Tale (Wittmann, 1979). But the clear dynamics of both the metals in different tissues is not fully understood. Dogfish Scyliorhinus canicula exposed to 10 ppm zinc for 3 weeks displayed an increase in zinc content of the gills, kidney and intestine (Crespo et al., 1981). An increase in copper concentration of the gills was also recorded which suggests that copper and hence zinc and other heavy metals, are excreted via gills when the kidney and intestine are overloaded (Crespo et al., 1981). Similarly in the present study after liver; the intestine and kidney of L. parsia recorded higher concentration of zinc. This indicates that the rate of uptake is greater than the rate of excretion of zinc.

Metallothionein-like protein i.e. nonthionein capable of binding zinc has been described in liver and kidney of Salmo gairdneri induced by 7 mg/kg injection of divalent zinc ion (Pierson, 1985). The accumulation of zinc in liver and kidney of L. parsia is likely by binding of zinc to metallothionein-like protein. Zinc accumulation in liver of Dogfish Scyliorhinus canicula following subacute contamination is descried by Flos et al. (1979).

Zinc accumulated in the gills, reaching a peak value on 4th day which did not increase although the treatment was prolonged upto 25 days with 15 ppm of zinc in the medium (Crespo et al., 1979). They conducted the experiments on Dogfish. Zinc accumulation in the storage organs (spleen, liver and pancreas) increased following the 7th day of treatment (Flos et al., 1979). Similarly in the present study except skin; in all the tissues the maximum accumulation of zinc was recorded on 14th day of exposure of $\frac{1}{10}$-th 96 hr LC50, most probably due to lesser

14th day of exposure to $\frac{1}{10}$ th 96 hr LC50, most probably due to lesser concentration in the medium. In the case of intestine and muscle similar results were obtained in all the exposure concentrations at 14th day. Subsequently the decrease in zinc content was noticed which needs further detailed studies to know the reasons. Crespo et al. (1981) reported marked increase in zinc content in kidney and intestine of Dogfish after treatment with 10 ppm zinc for 3 week, suggesting that these two organs may play an important role in the excretion of zinc. The decreased content of zinc in gills of L. parsia has already been discussed under "Bioaccumulation of copper in chronic exposures".

In the present study the intestine of L. parsia accumulated low concentration of zinc, exposed to zinc contaminations in experiments for 3 weeks than 2 weeks. The intestinal corpuscules limit the entry of metals through the intestinal wall and themselves excreted with the faeces (Noel-Lambot, 1981). The same reason can be attributed to the decrease of zinc in intestine of L. parsia in 3rd week of exposure.

Among teleosts, the specific sites of zinc accumulation, are viscera and gonad; muscle usually contain the lowest zinc residues (Eisler, 1981). According to Phillips (1977) least accumulation of metals take place in muscle tissue, which corroborates the findings of the present experimental study that the muscle accumulated less amount of zinc than other tissues. Similar results are also found in different field centres and discussed in Chapters 1 and 2.

The uptake of zinc by fish from the surrounding water can occur via three routes: gills, body surface (skin) and the alimentary canal, and also by temporary storage within tissues (Bryan, 1971; Murphy et al., 1978). The above reasons may be the fact to explain the increase of zinc in skin from initial to the 3rd week of exposure to different concentrations of metals.

Bioaccumulation Factor

The Bioaccumulation Factor in different tissues studied in different sublethal concentrations in different exposure periods are presented in Tables 114-119. Analyses of data for specific tissues, the grand mean values obtained from computer were:

Tissue	Value
Intestine	3004.48
Liver	3002.36
Skin	2809.66
Gills	2086.30
Kidney	2024.82
Muscle	821.43

As obtaind in other field centres including Cochin Backwater (Chapters 1 and 2) the muscle recorded the lowest Bioaccumulation Factor in experiments.

Liver: The Bioaccumulation Factor of zinc in liver of L. parsia obtained in experiments is presented in Table 114. The ANOVA indicated very

TABLE 114. **Bioaccumulation Factor of zinc in liver of L. parsia exposed to 1:1:1 ratio of lead nitrate: copper sulphate: zinc sulphate at different sub-lethal concentrations in different exposure periods**

Exposure period (weeks)	Control tissue factor	Sub-lethal concentrations			
		$\frac{1}{100}$ th 96 hr LC50		$\frac{1}{10}$ th 96 hr LC50	
		Tissue factor	% variation from control	Tissue factor	% variation from control
0	1941.0±564.2	1941.0± 564.2	0	1914.0±564.2	0
1	2001.5±118.3	411.0± 26.8	-79.5	68.8± 2.6	-96.6
2	1816.6±151.1	613.7± 44.2	-66.2	96.0± 2.9	-94.7
3	1919.3±101.8	698.7± 42.7	-63.6	88.4± 3.6	-95.4

± Standard deviation.

Between treatments ($P < 0.001$).

TABLE 115. **Bioaccumulation Factor of zinc in gills of L. parsia exposed to 1:1:1 ratio of lead nitrate: copper sulphate: zinc sulphate at different sub-lethal concentrations in different exposure periods**

Exposure period (weeks)	Control tissue factor	Sub-lethal concentrations			
		$\frac{1}{100}$ th 96 hr LC50		$\frac{1}{10}$ th 96 hr LC50	
		Tissue factor	% variation from control	Tissue factor	% variation from control
0	892.6±161.2	892.6±161.2	0	892.6±161.2	0
1	989.3± 88.2	111.3± 8.7	-88.8	11.9± 0.2	-98.8
2	1008.5±179.7	142.3± 8.9	-85.9	15.6± 1.3	-98.5
3	1070.3± 84.6	155.2± 10.2	-85.5	16.0± 0.9	-98.5

± Standard deviation.

Between treatments ($P < 0.001$).

TABLE 116. **Bioaccumulation Factor of zinc in kidney of L. parsia exposed to 1:1:1 ratio of lead nitrate: copper sulphate: zinc sulphate at different sub-lethal concentrations in different exposure periods**

Exposure period (weeks)	Control tissue factor	Sub-lethal concentrations			
		$\frac{1}{100}$ th 96 hr LC50		$\frac{1}{10}$ th 96 hr LC50	
		Tissue factor	% variation from control	Tissue factor	% variation from control
0	1721.9±273.9	1721.9±273.9	0	1721.9±273.9	0
1	1676.6±152.0	303.2± 11.6	-81.9	22.6± 2.3	-98.7
2	1433.3± 87.0	398.9± 25.6	-72.2	53.1± 2.4	-96.3
3	1434.6± 94.0	405.0± 60.5	-71.8	43.1± 1.7	-97.0

± Standard deviation.

Between treatments($P < 0.001$).

TABLE 117. **Bioaccumulation Factor of zinc in intestine of L. parsia exposed to 1:1:1 ratio of lead nitrate: copper sulphate: zinc sulphate at different sub-lethal concentrations in different exposure periods**

Exposure period (weeks)	Control tissue factor	Sub-lethal concentrations			
		$\frac{1}{100}$ th 96 hr LC50		$\frac{1}{10}$ th 96 hr LC50	
		Tissue factor	% variation from control	Tissue factor	% variation from control
0	1597.3±218.9	1597.3±218.9	0	1597.3±218.9	0
1	1768.1± 96.0	257.4± 14.7	-85.4	20.0± 1.1	-98.9
2	2056.4±168.3	371.8± 8.5	-81.9	101.5± 4.3	-95.1
3	2131.0±130.3	369.2± 15.4	-82.7	71.4± 7.0	-96.6

± Standard deviation.

