# Short-term Toxicity of Mercury in Relation to Water Hardness and Temperature to *Poecilia reticulata* (Peters) and Selected Indian Major Carp

# जलीय कठोरता एवं तापमान के सम्बन्ध में पारद की *पोयसिलिया रेटीकुलेटा* (पीटर्स) एवं चयनित भारतीय शफ़र मीन पर अंशकालिक विषाक्तता

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# Chapter - 1

# INTRODUCTION

# 1. INTRODUCTION

Water is a vital resource for the sustenance of life in general and aquatic life in particular. This stresses the need for conserving small fraction of water available for various human uses. Despite of the fact that freshwater is adequate however, its temporal and spatial distribution has been a matter of concern to the mankind. In view of this reason year 2003 has been declared as the year of freshwater by United Nation and Government of India as such.

Surface water in the form of lakes, reservoirs and such other water bodies are indeed life support systems. These are repository of valuable genetic material in the form of various types of aquatic flora and fauna. No life is possible without water. Out of total available water on earth, only three per cent is fresh water, and a small part of this is accessible for human consumption. This relatively small amount of fresh water that is available, is often threatened by pollution and a risk for use.

The term **'pollution'** broadly refers to any change in the natural quality of environment brought about by chemical, physical or biological factors. Environment pollution is unfavourable alteration of our surroundings due to direct or indirect activities of man. Various by-products of men's action are the pollutants, which increase with the rise in population.

Industrialisation and increase in the human population of large cities results in the rivers becoming the drains of waste material. Domestic wastes, sewage and industrial effluents are generally allowed to be added to the rivers without any pre-treatment, causing pollution of water. The waste products of some of the industries are extremely poisonous to the fish life, and cause depletion of fish population by the adverse changes in the physical, chemical, and biological properties of water. In addition to the industrial and domestic wastes, a large number of agricultural pesticides, insecticides and others have further increased the hazards of pollution of water.

In the present day living system the pollution is the major environmental problem associated with urbanization. The present day researches on environmental aspects are an alarm for human beings disclosing the hazardous effects of several

pollutants (insecticides, pesticides, weedicides, industrial effluents and heavy metals *etc.*) and also other chemicals. Pollution menance has gravely effected human race by way of pollutants generated from domestic sewage, agricultural pesticides, industrial wastes, the radioactive wastes, mining, refining and wastes caused by modern technological advances. **Water pollution** has been defined as contamination of water or such alteration of the physical, chemical or biological properties of water or discharges of any sewage or trade effluent or any other liquid, gaseous or solid substances into water that is likely to create a nuisance by way of physical appearance, odour, taste or render such water harmful and injurious to public health for the purpose of domestic, commercial, industrial, agricultural, aquaculture, or other legitimate uses or to health of animals and aquatic life's environment.

The environment has manifold infestation due to contaminants especially by the chemicals. The direct and indirect induced changes in the one or more components of ecosystem which are harmful or undesirable termed as '**pollutants**'. This created unnatural environment not only for men but also for his basic associates such as the fish, which serves as a high protein food for population, both poor and rich. The pollutants have entered in the environment through aimless disposal of wastes and consequently reaches the organisms either directly or through "food chain". The fish for the purpose, is the best experimental organisms to assess the quality of damages, which are likely to be transferred to population through its consumption as food, and hence needs careful surveillance.

A large number of chemicals and other complexes are produced by man and many of them reaches to the aquatic environment either directly or indirectly. The human civilization through ages have known that the water has great capacity to dissolve the chemicals and purify itself and perhaps the basis for disposal of sewage and discharges.

Excess of everything is bad. So it is a well known fact that essential nutrients are also toxic at high concentration and same may be essential nutrients when provided in low quantities. Metal salts are useful as therapeutic agents, whereas heavy metals are common pollutants to water bodies. The seriousness of heavy metal's contamination rests on the fact that they are generally water soluble, non-degradable, vigorous oxidizing agents and are strongly bonded to many biochemical

constituents inhibiting their function. Heavy metals are present in all phases of the environment – air, water and land. They are transmitted by direct ingestion through the food chain to higher organisms and ultimately to human beings.

The introduction of heavy metals into natural waters has shown to induce changes in the internal dynamics of aquatic ecosystem even in lesser concentrations. The heavy metal pollutants on entering into fish, effectively bring about changes in numerous bio-chemical and cytoenzymological reactions and modify the enzymatic system in fish, consequently bringing a variety of changes in metabolic activities which could cause even death.

Heavy metals constitute a heterogeneous group of elements. The periodic table and their form of electron configuration denote that a number of elements possess partially filled 'd' and 'f' shells (Cu, Cd, Hg, Pb, Zn, Mn, Ar, Fe *etc*). Such elements are known as heavy metals. Heavy metals may be categorised into two groups on the basis of their role played in animals. The first group consists of essential micronutrients like manganese, iron, cobalt, copper and zinc while the second group includes those metals which are not listed as micronutrients in the body such as mercury, cadmium, arsenic and lead *etc.*

Mercury is a silver white liquid metal solidifying at – 38.9°C, for a thin white ductile, malleable mass. It boils at 350.9°C, has a specific gravity of 13.6 and a vapour pressure of $1.2 \times 10^{-3}$ mm of mercury. It has three oxidation states (1) zero (elemental mercury), (2) +1 (mercurous compounds) and (3) +2 mercuric compounds. Mercury is widely distributed in the environment and biologically is a non-essential or non-beneficial element. Historically it was recognized to possess a high toxic potential and was a germicidal or fungicidal for medical and agricultural process. In the recent years mercury has been recognized as a toxic contaminant in the environment. Toxicity of mercury is related to its chemical forms. Liquid mercury appears to have little effect but mercury vapour is readily absorbed producing brain damage. Mercury I salts are relatively toxic as compared to mercury II salts, because of their low solubility. Mercury present in fish occurs almost entirely as methyl mercury. The WHO recommends a maximum daily intake of mercury by

humans from all sources of 43 $\mu g\ day^{-1}$ of which no more than 29 $\mu g\ day^{-1}$ should be methyl mercury. In lakes and streams mercury can be collected in the bottom deposits where it may retain for long periods of time.

Mercury is a dangerous pollutant among heavy metals. It is discharged in inorganic form from mercury and other metal mines. Man made sources of mercury include mining, refining, paper and pulp industry, acetylene, acetaldehyde synthesis, vinyl chloride synthesis, caustic soda industries using mercury cell, organo mercuric fungicides and seed disinfactants mercury electric appliances industries, phosphate rock process *etc.*

The study of gold site in the Migori Gold belt, Kenya, revealed that the concentrations of heavy metals mainly Hg, Pb and As are above acceptable level. The amount of mercury used by miners for gold amalgamation during peak mining periods varies from 150-200 kg per month. Mercury ions as a result of these industrial processes are generally discharged in the aquatic environment and become a major problem because of their toxicity, their persistent and tendency to accumulate in a aquatic organisms including fish and undergo food-chain amplification. In industrial effluents, bottom sediments metabolically convert it to methyl mercury, a highly persistent pollutant in the foodchain.

Mercury can be a part of both organic and inorganic compounds. A mercury discharged into lake, bays, rivers or estuaries as elemental mercury, inorganic divalent mercury, phenyl mercury or aloxalkyl mercury can be converted into methyl mercury compounds by natural processes. Methyl mercury is the most hazardous due to its high stability, its lipid solubility and also its possession of ionic properties that lead to a high ability to penetrate membranes in living organisms. There are certain organisms which are able to convert inorganic and organic forms of mercury into highly toxic methyl mercury ($CH_3\ H_g$) or dimethyl mercury ($CH_3\ H_g\ CH_3$) has made it clear that any form of mercury is highly hazardous to the environment.

The synthesis of methyl mercury by bacteria from inorganic mercury compounds present in water or sediment is the source of this molecule in aquatic environments. These processes can occur under both aerobic and anaerobic conditions but prefers to anaerobic condition. Since mercury is soluble it is readily

incorporated into organisms particularly in the aquatic environment and ultimately finds its way into higher trophic levels in the food-chain. Methyl mercury is particularly toxic to animals because it can readily pass the blood brain barrier causing injury to the cerebellum and cortex. The clinical symptoms of this damage are numbness, weakness in muscles, loss of vision, impairment of cerebral cortex resulting in coma and death.

Because of the non-degradable property of mercury, their toxicity, persistence and tendency to accumulate in aquatic organisms is long lasting. Like many environmental contaminants mercury undergoes bioaccumulation, where organisms can takeup contaminant more rapidly than their body can eliminate them thus the amount of mercury in their body accumulates over time. Biomagnification occurs when concentration of a material increases between two more trophic levels. It is a subset of trophic transfer in which the dietary transfer and accumulation of the contaminant is sufficient to increase contaminant concentrations at higher trophic levels. Mercury undergoes biotransformation and bioconcentration during its transfer through food chain.

Environmental pollution by heavy metals become widely recognized with the 'Minamata' disaster in Japan, between 1953 to 1960, when several thousands of people suffered mercury poisoning from eating fish caught in Minamata Bay which was receiving effluents containing mercury released from a vinyl chloride plant. Thereafter, the death of 450 Iraqui villagers in 1972 after eating the grains that was dusted with mercury containing pesticides. It lead to continuous approach and imposing strict water quality monitoring with respect to mercury contamination. There were similar cases reported in Pakistan, 1969; Guatemala, 1963, and Mexico, 1989. In 1970, the Norwegians found high mercury concentration in the fish from lake Saint-Clair. In Sweden, where poisoning of game birds and other wild life apparently by mercury treated seeds began to be noticed in 1960. The Swedish Medical Board in 1967 banned the sale of fish from about 40 lakes and rivers after it was found that fish caught in these waters contained high concentration of methyl mercury. This situation of mercury has assumed in such a sinister shape even mother's milk and eggs contain mercury.

The biochemical basis for the toxicological effects of mercury, both in its inorganic and organic forms, as well as a role in normal metabolism, if any, are probably dependent not only on dose but also on its interaction, inter alia, with thiol, selenide, phosphate, amino and carboxyl groups of such cellular components as amino acids, proteins, enzymes, nucleic acids and lipids.

Extra- and intracellular proteins, nucleic acids, membranes, mitochondria, mitotic apparatus *etc.* contain numerous ligands for mercury, including – SH groups. As a consequence, addition of mercury to these systems has resulted in derangements of their function. The multiplicity of variables involved in the in vitro intraction of mercury and its derivatives with ligands of amino acids, proteins, nucleic acids, and phospholipids also pertain to interactions of mercurials with sub-cellular and cellular components. However, the biological effects on cells and their components are more complex since additional variables such as, for example, the capability of mercurial to cross the cell membrances, distribute in cellular compartments, to bind to ligands whose availability may be altered by cellular metabolism *etc.* must be considered. Such considerations have direct bearing on the different effects of inorganic and organic mercury on the same biological system. Thus, while all mercury compounds are cytotoxic to cells in culture, the organic mercurials are more effective than inorganic ones. Thus, any biological effects of mercurials must be interpreted in terms of chemical state of mercury and its distribution, as well as the metabolism and composition of the cells involved.

Murcurials have significant effects on over-all cellular processes. Thus the alkyl mercury compounds decrease glucose transport across membranes decrease phosphate transport in the myocardial membrane, Na-K-ATPase and water permeability of red-cell membranes. In Addition, mercurials increase the lag in ATP-driven reduction of NAD by succinate in phosphorylating sub-mitochondrial particles from beef heart, while dithiothreitol eliminates the lag. In addition, accumulation of inorganic mercury in liver-cell lysosomes causes release of hydrolytic enzymes, a response that probably accounts for the resultant cellular toxicity. Mercury compounds produce chromosomal abnormalities and induce genetic and teratogenic effects.

The mercury based chemicals used in agriculture, industries, forestry, mining and related fields ultimately enters the water bodies and have fatal effects on fish fry and eggs, destroy spawning grounds and feeding areas, restrict migration, impairment of growth and reproduction. Mercury pollution also reduce resistance of fish to diseases and deteriorate quality of fish. This polluted water can affects fisheries by killing fish fauna, the food of fishes or making the fish unfit for human consumption. Pollutant like mercury remain in flesh of fish for a long time and transferred to human by way of food, causes harmful effects.

Variation in metabolic function in organism or changes in the physical and chemical properties of the ambient medium that indirectly affects the resident bioata in and around the water. Assessment of toxicity in aquatic ecosystem is conventionally done through testing procedure using fish as a test organisms. Fish have been a popular and useful test organism in aquatic toxicological studies with the logic, that if fish life is protected, the rest of the aquatic food chain is protected as well.

Mercury has high affinity for sulphur atom and easily attaches itself to the sulphur containing aminoacids of proteins. It also forms bond with haemoglobin and serum albumin, both of which contain sulphydral groups. Fresh fish flesh providesan excellent source of protein for human diet. This protein is relatively of high digestibility, biological and growth promotion values for human. Fishes are commercially important and serve as a high protein for the population both poor and rich.

Fishes are the most sensitive group among the endangered aquatic fauna in mercury toxication. Studies have also been also done on acute toxicity as well as physiological and biochemical effects on fishes. However, little information is available on toxic effects of mercury and its derivatives on offsprings, male and female sexes of fish. In view of this an attempt has been made to investigate the short-term (96 h) toxic effect of mercury in relation to water hardness and temperature to a freshwater fish, *Poecilia reticulata* (Peters) for collective (mixed population), sexwise (male and female) and young. The toxicity tests with mercury have also been performed on the fingerlings of selected commercially important Indian major carps. ie. *Catla catla*, *Labeo rohita* and *Cirrhinus mrigala*.

Various parameters such as median lethal concentration (LC50), 95% confidence limits and presumable harmless concentrations were computed from the toxicity test. Correlation coefficient (r) have also applied between different factors. The pathological changes at cellular level in relation to short-term toxicity at sub lethal concentration of mercury have also been studied in selected tissues of fingerlings of *Cirrhinus mrigala* (Ham.). Changes if any in behaviour and morphology of the test fishes with mercury toxicity during the short-term study have also been observed.

The main purpose of short-term bioassay tests with fish is to answer one or more of the following questions;

- Is it toxic?
- Toxic in What way?
- Does it vary in toxicity?
- Which fraction is more toxic?
- Is available dilution sufficient to protect fish?
- How effective are treatment methods in reducing toxicity?

The fish species, *Poecilia reticulata* (Peters) is selected for the present study considering many factors such as–they are easily cultured in the laboratory conditions, presence of sexual dimorphism, i.e. males and females are easily identified, and they are prolific ovo-viviparous, i.e. young are born alive and matures rapidly. Whereas *Catla catla* (Ham.), *Labeo rohita* (Ham.) and *Cirrhinus mrigala* (Ham.) are Indian major carps and have immense commercial importance in India and abroad.

**Objectives:**

(i) To study the short-term toxicity of mercury to a freshwater fish, *Poecilia reticulata* for collective (mixed population) and sexwise (male & female) in relation to water hardness and temperature.

(ii) To find out the toxicity of mercury to juveniles of guppies in relation to water hardness and temperature.

(iii) To study the short-term toxicity for selected fingerlings of commercially important Indian major carps.

(iv) To study the behavioural and pathological changes in Indian major carp.

# Chapter - 2

# REVIEW OF LITERATURE

# 2. REVIEW OF LITERATURE

The effects of mercury and its derivatives on man deserve special consideration because these were responsible to cause adverse effect of heavy metals released into the environment in large amounts. The first serious incident came to light occurred at Minamata Bay in Japan (1953). In this case, comparatively non-toxic inorganic mercury alongwith some methyl mercury was released in effluent by a chemical factory using mercuric sulphate catalysts in acetaldehyde production. The effluent entered a river running into Minamata Bay. In the sediments, the inorganic mercury was converted to methyl mercury. The accumulated mercury in shellfish and fish used to be consumed by the local inhabitant. Consequently by 1975, 116 people had died and many were left paralysed for life. Others suffered impairment of vision and hearing and other neurological symptoms. Prenatal poisoning of the foetus was observed even in the absence of symptoms in the mother. Since the Minamata Bay incident, another incidence occurred around the Agana River, Niigata, Japan. This led to 23 deaths. In both these cases, many domestic animals, fish, shellfish and seabirds were also affected.

In any aquatic ecosystem, fishes play an important role in the food chain. The effects of mercury toxicity on the biology of different fish species have been well documented. Hameed (1995) has investigated the maximum accumulation of mercury in liver followed by gill, brain, and muscle in the fish *Lethrinus nebulosus* (Forskal). Whereas, Mathieson and MeLusky (1995) have studied the accumulation of mercury contents in eel pout (*Zoarces viviparous*), pogge (*Agonus cataphractus*), dab (*Limanda limanda*), plaice (*Pleuronectes platessa*) and long rough dab (*Hippoglossides platessoides*). The authors (op. cit.) have reported that mercury concentrations were increased with length in pogge, eel pout and dab, whereas, mercury concentrations were decreased with length in plaice but showed no significant change with length in long rough dab. However, Pinkney *et al.* (1997) observed that similar size large mouth bass from both Deep Creek lake and the Piney Creek reservoir had 1.5-2.0 times more mercury than large mouth bass in ponds in the Maryland coastal plain. Further, Govindasamy *et al.* (1999) studied the availability of

heavy metals in the aquatic ecosystem and its impact on flora and fauna. Dutt *et al.* (2000) investigated the accumulation of mercury ranging from 0.68 to 4.36 ppm in liver, 3.28 to 4.41 ppm in Kidney, 36.78 to 49.22 ppm in gills and 0.47 to 0.86 ppm in intestine of *Puntius sophore*.

In a study Gilmour and Riedel (2000) surveyed the mercury concentrations in some freshwater fishes in Maryland. These authors (op. cit.) reported that mercury concentrations in such fishes were exceeded the limit (1.0 $\mu g\ g^{-1}$) as recommended by U.S. Food and Drug Administration (FDA) consumption advisory committee. Simon and Boudou (2001) reported that mercury organotropism in case of cray fish, *Astacus astacus* was specifically connected to the exposure conditions, especially at the biological barrier level according to the route of exposure: gill and carapace for the direct route and digestive tract including hepatopancreas for the trophic route with mercury contamination. Castro *et al.* (2002) noticed that regardless of size, all of the blue gill and yellow perch examined did not exceed the consumption advisory of 0.5 $\mu g\ g^{-1}$. But in contrast large mouth bass greater than 38 cm in the Piney Creek reservoir and Deep Creek lake had total mercury concentrations greater than 0.5 $\mu g\ g^{-1}$. Misra *et al.* (2002) have investigated the mercury concentrations in grass carp fish of upper lake were less than 0.6 $\mu g\ g^{-1}$ dry weight. According to them (op.cit.) the range of mercury was found in between 0.14 to 0.31 $\mu g\ g^{-1}$ in head portion, 0.034 to 0.085 $\mu g\ g^{-1}$ in abdomen portion, 0.001 to 0.321 $\mu g\ g^{-1}$ in tail portion and 0.263 to 0.563 $\mu g\ g^{-1}$ in liver of grass carp. Whereas, Michael *et al* (2002) studied the fish tissue quality near coastal areas of Gulf of Maxico. They (op.cit.) found that trace metal residues including mercury were statistically similar regardless of the collection site.

The metal pollutants on entering into fish, effectively bringing a variety of changes in metabolic activities which could cause even death. The effects of mercury at cellular level particularly in gills, liver, kidney and other vital organs of the certain fish species have also been studied. Simon (1953) reported that mercury compounds are detrimental to aquatic life including fishes effecting their enzyme system and architectural structure of their organs. Whereas, Wolf and Quimby (1962) studied the metal protective function of GSH in fish by investigating the importance of cellular GSH status for the cytotoxic response of the established fish cell line, RTG to six

divalent metal cations, ie. Hg, Cu, Cd, Zn, Pb and Ni. However, Jackim *et al.* (1970) observed a marked reduction in hepatic and alkaline phosphatases in Killifish after exposure to copper and mercury. Further, Hinton *et al.* (1973) and Kshirsagar (1975) have reported the inhibition in hepatic alkaline phosphatase activity by mercury poisoning in fishes. Hinton *et al* (1973) have also noticed decrease in acid and alkaline phosphatase activity in liver of channel catfish after methyl mercuric chloride poisoning.

Mukherjee and Bhattacharya (1975) studied the histopathological lesions in the hepatopancreas of *C. punctatus* and *C. batrachus* exposed to Hg, Cl, Cd, Phenol and factory effluent. Whereas, Kendall (1977) reported that in channel catfish (*Ictalurus punctatus*) a single peritoneal injection of 15 mg/kg of methyl mercury chloride cause deposition of mercury in the liver, necrosis of exocrine pancreatic and paranchyma cells, and inflammation at the hepatic capsular surface. However, Sastry and Gupta (1978) investigated the histopathological alteration in liver of *channa punctatus* after acute and chronic mercury toxicity. These authors (op.cit.) further reported that damage in liver was more severe in acute mercury toxicity. Khangarot and Somani (1980) have exposed fish *Puntius sophore* to high levels of dissolved inorganic mercury or methyl mercury (Me Hg) and suggested that mercury ions severely damage the gill and thus interferes the physiology of gas exchange and ion regulation.

Sharma and Davis (1980) stated that there is decrease in protein synthesis when fish was exposed to Me Hg. Further, Syversen (1981) has also reported that heavy metals in general interfere with protein synthesis. Saxena and Parashar (1983) studied the toxicity of six heavy metals, *viz.* Hg, Cd, Pb, Cr, Zn and Ni to *Channa punctatus* and found that Hg is the most toxic amongst all these metals. Verma and Tonk (1983) investigated the effect of a sublethal concentration of mercury on the proximate composition of liver muscle and ovary of *Notopterus notopterus*. These authors (op.cit.) found that protein metabolism was altered in *Notopterus notopterus* exposed to the mercury poisoning. Bhattacharya *et al.* (1984) reported a biphasic response of mercury, where the experiment was conducted for 30 days with a dose of 110 ppb of mercury on *Channa punctatus*. The hepatopathy in the *Channa punctatus* was noticed on Ist and 30th day of treatment, while on the 2nd and 15th day of

treatment, there was little degeneration. James *et al.* (1992) have also reported alteration of protein metabolism in a cat fish, *Heteropneustes fossilis* exposed to mixture of mercury and copper. Whereas, Stagg *et al.* (1992) have observed a significant correlation between gill $Na^+$, $K^+$-ATPase activity and muscle Hg concentration in flounder (*Platicthes flesus*) collected at polluted and clean sites in estuaries, and suggested that exposure to environmentally realistic Hg levels may affect ion regulation in estuarine fish.

