TOXICOLOGICAL EFFECTS OF COPPER AND MERCURY ON THE FISH *MACRONES GULIO* (HAMILOTON - BUCHANAN)

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

DOCTOR OF PHILOSOPHY
IN THE
FACULTY OF MARINE SCIENCES

BY
ASHA M.R.

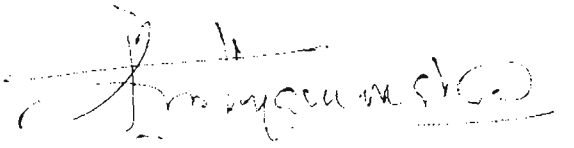
DEPARTMENT OF MARINE BIOLOGY, MICROBIOLOGY AND BIOCHEMISTRY
SCHOOL OF MARINE SCIENCES
COCHIN UNIVERSITY OF SCIENCE AND TECHNOLOGY
COCHIN - 682 016

2001

To my father

CERTIFICATE

This is to certify that this thesis is an authentic record of the research work carried out by **Smt.ASHA,M.R.,** under my scientific supervision and guidance in the School of Marine Sciences, Cochin University of Science and Technology, in partial fulfilment of the requirements for the degree of Doctor of Philosophy of the Cochin University of Science and Technology under the faculty of Marine Sciences, and no part thereof has been presented for the award of any other degree, diploma, or associateship in any University.



Dr. V.J.Kuttyamma,
Professor,
School of Marine Sciences,
Cochin University of Science and Technology,
Cochin - 682 016.

DECLARATION

I, **ASHA,M.R.** do hereby declare that this thesis entitled "Toxicological effects of Copper and Mercury on the fish *Macrones gulio* (Hamilton-Buchanan)" is a genuine record of the research work done by me under the scientific supervision of Dr.V.J. Kuttyamma, Professor, School of Marine Sciences, Cochin University of Science and Technology, and has not previously formed the basis of any degree, diploma, or associateship in any University.

Asha

Cochin - 682 016 **ASHA, M.R.**

ACKNOWLEDGEMENTS

I wish to express my deep sense of gratitude to my supervising teacher Dr.V.J.Kuttyamma, Professor, School of Marine Sciences, Cochin University of Science and Technology, for her guidance, encouragement and for scrutinising the manuscript and for suggesting improvements.

I thank professor Dr. N.R. Menon, Director, School of Marine Sciences for his constant encouragement throughout the research period.

I wish to record my sincere gratitude to Prof. Dr. A. Mohandas, Head, School of Environmental Studies, for his timely help, valuable suggestions and for providing me laboratary facilities. I am deeply indebted to Retired professor Dr.A.Antony, School of Marine Sciences, for critically going through the manuscript and for suggesting improvements.

I am thankful to the authorities of Cochin University of Science and Technology for providing me all the necessary facilities for the successful completion of my research work.

Sincere thanks are also due to all my colleques for their co-operation and timely suggestions during the course of my work. I am deeply indebted to my family members, for their constant encouragement throughout the research period.

The financial assistance received from the University Grants Commission by means of research fellowship is gratefully acknowledged.

PREFACE

Pollution of the aquatic environment has received considerable attention in recent years because it upsets the balance in the natural environment and also owing to its deleterious effects on human health and resources. In organisms including fish, usually negative consequence of pollution manifests as detrimental deviations from the normal state affecting behavioural, physiological and bio-chemical systems.

Living organisms with aquatic environment have a capability of contracting heavy metals in the body. Some metals are required in the life processes as essential elements. Copper and mercury are heavy metals. Copper is an essential element and it is known to accumulated by aquatic organisms. Though copper is a micronutrient, it is toxic to the animal above threshold levels. Mercury is a neural poison and it is harmful to the animal even at low concentrations. Uptake of dissolved copper and mercury is the principal pathway for the entry of metals into the food web.

A clear understanding of heavy metal toxicity on the aquatic organisms demands detailed investigations on the sublethal effects of heavy metals at various concentrations. Studies of sublethal effect of metals in fish aim at analysing the biological responses of an organism to metal exposure. Where these responses are analysed, it forms a basis of bioassay procedures. Hence an attempt was made to study the sublethal effects of copper and mercury on the fish Macrones gulio (Hamilton - Buchanan).

The thesis is presented in six chapters. The chapter I introduces the topic and outlines the aim and scope of the present investigation. The second chapter deals with the lethal toxicity of the two heavy metals copper and mercury on the experimental fish. In the third chapter the effects of copper and mercury on the blood parameters like haemoglobin, haematocrit and erythrocyte count are discussed. The fourth chapter describes the effect of copper and mercury on the glycogen and protein content of liver and muscle of the fish. The fifth chapter deals with the effects of copper and mercury on different body tissues (liver, kidney and gill) of the fish. The last chapter gives a summary of the thesis followed by a list of references.

CONTENTS

CHAPTER 1

GENERAL INTRODUCTION

Pollution is the general term associated with unfavourable alterations in the ecology, resulting in deleterious effects on human health and resources. It is an insidious and growing process which manifests itself only when the outflow of effluents exceeds the capacity of the receiving ecosystem of environment to recover. The various causes of pollution are the explosive growth of population, increasing urbanisation, rapid industrialisation, indiscriminate use of fertilizers and pesticides and lack of general awareness of environmental education. Pollution results in human health hazards and destruction of aquatic food resources like fishes, prawns, lobsters, molluscs and so on.

Marine pollution can be attributed to the discharge into seas and oceans of wastes from domestic, industrial, agricultural and navigational activities. In some cases the polluted material is discharged directly into the sea and in others it first reaches the rivers and estuaries and finally the sea. The alteration of the environment by human activites may be physical, chemical, biological or radio active. Chemical alteration of the environment appears to be the major problem which threatens the living system extensively. All organisms maintain their "internal milieu" more or less constant by making use of a variety of regulatory mechanisms. When the level of pollutants in the environment exceeds the assimilative capacity of the organisms it leads to physiologial and biochemical changes

finally resulting in mortality. Analysis of such variations in the body functions based on laboratory investigation is an important aspect of pollution research.

Heavy Metal Pollution

In recent years, concern has increased over heavy metal pollution, as all heavy metals are potentially harmful to most organisms at some level of exposure. The release of increasing quantities of heavy metals and their salts in terrestrial and aquatic environment and their accumulation in living and non-living systems endanger life. The nature of environment is of crucial importance when considering homeostatic mechanisms of an animal. Where as terrestrial animals are generally only exposed to metals in their diets, or in the air they breathe, aquatic organisms are also exposed to dissolved and particulate metals in the ambient medium.

Bio- availability of heavy metals in aquatic medium

Heavy metals may be considered as a threat to the environment and biota, only if these could be taken up by biota through body route. It is not possible to equate a given emperically defind fraction of water sample with the biological availability of a contaminant. Thus, both soluble and particulate fraction of water sample contain some components which are highly available to certain part of biota only. In the soluble fraction trace metal ions generally exhibit high biological availability. By contrast some chelates or complexes

present in solution may be unavailable. Pagenkopf et al. (1974) cited an example of this effect for copper in fresh waters and postulated that the effect of chelation may be responsible for the variation in the toxicity of most trace metals in hard and soft natural waters.

The particulate fraction can be divided into two groups, organic part and inorganic part. The bio-availability of trace metals attached to organic particulate matter is high and that of inorganic matter is low. All these factors make the availability of trace metals and their actual composition in water samples different.

Heavy metal uptake and bio - concentration

The mechanism of metal uptake has not yet been fully elucidated. The available evidence indicates that metals cross the cell membrane essentially by a passive transport process although endocytosis may also occur (Viarengo, 1985). Studies by Simkiss (1983) suggest that the metal complex goes through biological membranes as a lipophilic compound. Moreover in fish, metals like Hg, and Cd, are able to disrupt the ionic balance, altering the permeability characteristics of the cell membrane. Thus they effect passive ion movements as well as the active transport process either by directly inhibiting the activity of Na, K-dependent ATP ase or as a secondary effect by reducing the availability of ATP (Bouquegneau and Gilles, 1979). When metals cross the cell membrane the metals react with cytosolic

components and are usually complexed in different ways (by sulphydrylic binding, chelation, salt formation) to cytosolic compounds such as high affinity specific ligands, substrates, products of enzymatic activity or enzymes themselves. The form of the heavy metal (Ionic form, oxidised, reduced, complexed by organic susbstances, adsorbed to inorganic or organic particulate materials, acting singly or in combination with other cations) to which the organisms are exposed is extremely important in its over all toxicity to aquatic organisms and its uptake by them. Metals are taken up by aquatic organisms usually across respiratory surfaces and strongly bound by sulphydryl groups of proteins. Because of this ability, there is a tendency for them to be fixed in tissue and not to be excreted. In other words they have a long biological half life. Metal also changes the structure and enzymatic activities of proteins and cause toxic effects, evident at the whole organism level.

Requirements for a monitoring organism

Certain organisms have long been known to accumulate pollutants from the ambient water. Many bivalves have been known to accumulate a wide range of metals. Thus different heavy metals may be accumulated by aquatic biota at levels far above those found in the surrounding medium, thereby enabling to use selected organisms to monitor the levels of the metals in water bodies. They have several advantages over the classical methods of water or sediment analysis. The greatest advantage is that the biological availability of pollutant is measured directly.

In addition to this, such animals would produce a time averaged index of pollution availability. The much higher concentration of pollutants in the body, compared with those in the surrounding water makes it easier to analyse the samples, and the biochemical, haematological and enzymatic changes caused by pollutants are much more evident.

Industry and regulating authorities concerned with the environmental management increasingly recognize the need for biological monitoring to know changes, either deterioration or improvement in environmental quality. Numerous and varied biological responses have been suggested as potential techniques for monitoring biological impact of waste discharges to the aquatic environment.

Philips (1980) regarded bio-monitoring data more direct and suitable than water or sediment analysis because information on bio-availability and bio-magnification is included. Other reasons to use bio-monitoring in addition to physico - chemical monitoring are:-

(1) toxicity can not be estimated without testing biota, although the quantitative structure activity relationship (QSAR) techniques may give good approximation.

(2) biological response may be elicited by chemicals below analytical detection limits.

(3) biological responses to toxicity may be different in mixture (ecosystem, species, different chemicals etc.) than tested individually and

(4) Environmental quality strongly influences toxicity.

Criteria for standard test fish

Many authors have mentioned the desirability of using a standard fish species as a bioassay animal for reproducibility of test results (Marking, 1966; Lennon, 1967; Cairns, 1969; Sprague, 1970). In some western countries acute toxicity tests with a fish, Daphnia and an algal species have been made mandatory for the acceptance of new substances (Smeets, 1980). Though the species of fish that should be employed in such tests is not specified, the use of a standard fish has been suggested in order to prove the results, and to compare the results of one laboratory with another (Cairns, 1980). Furthermore, the results of tests conducted at different moments in the same laboratory can also be compared by employing a standard fish (Fogels and Sprague, 1977). The following criteria have been listed by Adelman and Smith (1976) for the choice of a standad test fish:-

1. It must have a constant response and have neither high nor low sensitivity to a broad range of toxicants tested under similar conditions.
2. It must be available throughout the year.
3. A constant size group of that species should be available all through the year.

4. It should be easy to collect, transport and handle.
5. The adults should be small enough to facilitate the conducting of acute or chronic tests without imposing undue difficulties in maintaining the recommended loading densities.
6. It should be possible to breed the species in the laboratory.
7. It should complete its life cycle within one year or less.

Suitability of Macrones gulio for the toxicological studies

Macrones gulio (Hamilton - Buchanan) selected for the present study, fulfils most of the criteria listed for a standard test fish. They are found in abundance in the rivers, lakes and backwaters of Kerala. They are commonly called as long - whiskers - catfish and belongs to the family Bagridae in the order Cypriniformes. It is an edible fish and therapeutic qualities and are recommended by physicians as diet during convalescence. The body of the fish is subcylindrical, head subconical, covered with skin and is without ordinary scale. The fins are soft rayed, pectorals and dorsal fin have sharp hardened spines. They posses small adipose fin with base shorter than dorsal. Colour brown shot with bronze dorsally, yellow shading to white ventrally. Fins dull orange with dark tips, caudal fin forked. They are more or less omnivorous in feeding habit. As it is a sturdy fish it can be easily maintained in the laboratory. Being euryhaline they can withstand a wide range of salinites and thrives well in freshwater as well as in brackish water. They are available

throughout the year. Considering all these factors, the fish, Macrones gulio has been selected for the present study.

Trace metals selected for the experiment

The term 'trace metals' identifies a large group of metallic elements which are present in living organisms in limited amounts. Trace metals are usually divided into two sub-classes. The first includes Fe, Mn, Cu, Mg, and Zn which are essential micronutrients. Such nutrient metals are usually key elements in metalloenzymes or co-factors in enzymatic reactions. However, the micronutrient when present above threshold levels becomes toxic. Cd, Hg, Cr, Pb etc. belong to the second category which is made up of metals without any established biological function and include the more important contaminants in the aquatic environment.

Mercury

The fact that inorganic mercurial compounds are transformed into biological active methyl mercury have prompted comprehensive investigation into environmental problems creatd by mercurials (Goldberg, 1980; Ray, 1984; Patel et al. 1985 a, b). Wobeser (1975) confirmed the ability of fish to concentrate Hg rapidly from water. Eventhough toxicity and bioaccumulation of the mercury in fishes have been studied by many authors, information on the Hg induced biochemical and haematological alterations in Macrones gulio is meagre. As most fishes are responsive to deleterious effects of mercury (Wobeser 1975; Panigrahi and Misra

1978; Christensen et al. 1977; Gill and Pant 1981; Dange, 1986a) the early detection of specific physiological abnormalities provide an indication of exposure prior to manifestation of any gross damage. The measurement of biochemical changes in blood and tissues of the fish under exposure to the toxicant may be used to predict effects upon chronic exposure.

Source of mercury pollution in the aquatic medium

The major sources of water contamination by mercury are effluents from chloralkali plants which use mercurials for producing chlorine and caustic soda. These factories discharge large quantities of mercury compounds into the aquatic medium. Agricultural application of mercury also contaminate water ways. Over 70 mercury compounds are known to have been used to control seed borne and soil borne fungal diseases. They get washed with rain and irrigation waters and pollute the water ways. Mercury metals and mercury compounds are also used in a variety of industrial application like electrical equipment manufacture, antifouling paint manufacture, industrial and scientific equipment manufacture, dental uses and laboratory uses. Miscellaneous uses include use as a catalyst and fungicide and slimicide in pulp and paper industry in pharmaceutical and cosmetic preparations and in amalgamation process. Burning of fossil fuels or sewage sludge, municipal wastes, Hg - containing fertilizer are also important sources of Hg. The release of Hg compounds from industrial liquid wastes has been shown to greatly influence the immediately adjacent environment of the source.

Effects of mercury on fish

Mercury has no known metabolic function in human beings and therefore even low concentration in the body may be considered potentialy harmful. Mercury in fish and seafood occurs mainly as methyl mercury and partly as inorganic mercury bound to organic molecules.

Mercury compounds also exert their action by altering the membrane structure, thereby seriously affecting the permeability character of these cell types. The inability of mercury-exposed fish to maintain its ionic balance could be attributed to either a decreased uptake of ions via gills or to an increased loss of ions via gills or kidney. As the gills function as the main route of mercury intake (Olson et al. 1973), the osmoregulatory function of this organ is likely to be affected.

When fishes are exposed to mecury in their environment, the gill tissue rapidly accumulates these compounds in such a manner that their concentration, reaches values exceedingly high by far those in other organs and tissues (Olson et al. 1973, 1978; Lock, 1979). This conspicuous accumulation is often accompanied by structural changes in the gill epithelium (Lindahl and Hell, 1970; Wobeser, 1975; Lock, 1979). Wobeser (1975) found that in rainbow trout exposed to mercuric chloride severe epithelial necrosis occurs.

Methylation of mercury drastically alters the properties of Hg. It loses polarity and ceases to behave as a typical metallic ion. It becomes much less water soluble and much more fat

soluble (Craig, 1986 a). This affects the environmental fate of Hg. Methyl mercury becomes mobile entering any substrate containing fat and is accumulated by aquatic organism directly from water. In fish methyl mercury may comprise 80% or more of total mercury and it occurs primarily in muscle as a cysteine complex (Craig, 1986 b). While energy is lost with each trophic transfer, methyl mercury is conserved and biomagnified in aquatic food chains.

Toxicologically increased fat solubility means not only rapid uptake and retention of mercury, but more rapid penetration of sensitive tissues, particularly the lipid membranes of neurons (Craig, 1986 a). The high affinity of methyl mercury for sulphydryl group causes significant neurotoxic effects by combining with cysteine containing protein.

The different Hg species have different path ways and routes in the environment. Many of the routes can be taken both by biologically mediated processes and by a biological process. Olson and Fromm (1973) found Hg in the gills of rainbow trout which had been exposed to inorganic Hg and suggested that Hg enters the gill across the general lammellar surface.

Copper

Copper and its compounds have been used by man since prehistroric times. Like other metals it is potentially toxic and is a widespread pollution of water (Slowey and Hood, 1970; Rehwoldt et al. 1971; Abdullah et al. 1972). Copper has been

added to marine culture system to control pathogens such as parasitic protozoan (Dempster, 1970). Numerous studies (Ozaki et al. 1971; Mckim and Benoit, 1971; O'Hara, 1971) have shown that addition of copper salts to natural water can seriously threaten aquatic life.

Source of copper pollution in the aquatic medium

The largest amount of copper enters the oceans as pollutant in river run offs from industrial and agricultural sources and appears to concentrate in near shore areas (Jernelov, 1975) posing serious water pollution problem (Pickering and Henderson, 1966; Rehwoldt et al. 1971). Copper is widely used as an algicide and in the treatment of diseases and parasitism in fishes. The latter application sometimes poses the problem of toxicity to the fish treated. Cronic copper poisoning can be a serious problem in domestic animals, since copper is stored in fish and other organisms, but the greatest hazard to fish is from acute poisoning (Cardeilhac, 1972; Takeshita, 1975).

Effects of copper on fish

Copper is an essential element for animals for the synthesis of haemoglobin, formation of bone, maintenance of myeline within nervous system and an essential component of key metalloenzymes. It is intimately associated and involved in normal hematopoiesis since it is essential for the absorption of iron available for haemoglobin (Hb) synthesis (Goodman and Gilman, 1956).

Both excess and deficiency of copper cause pathological changes. The behaviour of fish, flounder exposed to medium and high levels of copper was uncoordinated with spontaneous movement which was similar to Wilson's disease (Peisach et al. 1967). It seems possible that the symptoms shown by the flounder could be the result of copper acting on their central nervous system. Excessive copper intake causes hepatic necrosis, haemolytic anaemia, necrosis of kidney tubules and death of brain neurons. Copper also attacks various renal enzymes that mediate the resorption of several compounds. Copper has been shown to cause hemolytic anaemia by inhibiting erythrocytic glycolysis, denaturing Hb and oxidising glutathione (Fairbanks, 1967).

McKim and Benoit (1971) found that sublethal exposure of copper to brook trout decreased survival and growth in adult fish and reduced both number of viable eggs produced and hatchability. Baker (1968) found that effects of copper exposure resulted in fatty metamorphosis in the liver, necrosis in the kidney, destruction of haemopoietic tissue and gross changes in gill architecture of winter flounder. The respiratory system of fish seems to be the prime target at exposure to acute lethal levels of copper. The toxic effects of copper on fish are considered to be mainly attributed to copper ions (Pagen Kopf et al. 1974).

Gardner and LaRoche (1973) observed cellular changes attributable to copper in the mechanoreceptors of the lateral line canals in mummichihog Fundulus heteroclitus and atlantic

silver side, Menidia menidia, Renal lesions were observed in Fundulus heteroclitus. Hepatic and renal disorders have been reported for the winter flounder (Baker, 1968) while the metal was described as being both nephrotoxic and neurotoxic to the goldfish Carassius auratus (Vogel, 1959). Liver disorders have been reported in the mummichihog (Lett et al. 1976; Skadhauge, 1977; Drummond et al. 1973). Found that in the fish Sheephead, copper sulphate exposure resulted in the appearance of swollen and congested kidney, and gill lamellae of copper poisoned fish were blunt and thickend. They also observed the failure of osmoregulation in such fishes.

From these accounts it is clear that copper, though a micronutrient, is also a pollutant at high threshold levels. Hence an attempt was made to study the effects of both copper and mecury on some biological and biochemical activities of the fish Macrones gulio (Hamilton - Buchnan).

Objective of the present study

A continuing study of specific physiological, biochemical, metabolic and histological changes of aquatic organisms exposed for short periods to environmental stressors is essential to provide a rational basis for anticipating and understanding the ecological effects in the aquatic environment. Such studies may provide a sensitive method for predicting the effects of chronic exposure on survival, reproduction and growth. This would allow a relatively rapid evaluation of the chronic toxicity of a compound.

In the present study, sublethal effects of copper and mecury on the selected fish Macrones gulio were carried out. Physiological responses like disturbances in haematology, changes in biochemical composition and histological changes are the parameters chosen for the assessment of the sublethal effects.

In physiological studies of fish, haematology is often used as an index of the effects of xenobiotic compounds to these animals. The measurement of specific physiological and biochemical changes in the blood of fish exposed to sublethal concentrations of pollutants may provide a sensitive index in predicting the effects of chronic exposure on survival of the animal. Such analysis has considerable clinical importance in mammals. But in fishes such applications are only limited. A knowledge on the pathological effects of heavy metals on circulating blood elements and blood pigments can provide a frame work for simpler routine analysis of blood in fish toxicology. Heavy metal - induced haematological changes may be of some value in assessing the impact of exposure to these chemicals and may serve as tools for biological monitoring.

In almost all environmental stresses, the major share of stored energy in animals comes from the carbohydrate or glycogen reserves. Thus carbohydrates form the central point in energy production because of its great mobility in the living systems, together with its capacity to get compartmentalised within cells and tissues. The mobility is provided by glucose and

compartmentalisation by glycogen and glucose - 6 - phosphate. It is widely accepted that carbohydrate deposits in the form of glycogen in tissues like liver and muscle provide the immediate energy requirement in teleost fishes under a variety of stressors including exercise (Black et al. 1960, 1961, 1962), physical disturbance (Nakano and Tomilson, 1967), starvation (Black et al. 1966), environmental hypoxia (Heath and Pritchard, 1965; Narasimhan and Sundararaj, 1971), salinity changes (Bashamohideen and Parvatheswara Rao, 1972).

This sort of investigation will eventually open up a very interesting aspect of toxicology, the understanding of which would help to gain a better understanding of the effects of metals like copper and mercury on the physiological, biological and histological aspects of an organism.

As industrial effluent containing toxic heavy metals such as mercury, cadmium, lead, zinc, copper and arsenic are constantly discharged into our coastal environment causing serious threat to marine organisms and posing potential public health risk. It is necessary to understand the physiological and biochemical responses of locally available fishes and other organisms to environmental contaminants such as heavy metals. This kind of study may help to provide information on status, trends and source of risk to the aquatic ecosystem. The present investigation on the toxicological effect of copper and mercury on the fish Macrones gulio is done with a view to enhance the existing knowledge on pollution effects assessment in tropical waters.

CHAPTER 2

LETHAL TOXICITY STUDIES OF COPPER AND MERCURY

Excessive pollutants affect the type of organisms in the water which may lead to immediate death, depending on the strength of the poison and duration of exposure. The poison first debilitates the organism through physiological stress and this is often reflected first in the excitement and hyper activity, followed by coughing, gasping and other distress manifestations and finally, death. The physiological effect of the poison may be manifested in different ways on different species. For example, the function of the vital organ of the fish may be seriously impaired with irreparable damage to the liver, kidney or the spleen. In some cases, the respiratory processes may be adversely affected. This may arise as a result of gill damage which may at first appear as mucus secretions and latter tissue degeneration with fungus growth and other diseases.

The use of bioassays as part of a comprehensive approach to marine pollution assessment is widely accepted now a days. Toxicity is a biological response, which when quantified in terms of concentration of the toxicant can constitute the basis for a bioassay procedure. Bioassay tests are defined here as the estimation of the amount of biologically active substances by the level of their effects on test organism (Chapman and Long, 1983). Information generated from various toxicity tests can be of use in the management of pollution for different purposes like prediction of environment damage of waste, comparison of various toxicants, animals or test conditions and regulations of waste discharge.

The acute toxicity of poisons is determined in the laboratory on biological assays or bioassays, where the fish or other organisms are subjected to different concentration of the poison under controlled salinity, temperature and dissolved oxygen for a specified time; usually 48 or 96 hrs. The concentration in which one half of the organisms are killed in 96 hrs., say, is referred to as the 96 hrs median lethal concentration or LC 50. It is extremely important to note the species of organism tested and the conditions under which the bioassy tests are made, i.e., temperature, salinity, dissolved oxygen, pH and whether the tests are static (solutions changed only at specified intervals) or continuous flow. Usually the water quality criteria for protection of fish life are based on the LC 50 values (Mc Lee and Wolf, 1963; U.S. FWPCA. 1968). But it is quite clear that these can change from place to place, depending on such factors as temperature, salinity and dissolved oxygen concentration (Alderdice, 1963).

Static acute toxicity tests have been the primary tool for evaluating short term effects of pesticides and other foreign compounds on aquatic organisms (Nimmo, 1985). In general the LC 50 values from the static tests are greater than those from the flow through tests. However a well designed static test can be useful in determining acute toxicities for comparative studies or as indicator as for further acute or chronic tests (Nimmo, 1985).

Sprague (1969) has reviewed the problems of toxicity tests to fish. The incipient LC 50 (lethal concentration for 50

percent of individuals on long exposure) is recommended as the most useful criterion of toxicity. If this cannot be estimated, the 4 day LC 50 is a useful substitutes and often its equivalent (Sprague, 1969). Information needed for protection of aquatic life has been eloquently described by Tarzwell (1962 a, 1962 b, 1966), and tests provide some of the required answers. The period for which the LC 50 is determined is usually of considerable importance, values normally being much lower after 96 hr (Holden, 1973). In aquatic toxicology, the acute toxicity tests for fish enables estimation of the exposure concentration resulting in 50% mortality of test animals within 48 or 96 hr (expressed as LC 50 value). The numerical value of LC 50 has assumed special importance as an index of toxicity, but with the incorporation of highly persistent substances with high concerntration potentials and low water solubilities it can provide only marginal information (Ernst, 1980).

The acute toxicity data have been used in conjunction with so called safety factors of 0.1 - 0.01 to estimate safe concentration of chemicals for the protection of aquatic life during chronic exposure. However, these factors do not adequately consider the specific action of the individual substance (Ernst, 1980). The concept of specific application factor define the relationship between the acute and chronic toxicity of a chemical, the accurate estimate of the specific application factor for a chemical can be derived from maximum acceptable toxicant concentrations (MATC). Numerically the

application factor (AF) is Quotient of the MATC and the 96 hr LC 50. Application factors for some pesticides show that the highest concentration without any toxic effect may be more than two orders of magnitude lower than the 96 hr LC 50 (Hansen and Parish, 1977; Nimmo et al. 1977). The method for determination of medium of tolerance limit (TLM) was evolved long before (Hart et al. 1945; Doudoroff et al. 1957). Attempts were made to apply suitable factors to the TLM data for predicting long term safe concentrations. Burdick, (1957); Henderson and Pickering, (1957); Kimura and Matssushima, (1969); and Muirhead - Thomson, (1971) suggested that the factor to determine the long term safe concentration should be derived from simple short term bioassays since static bioassay stimulate most closely single or multiple applications of a pesticide to a lake or pond (Burdick, 1967).

Standard bioassay studies were conducted for 96 hrs period to obtain a measure of acute toxicity of copper and mercury on the fish Macrones gulio.

MATERIAL AND METHODS

This part of the investigation has centered mainly to know the toxic effects of copper and mercury individually at different lethal levels on the fish Macrones gulio.

There are various ways of investigating sublethal effects, and each techniques provides an insight into the physiology or behaviour of the organism in question (Waldichuk, 1979). Efforts

are made to evaluate the lethal and sublethal effects of heavy metals individually on the fish Macrones gulio.

Specimens were collected from an unpolluted area of the inland water system, confluent with Cochin backwaters. The animals were collected using castnets causing minimum stress, and then brought to the laboratary in large plastic buckets containing the water taken from the same site. Within half an hour they were brought to the laboratary. The environmental parameters of the collection area noted were, salinity 15±2‰, temperature 28±1 °C, PH 7.5 ± 0.5 and dissolved O_2 4.8 - 5.2 ml/l during the time of collection.

Laboratory Procedures

The animals brought to the laboratory were maintained in large tanks containing filtered unpolluted water of corresponding hydrographical parameters of the collection area. The fishes acclimated for one week and observed for mortality, disease symptoms or abnormal behaviour. During the acclimation period the fishes were fed with clam meat. The fishes were not fed 24h prior to the test experiment and during the entire period of the experiment. Only healthy fishes of immature stage of size range 10-13 cm in length were chosen for the experiment.

Toxicants

The toxicants selected for the experiment were copper and mercury.

Copper

Analar grade of copper sulphate (M.W.249.68) was the source of copper. The salt was dissolved in distilled water and added to achieve the required concentration.

Mercury

Standard solution of mercury were prepared using analar grade mercuric chloride (M.W.271.50) in distilled water and in amber colored bottles. Since mercury solutions are not stable for long periods, they were prepared fresh for each set of experiments and added to make up the required concentration.

Toxicant Concentration

The various concentrations of the toxicants are expressed in ppm in terms of the individual heavy metal (copper and mercury) formulation. Commercial formulations were used and calculated quantity was weighed out to give the desired concentrations in the test medium.

Bioassay for LC 50 determination

Static bioassay procedure given by APHA et al. (1981) and Ward Parish (1982) was followed in the experiment. For the study, filtered unpolluted water of corresponding hydrographical parameters was used (salinity $15 \pm 2‰$ temperature - $28 \pm 1\ ^{0}C$, pH - 7.5 ± 0.5 and dissolved oxygen > 90% saturation). After range finding tests, seven concentrations were used for each metal (Table 1).

Each experimental tank contained 50 litres of test solution with different concentrations of toxicants. Ten fishes were used for each test concentration of the toxicant. One tank was kept as control without metal solution. Duplicates were run for each metal concentration. The fishes were inspected every 12 hr for mortality and the water was renewed every 24 hr. Dead fishes if any were removed and recorded every 12 hr.

Data analysis

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

RESULTS

Behavioural response to metal exposure

During the lethal toxicity study behavioural response of the fish Macrones gulio to the metals (copper and mercury) were more or less similar.

Altered behaviour of the metal-exposed fish includes progressive exhibition of signs of tiredness, irregular erratic swimming, agitation, jerky movement, restlessness, frequent surfacing gulping of air, revolving, convulsions, extension of fins, accelarated ventilation with rapid rhythmic opercular and

Table 1

Cumulative % mortality of *Macrones gulio* exposed in copper and mercury

Metal	Conc ppm	Exposure time (Hours)							
		12	24	36	48	60	72	84	96
Control	0	0	0	0	0	0	0	0	0
Copper	0.04	0	0	0	0	0	0	0	0
	0.06	0	0	5	5	10	15	30	40
	0.08	0	0	10	10	15	40	75	90
	0.10	0	5	20	25	40	65	80	100
	0.12	0	10	35	60	70	85	90	100
	0.14	10	40	50	55	75	85	95	100
	0.16	15	40	60	80	100	100	100	100
Control	0	0	0	0	0	0	0	0	0
Mercury	0.05	0	0	0	0	0	0	0	0
	0.10	0	0	10	20	20	30	40	40
	0.20	0	0	10	40	50	60	70	80
	0.40	0	10	45	70	100	100	100	100
	0.60	0	10	45	75	100	100	100	100
	0.80	0	0	35	80	100	100	100	100
	1.00	0	10	40	90	100	100	100	100

Table 2

Lethal Concentrations of copper and mercury to *Macrones gulio*

Metal	Duration of Exposure	LC 84 mg/l	LC 50 and 95% confidence limits (mg/l)	LC 16 (mg/l)	Slope
Copper	48	0.165	0.125 (0.109 - 0.143)	0.088	1.370
	60	0.160	0.15 (0.086 - 0.128)	0.064	1.582
	72	0.130	0.088 (0.074 - 0.104)	0.060	1.472
	84	0.105	0.074 (0.061 - 0.090)	0.049	1.465
	96	0.076	0.063 (0.056 - 0.070)	0.053	1.203
Mercury	48	0.80	0.270 (0.177 - 0.410)	0.092	2.949
	60	0.45	0.202 (0.134 - 0.303)	0.090	2.236
	72	0.38	0.16 (0.093 - 0.275)	0.066	2.400
	84	0.31	0.135 (0.077 - 0.237)	0.051	2.485
	96	0.21	0.125 (0.09 - 0.171)	0.074	1.685

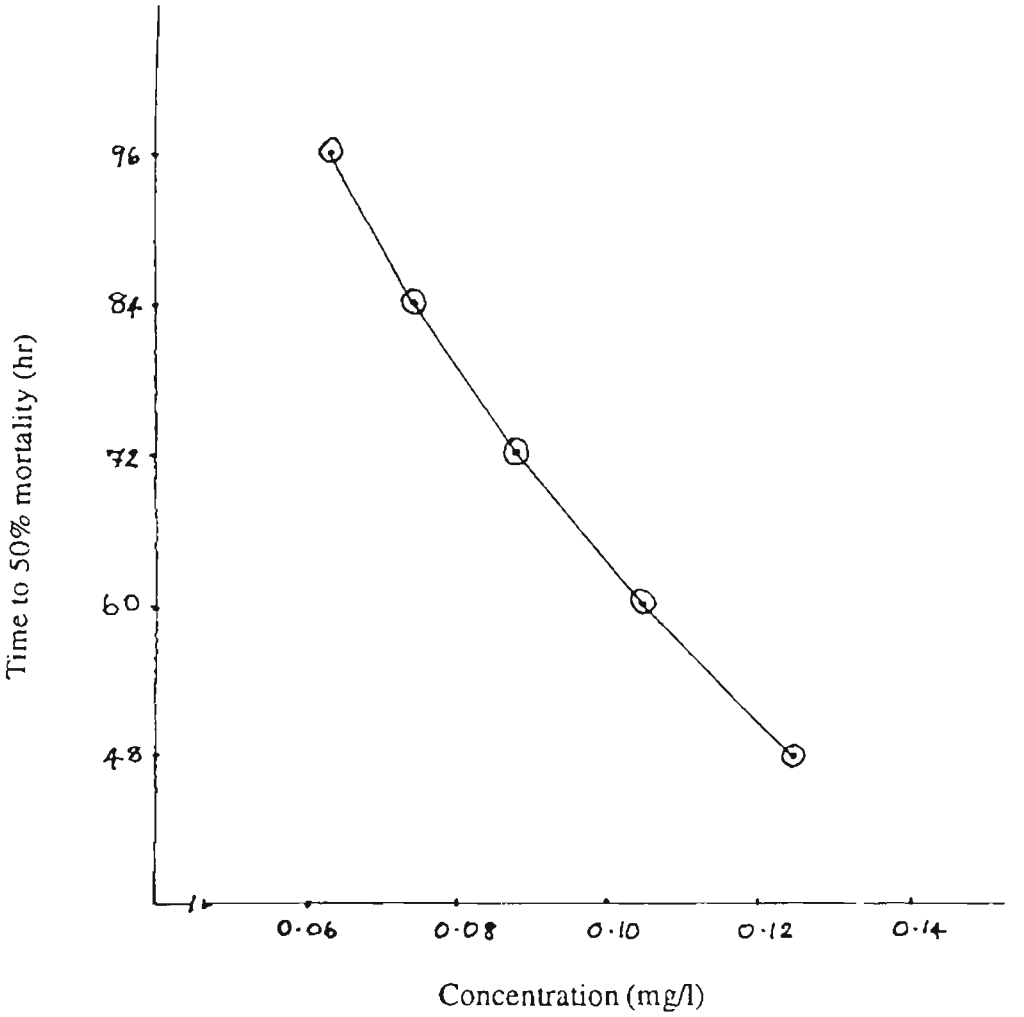


Figure 1 Toxicity curve for Copper.