Between treatments($P < 0.001$) and between periods ($P < 0.001$).

TABLE 118. **Bioaccumulation Factor of zinc in skin of L. parsia exposed to 1:1:1 ratio of lead nitrate: copper sulphate: zinc sulphate at different sub-lethal concentrations in different exposure periods**

Exposure period (weeks)	Control tissue factor	Sub-lethal concentrations			
		1/100 th 96 hr LC50		1/10 th 96 hr LC50	
		Tissue factor	% variation from control	Tissue factor	% variation from control
0	2385.0±282.3	2385.0±282.3	0	2385.0±282.3	0
1	2685.3±148.7	271.7± 16.9	-89.9	31.1± 1.4	-98.8
2	2598.3±167.7	282.9± 19.9	-89.1	45.1± 3.4	-98.3
3	2568.0±156.2	361.2± 9.5	-85.9	46.3± 2.0	-98.2

± Standard deviation.

Between treatments($P < 0.001$).

TABLE 119. **Bioaccumulation Factor of zinc in muscle of L. parsia exposed to 1:1:1 ratio of lead nitrate: copper sulphate: zinc sulphate at different sub-lethal concentrations in different exposure periods**

Exposure period (weeks)	Control tissue factor	Sub-lethal concentrations			
		1/100 th 96 hr LC50		1/10 th 96 hr LC50	
		Tissue factor	% variation from control	Tissue factor	% variation from control
0	510.0±26.3	510.0±26.3	0	510.0±26.3	0
1	521.1±35.9	53.0 ± 1.6	-89.8	6.7± 0.8	-98.7
2	478.6±34.6	116.2± 9.7	-75.7	14.0± 1.0	-97.1
3	491.5±44.1	102.6± 3.1	-79.1	9.8± 1.3	-98.0

± Standard deviation.

Between treatments($P < 0.001$).

high significant difference (at 0.1% level) between the treatments. The maximum mean Bioaccumulation Factor was calculated for control followed by $\frac{1}{100}$ th 96 hr LC50 and minimum in $\frac{1}{10}$ th 96 hr LC50. This indicated the decrease of Bioaccumulation Factor in liver with the increase of concentration in medium. No significant differences were seen among the exposure periods and the interactions of periods with treatments. A very good and highly significant negative relationship ($r = -0.822$) was obtained in relating the bioaccumulation in liver with its factor for zinc. This relationship was superimposed with the relation obtained for the same in Cochin Backwater and the combined graph from computer (Fig.16) showed the decline in "Bioaccumulation Factor" from 200 ppm dry wt.

Gills: Among the treatments the differences were seen at 0.1% F-value. The higher mean Bioaccumulation Factor was calculated for control followed by $\frac{1}{100}$ th 96 hr LC50 and $\frac{1}{10}$ th 96 hr LC50 (Table 115). This indicated the decrease in "Bioaccumulation Factor" in gills of *L. parsia* with the increase of concentration in medium showing a inversely proportional trend. No significant differences were seen among the exposure periods and the interactions of periods with treatments. The negative and "Poor relationship" ($r = -0.425$) was obtained from the correlation study for bioaccumulation of zinc in gills with its factor.

Kidney: Very high significant difference (at 0.1% level) was seen among the treatments. The high mean Bioaccumulation Factor was calculated for control followed by $\frac{1}{100}$ th 96 hr LC50 and $\frac{1}{10}$ th 96 hr LC50 (Table 116). This results proved the decrease in Bioaccumulation Factor in

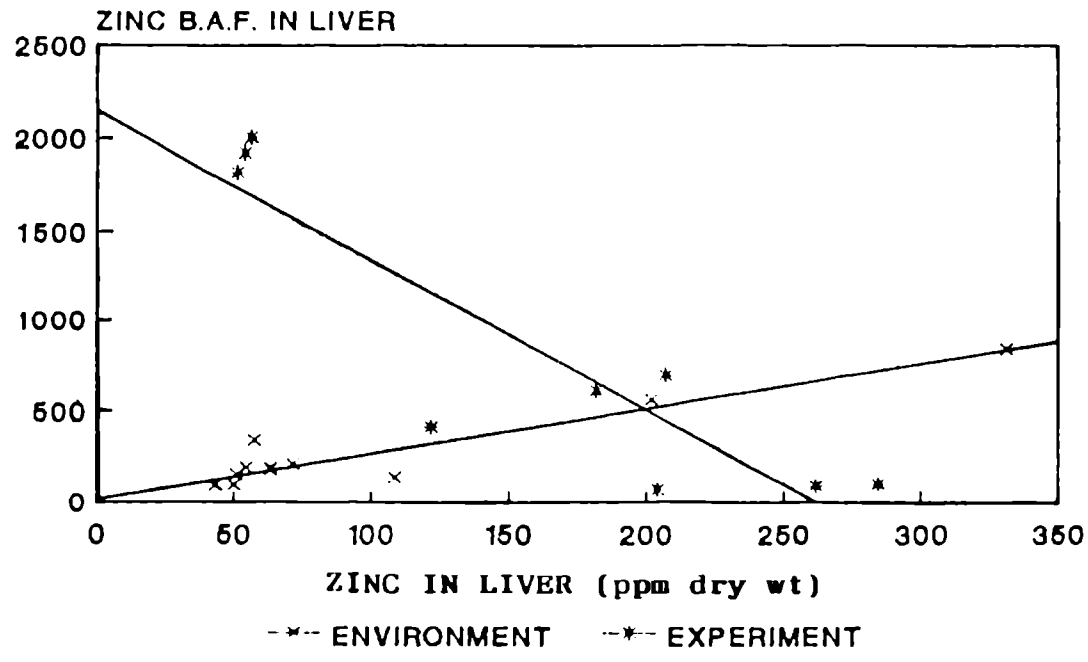


FIG. 16. Relationship between Bioaccumulation and Bioaccumulation Factor (B.A.F.) of zinc in Liver of L. parsia.

kidney for zinc with the increase in concentration in medium. No significant difference was seen between the exposure periods. The interactions of periods with treatments differed among themselves at 1% F-value. From correlation study the negative relationship (r = -0.690) was obtained between the bioaccumulation of zinc in kidney with that of Bioaccumulation Factor.

Intestine: Very high significant differenes (at 0.1% level) were seen among the treatments and exposure periods. But their interactions were seen significant at 5% F-value. The higher mean "Bioaccumulation Factor" was calculated and the trend was similar to all other tissues studied (Table 117). The gradual decrease in "Bioaccumulation Factor" was obtained with increase in the concentration of zinc in water. The high mean Bioaccumulation Factor was calculated for the intestine of L. parsia exposed for 3 weeks followed by 2 weeks and 1 week. In correlating the bioaccumulation of zinc with its factor in intestine a negative relationship was obtained (r = -0.566).

