

**MAHARANA PRATAP UNIVERSITY OF AGRICULTURE AND
TECHNOLOGY, UDAIPUR
RAJASTHAN COLLEGE OF AGRICULTURE, UDAIPUR**

CERTIFICATE – I

Dated: / /2005

This is to certify that **Miss. Pooja Chaudhary** has successfully completed the Comprehensive Examination held on 11/06/2005 as required under the regulation for the degree of **Master of Science in Agriculture.**

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RAJASTHAN COLLEGE OF AGRICULTURE, UDAIPUR

CERTIFICATE – II

Dated: / / 2005

This is to certify that the thesis entitled “**Investigation on Short-term Toxicity of Copper and Mercury in Relation to Selected Water Hardness and Temperature For a Freshwater Copepod**” submitted for the degree of **Master of Science in Agriculture** in the subject of **Limnology and Fisheries** embodies bonafide research work carried out by **Miss. Pooja Chaudhary** under my guidance and supervision and that no part of this thesis has been submitted for any other degree. The assistance and help received during the course of investigation have been fully acknowledged. The draft of this thesis was also approved by the advisory committee on

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CERTIFICATE – III

Dated: / / 2005

This is to certify that the thesis entitled “**Investigation on Short-term Toxicity of Copper and Mercury in Relation to Selected Water Hardness and Temperature For a Freshwater Copepod**” submitted by **Miss. Pooja Chaudhary** to the Maharana Pratap University of Agriculture and Technology, Udaipur in partial fulfilment of the requirements for the degree of **Master of Science in Agriculture** in the subject of **Limnology and Fisheries** after recommendation by the external examiner was defended by the candidate before the following members of the examination committee. The performance of the candidate in the oral examination on his thesis has been found satisfactory, we therefore, recommend that the thesis be approved.

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Dated: / / 2005

This is to certify that **Miss. Pooja Chaudhary** of M.Sc. (Ag.), Department of Limnology and Fisheries, Rajasthan College of Agriculture, Udaipur has made all corrections/modifications in the thesis entitled, **“Investigation on Short-term Toxicity of Copper and Mercury in Relation to Selected Water Hardness and Temperature For a Freshwater Copepod”** which were suggested by the external examiner and the advisory committee in the oral examination held on...../...../2005. The final copies of the thesis duly bound and corrected were submitted on...../...../2005 are enclosed herewith for approval.

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Investigation on Short-term Toxicity of Copper and Mercury in Relation to Selected Water Hardness and Temperature to a Freshwater Copepod

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ABSTRACT

In the present study short-term (96 h) toxicity of copper and mercury, separately and in combination in relation to selected water hardness and temperature to a freshwater copepod, Cyclops has been evaluated using static bioassay. Median lethal concentrations (LC50's) of copper and mercury, separately and in combination for Cyclops for the time intervals of 24, 48, 72 and 96 hours with water hardness of 520 mg/l, at a temperature of $18\pm 2^{\circ}\text{C}$ have been recorded highest as compared to waters of other hardness and at different temperatures (water hardness of 520 mg/l at a temperature of $30\pm 1^{\circ}\text{C}$ and water hardness of 270 mg/l at both the temperatures, i.e. 30 ± 1 and $18\pm 2^{\circ}\text{C}$). In comparison to mercury, copper was found less toxic to Cyclops.

The presumable safe concentrations for Cyclops ranged between 0.373-1.186 mg/l for copper and 4.011-18.695 $\mu\text{g/l}$ for mercury. These values ranged between 0.322-0.792 mg/l for copper in combination with mercury ions and 6.712-12.885 $\mu\text{g/l}$ for mercury in combination with copper ions at both the water hardness of 270 and 520 mg/l and at both the temperatures of 30 ± 1 and $18\pm 2^{\circ}\text{C}$. The safe dischargeable concentrations for copper and mercury separately and in combination ranged between 1.037-1.125 mg/l and 1.037-1.254 $\mu\text{g/l}$, respectively.

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1. INTRODUCTION

Water is essential for everything on our planet to grow and prosper. It is a vital resource for the sustenance of life in general and aquatic life in particular. 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. Out of total available water on earth, only three per cent is freshwater, and a small part of this is accessible for human consumption. Although we as humans recognize this fact, we disregard it by polluting our rivers, lakes and oceans. Subsequently, we are slowly but surely harming our planet to the point where organisms are dying at a very alarming rate. In addition to innocent organisms dying off, our drinking water has also become greatly affected due to continuous use to water for recreational purposes. In order to combat water pollution, we must understand the problems and become part of the solution.