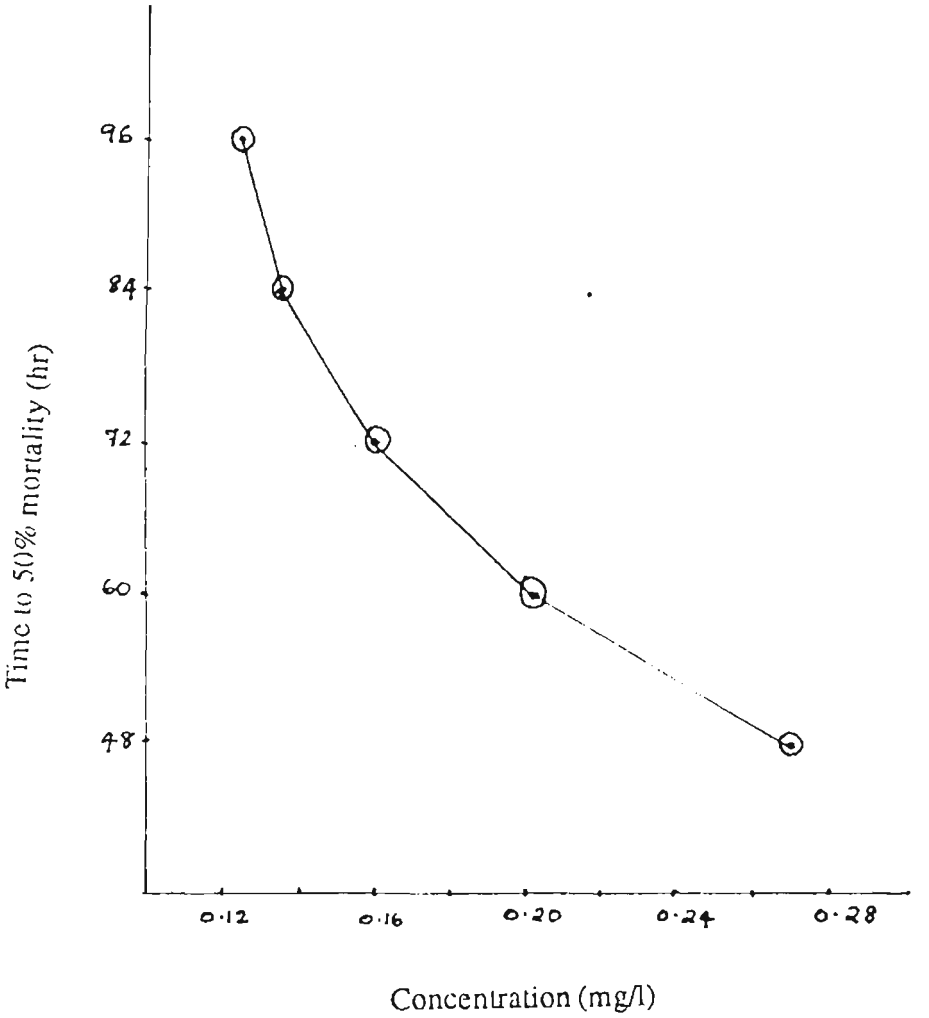


Figure 2 Toxicity curve for mercury.

mouth movements etc. Finally they lost their equilibrium and settled to the bottom before death. Water turned turbid and mucus oozed out of the dead fishes. The dead animals had blood clots on the gill surfaces with widely opened mouth and stretched gills. Air bubbles were seen trapped within the mucus.

Lethal toxicity of copper and mercury

The cumulative percentage mortality in different concentrations of copper and mercury are given in table 1 and LC 50 values are summarised in the table 2.

The concentration tested for the lethal toxicity of copper ranged from 0.04 ppm to 0.16 ppm and 96 hr LC 50 value worked out was 0.063 ppm.

The concentration tested for the lethal toxicity of mercury ranged from 0.05 ppm to 1.00 ppm. The 96 hr LC 50 value for mercury was 0.125 ppm.

Changes in LC 50 values with time are given in figures 1 and 2. In the figure the copper was found to be having steeper slope than mercury, which is an indication of acutely acting toxicity.

Discussion

The results of the acute toxicity studies of the present effort revealed that of the two metals tested, copper was more toxic than mercury.

The observed effect of copper on Macrones gulio which was found to be more toxic than mercury, appears to be is contrary to

what was observed in Tilapia nilotica (Nile Tilapia) by Somsiri (1982). He observed a higher 96 hr LC 50 value for Cu (58.1 mg/1) and lower values for Hg (3.71mg/1). A similar observation was made by Krishnakumari et al. (1983) in Therapon jarbua where 96 hr LC 50 value for copper was 4.5 mg/1 and for mercury it is 0.06 mg/1.

Acute toxicity on teleosts, have been conducted by several workers. The LC 50 values recorded for copper was 0.2 µg/1 for Fundulus heteroclitus (La Roche, 1974), 0.28 µg/1 for Onchorhynchus kishutch (Ma Carter et al. 1981) and 6.0 µg/1 for Tilapia mossambica (Balavenkatasubaiah et al. 1984). Those for mercury were 0.07 ml/1 (Roales and Perlmutter, 1984) in Trichogaster trichopterus and 0.03 ml/1 in Ictalurus punctatus (Nriagu, 1979).

These studies established that each toxicant affected different species of fishes in variety of manner. The effect on same fish can vary with environmental factors as observed by Dorfman (1977) in Fundulus sp.

Nriagu (1979) made an effort to compare the 96 hrs LC 50 values of mercury on different species of teleosts and observed that rainbow trout (Salmo giardneri) was the most sensitive (4.7 µg/1) species in relation to catfish, Ictalurus punctatus (30 µg/1) blue gill, Lepomis morochirus (88.7 µg/1). Lepomis microlophus (137.2 µg/1) and the large mouth bass, Micropterus salmoides (140 µg/1). Krishnakumari et al. (1983) found that

among the metals, Co, Ni, Cu, As, Pb, V, Hg and Zn, mercury was the most toxic and cobalt was the least to Therapon jarbua. The experimental LC 50 values would thus provide data on comparative effect of pollutants and are useful in identifying potentially toxic substance. In the present study the order of toxicity of Macrons gulio was Cu > Hg.

Concerning behavioural patterns, similar observations as in the present study have been reported by Eaton (1974), Spehar (1976), Nriagu (1979), Satchell (1984), Rai and Qayyam (1985), Lauren and Monald (1987) and James (1990). These abnormalities have been attributed to nervous impairment and blockade of nervous transmission between nervous system and various effector sites as a result of heavy metal treatment (James 1990).

CHAPTER 3

EFFECT OF COPPER AND MERCURY ON THE HAEMATOLOGICAL PARAMETER OF THE FISH *Marcrones gulio*

Fishes are susceptible to any change that may occur in the environment. It is expected that these changes would be reflected in the physiology of the fish, particularly in the values of haematological parameters and haematology has been used as an index of health status of a number of fish species. (Blaxhall, 1972).

Blood takes part directly and indirectly in almost all the activities of fish and that it can be a good indicator of stress conditions. The use of haematological parameters as indicators of sublethal stress can provide information on the physiological responses that the fish make to a changing environment. This is the result of close association of the circulatory system with the external environment and with tissues. Since haematological tests have been an important diagnostic tool in medicine for many years, it is speculated that they may be an equally valuable indicator of stress or disease condition of fish (Larsson, 1975).

Haematological changes have been detected in response to diseases, pollutants, surgical procedures, hypoxia etc. (Eisler and Edmunds, 1966; De wilde and Houston, 1967; Gardner and Yevich, 1969; Mckim et al. 1970; Soivio and Oikari, 1976; Duthie and Tort, 1985). Blood alteration or damage to the haemopoietic organs in these organisms may also be associated

with pathological conditions related to water - borne pollutants (Reichenback-Klinke, 1966; Gardner and Yevich, 1969; Saad et al. 1973). One of the important functions of blood is the tranportation of oxygen and carbondioxide in the body. The immature red blood corpuscles (RBC) is uniquely concerned with the synthesis of Hb. When the maturation is complete the RBC functions primarily in the transportation of haemoglobin (Hb).

The haemoglobin present in the red blood cell enables the blood to carry adequate amount of the gases to different tissues as the capacity of the Hb to carry these gases is very high. Hence an estimation of the Hb in the blood provides us information about the physiological status of the body. The increase or decrease of the haemoglobin content and RBC, variation of the packed cell volume (PCV) or haematocrit (Hct), mean cell haemoglobin concentration (MCHC) etc indirectly indicates the oxygen carrying capacity of the blood. Alternations of the haemotological parameters can be due to factors like retention of metabolites, metabolic problems, oxidation of Hb, increased or decreased erythropoiesis, haemodilution or haemoconcentration.

RBC count is a long cumbersome procedure which now a days is replaced by haemotocrit determination which express PCV (packed cell volume) as the percentage of the whole blood volume. Haematocrit provides a rapid approximation of the volume of circulating RBC and is used as a routine method for

haematological diagnosis of fish health in field studies. This method has the advantage of speed and simplicity and is suitable for the capillary blood.

Mckim et al. (1970) compared data for Hb, Hct, RBC count etc. of blood from male and female of fishes exposed to copper and found that no significant difference at 95% level. Bell (1968) also did not find any difference in the Hct Values between male and female fishes.

In Physiological studies of fish, haematology is often used as an index of the effect of xenobiotic compounds to these animals. The measurement of specific physiological and biochemical changes in the blood of fish exposed to sublethal concentrations of pollutants may provide a sensitive index in predicting the effects of chronic exposure on survival of the animals. Such analysis has considerable importance in mammals. But in fishes such applications are only limited.

In this chapter the effects of metals, copper and mercury on the Hb, Hct, MCV, MCH and MCHC values of Macrones quilo were described.

MATERIAL AND METHODS

Specimens of Macrones gulio were collected and acclimatized in the laboratory for a week. The collection procedure was the same that explained in Chapter II. The physico-chemical

parameters of the acclimatization tank was, salinity - 15±2‰, temperature - 28±1 °C pH - 7.5 ± 0.5 and dissolved oxygen > 90% saturation. The saturation of oxygen was maintained by giving aeration in the tank. During the acclimation period the fishes were fed with clam meat.

Fishes of immature stage with size range 10-13cm in length were selected for the experiment, irrespective of sex. The twenty four fishes were then transferred into each experimental tanks. Each tank contained 50 litres of water. For the study, filtered water containing corresponding hydrographical parameters of the collection area was used (salinity - 15±2‰, temperature 28 ± 1 °C, pH - 7.5 ± 0.5 and dissolved oxygen > 90% saturation). Based on the LC 50 values three different concentrations of metals, copper (0.001 ppm, 0.005 ppm and 0.01 ppm) and mercury (0.01 ppm, 0.015 ppm and 0.02 ppm) were added to the experimental tank. One tank was kept as control without metal solution and duplicates were run for each metal concentration. The test medium was renewed every 24 hrs. The physico-chemical parameters of the water were also measured. During the exposure of 15 days the fishes were fed with clam meat and feeding stopped 24h prior to each test experiment. The fishes were caught and immobilized with a hard blow on the head. Immediately the caudal peduncle of the fish was cut and blood was collected in small glass vials and were treated with heparin to prevent coagulation.

The different haematological analysis were carried out employing standard techniques (Hesser, 1960; Blaxhall and Daisley, 1973) unless specified.

Total Erythrocyte Count (TEC)

The techniques employed for the erythrocyte counts of fish blood were similar in most aspects to those used in mammalian counts except a change in RBC diluting fluid. Hendrick's RBC diluting fluid was used during the present study (Hendrick's 1952). The Hendricks fluid contained 10 gm of sodium sulphate, 2.5 gm of sodium chloride, 1.5 gm of sodium citrate and 50 ml of glacial acetic acid per 500 ml of distilled water. Neubauer type Haemocytometer was used for RBC counting. Total erythrocyte count is expressed in millions of RBC per cubic mm of blood.

Estimation of haemoglobin

Cyanomethaemoglobin method described by Ortho diagnostic systems (1986) was followed for estimating the haemoglobin content. To 0.02 ml of blood, 5 ml of Drabkin's reagent was added and stirred well. The potassium ferricyanide present in the reagent converts the haemoglobin iron from ferrous to ferric state to form haemoglobin and this in turn combines with potassium cyanide of the Drabkin's reagent to produce a stable pigment or the cyanomethaemoglobin which represents the sum of oxyhaemoglobin, carboxy haemoglobin and methaemoglobin. The cyanomethaemoglobin formed was measured spectrophotometrically at

540 nm. The calibration curve was prepared by the human haemoglobin standard provided with the reagent. The haemoglobin content is expressed as g%.

Measurement of haematocrit values
(or packed Cell volume - ht%)

Haematocrit value was measured by applying the method of McLeay and Gordon (1977). Blood was drawn into heparinised microhaematocrit tube (0.55 $\pm$ 0.05 mm diameter). One end of the tube was sealed and centrifuged in microhaematocrit centrifuge at 12000 rpm for 5 minutes. Haematocrit value was measured within 30 minutes of centrifugation and measured the red cell column using haematocrit counter provided along with the microhaematocrit centrifuge, and expressed as the percentage of whole blood.

Computation of erythrocyte constants

From the values of haemoglobin content (Hb%) haematocrit (Ht%) and total erythrocyte count (millions/mm^3) the following erythrocyte constants were calculated using the respective formula (Lamberg and Rothstein 1978).

Mean Corpuscular Volume (MCV)

MCV represents the average of individual erythrocytes in cubic microns (u^3) and computed by the formula

$$MCV = \frac{Ht\%}{RBC \text{ (in millions/ mm}^3\text{)}} \times 10$$

Mean Corpuscular Haemoglobin (MCH)

MCH represents the average weight of haemoglobin in individual erythrocytes in picograms (Pg) and calculated by the formula

$$MCH = \frac{Hb\%}{RBC \text{ (in million / mm}^3\text{)}} \times 10$$

Mean Corpuscular Haemoglobin Concentration

MCHC is the average haemoglobin concentration per 100 ml of packed erythrocytes in percent and computed by

$$MCHC = \frac{Hb\%}{Ht\%} \times 100$$

Data Analysis

The data have been subjected to statistical analysis using the student's `t' test to manifest the variation in comparison with the control. The variations were reported at three significant levles Viz. $P < 0.05$, 0.01 and 0.001.

RESULTS

Total erythrocyte count

Total erythrocyte count in mercury treated fishes are shown in the table 3 and Fig. 3. In fishes exposed to mercury the erythrocyte count did not vary from that of the control in 0.01 ppm. In 0.15 ppm and 0.02 ppm the erythrocytic count was significantly increased ($P < 0.001$).

Total erythrocyte count of copper dosed fishes are shown in table 4 and fig.4. Fishes exposed to 0.01 ppm copper registered a significant increase ($P < 0.01$) in erythrocyte count at all the time interval. In 0.005 ppm, the increase was only at 10th day of exposure.

Haemoglobin Concentration

Haemoglobin values of fishes exposed to mercury are given in table.5 and fig.5. The Haemoglobin values of fishes exposed to 0.01 ppm mercury showed an increase ($P <0.05$) at 24 hr. In 0.015 ppm the increase was at the 15th day of exposure. In 0.02 ppm mercury showed significant increase ($P<0.01$) at all the time intervel.

Haemoglobin values of fishes exposed to copper are given in table.6 and fig.6. In fishes exposed to copper the haemoglobin values did not vary from that of the control except in 0.01 ppm dosed fishes. In 0.01 ppm copper the haemoglobin values registered a significant increase ($P < 0.01$).

Table 3

Total erythrocyte count (as million/m³ of blood) in *Macrones gulio* exposed to different concentrations of mercury.

Conc. (ppm)	Time of exposure (days)			
	1	5	10	15
0	2.01 ± 0.08	2.01 ± 0.03	2.04 ± 0.24	2.13 ± 0.58
0.01	2.16 ± 0.15	2.25 ± 0.18*	2.40 ± 0.58	2.44 ± 0.24
0.015	2.60 ± 0.19***	2.62 ± 0.17***	2.80 ± 0.24***	2.99 ± 0.28*
0.02	3.19 ± 0.17***	3.32 ± 0.16***	3.44 ± 0.24***	3.66 ± 0.38***

Table 4

Total erythrocyte count (as millions/mm³ of blood) in *Macrones gulio* exposed to different concentrations of copper.

Conc. (ppm)	Time of exposure (days			
	1	5	10	15
0	2.66 ± 0.59	2.71 ± 0.43	2.81 ± 0.24	2.81 ± 0.57
0.001	2.93 ± 0.11	2.99 ± 0.28	2.99 ± 0.26	3.05 ± 0.46
0.005	2.04 ± 0.31	3.19 ± 0.17	3.21 ± 0.18*	3.22 ± 0.54
0.01	3.32 ± 0.16*	3.40 ± 0.19*	3.50 ± 0.33**	3.51 ± 0.18*

Values are the mean of six set of experiment ± SD *** P< 0.001, ** P < 0.01, * P< 0.05

Table 5

Concentration of Hb (as gm/ 100ml) in *Macrones gulio* exposed different concentrations of mercury.

Conc. (ppm)	Time of exposure (days)			
	1	5	10	15
0	7.02±0.85	7.51±0.98	7.81±0.34	8.10±0.66
0.01	8.22±0.38*	8.28±0.01*	8.40±0.85	8.51±1.37
0.015	8.65±0.32**	8.77±0.80	8.76±0.57*	8.94±1.27
0.02	9.14±0.57**	9.42±0.80**	9.60±0.94**	9.80±0.96*

Table 6

Concentration of Hb(as gm/10ml) in *Macrones gulio* exposed to different concentrations

Conc. ppm	Time of exposure (days)			
	1	5	10	15
0	8.50±1.09	8.79±1.04	8.86 ± 1.75	8.02± 1.70
0.001	9.10±1.12	9.58±1.46	9.68 ± 1.04	9.756±0.94
0.005	9.91±1.04	9.56±1.24	9.96 ± 0.97	10.96±0.91
0.01	10.49±0.67**	11.51±1.41**	11.76±1.14*	11.87±0.82**

Values are the mean of six sets of experiment ± SD *** P< 0.001, ** P< 0.01, * P< 0.05

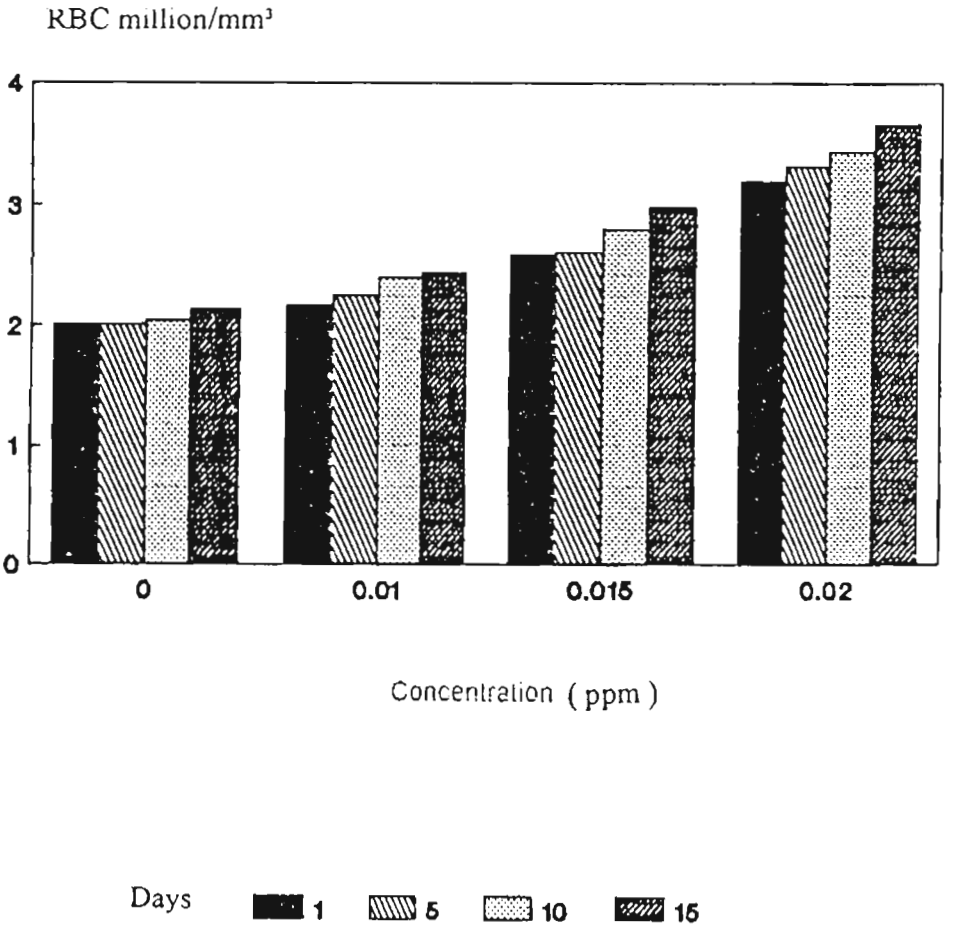


FIGURE 3 TOTAL ERYTHROCYTE COUNT IN *MACRONES GULIO* EXPOSED TO MERCURY.

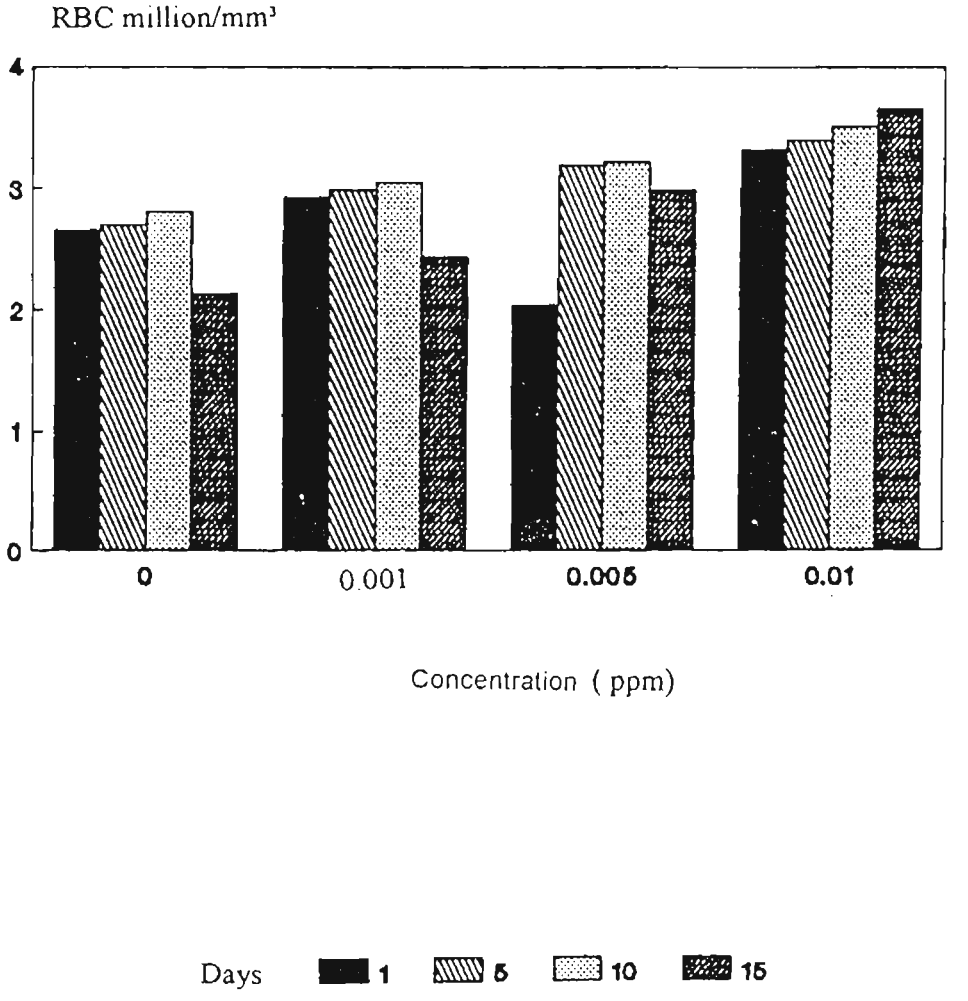


FIGURE 4 : TOTAL ERYTHROCYTE COUNT IN *MACRONES GULIO* EXPOSED TO COPPER.

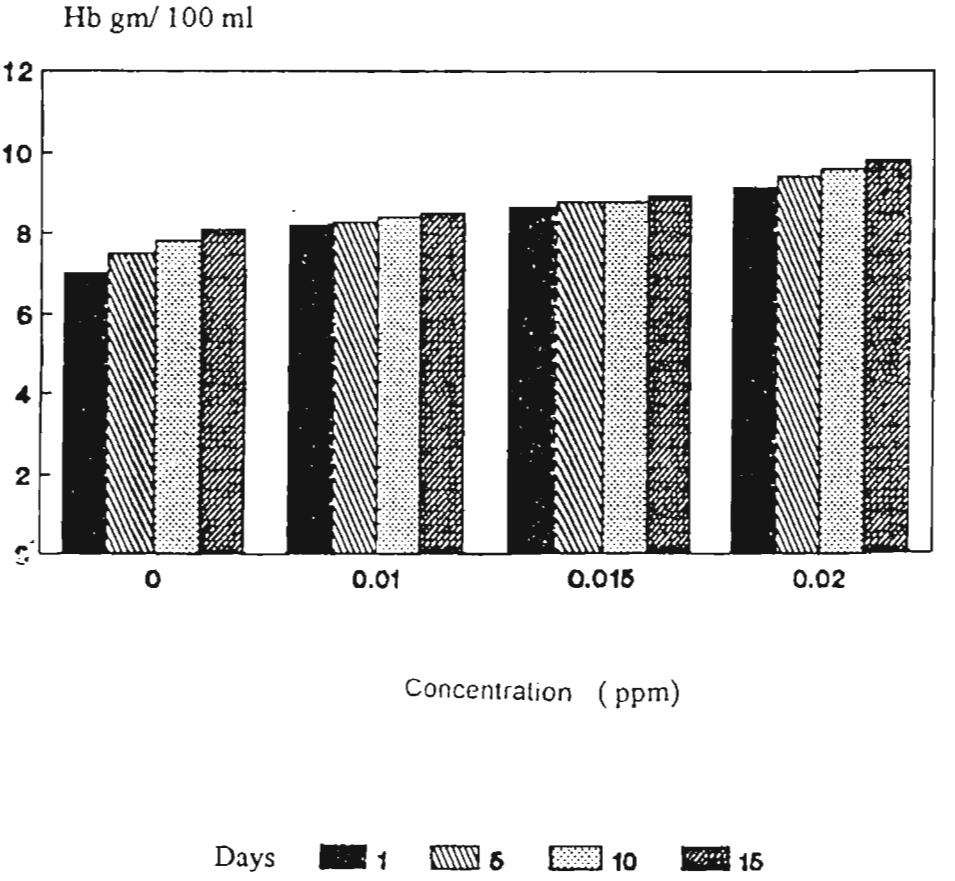


FIGURE 5 : HAEMOGLOBIN VALUES IN *MACRONUS GULIO* EXPOSED TO MERCURY.

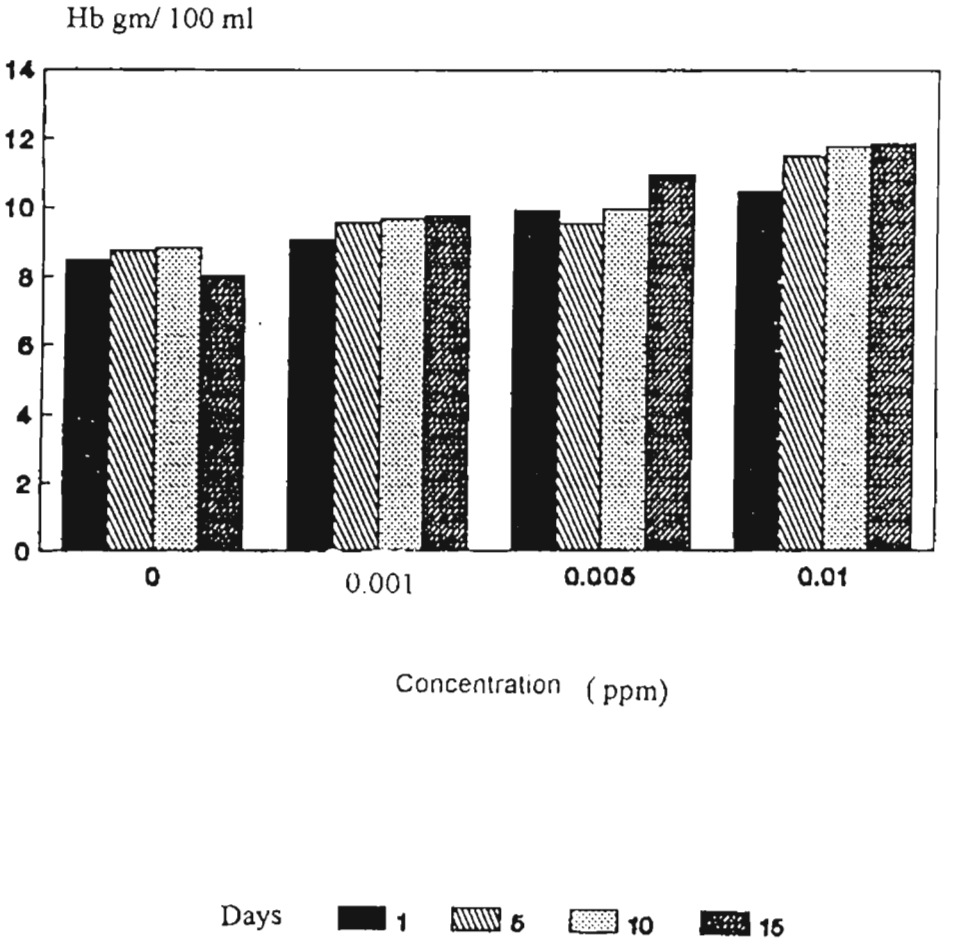


FIGURE 6 HAEMOGLOBIN VALUES IN *MACRONES GULIO* EXPOSED TO COPPER.

Haematocrit values

Hct values of fishes exposed to mercury are given in table 7 and fig.7 Hct values of fishes exposed to different concentration of mercury showed significant increase ($P < 0.05$) only in 0.02 ppm at 5 and 15 days. Hct values of fishes exposed to copper are given in table 8 and fig.8. Hct values of fishes exposed to copper did not show any significant variation.

Mean Corpuscular Haemoglobin Concentration

MCHC values of fishes exposed to mercury are given in table 9 and fig.9. MCHC values of fishes exposed to 0.015 ppm mercury shown significant increase ($P < 0.05$) at 5th day. MCHC value of fishes exposed to copper are given in table 10 and fig.10. Fishes exposed to 0.01 ppm registered a significant increase ($P < 0.05$) in MCHC values at 10th day.

Mean Corpuslar Haemoglobin Values

MCH values of fishes exposed to mercury are given in table 11 and fig.11.

MCH values in fishes exposed to 0.02 ppm mercury showed significant decrease ($P < 0.01$) at 1, 5, 10 and 15 days; in those fishes exposed to 0.015 ppm mercury showed significant decrease ($P < 0.01$) at 10 days when compared with the control.

Table 7

Haemocrit values (as %) in *Macrones gulio* exposed to different concentrations of mercury.

Conc. (ppm)	Time of exposure (days)			
	1	5	10	15
0	45.00± 2.39	45.25 ± 2.21	45.92 ± 4.1	45.42 ±2.76
0.01	45.13±1.44	46.50 ± 3.04	47.76 ±3.65	47.61±4.36
0.015	47.25±1.27	47.87 ± 7.34	48.81± 1.47	49.01±3.20
0.02	49.79±2.43*	49.86 ± 2.46*	50.08±2.38*	50.20±2.85*

Table 8

Haematocrit values (as %) in *Macrones gulio* exposed to different concentrations of copper.

Conc. (ppm)	Time of exposure (days)			
	1	5	10	15
0	48.24± 3.84	48.57± 3.03	48.74± 2.73	48.81 ±1.47
0.001	49.39±1.96	49.52± 1.86	49.62 ±2.48	48.92 ±3.20
0.005	50.08±2.38	50.23 ±4.99	50.25 ±2.86	50.56±2.54
0.01	50.87± 1.76	51.09 ±1.50	51.74 ±4.74	52.47 ±3.40

Values are the mean of six set of experiment ± SD ***P < 0.001 , ** P< 0.01, * P< 0.05

Table 9

Mean corpuscular haemoglobin concentration (as MCHC gm/100 ml packed RBC) in *Macrones gulio* expoed to different concentration of mercury.

Conc. (ppm)	Time of exposure (days)			
	1	5	10	15
0	15.69 ± 2.41	16.63±2.38	17.00 ±1.15	17.91±1.90
0.01	18.24 ± 1.06	17.75±1.85	17.74 ±2.75	17.84±2.00
0.015	18.31 ± 0.70*	18.80±4.10	17.97 ±1.10	18.19±2.10
0.02	18.26 ± 1.26	19.14±2.03	19.22 ±2.14	19.56±2.36

Table 10

Mean corpuscular haemoglobin concentration (as MCHC gm/100ml packed RBC) in *Macrones gulio* exposed to copper.

Conc. (ppm)	Time of exposure (days)			
	1	5	10	15
0	17.66±2.08	18.18±2.53	18.32±2.97	18.29±3.56
0.001	18.46±2.37	19.37±3.09	19.63±2.89	20.08±2.85
0.005	19.82±2.27	20.13±3.80	19.83±1.70	19.88±1.35
0.01	20.68±1.35*	22.51±2.65*	22.87±2.55*	22.80±2.99

Values are the mean of six set of experiment ± SD * P < 0.05, ** P < 0.01, *** P< 0.001

Table 11

Mean corpuscular haemoglobin (as picograins (pg)) in *Macrones gulio* exposed to different concentration of mercury.

Conc. (ppm)	Time of exposure (days)			
	1	5	10	15
0	35.02±4.31	38.15±4.52	38.62±3.53	41.26±12.18
0.01	38.26±1.88	37.05±5.56	37.05±9.67	34.91±4.78
0.015	33.45±2.41	33.72±4.36	31.48±3.01**	30.52±6.86
0.02	29.30±3.14*	28.39±2.14**	28.04±3.35**	27.30±5.78*

Table 12

Mean corpuscular haemoglobin (as picogram (pg)) in *Macrones gulio* exposed to different concentrations of copper.

Conc. (ppm)	Time of exposure (days)			
	1	5	10	15
0	33.62±7.77	32.87±5.59	33.05±3.36	34.04±10.22
0.001	31.13±3.75	32.31±5.42	32.39±2.94	32.82±5.99
0.005	33.22±6.67	31.19±3.12	32.99 ±6.09	32.29±6.87
0.01	31.62±1.81	33.86±3.70	33.79±3.40	33.38±2.45

Values are the mean of six set of experiment ± SD ***P < 0.001, ** P< 0.01, * P< 0.05

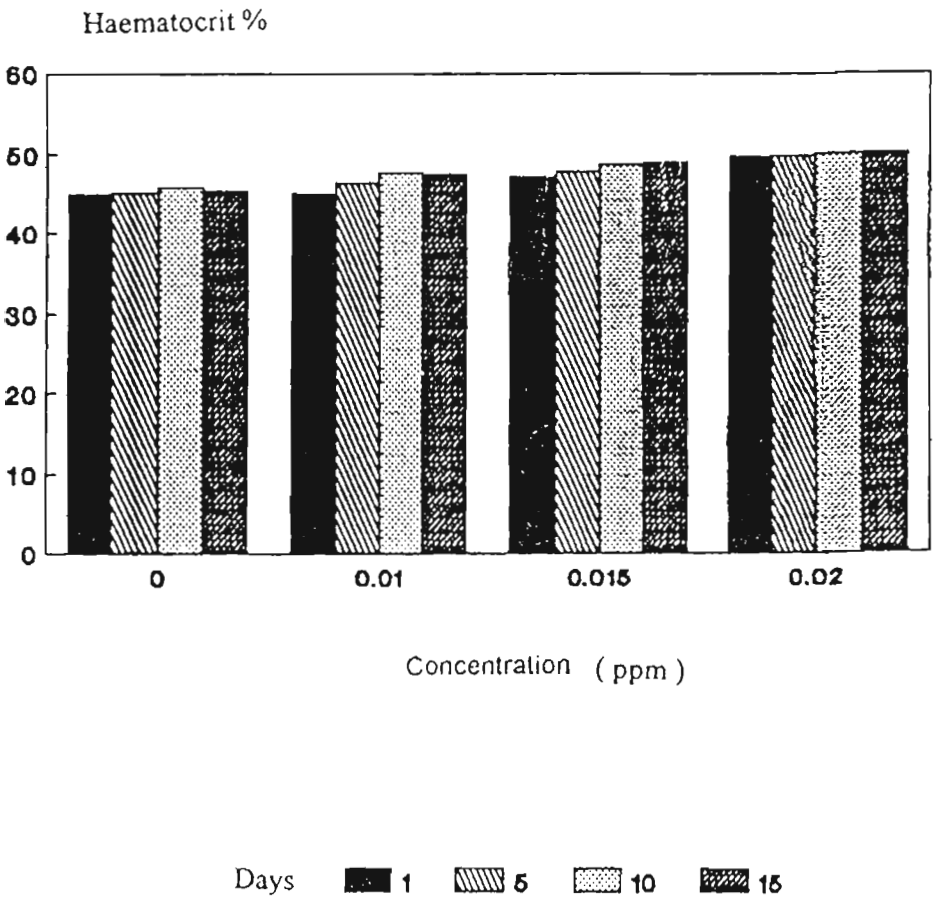


FIGURE 7 : HAEMATOCRITE VALUES IN *MACRONES GULIO* EXPOSED TO MERCURY.