However, Bose *et al.* (1993) studied time dependent distribution of [$^{203}$Hg] mercuric nitrate in the sub cellular fraction of rat and fish. These workers (op.cit.) have noticed various physiological and biochemical changes in these animals exposed to inorganic mercury. Further, Mustafa (1995) investigated the effects of mercury, chromium and nickel on some blood parameters in the carp, *Cyprinus carpio* and found that the blood hematocrit values were significantly increased after mercury exposure but levels of serum proteins were not significantly altered by any of the metal exposure. Gupta and Rajbanshi (1995) investigated significant changes in the gill architecture of *Rasbora daniconius* exposed to sub lethal concentration of 0.05 mg/l of mercury as $HgCl_2$ from 96 h static bioassay. The surface architecture of gill revealed severe changes such as: damage, fusion and clumping in the middle and distal parts of the primary lamellae; and swollen, deterioration and modification of arborizing ridges into more expanded surface area in the secondary lamellae. Gupta and Rajbanshi (op.cit.) have also discussed these changes in relation to mercury toxicity and respiratory physiology responsible for fish death. Prasad *et al.* (1995) also studied the histopathological alteration in the airbreathing organs of a catfish, *Heteropneustes fossilis* exposed to mercuric chloride and found that the epithelium of air breathing organ appeared to be less sensitive to the metal solutions as compared to gills. These authors (op. cit.) have also recorded noticeable effects such as shrinkage of epithelial layer, deposition of mucus in non-vascular areas of the suprabranchial cavity and the air sac, and dilated mucus cell opening in the distal portion of the air sac. Rana *et al* (1995) have noticed that mercury induces lipid per oxidation in the liver, kidney, brain and gill of a freshwater fish *Channa punctatus*. These workers (op. cit) further observed that the short exposures resulted in increased glutathion (GSH), but longer exposure reduced in all the tissues.

Ribeiro *et al.* (1995) evaluated the acute effects of $HgCl_2$ on epidermis of *Trichomyceterus brasiliensis* (Siluroidei : Trichomycteridae), when cell exposed to $HgCl_2$, different morphological alterations were observed in the epithelium structure, such as an increase in the lymphocytes number, hypertrophical epithelial cells at the surface, modified taste buds, obstruition of the goblet cells pore and high cellular proliferation. Moreover, the chemical nature of the goblet cells was not modified by the presence of $HgCl_2$ dissolved in water. These authors (op.cit.) also observed that all animals died within 24h after the contamination with inorganic mercury at concentration of 0.2 to 0.1 mg $HgCl_2$/l. Whereas, Shaffi (1995) has recorded the effect of mercury and lead for 24h and 48h on monoamine oxidase (MAO) activity in different regions of the brain telencephalon, cerebellum, diencephalon and medulla oblongata in *Labeo rohita* (Ham.), *Clarias batrachus* (L) and *Channa punctatus* (bloch.). However, Allen (1996) has noticed that different concentrations of mercury caused significant increase in hepatic GSH of *Oreochromis aureus* (Steindachner) exposed to mercury, cadmium and lead. Further, Mustafa (1996) reported that mercury caused highest depletion of glycogen in tissues (upto 96%) of *Cyprinus carpio* exposed to mercury, chromium and nickel.

Jagoe *et al.* (1996) investigated that the fish exposed to high concentrations of dissolved mercury (Hg) causes gill pathologies and interferes in ion and osmoregulation physiology. Gautam and Parihar (1996) have recorded the toxic effects of lead nitrate ($Pb\ No_3$) and mercuric nitrate ($Hg\ No_3$) on the activity of few lipids like phospholipids and neutral lipids in the liver and kidney of *Heteropneustes fossilis* and suggested that these two heavy metals interferes the protein and lipid metabolism in hepatic and nephric tissues. Ribeiro *et al.* (1996) further studied the lethal effects of inorganic mercury on cells and tissues of *Trichomyceterus brasiliensis* (Pisces : Siluroidei). Whereas, Banerjee and Bhattacharya (1997) evaluated the histopathological changes induced by chronic nonlethal levels of elsan, mercury and ammonia in the liver of *Channa punctatus* (Bloch). Mccrary and Heagler (1997) have studied on the use of simultaneous multiple species acute toxicity test to mosquito fish, *Gambusia affinis* to compare the relative sensitivities of aquatic organisms to mercury. Whereas Mondal *et al.* (1997) found that inorganic mercury binding to fish oocytes plasma membranes, induces steroidogenesis and translatable messenger RNA synthesis. Viarengo *et al.* (1997) investigated the *in vitro* effects of $Cu^{2+}$, $Hg^{2+}$ and

$CH_3Hg^+$ on the fish liver microsomal EROD activity. However, Devlin and Clary (1998) have studied on the *in vitro* toxicity of methyl mercury to fat head minnow cells, considering various parameters such as uptake, cell survival, cell morphology, total protein and induction of 70 K Da proteins. Low and Sin (1998) have suggested that the fish immunity was impaired by the action of mercury and selenium. However, *in vitro* lymphocytes proliferation tests show that mercury concentration lower than 0.045 mg/l $Hg^{2+}$ enhance the mitotic rate of kidney lymphocytes by approximately 30%. Further Sivaramakrishna and Radhakrishnaiah (1998) reported the impact of sublethal concentration of mercury on nitrogen metabolism in a fresh water fish, *Cyprinus carpio*. Whereas, Karuppasamy (1999) evaluated the effect of phenyl mercuric acetate (PMA) on the physiology, biochemistry and histology of selected organs of a freshwater fish, *Channa punctatus*. Dutt *et al.* (2000) worked on the accumulation of mercury (Hg) and metabolic disorder as changes in phosphatases (acid phosphatase, alkaline phosphatase and G-6-phosphatase) activity in different tissues of a freshwater fish, *Puntius sophore* induced of $1/5^{th}$ fraction of 96h LC50 of $HgCl_2$ and $CH_3HgCl$ after exposure of 5, 10 and 15 days. Mat-Jais and Mohamed (2000) studied the *in vitro* inhibition of acetylcholinesterase activity in *C. striatus* brain tissue by mercury; Hg, Cd, Pb, Ni and Zn; and the role of extra cellular calcium. Further Karuppasamy (2000) studied the protein and total amino acid content in liver, muscle, intestine, kidney, gill and brain of *Channa punctatus*, intoxicant with high (0.106 ppm) and low (0.040 ppm) concentration of phenyle mercuric acetate (PMA) during short (24, 72 and 120h) and long (10, 20 and 30 days) term exposure respectively.

Khadiga *et al.* (2002) reported that the *Oreochromis niloticus* is an endemic species in the river Nile and represents the main object of fishery in the Nile delta lake of which lake Maryut is the smallest and most polluted. These authors (op.cit.) found that the water concentration having ammonia, manganese, nickel, cadmium, lead and mercury proved hazardous to fish, whereas chromium, copper, iron, zinc, pH, alkalinity, hardness, phosphate, nitrate and nitrite always fall within acceptable levels. Physiologic evaluation of *O. niloticus* pointed out the improper growth, protein inadequacy and functional impairment in fish inhabitating the polluted area. Bogdan *et al* (2002) investigated the effect of mercury on the lipid composition of liver and muscles in the perch, *Perca fluviatilis*.

Large scale of mortality of spawn, fry and fingerlings in most of the water bodies has been reported due to discharge of metallic ions including mercury in the form of effluents from mining, paper industry and other electric appliances industries. Margaret *et al.* (1977) studied the physiological response of juvenile striped bass *Morone saxatilis* to low levels of cadmium and mercury. Whereas, Nelson *et al.* (1977) reported that survival of juvenile of bay scallops, *Argopectin irradians* was significantly affected by mercury concentration and by salinity, as well as by the interaction between temperature and salinity. Further, Bleau *et al.* (1996) exposed the juvenile of rainbow trout (*Oncorhynchus mykiss*) to mercury chloride – $HgCl_2$ (28 and 112 µg/l $Hg^{2+}$), and to methyl mercury chloride – $CH_3HgCl$ (6, 12 and 24 µg/l Hg) for 4, 72 and 168 h, to determine the effects of sublethal doses of these compounds on the hypothalamo – pituitary-inter renal and the hypothalamo – pituitary – thyroidaxes. However, Friedmann *et al.* (1996) reported that the mercury suppressed the plasma cortisol in juveniles (sex combined). These studies (op. cit.) also suggests that dietary methyl mercury might reduce juvenile survival by impairing growth and immune function. Buhl (1997) investigated the acute toxicity of four metal pollutants to larval and juvenile stages of endangered Colorado squafish (*Ptychocheilus lucius*), bony tail (*Gila elegaus*) and rozor back sucker (*Xyrauchen texanus*). According to him (op. cit.) the rank order of toxicity (96h LC50) of the metals to all species and life stages from most toxic to least toxic was : Mercury (57-168 µg/l) > cadmium (78 – 168 µg/l) > hexavalent chromium (32000 – 123000 µg/l) > lead (> 170000 µg/l). Zhou *et al.* (1998) reported that when larvae of *Fundulus heteroclitus* were raised in a solution of MeHg with or without embryonic pre-exposure, the Hg content in larvae was positively related to exposure concentration and exposure time.

Experiments conducted during the recent years have shown that besides insecticides, fungicides and fertilizers, heavy metallic ions including mercury are also adversely affects the reproduction and fertility performance, of certain fish species. Jagoe *et al.* (1996) studied the gill $Na^+$, $K^+$ - ATPase activity in different sexes of large mouth bass (*Micropterus salmoides*) from three reservoirs with different levels of mercury contamination. Reddy *et al.* (1997) reported that *in vivo* mercury significantly inhibit the ovarian maturation in the red swamp cray fish, *Procambarus clarkii*. These workers (op.cit.) further suggested that the cadmium and mercury may exert inhibitory effects on the fish species by directly inhibiting protein synthesis in

the ovaries, inhibiting 5-Hydroxytryptamine, stimulating GSH release and preventing the ovaries from responding to this hormone. Zarski *et al.* (1997) investigated the mercury distribution in the reproductive organs and muscles of breams and carps in different contaminated waters. Walter *et al.* (1998) revealed that there was no statistical significance ($p > 0.05$) when attempting to correlate mercury concentration with fish length or weight for either sex in smelt (*Oamerus mordax*) netted from Cayuga lake in central New York state. Chattopadhyay (2000) investigated the effect of mercury and methyl parathion on the ovaries of *Labeo rohita*. He (op. cit.) has observed some abnormalities like fall in State I : Stage II oocyte ratio and necrosis. Further, Friedmann *et al.* (2002) studied the effects of mercury on general and reproductive health of large mouth bass (*Micropterus salmoides*), and measured variety of health and reproductive indicators in male individuals collected from three bodies of water in new Jersey. Whereas, Sindhe *et al.* (2002) estimated the ovarian and hepatic protein, lipid and cholesterol contents in the fish *Notopterus notopterus* exposed to heavy metals including mercury at sub leathal concentrations.

The metal's toxicity and their accumulation in the aquatic animals and fishes may be enhanced by change in temperature. Vernberg and Vernberg (1972) reported that survival time of the adult fiddler crab, *Uca pugilator* was shortened by the addition of sublethal concentrations of mercury at certain salinity – temperature combinations. Jones (1973) noticed that low salinity and high temperature increases the mortality of the marine isopods, *Idotea neglecta* and *I. emarginatea*, during mercury exposure. Whereas, Vernberg *et al.* (1973) revealed that mercury increased the mortality of *U. pugilator* larvae at high and low temperature – salinity extremes. Further low salinity and high temperature enhanced mercury's toxicity to larvae of crab, *Uca pugilator*. Nelson *et al.* (1976) studied the toxicity of 4 heavy metals to juvenile bay scallops, *Argopecten irradians* during short-term exposure. These authors (op. cit.) recorded the following order of toxicity: Silver > mercury > Cadmium > arsenic. Further, Nelson *et al.* (1977) also reported that toxicity of mercury at low concentrations was enhanced by high temperature and low salinity in juvenile bay scallops, *Argopecten irradians*. Ayfer and Jacob (1995) reported that at high temperature, accumulation of mercury was increased in kidney and liver, and decreased in gill of *Cyprinus carpio* (L). John *et al.* (1996) investigated the rate of methyl mercury uptake by fish yellow perch, *Perca flavescens* in relation to seasonal

variation in environmental temperature, body size, diet and prey availability. Mathieson *et al.* (1996) noticed that liver somatic index (LSI) was significantly higher and liver mercury concentration was significantly lower in summer than in other season in eel pout, *Zoarces viviparus*. Zarski *et al.* (1997a, b) suggested that the season of the year does not significantly affect the mercury concentration in the tissues and organs of breams (*Abramis brama* L.). Reed and Bodaly (1998) investigated that orange lake was smaller, warmer, and had slower fish growth and higher mercury concentration in yearlings of yellow perch (*Perca flavescens*) and walleye (*Stizostedion vitreum*) than trout lake.

It is well known that the toxicity of heavy metals in aquatic animal is significantly changed with the change in water hardness. Pickering and Henderson (1966) recorded variations in LC50's in soft and hard water for the green sunfish, *Lepomis cyanellus*. Whereas, Takeda and Shimma (1977) have reported that dietary calcium reduced the toxicity of dietary zinc to rainbow trout. Further, Varanasi and Gmur (1978) have demonstrated that increased concentrations of calcium either in water or in the food reduces the lead (Pb) uptake from water in *Oncorhynchus kisutch*. Carroll *et al.* (1979) noticed that the calcium ion was the most effective component of hard water in protecting fish (*Salvelinus fontinalis*) against cadmium. Further Calamari *et al.* (1980) found that fish exposed to cadmium responded differently depending upon the hardness of water to which they had previously been acclimated. Chrost and Pinko (1980) have suggested that fly ash comprising mainly calcium sulphate, oxide and carbonate when applied to the soil of forest areas, reduces the lead and zinc concentration when it enters the water. Pascoe *et al.* (1986) demonstrated, toxicity tests with rainbow trout and confirmed that cadmium is less toxic in hard water (96h LC50 - 2.6 mg Cd/l) than soft water (96h LC50 - 1.3 mg Cd/l).

# Chapter - 3

# MATERIAL AND METHODS

# 3. MATERIAL AND METHODS

## Bioassay Method:

The basic routine bioassay method, which is widely applicable, constitute the simplest procedure. It is suitable for the detection and evaluation of acute toxicity and is not associated with excessive oxygen demand due to substances that are relatively stable and are not extremely volatile. The routine bioassay method is so designed that surface absorption of oxygen from the atmosphere plus some oxygen from the diluent generally provide an adequate amount of dissolved oxygen for the fish during the test period (APHA, 1989).

## Physico-Chemical Characteristics of Water:

The dechlorinated soft and hard water were used at different temperature for the evaluation of mercury toxicity for selected fish species. These water were also subjected to analyse their physical and chemical characteristics such as pH, temperature, conductivity, dissolved oxygen, total alkalinity, total hardness, free carbon dioxide, nitrates ($NO_3$-N) and phosphates ($PO_4$-P). Standard methods were followed for the determination of these parameters (APHA, 1989 and AOAC,1984).

## Toxicant Solution:

To commence with the assays for mercury toxicity, a common stock solution was prepared by dissolving appropriate amount of reagent grade of mercury salt ($HgSO_4$) in 1.0 litre of deionised diluent water. The diluent water was acidified by addition of 2-5 drops of 50 % hydrochloric acid for complete dissolution of mercury salt. The series of different concentrations of mercury (as microgram/litre) were prepared by adding the stock solution into the measured diluent water with the help of a micropipette. The series of concentrations of the mercury was based on the progressive bisection of intervals on a logarithmic scale (APHA, 1989 : Table 1).

## Exposure System:

Glass jars of 1 litre capacity and plastic tubs of 20 litre capacity were used for the evaluation of short-term (96 h) toxicity for *Poecilia reticulata* and Indian major carps fingerlings respectively (Photographs 1and 2).

## Biological Procedure:

Living specimens of mature *Poecilia reticulata* were collected from the local sources (Photograph 3). They were kept in cement cistern for culture and production of males, females and their juveniles. Whereas, fingerlings of Indian major carp, i.e. *Catla catla*, *Labeo rohita* and *Cirrhinus mrigala* were collected from Jaisamand fish farm of Rajasthan Tribal Development Cooperative Federation Limited (RTADCF), Udaipur (Photograph 4) and were reared in glass aquaria. During rearing the conventional food, i.e. rice bran and oilcake (1:1) was provided to the fishes. Before commencement of experiment, test fishes were first acclimatized for ten days in the laboratory conditions similar to those under which the toxicity tests were performed. During the period of acclimatization, the fishes were also fed with the same conventional feed. But during the course of bioassay tests, the fishes were not provided any food to avoid excretory waste products and change in metabolic rate, which may influence the toxicity of the test solution. Healthy specimens were selected for the bioassay tests.

## Length and Weight of Test Fish:

**In the present study average length and weight of the fishes were following:**

| Test fish | Length(cm) | Weight(gm) | Photograph (No.) |
|---|---|---|---|
| ***Poecilia reticulata* (Peters):** | | | |
| Males | 2.5 | 0.185 | 5th |
| Females | 3.2 | 0.297 | 5th |
| Juveniles | 1.5 | 0.030 | 5th |
| Mixed population | -do- | -do- | |
| **Indian major carps:** | | | |
| *Catla catla* (Ham.) | 3.7 | 0.825 | 6th |
| *Labeo rohita* (Ham.) | 5.4 | 1.930 | 7th |
| *Cirrhinus mrigala* (Ham.) | 5.8 | 2.352 | 8th |

## Water Sources:

Diluent water of different hardness used in the bioassay test were collected from different sources during the course of study. The water of low hardness, i.e. 270 mg/l was collected from the supply of Public Health and Engineering Department Udaipur, whereas the water of high hardness, i.e. 560 mg/l was collected from a bore well.

## Season of Exposure:

Short-term (96 h) bioassay for the evaluation of mercury toxicity for *Poecilia reticulata* and fingerlings of Indian major carps were conducted at different temperatures, i.e. 35 °C (summer) and 16 °C (winter) in relation to different water hardness.

## Behavioural Study:

Any change in the behaviour of test fishes, pronounced symptoms of intoxications and distress such as loss of equilibrium in the test fish were also recorded during the experimental period.

## Histopathological Study:

The treated fingerlings of *Cirrhinus mrigala* were carefully removed from a sublethal concentration of 240 µg/l of mercury and sacrificed immediately. The samples of gills, kidney and eye were excised carefully, washed in running water and fixed in 4% neutral formalin. The serial sections of 5-8 microns thickness were cut and stained using eosin and Mayre's haematoxylin. The histopathological changes if any in tissues of treated fish were observed carefully.

## Preliminary or Screening Test:

A preliminary exploratory investigation with different concentrations of toxicant solution was conducted by maintaining higher concentration of toxicant in the beginning and later lower concentration were tested so as to discover the critical concentration range for the fish. The test range for each bioassay study was taken between the highest concentration and the lowest concentration at which most, if not all, of the test fishes died or survived within a specified period of exposure, i.e. 24, 48, 72 and 96h.

## Full Scale Tests:

After preliminary examination, elaborate experiments for *Poecilia reticulata* (male, female, juveniles and mixed population) and fingerlings of Indian major carps (catla, rohu and mrigal) were conducted to evaluate the toxicity of mercury which was measured by testing various concentrations in the range known by the preliminary exploratory test.

The glass jars used for *Poecilia reticulata* (male, female, juvenile and mixed population ) were filled with one litre of toxicant solution. Whereas, plastic tubs used for fingerlings of catla, rohu and mrigal were filled with fifteen litre of toxicant solution. These experimental units were placed in three rows and each container was labelled with the details of the experiment such as concentration, name of fish, replicate number, date and time of the experiment (Photographs 1 and 2). The acclimatized fishes were transferred to these containers after about 20 minutes of the preparation of test solution.

Ten test fishes were placed in each experimental container. Proper controls were run simultaneously. The test solutions were renewed after every 24 h by fresh test solutions. The experiments were continued for a period of 96 h. The number of fishes died in each concentration of toxicant solution were observed carefully and recorded at the intervals of 24, 48, 72 and 96 h. The dead fishes were removed from the test solution after knowing the exact mortality which was observed by their respiratory and other body movements, either spontaneous or in response to mild mechanical stimulation by prodding the fish or gently pressing the tail with a smooth glass rod. The close observations of the reactions of the fishes during first 4 to 24 h gave an indication of the nature of toxicant and served as a guide for further examination.

## Parameters Applied for Reporting the Data:

### (i) Median lethal concentration (LC50):

The static bioassay tests were conducted to measure median lethal concentration (LC50). A concentration of toxicant at which 50 per cent of the test fishes survive for a specified time exposure. The LC50's were estimated

at different concentrations and time intervals (24, 48, 72 and 96 hours) for the mercury by probit analysis (Finney, 1971).

**(ii) 95 per cent confidence limits:**

The 95 per cent confidence limits, i.e. lower confidence limits (LCL) and upper confidence limits and their ratios (R= UCL/LCL) for each LC50 were also calculated (Finney, 1971). The 95 per cent confidence limits signifies the accuracy of the estimate that would be expected from replicate of the static bioassay carried out at the same time with exactly the same conditions.

**(iii) Relative sensitivities of test fishes:**

The relative sensitivities of the test fishes, i.e. *Poecilia reticulata* (male, female, juvenile and mixed population) and fingerlings of IMC (catla, rohu and mrigal) based on LC50's of mercury have also been estimated.

**(iv) Safe or harmless concentrations:**

Presumable safe or harmless concentrations of the mercury in short term toxicity test for the test fishes, i.e. *Poecilia reticulata* (male, female, juvenile and mixed population) and fingerlings of IMC (catla, rohu and mrigal) were calculated by using the following formula of Hart *et al.* (1945).

$C = (48 \text{ hours LC50} \times 0.3)/S^2$

$$\text{Where } S^2 = \frac{24 \text{ hour LC50}}{48 \text{ hour LC50}}$$

(C is the harmless concentration and S represents safe dischargeable concentration)

**(v) Statistical analyses:**

The LC50 values for all the test fishes were also tested between different water hardness and temperature for coefficient of correlation (r ) at 1% and 5% levels (Snedecor and Cochran, 1980).

