INTRODUCTION
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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-Flores 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 up to actinides (Nair, 1984). The heavy metals are normally regarded 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)$_2$ (90%) and CuCO$_3$ (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)$_2$, CuCO$_3$ and Cu$^{2+}$ at low pH values and Cu(OH)$_2$ above pH 7.2.

Zinc undergoes less complexation than the other metals. At the average pH 8.1, 17% remains uncomplexed, 62% Zn (OH)$_2$, 6.4% ZnCl$^+$ and 5.8% ZnCO$_3$. The ion pairs ZnSO$_4$ and ZnCl$_2$ are both about 4%. At higher pH nearly all of the total zinc is in the form of Zn(OH)$_2$.

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$_3$ and PbCl$^+$ are present in nearly equal amount and there is an appreciable amount of PbCl$_2$. However, as pH increases, PbCO$_3$ 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 \text{Km}^3 and
the sewage and effluents added by the rivers to the sea per is \(50 \times 10^6\text{m}^3\text{yr}^{-1}\).
The solid waste and garbage generated by coastal population per year is
\(33 \times 10^6\) 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 harbours, 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 (Creek, 1985) and copper fungicides (Deharrai, 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 (Nossal 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 vice 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 divided 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 impact 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 membrane 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* (Pragatheeewaran, 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 heavy 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 in turn 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 pathways 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). Noel-Lambot (1981) found white mucus corpuscles in the intestinal lumen of fishes Anguilla anguilla, Myxocephalus scorpius, Serranus cabrilla, Moena chrysalis, and Scorpaena sp. In fish intoxicated with CdCl₂, ZnCl₂ or CuCl₂ added to sea water, mucus corpuscles 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 biomonitoring 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 livelihood. This needs an urgent monitoring of the amount of pollutants entering into 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 (Hunspreugs, 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 Mangrove 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); in 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 Sankaranarayanan 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 distribution 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 5-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 appetite 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 exposure 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 lysosomal membrane 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. (1978) 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); Heteropneustes 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 nigro* (Jaffer and Ashraf, 1988). Concentrations of zinc and copper are reported in dogfish (Sanpera and Vallribera, 1983) and *Catostomus commersonii* (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 throughout 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, 1985b; 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 nilotica* (Somsiri, 1982); zinc to Rainbowtrout *Salmo gairdneri* (Kodama et al., 1982; Meisner and Hum, 1987), *Lebistes reticulatus* (Sehagal and Saxena, 1986), *Tilapia zillii* 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 toxicity 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