Skin: The Bioaccumulation Factor of zinc in skin of L. parsia obtained in the experiments is shown in Table 118. The highly significant (at 0.1% F-value) decrease was noticed for Bioaccumulation Factor from control to $\frac{1}{10}$ th 96 hr LC50. The highest mean factor was seen in the control followed by $\frac{1}{100}$ th 96 hr LC50 and $\frac{1}{10}$ th 96 hr LC50. The Bioaccumulation Factor showed the inverse relationship with zinc concentration in water (r = -0.651). No significant differences were seen among the exposure periods and the interactions of periods with treatments.

Muscle: From ANOVA it is seen very high significant difference (at 0.1% level) between the treatments. The highest mean Bioaccumulation Factor was calculated in the muscle of the fish in control followed by $\frac{1}{100}$ th 96 hr and $\frac{1}{10}$ th 96 hr LC50 (Table 119). The decrease in Bioaccumulation Factor was seen with the increase of the concentration in water medium during experimentation ($r = -0.529$). No significant difference was seen between the exposure periods. But the interactions of periods with treatments were seen significant at 5% F-value. The discussion is given in detail at the end of this chapter.

Lead

Accumulation in tissue

The accumulation of lead in different tissues of L. parsia exposed to different heavy metals and exposure periods are presented in Tables 120-125. The results indicated that the mean values obtained from computer were:

Tissue	Value
Kidney	556.70
Intestine	252.69
Liver	80.47
Gills	76.91
skin	47.99
Muscle	43.10

TABLE 120. **Accumulation of lead in liver of L. parsia exposed to 1:1:1 ratio of lead nitrate: copper sulphate: zinc sulphate at different sub-lethal levels in different exposure periods**

Exposure period (weeks)	Lead (ppm dry wt) in control tissue	Sub-lethal concentrations			
		$\frac{1}{100}$ th 96 hr LC50		$\frac{1}{10}$ th 96 hr LC50	
		Tissue content (ppm dry wt)	% variation from control	Tissue content (ppm dry wt)	% variation from control
0	7.66±0.62	7.66± 0.62	0	7.66±0.62	0
1	7.66±1.13	40.29±13.79	426.0	192.22±25.73	2409.3
2	7.71±0.66	49.22± 9.09	538.4	210.27±10.40	2627.2
3	10.51±1.20	55.01± 3.05	423.4	151.30±13.73	1339.6

± Standard deviation.

Between treatments($P < 0.05$) and between periods ($P < 0.001$).

TABLE 121. **Accumulation of lead in gills of L. parsia exposed to 1:1:1 ratio of lead nitrate: copper sulphate: zinc sulphate at different sub-lethal levels in different exposure periods**

Exposure period (weeks)	Lead (ppm dry wt) in control tissue	Sub-lethal concentrations			
		$\frac{1}{100}$ th 96 hr LC50		$\frac{1}{10}$ th 96 hr LC50	
		Tissue content (ppm dry wt)	% variation from control	Tissue content (ppm dry wt)	% variation from control
0	9.35±1.12	9.35± 1.12	0	9.35± 1.12	0
1	8.60±2.06	85.47± 4.35	893.8	109.30± 7.21	1170.9
2	7.40±1.87	84.49± 4.16	1041.8	11.77± 6.43	1450.9
3	5.84±1.49	109.32± 9.18	1771.9	166.98± 6.37	2759.2

± Standard deviation.

Between treatments($P < 0.001$) and between periods ($P < 0.001$).

TABLE 122. **Accumulation of lead in kidney of L. parsia exposed to 1:1:1 ratio of lead nitrate: copper sulphate: zinc sulphate at different sub-lethal levels in different exposure periods**

Exposure period (weeks)	Lead (ppm dry wt) in control tissue	Sub-lethal concentrations			
		1/100 th 96 hr LC50		1/10 th 96 hr LC50	
		Tissue content (ppm dry wt)	% variation from control	Tissue content (ppm dry wt)	% variation from control
0	133.62± 5.74	133.62± 5.74	0	133.62± 5.74	0
1	129.49±25.72	526.68±32.18	306.7	959.83±66.91	641.2
2	111.55±10.07	748.34±64.71	570.9	825.50±25.75	640.0
3	107.08± 6.37	1051.23±72.80	848.1	550.27±13.78	413.9

± Standard deviation.

Between periods ($P < 0.001$).

TABLE 123. **Accumulation of lead in intstine of L. parsia exposed to 1:1:1 ratio of lead nitrate: copper sulphate: zinc sulphate at different sub-lethal levels in different exposure periods**

Exposure period (weeks)	Lead (ppm dry wt) in control tissue	Sub-lethal concentrations			
		1/100 th 96 hr LC50		1/10 th 96 hr LC50	
		Tissue content (ppm dry wt)	% variation from control	Tissue content (ppm dry wt)	% variation from control
0	4.47± 2.25	4.47± 2.55	0	4.47± 2.55	0
1	6.17± 3.18	286.11±16.22	4537.1	140.40± 8.34	2176.2
2	7.95± 1.21	334.66±13.97	4109.6	660.98±18.39	8214.2
3	7.88± 2.62	335.50± 5.34	4157.6	575.54±51.80	7203.8

± Standard deviation.

Between treatments ($P < 0.001$) and between periods ($P < 0.001$).

TABLE 124. **Accumulation of lead in skin of L. parsia exposed to 1:1:1 ratio of lead nitrate: copper sulphate: zinc sulphate at different sub-lethal levels in different exposure periods**

Exposure period (weeks)	Lead (ppm dry wt) in control tissue	Sub-lethal concentrations			
		1/100 th 96 hr LC50		1/10 th 96 hr LC50	
		Tissue content (ppm dry wt)	% variation from control	Tissue content (ppm dry wt)	% variation from control
0	7.78±0.03	7.78± 0.03	0	7.78±0.03	0
1	8.35±0.65	35.21± 1.71	321.7	38.81±4.66	364.8
2	7.22±1.50	92.46±10.09	1180.6	94.09±6.00	1203.2
3	5.46±1.06	98.18± 5.55	1698.2	52.14±6.48	854.1

± Standard deviation.

Between treatments ($P < 0.001$) and between periods ($P < 0.001$).

TABLE 125. **Accumulation of lead in muscle of L. parsia exposed to 1:1:1 ratio of lead nitrate: copper sulphate: zinc sulphate at different sub-lethal levels in different exposure periods**

Exposure period (weeks)	Lead (ppm dry wt) in control tissue	Sub-lethal concentrations			
		1/100 th 96 hr LC50		1/10 th 96 hr LC50	
		Tissue content (ppm dry wt)	% variation from control	Tissue content (ppm dry wt)	% variation from control
0	19.63± 4.16	19.63± 4.16	0	19.63±4.16	0
1	17.28±3.82	30.04± 1.80	73.8	108.92±6.83	530.3
2	16.27±2.86	66.55± 6.12	309.0	35.38±4.42	117.5
3	15.14±1.39	26.55± 2.82	75.4	61.73±6.12	307.7

± Standard deviation.

Between treatments ($P < 0.001$) and between periods ($P < 0.001$).