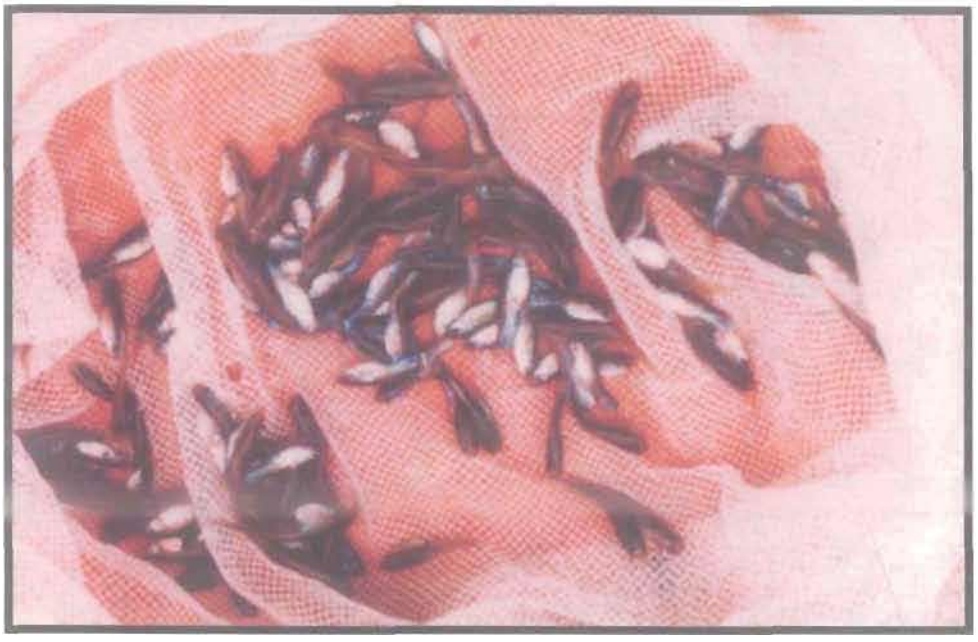
**Table 1 : Guide to the selection of test concentrations based on a logarithmic scale.***

| Test concentrations in mg/l, mg/g, or percent | | | | |
|---|---|---|---|---|
| Column 1 | Column 2 | Column 3 | Column 4 | Column 5 |
| 10.0 | | | | |
| | | | | 8.7 |
| | | | 7.5 | |
| | | | | 6.5 |
| | | 5.6 | | |
| | | | | 4.9 |
| | | | 4.2 | |
| | | | | 3.7 |
| | 3.2 | | | |
| | | | | 2.8 |
| | | | 2.4 | |
| | | | | 2.1 |
| | | 1.8 | | |
| | | | | 1.55 |
| | | | 1.35 | |
| | | | | 1.15 |
| 1.0 | | | | |

Reproduced from APHA (1989)

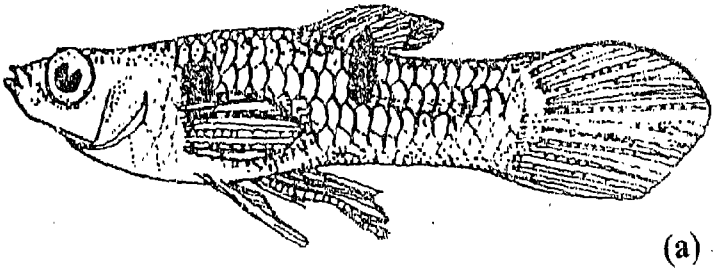

**Photograph 1:** Showing the battery of glass jar of 1 litre capacity for the short-term (96h) experiment conducted for *Poecilia reticulata* (male, female, juvenile and mixed population)

**Photograph 2:** Showing the battery of plastic tubs of 20 litre capacity for the short-term(96h) experiment conducted for the finger l ings of Indian major carp (catla, rohu and mrigal).

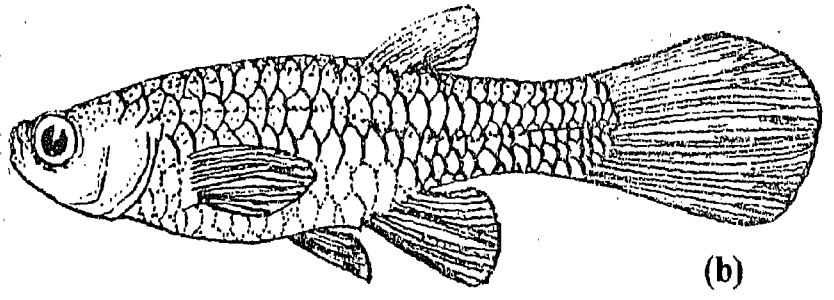


**Photograph 3:** Showing the catch of mature *Poecilia reticulata* collected from local ponds.

**Photograph 4:** Showing the catch of fingerlings of Indian major carps (catla, rohu and mrigal) collected from the nursery pond at Jaisamand fish farm of RTADCF, Udaipur.

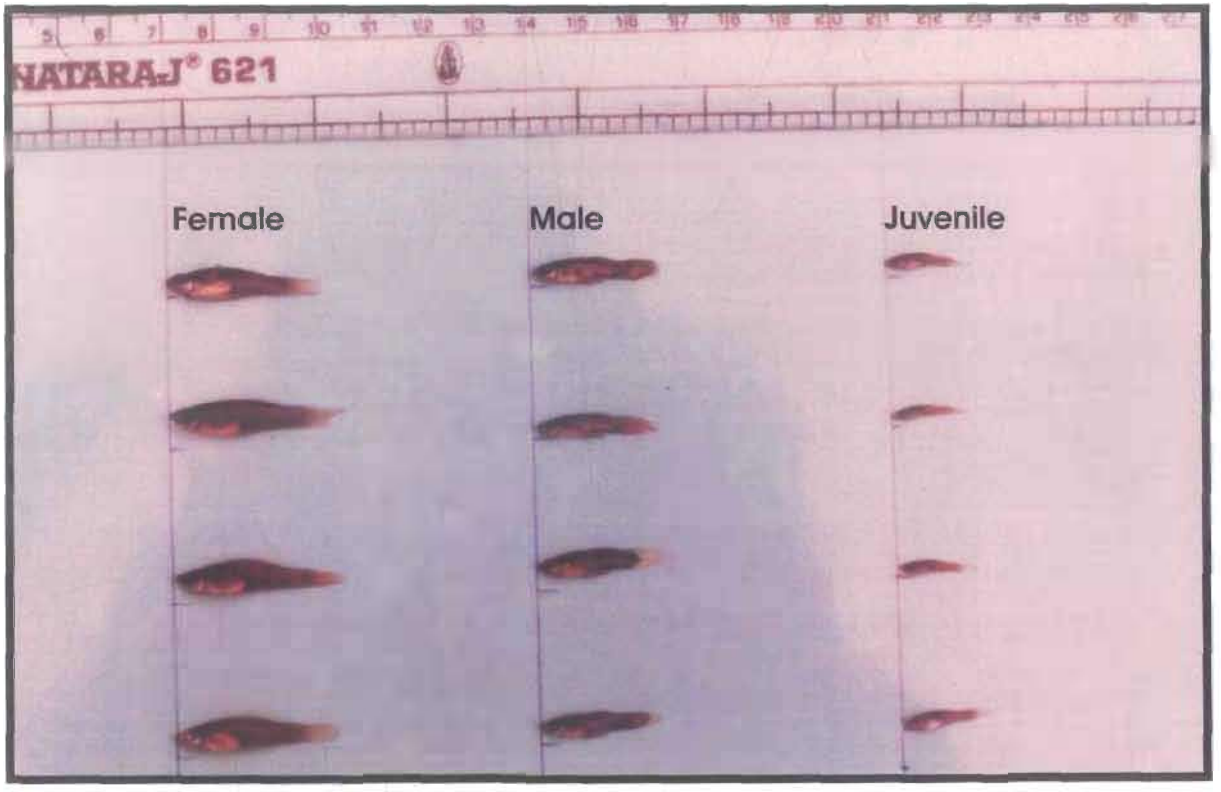


(a)

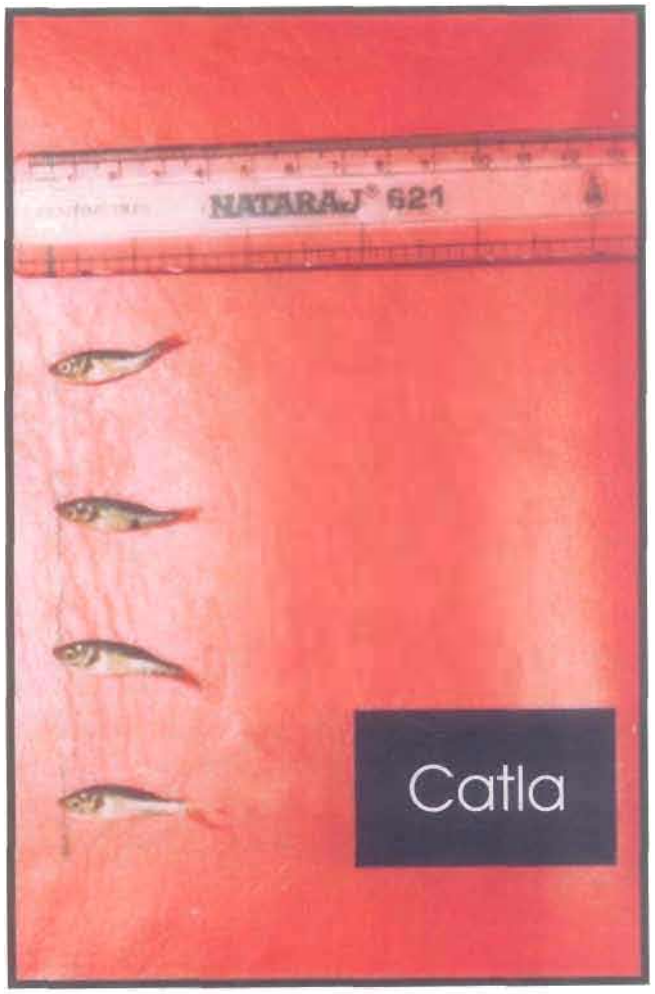


(b)

**Line diagram of male (a) and female (b) of *Poecilia reticulata***

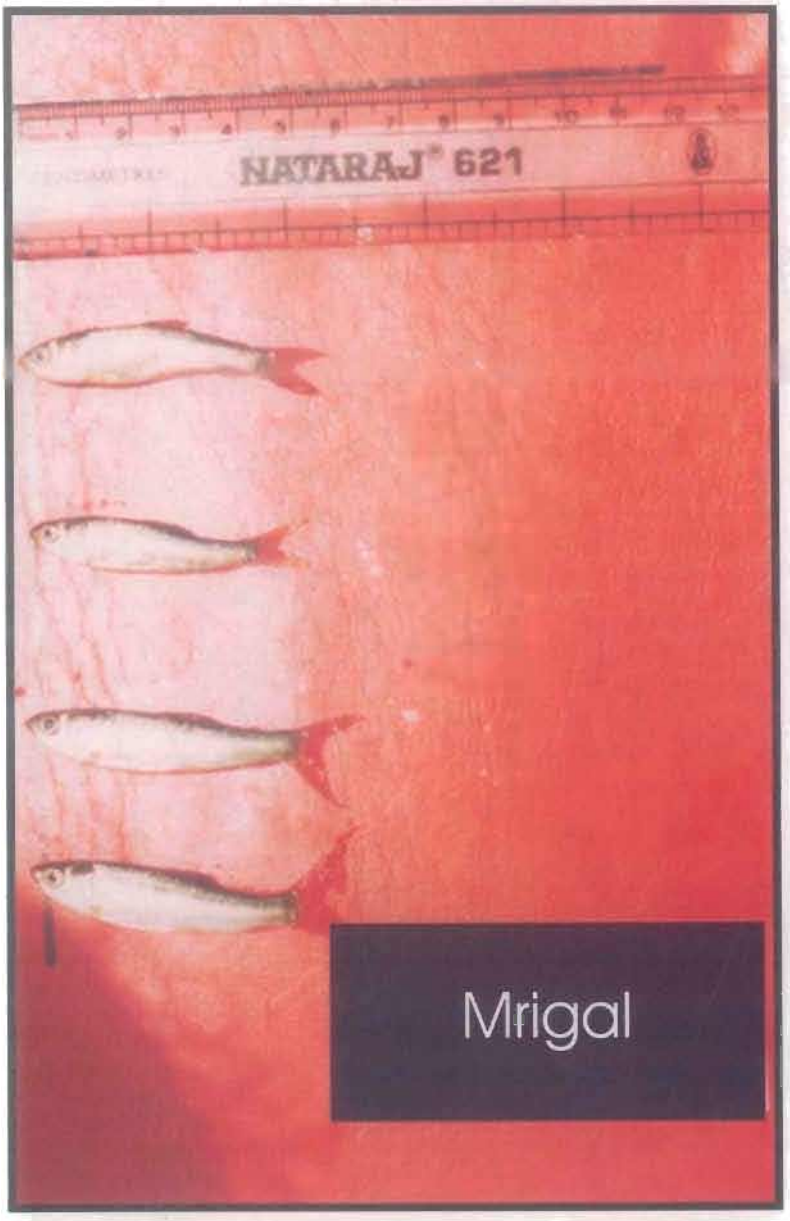



**Photograph 5:** Showing the test specimens of female, male & juvenile of *Poecilia reticulata* used in the short-term static bioassay (96h) for the evaluation of mercury toxicity.




**Photograph 6:** Showing the test specimens of the fingerlings of catla used in short-term static bioassay (96h) for the evaluation of mercury toxicity.


**Photograph 7:** Showing the test specimens of the fingerlings of rohu used in short-term static bioassay (96h) for the evaluation of mercury toxicity.




**Photograph 8:** Showing the test specimens of the fingerlings of mrigal used in short-term static bioassay (96h) for the evaluation of mercury toxicity.

# Chapter - 4

# RESULTS

# 4. RESULTS

## Physico-chemical Characteristics of the Water:

Physio-chemical characteristics of the diluent water of different hardness, i.e. 270 and 560 mg/l used for the determination of mercury toxicity for *Poecilia reticulata* (male, female, juvenile and collective) and fingerlings of Indian major carp (catla, rohu and mrigal) at two temperatures, i.e. 35 and 16 $^{0}$C have been summarized in table 1. Characteristics of the diluent water revealed that the same are standard one as in natural conditions it did not contain any toxic substances. However, few changes have been observed in the quality of water at two temperatures were mainly dissolved oxygen, total alkalinity, electrical conductivity and hardness.

## Concentrations of Mercury Used in Bioassay:

The test range of mercury concentrations selected for the study of static bioassay was based on the results of the preliminary or screening tests. Variations in the ranges of mercury concentration were observed with the change in water hardness and temperature. The concentrations of mercury (as µg/l) used for the evaluation of short-term (96h) toxicity with different water hardness and temperature for *Poecilia reticulata* and Indian major carps have been presented in table 2.

## Measurement of Acute Toxicity:

Change in acute toxicity in relation to seasonal variation is mainly due to change in water hardness and temperature. Short-term static bioassays for *Poecilia reticulata* (male, female, juvenile and mixed population) and fingerlings of Indian major carp (catla, rohu and mrigal) have been conducted to evaluate the toxicity of mercury ions in relation to water hardness and temperature. The per cent mortality of the test fishes with different test concentrations of mercury ions for the duration of 24, 48, 72 and 96 hours have been summarized in Appendix-1 (table 1 to 28).

## Median Lethal Concentrations (LC50):

LC50 is the concentration of toxicant at which 50 per cent of the test animals are able to survive for a specified period of exposure. '"The LC50 is merely a convenient reference point of expressing the acute lethal toxicity of a given pollutant to the average or typical fish" (Sprague, 1973). The LC50 values for mercury toxicity on varying water hardness and temperatures were computed by Probit analysis (Finney, 1971).

The bioassay study revealed that mercury ions were highly toxic not only to the *Poecilia reticulata* (male, female and juvenile) but also to the fingerlings of Indian major carp. The LC50 values for 24, 48, 72 and 96 hours for these test fishes were observed in micrograms/litre (tables 3 to 9 ; Fig. 5).

## LC50's for *Poecilia reticulata*:

### Male:

The LC50's of mercury for the male of *Poecilia reticulata* for different time intervals of 24, 48, 72 and 96h have been summarized in table 3. The LC50's were found to change significantly with change in water hardness and temperature. The 24, 48, 72 and 96h LC50's to male for mercury ions were observed as 91.422, 80.442, 71.555 and 69.080 µg Hg/l respectively with a water hardness of 270 mg/l at a temperature of 35°C. These values were considerably increased at a low temperature of 16°C with the same water hardness of 270 mg/l. Herein the LC50's of males for mercury ions were recorded as 267.101, 254.623, 232.115 and 216.184 µg Hg/l for 24, 48, 72 and 96h respectively.

The LC50's of male for mercury ions were further enhanced with an increase in water hardness. The 24, 48, 72 and 96h LC50's of male for mercury with a water hardness of 560 mg/l were noticed as 106.957, 94.363, 86.643 and 78.727 µg Hg/l respectively at a temperature of 35°C (table 3). Whereas, the LC50's of male for mercury ions with a water hardness of 560 mg/l at a temperature of 16°C were recorded highest as compared to LC50's with a water hardness of 560 mg/l at a temperature of 35°C and water hardness of

270 mg/l at both the temperatures, i.e. 35 and 16°C. Herein the LC50's of male for mercury were recorded as 297.906, 279.011, 257.975 and 231.643 µg Hg/l for the time intervals of 24, 48, 72 and 96h respectively.

**Female:**

LC50 values of mercury for female of *Poecilia reticulata* for different time intervals of 24, 48, 72 and 96 h have been summarized in table 4. The LC50's shows that female is less susceptible to mercury ions as compared to that of male. Further, LC50 values were also found to change with the change in water hardness and temperature. The 24, 48, 72 and 96h LC50 values for mercury ions to female were recorded as 124.184, 118.277, 105.930 and 86.262 µg Hg/l respectively with a water hardness of 270 mg/l at a temperature of 35°C. The LC50 values of mercury for female were increased about two times with the same water hardness of 270 mg/l at a low temperature of 16°C. The LC50's for mercury to female were found 279.335, 258.705, 240.775 and 218.775 µg Hg/l for 24, 48, 72 and 96h exposure respectively. The LC50's of mercury for female were also increased as in case of male with the increase in water hardness. The 24, 48, 72 and 96h LC50's of mercury for female with a water hardness of 560 mg/l and at a temperature of 35°C were noticed as 190.494, 172.163, 144.734 and 124.739 µg Hg/l respectively.

The LC50's of mercury for female with a water hardness of 560 mg/l at a temperature of 16°C were further increased in comparison to LC50's with water hardness of 560 mg/l at a temperature of 35°C and with water hardness of 270 mg/l at both the temperatures, i.e. 35 and 16°C. The 24, 48, 72 and 96 h values of mercury for female were recorded as 343.547, 304.134, 267.597 and 247.278 µg Hg/l respectively with water hardness of 560 mg/l at a temperature of 16°C.

**Juvenile:**

In general juveniles of *Poecilia reticulata* were found sensitive to mercury ions as compared to that of adults, i.e. male and female with both water hardness and temperatures. The 24, 48, 72 and 96 h LC50's of mercury for

juvenile of *Poecilia reticulata* have been presented in table 5. The lowest LC50 values for juvenile were recorded as 73.578, 60.261, 55.107 and 46.181 µg Hg/l for the duration of 24, 48, 72 and 96h respectively with a water hardness of 270 mg/l at a temperature of 35°C. The LC50 values increased approximately two times at a temperature of 16°C with the same water hardness of 270 mg/l. Herein the LC50's for juvenile were observed as 128.535, 122.280, 113,706 and 93.464 µg Hg/l for 24, 48, 72 and 96h respectively. The LC50's of mercury for juvenile were also increased with the increase in water hardness, i.e. 560 mg/l at both the temperatures. The 24, 48, 72 and 96h LC50's for juvenile with a water hardness of 560 mg/l at a temperature of 35°C were noticed as 80.717, 74.807, 68.878 and 61.200 µg Hg/l respectively. Whereas the LC50's for juvenile with the same water hardness, i.e. 560 mg/l at a temperature of 16°C were found 143.922, 131.609, 125.958 and 113.721 µg Hg/l for 24, 48, 72 and 96h respectively.

**Mixed Population:**

The LC50 value of mercury for mixed population of *Poecilia reticulata* for different time intervals of 24, 48, 72 and 96h have been depicted in table 6. The 24, 48, 72 and 96 h LC50's to mixed population were observed as 91.721, 78.884, 69.183 and 59.535 µg Hg/l respectively with a water hardness of 270 mg/l at a temperature of 35°C, whereas the LC50's of mercury ions for mixed population were noticed as 193.802, 176.950, 167.169 and 146.916 µg Hg/l for 24, 48, 72 and 96h respectively with the same water hardness at a temperature of 16°C. The 24, 48, 72 and 96h LC50's for mixed population with a water hardness of 560 mg/l at a temperature of 35°C were found as 106.844, 103.891, 95.303 and 83.918 µg Hg/l respectively. However, these values at the same water hardness of 560 mg/l at a temperature of 16°C were recorded as 217.611, 200.425, 180.175 and 167.861 µg Hg/l for the time intervals of 24, 48, 72 and 96 h respectively.

## LC50's for the fingerlings of Indian major carp:

*Catla catla:*

The 24, 48, 72 and 96h LC50's of mercury ions for the fingerlings of catla have been summarized in table 7. The fingerlings of catla were found most sensitive in comparison to the fingerlings of rohu and mrigal as revealed from the lower values of LC50. Further, variations in LC50 were also found with the change in water hardness and temperature. The 24, 48, 72 and 96 h LC50's for catla were observed as 59.969, 52.904, 46.921 and 43.922 µg Hg/l respectively with a water hardness of 270 mg/l at a temperature of 35°C. These LC50 values were also increased at a low temperature of 16°C with the same water hardness of 270 mg/l. Here the LC50's of mercury ions for catla fingerlings were observed as 83.288, 69.696, 60.677 and 52.755 µg Hg/l for 24, 48, 72 and 96 h respectively.

The LC50's of mercury ions for catla fingerlings were also increased with the increase in water hardness. The 24, 48, 72 and 96h LC50's of catla fingerlings with a water hardness of 560 mg/l at a temperature of 35 °C were observed as 75.578, 60.261, 57.418 and 47.707 µg Hg/l respectively. Whereas LC50's of mercury ions for catla fingerlings with a water hardness of 560 mg/l at a temperature of 16°C were recorded as 107.816, 91.357, 77.869 and 72.729 µg Hg/l for 24, 48, 72 and 96h respectively. Interestingly, the LC50 values of mercury ions for catla fingerlings with a water hardness of 560 mg/l at a temperature of 16°C were noticed highest as compared to LC50 values recorded with different water hardness and temperatures.

***Labeo rohita***:

The 24, 48, 72 and 96h LC50 values of mercury for fingerlings of rohu have been presented in table 8. In comparison to catla, fingerlings of rohu were found less sensitive as revealed from the higher LC50 values. The 24, 48, 72 and 96h LC50's for mercury ions to fingerlings of rohu were found as 289.470, 261.493, 218.239 and 194.612 µg Hg/l respectively with a water hardness of 270 mg/l at a temperature of 35°C. The values of LC50 of mercury for these fingerlings were found to increase at a temperature of 16°C with the same water hardness of 270 mg/l. These values were 297.822, 283.247, 273.06h

and 288.157 µg hg/l for 24, 48, 72 and 96h respectively. The data also indicate that LC50 values of mercury for the fingerlings of rohu were further increased with the increase in water hardness. The 24, 48, 72 and 96h LC50's for mercury to fingerlings of rohu with a water hardness of 560 mg/l at a temperature of 35°C were noticed as 293.130, 267.714, 250.347 and 222.069 µg Hg/l respectively. Whereas, LC50's of mercury ions for these fingerlings with a water hardness of 560 mg/l at a temperature of 16°C were recorded as 343.459, 318.939, 294.371 and 278.347 µg Hg/l for the durations of 24, 48, 72 and 96h respectively.