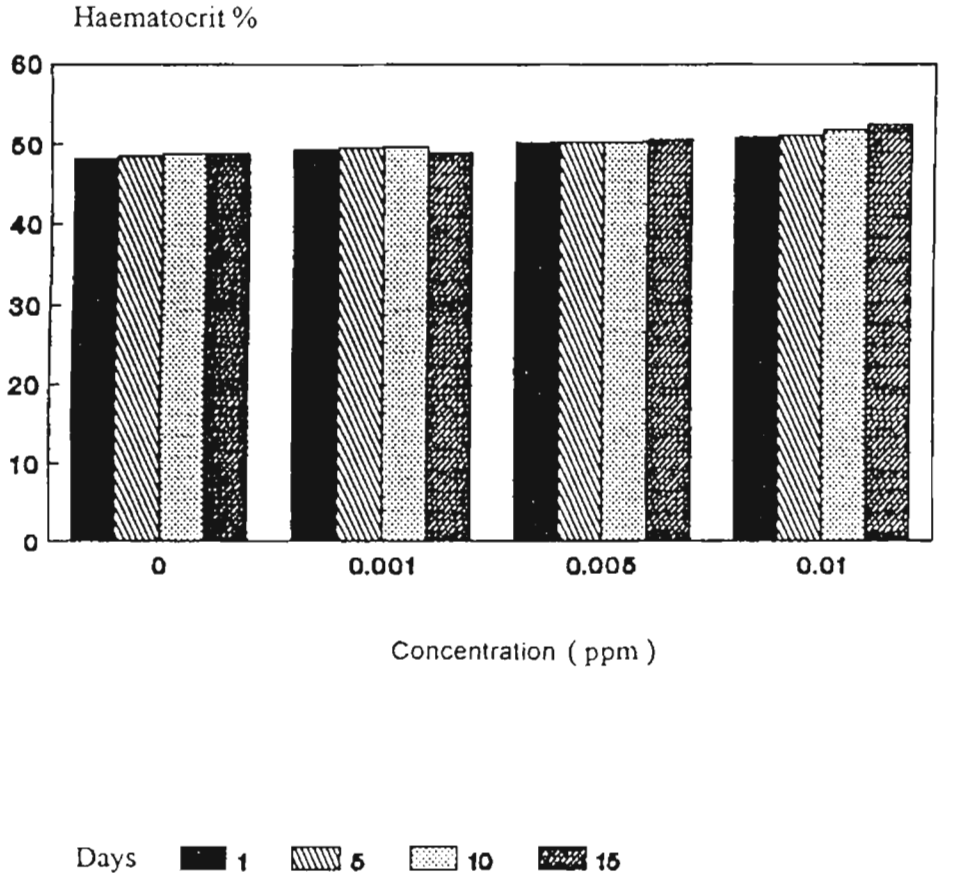


FIGURE 8 : HAEMATOCRITE VALUES IN *MACRONES GULIO* EXPOSED TO COPPER.

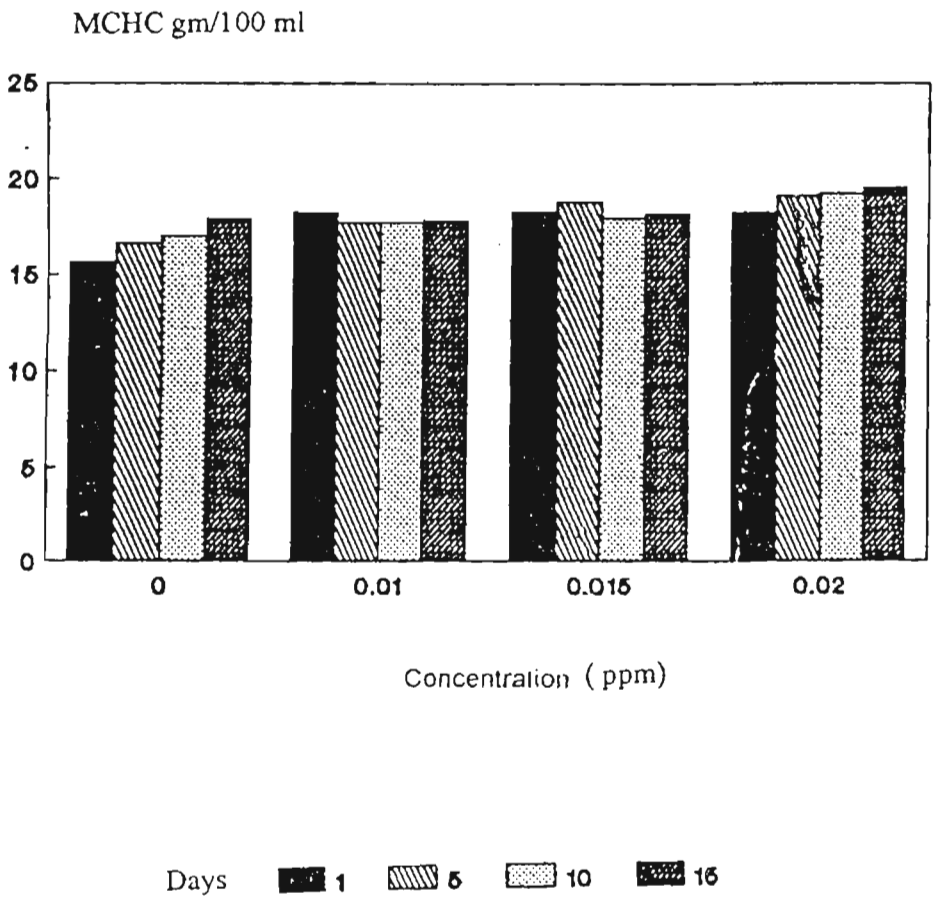


FIGURE 9 : MEAN CORPUSCULAR HAEMOGLOBIN CONCENTRATION IN *MICRONES GULIO* EXPOSED TO MERCURY

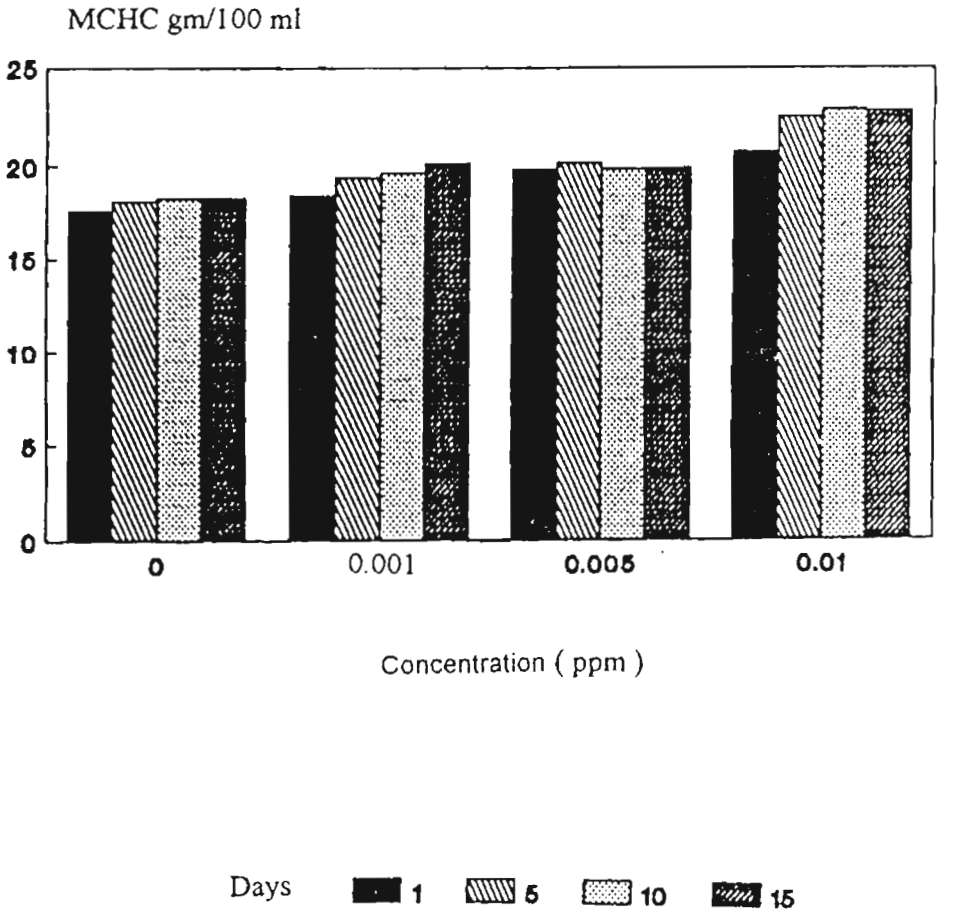


FIGURE 10 MEAN CORPUSCULAR HAEMOGLOBIN CONCENTRATION IN *MACRONUS GULIO* EXPOSED TO COPPER.

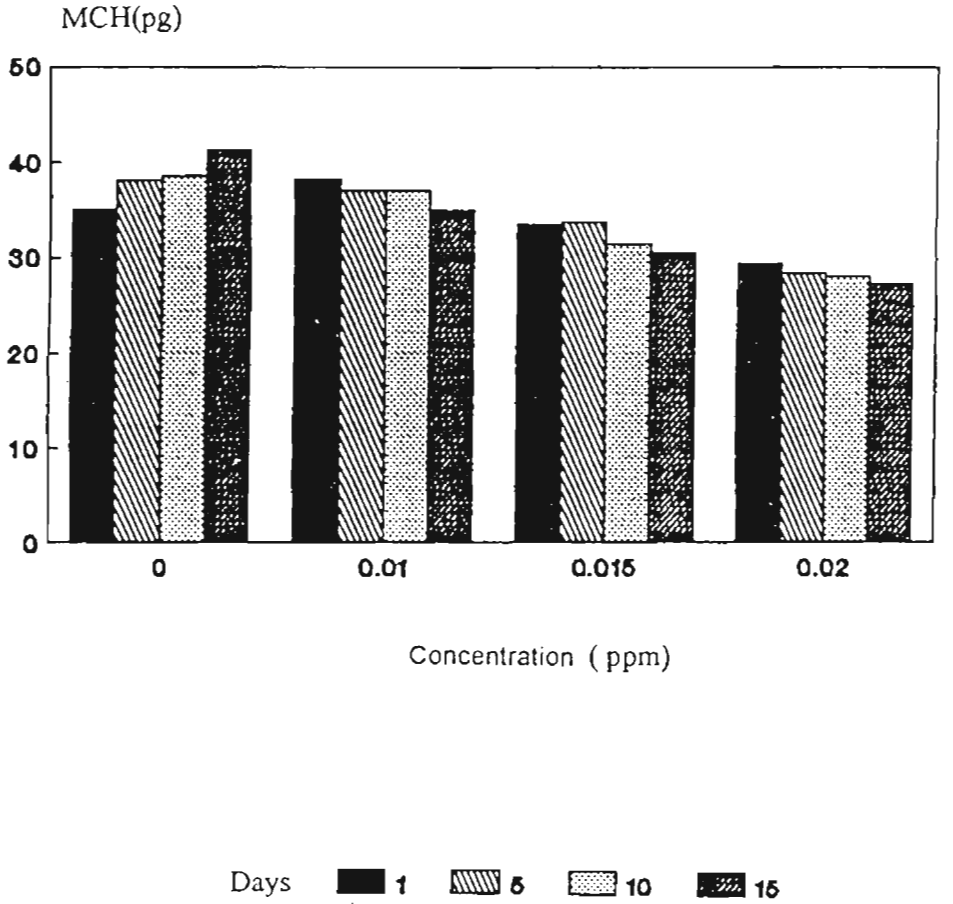


FIGURE11: MEAN CORPUSCULAR HAEMOGLOBIN IN *MACRONES GULIO* EXPOSED TO MERCURY.

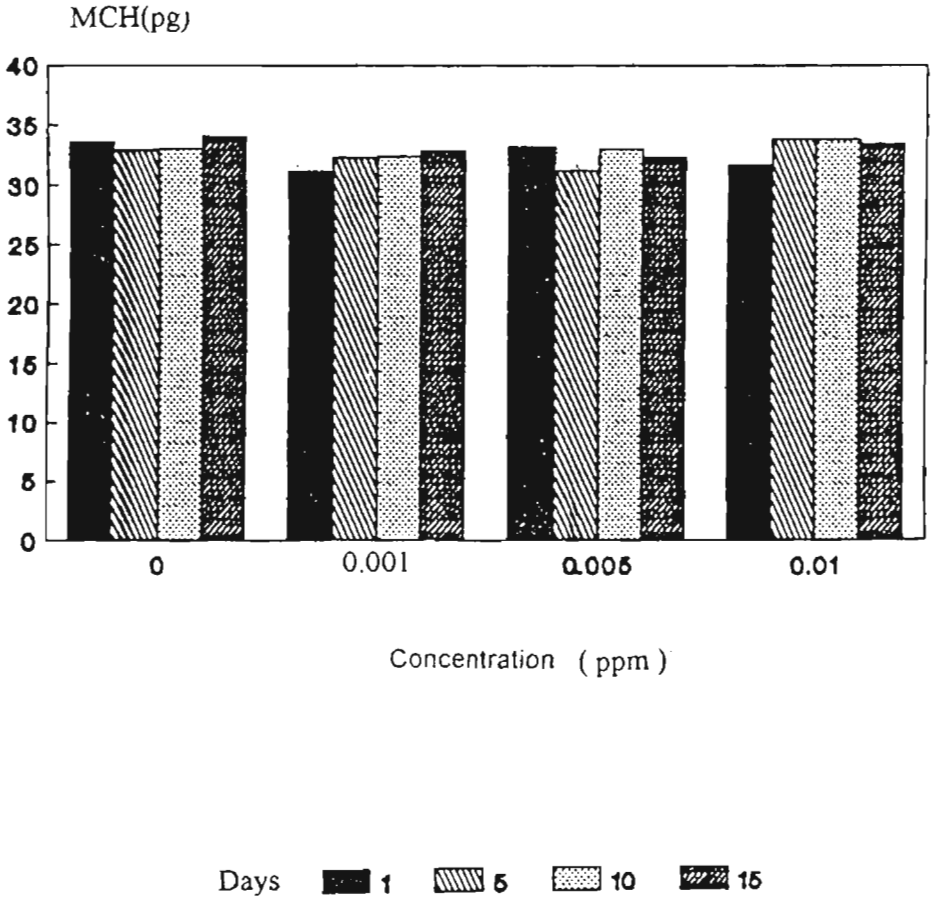


FIGURE 12 : MEAN CORPUSCULAR HAEMOGLOBIN IN *MACROMES GULIO* EXPOSED TO COPPER.

MCH values of fishes exposed to copper are given in table 12 and fig.12. MCH values of fishes exposed to different concentrations of copper did not vary from that of control.

Mean Corpuscular Volume

MCV values in fishes exposed to mercury are given in table 13 and fig.13.

MCV Values in fishes exposed to 0.015 ppm mercury showed significant decrease ($P < 0.01$) at 1, 5, 10 and 15 days when compared to control. In those fishes exposed to 0.02 ppm mercury showed significant decrease ($P < 0.001$) at 1, 5, 10 and 15 days when compared with that of the control. MCV values in fishes, exposed to copper are given in table 14 and fig.14. MCV values in fishes, exposed to 0.01 ppm mercury showed significant decrease ($P < 0.05$) at 5th day of exposure.

DISCUSSION

The result indicates an increase in haemoglobin content in fishes exposed to copper and mercury towards the later period of the experiment. There are reports of elevated Hb levles in brook trout exposed to copper (McFadden, 1965); in S. fontinalis exposed to methyl mercury (Christensen et al. 1977) ; in Channa punctatus exposed to mercury (Chitra and RamanaRao, 1986) and in dog fish exposed to cadmium (Tort and Torres, 1988).

Table 13

Mean corpuscular volume (as cubic microns μ^3) in *Macrones gulio* exposed to different concentrations of mercury.

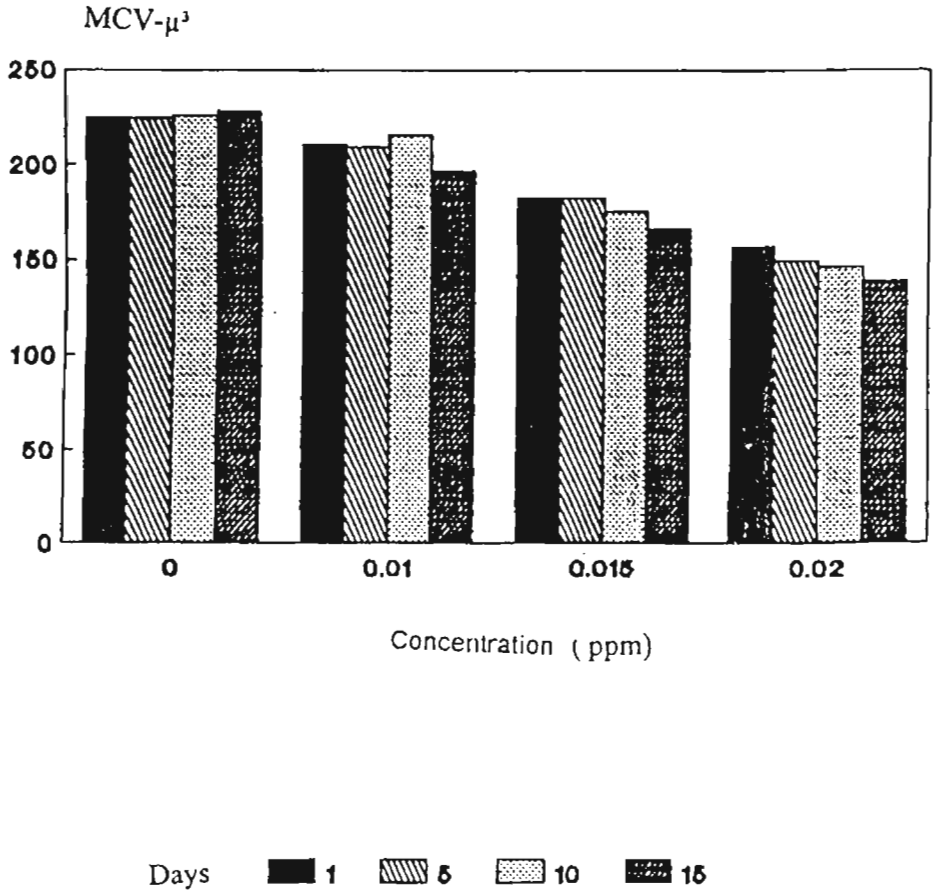
Conc. ppm	Time of exposure (days)			
	1	5	10	15
0	224.85 ± 20.53	224.28 ± 13.57	225.85 ± 12.21	228.13 ± 55.77
0.010	210.25 ± 14.41	208.76 ± 25.02	215.46 ± 70.58	196.26 ± 19.19
0.015	182.77 ± 13.15 **	182.72 ± 23.52 **	175.27 ± 14.20 ***	165.89 ± 20.48 *
0.02	156.69 ± 11.19 ***	149.41 ± 13.62 ***	146.21 ± 10.96 ***	138.49 ± 14.80 **

Table 14

Mean corpuscular volume (as cubic microns μ^3) in *Macrones gulio* exposed to different concentration of copper.

Conc. ppm	Time of exposure (days)			
	1	5	10	15
0	190.20 ± 37.53	181.46 ± 24.44	175.31 ± 20.33	179.36 ± 29.24
0.010	168.94 ± 5.22	166.8 ± 13.55	167.44 ± 20.47	163.72 ± 23.64
0.015	168.76 ± 18.28	158.48 ± 21.04	156.79 ± 10.93	161.08 ± 29.18
0.02	153.65 ± 10.01	150.73 ± 8.02 *	149.64 ± 22.96	150.29 ± 14.96

Values are the mean of six set of experiment ± SD *** P< 0.001, ** P < 0.01, * P< 0.05



Y.

FIGURE 13: MEAN CORPUSCULAR VOLUME IN *MICRONES GULIO* EXPOSED TO MERCURY.

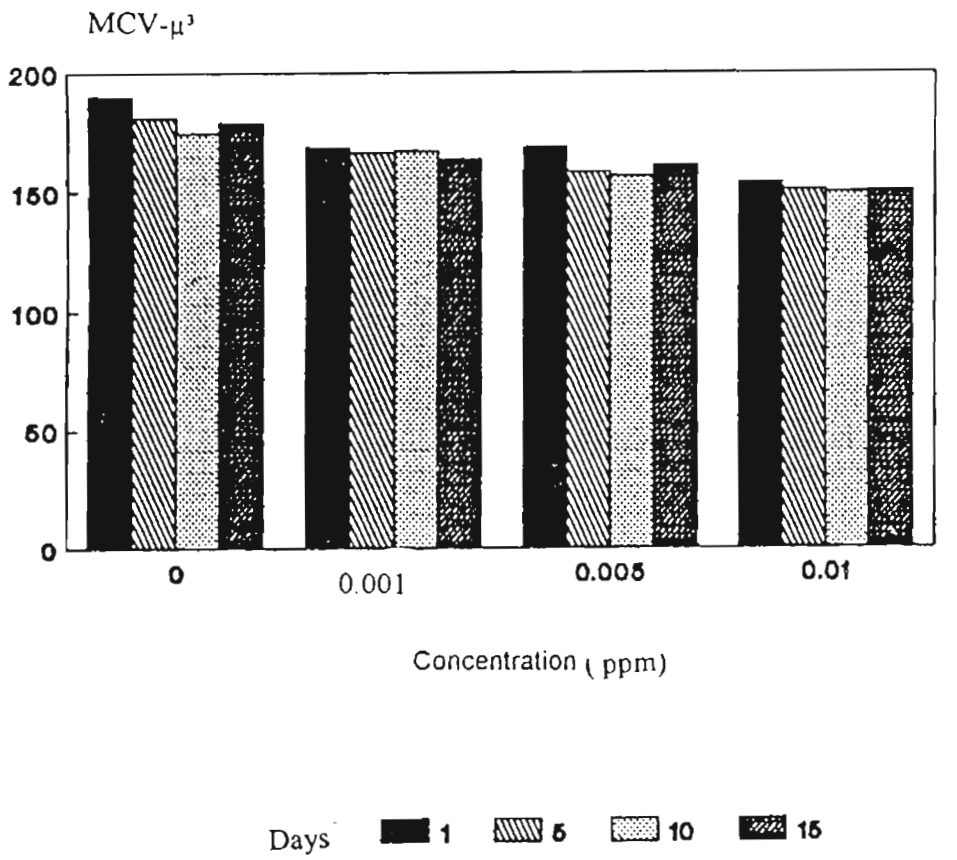


FIGURE 14 : MEAN CORPUSCULAR VOLUME IN *MACROMIS GULIO* EXPOSED IN COPPER.

In the present study haematocrit values showed an increase during the entire experimental period in fishes exposed to copper and mercury. In the early period of the experiment, an increase in the Hct values without any change in the Hb values may be due to Haemodilution. Haemodilution results in swelling of erythrocytes as reported in the dog fish. S. canicula in response to copper (tort et al. 1987) and hence an increase in Hct values without a change in Hb values.

Haemodilution has been interpreted as a mechanism which reduces the concentration of an irritating factor in the circulatory system (Smith et al. 1979). Decreased osmoregulation is the consequence of copper toxicity in fish (Leland and Kuwabara, 1985). Haemodilutin has been obserrved in Colisa fasciatus exposed to zinc by Mishra and Srivastava (1979,1980). One of the consequences of haemodilution is the decrease in plasma osmotic pressure as observed in Ictalurus punctatus in response to copper and zinc (Lewis and Lewis, 1971). An increase in the erythrocyte size is generally considered as a response against stress. The swelling of RBC would be a consequence of factors like high pCo_2, high lactate concentration or low PO_2 in the blood, leading to low ATP concentration, which would increase the oxygen affinity of blood (Soivio and Nikinmaa, 1981). Abrahamsson and Nilsson (1975) observed that the contraction of spleen of cod exposed to a stressed would release blood cell into the blood stream. A similar pattern has been detected in

Cyprinus carpio after cadmium exposure (Koyama and Ozaki, 1984), in which haematocrit decreases.

Helmy et al. (1978) reported a decrease in RBC count, Hb and Hct in the fish mullet exposed to copper and mercury. Similar effects were detected in flounder exposed to cadmium (Johansson - Sjobeck and Larsson, 1978); in winter flounder exposed to mercury (Dawson, 1979); in marine teleost, Aphanius dispar (Hilmy et al. 1980) and C. carpio exposed to cadmium and mercury (Beena and Viswaranjan, 1987). Panigrahi and Misra (1978, 1980) reported low Hb and RBC count associated with reduced respiratory rate in the fresh water fish Anabas scandens and Tilapia mossambica dosed with mercury.

Decrease in Hb was observed in perch in response to cadmium (Larsson, 1975); in brook trout exposed to lead (Christensen et al. 1977); in Anabas scandens dosed with mercury (Panigrahi, and Misra 1977); in S. mossambicus exposed to mercury (Aruna and Gopal, 1987); and in Clarias lazera intoxicated with copper (EL - Domiaty, 1987).

In the present study there was a significant increase in haemoglobin content and a corresponding increase in the haematocrit values in the later part of experiment. This may be due to the adaptation of the fishes body to metal stress. Haemodilution could be an initial reaction of the body to stress. Afterwards the living system rectified the imbalance by removing water from the blood. This could result in haemoconcentration.

Hilmy et al. (1980) reported that values of Hct, Hb and RBC count returned to control levels after an initial decrease in marine teleost A. dispar in response to mercury toxicity. Buckley (1976) also observed a partial recovery of Hb after a decline in Coho salmon exposed to treated water containing total residual chlorine ($TRCl_2$). He suggested (1) decreased haemolysis as a result of elimination of susceptible cells and decreased sensitivity of younger cells to oxidants and (2) compensatory erythropoiesis with the establishment of a balance between cell destruction and formation resulting in reduced number of circulating cells. But tort and Torres (1988) ruled out the hemolysis or RBC destruction as the RBC count increased in the fish after cadmium exposure. They postulated that the RBC count elevation was due to consequences of blood cell reserve combined with cell shrinkage, probably due to osmotic alterations of blood by the action of the metal. In addition, haemoglobin measurements by Tort and Torress (1988) in plasma showed no increase of extracellular haemoglobin. Torres et al. (1986) found that in fish subjected to confined stress condition, zinc treatment significantly decreased Hct and RBC count and the decrease was identical.

Gluth and Hanke (1985) postulated a biphasic response to pollutant in C. carpio consisting of water loss followed by a water gain in the blood. But in the present study the biphasic response observed in the copper dosed fish was just the reverse, that is, water gain followed by a water loss. Gill and Pant

(1981) also obtained a biphasic response similar to the findings in the present study. They observed a fall in haemoglobin, RBC following 1-3 weeks exposure to sublethal concentrations of mercury in the Puntius conchonius, but recorded an increase in Hb and RBC count after 8 weeks of exposure.

During the entire experimental period there was no significant variation in the mean copuscular haemoglobin concentration (MCHC) in the present study. Svobodova (1982) in C. carpio treated with copper and mercury; Gill and Pant (1985) in P. conchonius dosed with cadmium did not find any significant difference in MCHC values from that of control values. Because MCHC is the ratio of blood haemoglobin concentration to the Hct, it is not dependent on the blood volume or on the number of red cells per unit volume. This clearly indicates that the decrease of Hb noted in the present study was not due to haemolysis or unusual RBC destruction but caused by haemodilution. Similarly the increase in haemoglobin and a corresponding increase in Hct was due to either haemoconcentration or increased erythropoiesis or both.

The lack or decrease in Hb in mercury trated fishes could be due to increased production of urine which might remove the excess water present in the blood as a result of haemodilution. Lock et al. (1981) observed that increased water uptake by the gills did not result in the decreased haemotocrit values of mercury treated rainbow trout and instead there was an increase

in the haematocrit values. He suggested that the inflow of water is offset by an enhanced urine flow. The enzyme Na, K-ATPase appears to be involved in osmoregulatory transepithelial electrolyte transport in the gills, intestine and urinary bladder as well as in active sodium potassium exchange across all cell membranes (Schmidt - Neilsen, 1974). In a wide variety of tissue this enzyme is sensitive to mercurials and other sulphydryl reagent (Schwartz et al. 1975). Mercury may prevent the reabsorption of water across kidney and tubules, resulting in the increased urine flow and hence haemoconcentration.

The Hb content in both the copper and mercury dosed fishes increased at all time interval. There was an increase in the haematocrit values as well. The significant increase of Hb and Hct observed in copper and mercury treated fishes could have due to an increased production of RBC by the erythropoetic organs along with haemoconcentration. Mckim et al. (1970) in brook trout Salvelinus fontinalis and Svobodova (1982) in C. carpio exposed to copper reported a significant increase in RBC, Hct and Hb. The mean cell volume (MCV) and mean corpuscular haemoglobin concentration (MCHC) in C. carpio remain in without changes. This indicats that the incerase in Hb and Hct is due to an increase in RBC number. Svobodova (1982) explained the changes in the haematological parameters in the intoxicated carp as disorders in the oxidation process in the fish. An increase in Hb, Hct values were observed in S.fontinalis in response to copper (Mckim et al. 1970) in Ictalurus nebulosus exposed to

copper (Christensen et al. 1972) in C. fasciatus treated with nickel (Agrawal et al. 1979) and in rainbow trout dosed with copper (Wotten and Williams, 1980).

Haemoglobin levels were elevated in response to copper in brook trout (McFadden, 1965); in S. fontinalis in response to methyl mercury (Christensen et al. 1977); in Channa punctatus in response to mercury (Chitra and Ramana Rao 1986) and in dog fish in response to cadmium (Tort and Torres, 1988). Similarly Hct values increased in rainbow trout exposed to methyl mercury and in Mystus vitatus in response to copper and zinc (Singh and Singh, 1982).

The increase in Hb and Hct observed in the present study in metal dosed fishes may be an attempt by the body to counteract the low oxygen content of the blood. The low oxygen content may be due to the low oxygen carrying capacity of the blood or faulty gaseous exchange caused by damage to the gills.

It has been widely reported that many pollutants enter the RBC and either oxidise or denature the Hb by inhibiting the Glycolysis or metabolism of the hexose monophosphate shunt (HMPS). Fairbanks (1967) showed that copper penetrates the intact erythrocyte inhibiting glycolysis, denaturing Hb and oxidising glutathione. Chlorine also seemed to diffuse readily through gills oxidising Hb to methaemoglobin and disrupting erythrocyte membrane resulting to haemolysis (Zeitoun, 1977). Grothe and Eaton (1975) found a methaemoglobin (MHb) level of 30%

of total Hb. Formation of Methaemoglobin reduces the oxygen carrying capacity of the blood. Scarano et al. (1984) observed a decrease in Hb and increase in methaemoglobin in Seabass exposed to nitrate.

Asano and Hoikari (1987) stated toxic concentration of copper may cause cytotoxicity by its oxidant action and can affect the function of erythrocytic enzymes leading to oxidation of Hb, a disulphide formation of the membrane proteins and a decrease in the intracellular concentration of glutathione.

Hodson et al. (1980) studied the effects of water borne selenium on rain bow trout and found that even though the blood parameters decreased from the control levels by 30% the fish appeared to be compensating for these changes by increased erythropoiesis. Sahib et al. (1981) found that the exposure of fish to a sublethal concentration of malathion showed a consistent increase in the oxygen consumption up to 24 hr and later declined to 48% suggesting reduction of oxidative metabolism at the end of 48 hr. Panigrahi and Misra (1980) found that the uptake of oxygen decreased 27% in Tilapia mossambica exposed in mercury. Similarly Chlorine produced oxidants (CPO) reduced oxygen carrying capacity of the fish Leiostomus xanthurus (Middaugh et al. 1980).

A decrease in the oxygen carrying capacity may stimulate erythropoiesis in fish so that blood carries enough oxygen to meet the requirement of the body. The increase in Hb and Hct in

metal exposed fishes of the present study may be due to this phenomenon. An increased erythropoiesis may result in an increase in RBC count, Hb and Hct. An increase in RBC count or polycythemia in fishes after exposure to various toxicants were reported by many authors (Buckley et al. 1976, 1979; Agrawal et al. 1979; Juelich, 1979; Verma et al. 1981 C; Singh and Singh, 1982; Junjea and Mahajan, 1983; Lal et al. 1986; Haniffa et al. 1986; and Pant et al. 1987). Along with stimulation of erythropoiesis, a reduction in plasma volume and a mobilization of new erythrocytes into circulation could also have contributed to the increase in Hb and Hct. Erythrocyte recruitment was associated with depletion of spleenic RBC reserves which may be reflected in the erythrocyte count (Milligan and Wood, 1982). Lal et al. (1986) found that increase in RBC count was followed by a reduction in spleenosomatic index indicating a release of RBC from the spleen. Buckley (1976) and Buckley et al. (1979) had shown that there is an increase in the number of circulating immature erythrocytes when fishes were exposed to different pollutants. Buckley (1976) postulated that increased number of RBC in the circulatory system was an attempt by the body to meet the elevated demands for O_2 or CO_2 transport as a result of increased metabolic activity during stress or by a destruction of gill membrane causing faulty gaseous exchange. Nayak and Madhyastha (1980) found an erythropoietic response as evidenced by significant increase in the number of immature RBC.

Pollutants can influence the functioning of all parts of respiratory chain. Pollutant may not only restrict gas transfer, but their irritant effect can also interfere with ventilation (Hughes, 1981). Lindahl and Hell (1970) found that the gills from fishes exposed to mercurials show clear tissue injuries. The layer of epithelial cells is detached from the deeper layers. This causes faulty gaseous exchange. When gills from fish exposed to phenyl mercurial were studied, a decrease in the circulation of blood was observed in the secondary lamellae. This may either be the effect of decreased circulation of the blood in the secondary lamellae or diminished exchange between water and blood in the secondary lamellae or structural change in the haemoglobin molecule due to binding of phenyl mercury ions. Diffusing capacity of the gill is reduced, following the action of pollutants and consequently there is a fall in oxygen supply to the tissues which become hypoxic.

Wedmeyer (1971) explained the increased pituitary activity in formalin treated rainbow trout on the basis of a chemical adversely affecting gill function. Such an interference with gill function can be expected to reduce its respiratory role so that the Hb was increased in the treated fish to compensate the loss. This sort of compensatory reaction is known to occur in fishes infected by certain parasites (Kabata, 1970).

So a faulty gaseous exchange of gases as a result of damage to the gills by the action of metals or oxidation of haemoglobin

to MHB by various toxicants lowers the oxygen carrying capacity of the blood. Reaction to such a situation would be stimulating the erythropoietic tissue and increasing the Hb content of the blood. The increased Hb and Hct values observed in the metal treated fishes of the present study could be due to the increased erythropoiesis and Hb synthesis.

CHAPTER 4

EFFECT OF COPPER AND MERCURY ON THE GLYCOGEN AND PROTEIN CONTENT OF THE LIVER AND MUSCLE OF THE FISH *Macrones gulio*

Even though effects of environmental pollutants on the mortality of aquatic animals have been studied by many workers, very little is known about the disturbed physiological and biochemical processes within the organism following exposure to environmental pollutants. This is all the more important as contamination of natural water resources by heavy metals threaten fish culture and population (Mckim and Benoit, 1971; Christensen, 1975).

When heavy metal ions exceed a threshold concentration in the aquatic ecosystem, they act as pollutants and create stress in fish. Environmental pollution is reported as one of the major factors causing hypoxemia in animals (Black et al. 1962). The respiratory potential of an animal is an important physiological parameter to assess the toxic stress because it is a valuable indicator of energy expenditure in particular and metabolism in general. Basha et al. (1984) found that the activity levels of succinate dehydrogenase and malate dehydrogenase decreased in toxicant - exposed fish suggesting the prevalence of hypoxia pesticides, heavy metals and other xenobiotics are known to affect the oxygen consumption and metabolic path ways.