Liver: The analysis of variance obtained from computer for accumulation of lead in liver of L. parsia in the experiments (Table 120) showed significant difference (at 5% level) between the treatments. The high mean bioaccumulation was recorded in the liver of fishes exposed to $\frac{1}{10}$ th 96 hr LC50 followed by $\frac{1}{100}$ th 96 hr LC50 and control. The differences among the bioaccumulation at different exposure periods were seen at 0.1% F-value. The mean highest value was estimated in 2nd week followed by 1st and 3rd week. The interactions of periods with treatments were differed among themselves at 0.1% level.

Gills: The accumulation of lead in gills of L. parsia obtained in experiments is presented in Table 121. Very high significant differences ($P < 0.001$) were seen among the exposure periods, treatments and their interactions. The highest mean bioacumulation was calculated in $\frac{1}{10}$ th 96 hr LC50 followed by $\frac{1}{100}$ th 96 hr LC50 and control. Similarly the longer exposure period i.e. 3 weeks showed higher bioaccumulation in gills than the shorter ones i.e. 2nd and 1st week.

Kidney: From two-way ANOVA, no significant difference was seen among the treatments. But very high significant differences (at 0.1% level) were obtained among the exposure periods and the interactions of periods with treatments. It was found high mean bioaccumulation in kidney of fishes in 3rd week followed by 2nd week and 1st week (Table 122).

Intestine: Very high significant differences (at 0.1% F-value) were seen among the treatments, exposure periods and the interactions of periods

with treatments. The $\frac{1}{10}$ th 96 hr LC50 showed maximum mean bioaccumulation with $\frac{1}{100}$ th 96 hr LC50 and control. The 2nd week recorded high mean bioaccumulation of lead in intestine than 3rd and 1st week (Table 123).

Skin: The results in accumulation of lead in skin of L. parsia observed in different experiments, are presented in Table 124. The analysis of variance indicated very high significant differences (at 0.1% level) among the exposure periods, treatments and the interactions of periods with treatments. The mean bioaccumulation of lead in skin of fishes are found in the order i.e. $\frac{1}{100}$ th 96 hr LC60, $\frac{1}{10}$ th 96 hr LC50 and control. Similar to that of intestine, the high mean value was obtained in 2 weeks of exposure followed by 3rd week and 1st week.

Muscle: From two-way ANOVA for bioaccumulation of lead in muscle in the experiments (Table 125), very high significant differences (at 0.1% F-value) were obtained within treatments, exposure periods and the interactions. The least mean bioaccumulation was calculated in muscle of fishes of control and then by $\frac{1}{10}$ th 96 hr LC50 and $\frac{1}{100}$ th 96 hr LC50. The first week showed high mean bioaccumulation in the muscle exposed for 2 weeks and 3 weeks.

Discussion

The average lead levels in the majority of fishes collected from U.S. coastal waters were 0.3-0.7 ppm in muscle and 0.2-0.6 ppm in liver;

no pattern was observed for mean lead levels in whole fish although these tend to exhibit higher concentrations than muscle and liver, possibly due to lead accumulation in hard tissues such as bone (Hall et al., 1978). Similar observations were recorded by other investigators at various collection sites (Eisler, 1981). Maximum concentration of lead in gills of L. parsia in the present study has also already been discussed in Chapter 1. The lowest level of the same was seen for muscle and kidney in different field centres (Chapter 1) and Cochin Backwater (Chapter 2) respectively. This trend of accumulation was seen in tissues of fishes when the availability of lead from aquatic environment is low in (ppb) level. When L. parsia was experimented allowing them in higher concentrations of lead in water (ppm), the accumulation trend was seen interesting in its different tissues. The kidney accumulated the highest amount of lead and muscle the least. Rest of the tissues came in between the both. Many fold accumulations were observed in all the tissues in experiment than that observed in different study centres. It is concluded that the accumulation was the result of higher uptake of lead by the tissues/ organs than the excretion. Except the skin and muscle the other tissues of L. parsia showed comparatively higher accumulation of lead as a function in the experimental medium. But in all tissues the differential accumulation of lead were found over time. Lead concentration showed gradual increase in kidney and gills in different concentrations over time.

Lead is excreted via kidney and after overloading the gill plays an important role as an external route for detoxification (Crespo et al., 1981). In the present study also the continuous overloading of lead was

seen in the kidney of L. parsia over exposure time and at the same time increase in lead was also recorded in gills. It may be attributed to the view explained by Crespo et al. (1981). In conditions of acute metal stress (except mercury), the gill tissue secretes large quantities of mucus as an excretory mechanism (Bryan, 1971). The mucus cells are dialated and more abundant (Bryan, 1971) in fish exposed to heavy metals suggesting an increased activity. This activity may be associated with the excretion of metal bound to mucus. But lead, having an affinity to hard parts (Phillips, 1977; Wittmann, 1979) might have been associated to the gill arches increasing the lead content in the gills in the present investigation.

Human activities influence the lead content of marine teleosts. Geographical variations in lead levels among California tidepool fishes undoubtedly reflects contamination of the intertidal zone by atmospheric lead pollution (Alley et al., 1974). It established the relation between the lead in water and tissues. Similarly from the present study in most of the tissues the higher accumulations were noticed in fishes exposed to higher concentrations of lead. Lead concentrations in spotted Wolf-fish Anarhichas minor from the vicinity of Galena mine and an ore dumping plant in West Greenland increased significantly since mining started (Bollingberg and Johansen, 1979). Major sites of accumulation were in liver and kidney of Wolf-fish.

Distinct tissue-specific accumulation rates were found in Gillichthys mirabilis : spleen, gill, fin and intestine accumulated the greatest amount of lead, while liver and muscle accumulated the least amount of lead (Somero et al., 1977). Similarly in experiments with L. parsia accumulated

higher amount of lead in kidney and lower amount in muscle and skin. Skin is essentially impermeable to water and solutes (Bartholomew et al., 1972). Somero et al. (1977) suggested that the turnover of lead in these mucus covered tissues (skin, intestine and gills) was a result of lead complexing with mucus and subsequent loss of this complex when the mucus layer is sloughed. In the case of L. parsia the higher lead was seen in intestine (after kidney) of the fish and the lesser lead accumulation in muscle and skin. In the laboratory study conductedby Kralj-Klobucar and Spasojevic (1989) on one year old Carp Cyprinus carpio exposing them to lead nitrate, reported the maximum accumulation at liver and kidney and not detected in skin. The above findings, strengthen the results obtained from the present investigator.

The intra tissue competition for accumulation of different metals cannot be overlooked. A tissue never accumulates only one metal, where such competition will not arise. According to Eisler (1981) zinc content of seawater can modify uptake of lead and other heavy metals by fishes. For example, lead and cadmium are accumulated 10 fold at higher zinc levels (Harve et al., 1972). In the laboratory study with L. parsia exposed to the combination of metals copper, zinc and lead; the accumulation of zinc did not record many fold increase, but at the same time lead in tissues increased many folds as discussed earlier. The possible reason for the same may be the presence of zinc in experimental medium in substantial amount. From the study, it is concluded that zinc, being an essential element for organisms, might have been regulated by the body tissue and lead being an non-essential element, is not regulated by the organism thus increasing its content in the tissues/organs.