**Cirrhinus mrigala:**

The LC50 values of mercury for the fingerlings of mrigal have been presented in table 9. The LC50's for the fingerlings of mrigal for 24, 48, 72 and 96 h were noticed as 342.164, 306.050, 283.530 and 268.141 µg Hg/l respectively with a water hardness of 270 mg/l at a temperature of 35°C. Noticeable changes in LC50 value were also recorded with the variation in water hardness and temperature.The LC50's for the fingerlings of mrigal were observed as 392.569,358.341,341.174 and 308.456 µgHg/l for 24,48,72 and 96h respectively with the same water hardness i.e. 270 mg/l at a temperature of 16°C.

The LC50 values of mercury for the fingerlings of mrigal were further increased with the increase in water hardness. The 24, 48, 72 and 96h LC50's were observed as 378.826, 347.083, 304.405 and 281.449 µg Hg/l respectively with a water hardness of 560 mg/l at a temperature of 35°C. Whereas, the LC50's of mercury for the fingerlings of mrigal with a water hardness of 560 mg/l at a temperature of 16°C were recorded as 453.363, 383.142, 342.601 and 312.909 µg Hg/l for the time intervals of 24, 48, 72 and 96h respectively. The LC50 values of mercury showed that the fingerlings of mrigal were more hardy in comparison to fingerlings of catla and rohu.

**Relative Sensitivities of test fishes:**

The relative sensitivities of the test fishes based on LC50's of mercury have also been estimated (Appendix 2; Table 1 to 8). The results showed following trend of relative sensitivity for mercury ions to *Poecilia reticulata* : juvenile>male>female

(Fig.5). The relative sensitivity of mixed population of *Poecilia reticulata*, however, showed sometimes nearly equal to male and juvenile. Whereas, among the fingerlings of Indian major carp, following trend of relative sensitivity for mercury was recorded: Catla>rohu>mrigal (Fig. 5).

The relative sensitivity of test fishes was also studied in between different factors (Appendix 2; Table 5 to 8). All the test fishes were found more sensitive at high temperature and low water hardness as compared to low temperature and high water hardness as revealed from 24, 48, 72 and 96 h LC50 values. However, among the same water hardness, all test fishes were found more sensitive at high temperature.

**95 per cent Confidence Limits & Their Ratios:**

The 95 per cent confidence limits, i.e. lower confidence limit (LCL) and upper confidence limit (UCL) for LC50 values were also estimated for different periods of 24, 48, 72 and 96 hours (Tables 10 to 17). From the upper and lower confidence limits, ratio of confidence limits (R = UCL/LCL) were also calculated, which signify the test, i.e. smaller the ratio or smaller the spread between the 95 per cent confidence limits, the better the test.

***Poecilia reticulata*:**

The ratios of confidence limits, i.e. upper and lower confidence limits for median lethal concentrations of mercury ions for *Poecilia reticulata* (male, female, juvenile and mixed population) were ranged in between 1.309 to 1.608; 1.282 to 1.834; 1.236 to 1.900; and 1.235 to 1.965 for 24, 48,72 and 96h respectively with both the water hardness and temperature (Table 10 to13), signify the better performance of the bioassay test. However, insignificant higher ratio of confidence limit, i.e. 12.080, may be due to sensitivity variation of juvenile during 24h exposure. Slightly higher ratios of confidence limits, i.e. 2.718 and 2.295 were also indicated sensitivity variation to mixed population of *Poecilia reticulata* during 72 and 96h respectively with a water hardness of 270 mg/l at a temperature of 35°C.

Similarly, higher ratios of confidence limits, i.e. 2.031 and 2.193 for female and juvenile during 48 and 96h respectively were also recorded with a water hardness of 560 mg/l at a temperature of 35°C.

**Indian major carp:**

The ratios of confidence limits, i.e. upper and lower confidence limits for median lethal concentrations of mercury for the fingerlings of Indian major carp (catla, rohu and mrigal) were ranged in between 1.244 to 1.607; 1.241 to 1.646; 1.209 to 1.643; and 1.238 to 1.605 for 24, 48, 72 and 96 h respectively with both the water hardness and temperature (Table 14 to 17) also signify the betterness of the bioassay test. However, higher ratios of confidence limits, i.e. 2.221 and 2.229 were indicated sensitivity variation of the fingerlings of catla and mrigal to mercury ions during 24h of exposure with a water hardness of 270 mg/l at a temperature of 35°C.

The higher ratios of confidence limits, i.e. 3.469 and 2.264 were also indicated sensitivity variation of the fingerlings of catla during 24 and 96h respectively to mercury with the same water hardness, i.e. 270 mg/l at a temperature of 16°C. Further, insignificant ratio of confidence limit, i.e. 12.08 with a water hardness of 560 mg/l at a temperature of 35°C was also observed for the fingerlings of catla. Slightly higher ratios of confidence limits, i.e. 2.483 and 2.872 were also observed during 48 and 72h respectively with a water hardness of 560 mg/l at a temperature of 16°C.

**Safe Concentrations:**

The presumable safe or harmless concentrations of mercury ions for the test fishes were also determined at both the water hardness and temperature (Table 18). The safe concentrations of mercury for *Poceilia reticulata* were ranged in between 18.692 to 73.423 µgHg/l for male; 32.257 to 71.56 µgHg/l for female; 12.133 to 33.228 for juvenile and 17.516 to 51.042 µgHg/l for mixed population. Whereas, safe concentrations of mercury for the fingerlings of Indian major carps were ranged in between 12.133 to 19.689 µgHg/l for catla; 64.039 to 82.555 µgHg/l for rohu; and 73.510 to 89.585 µgHg/l for mrigal.

**Coefficient of correlation (r):**

The LC50 values for all the test fishes, i.e. *Poecilia reticulata* (male, female, juvenile and mixed population) and fingerlings of Indian major carp (catla, rohu and mrigal) were also tested between different water hardness and temperature for coefficient of correlation (r) at 1% and 5% levels. (See Appendix –3 ; Table-1). Significant positive correlations indicate uniform behaviour of different fishes in different water hardness and temperature.

**Behavioural changes:**

Behavioural and morphological changes of the test fishes, i.e. *Poecilia reticulata* (male, female and juvenile) and Indian major carp (catla, rohu and mrigal) were also recorded in response to different concentrations of mercury, water hardness and temperatures. These studies were helpful in finding out the nature and effect of mercury ions on the test specimens directly or indirectly. Remarkable changes in behaviour of the test fishes have been summarized in table 19 & 20.

**Histopathological changes resulting from bioassay of mercury ions:**

Gill, kidney and eye of the fingerlings of *Cirrhinus mrigala* exposed to a sublethal concentration of 240 µg/l of mercury for a period of 96h with a water hardness of 270 mg/l at a temperature of 35°C were also studied for histopathological changes (Figs 1-4).

The sublethal concentration of 240 µg/l of mercury brought about marked changes in the gill structure of the fingerlings of mrigal (Figs 1 & 2). The gill filaments showed severe pathological changes in the distal region as compared to that of middle region. The secondary lamellae as a whole, including the flanges of the pillar cells have been fused togather forming large spherical structure in the distal region of the gill filaments. The fusion was of the highest order and no distinction could be made among pillar cells, epithelial cells, blood cells and mucous cells in such region (Fig. 2). The blood cells were also found accumulate in these spherical structure. No apparent pathological changes were noticed in the proximal region of the gill filaments.

Kidney of the fingerlings of mrigal exposed to a sublethal concentration of 240 µg/l of mercury revealed various histopathological changes (Fig.3). The most remarkable histopathological change observed was the expansions of the renal tubule to such an extent that it covers the area of the lumen. Further, the cells lining the lumen also ruptured causing tubular necrosis. In lumen of the renal tubules cellular debris was also noticed. At places the glomeruli found engorged with blood. The haemopoitic tissue of the renal interstitium was also considerably reduced and degenerated.

The eye of the fingerlings of mrigal subjected to a sublethal concentration of 240 µg/l of mercury ions also revealed lesions in the periorbital tissue (Fig.4). The

choroids tissue showed considerable exudation by inflammatory cellular infiltration of the periorbital tissue. Whereas remarkable degeneration was seen in oedematous tissue or adjacent sclera of the periorbital tissue (Fig.4).

**Table-1 : Physico - chemical characteristics of the different diluent water used in short-term toxicity tests for mercury**

| Characteristics | Average values | | | |
|---|---|---|---|---|
| | Summer | | Winter | |
| **Water temperature (°C)** | **35** | **35** | **16** | **16** |
| Dissolved oxygen (mg/l) | 5.0 | 5.0 | 7 | 6.5 |
| pH | 8.5 | 8.6 | 8.0 | 8.2 |
| Free carbondioxide (mg/l) | Nil | Nil | Nil | Nil |
| Total alkalinity (mg/l) | 700 | 810 | 560 | 715 |
| **Total Hardness (mg/l)** | **270** | **560** | **270** | **560** |
| Electric conductivity (μ mhos/cm) | 830 | 820 | 835 | 830 |
| Nitrates $NO_3$-N (mg/l) | 1.61 | 1.69 | 1.57 | 1.61 |
| Phosphates $PO_4$-P (mg/l) | 0.04 | 0.05 | 0.03 | 0.05 |

**Table – 2 : Concentrations of mercury ( as µg/l) used in the 96h static bioassays with water hardness of 270 and 560 mg/l and at temperatures 35 and 16°C for *Poecilia reticulata* and fingerlings of Indian major carp.**

| Temperature | 35 °C | | 16 °C | |
|---|---|---|---|---|
| **Water hardness** | **270 mg/l** | **560 mg/l** | **270 mg/l** | **560 mg/l** |
| | **Hg (µg/l)** | **Hg (µg/l)** | **Hg (µg/l)** | **Hg (µg/l)** |
| ***Poecilia reticulata:*** | | | | |
| Male | 100 | 115 | 280 | 320 |
| | 87 | 100 | 240 | 280 |
| | 75 | 87 | 210 | 240 |
| | 65 | 75 | 180 | 210 |
| | 56 | 65 | 155 | 110 |
| | 00 | 00 | 00 | 00 |
| Female | 135 | 210 | 280 | 370 |
| | 115 | 180 | 240 | 320 |
| | 100 | 155 | 210 | 280 |
| | 87 | 135 | 180 | 240 |
| | 75 | 115 | 155 | 210 |
| | 00 | 00 | 00 | 00 |
| Juvenile | 75 | 87 | 135 | 155 |
| | 65 | 75 | 115 | 135 |
| | 56 | 65 | 100 | 115 |
| | 49 | 56 | 87 | 100 |
| | 42 | 49 | 75 | 87 |
| | 00 | 00 | 00 | 00 |
| Mixed population | 100 | 115 | 210 | 240 |
| | 87 | 100 | 180 | 210 |
| | 75 | 87 | 155 | 180 |
| | 65 | 75 | 135 | 155 |
| | 56 | 65 | 115 | 135 |
| | 00 | 00 | 00 | 00 |
| **Indian Major Carp:** | | | | |
| Catla | 65 | 75 | 87 | 115 |
| | 56 | 65 | 75 | 100 |
| | 49 | 56 | 65 | 87 |
| | 42 | 49 | 56 | 75 |
| | 37 | 42 | 49 | 65 |
| | 00 | 00 | 00 | 00 |
| Rohu | 320 | 320 | 320 | 370 |
| | 280 | 280 | 280 | 320 |
| | 240 | 240 | 240 | 280 |
| | 210 | 210 | 210 | 240 |
| | 180 | 180 | 180 | 210 |
| | 00 | 00 | 00 | 00 |
| Mrigal | 370 | 420 | 420 | 490 |
| | 320 | 370 | 370 | 420 |
| | 280 | 320 | 320 | 370 |
| | 240 | 280 | 280 | 320 |
| | 210 | 240 | 240 | 280 |
| | 00 | 00 | 00 | 00 |

**Table-3 : LC 50's (24, 48, 72 and 96 h) of male of *Poecilia reticulata* for mercury with different water hardness and temperatures**

| Water hardness / Temperature (°C) | Water hardness 270 mg/l | | | | Water hardness 560 mg/l | | | |
|---|---|---|---|---|---|---|---|---|
| | 24 h | 48 h | 72 h | 96 h | 24 h | 48 h | 72 h | 96 h |
| 35 | 91.422 | 80.442 | 71.555 | 69.080 | 106.957 | 94.363 | 86.643 | 78.727 |
| 16 | 267.101 | 254.623 | 232.115 | 216.184 | 297.906 | 279.011 | 257.975 | 231.643 |

**Table-4 : LC 50's (24, 48, 72 and 96 h) of female of *Poecilia reticulata* for mercury with different water hardness and temperature**

| Water hardness / Temperature (°C) | Water hardness 270 mg/l | | | | Water hardness 560 mg/l | | | |
|---|---|---|---|---|---|---|---|---|
| | 24 h | 48 h | 72 h | 96 h | 24 h | 48 h | 72 h | 96 h |
| 35 | 124.184 | 118.277 | 105.930 | 86.262 | 190.494 | 172.163 | 144.734 | 124.739 |
| 16 | 279.335 | 258.705 | 240.507 | 218.775 | 343.547 | 304.134 | 267.597 | 247.278 |

**Table-5 : LC 50's (24, 48, 72 and 96 h) of juveniles of *Poecilia reticulata* for mercury with different water hardness and temperature**

| Water hardness / Temperature (°C) | Water hardness 270 mg/l | | | | Water hardness 560 mg/l | | | |
|---|---|---|---|---|---|---|---|---|
| | 24 h | 48 h | 72 h | 96 h | 24 h | 48 h | 72 h | 96 h |
| 35 | 73.578 | 60.261 | 55.107 | 46.181 | 80.717 | 74.807 | 68.878 | 61.200 |
| 16 | 128.535 | 122.280 | 113.706 | 93.464 | 143.922 | 131.609 | 125.958 | 113.721 |

**Table – 6 : LC 50's (24, 48, 72 and 96 h) of mixed population of *Poecilia reticulata* for mercury with different water hardness and temperature**

| Water hardness / Temperature (°C) | Water hardness 270 mg/l | | | | Water hardness 560 mg/l | | | |
|---|---|---|---|---|---|---|---|---|
| | 24 h | 48 h | 72 h | 96 h | 24 h | 48 h | 72 h | 96 h |
| 35 | 91.721 | 78.884 | 69.183 | 59.535 | 106.844 | 103.891 | 95.303 | 83.918 |
| 16 | 193.802 | 176.950 | 167.169 | 146.916 | 217.611 | 200.425 | 180.175 | 167.861 |

**Table-7 : LC 50's (24, 48, 72 and 96 h) of fingerlings of *Catla catla* for mercury with different water hardness and temperature**

| Water hardness / Temperature (°C) | Water hardness 270 mg/l | | | | Water hardness 560 mg/l | | | |
|---|---|---|---|---|---|---|---|---|
| | 24 h | 48 h | 72 h | 96 h | 24 h | 48 h | 72 h | 96 h |
| 35 | 59.969 | 52.904 | 46.921 | 43.922 | 73.578 | 60.261 | 57.418 | 47.707 |
| 16 | 83.288 | 69.696 | 60.677 | 52.755 | 107.816 | 91.357 | 77.869 | 72.729 |

**Table-8 : LC 50's (24, 48, 72 and 96 h) of fingerlings of *Labeo rohita* for mercury with different water hardness and temperature**

| water hardness / Temperature (°C) | Water hardness 270 mg/l | | | | Water hardness 560 mg/l | | | |
|---|---|---|---|---|---|---|---|---|
| | 24 h | 48 h | 72 h | 96 h | 24 h | 48 h | 72 h | 96 h |
| 35 | 289.470 | 261.493 | 218.239 | 194.612 | 293.130 | 267.714 | 250.347 | 222.069 |
| 16 | 297.822 | 283.247 | 273.064 | 228.157 | 343.459 | 318.938 | 294.371 | 278.347 |

**Table-9 : LC 50's (24, 48, 72 and 96 h) of fingerlings of *Cirrhinus mrigala* for mercury with different water hardness and temperature**

| Water hardness / Temperature (°C) | Water hardness 270 mg/l | | | | Water hardness 560 mg/l | | | |
|---|---|---|---|---|---|---|---|---|
| | 24 h | 48 h | 72 h | 96 h | 24 h | 48 h | 72 h | 96 h |
| 35 | 342.164 | 306.050 | 283.530 | 268.141 | 378.826 | 347.083 | 304.450 | 281.449 |
| 16 | 392.569 | 358.341 | 341.174 | 308.456 | 453.363 | 383.142 | 342.601 | 312.909 |

**Table – 10 : 95 per cent confidence limits and their ratios for 24, 48, 72 and 96 h LC 50's of mercury for *Poecilia reticulata* (male, female, juvenile and mixed population) with water hardness of 270 mg/l at an average temperature of 35°C**

| Durations / Test animals | 24 h | | | 48 h | | | 72 h | | | 96 h | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | UCL | LCL | R | UCL | LCL | R | UCL | LCL | R | UCL | LCL | R |
| Male | 127.646 | 80.959 | 1.576 | 111.616 | 67.618 | 1.650 | 86.441 | 53.404 | 1.618 | 79.214 | 54.966 | 1.441 |
| Female | 173.783 | 110.069 | 1.578 | 166.944 | 103.769 | 1.608 | 124.575 | 93.759 | 1.328 | 94.683 | 73.941 | 1.280 |
| Juvenile | 740.387 | 61.286 | 12.080 | 83.468 | 50.714 | 1.645 | 64.655 | 45.798 | 1.411 | 52.134 | 33.109 | 1.574 |
| Mixed population | 118.732 | 82.307 | 1.442 | 116.537 | 63.520 | 1.834 | 86.532 | 31.836 | 2.718 | 69.317 | 30.193 | 2.295 |

UCL - Upper Confidence Limit; LCL - Lower Confidence Limit; R - Confidence Ratio (UCL/LCL)

**Table – 11 : 95 per cent confidence limits and their ratios for 24, 48, 72 and 96 h LC 50's of mercury for *Poecilia reticulata* (male, female, juvenile and mixed population) with water hardness of 270 mg/l at an average temperature of 16°C**

| Period / Test animals | 24 h | | | 48 h | | | 72 h | | | 96 h | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | UCL | LCL | R | UCL | LCL | R | UCL | LCL | R | UCL | LCL | R |
| Male | 354.138 | 241.181 | 1.468 | 356.522 | 225.189 | 1.583 | 270.526 | 209.173 | 1.293 | 245.570 | 193.112 | 1.271 |
| Female | 406.921 | 252.981 | 1.608 | 312.721 | 236.479 | 1.322 | 289.320 | 216.662 | 1.335 | 247.036 | 197.684 | 1.249 |
| Juvenile | 170.983 | 115.829 | 1.476 | 171.138 | 108.171 | 1.582 | 136.169 | 102.225 | 1.332 | 111.279 | 67.144 | 1.657 |
| Mixed population | 251.590 | 173.889 | 1.446 | 212.486 | 158.741 | 1.338 | 195.170 | 149.190 | 1.308 | 166.539 | 124.450 | 1.338 |

UCL - Upper Confidence Limit; LCL - Lower Confidence Limit; R - Confidence Ratio (UCL/LCL)

**Table – 12 : 95 per cent confidence limits and their ratios for 24, 48, 72 and 96 h LC 50's of mercury for *Poecilia reticulata* (male, female, juvenile and mixed population) with water hardness of 560 mg/l at an average temperature of 35°C**

| Period / Test animals | 24 h | | | 48 h | | | 72 h | | | 96 h | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | UCL | LCL | R | UCL | LCL | R | UCL | LCL | R | UCL | LCL | R |
| Male | 137.341 | 96.436 | 1.424 | 124.668 | 81.464 | 1.530 | 100.934 | 74.346 | 1.357 | 86.205 | 69.754 | 1.235 |
| Female | 267.917 | 168.159 | 1.593 | 291.026 | 143.272 | 2.031 | 173.103 | 102.991 | 1.680 | 143.692 | 77.030 | 1.965 |
| Juvenile | 108.624 | 72.078 | 1.507 | 104.873 | 64.425 | 1.627 | 105.253 | 55.393 | 1.900 | 84.290 | 38.434 | 2.193 |
| Mixed population | 128.298 | 97.941 | 1.309 | 146.282 | 91.577 | 1.597 | 116.303 | 84.481 | 1.376 | 93.456 | 74.044 | 1.262 |

UCL - Upper Confidence Limit; LCL - Lower Confidence Limit; R - Confidence Ratio (UCL/LCL)

**Table – 13 : 95 per cent confidence limits and their ratios for 24, 48, 72 and 96 h LC 50's of mercury for *Poecilia reticulata* (male, female, juvenile and mixed population) with water hardness of 560 mg/l at an average temperature of 16°C**

| Period / Test animals | 24 h | | | 48 h | | | 72 h | | | 96 h | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | UCL | LCL | R | UCL | LCL | R | UCL | LCL | R | UCL | LCL | R |
| Male | 382.593 | 268.488 | 1.424 | 345.546 | 250.226 | 1.380 | 289.603 | 234.246 | 1.236 | 269.075 | 189.464 | 1.420 |
| Female | 441.388 | 309.665 | 1.425 | 351.267 | 274.067 | 1.281 | 310.339 | 219.499 | 1.413 | 280.142 | 192.480 | 1.455 |
| Juvenile | 185.956 | 129.305 | 1.438 | 157.933 | 118.197 | 1.336 | 142.264 | 114.493 | 1.242 | 131.791 | 96.260 | 1.369 |
| Mixed population | 284.253 | 194.214 | 1.463 | 233.385 | 180.702 | 1.291 | 210.455 | 154.238 | 1.364 | 187.637 | 144.340 | 1.299 |

UCL - Upper Confidence Limit; LCL - Lower Confidence Limit; R - Confidence Ratio (UCL/LCL)

**Table – 14 : 95 per cent confidence limits and their ratios for 24, 48, 72 and 96 h LC 50's of mercury for the fingerlings of Indian major carps with water hardness of 270 mg/l at an average temperature of 35°C**

| Period / Test animals | 24 h | | | 48 h | | | 72 h | | | 96 h | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | UCL | LCL | R | UCL | LCL | R | UCL | LCL | R | UCL | LCL | R |
| Catla | 114.798 | 51.683 | 2.221 | 66.241 | 46.269 | 1.431 | 54.414 | 38.499 | 1.415 | 48.984 | 36.632 | 1.337 |
| Rohu | 409.602 | 254.868 | 1.607 | 326.718 | 228.511 | 1.429 | 240.239 | 190.625 | 1.260 | 219.552 | 150.788 | 1.456 |
| Mrigal | 657.224 | 294.814 | 2.229 | 373.350 | 271.427 | 1.375 | 333.715 | 245.579 | 1.358 | 303.946 | 230.667 | 1.317 |