The respiratory system of fish seems to be the prime target of many pollutants. When tissue of the animal do not receive sufficient oxygen they must either reduce the overall energy demand or respire anaerobically. Since glycogen is the ready

source of energy even in anaerobic condition, the depletion of glycogen from the tissue is expected to be an immediate manifestation of hypoxemia. During severe hypoxia, flounder reduces its oxygen consumption and partially compensates by increasing anaerobic energy metabolism based on fermentation of glycogen or glucose with lactic acid as the major anaerobic end product (Jorgensen and Mustafa, 1980). This strategy is also employed by other fishes such as carp (Johnston, 1975), goldfish (Vanden Thillart, 1982) and trout (Burton and Spehar, 1971). A decrease in the glycogen content confirms the prevelance of hypoxic condition at the tissue level since anoxia or hypoxia increases carbohydrate consumption (De Zwaan and Zandee, 1972) thereby creating a sort of stress in the fish even at the sublethal level, resulting in extra expenditure of energy.

The biochemical and physiological adaptations made by fish in response to change in environmental oxygen levels can be correlated to the ecology of the species. Species such as the European carp, Cyprinus carpio, which has been shown to live for several months in water of very low oxygen content (Blazka, 1958) are able to survive by reducing their oxygen uptake and changing to anaerobic metabolism (Johnston, 1975). Other species such as salmonids, adapted to environments of high oxygen tension, are less able to survive hypoxia (Itazawa, 1971). Since different fishes react differently to hypoxic situation, the procedure of using oxygen consumption as a yard - stick to measure the metabolic activities of the body may not produce satisfactory

results. Although a number of methods are now available to measure sublethal effects of pollutants (Sprague, 1971) most of them are long term and are not suitable for routine monitory programs.

Extensive investigations have revealed that different tissues of fish can sustain varying levels of anaerobic metabolism. In most teleosts fermentation of glucose to lactate provided the main source of energy under hypoxic condition (Heath and Pritchard, 1965; Burton and Spehar 1971). Black et al. (1961) found that the endurance of fast swimming fish is limited by the anaerobic energy released when stored glycogen is transformed by the Emden - Meyerhof cycle to form lactic acid within muscle cells. Johnston (1975) postulated that while skeletal muscle of fish, in common with most vertebrate tissues, responds to periods of anoxia by an increase in anaerobic glycogenolysis.

In almost all these circumstances the major share of stored energy comes from the carbohydrate or glycogen reserves. Thus carbohydrates form the central point in energy production because of great mobility in the living systems, together with its capacity to get compartmentalised within cells and tissues. The mobility is provided by glucose and compartmentalisation by glycogen and glucose-6-phosphate.

It is widely accepted that carbohydrate deposits in the form of glycogen in tissues like liver and muscle provided the

immediate energy requirements in teleost fishes under a variety of stressors including exercise (Black et al. 1960, 1961, 1962) physical disturbance (Nakano and Tomlinson, 1967), Starvation (Black et al. 1966), environmental hypoxia (Hea th and Pritchard, 1965; Narasimhan and Sundararaj, 1971), Salinity changes (Bashamohideen and Paravatheswara Rao, 1972).

Effects of environmental stress due to chemical pollution on tissue glycogen levels of fish have also been reported (Mc Ieay and Brown, 1975; Mazmanidi and Kovaleva 1975; Gill and Pant, 1981; Dange and Masurekar, 1982). These studies, involving exposure of fishes to different pollutants have indicated that the pollution stress stimulates glycogenolysis in fish tissue.

From a biochemical point of view, life is uniquely characterised by its association with protein. Tissue proteins as energy sources for fishes during thermal stress, spawning, and muscular exercise have been demonstrated by several investigators Idler and Clemens 1959). Though considerable information is available dealing with the determination of acute toxic levels of several pollutants and their influence on oxidative metabolism, studies on the tissue energy sources are relatively few.

Adrenocortical hormones are known to influence mammalian intermediary metabolism by stimulating protein metabolism (Long et al. 1940; Storer, 1967; Freeman and Idler, 1973). It is now known that these hormones are produced during stress. Hence the stress created by the exposure of metals interferes with the

intermediary metabolism and affect the protein content of the body.

Aspartate amino transferase (GOT) and alanine amino transferase (GPT) are known to play a strategic role in mobilizing L - Amino acid for gluconeogenesis and also function as links between carbohydrate and protein metabolism under altered physiological, pathological and induced environmental stress conditions (Nichol and Rosen 1963; Knox and Greengard, 1965; Harper et al. 1977).

Glycogen and protein present in liver and muscles provide energy to the body. Animal under stress deplete the energy sources at different rates. A study was conducted to examine the effect of copper and mercury on the glycogen and protein content of liver and muscle of the fish Macrones gulio.

MATERIAL AND METHODS

For the experiments the acclimatized fishes (the acclimatization procedure is given in Chapter II) were transferred into the experimental tanks. Only fishes of immature stage with size range 10-13 cm in length were used for the experiment, irrespective of sex. Each experimental tank contained filtered, unpolluted water (salinity 15±2‰ Temp. 28±1 °C, pH 7.5±0.5 and dissolved oxygen > 90% saturation). Twenty four fishes were transferred to each tanks which were treated with toxicant. Toxicant concentrations are for copper

(0.001, 0.005, 0.01) and for mercury (0.01, 0.015, 0.02). One tank was kept as control without metal solution and duplicates were run for each metal concentration. The test medium was renewed every 24 hr. The physico-chemical parameters were measured every 24 hr. (salinity - 15±2‰, pH - 7.5±0.5, temperature 28 °C±1 °C and dissolved oxygen > 90% saturation). During exposure period the fishes were fed with minced clam meat and feeding stopped 24h prior to each test experiment.

The fishes from each experimental tanks were caught at intervals of 1, 5, 10 and 15 days and immobilized with a hard blow on the head. Immediately, the body was cut open and the liver, and a piece of epoxial muscle from a definite area below the dorsal fin were removed and weighed accurately. The samples were homogenized in 5 ml of 10% Trichloro acetic acid (TCA) and centrifuged at 3000 rpm for 10 minutes.

Estimation of glycogen

The supernatant of the tissue extracted in TCA was used for the estimation of glycogen. The glycogen content of the extract was determined following the phenol sulphuric acid method of Montgomery (1957). 0.75 ml of the supernatant of the muscle tissue extract and 0.3 ml of the supernatant from the liver tissues extract was used for the glycogen estimation. To each sample 1.5 ml of 95% ethyl alcohol was added, mixed and kept overnight in a refrigerator. It was centrifuged at 3000 rpm for 15 minutes. The supernatant was very carefully decanted. The

precipitate was dissolved in 2 ml of distilled water and to this 0.1 ml 80% phenol was added. The mixture was shaken well, 5 ml of conc. H_2SO_4 was added forcefully to this mixture. It was then kept at room temperature for 30 minutes. After cooling to room temperature, the color developed was read at 490 mm in a spectrophotometer (Hitachi, light path 1 cm). The glycogen concentration of the samples were determined from the calibration curve prepared by employing oyster glycogen (Sigma) as the standard. The significant differece between controls and experimental fishes was determined using student's `t' test (Zar, 1974).

Estimation of Protein

The residue of the tissue extracts in TCA was used for the estimation of protein. The protein of the residue was estimated by the method of Lowry et al. (1951). The precipitated protein in the residue was dissolved in 5 ml NaOH. 0.3 ml of the liver sample and 0.2 ml of the muscle sample were used for the experiment. The samples were made up to 1 ml with distilled water. To these samples 5 ml of alkaline copper reagent was added and shaken well. After 10 minutes the blue colour developed were calculated from the standard graph prepared by using Bovine seram, albumin as the standard. The results were analysed statistically (student's `t' test).

STATISTICAL ANALYSIS

The data have been subjected to statistical analysis using the students `t' test to manifest the variation in comparison with the control. The variations were reported at three significant levels viz. $P < 0.05$, 0.01 and 0.001.

RESULTS

Results of the experiments are presented in tables 15-22 and figs. 15-22.

Glycogen content of the liver exposed to copper and mercury

In the liver of controls, the glycogen concentration showed a non - significant decline between days. In the liver of fishes exposed to all the concentrations (0.001 ppm, 0.005 ppm, 0.01 ppm) of copper there was a significant decrease ($P<0.001$) in the glycogen content at all the time intervals. The fishes exposed to 0.01 ppm of mercury the decrease of glycogen content started fifth day onwards and continue till the end of the experiment table 15-16 and fig.15 -16.

Glycogen content of muscle exposed to copper and mercury

Here also the control showed a non - significant decrease in the glycogen of the tissue. The fishes exposed to 0.001 ppm of mercury showed a significant decrease ($P<0.05$) of muscle glycogen at 1st, 5th and 15th days. The fishes exposed to 0.015 and 0.02 ppm of mercury showed a very significant decrease ($P<0.001$) at

Table 15 Glycogen content of the liver of *Macrones gulio* exposed to copper.

Conc ppm	Time of exposure (days)			
	1	5	10	15
Control	12.57 ± 0.15	11.24 ± 0.24	10.29 ± 0.22	10.59 ± 0.16
0.001	9.89 *** ± 0.44	8.48 *** ± 0.14	7.43 ** ± 2.15	6.74*** ± 0.24
0.005	8.14 *** ± 2.24	7.01 *** ± 0.13	6..23 *** ± 0.12	5.57*** ± 0.13
0.01	7.17 *** ± 0.21	6.90 *** ± 0.26	5.28*** ± 0.07	4.78 *** ± 2.40

Table 16 . Glycogen content of the liver of *Macrones gulio* exposed to mercury.

Conc ppm	Time of expsure (days)			
	1	5	10	15
Control	15.86 ± 2.18	14.76 ± 2.24	13.79 ± 2.32	13.44 ± 1.12
0.01	14.45 ± 0.93	11.5** ± 0.50	10.9 ** ± 0.77	9.06 *** ± 1.85
0.015	10.34 *** ± 1.97	10.2** ± 2.46	9.10** ± 2.21	8.2 ** ± 3.1
0.02	9±8 *** 0.52	8.48*** ± 0.14	7.14*** ± 2.12	6.13 *** ± 2.14

Values are the mean of ten sets of experiment ±SD
* P< 0.05, ** P< 0.01, ***P< 0.001

Table 17 Glycogen content of the muscle of *Macrones gulio* exposed to copper.

Conc ppm	Time of exposure (days)			
	1	5	10	15
Control	1. 54 ± 0. 52	1. 42 ± 0. 39	1. 33 ± 0. 44	1. 27 ± 0. 36
0. 001	1. 25* ± 0. 48	1. 01* ± 0. 07	0. 96 ± 0. 52	0. 78* ± 0. 41
0. 005	1. 22* ± 0. 53	1. 02 ± 0. 44	0. 91 ± 0. 43	0. 65*** ± 0. 08
0. 01	1. 00* ± 0. 06	0. 85* ± 0. 38	0. 79** ± 0. 11	0. 55** ± 0. 49

Table 18 Glycogen content of the muscle of *Macrones gulio* exposed to mercury.

Conc. ppm	Time of exposure (days)			
	1	5	10	15
Control	1. 97 ± 0. 26	1. 95 ± 0. 15	1. 88 ± 0. 15	1. 83 ± 0. 08
0. 01	1. 85 ± 0. 14	1. 75* ± 0. 11	1. 65 ± 0.35	1. 59* ± 0. 20
0. 015	1. 55* ± 0. 31	1. 22** ± 0. 53	1. 22** ± 0. 53	1. 06*** ± 0. 39
0. 02	1. 50*** ± 0. 04	1. 39* ± 0. 48	1. 00*** ± 0. 06	0. 96** ± 0. 52

Values are the mean of ten sets of experiment ± SD *P < 0. 05, ** P< 0. 01, *** P < 0. 001

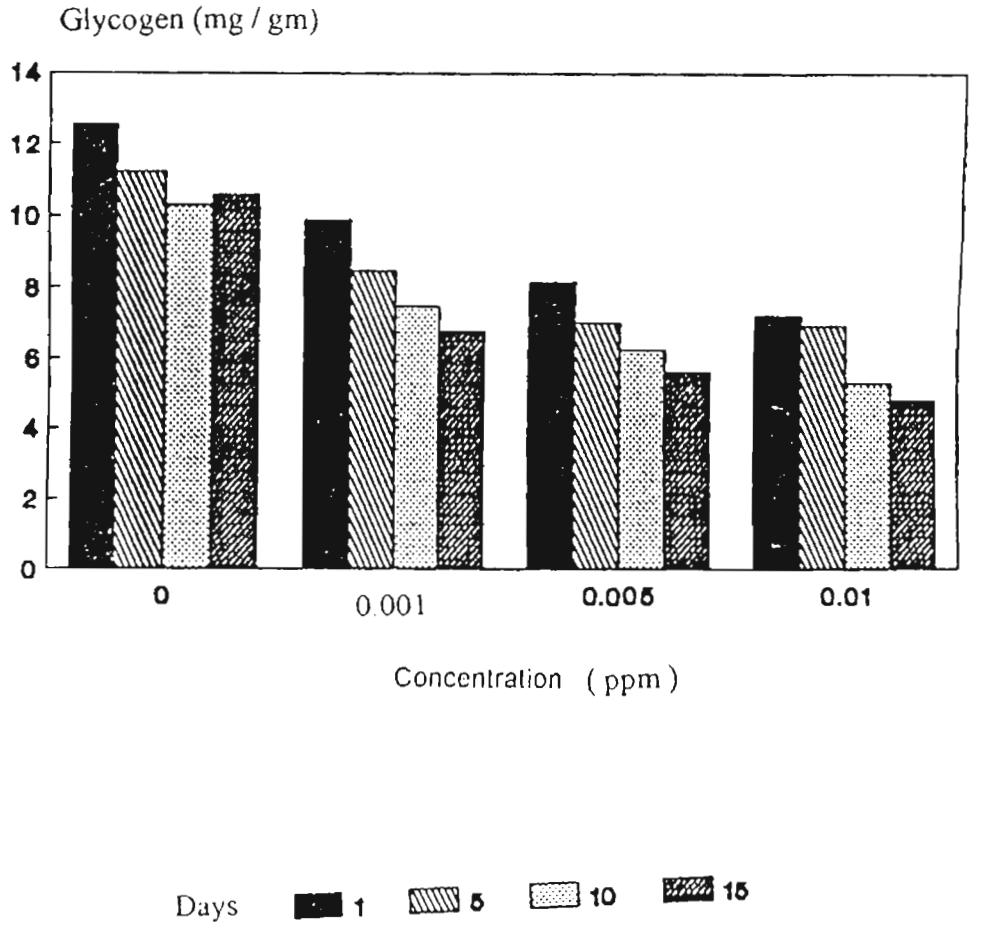


FIGURE 15 GLYCOGEN CONTENT OF THE LIVER OF *MACRONES GULIO* EXPOSED TO COPPER.

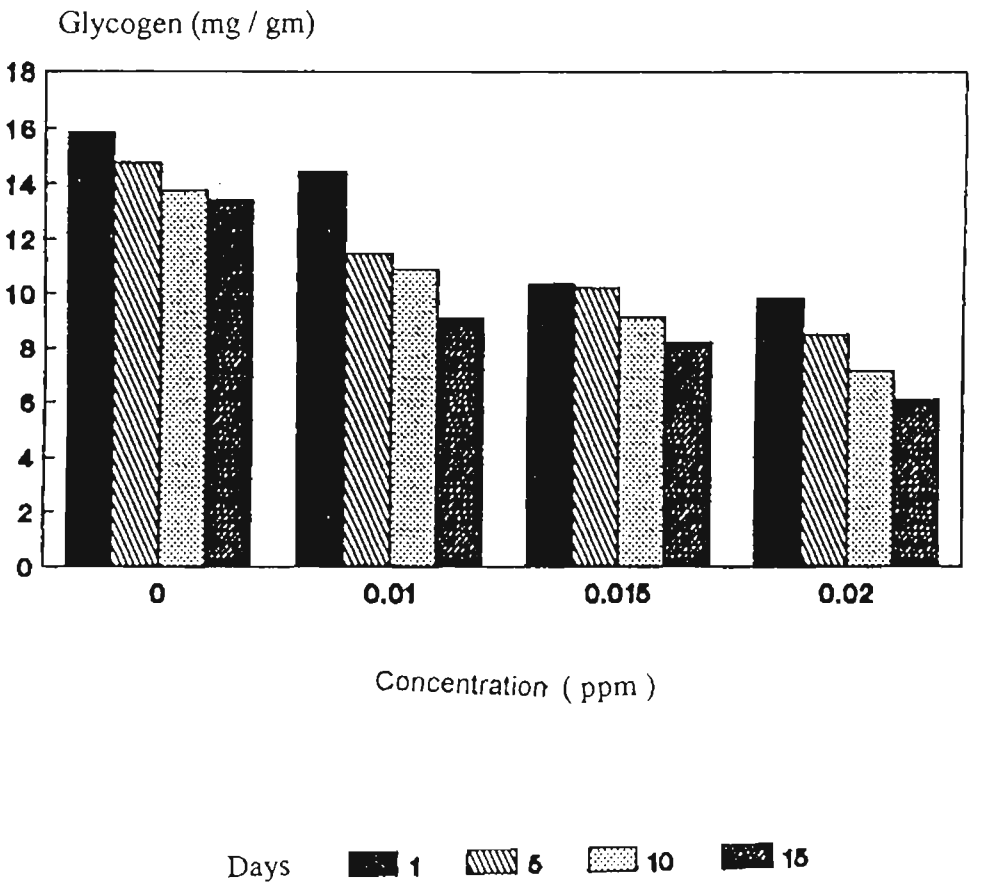


FIGURE 16 GLYCOGEN CONTENT OF THE LIVER OF *MICRONUS GULIO* EXPOSED TO MERCURY.

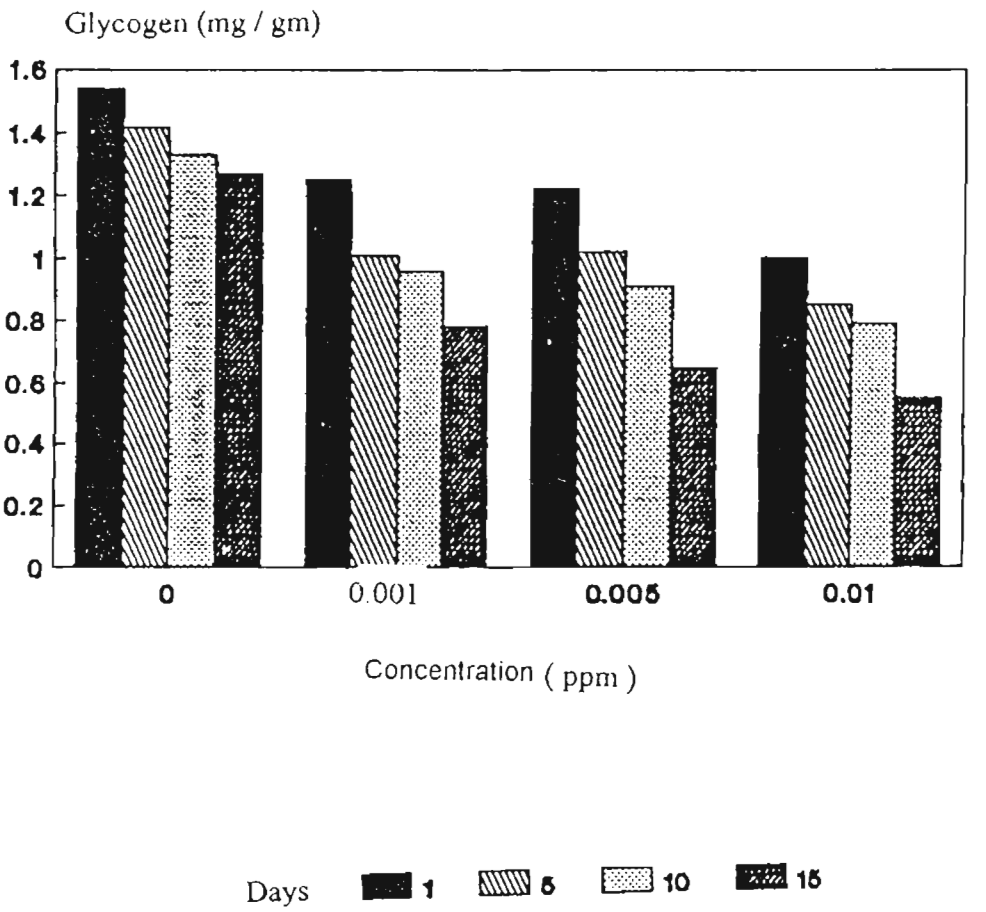


FIGURE 17 GLYCOGEN CONTENT OF THE MUSCLE OF *MACROMES GULIO* EXPOSED TO COPPER.

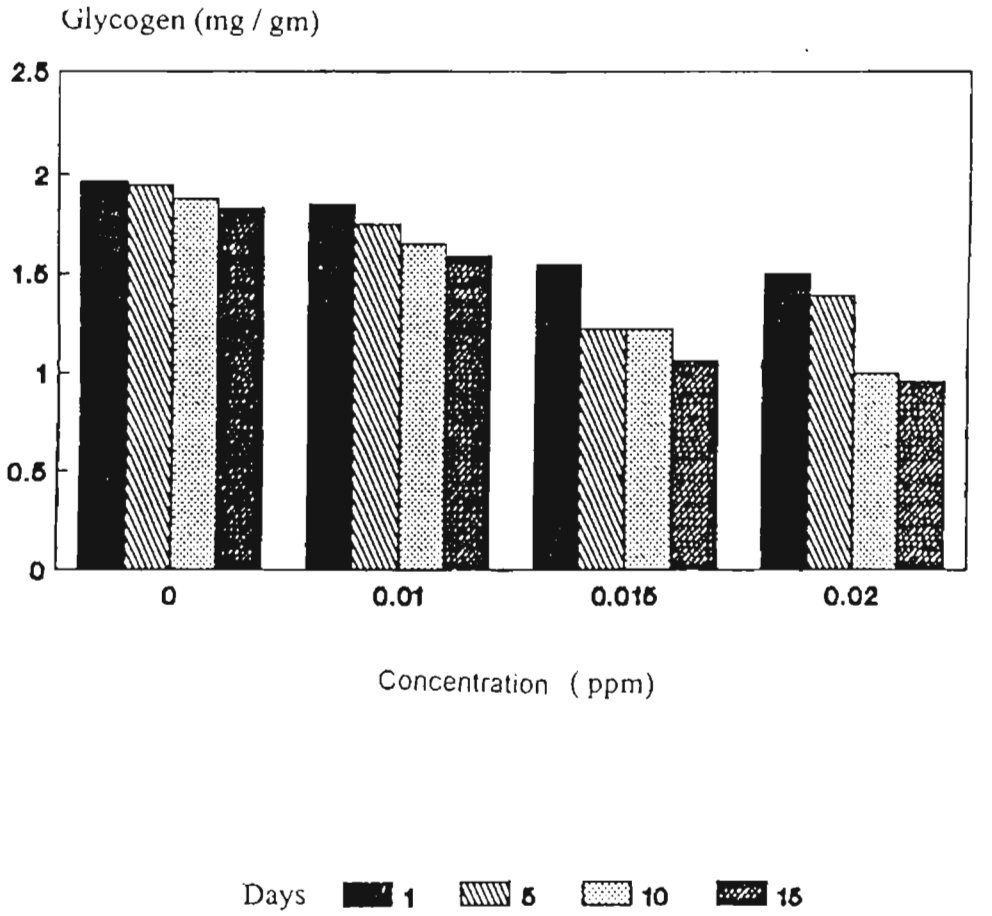


FIGURE 18 GLYCOGEN CONTENT OF THE MUSCLE OF *MUGIL CEPHALUS* EXPOSED TO MERCURY.

10th and 15th days. The fishes exposed to higher concentrations of copper the glycogen content in the muscle tissue showed a very significant decrease ($P<0.001$) at 10th day and 15th day table 17-18 and fig.17-18.

Protein content of liver exposed to copper and mercury

The lower concentration of copper did not effect the protein content. In those fishes exposed to lower concentration of copper (0.001 ppm), the protein content of liver did not vary significantly throughout the experiment from that of the controls and in liver exposed to higher concentrations of copper (0.005 ppm, 0.01 ppm), the significant decrease ($P<0.05$, $P<0.01$) in protein was observed at 5th, 10th and 15th days. But in the case of fishes exposed to 0.01 ppm, 0.015 ppm and 0.02 ppm of mercury showed a significant ($P<0.001$) decrease was observed in all the time intervals table 19-20 and fig.19-20.

Protein contents of muscle exposed to copper and mercury

The protein content of the muscle of the fishes exposed to lower concentration of copper did not show any significant variation from that of controls. But there was a significant decrease ($P<0.001$) in the protein level of muscle of the fishes treated with higher concentration of copper (0.01 ppm) at 5th and 10th days. The fishes exposed to mercury the protein content of muscle showed a non - significant variation in 0.01 ppm and 0.015 ppm. But in 0.02 ppm of mercury the protein content showed a significant decrease ($P<0.005$) at 15th days table 21-22 and fig.21-22.

Table 19 Protein content of the liver of *Macrones gulio* exposed to copper.

Conc ppm	Time of exposure (days)			
	1	5	10	15
Control	102.43 ± 10.05	104.65 ± 9.19	100.27 ± 8.52	100.25 ± 8.73
0.001	98.94 ± 10.12	96.77 ± 9.22	91.93 ± 10.17	84.68 ** ± 9.58
0.005	97.13 ± 11.61	93.96 * ± 8.69	80.95 ** ± 8.03	79.00 ** ± 11.06
0.01	95.34 ± 7.21	93.50 * ± 5.37	79.50 * ± 18.00	70.70 ** ± 16.90

Table 20 Protein content of the liver of *Macrones gulio* exposed to mercury.

Conc. ppm	Time of exposure (days)			
	1	5	10	15
Control	102.00 ± 4.00	100.27 ± 8.52	98.50 ± 3.40	98.8 ± 2.2
0.01	96.10 ** ± 2.8	94.70 ± 10.22	83.66** ± 9.58	80.50 *** ± 3.05
0.015	95.60 ** ± 2.5	88.19 * ± 9.36	81.36 *** ± 2.57	76.07*** ± 7.84
0.02	94.14 ± 9.76	81.00 ** ± 8.21	74.12 *** ± 2.50	70.00 *** ± 2.96

Values are the means of ten sets of experiment ± SD * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

Table 21 . Protein content of the muscle of *Macrones gulio* exposed to copper.

Conc. ppm	Time of exposure (days)			
	1	5	10	15
Control	146.20 ± 2.90	145.60 ± 3.70	145.40 ± 3.50	143.40 ± 24.50
0.001	138.15 ± 16.87	132.21 * ± 12.66	131.96 * ± 14.08	126.19 ± 14.43
0.005	133.26 ± 17.39	123.46 ** ± 16.71	122.75 ** ± 15.47	118.96 * ± 13.88
0.01	124.83 ** ± 16.53	120.70 *** ± 13.40	118.00 *** ± 4.30	116.68 * ± 14.62

Table 22 Protein content of the muscle of *Macrones gulio* exposed to mercury.

Conc. ppm	Time of exposure (days)			
	1	5	10	15
Control	132.76 ± 14.28	132.10 ± 14.66	131.96 ± 14.08	131.19 ± 15.76
0.01	130.17 ± 3.70	128.38 ± 13.93	122.27 ± 11.42	120.70 ± 13.40
0.015	129.70 ± 6.93	124.00 ± 4.10	118.16 ± 13.19	115.60 ± 15.37
0.02	126.19 ± 14.43	124.30 ± 13.78	116.68 ± 14.62	112.74 * ± 12.11

Values are the mean of ten sets of experiment± SD * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

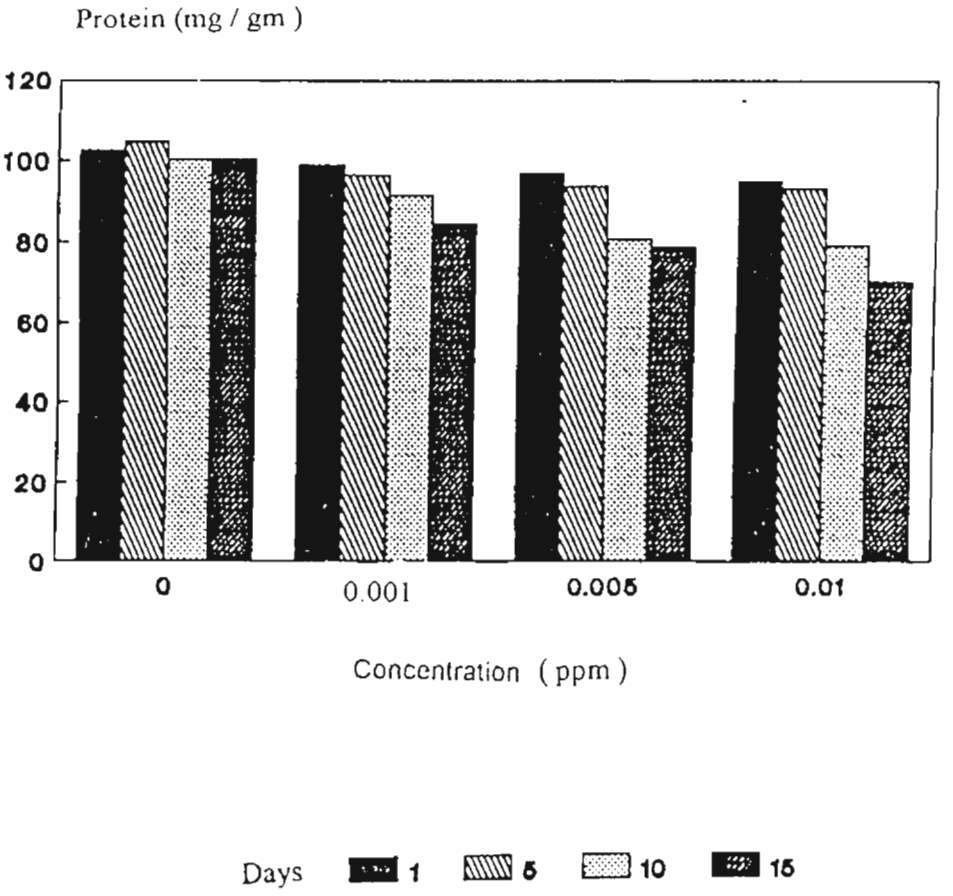


FIGURE 19: PROTEIN CONTENT OF THE LIVER OF *MACRONES GULIO* EXPOSED TO COPPER.

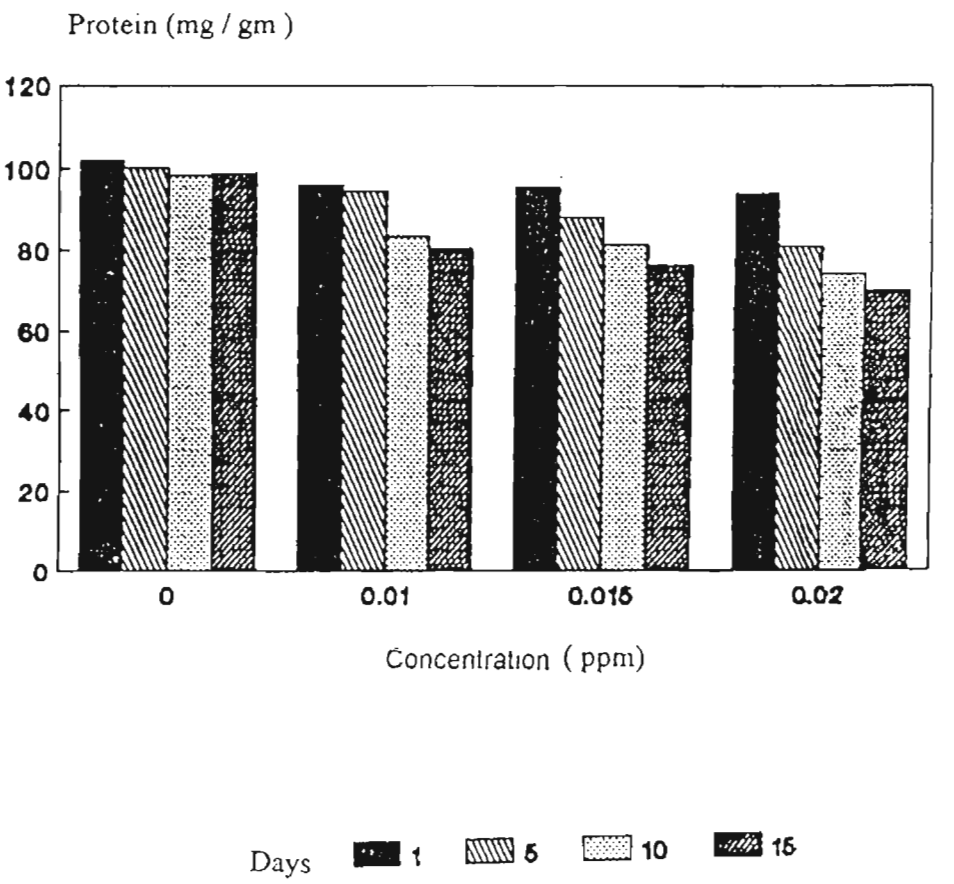


FIGURE 20: PROTEIN CONTENT OF THE LIVER OF *MACRONUS GULIO* EXPOSED TO MERCURY.

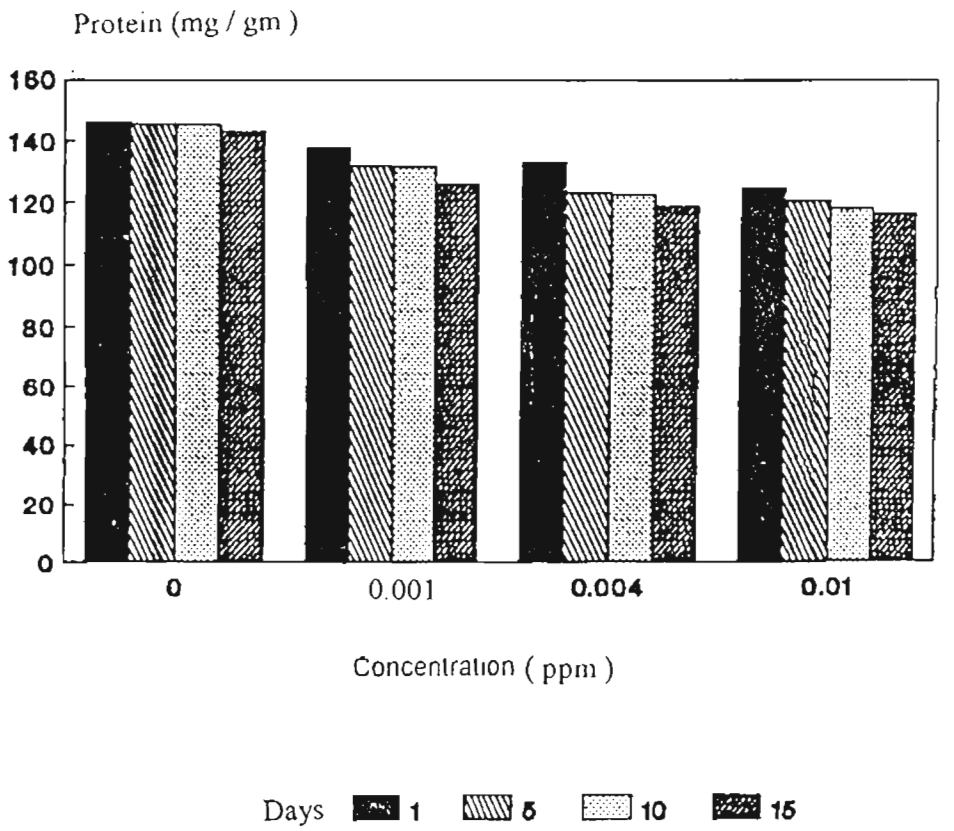


FIGURE 21 : PROTEIN CONTENT OF THE MUSCLE OF *MACRONEMS GULIO* EXPOSED TO COPPER.