Bioaccumulation Factor

The Bioaccumulation Factor in relation to water for different tissues of L. parsia exposed to different sublethal concentrations in different exposure periods are given in Tables 126-131. Mean Bioaccumulation Factors from the experiments found from computer analyses were:

Kidney	12314.57
Muscle	1642.79
Intestine	1238.07
Liver	920.35
Gills	843.15
Skin	788.00

Liver: The two-way ANOVA for the Bioaccumulation Factor of lead in liver of L. parsia in different experimental conditions (Table 126) indicated very high significant difference (at 0.1% level) between the treatments and high significant differences (at 1% level) among the exposure periods and the interactions of periods with treatments. The control showed high mean Bioaccumulation Factor followed by $\frac{1}{100}$ th 96 hr LC50 and $\frac{1}{10}$ th 96 hr LC50. This showed the decrease of Bioaccumulation Factor with the increase of concentration of lead in water. The high mean Bioaccumulation Factor was calculated for 3 weeks of exposure followed by 2nd weeks and 1st week. The relationship of accumulation of lead of Bioaccumulation Factor in liver produced a inverse relationship($r = -0.666$) between them.

TABLE 126. **Bioaccumulation Factor of lead in liver of L. parsia exposed to 1:1:1 ratio of lead nitrate: copper sulphate: zinc sulphate at different sub-lethal concentrations in different exposure periods**

Exposure period (weeks)	Control tissue factor	Sub-lethal concentrations			
		$\frac{1}{100}$ th 96 hr LC50		$\frac{1}{10}$ th 96 hr LC50	
		Tissue factor	% variation from control	Tissue factor	% variation from control
0	583.9±47.1	583.9±47.1	0	583.9±47.1	0
1	583.9±85.8	47.8±16.4	-91.8	22.8± 3.0	-96.1
2	587.7±49.9	58.3±10.8	-90.1	24.9± 1.3	-95.8
3	801.4±91.4	65.2± 3.6	-91.9	17.9± 1.6	-97.8

± Standard deviation.

Between treatments($P < 0.001$) and between periods ($P < 0.01$).

TABLE 127. **Bioaccumulation Factor of lead in gills of L. parsia exposed to 1:1:1 ratio of lead nitrate: copper sulphate: zinc sulphate at different sub-lethal concentrations in different exposure periods**

Exposure period (weeks)	Control tissue factor	Sub-lethal concentrations			
		$\frac{1}{100}$ th 96 hr LC50		$\frac{1}{10}$ th 96 hr LC50	
		Tissue factor	% variation from control	Tissue factor	% variation from control
0	500.7±60.1	500.7±60.1	0	500.7±60.1	0
1	460.9±110.2	71.2± 3.6	-84.6	9.1± 0.6	-98.0
2	396.4±100.2	70.4± 3.7	-82.2	9.6± 0.5	-97.6
3	312.9± 79.6	91.0± 7.7	-70.9	13.9± 0.5	-95.6

± Standard deviation.

Between treatments($P < 0.001$).

TABLE 128. **Bioaccumulation Factor of lead in kidney of L. parsia exposed to 1:1:1 ratio of lead nitrate: copper sulphate: zinc sulphate at different sub-lethal concentrations in different exposure periods**

Exposure period (weeks)	Control tissue factor	Sub-lethal concentrations			
		$\frac{1}{100}$ th 96 hr LC50		$\frac{1}{10}$ th 96 hr LC50	
		Tissue factor	% variation from control	Tissue factor	% variation from control
0	12090.7± 519.8	12090.7±519.8	0	12090.7±519.8	0
1	11716.7±2327.1	740.9± 59.3	-93.7	135.0± 9.4	-98.8
2	10093.4±1363.2	1052.6± 91.0	-89.6	116.1± 3.4	-98.8
3	9689.5± 576.9	1478.7±102.4	-84.7	77.4± 2.0	-99.2

± Standard deviation.

Between treatments($P < 0.001$).

TABLE 129. **Bioaccumulation Factor of lead in intestine of L. parsia exposed to 1:1:1 ratio of lead nitrate: copper sulphate: zinc sulphate at different sub-lethal concentrations in different exposure periods**

Exposure period (weeks)	Control tissue factor	Sub-lethal concentrations			
		$\frac{1}{100}$ th 96 hr LC50		$\frac{1}{10}$ th 96 hr LC50	
		Tissue factor	% variation from control	Tissue factor	% variation from control
0	337.5±192.6	337.5±192.6	0	337.5±192.6	0
1	465.7±240.5	392.9± 16.4	-27.9	16.5± 1.0	-96.5
2	600.1± 91.4	392.9± 16.4	-34.5	77.6± 2.2	-87.1
3	595.0±198.0	393.8± 6.3	-33.8	67.6± 6.1	-88.6

± Standard deviation.

Between treatments($P < 0.001$).

TABLE 130. **Bioaccumulation Factor of lead in skin of L. parsia exposed to 1:1:1 ratio of lead nitrate: copper sulphate: zinc sulphate at different sub-lethal concentrations in different exposure periods**

Exposure period (weeks)	Control tissue factor	Sub-lethal concentrations			
		$\frac{1}{100}$ th 96 hr LC50		$\frac{1}{10}$ th 96 hr LC50	
		Tissue factor	% variation from control	Tissue factor	% variation from control
0	781.4± 3.5	781.8± 3.5	0	781.8± 3.5	0
1	838.7± 65.0	55.0± 2.7	-93.4	6.0± 0.7	-99.3
2	725.2±150.6	144.4± 15.8	-80.1	14.7± 0.9	-98.0
3	548.6±106.2	153.4± 8.7	-72.0	8.2± 1.0	-98.5

± Standard deviation.

Between treatments($P < 0.001$).

TABLE 131. **Bioaccumulation Factor of lead in muscle of L. parsia exposed to 1:1:1 ratio of lead nitrate: copper sulphate: zinc sulphate at different sub-lethal concentrations in different exposure periods**

Exposure period (weeks)	Control tissue factor	Sub-lethal concentrations			
		$\frac{1}{100}$ th 96 hr LC50		$\frac{1}{10}$ th 96 hr LC50	
		Tissue factor	% variation from control	Tissue factor	% variation from control
0	1061.2±176.5	1061.2±176.5	0	1061.2±176.5	0
1	1198.0±264.5	32.4± 1.9	-97.3	11.7± 0.7	-99.0
2	1127.8±198.6	71.7± 6.6	-93.6	3.8± 0.5	-99.7
3	1049.7± 96.5	28.6± 3.0	-97.3	6.6± 0.7	-99.4

± Standard deviation.

Between treatments($P < 0.001$).

Gills: From two-way ANOVA, very high significant difference (at 0.1% level) was observed in Bioaccumulation Factor for gills between the treatments. The high mean Bioaccumulation Factor was calculated in the gills of the fishes in control and then $\frac{1}{100}$ th 96 hr LC50 and $\frac{1}{10}$ th 96 hr LC50 (Table 127). The decrease in the Bioaccumulation Factor was noticed with the increase in concentration in the medium. No significant difference was seen among the exposure periods and the interactions of the periods with the treatments. A good and nagative correlation ($r = -0.875$) was obtained between the bioaccumulation of lead and KB in gills. The results obtained in Cochin Backwater (Chapter 2) showed the positive relationship for bioaccumulation and KB. These two relationships were superimposed and the graph obtained from computer showed the concentration of 15 ppm dry wt as the break-up point for Bioaccumulation Factor for lead in gills (Fig. 17).