<table>
<thead>
<tr>
<th>Name of Estuary/Area</th>
<th>Stations</th>
<th>Place</th>
<th>Coast</th>
<th>Latitude (North)</th>
<th>Longitude (East)</th>
<th>Source of Freshwater</th>
<th>Source of pollution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Korapuzha Estuary</td>
<td>1-3</td>
<td>Elathur, Calicut</td>
<td>Southwest</td>
<td>11°21'</td>
<td>75°44'</td>
<td>Agala Puzha, Pannur Puzha</td>
<td>Coconut husk retting, coir industry, fishing, etc.</td>
</tr>
<tr>
<td>(Code I)</td>
<td></td>
<td></td>
<td></td>
<td>11°24'</td>
<td>75°46'</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cochin Backwater</td>
<td>4-6</td>
<td>Cochin</td>
<td>Southwest</td>
<td>9°58'</td>
<td>75°20'</td>
<td>River Periyar and Chalakudy at northern side, Meenanchil Pamba, Moovattupuzha, Achaneedi and Manimala at Southern side</td>
<td>Domestic waste, effluents from Petroleum Refinery, Fertilizer Plant, Caprolactam Plant, Cochin Port, Cochin City, Fisheries Harbour, etc.</td>
</tr>
<tr>
<td>(Code III)</td>
<td></td>
<td></td>
<td></td>
<td>10°10'</td>
<td>76°20'</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tuticorin Bay</td>
<td>7-9</td>
<td>Tuticorin</td>
<td>Southeast</td>
<td>8°45'</td>
<td>78°29'</td>
<td>Upper Odai Creek and Korampallam Creek</td>
<td>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.</td>
</tr>
<tr>
<td>(Code III)</td>
<td></td>
<td></td>
<td></td>
<td>8°48'</td>
<td>78°12'</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gulf of Mannar and Palay Bay</td>
<td>10-12</td>
<td>Mandapam</td>
<td>Southeast</td>
<td>9°15'</td>
<td>79°5'</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>(Code IV)</td>
<td></td>
<td></td>
<td></td>
<td>9°20'</td>
<td>79°13'</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ennore Creek</td>
<td>13-15</td>
<td>Ennore, Madras</td>
<td>Southeast</td>
<td>13°20'</td>
<td>79°29'</td>
<td>Kotaliar River</td>
<td>Madras Metropolitan sewage, effluents from Kothari Chemicals Ltd., Alkali Chemicals, Madras Fertilizers Ltd., Petrochemicals, Ennore Thermal Power Plant, Madras Refineries, fishing, etc.</td>
</tr>
<tr>
<td>(Code V)</td>
<td></td>
<td></td>
<td></td>
<td>13°23'</td>
<td>79°31'</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rusikulya Estuary</td>
<td>16-18</td>
<td>Ganjam, Orissa</td>
<td>Northeast</td>
<td>19°22'</td>
<td>85°02'</td>
<td>Rusikulya River</td>
<td>Effluents from Chloro-Alkali Plant, fishing, etc.</td>
</tr>
<tr>
<td>(Code VI)</td>
<td></td>
<td></td>
<td></td>
<td>19°54'</td>
<td>85°08'</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
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 H₂S 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 bar mouth was approximately 4 km.
FIG. 1. SAMPLE COLLECTION CENTRES ALONG THE EAST AND WEST COASTS OF INDIA
1. KORAPUZHA ESTUARY, 2. COCHIN BACKWATER, 3. TUTICORIN BAY,
4. MANDAPAM, 5. ENNORE CREEK AND 6. RUSIKULYA ESTUARY
St 1 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 barmouth approximately 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 *Eichornia 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 mangrove 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°09' to 78°12'E and from 8°45' to 8°48'N. The pollutant 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 x 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 outlet 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 coast 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 Rama-chandran et al. (1991) this station comes under fourth category of (Polluted) area and sewage production is $0.45 \times 10^4$ 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 Elmore 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 outlet 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 CaCO₃, 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. VI 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 parsie*, 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 HNO₃ 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 throughout 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 HNO₃ 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 HNO₃ 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 aseptically 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 CaCO₃.

**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 guidelines 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**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atomization</td>
<td>Flame</td>
</tr>
<tr>
<td>Lamp current</td>
<td>7.50 mA</td>
</tr>
<tr>
<td>Wavelength</td>
<td>324.80 nm</td>
</tr>
<tr>
<td>Slit</td>
<td>1.30 nm</td>
</tr>
<tr>
<td>Atomizer</td>
<td>Standard burner</td>
</tr>
<tr>
<td>Oxidant</td>
<td>Air</td>
</tr>
<tr>
<td>Oxidant pressure</td>
<td>1.60 kg/cm² (9.50 l/min)</td>
</tr>
</tbody>
</table>
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 (HClO₄) and nitric acid (HNO₃) 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 (HClO₄ : HNO₃ :: 10:2) and digested at 78 - 80°C for 12 hours. After cooling 3 drops of hydroxylammonium 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 filter 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 (\(\text{NH}_4\text{O}_3\) and \(\text{H}_2\text{O}_2\)) 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 Bioconcentration 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>
<thead>
<tr>
<th>Factor</th>
<th>Degrees of Freedom</th>
</tr>
</thead>
<tbody>
<tr>
<td>Factor A (year)</td>
<td>1</td>
</tr>
<tr>
<td>Factor B (Season)</td>
<td>2</td>
</tr>
<tr>
<td>Interaction of A and B</td>
<td>2</td>
</tr>
<tr>
<td>Factor C (Centre)</td>
<td>5</td>
</tr>
<tr>
<td>Interaction of A and C</td>
<td>5</td>
</tr>
<tr>
<td>Interaction of B and C</td>
<td>10</td>
</tr>
<tr>
<td>Factor D (between Surface and Bottom water)</td>
<td>1</td>
</tr>
<tr>
<td>Interaction of A and D</td>
<td>1</td>
</tr>
<tr>
<td>Interaction of B and D</td>
<td>2</td>
</tr>
<tr>
<td>Interaction of C and D</td>
<td>5</td>
</tr>
</tbody>
</table>
Sediment: The range, mean and standard deviation were calculated season-wise 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**