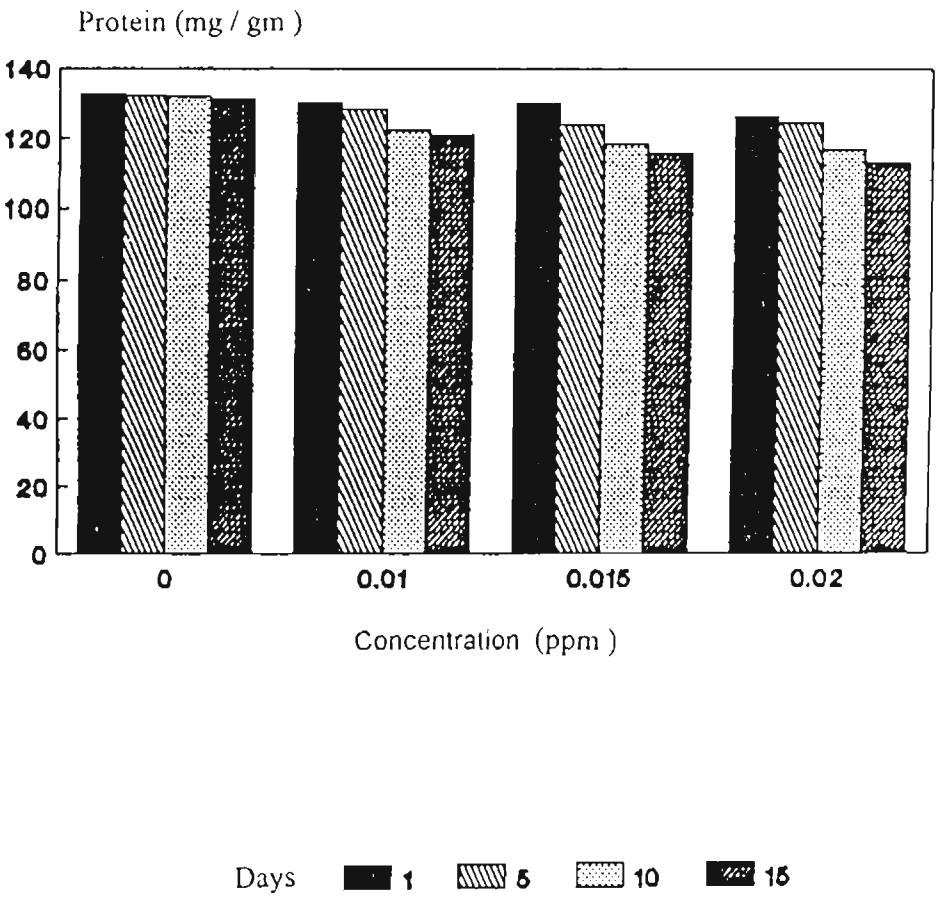


FIGURE 22 : PROTEIN CONTENT OF THE MUSCLE OF *MICRONEMS GULIO* EXPOSED TO MERCURY.

DISCUSSION

In the present study there were significant depletion of glycogen in the liver and muscles of fishes exposed to copper and mercury. Reactions of liver glycogen was more pronounced than that of the muscle. In the present study, similar to the observations by Dange (1986 a) the least extensive changes were seen in the copper dosed fishes.

When subjected to sublethal exposure, the energy reserves (glycogen and Protein) in the major reservoir (liver) were observed to have been depleted in different proportions. Besides liver, the glycogen reserves of the muscle too, was found to have been depleted on exposure of Macrones gulio to copper and mercury although this source was depended upon only after 48 hrs. of exposure. The glycogen and the protein reserves were comparatively less depleted.

The heavy metals tend to have a long residence time in the gut with the low permeability coefficient of divalent cations across the lipid bilayer of membranes, profuse binding on to the negative charges sites on the mucosal side of the gut increases the concentration of heavy metals in the lumen. This interferes with the normal process of nutrient absorption which explains the need for catabolism of stored energy (Farman Farmaian and Socci, 1984). Therefore carbohydrate deposits, in the form of glycogen in tissues like liver and muscle provide the immediate energy requirements in telecost fishes under different kinds of

stress (Dange, 1986a). The fact is upheld by the findings of Van Waarde (1988), who observed that different pollutants, when present in high concentrations in the medium, significantly affected the carbohydrate metabolism in Tilapia species. Ram and Sathyanesen (1987) reported that even in small concentrations emisan (mercury) was capable of inducing hepatic histopathological and biochemical alterations which could cause severe physio-metabolic disfunction leading to death. It was further pointed out that young fishes were more susceptible to emisan toxicity than the adults.

Significant decrease in the glycogen reserves of both liver and muscle has been reported in Heteropneustes fossilis in response to 25 and 50 ppm of mercury (Qayyam and Shaffi, 1977); in different fishes in response to Cadmium, copper and zinc (Shaffi, 1978 a, b; 1979 a, b; 1980 d;) in rainbow trout in response to cadmium (Larsson and Haux, 1980); in Heteropneustes fossilis in response to copper, cadmium and mercury (Srivastava, 1982) and in Notopterus notopterus in response to mercury (Verma and Tonk, 1983). Apart from these, glycogen depletion in the livery is reported in H. fossilis in response to lead (Shaffi and Quyyam, 1979); in Puntinus conchonius in response to mercury (Gill and Pant, 1981); In Channa punctatus in response to cadmium (Dubale and Shah, 1981); in C. punctatus in response to chromium (Sastry and Tyagi, 1982; Sastry and Sunitha, 1983) in Saratherodon mossambicus in response to 1.5 ppm mercury (Naidu

and Ramamurthy 1984) and in Clarias batrachus in response to lithium administration (Goel et al. 1985).

However there are also reports that various toxicants caused an increase in the glycogen level of various tissues of different fishes (Grant and Mehrle, 1973; Buckley et al. 1979; Bakthavathsalam and Reddy, 1982 b; Anderson et al. 1987; Nath and Kumar, 1987).

But in some fishes, glycogen, levels were not affected by mercury (Sastry and Rao, 1984) or copper (Tort et al. 1987).

Many studies have dealt with the rate and extent of tissue glycogen breakdown in fish following their exposure to situations demanding mobilization of internal energy stores, such as muscular activity, environmental hypoxia and salinity changes and reproductive conditions (Bashamohideen and Parvatheswara Rao, 1972). Tapti et al. (1985) concluded that mercury stress caused degenerative changes of the hepatopancreas where no death occurred. Similar depleted concentrations of energy reserves with increased enzyme activities have been reported by several investigators (Nriagu, 1979; Gill and Pant, 1981; Van Waarde, 1988).

The increased concentration of protein in E. maculatus after 48 hrs. exposure to copper is supported by the work of Hilmy et al. (1985). It is suggested that the transport and storage of these metal ions in an effort to detoxify it, required buffering

of the metal concentrations resulting in protein synthesis. Most of these proteins are continually degraded and resynthesized relatively quickly. Accordingly to Farman Farmaian et al. (1980), heavy metals inhibit amino acid absorption by the organism, by acting the luminal surface of microvillae membrane of the gut. This is more probable due to the specific binding of the Hg^{2+} to the exposed luminal sulphydryl groups of the carrier protein of the neutral aminoacids. Similar changes in the quantity of serum protein of a fresh water teleost Catla catla after mercury treatment were observed by Rai and Qayyam (1985) and Rai (1987). Disruption in pathways of intermediary metabolism, such as decreased activity of sulphydryl enzyme complexes, fatty acid synthetase or inhibition of protein synthesis on selenium exposure to teleost were observed by Hunn et al. (1987). Increased lactic acid concentrations observed after exposure to the metal suggests reduced consumption and high activity levels of succinates dehydrogenase with significant increase in lactate dehydrogenase activity. This indicated that the energy requirements in fishes were being met through anaerobic oxidation (Balavenkata subbaiah et al., 1984) due to impaired oxidative and transphosphorylative activities. A similar observation was made by Naidu and Ramamurthy (1984). Fort and Torres (1988) exposed dog fish, Scyliorhinus canicule to sublethal concentrations of cadmium and observed increase in glucose and lactic acid concentration.

In the present study, it was found that the fish became irritated after exposure to copper and mercury. They get irritated at the slightest provocation and were hyperactive and hyperactivity deplete the stored food materials present in muscle and liver. Many toxicants are known to oxidise glutathione, haemoglobin etc. damage cell membranes and organelles by lipid peroxidation, inhibit many enzymes and thus disrupt important physiological function of the body. The body requires large quantities of energy to produce substance like glutathione. Metallothionine, glucuronic acid and other substances to remove toxicants by activation, inactivation or conjugation and to repair damaged organelles and replace lost cell constituents.

So, in fishes exposed to pollutants the energy demand is very high. This increased energy demand is met by utilizing the stored glycogen in different tissues. The inccreased metabolic activity of the liver after heavy metal intoxication was reported by Maynard and Loosli (1962). Muscle glycogen is rapidly depleted during intense activity and glucose is mobilized from the liver glycogen stores to supply both raw materials and rebuilding muscles glycogen. Both glucogenolysis and glycogenesis involves a number of enzymes and is guided by hormones. Hormones increase glycogenolysis during stress to meet increased energy demand. The alterations in carbohydrate metabolism are produced indirectly by the environmental stresses through primary effect on the endocrine glands exciting them into releasing large amounts of hormones (Young and Chavin, 1965;

Oguri and Nace, 1966; Fagerlund, 1967; Nakano and Tomlinson, 1967, Grant and Mehrle, 1973, Assem et al. 1978; Schreck and Lorz, 1978; Hille, 1932; Gluth and Hanke, 1984).

Hormones control this alterations of carbohydrates metabolism mainly by suppressing insulin production and increasing the glucocorticoids and catecholamine synthesis. Impaired secretion of insulin by B-cells of pancreatic islets tend to decrease glycogen levels of the liver and muscle. Decreased insulin production after metal intoxication was observed in Caltus scarpius (Have, 1969) and rainbow trout (Wagner and Mckeown (1982). External toxic stimuli and stresors are known to elevate cortisol in fish (Schreck, 1981, Gluth and Hanke, 1984, 1985) and release corticosteroids into the blood which is followed by an elevation of blood glucose (Hille, 1982). In many fishes stress induced an elevation of catecholamines (Mazeaaud et al. 1977). Regardless of the nature of stress, either adrenaline or non-adrenaline are released into the blood and this increases blood glucose. To keep the glucose level in the blood relativity high, despite metabolic process following the influence of stressors, glycogenolysis is essential. The elevation in blood glucose may form a part of restorative process in which glucose is mobilized from the liver glycogen stores (Wardle, 1978). It enters the muscle cells supplying both raw materials and energy for rebuilding muscle glycogen. There are reports that the blood sugar levels are elevated in fish during acute exposure to a variety of

environmental alterations and exposure to toxicants (Holeton and Randall, 1967 Nakano and Tomlinson, 1971; Chavin and Young, 1970; Narasimhan and Sundararaj, 1971; Bashamohideen and Parvateswara Rao, 1972; Hunn, 1972; McLeay et al. 1972; Wedmeyer, 1972; 1973; Grant and Mehrle, 1973; McLeay, 1973; 1977; Hattingh, 1976; Soivio and Oikari, 1976; Mazeaud et al. 1977; Banerjee et al. 1978; Shaffi, 1978 b, 1980 a, c; Assem and Hanke, 1979 a,b, Itov and Murata, 1980; Jorgensen and Mustafa, 1980; Larsson and Haux 1980; Mukhopadhyay and Dehardrai, 1980, Gill and Pant, 1981; 1983; Srivastava and Singh, 1981, 1982, Sastry and Siddiqui, 1982, Sastry and Tyagi, 1982, Wagner and Mckeown, 1982; Mishra and Srivasthava 1983, 1984, Shrivasthava and Mishra, 1983; Cliff and Thurman, 1984; Gluth and Hanke, 1984; Goel et al. 1985; Sastry and Subhadra, 1985; Lal et al. 1986; Tewari et al. 1987). Phosphorylase is a regulatory enzyme of glycogen break down. Bhaskar and Govindappa (1986) found that in the red muscle of Tilapia mossambica limited to alkaline medium, a stepped up glycogen break down and an increased phosphorylase activity in the red muscle. They suggested that the phosphorylase activity could be responsible for depleted glycogen content. The depletion of glycogen observed in the present study could be due to this phenomenon also.

Normally the glucose molecule undergo glycolysis and the pyruvic acid formed enter tricarboxylic acid cycle (TCA cycle) releasing energy. But when there is a deficiency of oxygen, the pyruvic acid gets reduced to lactic acid. During hypoxia or

anoxia, the aerobic metabolism changes to anaerobic metabolism, as a result of which lactic acid is accumulated. So accumulation of lactic acid is a sign of anaerobic metabolism. Muscle glycogen depleted during intense activity and appears to be the principal source of lactic acid during anaerobic metabolism in lower vertebrates (Bennet, 1978). Accumulation of lactic acid in fishes due to many toxicants was reported by Grant and Mehrle (1973); McLeay and Brown (1975) Shaffi (1979 a, b, 1980 a, c); Sastry and Sidhiqui (1982, 1983); Srivasthava and Singh (1982); Sastry and Sunita, (1983); Cliff and Thurman (1984) Tort et al. (1984); Sastry and Subhadra (1985) and Bhaskara and Govindappa (1986). Thus lactic acid is produced in hypoxic tissue as a result of a switch from aerobic to anaerobic metabolism. This resulted in a decrease in the glycogen values of liver and muscle of fish.

Hypoxia and anoxia can result from the faulty gaseous exchange. Heavy metals are one class of pollutants which have a distruptive influence on the structural organization of the gill tissue. Hughes et al. (1979) have shown that exposure to pollutants causes a reduction in the morphological basis for diffusing capacity of the gills.

Lindahl and Hell (1970) demonstrated the effects of phenylmercuric hydroxide (PMOH) on the gill tissue and found that the superficial layer of the gill filaments appear somewhat detached from deeper parts. This reduces the diffusing capacity

and there is a fall in the oxygen supply to the tissues which becomes hypoxic. Lindahal and Hell (1970) found that oxygen consumption of gill is reduced by 30% after an exposure of the animal to PMOH. Davis (1973) found a decline in arterial oxygen tension in socckeye salmon following pulp mill effluent exposure.

Diffusing capacity of the gills is further reduced following the irritating action of pollutants which causes a secretion of mucus over the gills (Shaffi, 1978 b). Interference with gas transfer will reduce oxygen levels within the blood circulating to the brain where responses are initiated by the respiratory centre. The respiratory centre may coordinate cardiovascular changes and stimulate the hormonal system and erythropoietic tissue to take necessary steps to compensate for the decreased oxygen supply to the tissues. A decreased glycogen level of the body may be a step in that line.

Increase of anaerobic metabolism has been shown to be a rapid and clear response against depletion of energy caused by lack of oxygen (Van den Thillart, 1982). These response have also been observed after metal exposure [Burton et al. 1972; Hodson, 1976]. Basha et al. (1984) suggested the prevalence of hypoxic condition in the tissue and a reduction in the rate of oxidative metabolism at the mitochandrial level in Tilapia mossambica exposed to toxicant. In the present study also, copper and mercury may create such a condition in the fish Macrones gulio.

The significant decrease in the protein content of liver and muscles of metal dosed Macrones gulio occurred at the end of the exposure period, mainly at 10th and 15th day. This clearly indicates that the body utilises the glycogen stores first to meet the increased energy demand. When the glycogen stores were decreased, the body utilizes the protein for energy production. This is manifested as decrease in the protein content in different tissues and serum. There are many reports that the total protein in different tissues and serum of fish decreased after exposure to different toxicants (Grant and Mehrle, 1973; Camp et al. 1994; McLeay and Brown, 1974; Venktachari, 1974; Sakaguchi and Hamaguchi, 1975; Oikari and Soivio 1977, Goel and Garg, 1980; Panigrahi and Misra, 1980; Rath and Misra, 1980; Sharma and Davis, 1980; Dubale and Shah, 1981; Goel and Agrawal, 1981; Ramalingam and Ramalingam, 1982; Sastry and Siddiqui, 1983; Verma and Tonk, 1983; Awasthi et al. 1984; Gluth and Hanke, 1984, Naidu and Ramamurthy 1984, Verma et al. 1982, Sashikala et al. 1985; Katti Sathyanesan, 1986; Kumar and Ansari, 1986; Yamawaki et al. 1986; Reddy, 1987; Reddy and Bashamohideen, 198% and Seshagiri Rao et al. 1987).

Many toxicants interfere with protein synthesis or utilise the protein to meet the extra energy demand due to pollutant exposure. So, this affects growth and there are reports that growth has been retarded in fish by these pollutants (Waiwood and Beamish, 1978; Marathe and Deshumukh 1980; Buckley et al. 1982; Collvin, 1984). But there are instances in which total protein

actually increased in different tissues of fishes dosed with toxicants (Mc kim et al. 1970; Calabrese et al. 1975; Helmy et al. 1979; Sivaprasada Rao and Ramana Rao, 1979; Ito and Murata, 1980; Sahib et al. 1984 and Hilmy et al. 1985). But Mc Leay and Brown (1979) and Tort et al. (1987) reported that there was no change in the protein content after exposure to pollutant.

The decline in the liver and muscle protein would suggest an intensive proteolysis which in turn could contribute to the increase of free amino acids to be fed into FCA cycle as keto acids. Such a possibility is further strengthened by the investigation of Schafer (1967); Mehrle et al. (1971), Shakoori et al. (1976) which revealed both qualitative and quantitative variation in the tissue amino acids of fishes exposed to toxicants. In addition, studies by Bell (1968); Mc Kim et al. (1970); Lane and Scura (1970) Sakaguchi and Hamaguchi (1975) have also revealed marked variation in the activity of enzymes involved in transamination of fishes in similar situations. Sivaprasada Rao and Ramana Rao (1979) found that the decrease in glycogen is due to the immediate utilization in the tissue to meet the excess demands of the energy metabolism. They also suggested that high increase of amino acids they observed in Tilapia mossambica treated with methyl parathion is utilized for gluconeogenesis through the transamination and transdeamination reactions to supply the necessary keto acids to act as precursors for the maintenance of carbohydrate metabolism to meet the energy requirements during stress. There are reports that

transamination and transdeamination reactions are prominent under stress (Knox and Greengard, 1965; Harper et al. 1977).

Decreased protein content could possibly be due to protein breakdown which increased the aminoacid pool in the tissue. It is also reported that decreased protein moiety suggests damage to hepatic tissue and an intensive proteolysis (Rao, 1984) resulting in increased amount of free aminoacid to be fed into TCA cycle as keto acids. Loss of weight and elevation of nitrogenous compounds in tissues of fish exposed to benthiocarb were reported by Seshagiri Rao et al. (1983) in S. mossambicus.

The decrease in protein following exposure to Hg and Cu suggests their possible degradation by increased proteolysis. This increased proteolysis could be attributed to the damage caused to lysosomal membranes thus permitting the leakage of lysosomal enzyme into the cystol.

The lack of alteration of protein level of liver and muscle of Macrones gulio exposed to copper and mercury at 1st and 5th day (except in the liver of fishes exposed to higher concentration of Hg) could be that the body utilises the glycogen of these tissues in the initial period of exposure. The depletion of glycogen in the tissues of Macrones gulio after metal exposure prove this. These findings also support the concept of Fry (1971) that fishes tend to resist a changed situation for specific period, but will eventually succumb as a result of their inability to adapt. According to Umminger (1970)

carbohydrates represent the principal and immediate energy precursors for fishes exposed to stress condition while protein being the energy source to spare during chronic period of stress. Gluth and Hanke (1984) found that changes in plasma protein need time to occur and the reduction of protein can only be found after 70 hrs of exposure.

Radhaiah et al. (1987) observed that aminoacids in the kidney increase along with a decrease in the protein values. This proves that intense proteolytic activity in the tissues can increase aminoacids in the liver. Such an increase in amino acids after exposure to toxicant in different organisms were found by Girija (1984) and Rao (1984).

A defect in protein synthesis by the action of toxicant can also decrease the protein content in different tissues. An altered relationship between the ribosomes and the membranes of the endoplasmic reticulum may also produce a defect in protein synthesis. Rath and Misra (1980) examined the changes in nucleic acids and protein content in liver, muscle and brain of Tilapia mossambica exposed to the insecticide, dichlorvos. Post exposure studies revealed a significant decline in DNA, RNA content of the liver, muscle and brain. They observed that the liver exhibited a greater loss of protein than muscle. In the present study in Macrones gulio exposed to copper and mercury, also the liver showed a greater loss of protein than the muscle. Rath and Misra (1980) also found that the RNA/DNA ratio decreased in

exposed fish and it showed a passive correlation with protein. Usually RNA, and RNA/DNA ratio of a tissue are considered to indicate the intensity of protein synthesis (Misra and Patnaik, 1974). It is possible that mercury and copper may have influenced the protein synthesis in Macrones gulio by inhibiting RNA synthesis.

In view of the significant correlation of RNA and protein, a deficient synthesis of any type of RNA should have its reflection in a corresponding failure of protein synthesis. The reduction in the amount of RNA and the lesion of its functional capacity brings about a failure of protein synthesis (De Bruin, 1976 a).

It has been demonstrated that the cells of metal exposed organisms, Cd, Hg and Cu are able to reduce the protein synthesis (Viarengo et al. 1980) not only by reducing the rate of RNA synthesis but also by influencing the attachment of polyribosomes to the rough endoplasmic reticulum and probably damaging the ribosomes themselves.

The results of the present study shows a decrease in protein content in the liver and muscle of Macrones gulio exposed to metals copper and mercury and this may be due to the decreased protein synthesis and an increased proteolytic activity.

CHAPTER 5

EFFECT OF COPPER AND MERCURY ON DIFFERENT TISSUES (LIVER, KIDNEY & GILL) OF THE FISH *Macrones gulio*

Histopathological alterations in animal tissues have been identified as meaningful indicators of cellular responses to pollutant induced stress. Adverse biochemical and physiological changes in an organism finally results in histopathological alterations. The advantage of cellular kidneys over other approaches lies in the fact that it is possible to identify organells, cells or organs involved as pollution targets. Histopathology is one of the methods for assessing both short term and long term xenobiotic effects. Various cytological and cytochemical techniques have been employed in investigating pollutant induced alterations in cellular structure as well as function. As such, histopathology offers a great deal to aquatic toxicological studies. According to Hinton and Lauren (1990), histopathological approaches should be obligatory components of environmental assessments and may be used to formulate monitoring systems. However, it is essential that routine histopathological studies discriminates between toxicant induced lesions and normal variations in cellular structure.

Primary effects of pollutants may be exerted at the enzyme level or at some cell function such as permeability of membranes. These in turn affect cell integrity, ultra structure and gross function such as energy budget. When these alterations are severe enough they lead to the death of cells resulting in histological lesions. As organs constitute different types of cells and impairment of one or more of these results in change in

functions of organs, the impact may be reflected on the organism's growth, reproduction etc., and ultimately affect the population. The spectrum of levels of integration (Heath, 1987) clearly shows that more specific action are observed at lower levels.

Two ways of assessing marine pollution are by measuring the chemical contaminants in the various components of the ecosystem and by ecological monitoring of the marine communities. A more rapid and direct approach is by looking for effects of pollutants on living organisms in the field and to some extent, in the laboratary. A measure of the biological effect of a pollutant on individual organisms should generally be quantifiable and more or less proportional to the levels of pollutant. Acute cell death without somatic death leads to a series of reactions important for the recovery of a tissue or organ. These may also serve as biological indices of environmental stress effect (Hinton and Lauren, 1990).

Basically, there is a fundamental unity between the structural organization and various functions of the cellular components. An interference to the balanced situation affecting the basic structure may have far reaching effects on the stipulated functions of the organelles. This has been well illustrated by studies on different organells of various organisms which clearly show structural and related functional alterations when exposed to xenobiotics. Viarengo et al. (1980)

and Viarengo (1985) have demonstrated that metals like copper, mercury and cadmium reduce the RNA synthesis, influence the attachement of only ribosomes to rought endoplasmic reticulum and probably damage the ribosomes themselves. Accumulation of heavy metals has been reported in lysosomes, the subcellular organelles involved in the degradation of endocytosed nutrients. Heavy metal accumulation stimulates lipid peroxidation process and formation of lipofuscin granules. The lipofuscian granules in turn may trap toxic metals in a relatively stable form and may subsequently get eliminated by exocytosis of the residual bodies (Viarengo, 1985). Among the different methods of storage and detoxification of heavy metals developed by aquatic mestebration are metal compartmentation in membrane limited vesicles and formation of inorganic endo cellular precipitates. According to Viarengo (1985) mercury is able to disrupt the ionic balance in fish by altering permeability characteristic of the cell membranes thus affecting both passive and active transport processes. Thus the impact of enobiotics at both the cellular and subcellular levels of organization.

Report on cellular and subcellular responses of marine organisms to a variety of pollutants include neoplastic lesions in fish and non-neoplastic abnormalities in crabs (Malins et al. 1984), hepatopancreatic epithelial reduction in bivalve molluscs (Lowe et al. 1981; Couch, 1984), lysosomal disruption in mussels (Pickwell and Steinert, 1984, Moore et al. 1984) etc. Pollutant induced cell injury provides a highly sensitive indication of

environmental impact on the structural-functional organization. Gills are particularly vulnerable to environmental toxicants because of their external location and close contact with water and because of their permeability which makes them the principal sights of the uptake of toxicants from the medium (Roberts, 1978). Damage to gills has, for this reason, been studied in a large variety of fish exposed to various kinds of environmental pollutants at the light microscopic and electron microscopic levels (Eller, 1975., Jagoe and Haines, 1983).

Since the liver of teleosts is important in the maintenance of internal homeostasis and the metabolism of xenobiotics (Chambers and Yarbrough, 1976) and has also be shown to accumulate foreign compounds (Stathan et al. 1978) and to be susceptible to damage by toxic agent (Racicot et al. 1975; Gingerich et al. 1978) the functional integrity of the liver in fish can be affected by enobiotics (Gingerich, 1982).

The liver is specifically affected by a large number of chemical agents. The liver of mammals act as a major organ for colpper storage as also many other metals. Backstorm (1967) observed that liver is one of the most important mercury accumulating organs in animals treated with phenyl mercurials. According to Buck (1978) liver is the first line of defence against copper poisoning. Copper becomes toxic only when the high binding capacity of the liver is exceeded and copper is released into blood stream. In fish also, liver is the major storage organ for copper (Buckley et al. 1982, Shearer, 1984).

El-Domiaty (1987) found that highest concentration of copper was in the liver and kidney of Clarias lazera after exposure to copper and suggested that the liver and kidney are vital organs in the regulation of metals and there are detoxication, centres. Kidney is second only to the gills as an affector organ in ionic regulation and played an important role in the removal of the heavy metals from the body. According to Adamson (1967) the gill of fish is a poor excretory unit whereas kidney is capable of active excretion of many biotransformed derivatives of toxicants. Hence, examination of the changes in the biochemical composition and enzymatic activity in the liver and kidney are essential to understand the detoxification mechanisms in these organs.

The present study describes the histopathological changes produced in the gill, liver and kidney of the cat fish Macornes gulio maintained in laboratory when exposed to various concentrations of mercury and copper.

MATERIAL AND METHODS

Collection and acclimatization of fishes were similar as described Chapter 2. Fishes of immature stage with size range 10-13cm in length irrespective of sex were selected for the experiment. Twenty four fishes were transferred to each experimental tank contained 50 litres of water. Fishes were exposed separately to copper and mercury. For the study filtered unpolluted water was used (salinity 15±2‰, temperature 28±1 ^{o}C, pH - 7.5±0.5 and dissolved oxygen > 90% saturation). Toxicant

concentration for copper was 0.01 ppm and for mercury 0.02 ppm. One tank was kept as control without metal solution and duplicates were run for each metal concentration. The test medium was renewed every 24 hr. The physico-chemical parameters were measured every 24 hr. Fishes were fed with clam meat during the exposure period and feeding stopped 24h prior to each test experiment.

The techniques for histological study and staining procedures were mainly adopted from the methods described by Bucke (1972) and Bullock (1978). Samples for the study were collected on the 1st, 5th, 10th and 15th day. The fishes were caught from the tank and immobilized. The kidney, liver and gill tissues were dissected out and fixed in Bouins fixative for 24 hrs. After fixation the tissues were graded in ascending alcohol series and cleared in xylene. The gill tissue was decalcified in 3% nitric acid before alcohol grading. The tissue was embedded in paraffin wax after proper paraffin infiltration. The sections were cut at 5 u thickness using a rotary microtome and the sections were examined under microscope. Delafield's Haematoxylin staining methods was used.

Delafield's haematoxylin

Dissolve 4g of haematoxylin in 25ml absolute ethyl alcohol. Mix gradually into 400ml ammonia, alum, $NH_4Al(SO_4)_2 12H_2O$, saturated aqueous (approximately 1 part alum to 11 parts distilled water). Leave exposed to light in a flask with a

cotton plug for 3 - 5 days and filter. To the filtrate add 100 ml glycocerine and 100 ml methyl alcohol.

Eosin

Eosin Y	-	1 g
70% ethyl alcohol	-	1000 ml
Glacial acetic acid	-	5 ml

Dilute with equal volume of 70% alcohol for use and added 2-3 drops of acetic acid.

Procedure

1. Deparaffinize and run slides down to water
2. Stain in Delafield's hematoxylin, until slides are well over stained : 15-20 minutes.
3. Wash in running water : 3-5 minutes.
4. Transfer to 70% alcohol
5. Concentration in eosin : 1-2 minutes
6. Transfer to 70% alcohol : 100 more drops
7. Transfer to 95% alcohol : few drops
8. Dehydrate, clear in xylene and mount in DPX.

RESULTS

Liver Control

Liver of the control did not reveal any major alterations (Plate 1). Normal architecture of parenchyma was altered very little. The hapatocytes were polyhedral in shape having a

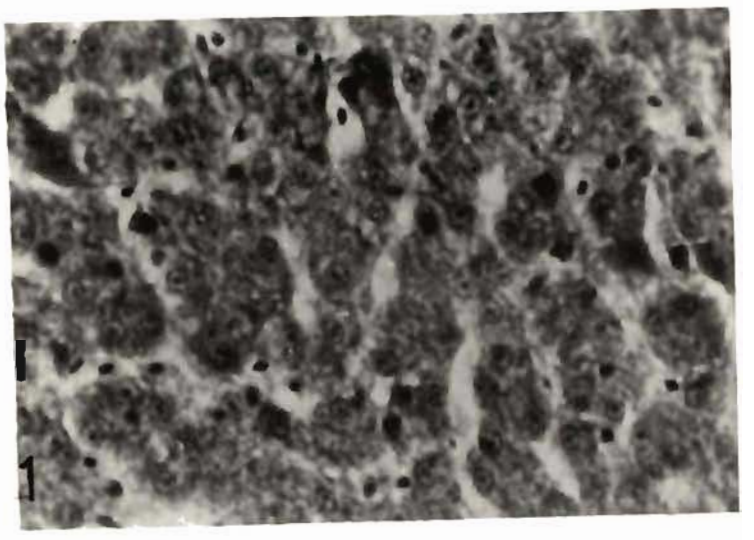


Plate 1. Liver hepatocytes from control fish. H&E X320

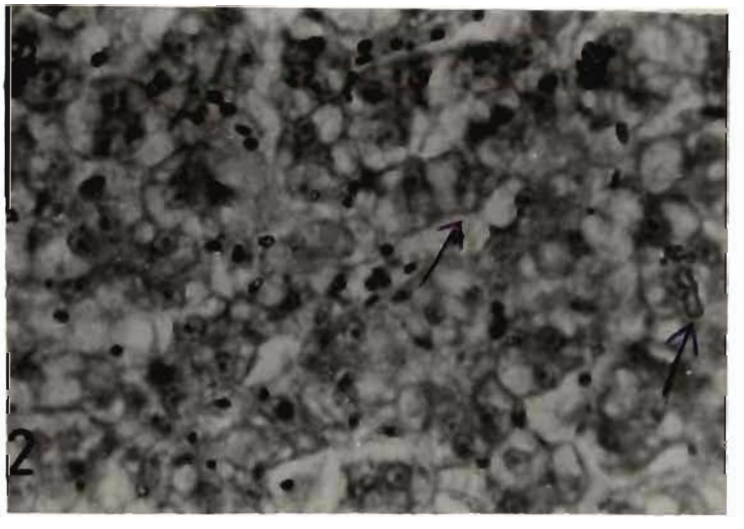


Plate 2. Liver of copper treated fish showing extensive vacuolation of hepatic cells - H&E X320

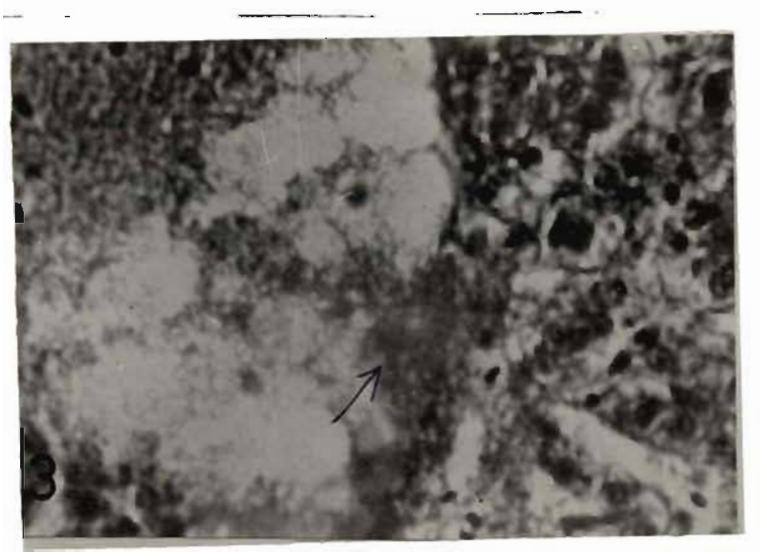


Plate 3. Liver of copper treated fish showing intravascular coagulation of blood. H&E X320

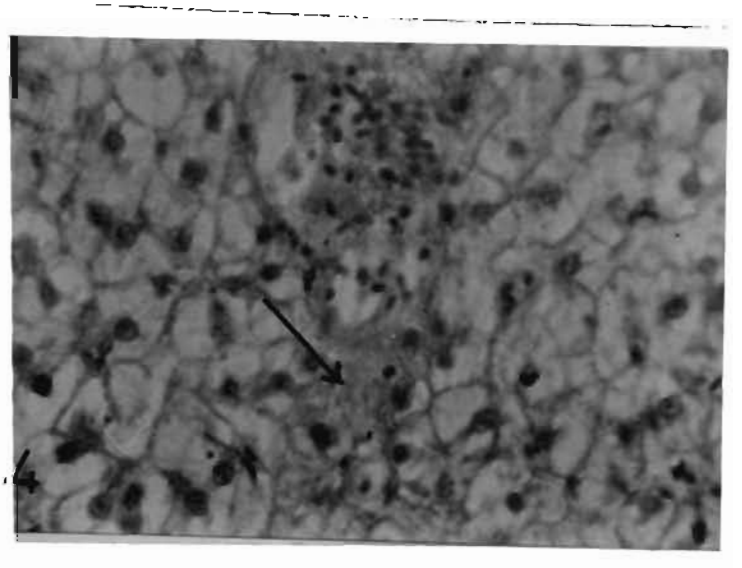


Plate 4. Liver of copper treated fish exhibiting intravascular coagulation and perivascular fibrinous exudate (arrow), note also the extensive vacuolation of hepatic cells. H&E X320

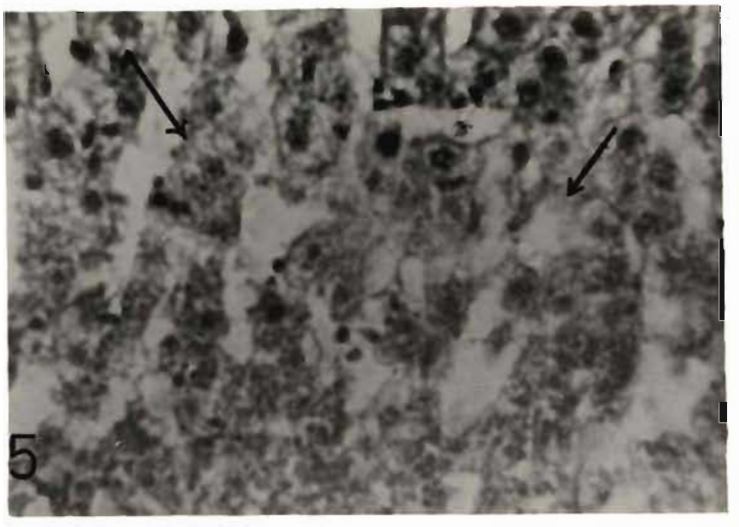


Plate 5. Liver of mercury treated fish showing of the necrotic region (arrows). H&E X320

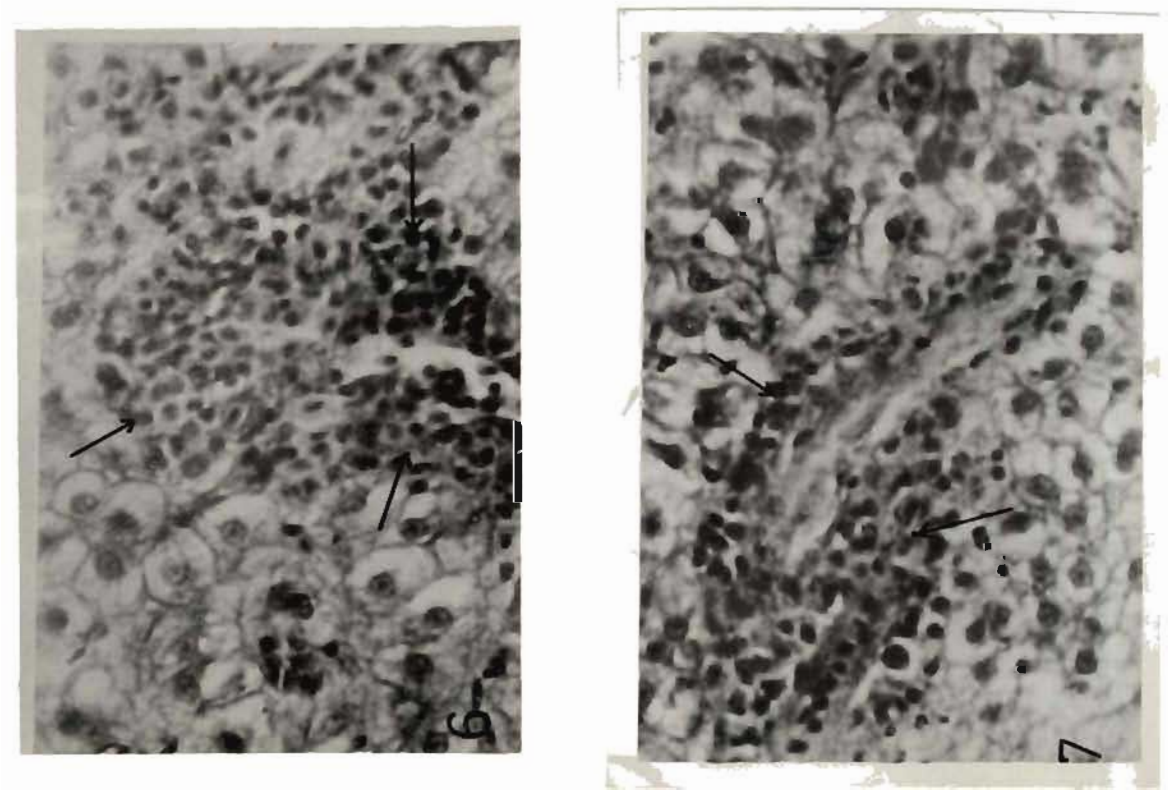


Plate 6. and Plate 7_Liver of mercury treated fish showing accumulation of mononuclear cells in perivascular region of hepatic parenchyma (arrows). H&E X320

central vesicular nucleus. The hepatocytes formed irregular cords which were separated by sinusoids lined with endothelial cells.