The term 'pollution' broadly refers to an undesirable change in the physical, chemical or biological characteristics of our air, land and water that may or will harmfully affect human life or that of desirable species, our industrial process, living conditions and cultural assets or deteriorate our raw material resources. 'Environmental pollution' is unfavourable alteration of our surroundings due to direct or indirect activities of man. 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 menace has gravely affected human race by way of pollutants generated from domestic sewage, agricultural pesticides, industrial wastes, radioactive wastes, mining, refining and wastes caused by modern technological advances.

Water pollution has also 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, or other legitimate uses or to health of animals and aquatic life's environment. When it is unfit for its intended use, water is considered polluted. There are two main phases of water pollution.

- (i) Early phase - in which the threat comes from humans living in close contact with contaminated water.
- (ii) Developed phase – in which the pollution becomes at risk from toxic and hazardous substances associated with modern agriculture and industry.

Industrialization 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 pretreatment, causing pollution 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. Two types of water pollutants exist, point source and non-point source.

Point sources of pollution occur when harmful substances are emitted directly into a body of water. A non-point source delivers pollutants indirectly through environmental changes. Non point sources are much more difficult to control. The direct and indirect induced changes in the one or more components of ecosystem which are harmful or undesirable are termed as 'pollutants'. The categories of water pollutants are:

- (i) Disease causing agents
- (ii) Oxygen demanding wastes
- (iii) Water soluble inorganic chemicals
- (iv) Excess plant nutrients leading to eutrophication.
- (v) Organic chemicals
- (vi) Sediments
- (vii) Radioactive particles
- (viii) Heat

The pollutants have entered in the environment through aimless disposal of wastes and consequently reaches the organisms either directly or through 'food-chain'.

The introduction of heavy metals into natural water have 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.

Living organisms also require trace amounts of some heavy metals, including copper, cobalt, iron, manganese, molybdenum, vanadium, strontium and zinc for normal growth and to maintain metabolic activity but excessive levels can be detrimental to the organism. Other heavy metals such as mercury, lead and cadmium have no known vital or beneficial effect on organisms and their accumulation over time in the bodies of animals can cause serious illness. 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 bounded to many biochemical constituents inhibiting their function. Biological and physical factors in the environment can change the chemical form of the heavy metal. These changes may produce marked differences in the reactivity of trace elements with biological and non-biological sites in the environment. The effects of toxic element in the environment depends on the concentration of the heavy metal and its chemical form. The metals are introduced into aquatic system as a result of weathering of soils and rocks, from volcanic eruptions and from a variety of human activities, involving the mining, processing or use of elements and/or substances that contain element contaminants.

Environmental pollution by heavy metals becomes 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 Chisso's chemical works, a vinyl chloride plant. In 1970, the Norwegians found high mercury concentration in the fish from lake Saint-Clair. In Sweden, 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.

Mercury is a naturally occurring element that can be found throughout the environment. It is a silver white liquid metal solidifying at -38.9°C . It boils at 350.9°C , has a specific gravity of 13.6 and a vapour pressure of 1.2×10^{-3} mm of mercury. It has three oxidation states (i) zero (elemental mercury), (ii) +1 (mercurous compounds) and (iii) +2 (mercuric compounds). Historically it was recognized to possess a high toxic potential and was a germicidal or fungicidal for medical and agricultural processes. In recent years mercury has been recognized as a toxic contaminant in the environment. Toxicity of mercury is related to its chemical forms. Human activities, such as burning coal and using mercury to manufacture certain products have increased the amount of mercury in many parts of the environment, including the atmosphere, lakes and streams. 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 disinfectants, mercury electric appliances industries, phosphate rock process *etc.* 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 persistence and tendency to accumulate in aquatic organism including fish and undergo food chain amplification.

The primary way people are exposed to mercury is by eating fish containing methyl mercury. Mercury in the atmosphere is eventually deposited to the earth's surface either through dry or wet deposition (rain/snow). When mercury falls from the air or runs off the ground into the water, certain micro-organisms in soils and sediments convert some part of it into methyl mercury, a highly toxic form of mercury. 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. 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. Small organisms take up methyl mercury as they feed. When animals higher up the food

chain eat the smaller ones, they also take in the methyl mercury. As this process (known as bioaccumulation) continues, levels of methyl mercury increases up the food chain. Fish that are higher in the food chain, such as sharks and sword fish have much greater methyl mercury concentrations than fish that are lower on the food-chain. This is true for both saltwater and freshwater fish. Because of the non-degradable property of mercury, their toxicity, persistence and tendency to accumulate in aquatic organisms is long lasting.