UCL - Upper Confidence Limit; LCL - Lower Confidence Limit; R - Confidence Ratio (UCL/LCL)

**Table – 15 : 95 per cent confidence limits and their ratios for 24, 48, 72 and 96 h LC 50's of mercury for the fingerlings of Indian major carps with water hardness of 270 mg/l at an average temperature of 16°C**

| Period / Test animals | 24 h | | | 48 h | | | 72 h | | | 96 h | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | UCL | LCL | R | UCL | LCL | R | UCL | LCL | R | UCL | LCL | R |
| Catla | 244.844 | 70.562 | 3.469 | 96.591 | 58.659 | 1.646 | 72.093 | 43.859 | 1.643 | 61.343 | 27.087 | 2.264 |
| Rohu | 358.515 | 272.691 | 1.314 | 351.915 | 254.685 | 1.381 | 325.635 | 245.900 | 1.324 | 257.618 | 194.138 | 1.326 |
| Mrigal | 500.047 | 355.193 | 1.407 | 406.767 | 327.532 | 1.241 | 377.643 | 312.263 | 1.209 | 348.805 | 265.786 | 1.312 |

UCL - Upper Confidence Limit; LCL - Lower Confidence Limit; R - Confidence Ratio (UCL/LCL)

**Table – 16 : 95 per cent confidence limits and their ratios for 24, 48, 72 and 96 h LC 50's of mercury for the fingerlings of Indian major *carps with water hardness of 560 mg/l at an average temperature of 35°C***

| Period / Test animals | 24 h | | | 48 h | | | 72 h | | | 96 h | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | UCL | LCL | R | UCL | LCL | R | UCL | LCL | R | UCL | LCL | R |
| Catla | 740.387 | 61.286 | 12.080 | 83.468 | 50.714 | 1.645 | 71.113 | 48.049 | 1.480 | 54.324 | 33.834 | 1.605 |
| Rohu | 407.556 | 259.941 | 1.567 | 310.712 | 241.839 | 1.284 | 291.342 | 220.547 | 1.320 | 243.945 | 196.971 | 1.238 |
| Mrigal | 434.748 | 349.240 | 1.244 | 396.818 | 314.593 | 1.261 | 337.805 | 267.985 | 1.260 | 307.790 | 246.219 | 1.250 |

UCL - Upper Confidence Limit; LCL - Lower Confidence Limit; R - Confidence Ratio (UCL/LCL)

**able – 17 : 95 per cent confidence limits and their ratios for 24, 48, 72 and 96 h LC 50's of mercury for the fingerlings of Indian major carp with water hardness of 560 mg/l at an average temperature of 16°C**

| Period / Test animals | 24 h | | | 48 h | | | 72 h | | | 96 h | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | UCL | LCL | R | UCL | LCL | R | UCL | LCL | R | UCL | LCL | R |
| Catla | 54.324 | 33.834 | 1.605 | 175.093 | 70.492 | 2.483 | 94.891 | 33.030 | 2.872 | 83.601 | 46.621 | 1.793 |
| Rohu | 480.380 | 305.068 | 1.574 | 414.888 | 282.326 | 1.469 | 353.732 | 258.569 | 1.368 | 324.038 | 239.098 | 1.355 |
| Mrigal | 580.253 | 409.253 | 1.417 | 465.277 | 331.547 | 1.403 | 386.509 | 284.572 | 1.358 | 349.052 | 244.298 | 1.428 |

UCL - Upper Confidence Limit; LCL - Lower Confidence Limit; R - Confidence Ratio (UCL/LCL)

**Table -18 : Presumable Safe or harmless concentration ( C)* and safe dischargeable concentration (S)* of mercury ( μg/l) for *Poecilia reticulata* (male, female, juvenile and mixed population) and fingerlings of Indian major carp, *Catla catla, Labeo rohita* and *Cirrhinus mrigala* with different water hardness and temperature.**

| Test fish | Temperature 35 °C | | | | Temperature 16 °C | | | |
|---|---|---|---|---|---|---|---|---|
| | Water hardness 270 mg/l | | Water hardness 560 mg/l | | Water hardness 270 mg/l | | Water hardness 560 mg/l | |
| | C | S | C | S | C | S | C | S |
| ***Poecilia reticulata:*** | | | | | | | | |
| Male | 18.692 | 1.136 | 22.047 | 1.133 | 69.442 | 1.049 | 73.423 | 1.067 |
| Female | 32.257 | 1.049 | 42.196 | 1.106 | 66.619 | 1.079 | 71.56 | 1.129 |
| Juvenile | 12.133 | 1.220 | 19.280 | 1.079 | 33.228 | 1.051 | 33.039 | 1.093 |
| Mixed population | 17.516 | 1.162 | 29.486 | 1.028 | 44.274 | 1.095 | 51.042 | 1.085 |
| **Indian major carp:** | | | | | | | | |
| Catla | 12.360 | 1.133 | 12.133 | 1.220 | 14.642 | 1.195 | 19.689 | 1.180 |
| Rohu | 64.039 | 1.106 | 67.040 | 1.094 | 76.899 | 1.051 | 82.555 | 1.076 |
| Mrigal | 73.510 | 1.118 | 87.426 | 1.091 | 89.585 | 1.095 | 82.101 | 1.183 |

* μg/l

**Table-19 : Behavioural and morphological changes in the *Poecilia reticulata* (male, female and juvenile) during short-term toxicity tests of mercury.**

| Male | Female | Juvenile |
|---|---|---|
| 1. On introducing into the mercury solution, fish showed much excitement with increasing opercular movements in the higher concentrations. | 1. On introducing into the toxicant solution fish showed less excitement than male in the higher concentrations. | 1. juvenile also showed much excitement on introducing into the toxicant solution of higher concentrations. |
| 2. After 24 h besides excitement, fishes were moving rapidly up and down. | 2. After 48 h besides excitement, fishes were moving rapidly up and down with jerky movement. | 2. Juvenile showed excitement by moving up and down during 24 h. |
| 3. At the time of loss of equilibrium, fishes moved laterally side by side in the container. | 3. The fishes moving laterally and rubbing their body with glass jar at the time of loss of equilibrium. | 3. Juvenile showed rolling movement at the time of loss of equilibrium. |
| 4. After loss of equilibrium, fish stood in slanting position with decreasing opercular movement | 4. After loss of equilibrium few of the test specimens showed backward movement. | 4. After loss of equilibrium, juvenile showed rapid opercular movement. |
| 5. Test specimens showed large secretion of mucus from the body surface and gills | 5. Test specimens also showed large secretion of mucus from the body surface and gills. | 5. Juvenile also showed secretion of mucus from the body surface and gills. |
| 6. At the time of death, fish settled down on the bottom. | 6. At the time of death, body of the test fishes became arc shaped. | 6. Juvenile also showed arc shaped body at the time of death. |

**Table - 20 : Behavioural and morphological changes in the fingerlings of Indian major carps(catla, rohu and mrigal) during short-term toxic tests of mercury.**

| Catla | Rohu | Mrigal |
|---|---|---|
| 1. On introducing into the toxicant solution, test specimens showed much excitement. | 1. On introducing into the toxicant solution test specimens also showed slight excitement. | 1. Test specimens in the toxicant solution were much excited and tried to jump out. |
| 2. After 5-6 h of introduction of specimens into the toxicant solution, about 50-60% test fishes in all the mercury concentrations came to surface. | 2. After 5-6 h fishes were observed to move ups and down with some irritation. | 2. After 5-6 h test fishes were observed with normal movements. |
| 3. At the time of loss of equilibrium, fishes stood laterally with fast opercular movement. | 3. At the time of loss of equilibrium, fishes stood laterally and sometimes moved side by side. | 3. Test specimen came to water surface and float. |
| 4. At the time of death, fishes laid down laterally on the bottom side by side. | 4. At the time of death, fishes laid down laterally on the bottom side by side. | 4. At the time of loss of equilibrium, test fishes stood in slanting position with decrease in opercular movement. |
| 5. Test fishes secreted mucus from the body surface and gills. | 5. Test fishes also secreted excess mucus from the body surface and gills | 5. Test fishes also secreted mucus from the body surface and gills |
| 6. Colour of the skin changed to yellowish in the belly region | 6. Colour of the skin was not changed | 6. Colour of the skin was not changed |
| 7. After death no change in body shape was noticed | 7. After death no change in body shape was noticed. | 7. After death swellings were observed in the abdominal region. |

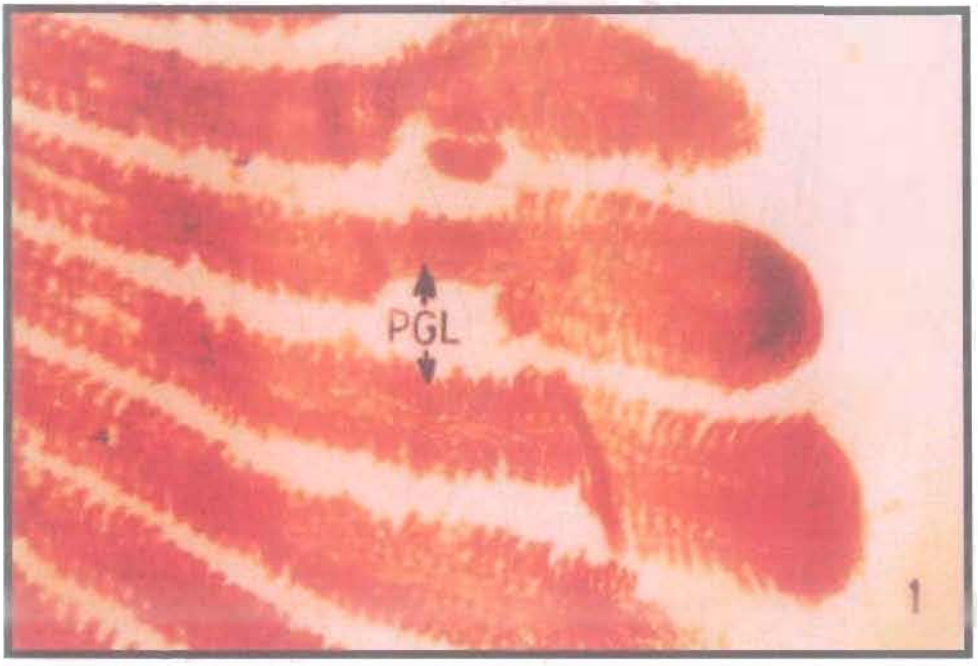



**Fig. 1:** Photomicrograph of the section of gill filament of *Cirrhinus mrigala* fingerlings treated with a sublethal concentration of 240µg/1 of mercury for 96h. (X-70). PGL-Primary gill lamellae showing degeneration in the middle and distal region.

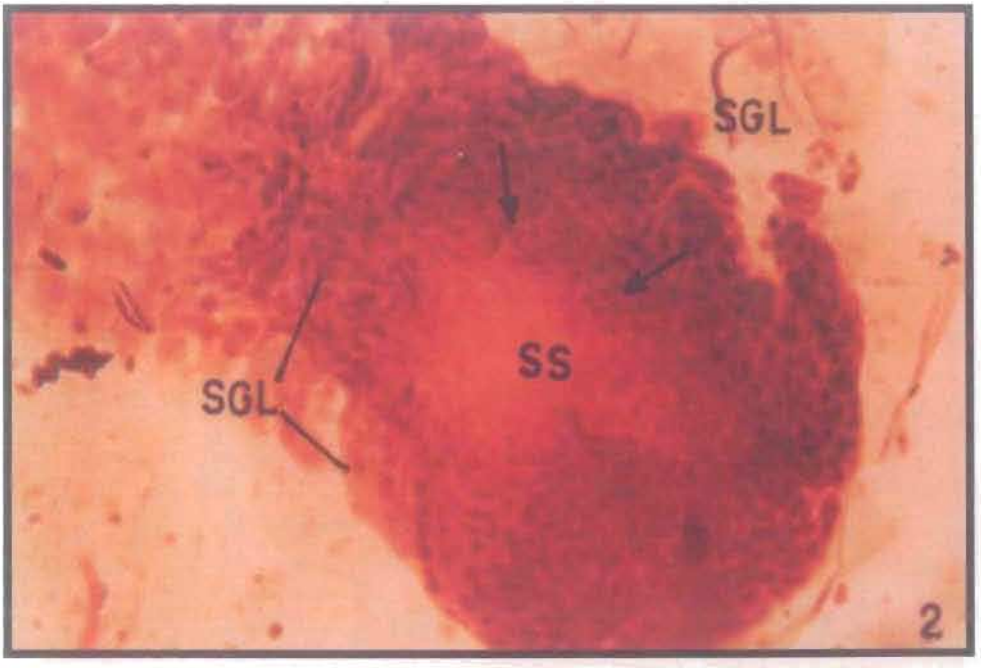



**Fig. 2:** Photomicrograph of the section of distal region of the gill filament of *Cirrhinus mrigala* fingerlings treated with a sublethal concentration of 240 µg/1 of mercury for 96h. (X-700) SS- Spherical structure showing complete degeneration and fusion of pillar cells, epithelial cells, mucous producing gland cells and blood cells in the secondary gill lamellae (SGL), Arrows indicate blood cells accumulated inside the spherical structure.

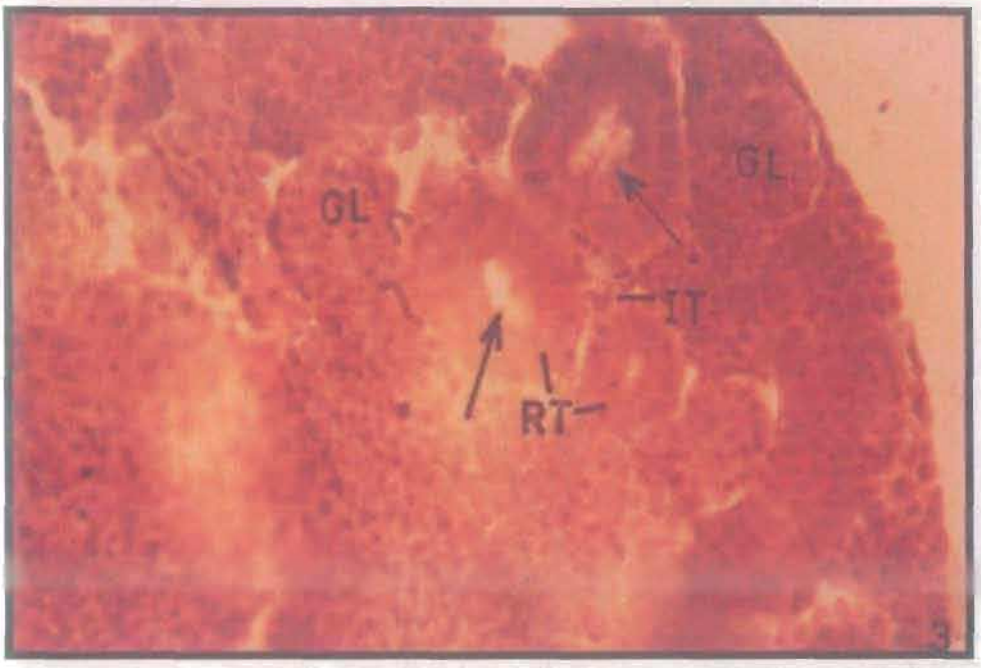



**Fig. 3:** Photomicrograph of the section of kidney of *Cirrhinus mrigala* fingerlings treated with a sublethal concentration of 240 µg/1 of mercury for 96h (X-700) RT- Renal tubule expanded, Arrows- indicate necrosis in epithelial lining the lumen, GL- Degeneration in the glomerulus, IT- Degeneration in institium tissue.

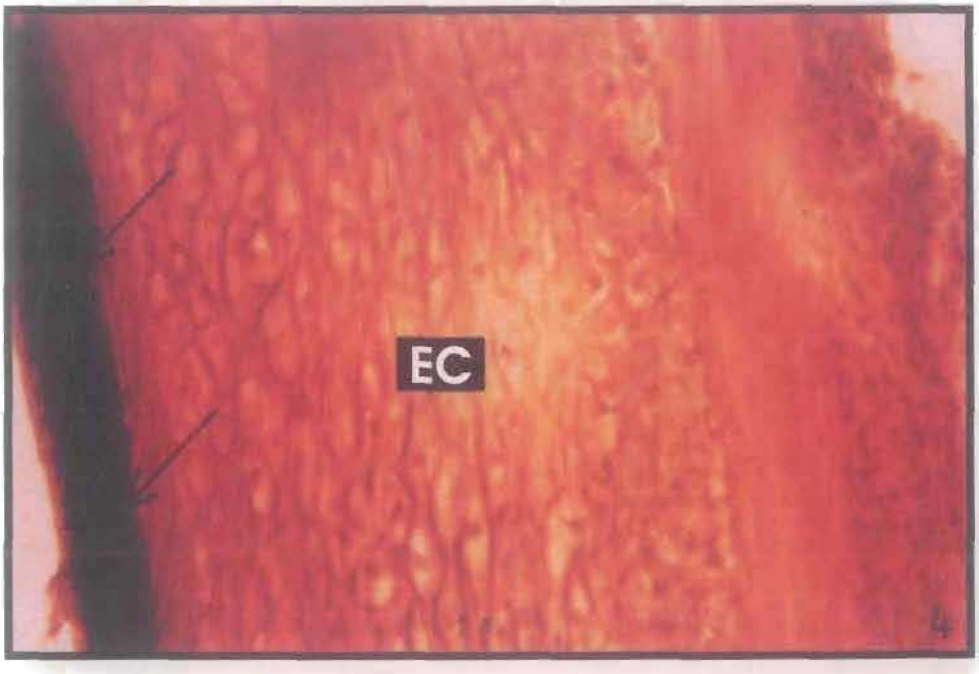
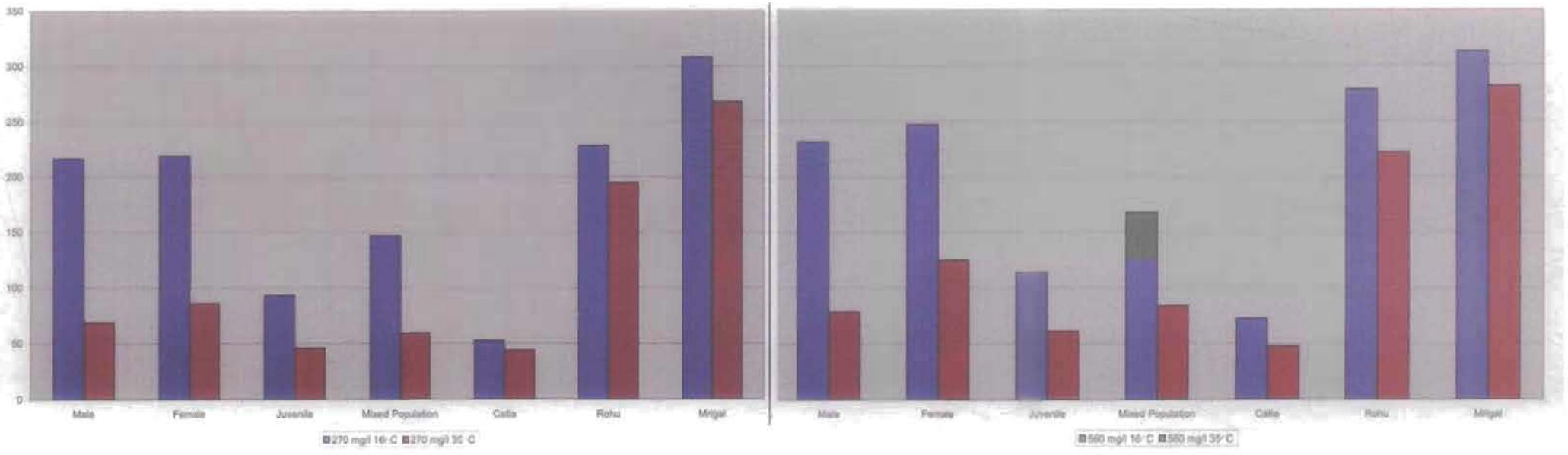



**Fig. 4:** Photomicrograph of the section of periorbital region of the eye of *Cirrhinus mrigala* fingerlings treated with a sublethal concentration of 240 µg/1 of mercury for 96h. (X-700) EC- Exudated choroid region, Arrows - Degenerated oedematous tissue or adjacent sclera.

Fig. 5 : Histogram showing comparison of 96h LC50 values for *Poecilia reticulata* (male, female, juvenile & mixed population) and Indian major carp (catla, rohu & mrigal) fingerlings for mercury ions at different water hardness and temperature.



# Chapter - 5

# DISCUSSION

# 5. DISCUSSION

In the present study short-term (96h) toxicity of mercury ions in relation to different water hardness and temperature has been evaluated for *Poecilia reticulata* (Peters) and fingerlings of Indian major carp, i.e. catla, rohu and mrigal. Results of the toxicity test were expressed as median lethal concentration (LC50), 95 per cent confidence limits and their ratios, relative sensitivity between different biological and physical factors, and presumable safe concentration. Besides these behavioural and histopathological studies have also been made in the test fishes in response to mercury ions. The median lethal concentration is widely used for measuring the acute toxicity as they are more reproducible in comparison to other values (Pickering and Henderson, 1966). From the results following observations have been made:

1. The magnitude of mercury toxicity was found high to *Poecilia reticulata* (male, female, juvenile and mixed population) and fingerlings of Indian major carp (catla, rohu and mrigal) as revealed from LC50 values.
2. However, significant difference in LC50 values for mercury ions was observed in test fishes.
3. Further, LC50 values for each test fish were changed with the change in water hardness and temperature.
4. The order of sensitivity for *Poecilia reticulata* to mercury ions was recored as juvenile > male > female.
5. Whereas, order of sensitivity for the fingerlings of indian major carp to mercury ions was observed as : catla > rohu > mrigal.
6. Significant variations in presumable safe or harmless concentrations for mercury ions were also noticed for *Poecilia reticulata* (male, female, juvenile and mixed population) and fingerlings of Indian major carp (catla, rohu and mrigal).
7. Test specimens were also showed abnormal behaviour and histopathological lesions in selected tissues in response to mercury ions.

The results of LC50 values in the present study are based on the nominal concentrations of mercury ions showed that it is relatively more toxic in water of low

hardness at higher temperature as compared to that of water of high hardness at both the temperature.