Liver exposed to Copper

The liver tissue after 5 days copper exposure revealed vacuolation of hepatocytes and condensation of nuclear chromatin. The samples collected 10th day showed intravascular coagulation of blood and focal necrosis of hepatic parenchyma in addition to vascular changes. The samples taken on 15th day, showed very extensive vacuolation of hepatic cells with several foci of coagulative necrosis and mononuclear cell accumulation and perivascular mononuclear cell infiltration and intravascular coagulation of blood were also observed. Hepatic nuclei were either swollen or pyknotic (Plates 2, 3 and 4).

Liver exposed mercury

Liver of mercury treated fish 5th day exposre exhibits intravascular coagulation. Many of the blood vessels appeared more permeable and exhibited fibrinous exudate in perivascular region. The extensive vocuolation of hepatic cells were also noticed. After 10th day of exposure the liver of treated fish showed the focal necrosis of hepatic parenchyma. After 15th day of exposure the liver of treated fish showed accumulation of mononuclear cells in perivascular region of hepatic parenchyma (Plates 5, 6 and 7).

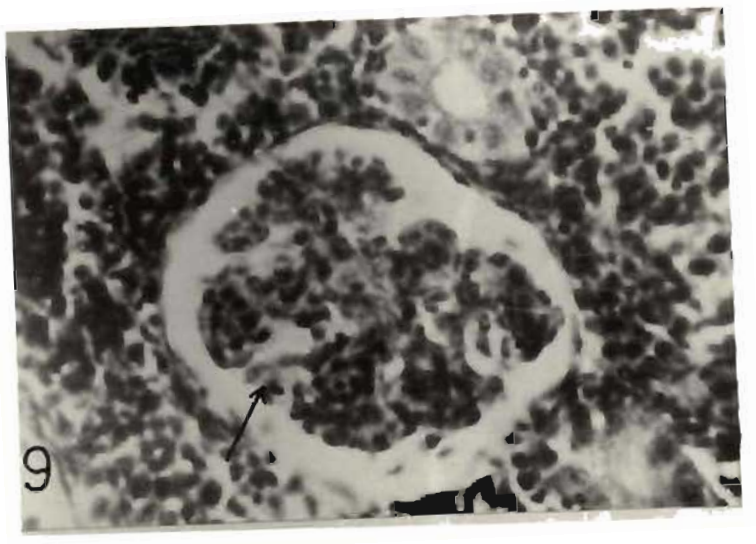


Plate 9. Section of kidney from control fish showing congested glomerulus (arrow), haemopoietic tissue and tubules. H&E X320

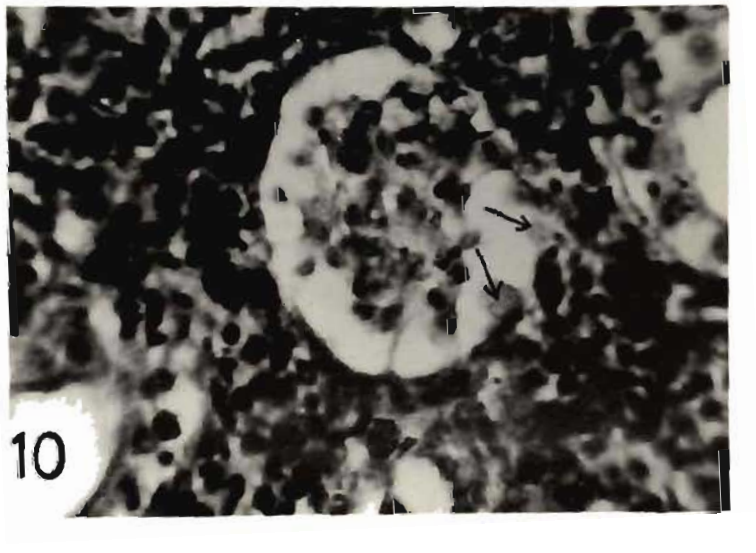


Plate 10. Kidney from copper treated fish showing increased permeability of glomerulus and accumulation of exudate in Bowman's capsule (arrows).

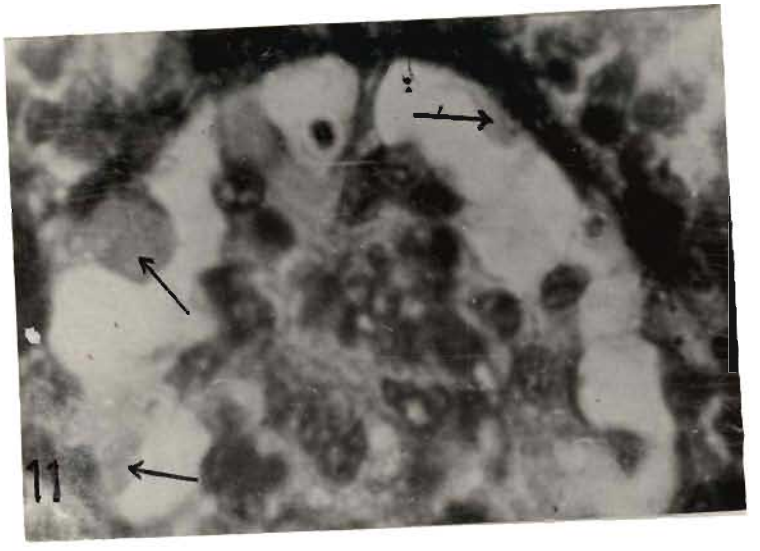


Plate 11. High power view of Fig. 10. H&E X800.

Plate 12. Section of kidney from copper treated fish showing extensive thickening of capillaries (arrows). H&E X400.

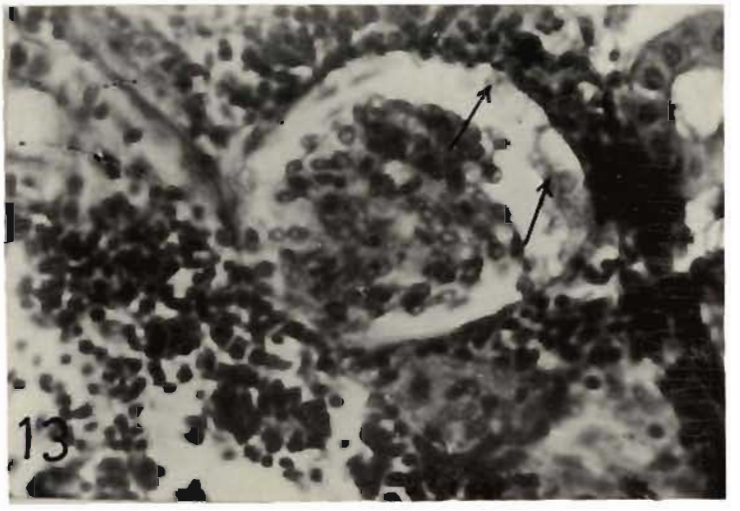


Plate 13. Copper treated fish glomerulus showing increased celluarity, note also accumulation of exudate in Bowman's capsule (arrows). H&E X400.

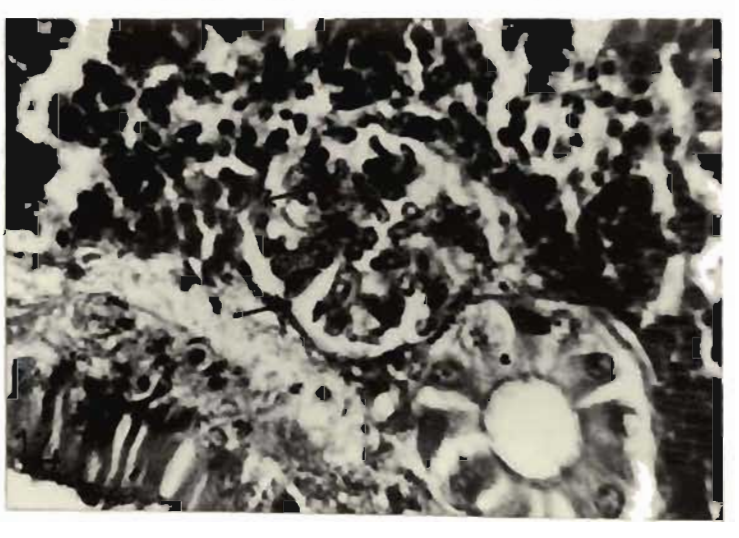


Plate 14. Glomerulus showing sclerotic changes and adhesion (arrows). H&E X400.

Kidney Control

The kidneys were composed of excretory, haemopoietic and reticuloendothelial tissues. The nephrons consisted of a well vascularised glomeruli which were congested. The glomeruli were surrounded by Bowman's capsule which were lined by squamous epithelial cells. The Bowman's capsule continued through a ciliated neck. The proximal segments, one with a prominent brush border and other with basal striations which were separated by a ciliated segment were seen. In addition to these tubules there were distal segments which connected second proximal segments to the collecting ducts. The Proximal ducts were more eosinophilic in staining and the proximal segment of the tubule were lined by low columnar epithelium with indistinctive borders. Interstitial space was occupied by actively dividing haemopoietic tissue and elements of adrenal tissues. Numerous melanomacrophage centres were also seen (Plate 9).

Kidney exposed to copper

The samples collected on 24 hr revealed no change in glomeruli from that of control. However, swelling of epithelial cells of proximal segments with cast in the lumen were observed. On 5th day, many of the glomeruli showed more permeability and Bowman's capsule continued proteinaceous fluid and heamogenous eosinophilic materials. Some of the glomeruli revealed increased cellularity and sclerotic changes. In addition to necrosis of cells of proximal tubules, swelling of the cells and cast in the

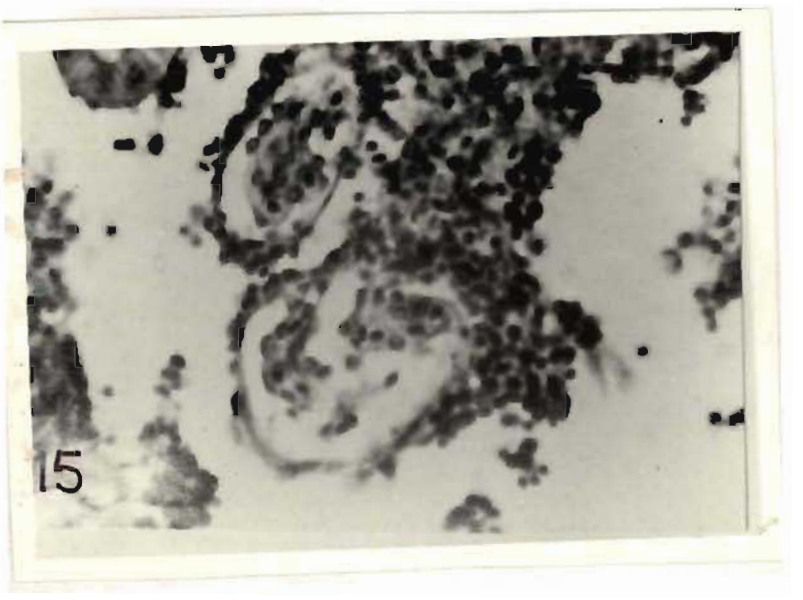


Plate 15. Copper treated kidney glomeruli showing the sclerotic changes, note also the thickening of Bowman's capsule. H&E X320

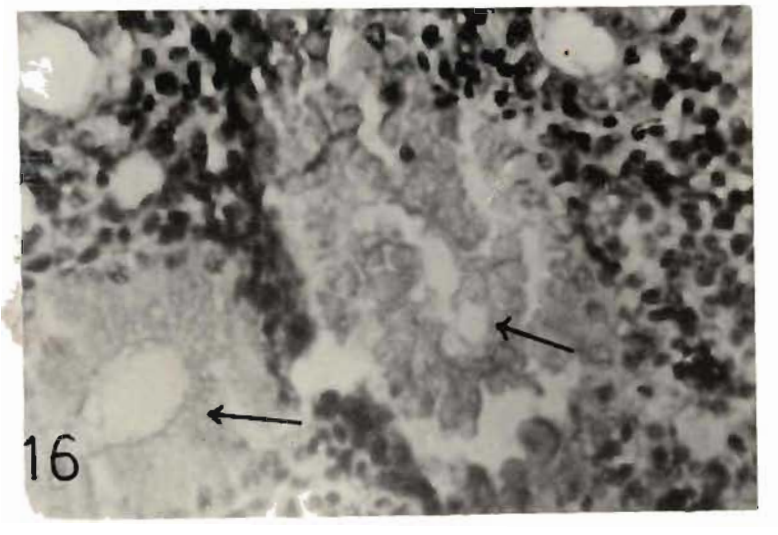


Plate 16. Section of kidney from mercury treated fish showing necrosis of tubules (arrows). H&E X320

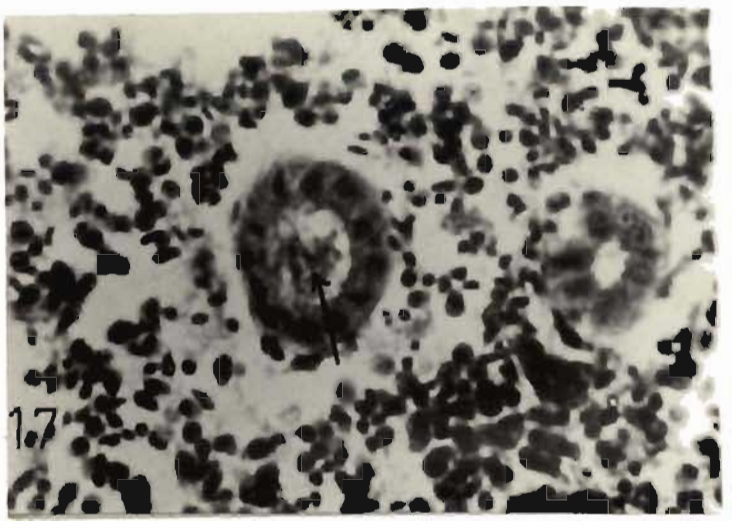


Plate 17. Section of kidney from mercury treated fish depicting appearence of cast in tubule (arro
H&E X320

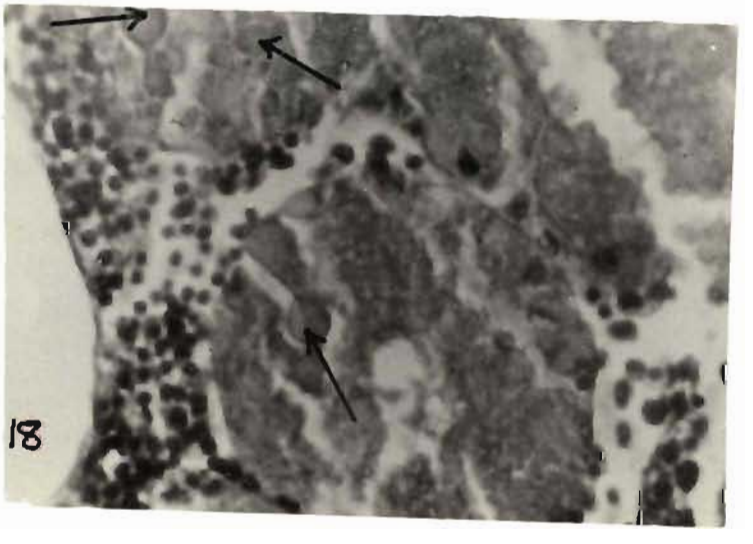


Plate 18. Section of necrosed tubule showing presence of hyaline droplets (arrows). H&E X320.

lumen were also noticed. By 10th day exposure in many glomeruli the capillaries appeared highly thickend. A few Glomeruli revealed mesenchymal proliferation and also increased permeability. On 15th day of exposure the glomerulus showed sclerotic changes and adhesion (Plates 12, 13, 14 and 15).

Kidney exposed to mercury

The kidney's on 5th day revealed must severe changes in tubules. There were only very mild changes in glomeruli. The tubular epithelial cells were either degenerated or had undergone severe extensive necrosis. The lumen of tubule contained hyaline cast. By 10th day, considerable changes observed in glomeruli and tubules. The glomerular changes were characterised by accumulation of proteinaceous fluid in Bowman's space, the thickening of Glomerular capillaries, mesenchymal cell proliferation and adhesion of visceral and parietal layers; and periglomerular fibrosis. In 15th day samples tubular epithelial cells were necrosed and almost all the tubules contained hyaline casts (Plates 16, 17 and 18).

Gill Control

The control gill had structure very similar to normal gill. The gill arch was covered by typical epidermal tissue which at the origin of primary lamellae was much thicker and endowed with mucus cells. Below the epidermis there was an array of lymphoid tissue consisting of lymphocytes and large cells containing

Plate 19. Gill of control fish H&EX400

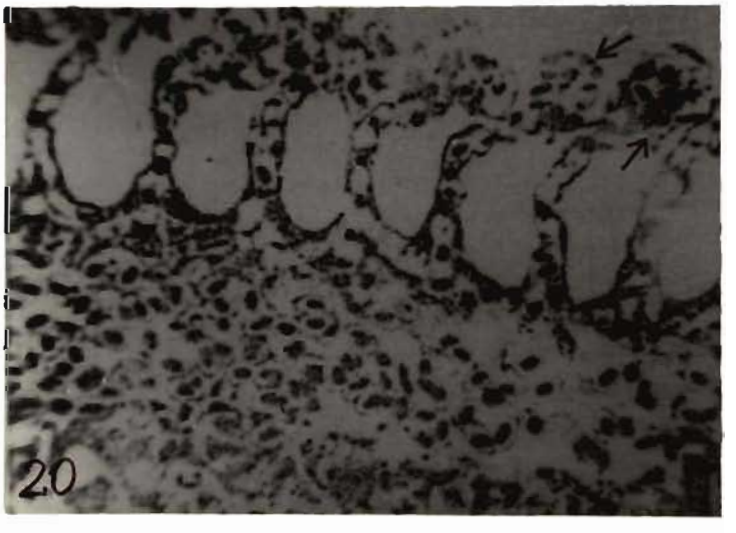


Plate 20. Gill of mercury treated fish showing hyperplasia in the tip of secondary lamellae H&EX400.

Plate 21. Gill of mercury treated fish showing advanced stage of hyperplasia resulting fusion of adjacent lamellae. H&EX400

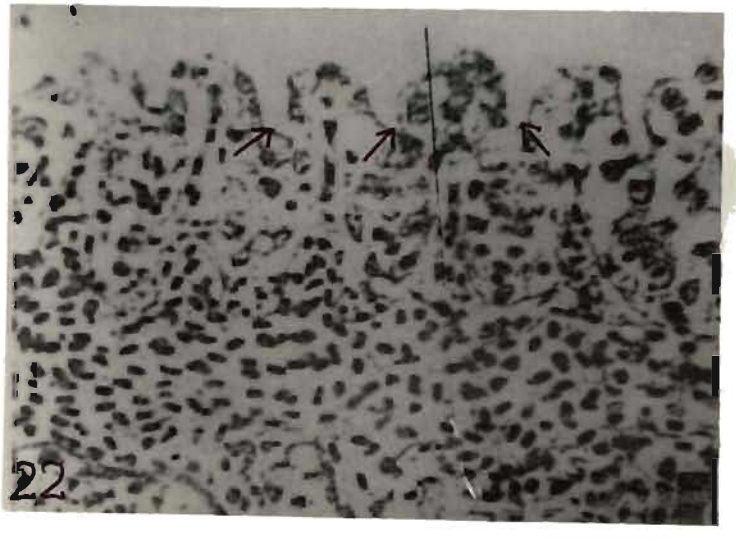


Plate 22. Gill of mercury treated fish showing complete fusion of lamellae. H&EX400

eosinophile granules. The primary lamellae had so many lateral projections, the secondary lamellae which were covered with epithelial cells - one layer thick which was supported and protected by the pillar cells. The pillar cells form the lining of blood sinuses or lamellar sinus which connect the afferent and efferent lamellar arteries (Plate 19).

Gill exposed to mercury

The change in the mercury treated fish after 24 hrs swelling at the tip of the secondary lamellae followed by hypertrophy and hyperplasia. As the exposure continued further for 5 days the increased hyperplasia was accompanied by fusion of adjacent lamellae, and the epithelium was lined by several layers of cells instead of a single layer. On the 10th day the entire inter - lamellar space was filled with hyperplastic epithelium and the lamellar structure of the gill was completely lost. As the exposure continued for 15 days disintegration of the hyperplastic epithelium started. There was cytoplasmic vacuolation and the nuclei were either karyorhectic or pycnotic. Hemorrhage and lymphocytic infiltration was also observed (Plates 20, 21, 22 and 23).

Gill exposed to copper

The histopathological changes in the gills of the copper treated fishes are shown in plates 24, 25, 26 and 27. After 24 hr of exposure the copper treated fish showed hypertrophy and

Plate 23. Gill of mercury treated fish showing vacuolation and desquamation in lamellae. H&EX400.

Plate 24. Gill of copper treated fish showing hypertrophy and hyperplasia in secondary lamellae. H&EX400.

Plate 25. Gill of copper treated fish showing fusion of adjacent lamellae. H&EX400.

Plate 26. Gill of copper treated fish showing widespread necrosis and vacuolation. H&EX400.

Plate 27. Gill of copper treated fish showing advanced stage of degenaration H&EX400.

hyperplasia in secondary lamellae of the gill. The fusion of the adjacent lamellae and observed in the gills of copper treated fish after 5th day of exposure. As the exposure continued further for 10 days the widespread necrosis and vacuolation was observed in the gill tissue of the fish. On the 15th day the cells showed advanced stage of degeneration (Plates 24, 25, 26 and 27).

DISCUSSION

Histopathological studies of controls did not show any major alterations. The structure of liver was very similar to that described for normal fish liver by Varichak (1988), Ferguson (1974), Ellis et al. (1976), Hinton and Pool (1976) and Ellis et al. (1978). In treated fishes the liver samples revealed vacuolation of hepatic cell. This vacuolation was present in all the treated groups throughout the experimental period and increase in severity in proportion to the time of exposure and dose. Crandall and Goodnight (1963) also reported the same type of changes in hepatic parenchyma by prolonged exposure of zinc in fishes. Kumar and Pant (1981), Sultan and Khan (1981), Wester and Canton (1986, 1987), Ansari and Kumar (1987), Jambulingam (1988) have also reported hepatic vacolation in toxic condition associated with zinc, mercury, copper, cdamium etc. This study also supports the same observations.

Liver suffers from lipid accumulation in a number of conditions like deficiency of tocopherol lipotropic factors,

excessive fat in the diet and toxic damage to the liver which interfere with transport and metabolism of fat or protein synthesis in liver. Many of the toxins like antimony, arsenic, cadmium, carbon tetrachloride etc. were reported to have caused fatty liver in higher vertebrates (Runnells et al. 1965; Meiss et al. 1982; Jones and Hunt, 1983).

The heavy metals cadmium, zinc, manganese and calcium were reported to have effect on respiratory metabolism and protein synthesis of hepatic cells in fishes (Hilt ibran, 1971; Shukla and Pandey, 1986). In the present study, due to toxicants the changes became more apparent and severe as the concentration and period of exposure increased. It is believed that the injurious effect of the toxin might have produced these changes. Severe necrotic changes were observed in the liver of fish exposed to lethal concentration of copper and zinc by Kumar and Pant (1981). Necrosis of hepatocytes was a common finding in many of the studies involving heavy metal pollution and other toxic condition in fishes (Bhattacharya et al. 1985; Cruz and Tamse, 1986; Jambulingam, 1988).

Intravascular coagulation and perivascular accumulation of fibrinous material observed in this study, have also been reported by some workers like Di Michele and Taylor (1978). Fish generally has very high count of thrombocytes in blood, hence, fish blood clot very rapidly (Ellis et al. 1978). A number of workers have reported vascular damage as well as poor development

of blood vessels in the liver of animals exposed to heavy metal toxicity and other toxic conditions (Crandall and Goodnight, 1963; Ellis et al. 1976). Increased perimiability of vascular and intravascular clotting were observed in the present study also. The changes probably may be due to the vascular damage which might have initiated the clotting and exudation of fibrin.

Generally, necrosis is accompanied by inflamatory reaction and accumulation of leucocytes in the periphery of those affected areas (Jones and Hunt, 1983). The leucocytic infiltration which was observed in present sudy may be due to an inflammatory response against the necrotic tissue. Narain and Singh (1991) reported the acute thiodan toxicity in liver causes extensive degeneration of cytoplasm and pyknosis of nuclei. Focal degeneration of liver cells due to mercury toxicity was reported by Bano and Hasan (1990).

In the control group the structure of kidney was very similar to the euryhaline fish which was described by Ellis et al. (1978). Occassionally some tubules showed degenerative changes and many glomeruli appeared, congested. Since the changes were mild in nature they were considered not very significant.

In toxicant exposed fishes considerable changes were observed in glomeruli and tubules. Glomerular changes consisted of increased permeability leading to accumulation of

proteinaceous fluid in Bowman's capsule, thickening of capillaries, mesenchymal cell proliferation and sclerotic changes in glomeruli. Initially the changes in glomeruli were characterised by increased permeability indicating vascular damage to the glomerular capillaries. These glomerular changes were morphologically very similar to changes described for glomerulonephritis, in other vertebrates (Cassey et al. 1979; Slauson and Lewis, 1979; George and Somvanshi 1984). Jones and Hunt, 1983; classified Glomerulonephritis as membranous, acute proliferative, membrano proliferative and chronic sclerosing glomerulonephritis. It is believed that glomerular injury results from immunologically mediated inflammatory reaction at glomeruli. This include deposition of circulating antigen antibody complexes, auto immune reaction and compliment activation (Heyman et al. 1959; Lewis et al. 1963, 1965; Cassey et al. 1979 Slauson and Lewis, 1973 and Jones and Hunt, 1983). Glomerulonephritis is a frequent condition observed in fishes during histopathological examinations. The changes like thickening of glomerular capillaries, hyalinisation of capillaries, dilation of glomerular capillaries, shrinkage of glomeruli and dilation of Bowman's capsule were observed in experimental toxic studies on zinc, copper, cadmium, $KMNO_4$, B-hexachlorocyclohexane etc, in fishes including milk fish (Kumar and Pant, 1981; Sexena, 1981; Cruz and Tamse, 1986; Wester and Canton, 1986).

In the present study tubules had undergone degenerative and necrotic changes depending on concentrarion of toxion and period of exposure. These changes were more severe in proximal tubules and consisted of swelling appearence of hyaline droplets and complete necrosis of epithelial cells. The lumen contained hyaline casts. A large number of chemical poisons like cadmium, copper, mercury, arsenic, bismuth, chromium, potasium, dichromatic, etc; were reported to have produced same conditions in higher animals (Runnells et al. 1965, Jones and Hunt; 1983). The hyaline droplets, which appeared in many tubular epithelial cells were reported to have occurred in kidneys where protein leakage through glomeruli occurred (Jones and Hunt, 1983). In this case also glomeruli were damaged and Bowman's capsule contained the proteinaceous fluid. Tubular degeneration and necrotic conditions were common findings in many experimental studies involving chemicals and insecticide toxin in different groups of fishes (Gardner and Yevich, 1970; Koyama and Itazawa, 1977 Kumar and Pant, 1981; Cruz and Tamse, 1986; Forlin et al. 1986).

The dilation of the lamina of the kidney tubules and necrosis of tubules as observed in the present investigation after chloropyrifos treatment have been reported from various fish exposed to pollutants (Kumar and Pant, 1981, 1984; Srivastava and Srivastava 1981; Casillas et al. 1983; Sukumar and Karpagaganapathy, 1986; Gill et al. 1988). The chloropyrifos treated fish, the glomeruli are shrunken and the blood cells in

the glomerular tuft become vacuolated (Sanjay et al. 1990). Bano and Hasan (1990) studied about the histopathological lesions in the body organs of catfish (Heteropnuestes fossilis) following mercury intoxication. Focal degeneration of liver cells and disorganization of hepatic cords were found. Furthermore centriloblular atrophy and some compensatory hepatic cells were also observed.

Physiological, histological and ultra structural studies have shown that heavy metal ions interfere with respiration and osmoregulation by disrupting the structure of gill cells in fishes (Eisler and Gardner 1973; Jones, 1975). Hypertrophy, hyperplasia and mucus production on gills are associated with prolonged, exposure to chronic levels of pesticides (Cope, 1965; Eller, 1969), heavy metals (Skidmore and Tovell, 1972; Gardner and Yevich, 1970), un-ionized ammonia (Flis, 1968) and other water borne irritants (Eller, 1975). Severe hypertrophy, hyperplasia and necrosis were seen in the gills of fish exposed to formalin (Wedemeyer, 1971). Oversecretion of mucus cells, fusion of secondary gill lamellae from the pillar cells and occurrence of necrotic cells were seen in the gill of fish Heteropnuestes fossilis exposed to chlorpyrifos (Srivastava et al. 1989).

The effects of toxicants in the cellular structure on different tissues of the fish Macrones gulio treated with copper and mercury observed in the present study were mainly the

hyperplasia and necrosis of liver cells, proliferation and degeneration and oedema of lamellar epithelial cells of the gills and dialation of glomerular capillaries and proliferation of glomeral cells of kidney. In general the structural changes increased in severity in proportion to the time of exposure and dose. Such changes were also reported earlier by Skidmore and Tovell, 1972; Kumar and Pant, 1981; Sultan and Khan, 1981; Cruz and Tamse, 1986. after exposing the fishes to heavy metals including copper and mercury.

SUMMARY

The present study embraces investigations on the sublethal effects of copper and mercury on the fish, Macrones gulio (Hamilton-Buchanan). The fishes were exposed to three sublethal concentrations of each metal and the changes in glycogen, protein, haemoglobin, haematocrit, erythrocyte count and histopathology of liver, kidney and gill, during metal exposure were monitored.

Chapter 1. In this chapter, a broad outline of heavy metal uptake, requirement of a suitable bio - monitoring organism, criteria for a standard test fish, and suitability of Macrones gulio for the toxicological study are given.

Chapter 2. This chapter deals with the lethal toxicity bioassays to find the 96 hr LC 50 of copper and mercury for the fish Macrones gulio. The experimental results indicated that of the two metals tested, copper was more toxic than mercury. The LC 50 of copper is 0.05 ppm and LC 50 of mercury is 0.13 ppm. During the lethal toxicity study, behavioural response of the fish to the metals copper and mercury were more or less similar.

Chapter 3. The effect of copper and mercury on the haemoglobin, haematocrit, erythrocyte count, MCV, MCH and MCHC was studied. There was an increase in haemoglobin content in copper and mercury exposed fishes. This increase might be an attempt of the body to increase the oxygen carrying capacity of the blood as hypoxia generally occurs in metal intoxicated fishes.

Chapter 4. The glycogen and protein contents of liver and muscle after exposure to copper and mercury were studied. There was a significant decrease of glycogen in the liver and muscle of metal treated fishes. A decrease in protein content in the liver and muscles was observed during the later period of the experiment. During metal intoxication, the energy requirements goes up owning to the detoxification and tissue repair processes. This increased energy demand and the hypoxic condition usually associated with metal exposure, results in the depletion of glycogen reserves. When the glycogen reserves decrease, the protein in the tissue are utilized for energy production as there is a decrease in the protein content towards the end of the experimental period.

Chapter 5. The histopathological changes of the tissues like liver, kidney and gill after exposure to copper and mercury were studied. Vacuolation of hepatocytes, condensation of nuclear chromatin, intravascular coagulation of blood, focal necrosis of hepatic parenchyma were the changes noted in liver following heavy metal (copper and mercury) exposure. The changes in kidney included swelling of epithial cells of proximal segment with cast in the lumen, cellularity and sclerotic changes in glomeruli, necrosis of cells of proximal tubules, the thickening of capillaries. The changes in the gill of copper and mercury treated fish were swelling at the tip of the secondary lamellae followed by hypertrophy and mild hyperplasia. The other changes are fusion of the adjacent lamellae, necrosis of the gill epithelium.

Important and relevant papers dealing with the subject matter of study have listed under reference.

REFERENCES

References

ABDULLAH, M.I., ROYLE, L.G. and MORRIS, A.W. (1972) Heavy metal concentration in coastal waters. Nature, 235 : 158 - 160.

ABRAHAMSSON, T. and NILSSON, S. (1975) Effect of nerve sectioning and drugs on the cathecholamine content in the spleen of cod *Gadus morhua*. Comp: Biochem. Physiol., 51 : 231 - 233.

ADAMSON,R.H. (1967) Drug metabolism in marine vertebrates. Fed. Proc Fedn. Am Socs. exp. Biol., 26 : 1047 - 1055.

AGRAWAL, S.J., SRIVASTAVA, A.K. and CHAUDHRY, H.S. (1979) Haematological effects of nickel toxicity on a fresh water teleost *Colisa fasciatus*. Acta. Pharmacol Toxicol., 45 : 215 - 270.

ALDERDICE, D.F. (1963) Some effects of simultaneous variation in salinity, temperature and dissolved oxygen on the resistance of young *Coho salmon* to a toxic substance. J. Fish. Res. Bd. Can. 20 : 525 - 550.

ANDERSON, T., BENGTSSON, B.E., FOERLIN, L., HAERDIG, J. and LARSSON. A. (1987) Longterm effects of bleached kraft mill effluent on carbohydrate metabolism and hepatic xenobiotic biotransformation enzymes in fish. Ecotoxicol: Environ. Saf., 13 : 53 - 60.