<table>
<thead>
<tr>
<th>Factor</th>
<th>Degrees of Freedom</th>
</tr>
</thead>
<tbody>
<tr>
<td>Factor A (year)</td>
<td>1</td>
</tr>
<tr>
<td>Factor B (Season)</td>
<td>2</td>
</tr>
<tr>
<td>Interaction of A and B</td>
<td>2</td>
</tr>
<tr>
<td>Factor C (Centre)</td>
<td>5</td>
</tr>
<tr>
<td>Interaction of A and C</td>
<td>5</td>
</tr>
<tr>
<td>Interaction of B and C</td>
<td>10</td>
</tr>
<tr>
<td>Factor D (Station)</td>
<td>2</td>
</tr>
<tr>
<td>Interaction of A and D</td>
<td>2</td>
</tr>
<tr>
<td>Interaction of B and D</td>
<td>4</td>
</tr>
<tr>
<td>Interaction of C and D</td>
<td>10</td>
</tr>
</tbody>
</table>

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 centre-wise and season-wise 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

<table>
<thead>
<tr>
<th>Factor</th>
<th>Degrees of Freedom</th>
</tr>
</thead>
<tbody>
<tr>
<td>Factor A (year)</td>
<td>1</td>
</tr>
<tr>
<td>Factor B (Season)</td>
<td>2</td>
</tr>
<tr>
<td>Interaction of A and B</td>
<td>2</td>
</tr>
<tr>
<td>Factor C (Centre)</td>
<td>5</td>
</tr>
</tbody>
</table>

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$_2$O), zinc sulphate (ZnSO$_4$ 7H$_2$O) 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 toxicant 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 of 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):

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

\[
95\% \text{ confidence limit (} f_{\text{LC50}} \text{)} = S \frac{2.77}{\sqrt{N}}
\]
Where \( N \) = total number of organisms tested at those exposure concentrations whose 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 \( \text{viz. CuSO}_4 \ 5\text{H}_2\text{O, ZnSO}_4 \ 7\text{H}_2\text{O and Pb (NO}_3\text{)}_2 \) 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 \( \text{CuSO}_4 \ 5\text{H}_2\text{O, ZnSO}_4 \ 7\text{H}_2\text{O and Pb(NO}_3\text{)}_2 \) 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 comparison with the results obtained from different environments i.e., centres.

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

(Arranged in alphabetical 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 an 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.
<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
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<tbody>
<tr>
<td>Fiducial</td>
<td>A line or point established accurately as a basis of reference.</td>
</tr>
<tr>
<td>Heavy metals</td>
<td>The metals having specific gravity of 5.0 or above. In the text the copper, zinc and lead together referred as heavy metals.</td>
</tr>
<tr>
<td>Highly significant</td>
<td>At 1% F-value</td>
</tr>
<tr>
<td>KB</td>
<td>Bioaccumulation Factor</td>
</tr>
<tr>
<td>LC50</td>
<td>The concentration of a substance which is lethal to 50% of the test animals.</td>
</tr>
<tr>
<td>Linear-correlation</td>
<td>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.</td>
</tr>
<tr>
<td>Log-log paper</td>
<td>Paper ruled with two sets of mutually perpendicular, parallel lines spaced according to the logarithms of consecutive numbers rather than the numbers themselves.</td>
</tr>
<tr>
<td>Negative correlation</td>
<td>An increase in the value of one variable is followed by the decrease in the value of the other variable.</td>
</tr>
<tr>
<td>Positive correlation</td>
<td>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 decrease in the other variable.</td>
</tr>
<tr>
<td>Probit paper (Probability paper)</td>
<td>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 &quot;normal&quot; and the cumulative diagram is a straight line.</td>
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<tr>
<td>Response</td>
<td>The value of some measurable quantity after a treatment has been applied.</td>
</tr>
<tr>
<td>Significant</td>
<td>At 5% F-value</td>
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<tr>
<td>Standard deviations</td>
<td>The root of the average of the squares of the differences from their mean, $\bar{x}$, of a number, n, of observations, x.</td>
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$$SD = \frac{1}{n} \sum (x - \bar{x})^2$$
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<thead>
<tr>
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<tr>
<td>Sublethal</td>
<td>Only slightly less than lethal.</td>
</tr>
<tr>
<td>Threshold value</td>
<td>The minimum input that produces a corrective action in an automatic control system.</td>
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<tr>
<td>Very highly significant</td>
<td>At 0.1% F-value</td>
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