Kidney: From ANOVA for the Bioaccumulation Factor of lead in kidney of L. parsia exposed to different sublethal concentrations for varying periods (Table 128), very high significant difference was seen among the treatments at 0.1% F-value. The high mean Bioaccumulation Factor was seen in kidney of fishes in control followed by $\frac{1}{100}$ the 96 hr LC50 and $\frac{1}{10}$ th 96 hr LC50. The decrease in Bioaccumulation Factor was seen with the increease in concentration of lead in the medium. No significant difference was seen among the exposure periods and th interactions of periods with treatments. A good and negative relationship ($r = -0.759$) was obtained between the bioaccumulation of lead and the Bioaccumulation Factor in kidney. A good positive relationship was also obtained in Cochin Backwater and discussed earlier. The relationships

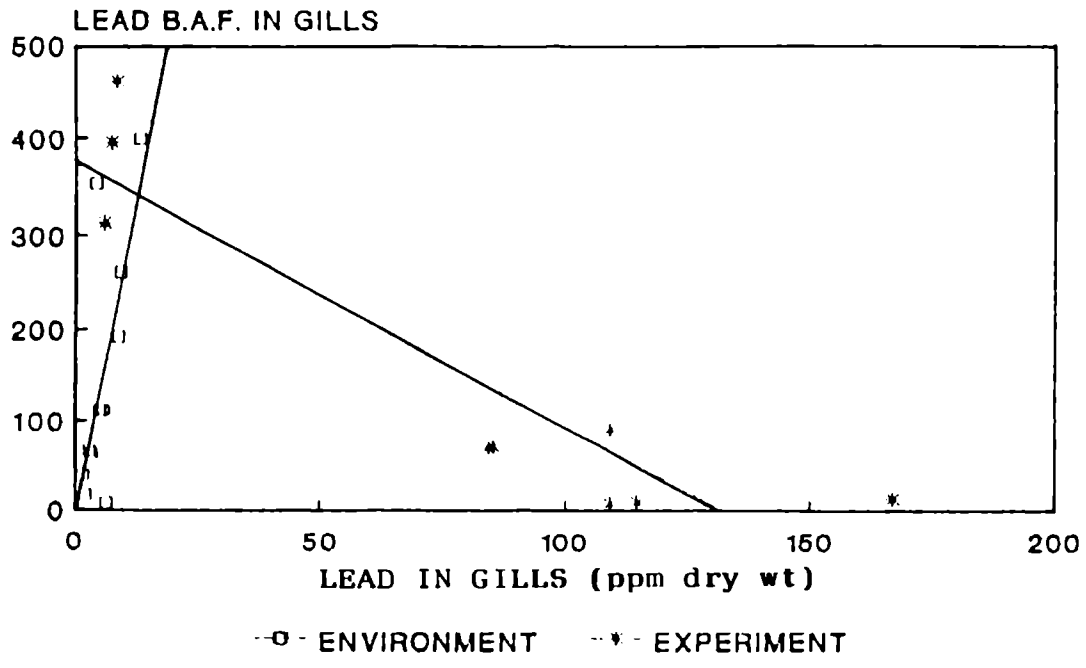


FIG. 17. Relationship between Bioaccumulation and Bioaccumulation Factor (B.A.F.) of lead in Gills of L. parsia.

found between the bioaccumulation and its factor (KB) in the laboratory experiments and in the stations at Cochin Backwater was contradicting each other, i.e. negative relation in the laboratory tests and positive relation in the Cochin Backwater. They were superimposed (Fig. 18) to get the break-up concentration in the computer, where the Bioaccumulation Factor for lead in kidney starts declining. The concentration found was 7 ppm dry wt.

Intestine: The analysis of variance obtained from computer showed very high significant difference (at 0.1% level) between the treatments. The mean Bioaccumulation Factor was high as calculated for lead in intestine of fishes in control followed, by $\frac{1}{100}$ th 96 hr LC50 and $\frac{1}{10}$ th 96 hr LC50 (Table 129). The Bioaccumulation Factor was seen decreasing with increasing of concentration in water. No significant difference was seen among the exposure periods and the interactions of periods with treatments. From the correlation study the negative relationship ($r = -0.647$) was obtained between the bioaccumulation of lead and the Bioaccumulation Factor in intestine.

Skin: The very high significant differences (at 0.1% F-value) were seen among the treatments. The mean Bioaccumulation Factor was seen high in the skin of fishes in control followed by $\frac{1}{100}$ th 96 hr LC50 and $\frac{1}{10}$th 96 hr LC50 (Table 130). The increase in concntration of lead in the experimental water coincided with the decrease of Bioaccumulation Factor. Between the exposure periods and the interactions of the periods with treatments, the differenc was seen monsignificant. The reverse relationship ($r = -0.610$) was obtained from the correlation study between the bioaccumulation and the Bioaccumulation Factor of lead in skin.

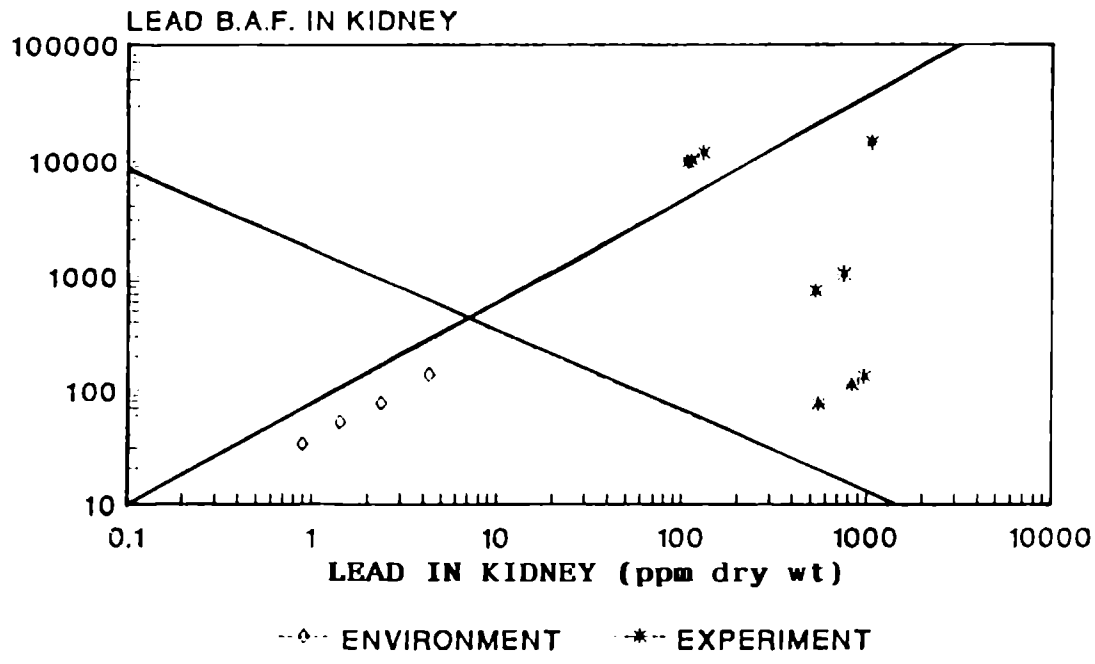


FIG. 18. Relationship between Bioaccumulation and Bioaccumulation Factor (B.A.F.) of lead in Kidney of L. parsia.