The amount of methyl mercury in fish in different water bodies is a function of number of factors including the amount of mercury deposited from the atmosphere, local non-air release of mercury, naturally occurring mercury in soils, the physical, biological and chemical properties of different water bodies and the age, size and type of food the fish eats.

High levels of mercury in the blood stream of unborn bodies and young children may harm the developing nervous system. Methyl mercury is particularly toxic 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.

Maximum permissible concentration (MPC) of mercury in natural waters for the protection of human health is 0.144 mg m^{-3} . The WHO recommends a maximum daily intake of mercury by humans from all sources of $43 \text{ } \mu\text{g day}^{-1}$ of which no more than $29 \text{ } \mu\text{g day}^{-1}$ should be methyl mercury. The maximum level of mercury in drinking water permitted by EPA is 2 parts per billion.

The organic forms of mercury are generally more toxic to aquatic organisms than inorganic forms. Aquatic plants are affected by mercury in the water at concentration approaching 1 mg/l for inorganic mercury, but at much lower concentration of organic mercury. Aquatic invertebrates vary greatly in their susceptibility to mercury. Generally, larval stages are more sensitive than adults ($1\text{-}10 \text{ } \mu\text{g/l}$ normally causes acute toxicity). Toxicity is affected by temperature, salinity, dissolved oxygen and water hardness.

Copper is also one of the earliest known metal having atomic number 29 and atomic weight 63.54. It is reddish, lustrous, ductile and malleable metal which becomes dull when exposed to air. Copper and its compounds are common in environment. Copper occasionally occurs natively and is found in many minerals such as cuprite, malachite, azurite *etc.*

Most copper released from human activities comes from disposal of coal, ash residue and spreading of municipal and industrial wastes on land. copper is widely distributed in water since it is a naturally occurring element. Both natural and anthropogenic sources contribute copper to water, natural weathering of soil, atmospheric deposition and discharges from industry and wasteland treatment plants. Different copper fractions in water have also been reported as:

- (i) Dissolved copper – copper in an unacidified sample that passes through a 0.45 μ m membrane filter.
- (ii) Total copper - copper in an unfiltered sample after vigorous digestion with a concentrated acid or the sum of dissolved copper and suspended copper.
- (iii) Acid extractable copper- copper in unfiltered sample after treatment with a hot dilute mineral acid or after the addition of a dilute mineral acid, and
- (iv) Suspended/residue copper – total copper in an unacidified sample retained by 0.45 μ m membrane filter.

Cupric ion is the main toxic form of copper. Cupric ion in water is bound (complexed) with inorganic and organic compounds, which reduces cupric ion concentration (and its toxicity) substantially. The effects of copper on freshwater organism are numerous. Copper levels in river water range from 0.0 to 400 μ g/l, with a medium of 10 μ g/l. Dissolved copper levels in uncontaminated freshwaters usually range from 0.5 to 1 μ g/l, increasing to ≥ 2 μ g/l in urban areas.

One may be exposed to these toxic metals in any of its forms under different situations. The factors that determine how severe the health effects are from exposure include:

- (i) The chemical form-elemental (metallic), inorganic compound or organic compound.
- (ii) The dose - how much.
- (iii) The duration of exposure – how long.
- (iv) The route of exposure – eating, breathing, injecting, touching.
- (v) Other chemical exposures.
- (vi) The specific characteristics of the person – age, health *etc.*

Toxicity tests are necessary in water pollution evaluations because chemical and physical tests alone are not sufficient to assess potential effects on aquatic biota. Pickering and Heanderson (1965) suggest that the estimation of water quality criteria for protection of all life stages of aquatic organisms should be derived from chronic toxicity. The pollutants attack non-target organisms simultaneously and quite often effect of a single compound is different from that when it is present together with other chemicals at comparable concentration.

Toxicities of metals in a mixture produce an integrated response in test organisms that can be quantified as an interaction. The concepts of synergistic, additive and antagonistic toxicity are important to modelling the effects and developing criteria for multiple toxicants in the aquatic environment.

In view of this an attempt has been made to investigate the short-term (96 h) toxicity of copper and mercury separately and in combination to a freshwater copepod (*Mesocyclops leuckart*) in relation to selected water hardness and temperature using static bioassay test. The main purpose of acute bioassay tests with a copepod (*Mesocyclops leuckart*) 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 is sufficient to protect plankton?
- How effective are treatment methods in reducing toxicity?