ANSARI, B.A. and KUMAR.K. (1987) Malathion toxicity : Pathological changes in the liver of zebrafish, *Byachydaniorerio* (cyprinidae). Bol. Fisiol.anim., Sao paulo., 11 : 27 - 34.

ARUNA, D. and GOPAL, V. (1987) Toxic effects of sublethal level of mercury on haematological parameters. Indian J. Environ. Health., 29 : 52 - 56.

ASANO, R. and HOIKARI, S. (1987) The effect of copper on intact swine erythrocytes. Comp. Biochem. Physiol., 86 : 443 - 447.

ASSEM, H., and HANKE, W. (1979 a) Concentrations of carbohydrates during osmotic adjustment of the euryhaline teleost *Tilapia mossambica*. Comp. Biochem. physiol., 64 : 5 - 16.

ASSEM, H. and HANKE, W. (1979 b) Volume regulation of muscle cells on the euryhaline teleost - *Tilapia mossambica*. Comp. Biochem. Physiol., 64 : 17 - 23.

ASSEM, H., MASER, C., PAUL, R., SCHROCK, H. WEHE, E. and HANKE, W. (1978) Hormones and osmomineral regulation in fish and amphibia. In: Comparative Endocrinology. (Eds. Gallard, P.J. and Boer, H.H.) Elsevier/North - Holland, Amsterdam.

AWASTHI, M., SHAH, P., DUBALE, M.S. and GADHIA, P. (1984) Metabolic changes induced by organophosphates in the piscine organs. Environ. Res., 35 : 320 - 325.

BACKSTROM, J. (1967) Distribution of mercuric compounds in fish and birds Oikos. Suppl., 9 : 30 - 31.

BAKER, J.T.P. (1968) Histological and electron microscopical observations on copper poisoning in the winter flounder (*Pseudopleuronectes americanus*). J. Fish. Res. Bd. Can., 26 : 2785 - 2793.

BAKTHAVATHSALAM, R. and REDDY, Y.S. (1982 a) Intoxication effects of Lindane (δ-BHC) on the activities of asparatate and alanine transferases of *Anabas testudinus* (Bloch.). Pestic. Biochem. Pysiol., 18 : 226 - 234.

BALAVENKATASUBHAIAH, M., RANI, A.V., GEETHANJALI, K.R., PURUSHOTHAM, K.R. and RAMAMURTHI, R. (1984) Effect of cupric chloride on oxidative metabolism in the fresh water teleost, *Tilapia mossambica*. Ecotoxicol. Environ. Saf., 8 : 289 - 293.

BANERJEE, S.K., DASTIDAR, S.G., MUKHOPADHYAY, P.K. and DEHADRAI, P.V. (1978) Toxicity of Cadmium : a comparative study in the air - breathing fish *Clarias batrachus* (Linn.) and in the non air breathing one *Tilapia mossambica* (Peters). Ind. J. Exp. Biol., 16 : 1274 - 1277.

BANO, Y. and HASAN, M.B. (1990) Histopathological lesions in the body organs of catfish *Hetropneustes fossilis* following mercury intoxication. J. Environ. Sci. Health, Part B Vol. B25, no. 1. pp. 67 - 85.

BASHA, S.M., PRASADA RAO, K.S., SAMBASIVA RAO, K.R.S. and RAMANA RAO, K.V. (1984) Respiratory potentials of the fish *Tilapia mossambica* under malathion, carbaryl and lindane intoxication. Bull, Environ. Contam. Toxicol., 32 : 570 - 574.

BASHAMOHIDEEN, M. and PARVATHESWARA RAO, V. (1972) Adaptations to osmotic stress in the fresh water euryhaline teleost *Tilapia mossambica* for changes in blood glucose, liver glycogen and muscle glycogen levels. Mar. Biol., 16 : 68 - 74.

BEENA, S. and VISWARANJAN, S. (1987) Effect of cadmium and mercury on the haematological parameters of the fish *Cyprinus carpio*. Environ. Ecol., 5 : 726 - 732.

BELL, G.R. (1968) Distribution of transaminases (Amino transferases) in the tissue of pacific salmon (*Oncorhynchus*) with emphasis on the properties and diagnostic use of glutamic - oxaloacetic transaminase. J.Fish. Res. Bd. Can., 25 : 1247 - 1268.

BENNET, A.F. (1978) Activity of metabolism of the lower vertebrates. A. Rev. Physoil., 400 : 447 - 469.

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

BHATTACHARYA, T., RAY.A.K. and BHATTACHARYA, S. (1985) Histopathological lesions in the hepatopancreas of *Channa punctatus* (Bloch) exposed to mixtures of mercuric chloride and phenol and a factory effluent. matsya 11 : 1 - 4.

BLACK, E.C. BOSOMWORTH, N.J. and DOCHERTY, G.E. (1966) Combined effect of Starvation and severe exercise on glycogen metabolism of rainbow trout, *Salmo gairdneri*. J. Fish. Res. Bd. Can., 32 : 1461 - 1463.

BLACK, E.C., CONNOR, A.R. and PARKET, R.R. (1961) Some aspects of carbohydrate metabolism in fish. In : Comparative physiology of carbohydrate metabolism in heterothermic animal. (Ed. Martin, A.W.) Univ. Washington Press. P.89 - 122.

BLACK, E.C., CONNOR, A.R., HARSHIP, A.R. and CHIU, W. (1960) Alterations in glycogen glucose and lactate in rainbow and kamloops trout (*Salmo gairdneri*) following muscular activity. J. Fish Res. Bd. Can., 17 : 487 - 500.

BLACK, E.C., CONNOR, A.R., LAM, K.C and CHIU, W. (1962) Changes in glycogen, pyruvate and lactate in rainbow trout (*Salmo gairdneri*) during and following muscular activity. J. Fish. Res. Bd. Can., 19 : 409 - 436.

BLAXHALL, P.C. and DAISELY, K.W. (1973) Routine haematological methods for use with fish blood. J. Fish. Biol. 5 : 771 - 781.

BLAXHALL, P.C. (1972) The haematological assessment of the health of freshwater fish: J. Fish Bio., 4 : 593 - 605.

BLAZKA, P. (1958) The anaerobic metabolism of fish. Physiol. Zool., 31 : 117 - 128.

BOUQUEGNEAU, J.M; and GILLES, R. (1979) Osmoregulation and pollution of the aquatic medium. In : Metabolism of osmoregulation in animals. (Ed. Gills, R.) P. 563 - 580, New York.

BUCK, W.B. (1978) Copper / Molybdenum toxicity in animals. In : Toxicity of heavy metals in the environment. (Ed. Oehme, F.W.) Marcel Dekker, Inc. New York, Part I : 491 - 515.

BUCKLEY, J.A. (1976) Heinz body hemolytic anemia in coho salmon (*Oncorhynchus kisutch*), exposed to chlorinated waste water. J. Fish. Res. Bd. Can., 34 : 215 - 224.

BUCKLEY, J.A., WHITEMORE, C.M and MATSUDA, R.I. (1976) Changes in blood chemistry and blood cell morphology in coho salmon (*Oncorhynchus kisutch*) following exposure to sublethal levels of total residual chlorine in municipal waste water.J.Fish.Res.Bd.Can., 33 : 776 - 778.

BUCKLEY, J.A., ROCHE, M., MC CARTER, J.A., RENDELL, C.A. and MATHESON, A.T. (1982) Chronic exposure of coho salmon to sublethal concentration of copper - 1 Effect on growth, on accumulation and copper tolerance. Comp. Biochem. Physiol., 72 : 15 - 19.

BUCKLEY, J.A, WHITEMORE, C.M and LIMING, B.D (1979) Effects of prolonged exposure to ammonia on the blood and liver glycogen of Coho salmon (*Oncorhynchus kisutch*). Comp. Biochem. Physiol., 63 : 297-303.

* BURDICK, G.E. (1957) Fish Game - Journal New York 4 : 102 - 108.

* BURDICK, G.E. (1967) Ame., Fish. Sec. Sp. public. 4 : 37.

BURTON, D.T., JONES, A.H. and CAIRNS, J.Jr. (1972) Acute zinc toxicity to rainbow trout (*Salmo gairdneri*): Confirmation of the hypothesis that death is related to tissue hypoxia. J. Fish. Res. Bd. Can., 29 : 1463 - 1466.

BURTON, D.T. and SPEHAR, A.M. (1971) A revaluation of the anaerobic endproducts of fresh water exposed to environmental hypoxia. Comp. Biochem. Physiol., 40 : 945 - 954.

* CAIRNS, J. (Jr.) (1969) Fish bioassays - reproducibility and rating. Rev. Biol. (Lisbon), 7 : 7 - 12.

CAIRNS, J.(Jr.) (1980) Developing toxicity testing capability for Australia. Water, 7 : 14 - 16/

CALABRESE, A., THURBERG, F.P., DAWSON, M.A. and WENZLOFF, D.R. (1975) Sublethal physiological stress induced by cadmium and mercury in the winter flounder *Psuedopleuronectes americanus*. In : Sublethal effects of toxic chemicals on aquatic animals. (Eds. Koeman, J.H. and Strik, J.J.T.W.A.), Amsterdam. Elsevier. P. 15 - 21.

CAMP, B.J., HEJTMANCIK, E., ARMOUR, C., and LEWIS D.H. (1974) Acute effects of Aroclor, R.1254 (PCB) on *Ictalurus-punctatus* (Catfish). Bull. Environ. Contam. Toxicol., 12 : 204 - 208.

CARDEILHAC, P.T. (1972) Mineral metabolism. In: Text book of veterinary physiology. (Ed. Breazile, J.E) Lea and Febiger, Philadelphia. P. 448 - 457.

CASILLAS, E., MYERS, M. and AMES, W.E. (1983) Relationship of serum chemistry values to liver and kidney histopathology in English sole *(Porophrys vetulus)* after acute exposure to carbon tetrachloride. Aquat. Toxicol. 3 : 61 - 78.

CASSEY, H.M., AIYERS, K.M. and ROBINSON, F.R. (1979). The urinary system. In pathology of laboratory Animals. vol. 1. (ed.) Benirschke, K. Garner, F.M. and Jone, T.C. Springer Verlag, New York.

CHAMBERS, J.E. and YARBROUGH, J.D. (1976) Xenobiotic transformation systems in fishes. Comp. Biochem. Physiol., 55C : 77 - 84.

CHAPMAN, P.M. and LONG, E.R. (1983) The use of bioassays as a part of a comprehensive approach to marine pollution assessment (View point). Mar. Pollut. Bull. 14(3) : 81 - 84.

CHAVIN, W. and YOUNG, J.E. (1970) Factors in the determination of normal serum glucose levels of goldfish, *Carassius auratus*. Comp. Biochem. Physiol., 33 : 629 - 653.

CHITRA,T. and RAMANA RAO, J.V. (1986) Temperature induced changes in certain haematological parameters in mercury trated *Channa punctatus* (Bloch). Com. Physiol. Ecol., 11 : 49 - 53.

CHRISTENSEN, G.M. (1975) Biochemical effects of methyl mercuric chloride, cadmium chloride and lead nitrate on embryos and alevins of the brook trout, *Salvelinus fontinalis*. Toxicol. Appl. Pharmacol., 32 : 191 - 197.

CHRISTENSEN, G.M., HUNT, E. and FIANT, J. (1977) The effect of methyl mercuric chloride cadmium chloride and lead nitrate on six biochemical factors of the brook trout, *Salvelinus fontinalis*. Toxicol. Appl. Pharmacol., 42 : 523 - 530.

CHRISTENSEN, G.M., MCKIM, J.M., BRUNGS.W.A. and HUNT. E.P. (1972) Changes in the blood of brown bullhead *Ictalurus nebulosus* (Lesuer) following short and long term exposure to copper (ii) Toxicol. Appl. Pharmacol., 23 : 417 - 427.

CLIFF, G. and THURMAN, G.D. (1984) Pathological and physiological effects of stress during capture and transport in the juvenile dusky shark, *Carcharhinus abscurus*. Comp. Biochem. Physiol., 78 : 167 - 173.

COLLVIN, L. (1984) The effects of copper on maximum respiration rate and growth rate of perch, *Perca fluviatilis*. L. Water. Res., 18 : 139 - 144.

COPE, O.B. (1965) Some responses of fresh water fishes to herbicides. proceeding of the southern weed conference. 18, 439 - 445.

COUGH, J.A. (1984) Atrophy of diverticular epithelium as an indicator of environmetal irritants in the oyster *Crassostrea viriginica. Mar.* Environ. Res. 14 : 525 - 526.

CRAIG, P.J. (1986 a) Occurance and pathways of organometallic compounds in the environment, general considerations. In : organometallic compounds in the environment, Principles and reactions. (Ed. Craig, P.J.) Longman, Essex. U.K. P. 368.

CRAIG, P.J. (1986 b) Organomercury compounds in the environment, Chapter II In : Organometallic compounds in the environment; principles and reactions. (Ed. Craig, P.J.) Longman, Essex. U.K. P. 368.

CRANDALL, C.A. and GOODNIGHT, C.J. (1963) The effects of sublethal concentrations of several toxicants to the common guppy, *Lebistes reticulatus*. Trans. Am. Microscop. Soc., 82 : 59 - 73.

CRUZ, E.R. and TAMSE, C.T. (1986). Histopathological response of milkfish *Chanos chanos* Forskal fingerlings to potassium permanganate. Fish path., 21 (3) : 151 - 159.

DANGE, A.D. (1986 a) Changes in carbohydrate metabolism in Tilapia, *Oreochromis* (*Sarotherodon*) *mossambicus*, during short term exposure to different types of pollutants. Environ. pollut., 41 : 165 - 177.

DANGE, A.D. and MASUREKAR, V.B. (1982) Naphthalene induced changes in carbohydrate metabolism in *Sarotherodon mossambicus* (Pisces : Cichlidae) Hydrobiologia, 94 : 163 - 172.

DAVIS, J.C. (1973) Sublethal effects of bleached kraft pulp mill effluent on respiration and circulation in sockeye salmon (*Oncorhynchus nerka*). J.Fish. Res. Bd. Can., 30 : 369 - 377.

DAWSON, M.A. (1979) Hematological effects of long term mercury exposure and subsequent period of recovery on the winter flounder, *Pseudopleuronectes americanus*. In : marine pollution : functional response. Proceedings of the symposium pollution and physiology of marine organisms. (Eds. vernberg, W., Calabrese, A., Thurberg, F.P. and Vernberg.F.J.) Academic Press, New York, U.S.A, P. 171 - 182.

* DE BRUIN, A. (1976 a) Biochemical toxicology of environmental agents, Elsevier, Amsterdam P. 612.

DE WILDE, M.A. and HOUSTON A.H. (1967) Haematological aspects of the thermoaclimatory process in the rainbow trout, *Salmo gairdneri*. J. Fish. Res. Bd. Can., 24 : 2267 - 2281.

DE ZWAAN., A and ZANDEE, D.I. (1972) The utilization of glycogen and accumulation of some intermediates in anerobiosis in *Mytilus edulis*. Comp. Physiol. Biochem., 43 : 47 - 54.

DEMPSTER, R.P. (1970) The use of copper sulphate as a mediament for aquarium fishes and as an algicide in marine mammal water systems. Marine Aquarist, 1 : 24 - 26.

DI MICHELE, L. and TAYLOR, M.H. (1978) Histopathological and physiological response of *Fundulus heteroclitus* to naphthalene exposure. J. Fish. Res. Board Can., 35 : 1060 - 1066.

DORFMAN, J. (1977) Tolerance of *Fundulus heteroclitus* to different metals in salt water. Bull. N.J. Acad. Sci. 22 : 221 - 223.

* DOUDORFF, P., ANDERSON, B.G., BUDRIK, G.E; GATSTOFF, P.S., HART, W.B., PATRICK, B. STRONG, E.R., SURFER, E.W. and VANHORN, W.M. (1957) Bioassay methods for the evaluation of acute toxicity of industrial waste fish. Sew Industrial waste. 23 : 1380 - 1397.

DRUMMOND, R.A., SPOOR, W.A. and OLSON, G.F. (1973) Some short term indicators of sublethal effects of copper on brook trout, *Salvelinus fontinalis*. J. Fish. Res. Bd. Can., 30 : 698 - 701.

DUBALE, M.S. and SHAH, P. (1981) Biochemical alterations induced by Cd in the liver of *Channa punctatus*. Environ. Res., 26 : 110 - 118.

DUTHIE, G.G and TORT, L. (1985) Effect of dorsal aortic cannulation on the respiration and haematology of the Mediterranean dog fish *Scyliorhinus canicula*. L.Comp. Biochem. Physiol., 81 : 879 - 883.

EATON, J.G. (1970) Chronic malathion toxicity to the blue gill, *Lepomis macrochirus* Ref. Wat. Res. 4 : 673 - 684.

EISLER, R and EDMUNDS, P.H. (1966) Effect of endrin on blood and tissue chemistry of marine fish. Trans. Am. Fish. Soc., 95 : 153 - 159.

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

EL - DOMIATY, N.A. (1987) Stress response of juvenile *Clarias lazera* elicited by copper. Comp. Biochem. physiol., 88 : 259 - 262.

ELLER, L.L. (1969) Pathology of readear sunfish exposed to hydrothol 191, Transactions of the American fisheries Society 98, 52 - 59.

ELLER, L.L. (1975) Gill lesions in fresh water teleosts. In: The pathology of fish (Ed. by W.E. Ribelin and G. Migaki). P. 305 - 330. The university of wisconsin Press, madision, Wsconsin.

ELLIS, A.E., MUNRO, A.L.S, and ROBERTS, R.J. (1976) Defence mechanisms in fish I. A study of the phagocytic system and the fate of intraperitonially injected particulate material in the plaice (*Pleuronectes plattessa*). J. Fish Biol., 8 : 67 - 78.

ELLIS, A.E., ROBERTS, R.J. and TYTLER, P. (1978) The anatomy and physiology of teleosts. In fish Pathology (ed.) R.J. Roberts Bailliere Tindall, London. pp. 13 - 54.

ERNST, W. (1980) Effects of pesticides and related organic compounds in the sea. Helgolander wiss. Meeresunter-suchuncen 33 : P.214 - 225.

FAGERLUND, U.H.M. (1967) Plasma cortisol concentration in relation to stress in adult sockeye salmon during the fresh water stage of their life cycle. Gen. Comp. Endocrinol., 8 : 197 - 207.

FAIRBANKS, U.F. (1967) Copper sulphate - induced haemolytic anaemia. Arch. Intern. Med., 120 : 428 - 436.

FARMAN FARMAIAN and SOCCI, R. (1984) Inhibitation of essential aminoacid absorption in marine fishes by mercury. Mar. Environ. Res. 14 : 185 - 199.

FARMAN FARMAIAN, A and SOCCI, R. and POLIDOSE, T. (1980) Mechanism of heavy metal inhibition of aminoacid transport in inestine of marine fish. Biol. Bull. 159 : 458.

FERGUSON, H.W. (1974) Ph. D. Thesis, University of Stirling.

FLIS. J. (1968) Anatomic - histopathological changes induced in carp (*Cyprinus carpiol. L*) by ammonia water I. Effects of toxic concentrations. Acta Hydrobiologica 10 : 205 - 224.

FOGELS, A. and SPRAGUE, J.B. (1977) Comparative short term tolerance of zebra fish, flag fish and rainbow trout to five poisons including potential reference toxicants. Water Res., 11 : 811 - 817.

FORLIN, F., HAUX, C., KARLSSON - NORRGREN, L. and RUNN, P. (1986) Biotransformation enzyme activities and histopathology in rainbow trout, *Salmo gairdneri*, treated with cadmium. Aquat. Toxicol. 8 : 51 - 64.

FREEMAN, H.L. and IDLER, A. (1973) Effects of corticosteroids on liver transaminases in two salmonids, the rainbow trout (*Salmo gairdneri*) and the brook trout (*Salvelinus fontinalis*) Gen. Comp. Endocrinol., 20 : 69 - 75.

FRY, F.E.J (1971) The effect of environmental factors on the physiology of fish. In : Fish physiology Vol. VI. (Eds. Hoar, W.S. and Randall, D.J.). Academic Press Inc., New York, p. 1 - 98.

GARDNER G.R. and YEVICH, P.P. (1969) Studies on the blood morphology of three estuarine cyprinodontiform fishes. J. Fish. Res. Bd. Can., 26 : 433 - 447.

GARDNER, G.R. and LA ROCHE, G. (1973) Copper induced lesions in estuarine teleosts. J. Fish. Res. Bd. Can., 30 : 363 - 368.

GARDNER, G.R. and YEVICH, P.P. (1970). Histological and haematological responses of an estuarine teleost to cadmium, J. Fish. Res. Board Can., 27 : 2185 - 2196.

GEORGE, K.C. and SOMVANSHI, R. (1984). Spontaneous glomerulonephritis in rabbits (*Oryctolagus caniculus*) Kerala J. Vet. Sci., 15 (2) : 126 - 130.

GILL, T.S. and PANT, J.C. (1981) Effects of sublethal concentration of Hg in a teleost *Puntius conchonius* : Biochemical and Haemtlogical responses. Indian J. Exp. Biol., 19 : 571 - 573.

GILL, T.S. and PANT, J.C. (1983) Cadmium toxicity : Inducement of changes in blood and tissue metabolites in fish. Toxicol., Lett., 18 : 195 - 200.

GILL, T.S. and PANT, J.C. (1985) Erythrocytic and leukocytic responses to cadmium poisoning in a freshwater fish, *Puntius conchonius* Ham. Environ. Res., 36 : 327 - 337.

GILL, T.S., PANT, S.C. and PANT, J. (1988) Gill, liver and kidney lesions associated with experimental exposure to carbaryl and dimethoate in the fish (*Puntius conchonius* (Hamilton)). Bull. Environ. Contam. Toxicol. 41 : 71 - 78.

GINGERICH, W.H. (1982) Hepatic toxicology of fishes. In : Aquatic Toxicology (Ed. Weber. L.J.) Raven Press, New York, Vol. I : 55 - 105.

GINGERICH, W.H., WEBER, L.J. And LARSSON, R.E. (1978) Carbon tetrachloride induced retention of sulphobromopthalien in the plasma of rainbow trout. Toxicol. Appl. Pharmacol., 43 : 147 - 158.

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

GLUTH, G. and HANKE, W. (1985) A comparison of physiological changes in carp, *Cyprinus carpio* induced by several pollutants at sublethal concentration I. The dependency on exposure time. Ecotoxicol. Environ. Saf., 9 : 179 - 188.

GOEL, B.B. and AGRAWAL, V.P. (1981) Tannic acid biochemical changes in the liver of two teleost fishes, *Clarius batrachus* and *Ophiocephalus punctatus*. Ecotoxicol. Environ. Saf., 5 : 418 - 423.

GOEL, K.A. and GARG, V. (1980) 2, 3', 4 - Triamino azobenzene - induced haematobiochemical anomalies in fish (*Channa punctatus*). Bull. Environ. Contam. Toxicol., 25 : 136 - 141.

GOEL, K.A., SHARMA, S.D. and MAYA (1985) Biochemical and enzymological changes in liver and kidney of *Clarias batrachus* following lithium intoxication. Arch. Ind. Hyg. Toxicol., 36 : 249 - 253.

GOLDBERG, E.D. (1980) In : The international mussel watch. (Ed.) National Academy of Sciences, Washington, D.C.

GOODMAN, A.G. and GILMAN, F. (1956) In : The Pharmacological basis of therapeutics. Macmillan Co., New York.

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

GROTHE, D.R. and EATON, J.W. (1975) Chlorine induced mortability in fish. Trans. Am. Fish. Soc., 104 : 800 - 802.

HANIFFA, M.A., MURUGESAN, A.G. and PORCHELVI, M. (1986) Haematological effects of distillary and paper mill effluents on *Heteropneustes fossilis* (Bloch) and *Sarotherodon mossambicus* (Peters). Proc. Indian Aca. Sci. Anim. Sci., 95 : 155 - 161.

HANSEN, D.J. and PARRISH, P.K. (1977) Suitability of sheeps-head minnow (*Cyprinodon variegatus*) for life cycle toxicity test. In Aquatic toxicology and hazard evaluation Eds. F.L. Meyer and J.L. Hamelink, ASTM. Philadephia P. 117 - 126.

* HARPER, H.A., RODWELL, V.W. and MAYES, P.A. (1977) In : A review of physiological chemistry. (Ed. Harper, H.A.) 16th Ed. Lange Medical Publications. California, U.S.A.

* HART, W.B., DOUDOROFF, P. and GREEN BANK, J. (1945) Waste control Lab of the Atlantic Refining Co. 317.

HATTINGH, J. (1976) Blood sugar as an indicator of stress in the fresh water fish, *Labeo capensis* (Smith). J. Fish Biol., 10 : 191 - 195.

HAVE, N. (1969) Sulphydryl inhibitors and pancreatic islets tissue. Acta. Endocrin. Suppl., 139 : 1 - 231.

HEATH, A.G. and PRITCHARD, A.W. (1965) Effects of severe hypoxia on carbohydrate energy stores and metabolism of two species of freshwater fishes. Physiol. Zool., 38 : 325 - 334.

HEATH, A.G. (1987) Water pollution and fish physiology. CRC Press. Inc, Boca Raton, Florida. pp 235.

HELMY, M.M., LEMKE, A.E., JACOB, P.G. and OOSTDAM, B.L. (1978) Effects of some trace elements on the blood of Kuwait mullets, *Liza macrolepis* (Smith). J. Exp. Mar. Biol. Ecol., 34 : 151 - 161.

HELMY, M.M., LEMKE, A.E., JACOB, P.G. and AI - SULTAN, Y.Y. (1979) Haematological changes in Kuwait mullet, *Liza marcolepis* (Smith), induced by heavy metals. Indian J. Mar. Sci., 8 : 278 - 281.

HENDRICKS, L.J. (1952) Erythrocyte counts and haemoglobin determinations for two species of sucker's, genus *Catostomus* from colorodo, copeia 4 : 265 - 266.

HESSER, E.F. (1960) Methods for routine fish Haematology. Prog. Fish. Cult., 22 : 164 - 171.

* HEYMAN, W., et al. (1959). Production of nephrotoxic syndrome in rats by Freund's adjusva and rat kindney suspension. Proc. Soc. Exp. Biol. Med., 100 : 600 - 1959.

HILLE, S. (1982) A literature review of blood chemistry of rainbow trout, *Salmo gairdneri* Rich. J. Fish Biol., 20 : 535 - 569.

HILMY, A.M., SHABANA, M.B. and DAABEES, A.Y. (1985) Effects of cadmium toxicity upon the in vivo in vitro activity of proteins and five enzymes in blood serum and tissue homogenates of *Mugil cephalus*. Comp. Biochem. Physiol., 81 : 145 - 153.

HILMY, A.M., SHABANA, M.B. and SAID, M.M. (1980) Blood chemistry levels after acute and chronic exposure to $HgCl_2$ in the Killifish *Aphanius dispar* (Rupp.) Water Air Soil Pollut., 14 : 409 - 417.

HILTIBRAN, R.S. (1971). Effects of cadmium, zinc, manganese and calcium on oxygen and phosphate metabolism of bluegill liver mitrochondria. J. Water Poll. Control Fed., 43 : 818 - 823.

HINTON, D.E. and POOL, C.R. (1976) Ultrastructure of the liver of channel catfish *Ictalurus punctatus* (Rafinesque). J. Fish Biol., 8 : 209 - 220.

HINTON, D.E. and LAUREN, D.J. (1990) Integrative histopathological approaches to detecting effects of environmental stressors on fishes. In. Biological indictors of stress of fish. Adams. S.M. (Ed). American fisheries society Symposium. 8 : 51 - 66.

HODSON, P.V., HILTON, J.W., BLUNT, B.R. and SLINGER, S.J. (1980) Effects of dietary ascorbic acid on chronic lead toxicity to young rinbow trout (*Salmo gairdneri*). Can. J. Fish. Aquat. Sci., 37 : 170 : 176.

HODSON, P.V., (1976) Temperature effects on lactate - glycogen metabolism in zinc - intoxicated rainbow trout (*Salmo gairdneri*). J. Fish Res. Bd, Can., 33 : 1393 - 1397.

HOLDEN, A.V. (1973) Effects of pesticides on fish : In "Environmental pollution by pesticides" Ed. C.A. Edwards.p. 213 - 253. Plenum Trans. London.

HOLETON, G.F. and RANDALL, D.J. (1967) The effect of hypoxia upon the partial pressure of gases in the blood and water afferent and efferent to the gills of rainbow trout. J. Exp. Biol., 46 : 317 - 327.

HUGHES, G.M. (1981) Effects of low oxygen and pollution on the respiratory system of fish. In : Stress and fish (Ed. Pickering, A.D.), Academic Press, New York, p. 121 - 146.

HUGHES, G.M., PERRY, S.F. and BROWN, V.M. (1979) A morphometric study of effects of nickel, chromium and cadmium on the secondary lamellas of rainbow trout gills. Wat. Res., 13 : 665 - 679.

HUNN, J.B., HAMILTON, S.J. and BUCKLER, Dr. (1987). Toxicity of sodium selenite to rainbow trout fry. Wat. Res. 21 : 233 - 238.

IDLER, P.R. and CLEMENS, W.A. (1959) The energy expenditure of Fraser River sockeye salmon during the spawining migration to chilka and stuart lakes. Intl. Pac. Salmon Fish Comm. Prog. Rep., 6 : 1 - 80.

ITAZAWA, Y. (1971) An estimation of minimum level of dissolved oxygen in water required for normal life in fish. Bull. Jap. Soc. Sci. Fish., 37 : 273 - 276.

JAGOE, C.H. and HAINES, T.A. (U.S. Fish wildl. Serv., Columbia Natl. Fish. Res. Lab. Field Res. Stn., Dep. Zool., Univ. Marine, Orono, ME 04469, U.S.A.) Trans. Am Fish. Soc. vol. 112, no.5, Sep. 1983, pp : 689 - 695. En., en.

JAMBULINGAM, M. (1988). Sublethal effects of Endosulphan and cadmium on histological organization and hexokinase activity in *Oreochromis* (Tilapia) *mossambicus* (Peters). M. Phil thesis Annamalai University.

JAMES, R. (1990) Individual and combined effects of heavy metals on behaviour and respiratory responed to Oreochromis mossambicus. Indian J. Fish. 32 : 139 - 143.

JERNELOV, A. (1975) Heavy metals in the marine environment. In : Discharge of sewage from sea outfalls. (Ed. Gameson, A.L.H.), Pergamon Press, New York, 12 : 115 - 122.

JOHANSSON - SJOBECK, M.L. and LARSSON, A. (1978) The effect of cadmium on the hematology and on the activity of & - aminolevulinic acid dehydratase (ALA -D) in blood and hematopoietic tissues of the flounder, *Pleuronectes flesus* L. Environ. Res., 17 : 191 - 204.

JOHNSTON, I.A. (1975) Anaerobic metabolism in the carp (*Carassius carassius*) Comp. Biochem. Physiol., 51 : 235 - 241.

JONES, T.C. and HUNT, R.D. (1983) Veterinary pathology. 5th Ed. Lea and Febiger. Philadelphia U.S.A.

JONES, M.B. (1975) Synergistic effects of salinity, temperature and heavy metals on mortality and osmoregulation in marine and estuarine isopodes (Crustacea). Mar. Biol. 30 : 13 - 20.

JORGENSEN, J.B. and MUSTAFA, T. (1980) The effect of hypoxia on carbohydrate metabolism in flounder (*Platichthys flesus* L.) Utilization of glycogen and accumulation of glycolytic endproducts in various tissues. Comp. Biochem. Physiol., 67 : 243 - 248.

JUELICH, F. (1979) Haematological study of rainbow trout (*Salmo gairdneri*) and carp (Cyprinus carpio) after exposure to sublethal concentration of pesticides Rogor, Benomyl, Pomuran and Domatol - Spezial. Z. Angew. Zool., 66 : 475 - 504.

JUNEJA, C.J. and MAHAJAN, C.J. (1983) Haematological and heamatopoietic changes in fish *Channa punctatus* due to mercury pollution in water. Indian J. Anim. Res., 17 : 63 - 71.

KABATA, Z. (1970) Crustacea as enemies of fishes. In : Diseases of fishes. (Eds. Snieszko, S. and Axelord, H.R) T.F.H. Publications, New Jersey, U.S.A: P. 171.

KATTI, S.R. and SATHYANESAN, A.G. (1986) Lead nitrate induced changes in the brain constituents of freshwater fish, *Clarias batrachus*. Neurotoxicology 7 : 47 - 52.

KIMURA, S. and MATSSUSHIMA, M (1969) Bull. of Freshwater fish. Re. Lab. 19(2) : 121 - 135 cited from pesticides and freshwater fauna Muirhead - Thomson R.C. Academic Press. 1971.

* KNOX, W.E. and GREENGARD, O. (1965) An introduction to enzyme physiology. (Ed. Weber, G.) Pergamon Press, New York, No. 3, 247.

KOYAMA, J. and OZAKI, H. (1984) Hematological changes of fish exposed to low concentrations of cadmium in the water. Bull. Jap. Soc. Sci. Fish., 50 : 199 - 203.

KOYAMA, J. and ITAZAWA, Y. (1977) Effects of oral administration of cadmium on fish - II Results of Morphological examination. Bull. Jap. Soc. Sci. Fish., 43(5) : 527 - 533.

* KRISHNA KUMARI, L., VARSHNEY, P.K., GAJHIYE, S.N., GOVINDAN, K. and NAIR, V.R. (1983) Toxicity of some metals on the fish, *Therapon jarbua* (Forsskal, 1775). Indian J. Mar. Sci. 12 : 64 - 66.

KUMAR, K. and ANSARI, A.B. (1986) Malathion toxicity : Effect on the liver of the fish *Brachydanio rerio* (Cyprinidae) Ecotoxicol. Environ. Saf., 12 : 199 - 205.

KUMAR, S. and PANT, S.C. (1981) Histopathological effects of acutely toxic levels of copper and zinc on gills, liver and kidney of *Puntius conchonius* (Ham). Ind. J. Exp. Biol., 19 : 191 - 194.

LA ROCHE, G. (1974) In : Industrial pollution. Ed. by 1/sax (Van nostrend Reinhold Co, New York) : P - 101.

LAL, B., SINGH, A., KUMARI, A. and SINGHA, N. (1986) Biochemical and haematological changes following malathion treatement in the freshwater catfish *Hetropneustes fossilis* (Bloch). Environ. pollut., 42 : 151 - 156.

LAMBERG, S.L. and ROTHSTEIN, R., (1978) Haematology and Urynalysis.A.V.I. publishing company, connecticut, U.S.A.

LANE, C.E. and SCURA, E.D. (1970) Effects of dieldrin on glutamic oxaloacetic transaminase in *poecilia latipinna* (Sailfin mollie). J. Fish. Bd. Can., 27 : 1869 - 1871.

LARSSON, A. (1975) In : Sublethal effects of toxic chemicals on aquatic animals (Ed. Koeman, J.H. and Strik. J.J.T.W.A.). Elsevier. Sci. Publ. Co. Amsterdam 3 - 13.

LARSSON, A. and HAUX, C. (1980) Effects of Cadmium carbohydrate metabolism in fish. F.S.B.I. International symposium "Stress in Fish". University of East Anglia. Norwich.

LAUREN, D.J. and MC DONALD, D.G. (1987) Acclimation to copper by rainbow trout, *Salmo gairdneri*: Bio chemistry. Can. J. Fish Aquat. Sci. 44 : 105 - 111.

LELAND, H.V. and KUWABARA.J.S. (1985) Trace metals. In : Fundamentals of aquatic toxicology. (Eds. Ranch, G.M. and Petrocelli, S.R.) Hemisphere, Publishing Corporation, Washington, P. 375 - 415.

LENNON, R.E. (1967) Selected strains of fish as bioassay animals. Prog. Fish. Cult., 29 : 129 - 132.

LETT, P.F., FARMER, G.J. and BEAMISH, F.W.H. (1976) Effect of copper on some aspects of the bioenergetics of rainbow trout (*Salmo gairdneri*). J. Fish. Res. Bd. Can., 33 : 1335 - 1342.

LEWIS, S.D. and LEWIS, V.M. (1971) The effect of zinc and copper on the osmolality of the blood serum of the channel catfish, *Ictalurus punctatus* Rafinesque and golden shiner, *Notemigonus crysoleucas* Mitchell. Trans. Am. Fish. Soc., 100 : 639 - 643.

* LEWIS, R.M. SCHWARTS, R.S. and GILMORE, C.E. (1963) A syndrome of auto immune haemolytic anemia and thrombocytopenia in dogs. Sci. Proc. AVMA., 140 - 163.