The 96h LC50 of mercury with water hardness of 560 mg/l at a temperature of 16°C for *Poecilia reticulata*, i.e. male, female juvenile and mixed population has been recorded maximum, i.e. 231.643, 247.278, 113.721 and 167.861 µg Hg/l respectively. However for the fingerlings of indian major carp, i.e. catla, rohu and mrigal, it was 72.729, 278.347 and 312.909 µg Hg/l respectively. These 96h LC50 values of mercury for such fishes were considerably higher than those reported for *Rasbora daniconius* (LC50-0.08 mg Hg/l; Gupta and Rajbanshi, 1995), *Notemigonus crysoleuces* and *Gambusia affinis* (LC50 – 16.75 and 52.62 µg Hg/l respectively ; Mccrary and Heagler, 1997). However 96h LC50 values of mercury with water hardness of 270 mg/l at a temperature of 35°C for *Poecilia reticulata, i.e.* male, female, juvenile and mixed population have been found minimum, i.e. 69.080, 86.262, 46.181 and 59.535 µgHg/l respectively. For fingerlings of Indian major carp, i.e. catla, rohu and mrigal, these minimum LC50 values were : 43.922, 194.612 and 268.141 µg Hg/l respectively. Similar trend of 96h LC50 values of copper for *Heteropneustes fossilis* has been recorded by Gupta and Rajbanshi (1995). Gupta and Rajbanshi (op. cit.) have also observed maximum LC50's (10.49 mg Cu/l) in may (temp. 31.3 ± 1.5°C), and minimum LC50 (9.44 mg Cu/l) in November (temperature 22 ± 1°C). It is interesting to note that 96h LC50 values of mercury with both the water hardness were lower as compared to that of cadmium ions in hard water : fathead minnow (0.56 – 1.01 mg Cd/l ; Pickering and Henderson, 1965); *Fundulus heteroclitus* (Voyer, 1975; Dorfman, 1977), *Heteropneustes fossilis* and *Channa punctatus* (14.60 – 20.60 mg/l and 6.81 – 7.40 mg/l; Gupta and Rajbanshi, 1991)and rainbow trout (96h LC50 – 2.6 mg/l; Pascoe *et al.*, 1986).

Buhl (1997) studied the toxic effects of four metals, viz. mercury, cadmium, hexavalent chromium and lead to larvae and juvenile stages of endangered colarado squafish (*Ptychocheilus eucius*), bony tail (*Gila elegans*) and razor back sucker (*Xyrouchen texanus*). He (op. cit.) found that larvae of each species were as sensitive or more sensitive than juvenile to cadmium, hexavalent chromium and mercury ions. Further Sukhovaskaya *et al.* (2001) observed that male of perch are more sensitive than female to elevated mercury concentrations during chronic exposure. Weir and

Walter (1976) found that immature (*Physa gyrina*) were three times more sensitive than mature one to cadmium. Results of the present study also showed similar trend of relative sensitivity for mercury ions to *Poecilia reticulata* : juvenile > male > female. Whereas, among the fungerlings of Indian major carp, following trend of relative sensitivity for mercury ions was recorded : catla > rohu > mrigal. Buhl (1997) also compared the results for juvenile of colarado squa fish, bony tail and razor back sucker with toxicity values reported for other freshwater fishes and found that their sensitivity to hexavalent chromium and mercury is similar to that of other cyprinids. Organism physiology may play an important role in organism sensitivities to mercury ions. According to Pickering and Fowler (1986), Fowler (1987), Olsson and Haux (1986), metal – binding protiens (e.g. metallothioneins) act as sinks or sequester metals such as zinc, copper, cadmium and mercury in organism tissues. Further, Luoma (1983) suggested that the quantity of metal transferred into biological tissues is influenced by both the physiological state of the organism and by biological factors involved in metal metabolism.

In the present study alterations in the behaviour of the test fishes such as abnormal behaviour, change in colour of the skin and mortality have been used as parameters to assess the toxicity of mercury ions. In higher concentrations of mercury, male of *Poecilia reticulata* moved laterally side by side in the container at the time of loss of equilibrium. Whereas female was rubbing their body with container. However, juvenile showed rolling movement at the time of loss of equilibrium. At the time of death, male showed no change in body shape, whereas the body of female and juvenile become arc shaped at the time of death. Gunstrom (1973 cited in : Weis and Weis, 1977) reported loss of equilibrium in coho salmon with abnormal behaviour including curling up of the test specimen exposed to lead. Whereas, Weis and Weis (1977a) reported a condition in which fry remained in the curled up embryonic position in lead treated *fundulus heteroclitus.*

Eaton (1974) and Benoit (1976) have observed following changes in behaviour; uncontrolled swimming movement, convulsions, loss of equilibrium and apparent coma in cadmium-exposed blue gills. Similar results were observed by Cearley (1971) in the bass and blue gills exposed to cadmium and silver. whereas Gupta and Rajbanshi (1991) reported that *Heteropneustes fossilis* exposed to

cadmium ions were laid down laterally on the bottom at the time of loss of equilibrium and death.

In present study behaviour of the fingerlings of Indian major carp was also altered when they were introduced to higher concentrations of mercury. Catla showed loss of equilibrium and lies laterally with fast opercular movement. Further, colour of the skin also changed to yellowish in the belly region at the time of death. Whereas rohu lies laterally and moved side by side, but mrigal stood in slanting position with decrease is opercular movement. Mrigal also showed swellings in the abdominal region, at the time of death. Gupta and Rajbanshi (1991) have also observed slanting position, decrease in opercular movement and loss of equilibrium in *Heteropneustes fossilis* treated with copper ions. Further, Gupta and Rajbanshi (op. cit.) reported that when *Heteropneustes fossilis* exposed to cadmium ions, colour of skin was changed from reddish to white in the belly region, and at the time of loss of equilibrium laid down on the bottom in the shape of ' , ' with less opercular movement. MacLeod and Pessah (1973) noticed visible signs of mercury poisoning in rainbow trout *Salmo gairdneri* such as loss of equilibrium, frequent surfacing and sinking and periodic bursts of erratic swimming.

Weis and Weis (1977a) studied the behaviour in lead treated killifish. These authors (op. cit.) observed that killifish, treated with lead exhibited lordosis, twitch and respond to tactile stimulation, but when relaxed return to curled up position. Whereas, Wiawood and Beamish (1978) noticed that copper affected highly the swimming performance of rainbow trout, *Salmo gairdneri* on exposure of five days. Cearley (1971) attributed that changes in behaviour may be due to inhibition of acetylcholinesterase resulting in paralysis and depression of the respiratory centre owing to which the fish collapsed. The present results agreed with the suggestions of cearley (1971) for significant changes in the behaviour and death of test fishes exposed to mercury ions during 96 hours static bioassay.

In the present investigation presumable safe or harmless and dischargeable concentrations have also been determined for mercury ions for their safe use in the waters for the test fishes as well as for other aquatic organisms also. The safe concentrations of mercury for *Poecilia reticulata* were ranged in between 18.692 to

73.423 µg/l for male; 32.257 to 71.56 µg/l for female ; 12.133 to 33.228 µg/l for juvenile and 17.516 to 51.042 µg/l for mixed population. Whereas, safe concentrations of mercury for the fingerlings of Indian major carp were ranged in between 12.133 to 19.689 µg/l for catla; 64.039 to 82.555 µg/l for rohu and 73.510 to 89.585 µg/l for mrigal at both the water hardness and temperature.

The values of presumable safe and dischargeable concentration obtained are interesting and significant since they are not constant with the succeeding experiments for both the temperature and water hardness. Davies *et al.* (1979), Mckim *et al.* (1979) and Gupta *et al.* (1994) also reported similar pattern of safe concentration for different metallic ions separately and in combination for different fish species. The 24h and 48 hours LC50 values in the present study varied greatly for the calculation of presumable safe concentration or harmless concentration. Probably some of the variation may be due the chemical reaction between mercury salt and diluent water and this in turn affect the sensitivity of the test fishes. The bioassays which were conducted with a diluent water of 270 mg/l hardness as $CaCO_3$ at a temperature of 35°C revealed that mercury ions were highly toxic for the test fishes. The present results agreed with Carroll *et al.* (1979) who suggested that calcium ions was most effective component of hard water in protecting fish brook trout against cadmium. Andrews (cited in : Pickering and Gast, 1972) held the view that cadmium was more acutely toxic at a higher pH. Voyer (1975) emphasized that resistance of mumichog, *Fundulus heteroclitus* in acute cadmium poisoning was not influenced by reduction in dissolved oxygen of about 4 mg/l and a salinity of 10 to 32%. Lloyd (1962) was of the opinion that the resistance of rainbow trout, *Salmo gairdneri* to copper, lead and zinc was inversely related to ambient oxygen level. Amend *et al.* (1969) observed the increased rate of death in rainbow trout which was subjected to ethyl mercury phosphate to a lower dissolved oxygen concentrations. Hasselrot (1968), Amend *et al.* (1969) and MacLeod and Pessah (1973) suggested that fishes are less tolerant to mercury in the water at warmer temperatures.

In a number of studies it has been suggested that no effect concentration become constant throughout the year for several successive generations of brook trout exposed to copper, lead and mercury (McKim and Benoit, 1974; Benoit *et al.*, 1976 : Holcombe *et al.*, 1976; McKim *et al.*, 1976). Mount and Stephan (1969) have

suggested that the application factor for a known toxicant, experimentally determined for one species of fish in a water, can be applied to other waters and species, i.e. the maximum safe concentration of a given toxicant could be estimated by determining the 96h LC50 value for the species in concerned water and multiplying the above by the previously determined application factor for that toxicant. In the present study safe concentrations of the mercury have been calculated by following formula propounded by Hart, Doudoroff and Greenbank (1945) for the calculation of safe concentration of chemicals, but the formula is used mainly for acute mortality test : C= (48h LC50 x 0.3)/S,[2] where $S^2$ = 24h LC50/ 48h LC50 (C is the harmless concentration and S represents safe dischargeable concentration).

Histopathological lesions were also studied in the gills, kidney and eyes of the fingerlings of *Cirrhinus mrigala* to evaluate the toxicity of a sublethal concentration of 240 µg/l of mercury. The mercury ions induced severe histopathological changes in gills such as degeneration in the middle and distal region in the primary lamellae. In the distal region spherical structure was formed due to degeneration and fusion in pillar cells, epithelial cells and mucus producing gland cells alongwith large number of blood cells of the secondary gill lamdlae. Khangarot and Somani (1980) have also exposed fish *Puntius sophore* to high levels of dissolved inorganic Hg or methyl mercury (MeHg) and suggested that mercury ions severly damaged the gill. Gupta and Rajbanshi (1995) have also studied the surface architecture of the gill of *Rasbora daniconius* treated with a sub lethal concentration of mercury (0.05 mg/l). These authors (op. cit.) also observed significant changes such as damage, fusion and clumping in the middle and distal parts of the gill lamellae resulting into considerable decrease in the over all surface area. Further, these authors (op. cit.) also reported swollen and disarrayed position in the secondary lamellae; and extensive mucus, blood cells and cellular worn-off were seen accumulated on the gill filament and in the spaces between secondary lamellae. Whereas Ribeiro *et al.* (1996) reported an increased proliferation in the interlamellar region lead to a thickening of the secondary lamellae of the gills of *Trichomycterus brasiliensis* exposed to mercury ions.

It is suggested that severe damage in gill of fish exposing to high levels of dissolved inorganic mercury or methyl mercury (Me Hg), interfered with

physiological processes involving the gills, including gas exchange and ions regulation (Renfro *et al.*, 1974; Borquegneau, 1977; Lock *et al.*, 1981; Stinson and Mallat, 1989; Gupta and Rajbanshi, 1995). Skidmore (1970), Eisler (1971), Burton *et al.* (1972), Bilinski and Jonas (1973), have also described the deleterious effect of heavy metals and suggested that the death of fish in acute poisoning was due to disruption of respiratory process caused by the damage of gill epithelium. Further, Skidmore (1970) suggested that the respiratory handicap imposed by lifting of the epithelium (as swellings) must out – weigh any protective effect against pollutant uptake in the later stage of acute poisoning. However Skidmore and Tovell (1972) in an electron microrscopic study of the effect of zinc on trout gills, found that although the gill reaction was primarily an inflammatory response, the chloride cells became detached early in a characteristic and prominent manner and the pillar cells became vacuolated.

Burton *et al.* (1972) demonstrated that tissue hypoxia precedes death when maximum gill ventilation is no longer efficient to supply the oxygen needs of trout because of gill deterioration due to which the gas exchange system is altered. Gardner and Yevich (1970), Skidmore and Tovell (1972), Bilinski and Jonas (1973), Gardner and LaRoche (1973) and Gupta and Rajbashi (1981, 1982, 1988 a & b and 1995) have the same opinion that the depression in respiratory activity is a common feature in acute metallic poisoning caused due to alterations in the cellular components of the gills of the fishes. The conclusion were drawn on the basis of the present findings and the information available from the study of other workers (op. cit.). The toxic substances affecting the gill mainly based on certain principles : I – the spaces between the gill filament become filled with precipitate, so that the water flowing through branchial chambers unable to reach the cells of the gill filaments, II- the spaces between the gill filaments completely block due to which circulation of the blood in the capillaries was affected, III- the affected blood circulation, in turn, led to heart block or heart action dropping to about half of the normal rate.

The pathological changes noticed in the kidney of mrigal fingerlings by a sublethal concentration of mercury include degeneration in the glomerulus and institium tissue, necrosis in epithelium lining the lumen and expansion in renal tubules which possibly caused the impairment of kidney function. Sastry and

Agrawal (1977) have also noticed desquammation of the tubular cells, enlargement and pycnosis of nuclei, shrinkage of glomerular network, rupture of the glomerular wall and haemorrhages in the interstitial haematopoietic tissue in the kidney of *Cirrhinus mrigala* treated with mercuric chloride. Whereas Ribeiro *et al.* (1996) have reported that the kidney of *Trichomycterus brasiliensis* treated with mercury were disorganised and tubule cells decrease in number and change in size.

Gupta and Rajbanshi (1979 & 1986) have observed pathological symptoms in kidney of *Heteropneustes fossilis* and *Channa punctatus* exposed to different concentrations of copper include modifications in renal tubules by expansion and necrosis in the outer and inner epithelial lining and accumulation of cellular debris. Further, Gupta and Rajbanshi (1982 & 1988b) have also revealed notable pathological changes in the kidney of *Heteropneustes fossilis* and *Channa punctatus* for cadmium ions include accumulation of cellular debris in bowman's capsule and expansion of renal tubules towards inner and outer sides. Presumably, the observed pathological alterations in the kidney of mrigal fingerlings may be due to the internal exhaustion that brought about a change in the metabolic activity as a result of interaction between metallic ions and renal tissue through blood, resulting in disorder of the divalent ions as also suggested by Rasquin and Rasenbloom (1954). Trump and Bulger (1967), Kendell (1977), and Trump *et al.* (1975) in their superb studies have described both light microscopical and ultrastructural changes in the tubules associated with mercury poisoning in the southern flounder. Such studies have shown the main effects of levels of mercury as binding S-H groups to the protein of cell membranes, thus inhibiting enzyme system.

In the present investigation eye of the mrigal fingerlings exposed to a sublethal concentration of 240 µg/l of mercury ions also revealed histopathological lesions in the periorbital region include exudation by inflammatory cellular infiltration in choroids tissue and remarkable degeneration in oedematous tissue or adjacent sclera. Weis and Weis (1977b) studied the development of killifish in the methyl mercury – exposed groups revealed a spectrum of optic and cephalic abnormalities, the appearance of which correlated with dose level and length of time exposure. These authors (op. cit., 1977a) further reported that in even more severe cases the entire axis is reduced in size and the embryo can consist of only a cyclopic head.

anophthalmic head, or, in some cases no axis at all. Ribeiro *et al.* (1996) have recorded that optic nerve of *Trichomycterus brasiliensis* in mercury concentrations show disorganised disposition of axons and mainly disruption and dissociation of myelin sheath, leading to decrease in motility and coordination. The pathological lesion in the periorbital region of the eye may in turn produce abnormalities in the optic nerve and cephalic region of the brain leading to decrease in motility and coordination in the fingerlings of mrigal.

The results of the present investigation suggest that further study is needed to determine chronic and/or long-term extensive effects of mercury for survival, growth and reproduction in the context of various physiological and biochemical indices for the fishes. The investigation is also needed to assess the impact of stresses caused due to mercury ions on fish diversity in the aquatic ecosystem. The studies on biomonitoring are also required at different trophic levels for those aquatic ecosystem which are particularly affected due to mercury pollution.

# Chapter - 6

# SUMMARY

# 6. SUMMARY

In the present study an attempt has been made to investigate the short-term (96h) toxic effects of mercury in relation to water hardness and temperature to a freshwater fish, *Poecilia reticulata* for collective (mixed population), sex wise (male and female) and young fish (juvenile), considering various parameters such as LC50, 95% confidence limit and their ratios, relative sensitivities and presumable safe or harmless concentrations. The toxicity tests with mercury have also been performed for selected fingerlings of commercially important fish species of Indian major carp, i.e. *Catla catla*, *Labeo rohita* and *Cirrhinus mrigala*. The behavioural, and histopathological changes in the test organisms in response to mercury ions have also been studied.

The static bioassay study revealed that the mercury ions were highly toxic not only to the *Poecilia reticulata* (male, female, juvenile and mixed population) but also to the fingerlings of Indian major carp (catla, rohu and mrigal). The LC50's of mercury for these test fish, for the time intervals of 24, 48, 72 and 96 hours were found to change significantly with the change in water hardness and temperature. The highest 96 h LC50 values of mercury for the male, female and juvenile of *Poecilia reticulata* were noticed as : 231.643, 247.278 and 113.721 µg/l respectively with water hardness of 560 mg/l at a temperature of 16°C. Whereas the lowest 96h LC50 values of mercury for these fishes were recorded as:69.080, 86.262 and 46.181 µg/l respectively with water hardness of 270 mg/l at a temperature of 35°C. However, the highest 96h LC50 values of mercury for the fingerlings of Indian major carp, i.e. catla, rohu and mrigal were noticed as : 72.729, 278.347 and 312.909 µg/l

respectively with water hardness of 560 mg/l at a temperature of 16°C. Whereas the lowest 96h LC50 values of mercury for these test fishes were recorded as : 43.922, 194.612 and 268.141 µg/l respectively with water hardness of 270 mg/l at a temperature of 35°C. On the basis of LC50 values it is concluded that all the test fishes were found most resistant with water hardness of 560 mg/l at a temperature of 16°C as compared to that of water hardness of 560 mg/l at a temperature of 35°C and water hardness of 270 mg/l at both the temperatures, i.e. 35 and 16°C.

Relative sensitivities based on LC50's of mercury for test fishes have also been estimated. The results showed following trend of relative sensitivity of mercury ions for *Poecilia reticulata* : Juvenile > male > female. The relative sensitivity of mixed population, however, showed approximately equal to male and juvenile. Whereas in case of fingerlings of Indian major carp, following trend for relative sensitivity of mercury ions was recorded : catla > rohu > mrigal.

The 95 per cent confidence limits, i.e. lower confidence limit (LCL) and upper confidence limit (UCL) and their ratios for LC50 values were also estimated. The smaller ratio or smaller spread between 95 per cent confidence limits recorded for different periods of 24, 48, 72 and 96h in static bioassay indicates the betterness of the tests.

In the present investigations presumable safe or harmless concentrations of mercury ions were also determined. The safe concentrations of mercury ions for *Poecilia reticulata* were ranged in between :18.692 to 73.423 µg/l for male ; 32.257 to 71.560 µg/l for female ; 12.133 to 33.228 for juveniles and 17.516 to 51.042 µg/l for mixed population with water hardness of 270 and 560 mg/l at the temperature of 35 and 16°C. Whereas safe concentrations of mercury for the fingerlings of Indian major

carp were ranged in between : 12.133 to 19.189 µg/l for catla; 64.039 to 82.555 µg/l for rohu ; and 73.510 to 89.585 µg/l for mrigal for both the water hardness and temperatures.

Changes in the behaviour of test fishes in response to different test concentrations of mercury were also observed. In higher concentrations of mercury, male of *Poecilia reticulata* showed loss of equilibrium and move laterally side by side in the container. Whereas female was rubbing their body with the glass jar. However, juvenile showed rolling movement at the time of loss of equilibrium. At the time of death, male showed no change in body shape whereas the body of female and juvenile become arc shaped at the time of death. In higher concentrations of mercury, catla showed loss of equilibrium and stood laterally with fast opercular movement. At the time of death, colour of the skin changed to yellowish in the belly region. Whereas rohu stood laterally and moved side by side, but mrigal stood in slanting position with decrease in opercular movement. Mrigal also showed swelling in the abdominal region at the time of death.

Histopathological changes in the selected tissues such as gills, kidney and eye of the fingerlings of *Cirrhinus mrigala* exposed to a sublethal concentration of 240 µg/l of mercury were also studied. The primary gill lamellae showed degeneration in the middle and distal region. In the distal region of the gill filament, spherical structure showing complete degeneration and fusion of pillar cells, epithelial cell, mucous producing gland cells and blood cells in the secondary gill lamellae. Degeneration in the glomerulus and institium tissue was also observed. Further, necrosis in epithelium lining the lumen and expansion in renal tubules were also seen in the mercury treated fingerlings. Whereas periorbital region of the eye of mrigal

showed exudation in choroids layer and degeneration in oedematous tissue or adjacent sclera.

The physico-chemical characteristics of the experimental waters of different water hardness were also analysed and discussed in relation to toxicity of mercury at two different temperatures.

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# ABSTRACT

## (ENGLISH & HINDI)

# SHORT-TERM TOXICITY OF MERCURY IN RELATION TO WATER HARDNESS AND TEMPERATURE TO *Poecilia reticulata* (Peters) AND SELECTED INDIAN MAJOR CARP

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## ABSTRACT

In the present study short-term (96h) toxicity of mercury in relation to water hardness and temperature to *Poecilia reticulata* (male, female, juvenile and mixed population) and selected Indian major carp, i.e. catla, rohu and mrigal has been evaluated using static bioassay. Median lethal concentrations (LC50's) of mercury for these test fishes for the time intervals of 24, 48, 72 and 96 hours with water hardness of 560 mg/l, at a temperature of 16°C have been recorded highest as compared to that of water hardness of 560 mg/l, at a temperature of 35°C and water hardness of 270 mg/l at both the temperature, i.e. 35 and 16°C. Among the *Poecilia reticulata*, following trend of sensitivities have been recorded for the mercury ions : juvenile > male > female. Whereas, among the fingerlings of Indian major carp, following trend of sensitivity to mercury ions have been observed : catla > rohu > mrigal.