The short-term toxicity tests were conducted using static bioassays with different concentrations of metallic ions, *viz.*, Cu and Hg and Cu : Hg in relation to

hardness and temperature in order to establish the toxic values with environmental variables. The median lethal concentrations (LC50's) for different time intervals, presumable safe or harmless and dischargeable concentrations of copper and mercury separately and in combination have been determined from the per cent mortality of Cyclops in static bioassays.

The group copepod under the present investigation have been selected considering many factors such as its easy culture and maintenance in laboratory conditions and also responsiveness to various environmental variable.

OBJECTIVES

- (i) To study the acute toxicity of copper and mercury separately in relation to selected water hardness and temperature for the zooplankter copepod.
- (ii) To study the acute toxicity of copper and mercury in combination in relation to selected water hardness and temperature for the zooplankter copepod.
- (iii) To determine the safe and dischargeable concentrations of these heavy metals for the zooplankter copepod.

2. REVIEW OF LITERATURE

Zooplankters are often cited more sensitive to heavy metals as compared to fish. There have been limited reports on the toxicity of heavy metals including copper and mercury for the fish food organisms, i.e. zooplankton. Biesinger and Christensen (1972) have studied the effects of various metals on survival, growth, reproduction and metabolism of *Daphnia magna*. Whereas, Baudovin and Scoppa (1974), McIntosh and Keven (1974), Marshall and Mellinger (1978) have observed the sensitivity of metals to zooplankton. Tabata (1969) and Black *et al.* (1975) opined that hardness has a strong influence on the toxicity of heavy metals, i.e. increasing hardness increases the value of median effective concentrations to aquatic organisms. Buikema *et al.* (1977) have observed rotifer sensitivity to combinations of inorganic water pollutants. Moolenaar and Kenga (1979) have suggested that aquatic animals especially *Daphnia* and other zooplankters are more sensitive indicators of toxic effects than plants and fishes. Joseph *et al.* (1981) have found significant effect of trace elements on plankton production. Balog and Sholanki (1984) used crustacean zooplankters for the evaluation of heavy metals in lake Balaton, Hungary. Further, Lalande and Alloul (1984) studied the acute toxicity of cadmium, copper, mercury and zinc to *Tropocyclops prasinus* and observed that metals when mixed together had a pronounced synergistic action than the individual action of these metals. Munawar *et al.* (1985) have investigated comparative studies on copepods and cladocerns of lake Ontario of Canada. These authors (*op. cit.*) opined that predatory cyclopoid copepods are far less sensitive to metals than filter feeding. Govindasamy *et al.* (1999) studied the availability of heavy metals in the aquatic ecosystem and its impact on flora and fauna.

Barnes and Stanbury (1948) have studied toxic action of copper and mercury salts on the harpacticoid copepod, *Nitocra spinipes*. Whereas, Biesinger and Christensen (1972) reported that 2 weeks LC50 values of Zn, Pb, Cu and Cd were 158, 300, 44 and 5 µg/l respectively, affecting the survival and reproduction potential of *Daphnia magna*. Further, Anderson and Weber (1975) have studied the toxicity to aquatic populations of mixtures containing heavy metals. Then, Arnott and Ahsanullah (1978) have studied acute toxicity of Cu, Cd and Zn to crab, *Paragrapsus quadridentus*. Further, Winner (1976, 1977, 1986) has studied the bioaccumulation acute and chronic toxicities of Cu and Cd to a freshwater *Daphnia* and also related chronic toxicity of cadmium with water hardness to *Daphnia pulex*. Also, Wong *et al.* (1977) have studied effect of zinc and copper salts on common

carp, *Cyprinus carpio* and grass carp, *Ctenopharyngodon idella*. Christopher and Robert (1983) have also studied the effects of Cu and Cd on the survival and growth of *Daphnia pulex*. Jindhal and Verma (1990) have carried out acute toxicity test using heavy metals, viz., Cd, Cu, Hg, Ni and Zn on adult specimens of *Daphnia pulex*. Further, Gupta and Rajbanshi (1991) have observed toxicity of copper and cadmium to *Heteropneustes fossilis* (Bloch.). Whereas, Le *et al.* (1994) studied the uncoupling of phytoplankton production in freshwaters affected by inorganic nutrient limitation. Mustata and Mustata (2001) have analysed effects of manganese, cadmium and copper toxicity on some Amphipoda.