Muscle: Table 131 presents the Bioaccumulation Factor of lead in muscle of L. parsia in different experimental conditions and exposure periods. The treatments were differed among themselves at 0.1% F-Value. The mean Bioaccumulation Factor was calculated in muscle of L. parsia in control and it was high followed by $\frac{1}{100}$ th 96 hr LC50 and $\frac{1}{10}$ th 96 hr LC50. The decrease in Bioaccumulation Factor was seen with the increase of concentration of lead in experimental medium. The exposure periods and the interactions of periods with treatments did not differ significantly. The poor negativerelationship (r = -0.396) was obtained from the correlation study between the bioaccumulation of lead and its factor (KB) in muscle of L. parsia.

Discussion

The Bioaccumulation Factor of copper, zinc, and lead in different tissues of fishes and other marine organisms collected from the fields is already discussed in Chapter 1 and 2. The publishedliterature or reports regarding Bioaccumulation Factor are scanty on fishes and its tissues and organs.

Bioaccumulation (ppm) was determined by calculating a Bioaccumulation Factor $\frac{\text{PCB in whole fish}}{\text{PCB in water}}$ for Fathead minnows in laboratory (Peterson and Guiney, 1979). It was found 32,000 - 274,000; 60,000 - 120,000; 46,000 - 307,000 and 160,000 - 270,000 for Aroclor 1242, 1248, 1254 and 1260 respectively. The Bioaccumulation Factor was increased as the duration of exposure increased. Their findings demonstrated that

that to achieve a steady state concentration of PCBs in fish, when exposed to the compounds in water, a long duration of exposure was needed. The present study for Bioaccumulation Factor in different tissues of L. parsia exposd to metals in combination revealed interesting findings similar to that of PCBs and discussed.

An attempt was made to study th Bioaccumulation Factor of heavy metals in different tissues/organs of L. parsia in the experiments. These findings were correlated with that of bioaccumulation of same metals in the tissues. The Bioaccumulation Factors for different metals in different tissues of L. parsia showed positive relationship with the contents observed in different field centres and elaborately discussed in Chapter 1 and 2. In the laboratory experiments the same was seen significantly negative indicating that the increase of bioaccumulation with increase of concentration will reduce the Bioaccumulation Factor. In the environments the metals are available in low amount i.e. in parts per billion (ppb). The same were added in the experimental medium in higher amount i.e. in parts per million (ppm). When the level of metals were more in medium, the accumulation was seen higher in all tissues. At the same time the factor (KB) decreased. It is concluded that the increase in Bioaccumulation was "less than proportionately" and the same in the environments was "more than proprtionately" as revealed from the computer graphs. Most of the significant findings from the environments and experiments for bioaccumulation and its factor were superimposed (Figs. 15, 16, 17 and 18) to find out the level of respective heavy metal where the factor (KB) declined for a specific tissue. This proved that the tissues cannot accumulate metals beyond certain limits even though the same

is available in the environment abundantly. In most of the cases, the significant differences were not seen between the exposure periods. This definitely shows that the saturation limit of a tissue for heavy metal was reached within a short period of 14 days exposure. In all the cases for Bioaccumulation Factor for copper, zinc and lead varied significantly in different exposure concentrations. At lower concentrations (i.e. in control) the Bioaccumulation Factor was seen higher than $\frac{1}{100}$th 96 hr LC50 and $\frac{1}{10}$th 96 hr LC50.

Application Factor

Acute toxicity tests can provide meaningful comparison of toxicant lethality between animals or toxicants or test conditions. It can reliably monitor changes in the lethality of complex mixtures to an organism. But the acute toxicity test (bioassay), by itself, cannot adequately predict a concentration of toxicant unlikely to harm a population or ecosystem. When the question of interest is the prediction of a concentration unlikely to cause dreadful effects to a complex ecosystem and its population, additional informations for that purpose are essential. In most of the predictions, the "Application Factor" is actually used along with LC50 values. According to National Academy of Sciences and National Academy of Engineering (NAS/NAE, 1973) the "Application Factors" vary from 0.1 to 0.0001. The more conservative numbers are for bioaccumulative substances. But the estimates of "Safe" concentrations in environments can not be guaranted as valid (Buikema et al., 1982). An Application Factor ranging from 0.030 to 0.099 was calculated for zinc conducting

bioassay on Guppy Poecilia reticulata (Pierson, 1981). The Environmental Protection Agency (EPA) guidelines indicated that lead concentrations in both marine and freshwater systems should not exceed 1% of the 96 hr TLm i.e. 96 hr LC50 for sensitive species (Laws, 1981). In planning the present study, the 0.01 ($\frac{1}{100}$th 96 hr LC50) was first predicted to be the "Application Factor" for copper, zinc and lead. After statistical analyses of data for accumulation of Cu, Zn and Pb in different tissues of L. parsia, it is concluded that at that concentration the metals accumulated significantly over the tissues of the control animal. If it could have been nonsignificant with the accumulation of control animal, 0.01 could have been forecasted as the Application Factor for copper, zinc and lead. These experiments also proved that, 0.01 can not be used as an "Application Factor". Thus 0.001 or 0.0001 may be used along with 96 hr LC50 to calculate "safe" limit for environments (NSA/NAE, 1973).

All these above results and salient findings in Chapter 1 and 2 of Thesis are supported beyond doubt by the findings from Chapter 3.

From the whole laboratory studies under Chapter 3, it is concluded that most of the findings are significant as they are first from this type of investigation from India and are similar to the earlier observations elsewhere in the world. The mode of entry of copper, zinc and lead into the fish body, their dynamics and other regulatory measures and mechanisms are yet to be clearly understood by thorough experimental and radio isotope incorporation studies through "Autoradiography" or "EDAX by Electron Microscopy". Hence the results from this study form a basis for future research.

SUMMARY

SUMMARY

This thesis entitled "Effects of some heavy metals copper, zinc and lead on certain tissues of Liza parsia (Hamilton and Buchanan) in different environments" and the results obtained herein are based on the detailed investigations carried out by the candidate from january 1991 to December 1992 at 6 different estuaries and coastal waters along the east and west coasts of Peninsular India.

The investigations and the results are dealt with in three chapters preceded by "Preface", "Introduction", "Review of Literature" and "Materials and Methods".

The Chapter 1 deals with "Heavy metals copper, zinc and lead in water, in sediment and in different tissues of Liza parsia and their interrelationships with environmental conditions" at six different centres.

The significant findings in "Chapter 1" are:

1. The environmental parameters' salinity and total hardness have shown close relationship with heavy metals content in water.

Copper

2. Very high "significant" differences are observed for copper in water between centres, seasons and between surface and bottom waters.

3. The mean copper content in water at all the centres are found above the World Health Organisation (WHO) standard (3.0 ppb) and below the Environmental Protection Agency's (EPA of United States) "safe limit" (25.0 ppb).