* LEWIS, R.M. SCHWARTS, R.S. and GILMORE, C.E. (1965) Autoimmune disease in domestic animals. Ann. N.Y.Acad. Sci., 124 : 178 - 200.

LINDAHL, P.E. and HELL, C.E.B. (1970) Effects of short - term exposure to *Leuciscus rutilus* L. (Pisces) to phenyl mercuric hydroxide. Oikos. 21 : 267 - 275.

LITCHFIELD, J.T., and WILCOXON, F. (1949) A simplified method of evaluating dose - effectexperiments. J. pharmac. exp. Ther. 96 : 99 - 113.

* LOCK, R.A.C. (1979) Effects of mercuric chloride and methyl mercuric chloride on mucus production and osmoregulatory function of the gills in rainbow trout, *Salmo gairdneri* Richardson. Doctral disseration, University of Nijmegen, The Netherlands.

LOCK, R.A.C. CRUIJSEN, P.M.J.M. and VAN OVERBEEKE, A.P. (1981) Effects of mercuric chloride and methyl mercuric chloride on the osmoregulatory functions of the gills in the rainbow trout, *Salmo gairdneri* Richardson. Comp. Biochem. Physiol., 68 : 151 - 159.

LONG, C.N.H., KATZIN, B. and FRY, E.G. (1940) The adrenal cortex and carbohydrate metabolism. Endrcrinol., 26: 309 - 334.

LOWE, D.M., MOORE, M.N. and CLARKE, K.R. (1981) Effects of oil on digestive cells in mussles quantitative alterations in cellular and lysosomal structure, Aquat. Toxicol. 1 : 213 - 226.

MACARTER, J.A., MATHESON, A.T., ROCH, M., OLAFSON, R.W. and BUCKLEY, J.T. (1981) Chronic exposure of coho salmon to sublethal concentration of copper. 2. Distribution of copper between high and low molecular weight protein in liver cytosol and the possible role of metallothionein in detoxification. Comp. Brochem. Physiol. 72 C : 21 - 26.

MALINS, D.C., MC CAIN, B.V.B., MYERS, M.S., BROWN, D.W., SPARS, A.K., MORADO, J.F. and HODINGS, H.O. (1984) Toxic chemicals and abnormalities in fish and shell fish from urban bays of puget sound. Mar. Environ. res. 14 : 527 - 528.

MARATHE, V.B. and DESHMUKH, S.S (1980) Effect of copper contaminated food on the growth (blomass) of *Channa gachua* (Ham). Proc. Indian Acad. Sci. (Anim. Sci.)., 89 : 403 - 406.

MARKING, L.L. (1966) Evaluation of P,P' - DDT as a reference toxicant in bio assays. U.S. Bur. Sport. Fish. Wildl. Resources Publ., 14 : 10.

* MAZEAUD, M.M., MAZEAUD, F. and DONALDSON, E.M. (1977) Primary and Secondary effects of stress in fish. Some new data with a general review. Trans. Am. Fish. Soc., 106 : 201 - 212.

MAYNARD, L.A. and LOOSLI.J.K. (1962) The Nutrients and their metabolism. In : Animal Nutrition, McGraw - Hill, New York, P.67 - 85.

MAZMANIDI, N.D. and KOVALEVA, G.I. (1975) The effects of dissolved petroleum products on some of the aspects of carbohydrate metabolism in fishes and invertebrates. J. Ichthyology, 15 : 811 - 816.

* MCFADDEN, T.W. (1965) Copper and Zinc poisoning in brook trout (*Salvelinus fontinalis* Mitchell), M.S. Thesis, Uni. of Manitoba, Winnipeg, manitoba.

MCKIM, J.M. and BENOIT, D.A. (1971) Effects of long term exposure to copper on survival, growth and reproduction of brook trout (*Salvelinus fontinalis*) J. Fish. Res. Bd. Can., 28 : 655 - 662.

MCKIM, J.M., CHRISTENSEN, G.M. and HUNT, E.P. (1970) Changes in the blood of brook trout (*Salvelinus fontinalis*) after short term and long term exposure to copper. J. Fish. Res. Bd. Can., 27 : 1883 - 1889.

MCLEAY, D.J. (1973) Effects of 12 h and 25 day exposure to kraft pulp mill effluent on the blood and tissues of juvenile coho salmon (*Oncorhynchus kisutch*). J. Fish. Res. Bd. Can. 30 : 395 - 400

MCLEAY, D.J. (1977) Development of a blood sugar bioassay for rapidly measuring stressful levels of pulpmill effluent to salmonid fish. J. Fish. Res. Bd. Can., 34 : 477 - 485.

MCLEAY, D.J. and BROWN, D.A. (1974) Growth stimulation and biochemical changes in juvenile coho salmon (*Oncorhynchus kisutch*) exposed to bleached kraft pulp mill effluent for 200 days. J. Fish. Res. Bd. Can., 31 : 1043 - 1049.

MCLEAY, D.J. and BROWN, D.A. (1975) Effects of acute exposure to bleached kraft pulp mill on carbohydrate metabolism of juvenile coho salmon (*Oncorhynchus kisutch*) during rest and excercise. J. Fish. Res. Can., Bd. Can., 32 : 753 - 760.

MCLEAY, D.J., and BROWN, D.A. (1979) Stress and chronic effects of untreated and treated bleached kraft pulp mill effluent on the biochemistry and stamina of juvenile coho salmon (*Oncorhynchus kisutch*) J. Fish. Res. Bd. Can., 36 : 1049 - 1069.

MCLEAY, D.J., HOWARD, T.E. and WALDEN, C.C. (1972) sublethal effects of bleached kraft mill effluents to fish. Can. For. Serv. Ottawa, Ont., CPAR Rep., 5 - 9.

MEHRLE, P.M., STALLING, D.L. and BLOOMFIELD, R.A. (1971) Serum aminoacids in rainbow trout as affected by DDT and dieldrin. Comp. Biochem. Physiol., 38 : 373 - 377.

MEISS, R., ROBENEK, H., RASSAT. J., THEMANN, H. and RECICHERT, B. (1982) Ultrastructural alterations in the hepatic parenchyma of the rat following acute cadmium intoxication Arch. Environ. Contam. Toxicol., 11 : 283 - 289.

MIDDAUGH, D.P., BURNETT, L.E. and COUCH, J.A. (1980) Toxicological and physiological responses of the fish, *Leiostomus xanthurus*, exposed to chlorine produced oxidants. Estuaries, 3 : 132 - 141.

* MILLIGAN, C.L. and WOOD, C.M. (1982) Disturbances in haematology, fluid volume distribution and cardiovascular function associated with low environmental P^H in the rainbow trout *Salmo gairdneri* In : Acid Rain Fisheries : Proceeding of an international symposium on acidic rain fishery impacts on North - eastern and North America. (Ed. Johnson, R.E.) American Fisheries Soc., Bethedsa, U.S.A.P. 335.

MISHRA, J. nad SRIVASTAVA, A.K. (1983) Malathion induced hematological and biochemical changes in the Indian catfish *Hetropneustes fossilis* Environ. Res., Vol. 30 : 393 - 398.

MISHRA, J. and SRIVASTAVA, A.K. (1984) Effects of chlordane on the blood and tissue chemistry of a teleost fish, *Hetropneustes fossilis*. Cell. Mol. Biol. Vol. 30 : 519 - 523.

MISHRA, S. and SRIVASTAVA, A.K. (1979) Haematology as an index of sublethal toxicity of zinc in a freshwater teleost. Bull. Environ. Contam. Toxicol., 22 : 695 - 698.

MISHRA, S and SRIVASTAVA, A.K. (1980) The acute toxic effects of copper on the blood of a teleost. Ecotoxicol. Environ. Saf., 4 : 191 - 194.

MOORE, M.N., WIDDOWS, J., CLEARY, J.J., PIPE, R.K., SALKELD, P.N., DONKIN. P., FARRAR, S.V., EVANS, S.V. and THOMSON. P.E. (1984) Responses of the mussel *Mytilus edulis* to copper and phenanthrene. Interactive effects. Mar. Environ. Res. 14 : 167 - 183.

MUIRHEAD - THOMSON.R.C. (1971) Pesticides and Freshwater fauna. Acad. Press. Lond.

MUKHOPADHYAY, P.K. and DEHARDRAI, P.V. (1980) Biochemical changes in the air - breathing catfish *Clarias batrachus* (Linn) exposed to malathion. Environ. pollut., Ser, A, 22 : 149 - 158.

NAIDU, K.A., and RAMAMURTHI, R. (1984) Acute effect of mercury toxicity on some enzymes in liver of teleost *Sarotherodon mossambicus*. Ecotoxicol. Environ. Saf., 8 : 215 - 218.

* NAKANO, T. and TOMLINSON, N. (1967) Catecholamine and carbohydrate concentration in rainbow trout (*Salmo gairdneri*) in relation to physical disturbances J. Fish. Res. Bd. Can., 24 : 1701 - 1715.

NARAIN, A.S. and SINGH, B.B. (1991) Histopathological lesions in *Hetropneustes fossilis* subjected to acute thiodan toxicity. Acta. Hydrochem. Hydrobiol. Vol. 19, no : 2 PP 235 - 243.

NARASIMHAN, P.V. and SUNDARARAJ. B.I. (1971) Effects of stress on carbohydrate metabolism in the teleost *Notopterus notopterus* (Pallas) J. Fish Biol., 3 : 441 - 451.

NATH, K. and KUMAR, N. (1987) Toxicity of Manganese and its impact on some aspects of carbohydrate metabolism of a freshwater teleost, *Colisa fasciculatus*. Sci. Total Environ., 67 : 257 - 262.

NAYAK, R.R. and MADHYASTHA, M.N. (1980) Bioassay studies with DDT with reference to some behavioural and haematological parameters of *Rasbora daniconius* (Ham). Proceedings of the seminar on management of environment February, Bombay, P.243 - 250.

NICHOL, C.A. and ROSEN, F. (1963) In : Advances in enzyme regulation (Ed. Weber, B) Pergamon Press, New York, P. 341.

NIMMO, D.R. (1985) Pesticides : In fundamentals of aquatic toxicology methods and application. Eds. Gary, M. Rand, Sam, R. Petrocelli, 335 - 373. Hemisphere publishing corporation, Washington.

NIMMO, D.R., BAHNER, L.H., RIGBY, R.A., SHEPARD, J.M. and WILSON, A.J. Jr. (1977) *Mysidopsis fahia*. An estuarine species suitable for life cycle toxicity test to determine the effects of a pollutant. In: Aquatic toxicology and hazard evaluation. ASTM. Philadelphia. Ed: F.L. Meyer, J.L. Hamelink, pp. 109 - 110.

NRIAGU, J.O. (1979) The biochemistry of mercury in the environment. Elsevier North - Holland Biochemical Press.

OGURI, M. and NACE, P.F. (1966) Blood sugar and adrenal histology of the goldfish after treatment with mammalian adrenocorticotrophic hormone. Chesapeake Sci., 7 : 198 - 202.

O'HARA, J. (1971) Alterations in oxygen consumption by blue gills exposed to sublethal treatment with copper. Water Res., 5 : 321 - 327.

OIKARI, A and SOIVIO, A (1977) Physiological conditions of fish exposed to water containing pulp and paper industry wastes and sewage. In : Proceedings of symposium of the European Inland Fisheries Advisory commission (Ed. Alabaster, J.S.), Helsinki, Finland, Applied Science Publishers, London, P. 89 - 96.

OLSON, K.R. and FROMM, P.O. (1973) Mercury uptake and ion distribution in gills of rainbow truot (*Salmo gairdneri*) tissues scans with electron microscope. J. Fish. Res. Bd. Can., 30 : 1575 - 1578.

OLSON, K.R. and BERGMANN, H.L. and FROMM, P.O. (1973) Uptake of methyl mercuric chloride and mercuric chloride by trout. A study of uptake pathways into the whole animal and uptake by erythrocytes in vitro. J. Fish. Res. Bd. Can., 30 : 1239 - 1299.

OLSON, K.R., SQUIBB, K.S. and COUSINS, R.J. (1978) Tissue uptake subcellular distribution and metabolism of $^{14}CH_3$ Hgcl, and $^{14}CH_3$, ^{203} Hgcl by rainbow trout, *Salmo gairdneri*. J. Fish. Res. Bd. Can., 35 : 381 - 390.

OZAKI, H.K., EMATSU, U. and TANAKA, K. (1971) Survival and growth of gold fish and carp in dilute solutions of copper sulphate. Jap. J. Ichthyol., 17 : 166 - 172.

PAGENKOPF, G.K., RUSSO, R.C. and THURSTON, R.V. (1974) Effect of complexation on toxicity of copper to fishes. J. Fish. Res. Bd. Can., 31 : 461 - 465.

PANIGRAHI, A.K. (1977) Toxicological effects of mercury poisoning of a freshwater fish Anabas and its ecological implications. Proc. 64th session of Indian Science congress Ab., 99 : 209.

PANIGRAHI, A.K. and MISRA, B.N. (1978) Toxicological effects of mercury on a freshwater fish *Anabas scandens*, Cur. and Val. and their ecological implications. Environ. Pollution, 16 : 31 - 39.

PANIGRAPHI, A.K. and MISRA, B.N. (1980) Toxicological effects of a sublethal concenuration of inorganic mercury on the freshwater fish *Tilapia mossambica* Peters. Arch. Toxicol., 44 : 269 - 278.

PANT, J., TEWARI, H and GILL, T.S. (1987) Effects of aldicarb on the blood and tissue of a freshwater fish. Bull. Environ. Contam. Toxicol., 38 : 36-41.

PATEL, B., BANGERA, V.S., PATEL, S. and BALANI, M.C. (1985 a) Heavy metals in the Bombay harbour area. Mar. Pollut. Bull., 16 : 22 - 28.

PATEL, B., BALANI, M.C. and PATEL, S. (1985 b) Sponge sentinel of heavy metals. Sci. Tot. Environ., 41 : 143 - 152.

PEISACH, J., AISEN, P. and BLUMBERG, W.E. (1967) The biochemistry of copper. Academic Press, New York, P. 475 - 503.

PHILIPS, D.J.H. (1980) Quantitative aquatic biological indicators. In : pollution Monitoring series. (Ed. Mellanby, K.), Applied Science Publishers, P. 488.

PICKERING, Q.H. and HENDERSON, C. (1966) The acute toxicity of some heavy metals to different species of warm water fishes. Air water pollut. Int. J., 10 : 453 - 463.

QAYYUM, M.A and SHAFFI, S.A. (1977) Changes in tissue glycogen of a fresh water catfish *Heteropneustis* fossilis (Bloch) due to mercury intoxication Curr. Sci, 46 : 652 - 653.

RACICOT, J.G., GAUDET, M and LERACY, C. (1975) Blood and liver enzymes in rainbow trout (*Salmo gairdneri*) with emphasis on their diagnostic use. Study of CCl_4 toxicity and care of Aeromonas infection. J. Fish Biol., 7 : 825 - 835.

RADHAIAH, V., GIRIJA, M. and JAYANTHA RAO, K. (1987) Changes in selected biochemical parameters in the kidney and blood of the fish *Tilapia mossambica* (Peters), exposed to heptachlor. Bull. Environ. Contam. Toxicol., 39 : 1006 - 1011.

RAI, R. (1987). Response of serum protein in a fresh water fish to experimental mercury poisoning J. Environ. Biol. 8 (2) 225 - 228.

RAI, R. and GAYYUM, M.A. (1985) Biochemical responses of blood to copper toxicity in a fresh water teleost, *Catla catla* (Ham). Int. J. Acad. Icthyost. Modinagar. 6 : 145 - 147.

RAM, R.N. and SATHYANESAN, A.G. (1987) Effects of long - term exposure to cythion on the reproduction of the teleost fish *Channa punctatus* (Bloch). Environ. pollu. 44 : 49 - 60.

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

RATH, S. and MISRA, B.N. (1980) Changes in nucleic acids and protein content of *Tilapia mossambica* exposed to dichlorvos (DDVP). Indian J. Fish., 27 : 76 - 81.

RAY, S. (1984) Bioaccumulation of cadmium in marine organism. Experientia, 40 : 14 - 23.

REDDY, P.M. (1987) Toxic impact of malathion on the branchial protein metabolism of freshwater fish *Cyprinus carpio* Environ. Ecol., 5 : 368 - 370.

REDDY, P.M. and BASHAMOHIDEEN, M. (1987) Biochemical changes in the kidney and intestine of freshwater fish *Cyrinus carpio* exposed to malathion. Environ. Ecol., 5 : 378 - 380.

REHWOLDT, R., GERALD, B. and NERRIE, B. (1971) Acute toxicity of copper, nickel and zinc ions to some Hudson river fish species. Bull. Enviorn. Contam. Toxicol., 6 : 445 - 448.

REICHENBACH - KILNKE, H.H. (1966) The blood components of fish with relation to parasite infections and water pollution. Bull. Off. Int. Epizool., 65 : 1039 - 1054.

ROALES, R.R. and PERLMUTTER, A. (1984) Toxicity of methyl mercury and copper applied singly or jointly to the blue gourami, *Trichogaster trichopterus*. Bull Environ. contam, toxicol. 12 : 633 - 639.

ROBERTS, R.J. (1978) The pathophysiology and systematic pathology of teleosts. In : Fish pathology (Ed.) R.J. Roberts Eailliere Tindall, London. PP : 55 - 91.

RUNNELLS, R.A., MONLUX, W.S. and MONLUX, A.W. (1965) Principles of veterinary pathology Ames, The lowa state University press.

SAAD, M.A.M., EZZAT, A.A. and SHABANA, M.B. (1973) Effect of pollution on the blood characteristics of *Tilapia zillii*. Water Air Soil Pollut., 2 : 171 - 179.

SAHIB, I.K.A., JAGNATHA RAO, K.S. and RAMANA RAO, K.V. (1981) Effect of malathion exposure on some physical parameters of whole body and on tissue cations of teleost, *Tilapia mossambica* (Peters). J. Biosci., 3 : 17 - 21.

SAHIB, I.K.A., RAO, K.R.S.S. and RAO, K.V.R. (1984) Effect of malathion on protein potentiality of the tissue of teleost, *Tilapia mossambica* (Peters), as measured through incorporation of (^{14}C) amino acids. Toxicol. Lett., 20 : 63 - 67.

SAKAGUCHI, H. and HAMAGUCHI, A. (1975) Physiological changes in the serum and hepatopancreas of yellow tail injected with carbontetrachloride, Bull. Jap. Soc. Sci. Fish., 41 : 283 - 290.

SANJAY, K., SRIVASTAVA; PUSPHA RANI., TIWARI and AJAI.K.SRIVASTA (1990) Effect of chlolorpyrifans on the kidney of freshwater catfish *Heteropneustes fossilis* Bull. Environ. Contam. Toxicol. 45 : 748 - 756.

SASTRY, K.V. and RAO, D.R. (1984) Effect of mercuric chloride on some histochemical and physiological parameters of the freshwater murrel, *Channa punctatus*. Environ. Res., 34 : 343 - 350.

SASTRY, K.V. and SIDDIQUI.A.A. (1982) Chronic toxic effects of the carbamate pesticide sevin on carbohydrate metabolism in a freshwater snakehead fish *Channa punctatus*. Toxicol. Lett., 14 : 123 - 130.

SASTRY, K.V. and SUBHADRA, K. (1985) In vivo effects of cadmium on some enzyme activities in tissue of the freshwater catfish *Heteropneustes fossilis*. Environ. Res., 36 : 32 - 45.

SASTRY, K.V. and SIDDIQUI, A.A. (1983) Metabolic changes in the snakehead fish *Channa punctatus* chronically exposed to endosulfan. Water Air Soil Pollut., 19 : 133 - 141.

SASTRY, K.V. and SUNITHA, K. (1983) Enzymological and biochemical changes produced by chromium exposure to a teleost fish *Channa punctatus*. Toxicol. Lett., 16 : 9 - 15.

SASTRY, K.V. and TYAGI, S. (1982) Toxic effects of chromium in a freshwater teleost, *Channa punctatus*. Toxicol. Lett., 11 : 17 - 21.

SATCHELL, G.H. (1984). Respiratory toxicology of fishes. Aquatic toxicology. 2 : 1 - 50.

SAXENA, O.P. (1981) Cadmium - induced neoplasis in *Channa punctatus* (Bloch) Current Sci., 50 (16) 735 - 736.

SCARANO, G., SAROGLIA, M.G., GRAY, R.H. and TIBALDI, E. (1984) Haematological response of seabass *Dicentrarchus labrax* to sublethal nitrite exposure trans. Am. Fish. Soc., 113 : 360 - 364.

SCHMIDT - NIELSON, B. (1974) Osmoregulation : Effect of salinity and heavy metals. Fed. Proc., 33 : 2137 - 2146.

SCHRECK, C.B. (1981) Stress and compensation in teleostean fishes : Response to social and physical factors. In: stress in fish (Ed. pickering, A.) Academic Press, London, P. 295 - 321.

SCHRECK, C.B. and LORZ, H.W. (1978) stress response of coho salmon (*Oncorhynchus kisutch*) elicited by cadmium and copper and potential use of cortisol as an indicator of stress. J. Fish. Res. Bd. Can., 35 : 1124 - 1129.

SCHWARTZ, A., LINDENMAYER, G.E. and ALLEN, J.C. (1975) The sodium - pottaaium adenosine triphosphate Pharmacologic, Physiological and Biological aspects. Pharamacol. Rev., 27 : 3 - 134.

SESHAGIRI RAO, K., MOORTHY, K.S. NAIDU, K.D., CHETTY, C.S. and SWAMI, K.S. (1983) Changes in nitrogen metabolism in tissue of fish (*Sarotherodon mossmbicus*) exposed to benthiocarb. Bull. Environ. Contam. Toxicol., 30 : 473 - 478.

SESHAGIRI RAO, K., MOORTHY, K.S., REDDY, B.K., SWAMI, K.S. and CHETTY, C.S. (1987) Effect of Benthiocarb on protein metabolism of freshwater teleost *Sarotherodon mossambicus*. Indian J. Environ. Health., 29 : 45 - 51.

SHAFFI, S.A. (1978 a) Cadmium intoxication on tissue glycogen content of three freshwater teleosts. Curr. Sci., 47 : 868 - 870.

SHAFFI, S.A. (1978 b) Variations in tissue glycogen content, serum lactate and glucose levels due to copper intoxication in three freshwater teleosts, Curr. Sci., 47 : 954 - 956.

SHAFFI, S.A. (1979 a) The acute toxicity of heptachlor for freshwater fishes. Toxicol. Lett., 4 : 31 - 37.

SHAFFI, S.A. (1979 b) Lead toxicity biochemical and physiological imbalance to nine freshwater teleosts. Toxicol. Lett., 4 : 155 - 161.

SHAFFI, S.A. (1980 a) The acute industrial effluent toxicity to freshwater fish. Toxicol. Lett., 5 : 183 - 190.

SHAFFI, S.A. (1980 c) Ammonia toxicity : Metabolic disorder in nine freshwater teleosts. Toxicol. Lett., 6 : 349 - 356.

SHAFFI, S.A. (1980 d) Zinc intoxication in some freshwater fishes. 1. Variations in tissue energy reserve. An. Limnology, 16 : 91 - 97.

* SHAFFI, M. and QAYYUM, M.A. (1979) Lead intoxication (effects) on tissue glycogen content in a freshwater catfish *Heteropneustes fossilis* (Bloch). Zool. Jahrb, Abt. Anat. Ontog. Tiere, 101 : 404 - 406.

* SHAKOORI, A.R., SALEEM, A.Z. and MUHAMMED, S.A. (1976) Effect of Malathion, dieldrin and endrin on blood serum proteins and free aminoacids pool of *Channa punctatus* (Bloch) Pak. J. Zool., 8 : 124 - 134.

SHARMA, D.C. and DAVIS, P.S. (1980) Effects of methyl mercury on protein synthesis in liver of the European carp *Cyprinus carpio* Indian. J. Expt. Biol., 18 : 1054 - 1055.

SHEARER, K.D. (1984) Changes in elemental composition of hatchery reared rainbow trout, *Salmo gairdneri*, associated with growth and reproduction. Can., J. Fish. Aquat. Sci., 41 : 1592 - 1600.

SHUKLA, J.P. and PANDEY., K. (1986) Zinc induced changes in the nucleic acids and protein metabolism in the fingerlings of a freshwater murrel, *Channa punctatus*. Acta hydrochim, hydrobiol., 14(2) : 195 - 197.

SIMKISS, K. (1983) Lipid solubility of heavy metals in saline solution. J. Mar. Biol. Asso. U.K., 63 : 1 - 9.

SINGH, S.R. and SINGH, B.R. (1982) Effect of copper and zinc sulphate on the blood parameters of *Mystus vittatus* (Bloch). Matsya, 8 : 1-6.

SIVA PRASADA RAO, K. and RAMANA RAO, K.V. (1979) Effect of sublethal concentration of methyl parathion on selected oxidative enzymes and organic constituents in the tissues of the freshwater *Tilapia mossambica* (Peters). Curr. Sci., 48 : 526 - 528.

SKADHAUGE, E. (1977) Excretion in lower vertebrates. Function of gut, cloaca and bladder in modifying the composition of urine. Fed. Proc. Fed. Am. Soc. Exp. Biol., 36 : 2487 - 2492.

SKIDMORE, J.F. and TOVELL, P.W. (1972) Toxic effects of zinc sulphate on the gills of rainbow trout. Water Res., 6 : 217 - 2230.

SLAUSON, D.D. and LEWIS, R.M. (1979) comparative pathology of glomerloephritis in animals. Vet. path., 16 : 35 - 164.

SLOWEY, J.F. and HOOD, D.W. (1970) Copper, manganese and zinc concentration in gulf of Mexico waters. Geochim. Cosmochim. Acta., 35 : 121 - 138.

SMEETS, J. (1980) Tests and notification of chemical substance in current legislation. Ecotoxicol. Environ. Saf., 4 : 103 - 113.

SMITH, G.L., HATTINGH, J. and BURGER, A.P. (1979) Haematological assessment of the anaesthetic MS 222 in natural and neutralized form in three fresh water fish species. Inter species differences. J. Fish., 15 : 633 - 643.

SOIVIO, A. and OIKARI, A. (1976). Haematological effects of stress on a teleost *Esox lucius* L.J. Fish Biol., 8 : 397 - 411.

SOIVIO, A. and NIKINMAA, M. (1981) The swelling of erythrocytes in relation to the oxygen affinity of the blood of the rainbow trout *Salmo gairdneri* Richardson. In : Stress and fish. (Ed. Pickering, A), Academic Press, London, P. 103 - 119.

SOMSIRI, C. (1982) Acute toxicity of mercury, copper and zinc to the Nile Tilapia. Thai - Fish. Gaz. 35 : 313 - 318.

SPEHAR, R.L. (1976) Cadmium and zinc toxicity to flag fish (*Jordenella fleridae*) J. Fish Res. oard. Can. 33 : 1939 - 1945.

SPRAGUE, J.B. (1970) Measurement of pollutant toxicity to fish II., Utilizing and applying bioassay result. Water Res., 4 : 3 - 32.

SPRAGUE, J.B. (1971) Measurement of pollutant toxicity to fish III. Sublethal effects and safe concentrations. Water Res., 5 : 245 - 266.

SPRAGUE, J.B. (1969) Measurement of pollutant toxicity to fish I. Bioassay methods for acute toxicity. Water Research Pergamon Press, 3 : 793 - 821.

SRINIVASTAVA, D.K. (1982) Comparative effects of copper, cadmium and mercury on tissue glycogen of the catfish, (*Hetropneustes fossilis*). (Bloch). Toxicol. Lett., 11 : 135 - 139.

SRIVASTHAVA, A.K. and MISHRA, J. (1983) Effect of fenthion on the blood and tissue chemistry of a teleost fish *(Hetreopneustes fossilis*). J. Comp. Pathol., 93 : 27 - 32.

SRIVASTHAVA, A.K. and SINGH, N.N. (1981) Effects of endosulfan on fish carbohydrate metabolism. Ecotoxicol. Environ. Saf., 5 : 412 - 417.

SRIVASTHAVA, A.K. and SINGH, N.N. (1982) Acute toxicity of propoxur on carbohydrate metabolism of Indian catfish (*Heteropneustes fossilis*). Toxicol. Lett., 11 : 31 - 34.

SRIVASTHAVA, O.P. and SRIVASTAVA, G.J. (1981) Urea - induced histopathology in kidney of the tropical freshwater teleost *Channa punctatus* (Bloch). Bull. Soc. Sci. Vet. Med. Comparee. 83 : 213 - 217.

STATHAM, C.N., CROFT, W.A. and LECH, J.J. (1978) uptake, distribution and effects of carbon tetrachloride in rainbow trout (*Salmo gairdneri*). Toxicol. Appl. Pharmacol., 45 : 131 - 140.

STORER, J.H. (1967) Starvation and effect of cortisol in the goldfish (*Carassius auratus L.*) Comp. Biochem. Physiol., 20 : 939 - 948.

SUKUMAR, A and KARPAGAGANAPATHY, P.R. (1986) Renal dysfunction in a freshwater fish, *Colisa lalia* after carbofuran intoxication. In : Environment and Ecotoxicology : (eds. Dalela, R.C., Sahai, Y.N. and Gupta, S. Acad. Environ. Biol. India. p : 243 - 247.

SULTAN, S. and KHAN, S.M. (1981) Histopathological studies on the liver and gills in *Carassius auratus* exposed to copper sulphate. Ind. J. Fish., 30 (1) : 96 - 98.

SVOBODOVA, Z. (1982) Changes in some haematological parameters of the carp after intoxication with $CuSo_4$. 5 H2O. Bull. Vyzk. Ustav. Ryb. Hydrobiol., 18 : 26 - 29.

TAKESHITA, G.Y. (1975) Copper and its use. Marine Aquarist., 5 : 5 - 12.

TAPTI, B., ROY A.K. and SHELLEY, B. (1985). Histopathological lesions in the hepatopancreas of *Channa punctatus* (Bloch) exposed to mixtures of mercuric chloride and phenol and a factory effluent. matsya II : 1 - 4.

TARZWELL, C.M. (1962 a) The need and value of water quality criteria with special reference to aquatic life. Can. Fish. Cult. 31, 35 - 41.

TARZWELL, C.M. (1962 b) Development of water quality criteria for aquatic life J. Wat. Pollut. Control Fed. 34, 1178 - 1185.

TARZWELL, C.M. (1966) Water quality requirements for aquatic life. In proc. nat. Symposium on water quality, Ann, Arbor, Michigan, U.S.A. pp. 185 - 197; University of Michigan, School of public Health, continued education series No : 161.

TEWARI, H., GILL, T.S. and PANT, J. (1987) Impact of chronic lead poisoning on the hematological and biochemical profiles of fish, *Barbus conchonius* (Ham). Bull. Environ. Contam. Toxicol., 38 : 748 - 752.

TORRES, P., TORT, L., PLANAS, J. and FLOS, R. (1986) Effects of confinement stress and additional zinc treatment on some blood parameters in the dogfish *Scyliorhinus canicula*. Comp. Biochem. physiol., 83 : 89 - 92.

TORT, L. and TORRES, P. (1988) The effects of sublethal concentrations of cadmium on haematological parameters in the dogfish, *Scyliorhinus canicula*. J. Fish Biol., 32 : 277 - 282.

TORT, L., TORRES, P. and FLOS, R. (1987) Effects on dogfish haematology and liver composition after acute copper exposure. Comp. Biochem. Physiol., 87 : 349 - 353.

TORT, L., TORRES, P. and HIDALGO, J. (1984) Short - term cadmium effects on gill tissue metabolism. Mar. pollut. Bull., 15 : 448 - 450.

UMMINGER, B.L. (1970) Physiological studies on supercooled killifish *Fundulus heteroclitus* III Carbohydrate metabolism and survival at subzero temperature. J. Exp. Zool., 173 : 159 - 174.

VAN DEN THILLART, G. (1982) Adaptations of fish energy metabolism to hypoxia and anoxia. Molec. Physiol., 2 : 49 - 61.

VAN WAARDE, A. (1988). Biochemistry of non - protein nitrogenous structural alterations in fish epidermal mucus produced by water drain lead and mercury. Nature London. 258 : 431 - 432.

VARICHAK, T. (1938) Studies on endothelial cells in the liver of fishes *Z. Zellforsch.* mirkrosk. Anat., 27 : 46 - 51.

VENKTACHARI, S.A.T. (1974) Effects of salinity adaptation on the nitrogen metabolism in freshwater fish *Tilapia mossambica* I. Tissue protein and aminoacid level. Mar. Biol., 24 : 57 - 63.

VERMA, S.R. and TONK, I.P. (1983) Effects of sublethal concentrations of mercury on the composition of liver muscle and ovary of *Notopterus notopterus*. Water Air Soil Pollut., 20 : 287 - 292.

VERMA, S.R. RANI, S. and DALELA, R.C. (1981 c) Effects of phenolic compounds in vivo blood parameters of a fish *Notopterus notopterus*. J. Environ. Sci. Health Party-B, 16 : 273 - 282.

VIARENGO, A. (1985) Biochemical effects of Trace metals. Mar. Pollut. Bull., 16 : 153 - 158.

VIARENGO, A., PERTICA, M., MANCINELLI, G., CAPELLI, R. and ORUNESU, M. (1980) Effects of copper on the uptake of amino acids on protein synthesis and on ATP content in different tissues of *Mytilus galloprovincialis* lam. Mar. Environ. Res., 4 : 745 - 752.

VIARENGO, A. (1985) Biochemical effects of trace metals. Mar. Pollut. Bull. 16 (4) : 153 - 158.

VOGEI, S.F. (1959) The deposition of exogenous copper under experimental conditions with observations on its neurotoxic and nephhrotoxic properties in relation to Wilson's disease. J. Exp. Med., 110 : 801 - 809.

WAGNER, G.F. and MCKEOWN, B.A. (1982) Changes in plasma insulin in carbohydrate metabolism of zinc - stressed rainbow trout *Salmo gairdneri*. Can. J. Zool, 60 : 2079 - 2084.

WAIWOOD, K.G. and BEAMISH, F.W.H. (1978) Effects of copper, pH and hardness on the critical swimming performance of rainbow trout (*Salmo gairdneri* Richardson) Water Res., 12 : 611 - 619.

WEDEMEYER, G. (1971) The stress of formalin treatments in rainbow trout (*Salmo gairdneri*) and coho salmon (*Oncorhynchus kisutch*). J. Fish. Res. Bd. Can., 28 : 1899 - 1904.

WEDEMEYER, G. (1973) Some physiological aspects of sublethal heat stress in the juvenile steelhead trout (*Salmo gairdneri*) and coho salmon (*Oncorhynchus kisutch*). J. Fish. Res. Bd. Can., 30 : 831 - 834.

WEDEMEYER, G. (1972) Some physiological consequences of handling stress in the juvenile coho salmon and steelhead trout. J.Fish. Res. Board Can., 29 : 1780 - 1783.

WESTER, P.W. and CANTON, J.H. (1986) Histopathological study of *Oryzias latipes* (Medaka) after long term hexachlorocyclohexane exposure. Aquat. Toxicol., 9 : 21 - 45.

WESTER, P.W. and CANTON, J.H. (1987) Histopathological study of *poecilia reticulata* (guppy) after long - term exposure to bis (tri - n - butyltin) oxide (TBTO) and di - n butyltindichloride (DBTC). Aquat. Toxicol., 10 : 143 - 165.

WOBESER, G. (1975) Acute toxicity of methyl mercury chloride and mercuric chloride for rainbow trout (*Salmo gairdneri*) fry and fingerlings. J. Fish Res. Bd. Can., 32 : 2005 - 2013.

WOTTEN, R. and WILLIAMS, H.A. (1980) Some effects of therapeutic with copper sulphate and formalin in rainbow trout. (*Salmo gairdneri*). Presented at F.S.B.I. symposium "Stress in Fish" University of East Anglia, Norwich.

YAMAWAKI, K., HASHIMOTO, W., FUJII, K., KOYAMA, J., IKEDA, Y. and OZAKI, H. (1986) Hemochemical changes in carp exposed to cadmium concentrations. Bull. Jap. Soci. Fish., 52 : 459 - 466.

YOUNG, J.E. and CHAVIN, W. (1965) Effects of glucose, epinephrin or glucagon upon serum glucose levels in the goldfish *Carassius carassius* L. Am. Zool., 5 : 688 - 689.

ZEITOUN, I.H. (1977) The effect of chlorine toxicity on certain blood parameters of adult rainbow trout (*Salmo gairdneri*). Environ. Biol. Fishes. 1 : 189 - 195.