The presumable safe concentrations of mercury for *Poecilia reticulata* ranged in between :18.692 to 73.423 µg/l for male; 32.257 to 71.56 µg/l for female; 12.133 to 33.228 for juvenile and 17.516 to 51.042 µg/l for mixed population. Whereas, presumable safe concentrations for fingerlings of Indian major carp ranged in between:12.133 to 19.689 µg/l for catla ; 64.039 to 82.555 µg/l for rohu and 73.510 to 89.585 µg/l for mrigal with both the water hardness of 270 and 560 mg/l and at both the temperatures of 35 and 16°C. The test fishes in mercury solution have also showed pronounced symptoms of stress in their behaviour. Histopathological changes in the selected tissues such as gills, kidney and eyes of fingerlings of *Cirrhinus mrigala* have also revealed the toxic nature of mercury ions.

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# जलीय कठोरता एवं तापमान के सम्बन्ध में पारद की पोयसिलिया रेटीकुलेटा (पीटर्स) एवं चयनित भारतीय शफ़र मीन पर अंशकालिक विषाक्तता

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## अनुक्षेपण

इस शोधकार्य के अंतर्गत, जलीय कठोरता एवं तापमान के संबंध में पारद की, पोयसिलिया रेटीकुलेटा (नर, मादा, तरूण एवं मिश्रित जनसंख्या) एवं चयनित भारतीय शफ़र मीन जैसे – कतला, रोहू एवं मृगल पर स्थिर जैविक विश्लेषण द्वारा अंशकालिक (96 घंटे) विषाक्तता का विश्लेषण किया गया। उपरोक्त प्रयोग में ली गई मछलियों के प्रतिरूप के लिए मध्यम मृत्युकर सान्द्रता (LC50) 24, 48, 72 एवं 96 घण्टों के समय अन्तराल पर 560 मिलीग्राम जलीय कठोरता एवं 16 डिग्री सेन्टीग्रेड तापमान पर, जलीय कठोरता 560 मिलीग्राम 35 डिग्री सेन्टीग्रेड तापमान एवं जलीय कठोरता 270 मिलीग्राम/लीटर और दोनों तापमानों 35 डिग्री सेन्टीग्रेड एवं 16 डिग्री सेन्टीग्रेड की तुलना में उच्चतम पाई गई।

प्रयोग में ली गई पोयसिलिया रेटीकुलेटा मछलियों में पारद के प्रति संवेदनशीलता का झुकाव निम्नानुसार जैसे : तरूण > नर > मादा, जबकि भारतीय शफ़र मीन की अंगुलिकाओं पर यह झुकाव कतला > रोहू > मृगल के क्रम में पाया गया।

पोयसिलिया रेटीकुलेटा के लिए पारद की अनुमानित सुरक्षित सांन्द्रता 18.692 से 73.423 माइक्रो ग्राम/लीटर नर के लिए, 32.257 से 71.561 माइक्रो ग्राम/लीटर मादा के लिए, 12.133 से 33.228 माइक्रो ग्राम/लीटर तरूण के लिए, एवं 17.516 से 51.042 माइक्रो ग्राम/लीटर मिश्रित जनसंख्या के लिए पायी गई, जबकि भारतीय शफ़र मीन की अंगुलिकाओं के लिए, अनुमानित सुरक्षित सांन्द्रता 12.133 से 19.689 माइक्रों ग्राम/लीटर कतला के लिए, 64.039 से 82.555 माइक्रों ग्राम/लीटर रोहू के लिए, एवं 73.510 से 89.588 माइक्रो ग्राम/लीटर मृगल के लिए, दोनों जलीय कठोरताओं (270 एवं 560 मिलीग्राम/लीटर ) के साथ दोनों तापमानों (35 एवं 16 डिग्री सेन्टीग्रेड) पर पायी गयी।

पारद के घोल में प्रयोग में ली गई मछलियों ने अपने व्यवहार में दबाव के निर्णायक लक्षण भी दर्शाये। सिरहिनस मृगला की अंगुलिकाओं के चयनित उत्तकों जैसे गलफड़े, गुर्दा एवं आंखों में उत्तक रोगीय परिवर्तनों द्वारा पारद की विषाक्तता की प्रकृति भी दर्शायी।

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# APPENDIX-1

**Table-1 : Number and per cent ( ) of mortality of male of *Poecilia reticulata* in different concentrations of mercury during 96 h static bioassay with water hardness of 270 mg/l at a temperature of 35°C.**

| S.No. | Hg (µg/l) | No. and percentage of test animals | Number and per cent mortality of male | | | |
|---|---|---|---|---|---|---|
| | | | 24 h | 48 h | 72 h | 96 h |
| 1. | 100 | 10 (100%) | 6 (60) | 7 (70) | 8 (80) | 9 (90) |
| 2. | 87 | 10 (100%) | 4 (40) | 6 (60) | 7 (70) | 7 (70) |
| 3. | 75 | 10 (100%) | 3 (30) | 4 (40) | 5 (50) | 6 (60) |
| 4. | 65 | 10 (100%) | 2 (20) | 3 (30) | 4 (40) | 4 (40) |
| 5. | 56 | 10 (100%) | 0 (00) | 2 (20) | 3 (30) | 3 (30) |
| 6. | 00 (Control) | 10 (100%) | 0 (00) | 0 (00) | 0 (00) | 0 (00) |

**Table-2 : Number and per cent ( ) of mortality of female of *Poecilia reticulata* in different concentrations of mercury during 96 h static bioassay with water hardness of 270 mg/l at a temperature of 35°C.**

| S.No. | Hg (µg/l) | No. and percentage of test animals | Number and per cent mortality of female | | | |
|---|---|---|---|---|---|---|
| | | | 24 h | 48 h | 72 h | 96 h |
| 1. | 135 | 10 (100%) | 6 (60) | 7 (70) | 8 (80) | 10 (100) |
| 2. | 115 | 10 (100%) | 4 (40) | 4 (40) | 6 (60) | 9 (90) |
| 3. | 100 | 10 (100%) | 2 (20) | 3 (30) | 4 (40) | 7 (70) |
| 4. | 87 | 10 (100%) | 2 (20) | 2 (20) | 3 (30) | 5 (50) |
| 5 | 75 | 10 (100%) | 0 (00) | 1 (10) | 1 (10) | 3 (30) |
| 6. | 00 (Control) | 10 (100%) | 0 (00) | 0 (00) | 0 (00) | 0 (00) |

**Table-3 : Number and per cent ( ) of mortality of juvenile of *Poecilia reticulata* in different concentrations of mercury during 96 h static bioassay with water hardness of 270 mg/l at a temperature of 35°C.**

| S.No. | Hg (µg/l) | No. and percentage of test animals | Number and per cent mortality of Juvenile | | | |
|---|---|---|---|---|---|---|
| | | | 24 h | 48 h | 72 h | 96 h |
| 1. | 75 | 10 (100%) | 5 (50) | 7 (70) | 8 (80) | 10 (100) |
| 2. | 65 | 10 (100%) | 4 (40) | 6 (60) | 7 (70) | 8 (80) |
| 3. | 56 | 10 (100%) | 3 (30) | 4 (40) | 5 (50) | 7 (70) |
| 4. | 49 | 10 (100%) | 2 (20) | 3 (30) | 4 (40) | 6 (60) |
| 5 | 42 | 10 (100%) | 1 (10) | 2 (20) | 2 (20) | 4 (40) |
| 6. | 00 (Control) | 10 (100%) | 0 (00) | 0 (00) | 0 (00) | 0 (00) |

**Table-4 : Number and per cent ( ) of mortality of mixed population of *Poecilia reticulata* in different concentrations of mercury during 96 h static bioassay with water hardness of 270 mg/l at a temperature of 35°C.**

| S.No. | Hg (µg/l) | No. and percentage of test animals | Number and per cent mortality of mixed population | | | |
|---|---|---|---|---|---|---|
| | | | 24 h | 48 h | 72 h | 96 h |
| 1. | 100 | 10 (100%) | 6 (60) | 7 (70) | 8 (80) | 9 (90) |
| 2. | 87 | 10 (100%) | 4 (40) | 6 (60) | 6 (60) | 8 (80) |
| 3. | 75 | 10 (100%) | 3 (30) | 4 (40) | 6 (60) | 8 (80) |
| 4. | 65 | 10 (100%) | 1 (10) | 4 (40) | 5 (50) | 6 (60) |
| 5 | 56 | 10 (100%) | 0 (00) | 2 (20) | 3 (30) | 4 (40) |
| 6. | 00 (Control) | 10 (100%) | 0 (00) | 0 (00) | 0 (00) | 0 (00) |

**Table- 5 : Number and per cent ( ) of mortality of the fingerlings of Catla in different concentrations of mercury during 96h static bioassay with water hardness of 270 mg/l at a temperature of 35°C**

| S.No. | Hg (µg/l) | No. and percentage of test animals | Number and per cent of mortality of Catla fingerlings | | | |
|---|---|---|---|---|---|---|
| | | | 24 h | 48 h | 72 h | 96 h |
| 1. | 65 | 10 (100%) | 6 (60) | 7 (70) | 8 (80) | 9 (90) |
| 2. | 56 | 10 (100%) | 4 (40) | 6 (60) | 7 (70) | 8 (80) |
| 3. | 49 | 10 (100%) | 3 (30) | 4 (40) | 6 (60) | 7 (70) |
| 4. | 42 | 10 (100%) | 2 (20) | 3 (30) | 4 (40) | 5 (50) |
| 5 | 37 | 10 (100%) | 1 (10) | 1 (10) | 2 (20) | 2 (20) |
| 6. | 00 (Control) | 10 (100%) | 0(00) | 0(00) | 0(00) | 0(00) |

**Table- 6: Number and per cent ( ) of mortality of the fingerlings of Rohu in different concentrations of mercury during 96h static bioassay with water hardness of 270 mg/l at a temperature of 35°C**

| S.No. | Hg (µg/l) | No. and percentage of test animals | Number and per cent of mortality of Rohu fingerlings | | | |
|---|---|---|---|---|---|---|
| | | | 24 h | 48 h | 72 h | 96 h |
| 1. | 320 | 10 (100%) | 6 (60) | 7 (70) | 10 (100) | 10 (100) |
| 2. | 280 | 10 (100%) | 5 (50) | 6 (60) | 8 (80) | 9 (90) |
| 3. | 240 | 10 (100%) | 3 (30) | 4 (40) | 6 (60) | 7 (70) |
| 4. | 210 | 10 (100%) | 1 (10) | 3 (30) | 5 (50) | 6 (60) |
| 5 | 180 | 10 (100%) | 1 (10) | 1 (10) | 2 (20) | 4 (40) |
| 6. | 00 (Control) | 10 (100%) | 0 (00) | 0 (00) | 0 (00) | 0 (00) |

**Table- 7 : Number and per cent ( ) of mortality of the fingerlings of Mrigal in different concentrations of mercury during 96h static bioassay with water hardness of 270 mg/l at a temperature of 35°C**

| S.No. | Hg (µg/l) | No. and percentage of test animals | Number and per cent of mortality of Mrigal fingerlings | | | |
|---|---|---|---|---|---|---|
| | | | 24 h | 48 h | 72 h | 96 h |
| 1. | 370 | 10 (100%) | 6 (60) | 7 (70) | 8 (80) | 9 (90) |
| 2. | 320 | 10 (100%) | 4 (40) | 6 (60) | 7 (70) | 7 (70) |
| 3. | 280 | 10 (100%) | 3 (30) | 4 (40) | 4 (40) | 5 (50) |
| 4. | 240 | 10 (100%) | 2 (20) | 2 (20) | 3 (30) | 4 (40) |
| 5 | 210 | 10 (100%) | 1 (10) | 1 (10) | 2 (20) | 2 (20) |
| 6. | 00 (Control) | 10 (100%) | 0 (00) | 0 (00) | 0 (00) | 0 (00) |

**Table-8 : Number and per cent ( ) of mortality of male of *Poecilia reticulata* in different concentrations of mercury during 96 h static bioassay with water hardness of 560mg/l at a temperature of 35°C.**

| S.No. | Hg (µg/l) | No. and percentage of test animals | Number and per cent of mortality of male | | | |
|---|---|---|---|---|---|---|
| | | | 24 h | 48 h | 72 h | 96 h |
| 1. | 115 | 10 (100%) | 6 (60) | 7 (70) | 8 (80) | 10 (100) |
| 2. | 100 | 10 (100%) | 4 (40) | 6 (60) | 7 (70) | 8 (80) |
| 3. | 87 | 10 (100%) | 2 (20) | 4 (40) | 5 (50) | 7 (70) |
| 4. | 75 | 10 (100%) | 1 (10) | 2 (20) | 3 (30) | 4 (40) |
| 5. | 65 | 10 (100%) | 0(00) | 2 (20) | 2 (20) | 2 (20) |
| 6. | 00 (Control) | 10 (100%) | 0(00) | 0 (00) | 0 (00) | 0 (00) |

**Table-9 : Number and per cent ( ) of mortality of female of *Poecilia reticulata* in different concentrations of mercury during 96 h static bioassay with water hardness of 560 mg/l at a temperature of 35°C.**

| S.No. | Hg (µg/l) | No. and percentage of test animals | Number and per cent of mortality of female | | | |
|---|---|---|---|---|---|---|
| | | | 24 h | 48 h | 72 h | 96 h |
| 1. | 210 | 10 (100%) | 6 (60) | 7 (70) | 8 (80) | 9 (90) |
| 2. | 180 | 10 (100%) | 4 (40) | 5 (50) | 7 (70) | 9 (90) |
| 3. | 155 | 10 (100%) | 3 (30) | 4 (40) | 6 (60) | 7 (70) |
| 4. | 135 | 10 (100%) | 2 (20) | 3 (30) | 4 (40) | 6 (60) |
| 5. | 115 | 10 (100%) | 0(00) | 2 (20) | 3 (30) | 4 (40) |
| 6. | 00 (Control) | 10 (100%) | 0(00) | 0(00) | 0(00) | 0 (00) |

**Table-10 : Number and per cent ( ) of mortality of juvenile of *Poecilia reticulata* in different concentrations of mercury during 96 h static bioassay with water hardness of 560 mg/l at a temperature of 35°C.**

| S.No. | Hg (µg/l) | No. and percentage of test animals | Number and per cent of mortality of Juvenile | | | |
|---|---|---|---|---|---|---|
| | | | 24 h | 48 h | 72 h | 96 h |
| 1. | 87 | 10 (100%) | 6 (60) | 7 (70) | 7 (70) | 8 (80) |
| 2. | 75 | 10 (100%) | 4 (40) | 5 (50) | 6 (60) | 6 (60) |
| 3. | 65 | 10 (100%) | 2 (20) | 3 (30) | 4 (40) | 5 (50) |
| 4. | 56 | 10 (100%) | 1 (10) | 2 (20) | 3 (30) | 4 (40) |
| 5. | 49 | 10 (100%) | 0(00) | 1 (10) | 2 (20) | 3 (30) |
| 6. | 00 (Control) | 10 (100%) | 0(00) | 0 (00) | 0 (00) | 0 (00) |

**Table-11 : Number and per cent ( ) of mortality of mixed population of *Poecilia reticulata* in different concentrations of mercury during 96 h static bioassay with water hardness of 560 mg/l at a temperature of 35°C.**

| S.No. | Hg (µg/l) | No. and percentage of test animals | Number and per cent of mortality of mixed population | | | |
|---|---|---|---|---|---|---|
| | | | 24 h | 48 h | 72 h | 96 h |
| 1. | 115 | 10 (100%) | 6 (60) | 6 (60) | 7 (70) | 9 (90) |
| 2. | 100 | 10 (100%) | 4 (40) | 5 (50) | 6 (60) | 7 (70) |
| 3. | 87 | 10 (100%) | 2 (20) | 3 (30) | 4 (40) | 6 (60) |
| 4. | 75 | 10 (100%) | 0(00) | 1 (10) | 2 (20) | 4 (40) |
| 5. | 65 | 10 (100%) | 0(00) | 1 (10) | 1 (10) | 1 (10) |
| 6. | 00 (Control) | 10 (100%) | 0(00) | 0(00) | 0(00) | 0(00) |

**Table-12 : Number and per cent ( ) of mortality of the fingerlings of Catla in different concentrations of mercury during 96h static bioassay with water hardness of 560 mg/l at a temperature of 35°C**

| S.No. | Hg (µg/l) | No. and percentage of test animals | Number and per cent of mortality of Catla fingerlings | | | |
|---|---|---|---|---|---|---|
| | | | 24 h | 48 h | 72 h | 96 h |
| 1. | 75 | 10 (100%) | 5 (50) | 7 (70) | 8 (80) | 9 (90) |
| 2. | 65 | 10 (100%) | 4 (40) | 6 (60) | 6 (60) | 8 (80) |
| 3. | 56 | 10 (100%) | 3 (30) | 4 (40) | 4 (40) | 7 (70) |
| 4. | 49 | 10 (100%) | 2 (20) | 3 (30) | 4 (40) | 6 (60) |
| 5. | 42 | 10 (100%) | 1 (10) | 2 (20) | 2 (20) | 3 (30) |
| 6. | 00 (Control) | 10 (100%) | 0(00) | 0(00) | 0(00) | 0(00) |

**Table-13 : Number and per cent ( ) of mortality of the fingerlings of Rohu in different concentrations of mercury during 96h static bioassay with water hardness of 560 mg/l at a temperature of 35°C**

| S.No. | Hg (µg/l) | No. and percentage of test animals | Number and per cent of mortality of Rohu fingerlings | | | |
|---|---|---|---|---|---|---|
| | | | 24 h | 48 h | 72 h | 96 h |
| 1. | 320 | 10 (100%) | 6 (60) | 7 (70) | 8 (80) | 10 (100) |
| 2. | 280 | 10 (100%) | 4 (40) | 6 (60) | 6 (60) | 8 (80) |
| 3. | 240 | 10 (100%) | 3 (30) | 4 (40) | 5 (50) | 6 (60) |
| 4. | 210 | 10 (100%) | 2 (20) | 2 (20) | 3 (30) | 4 (40) |
| 5. | 180 | 10 (100%) | 0(00) | 0 (00) | 1 (10) | 2 (20) |
| 6. | 00 (Control) | 10 (100%) | 0(00) | 0 (00) | 0(00) | 0(00) |

**Table-14 : Number and per cent ( ) of mortality of the fingerlings of Mrigal in different concentrations of mercury during 96h static bioassay with water hardness of 560 mg/l at a temperature of 35°C**

| S.No. | Hg (µg/l) | No. and percentage of test animals | Number and per cent of mortality of Mrigal fingerlings | | | |
|---|---|---|---|---|---|---|
| | | | 24 h | 48 h | 72 h | 96 h |
| 1. | 420 | 10 (100%) | 7 (70) | 8 (80) | 9 (90) | 10 (100) |
| 2. | 370 | 10 (100%) | 4 (40) | 6 (60) | 8 (80) | 9 (90) |
| 3. | 320 | 10 (100%) | 3 (30) | 4 (40) | 6 (60) | 7 (70) |
| 4. | 280 | 10 (100%) | 0 (00) | 1 (10) | 3 (30) | 4 (40) |
| 5. | 240 | 10 (100%) | 0 (00) | 1 (10) | 2 (20) | 3 (30) |
| 6. | 00 (Control) | 10 (100%) | 0(00) | 0(00) | 0(00) | 0(00) |

**Table-15 : Number and per cent ( ) of mortality of male of *Poecilia reticulata* in different concentrations of mercury during 96 h static bioassay with water hardness of 270 mg/l at a temperature of 16°C.**

| S.No. | Hg (µg/l) | No. and percentage of test animals | Number and per cent of mortality of Male | | | |
|---|---|---|---|---|---|---|
| | | | 24 h | 48 h | 72 h | 96 h |
| 1. | 280 | 10 (100%) | 5 (50) | 6 (60) | 7 (70) | 8 (80) |
| 2. | 240 | 10 (100%) | 4 (40) | 4 (40) | 6 (60) | 7 (70) |
| 3. | 210 | 10 (100%) | 2 (20) | 3 (30) | 4 (40) | 5 (50) |
| 4. | 180 | 10 (100%) | 0(00) | 2 (20) | 2 (20) | 2 (20) |
| 5. | 155 | 10 (100%) | 0(00) | 0(00) | 0(00) | 1 (10) |
| 6. | 00 (Control) | 10 (100%) | 0(00) | 0(00) | 0(00) | 0(00) |

**Table – 16 : Number and per cent ( ) of mortality of female of *Poecilia reticulata* in different concentrations of mercury during 96 h static bioassay with water hardness of 270 mg/l at a temperature of 16°C.**

| S.No. | Hg (µg/l) | No. and percentage of test animals | Number and per cent of mortality of female | | | |
|---|---|---|---|---|---|---|
| | | | 24 h | 48 h | 72 h | 96 h |
| 1. | 280 | 10 (100%) | 5 (50) | 6 (60) | 7 (70) | 9 (90) |
| 2. | 240 | 10 (100%) | 2 (20) | 4 (40) | 5 (50) | 6 (60) |
| 3. | 210 | 10 (100%) | 1 (10) | 2 (20) | 3 (30) | 4 (40) |
| 4. | 180 | 10 (100%) | 0(00) | 0(00) | 2 (20) | 2 (30) |
| 5. | 155 | 10 (100%) | 0(00) | 0(00) | 0(00) | 1 (10) |
| 6. | 00 (Control) | 10 (100%) | 0(00) | 0(00) | 0(00) | 0(00) |

**Table-17 : Number and per cent ( ) of mortality of juvenile of *Poecilia reticulata* in different concentrations of mercury during 96 h static bioassay with water hardness of 270 mg/l at a temperature of 16°C.**

| S.No. | Hg (µg/l) | No. and percentage of test animals | Number and per cent of mortality of Juvenile | | | |
|---|---|---|---|---|---|---|
| | | | 24 h | 48 h | 72 h | 96 h |
| 1. | 135 | 10 (100%) | 5 (50) | 6 (60) | 7 (70) | 8 (80) |
| 2. | 115 | 10 (100%) | 4 (40) | 4 (40) | 5 (50) | 7 (70) |
| 3. | 100 | 10 (100%) | 2 (20) | 3 (30) | 4 (40) | 6 (60) |
| 4. | 87 | 10 (100%) | 0(00) | 2 (20) | 2 (20) | 4 (40) |
| 5. | 75 | 10 (100%) | 0(00) | 0(00) | 0(00) | 3 (30) |
| 6. | 00 (Control) | 10 (100%) | 0(00) | 0(00) | 0(00) | 3 (30) |