4. Highly "significant" differences in copper in sediment among different centres, stations and in seasons are noticed.

5. Copper in sediments of the Cochin Backwater and Ennore Creek is recorded more than the "MESS-1" (29.3 ppm dry wt) and "BCSS-1" (23.4 ppm dry wt) standards.

6. In L. parsia, the highest content of copper is seen in liver and the lowest in muscle. Differences in copper in liver and intestine are encountered between centres and in ovary between seasons. No good and "significant" relations are reported between content of copper in tissues and water, and between tissues and sediment. Good and "significant" relations are found for copper between muscle and ovary, and muscle and skin.

7. All the tissues recorded higher level of copper compared to the "Standard Reference Material (SRM) of IAEA/Monaco" (4.5 ppm dry wt).

8. The "Bioaccumulation Factor" (KB) calculated for copper is seen the highest in liver, and the lowest in gills. Centrewise difference is seen for copper in liver only. The positive relation is established between the KB and the metal content for each specific tissue.

Zinc

9. "Significant" differences in zinc in water in different seasons, centres, and surface and bottom samples are reported. Zinc is related inversely, to salinity and total hardness, but it is "significant" with very good correlation coefficient (r = 0.707) with copper during postmonsoon.

In all the centres, zinc in water is found above WHO standard (5.0 ppb), but below the EPA "safe level" (100 ppb).

10. The difference in zinc in sediments is seen at 0.1% level between seasons, centres and stations. Zinc in water and sediment are related at 1% level with coefficient 0.744.

11. The sediment of Cochin Backwater has shown higher zinc than "MESS-1" (144 ppm dry wt) and "BCSS-1" (117 ppm dry wt) standards. The Cochin Backwater and Ennore Creek in their sediments show higher zinc than USGS rock standards (66.7-102.0 ppm dry wt).

12. The ovary and muscle of L. parsia show highest and lowest content of zinc respectively. Zinc in liver and intestine show difference between seasons and ovary, skin and muscle among the centres. Good and "significant" relation (r = 0.692 and at 1% level) is found for zinc in liver with gills.

13. Zinc content in all the tissues is higher than SRM/IAEA/Monaco standard (33.0 ppm dry wt).

14. As in the case of zinc in tissue, the "Bioaccumulation Factor" for ovary recorded highest and the muscle lowest. The differences between centres in Bioaccumulation Factor are found in liver, kidney, intestine, skin and muscle. The KB has shown positive relation to the zinc content in tissues.

Lead

15. No "significant" variation is found in lead in water between seasons, centres and surface and bottom samples. The difference was found only between years with 1992 over 1991.

16. Except certain collections at Ennore Creek, the mean lead concentrations in water at all centres are found below EPA safe limit for lead (100 ppb).

17. The "significant" differences are reported for lead in sediments in different years, seasons, centres and stations.

18. The sediment of Cochin Backwater is found polluted in comparison with USGS rock (31.3 ppm dry wt), "MESS-1" (42.9 ppm dry wt) and "BCSS-1" (19.7 ppm dry wt) standards.

19. The gills of L. parsia show highest lead content than other tissues studied. The 10% "significance" in lead content in liver of L. parsia between seasons at six centres are observed. No differences between centres in lead is observed in tissues. Very highly "significant" and good relations are observed in lead between water and

intestine, kidney and intestine, muscle and liver, muscle and gills, muscle and skin, and kidney and water.

20. The KB showed good positive relationships with lead content in all tissues.

General

21. Zinc is found more than copper and lead in liver, kidney, intestine, skin and muscle. In ovary the lead is more than copper and zinc; and in gills zinc is more than lead and copper.

22. The "Bioaccumulation Factor" is found high for zinc followed by copper and lead in all the tissues.

Monthly samples from Cochin Backwater during June 1991 to May 1992 were collected in the same pattern as in Chapter 1 to find out the monthly fluctuations in heavy metals in water, sediment and tissues, and water quality to compare the results obtained in Chapter 1 and to know whether the results in Chapter 2 "Monthly and seasonal variations in copper, zinc and lead in water, sediment and different tissues of Liza parsia in Cochin Backwater" substantiate and corroborate the findings of the Chapter 1.

The important results and findings from Chapter 2 are:

23. "Significant" monthly variations in copper, zinc and lead in water are ascertained in Cochin Backwater.

24. Only lead shows monthly "significant" variation in sediment

25. The differences in seasons in copper in skin, intestine and kidney are found "significant". The seasonal variation in zinc is also observed for gills, kidney, intestine and skin of L. parsia at Cochin Backwater.

Very good relations with higher significance are obtained on zinc content in sediment to that of gills, skin and intestine, which proves the brousing habit of L. parsia in sediment of Cochin backwater rich in zinc. The lead in intestine shows seasonal variation at 10% F-value. The direct bearings are established for lead in water with muscle and ovary, and found significant at 1% level.

26. The "Bioaccumulation Factor" for all three heavy metals is reported less in muscle of L. parsia. The intestine shows seasonal differene for KB at 10% F-value for lead in Cochin Backwater.

The Chapter 3 "Bioassay and chronic exposure studies on Liza parsia with heavy metals copper, zinc and lead" deals with the laboratory experiments on the test animal L. parsia to different concentrations of copper, zinc and lead to compare with that from the field observations. The experiments have shown very interesting and significant findings. The results and discussion in chapter 3 includes the "Bioassay", "Toxic unit" and "Chronic exposures". Some of the important findings are:

27. Based on 12 hr LC50, copper is found more toxic than zinc and lead. But, after 12 hrs of exposure, zinc become toxic followed by copper and lead.

28. The toxic unit (1.3489) observed here for copper, zinc and lead is greater than "unit" (1.0) showing the synergistic effect of heavy metals on L. parsia.

29. As discussed in Chapter 1 and 2, the accumulation of heavy metals in different tissues, is found almost similar in chronic exposures. The accumulation of heavy metals in tissues is seen as a function of concentration of metals in medium. Differential accumulation of metals is noticed in tissues over exposure time. Many fold increase in lead is seen in tissues of L. parsia in experiments and proved being a nonessential element corroborate the reports available. The lead is not regulated in the body.

30. The inverse relation observed between the "Accumulation" of metals in tissues with that of "Bioaccumulation Factor" establishes that the accumulation of a metal in tissues is not proportional to the increase of metal in the medium when applied even in higher doese.

These relationships are utilised to get the composite graph by supperimposing them to the results obtained in field studies. To get the composite graph, only the highly significant and good correlat ted results are utilized, as is the principle in statistics. These graphs are obtained for copper in kidney, zinc in liver and lead in gills and kidney. These composite graphs are well elucidated

in the Thesis and give the idea to what level a particular metal will be accumulated in a tissue in relation to the availability in the medium.

31. Based on the accumulation of heavy metals in different tissues of L. parsia and as significant differences are obsersed between the control and $\frac{1}{100}$th 96 hr LC50, it is evident from the results that the 0.01 fraction of 96 hr LC50 for copper, zinc and lead cannot be used as "Application Factor".

A list of literature referred and cited in the text and discussions is included in the "References" at the end.

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