**Table-18 : Number and per cent ( ) of mortality of mixed population of *Poecilia reticulata* in different concentrations of mercury during 96 h static bioassay with water hardness of 270 mg/l at a temperature of 16°C.**

| S.No. | Hg (µg/l) | No. and percentage of test animals | Number and per cent of mortality of mixed population | | | |
|---|---|---|---|---|---|---|
| | | | 24 h | 48 h | 72 h | 96 h |
| 1. | 210 | 10 (100%) | 6 (60) | 7 (70) | 8 (80) | 9 (90) |
| 2. | 180 | 10 (100%) | 4 (40) | 5 (50) | 6 (60) | 7 (70) |
| 3. | 155 | 10 (100%) | 2 (20) | 4 (40) | 4 (40) | 6 (60) |
| 4. | 135 | 10 (100%) | 1 (10) | 2 (20) | 2 (20) | 4 (40) |
| 5. | 115 | 10 (100%) | 0(00) | 0(00) | 1 (00) | 2 (20) |
| 6. | 00 (Control) | 10 (100%) | 0(00) | 0(00) | 0(00) | 0(00) |

**Table-19 : Number and per cent ( ) of mortality of the fingerlings of Catla in different concentrations of mercury during 96 h static bioassay with water hardness of 270 mg/l at a temperature of 16°C.**

| S.No. | Hg (µg/l) | No. and percentage of test animals | Number and per cent of mortality of Catla fingerlings | | | |
|---|---|---|---|---|---|---|
| | | | 24 h | 48 h | 72 h | 96 h |
| 1. | 87 | 10 (100%) | 6 (60) | 7 (70) | 8 (80) | 9 (90) |
| 2. | 75 | 10 (100%) | 3 (30) | 6 (60) | 7 (70) | 8 (80) |
| 3. | 65 | 10 (100%) | 3 (30) | 4 (40) | 6 (60) | 7 (70) |
| 4. | 56 | 10 (100%) | 2 (20) | 3 (30) | 4 (40) | 6 (60) |
| 5. | 49 | 10 (100%) | 1 (10) | 2 (20) | 3 (30) | 4 (40) |
| 6. | 00 (Control) | 10 (100%) | 0(00) | 0(00) | 0(00) | 0(00) |

**Table-20 : Number and per cent ( ) of mortality of the fingerlings of Rohu in different concentrations of mercury during 96 h static bioassay with water hardness of 270 mg/l at a temperature of 16°C.**

| S.No. | Hg (µg/l) | No. and percentage of test animals | Number and per cent of mortality of Rohu fingerlings | | | |
|---|---|---|---|---|---|---|
| | | | 24 h | 48 h | 72 h | 96 h |
| 1. | 320 | 10 (100%) | 6 (60) | 7 (70) | 7 (70) | 9 (90) |
| 2. | 280 | 10 (100%) | 4 (40) | 4 (40) | 5 (50) | 7 (70) |
| 3. | 240 | 10 (100%) | 2 (20) | 3 (30) | 4 (40) | 6 (60) |
| 4. | 210 | 10 (100%) | 0(00) | 2 (20) | 2 (20) | 4 (40) |
| 5. | 180 | 10 (100%) | 0 (00) | 0(00) | 0(00) | 2 (20) |
| 6. | 00 (Control) | 10 (100%) | 0 (00) | 0 (00) | 0(00) | 0(00) |

**Table-21 : Number and per cent ( ) of mortality of the fingerlings of Mrigal in different concentrations of mercury during 96 h static bioassay with water hardness of 270 mg/l at a temperature of 16°C.**

| S.No. | Hg (µg/l) | No. and percentage of test animals | Number and per cent of mortality of Mrigal fingerlings | | | |
|---|---|---|---|---|---|---|
| | | | 24 h | 48 h | 72 h | 96 h |
| 1. | 420 | 10 (100%) | 6 (60) | 7 (70) | 8 (80) | 9 (90) |
| 2. | 370 | 10 (100%) | 4 (40) | 6 (60) | 7 (70) | 7 (70) |
| 3. | 320 | 10 (100%) | 2 (20) | 4 (40) | 4 (40) | 5 (50) |
| 4. | 280 | 10 (100%) | 1 (10) | 1 (10) | 2 (20) | 4 (40) |
| 5. | 240 | 10 (100%) | 0 (00) | 0(00) | 0 (00) | 2 (20) |
| 6. | 00 (Control) | 10 (100%) | 0 (00) | 0(00) | 0 (00) | 0 (00) |

**Table-22 : Number and per cent ( ) of mortality of male of *Poecilia reticulata* in different concentrations of mercury during 96 h static bioassay with water hardness of 560mg/l at a temperature of 16°C.**

| S.No. | Hg (µg/l) | No. and percentage of test animals | Number and per cent of mortality of Male | | | |
|---|---|---|---|---|---|---|
| | | | 24 h | 48 h | 72 h | 96 h |
| 1. | 320 | 10 (100%) | 6 (60) | 7 (70) | 8 (80) | 8 (80) |
| 2. | 280 | 10 (100%) | 4 (40) | 4 (40) | 6 (60) | 7 (70) |
| 3. | 240 | 10 (100%) | 2 (20) | 4 (40) | 5 (50) | 6 (60) |
| 4. | 210 | 10 (100%) | 1 (10) | 2 (20) | 2 (20) | 4 (40) |
| 5. | 180 | 10 (100%) | 0(00) | (00) | 0(00) | 2 (20) |
| 6. | 00 (Control) | 10 (100%) | 0 (00) | 0(00) | 0(00) | 0 (00) |

**Table-23 : Number and per cent ( ) of mortality of female of *Poecilia reticulata* in different concentrations of mercury during 96 h static bioassay with water hardness of 560mg/l at a temperature of 16°C.**

| S.No. | Hg (µg/l) | No. and percentage of test animals | Number and per cent of mortality of Female | | | |
|---|---|---|---|---|---|---|
| | | | 24 h | 48 h | 72 h | 96 h |
| 1. | 370 | 10 (100%) | 6 (60) | 7 (70) | 8 (80) | 9 (90) |
| 2. | 320 | 10 (100%) | 4 (40) | 6 (60) | 7 (70) | 8 (80) |
| 3. | 280 | 10 (100%) | 2 (20) | 5 (50) | 6 (60) | 6 (60) |
| 4. | 240 | 10 (100%) | 1 (10) | 2 (20) | 4 (40) | 5 (50) |
| 5. | 210 | 10 (100%) | 0 (00) | 0(00) | 2 (20) | 3 (30) |
| 6. | 00 (Control) | 10 (100%) | 0 (00) | 0(00) | 0(00) | 0(00) |

**Table-24 : Number and per cent ( ) of mortality of Juvenile of *Poecilia reticulata* in different concentrations of mercury during 96 h static bioassay with water hardness of 560mg/l at a temperature of 16°C.**

| S.No. | Hg (µg/l) | No. and percentage of test animals | Number and per cent of mortality of Juveniles | | | |
|---|---|---|---|---|---|---|
| | | | 24 h | 48 h | 72 h | 96 h |
| 1. | 155 | 10 (100%) | 6 (60) | 7 (70) | 8 (80) | 8 (80) |
| 2. | 135 | 10 (100%) | 4 (40) | 5 (50) | 6 (60) | 7 (70) |
| 3. | 115 | 10 (100%) | 2 (20) | 4 (40) | 4 (40) | 6 (60) |
| 4. | 100 | 10 (100%) | 1 (10) | 2 (20) | 2 (20) | 3 (30) |
| 5. | 87 | 10 (100%) | 0(00) | 0(00) | 0 (00) | 2 (20) |
| 6. | 00 (Control) | 10 (100%) | 0 (00) | 0(00) | 0 (00) | 0 (00) |

**Table-25 : Number and per cent ( ) of mortality of mixed population of *Poecilia reticulata* in different concentrations of mercury during 96 h static bioassay with water hardness of 560mg/l at a temperature of 16°C.**

| S.No. | Hg (µg/l) | No. and percentage of test animals | Number and per cent of mortality of mixed population | | | |
|---|---|---|---|---|---|---|
| | | | 24 h | 48 h | 72 h | 96 h |
| 1. | 240 | 10 (100%) | 6 (60) | 7 (70) | 8 (80) | 9 (90) |
| 2. | 210 | 10 (100%) | 5 (50) | 6 (60) | 7 (70) | 8 (80) |
| 3. | 180 | 10 (100%) | 2 (20) | 4 (40) | 5 (50) | 6 (60) |
| 4. | 155 | 10 (100%) | 2 (20) | 2 (20) | 3 (30) | 4 (40) |
| 5. | 135 | 10 (100%) | 0 (00) | 0(00) | 2 (20) | 2 (20) |
| 6. | 00 (Control) | 10 (100%) | 0 (00) | 0(00) | 0 (00) | 0(00) |

**Table-26 : Number and per cent ( ) of mortality of the fingerlings of Catla in different concentrations of mercury during 96 h static bioassay with water hardness of 560 mg/l at a temperature of 16°C.**

| S.No. | Hg (µg/l) | No. and percentage of test animals | *Number and per cent* of mortality of Catla fingerlings | | | |
|---|---|---|---|---|---|---|
| | | | 24 h | 48 h | 72 h | 96 h |
| 1. | 115 | 10 (100%) | 6 (60) | 7 (70) | 8 (80) | 9 (90) |
| 2. | 100 | 10 (100%) | 4 (40) | 6 (60) | 7 (70) | 8 (80) |
| 3. | 87 | 10 (100%) | 3 (30) | 4 (40) | 6 (60) | 7 (70) |
| 4. | 75 | 10 (100%) | 3 (30) | 3 (30) | 4 (40) | 5 (50) |
| 5. | 65 | 10 (100%) | 2 (20) | 3 (30) | 4 (40) | 4 (40) |
| 6. | 00 (Control) | 10 (100%) | 0(00) | 0(00) | 0(00) | 0(00) |

**Table-27 : Number and per cent ( ) of mortality of the fingerlings of Rohu in different concentrations of mercury during 96 h static bioassay with water hardness of 560 mg/l at a temperature of 16°C.**

| S.No. | Hg (µg/l) | No. and percentage of test animals | Number and per cent of mortality of Rohu fingerlings | | | |
|---|---|---|---|---|---|---|
| | | | 24 h | 48 h | 72 h | 96 h |
| 1. | 370 | 10 (100%) | 6 (60) | 7 (70) | 8 (80) | 8 (80) |
| 2. | 320 | 10 (100%) | 4 (40) | 5 (50) | 6 (60) | 7 (70) |
| 3. | 280 | 10 (100%) | 2 (30) | 3 (30) | 4 (40) | 5 (50) |
| 4. | 240 | 10 (100%) | 2 (20) | 2 (20) | 2 (20) | 3 (30) |
| 5. | 210 | 10 (100%) | 0 (00) | 1 (10) | 2 (20) | 2 (20) |
| 6. | 00 (Control) | 10 (100%) | 0(00) | 0(00) | 0(00) | 0(00) |

**Table-28 : Number and per cent ( ) of mortality of the fingerlings of Mrigal in different concentrations of mercury during 96 h static bioassay with water hardness of 560 mg/l at a temperature of 16°C.**

| S.No. | Hg (µg/l) | No. and percentage of test animals | Number and per cent of mortality of Mrigal fingerlings | | | |
|---|---|---|---|---|---|---|
| | | | 24 h | 48 h | 72 h | 96 h |
| 1. | 490 | 10 (100%) | 6 (60) | 8 (80) | 9 (90) | 10 (100) |
| 2. | 420 | 10 (100%) | 4 (40) | 6 (60) | 7 (70) | 8 (80) |
| 3. | 370 | 10 (100%) | 2 (20) | 4 (40) | 6 (60) | 7 (70) |
| 4. | 320 | 10 (100%) | 1 (10) | 3 (30) | 5 (50) | 5 (50) |
| 5. | 280 | 10 (100%) | 0 (00) | 2 (20) | 2 (20) | 4 (40) |
| 6. | 00 (Control) | 10 (100%) | 0(00) | 0(00) | 0(00) | 0(00) |

***

# APPENDIX-2

**Table-1 : Relative Sensitivity ( ) among the test fishes of *Poecilia reticulata* and Indian major carp's fingerlings based on LC50 values for 24 h at different water hardness and temperatures.**

| Temperature (°C) | 35 | | 16 | |
|---|---|---|---|---|
| Water Hardness (mg/l) | 270 | 560 | 270 | 560 |
| ***Poecilia reticulata:*** | | | | |
| Males | 91.422 (1) | 106.957 (1) | 267.101 (1) | 297.906 (1) |
| Females | 124.184 (1.35) | 190.494 (1.78) | 279.335 (1.04) | 343.547 (1.15) |
| Juveniles | 73.578 (0.80) | 80.717 (0.75) | 128.535 (0.48) | 143.922 (0.48) |
| Mixed population | 91.721 (1.00) | 106.844 (0.99) | 193.802 (0.72) | 217.611 (0.73) |
| **Indian Major Carps:** | | | | |
| Catla | 59.969 (1) | 73.578 (1) | 83.288 (1) | 107.816 (1) |
| Rohu | 289.470 (4.82) | 293.130 (3.98) | 297.822 (3.57) | 343.459 (3.18) |
| Mrigal | 342.164 (5.70) | 378.826 (5.14) | 392.569 (4.71) | 453.363 (4.20) |

**Table-2 : Relative Sensitivity ( ) among the test fishes of *Poecilia reticulata* and Indian major carp's fingerlings based on LC50 values for 48 h at different water hardness and temperatures.**

| Temperature (°C) | 35 | | 16 | |
|---|---|---|---|---|
| Water Hardness (mg/l) | 270 | 560 | 270 | 560 |
| ***Poecilia reticulata:*** | | | | |
| Males | 80.442 (1) | 94.363 (1) | 254.623 (1) | 279.011 (1) |
| Females | 118.277 (1.47) | 172.163 (1.82) | 258.705 (1.01) | 304.134 (1.09) |
| Juveniles | 60.261 (0.74) | 74.807 (0.79) | 122.280 (0.48) | 131.609 (0.47) |
| Mixed population | 78.884 (0.98) | 103.891 (1.10) | 176.950 (0.69) | 200.425 (0.71) |
| **Indian Major Carps:** | | | | |
| Catla | 52.904 (1) | 60.261 (1) | 69.696 (1) | 91.357 (1) |
| Rohu | 261.493 (4.94) | 267.714 (4.44) | 283.247 (4.06) | 318.938 (3.49) |
| Mrigal | 306.050 (5.78) | 347.083 (5.75) | 358.341 (5.14) | 383.142 (4.19) |

ble-3 : **Relative Sensitivity ( ) among the test fishes of *Poecilia reticulata* and Indian major carp's fingerlings based on LC50 values for 72 h at different water hardness and temperatures.**

| Temperature(°C) | 35 | | 16 | |
|---|---|---|---|---|
| Water Hardness (mg/l) | 270 | 560 | 270 | 560 |
| ***Poecilia reticulata:*** | | | | |
| Males | 71.555(1) | 86.643 (1) | 232.115 (1) | 257.975 (1) |
| Females | 105.930 (1.48) | 144.734 (1.67) | 240.507 (1.03) | 267.597 (1.03) |
| Juveniles | 55.107 (0.77) | 68.878 (0.79) | 113.706 (0.48) | 125.958 (0.48) |
| Mixed population | 69.183 (0.96) | 95.303 (1.09) | 167.169 (0.72) | 180.175 (0.69) |
| **Indian Major Carps:** | | | | |
| Catla | 46.921(1) | 57.418 (1) | 60.677 (1) | 77.869 (1) |
| Rohu | 218.239 (4.65) | 250.347 (4.36) | 273.064 (4.50) | 294.371 (3.78) |
| Mrigal | 283.530 (6.04) | 304.450 (5.30) | 341.174 (5.62) | 342.601 (4.39) |

**Table-4 : Relative Sensitivity ( ) among the test fishes of *Poecilia reticulata* and Indian major carp's fingerlings based on LC50 values for 96 h at different water hardness and temperatures.**

| Temperature(°C) | 35 | | 16 | |
|---|---|---|---|---|
| Water Hardness (mg/l) | 270 | 560 | 270 | 560 |
| ***Poecilia reticulata:*** | | | | |
| Males | 69.080 (1) | 78.727 (1) | 216.184 (1) | 231.643 (1) |
| Females | 86.262 (1.24) | 124.739 (1.58) | 218.775 (1.01) | 247.278 (1.06) |
| Juveniles | 46.181 (0.66) | 61.200 (0.77) | 93.464 (0.43) | 113.721 (0.49) |
| Mixed population | 59.535 (0.86) | 83.918 (1.06) | 146.916 (0.67) | 167.861 (0.72) |
| **Indian Major Carps:** | | | | |
| Catla | 43.922 (1) | 47.707 (1) | 52.755 (1) | 72.729 (1) |
| Rohu | 194.612 (4.43) | 222.069 (4.65) | 228.157 (4.32) | 278.347 (3.82) |
| Mrigal | 268.141 (6.10) | 281.449 (5.89) | 308.456 (5.84) | 312.909 (4.30) |

Table-5: Relative Sensitivity ( ) among the test fishes of *Poecilia reticulata* and Indian major carp's fingerlings based on LC50 values for 24 h at different water hardness and temperatures.

| | Relative sensitivity ( ) between some water hardness at different temperatures | | | |
|---|---|---|---|---|
| **Water hardness (mg/l)** | **270** | **270** | **560** | **560** |
| **Temperature (°C)** | **35** | **16** | **35** | **16** |
| ***Poecilia reticulata:*** | | | | |
| Males | 91.422 (1) | 267.101 (2.92) | 106.957 (1) | 297.906 (2.78) |
| Females | 124.184 (1) | 279.335 (2.24) | 190.494 (1) | 343.547 (1.80) |
| Juveniles | 73.578 (1) | 128.535 (1.74) | 80.717 (1) | 143.922 (1.78) |
| Mixed population | 91.721 (1) | 193.802 (2.11) | 106.844 (1) | 217.611 (2.03) |
| **Indian major carp:** | | | | |
| Catla | 59.969 (1) | 83.288 (1.38) | 73.578 (1) | 107.816 (1.46) |
| Rohu | 289.470 (1) | 297.822 (1.02) | 293.130 (1) | 343.459 (1.17) |
| Mrigal | 342.164 (1) | 392.569 (1.14) | 378.826 (1) | 453.363 (1.19) |

**Table-6 : Relative Sensitivity ( ) among the test fishes of *Poecilia reticulata* and Indian major carp's fingerlings based on LC50 values for 48 h at different water hardness and temperatures.**

| | **Relative sensitivity ( ) between some water hardness at different temperatures** | | | |
|---|---|---|---|---|
| **Water hardness (mg/l)** | **270** | **270** | **560** | **560** |
| **Temperature (°C)** | **35** | **16** | **35** | **16** |
| ***Poecilia reticulata:*** | | | | |
| Males | 80.442 (1) | 254.623 (3.16) | 94.363 (1) | 279.011 (2.95) |
| Females | 118.277 (1) | 258.705 (2.18) | 172.163(1) | 304.134 (1.76) |
| Juveniles | 60.261 (1) | 122.280 (2.02) | 74.807 (1) | 131.609 (1.75) |
| Mixed population | 78.884 (1) | 176.950 (2.24) | 103.891(1) | 200.425 (1.92) |
| **Indian major carp:** | | | | |
| Catla | 52.904 (1) | 69.696 (1.31) | 60.261(1) | 91.357 (1.51) |
| Rohu | 261.493 | 283.247 (1.08) | 267.714 (1) | 318.938 (1.19) |
| Mrigal | 306.050 | 358.341 (1.17) | 347.083 (1) | 383.142 (1.10) |

**Table-7 : Relative Sensitivity ( ) among the test fishes of *Poecilia reticulata* and Indian major carp's fingerlings based on LC50 values for 72 h at different water hardness and temperatures.**

| | Relative sensitivity ( ) between some water hardness at different temperatures | | | |
|---|---|---|---|---|
| **Water hardness(mg/l)** | **270** | **270** | **560** | **560** |
| **Temperature (°C)** | **35** | **16** | **35** | **16** |
| ***Poecilia reticulata:*** | | | | |
| Males | 71.555 (1) | 232.115 (3.24) | 86.646 (1) | 257.975 (2.97) |
| Females | 105.930 (1) | 240.507 (2.27) | 144.734 (1) | 267.597 (1.84) |
| Juveniles | 55.107 (1) | 113.706 (2.06) | 68.878 (1) | 125.958 (1.82) |
| Mixed population | 69.183 (1) | 167.169 (2.41) | 95.303 (1) | 180.175 (1.89) |
| **Indian major carp:** | | | | |
| Catla | 46.921 (1) | 60.677 (1.29) | 57.418 (1) | 77.869 (1.35) |
| Rohu | 218.239 (1) | 273.064 (1.25) | 250.347 (1) | 294.371 (1.17) |
| Mrigal | 283.530 (1) | 341.174 (1.20) | 304.450 (1) | 342.601 (1.12) |

**Table-8 : Relative Sensitivity ( ) among the test fishes of *Poecilia reticulata* and Indian major carp's fingerlings based on LC50 values for 96 h at different water hardness and temperatures.**

| | Relative sensitivity ( ) between some water hardness at different temperatures | | | |
|---|---|---|---|---|
| **Water hardness (mg/l)** | **270** | **270** | **560** | **560** |
| **Temperature (°C)** | **35** | **16** | **35** | **16** |
| ***Poecilia reticulata:*** | | | | |
| Males | 69.080 (1) | 216.184 (3.12) | 78.727 (1) | 231.643 (2.94) |
| Females | 86.262 (1) | 218.775 (2.53) | 124.739 (1) | 247.278 (1.98) |
| Juveniles | 46.181 (1) | 93.464 (2.02) | 61.200 (1) | 113.721 (1.85) |
| Mixed population | 59.535 (1) | 146.916 (2.46) | 83.918 (1) | 167.861 (2.00) |
| **Indian major carp:** | | | | |
| Catla | 43.922 (1) | 52.755 (1.20) | 47.707 (1) | 72.729 (1.52) |
| Rohu | 194.612 (1) | 228.157 (1.17) | 222.069 (1) | 278.347 (1.25) |
| Mrigal | 268.141 (1) | 308.456 (1.15) | 281.449 (1) | 312.909 (1.11) |

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# APPENDIX-3

**Table-1 : Coefficient of correlation (r ) of LC50 values for the test fishes between different water hardness and temperature**

| Factors | Water hardness 270 mg/l; temperature 35 °C | Water hardness 560 mg/l ; temperature 35 °C | Water hardness 270 mg/l ; temperature 16 °C | Water hardness 560 mg/l ; temperature 16 °C |
|---|---|---|---|---|
| **Water hardness 270 mg/l ; temperature 35 °C** | - | 0.99 ** | 0.82 * | 0.82 * |
| **Water hardness 560 mg/l ; temperature 35 °C** | 0.99 ** | - | 0.85 * | 0.86 * |
| **Water hardness 270 mg/l ; temperature 16 °C** | 0.82 * | 0.85 * | - | 0.99 ** |
| **Water hardness 560 mg/l ; temperature 16 °C** | 0.82 * | 0.86 * | 0.99 ** | - |

* Significant at 5% level

** Significant at 1% level