Black *et al.* (1975) have presented annotated list of copper concentrations which were found harmful to aquatic organisms. Whereas, Andrew *et al.* (1977) have investigated the effects of inorganic complexing on the toxicity of copper to *Daphnia magna*. Winner *et al.* (1977) demonstrated that copper inhibits reproduction of daphnids only when these were maintained on nutritionally inadequate diet. Borgmann and Ralph (1984) have assured the toxicity of copper on freshwater zooplankton. Whereas, Nicola *et al.* (1988) have explored crustaceans for the chronic toxicity studies of copper. Willams *et al.* (1991) have studied copper toxicity to fishes and shrimp. Further, Warts and Perschbacher (1994) have reported effects of bicarbonate alkalinity and calcium on the acute toxicity of copper to juvenile channel catfish (*Ictalurus punctatus*). Havens (1994) studied structural and functional responses of a freshwater plankton community to acute copper stress. Further, Erickson *et al.* (1997) studied the effects of acclimation period on the relationship of acute copper toxicity to water hardness for fathead minnows. Whereas, Brown *et al.* (1998) have assessed the heavy metal toxicity of seawater. Hall *et al.* (1998) have also assessed the ecological risk of copper and cadmium in the surface waters of Chesapeake Bay watershed. Perschbacher and Warts (1999) studied the effects of calcium and magnesium hardness on acute copper toxicity to juvenile channel catfish, *Ictalurus punctatus* in low alkalinity environments and suggested a calcium specific mechanism with respect to acute copper toxicity in channel catfish.

Sherba *et al.* (2000) have studied the sublethal copper toxicity and food response in the freshwater crayfish, *Cambarus bartonii*. Whereas, Santore *et al.* (2001) have developed a biotic ligand model to explain and predict the effects of water chemistry on the acute toxicity of metals to aquatic organisms. La Breche *et al.* (2002) have also evaluated the copper toxicity to larval *Mercenaria mercenaria*. Further, James and Sampath (2003) have evaluated the effect of zeolite on the removal of copper at 2 different levels (sublethal and median lethal) from water and tissues of *Oreochromis mossambicus* (Peters.). Whereas, Naddy *et al.* (2003) have examined whether the sensitivity of

Ceriodaphnia dubia to copper toxicity was influenced by the hardness of the water in which they are reared. Carvalho *et al.* (2004) have studied the hepatic metallothionein in a teleost fish, *Prochilodus scrofa* exposed to copper at pH 4.5 and pH 8.0. Kaiser *et al.* (2004) have suggested that since the effects of copper on marine organisms are difficult to predict due to its interactions with other water quality parameters, the behaviour and condition of the rock anemone, *Aiptasia* could be used as an indicator to predict their population growth in marine hatchery systems. Whereas, Long *et al.* (2004) characterized the acute toxicity of copper to *Daphnia magna* in waters with low hardness and low pH.

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 which 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 was consumed by the local inhabitants. Consequently by 1975, 116 people had died and many were left paralyzed for life. 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.

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* (Forsk.). 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*).

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. Simon and Boudou (2001) reported that mercury organotropism in case of crayfish, *Astacus astacus* was specifically connected to the exposure conditions, especially at the biological barrier level according to the route of exposure. 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 $\mu\text{g g}^{-1}$. 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 $0.5 \mu\text{g g}^{-1}$. Misra *et al.* (2002) have investigated the mercury concentrations in grass carp fish of upper lake were less than $0.6 \mu\text{g g}^{-1}$ dry weight. Kumar and Gupta (2003) studied short-term (96 h) toxicity of mercury in relation to water hardness and temperature to *Poecilia reticulata* (male, female, juvenile and mixed population) and selected Indian major carps, i.e. catla, rohu and mrigal using static bioassay. Kumar and Gupta (2006) have also studied the acute toxic effects of mercury to the fingerlings of Indian major carps (catla, rohu and mrigal) in relation to water hardness and temperature.

The metal pollutants on entering into fish, effectively bring a variety of changes in metabolic activities which could cause even death. Simon (1953) reported that mercury compounds are detrimental to aquatic life including fishes affecting 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, i.e. Hg, Cu, Cd, Zn, Pb and Ni. However, Jackim *et al.* (1970) observed a marked reduction in hepatic and alkaline phosphates in killifish after exposure to copper and mercury. Further, Hinton *et al.* (1973) have reported the inhibition in hepatic alkaline phosphatase activity by mercury poisoning in fishes.

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. 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 interfere 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*. 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*. James *et al.* (1992) have also reported

alternation of protein metabolism in a catfish *Heteropneustes fossilis* exposed to mixture of mercury and copper.

Mustafa (1995) investigated the effects of mercury, chromium and nickel on some blood parameters in the carp, *Cyprinus carpio*. Gupta and Rajbanshi (1995) investigated significant changes in the gill architecture of *Rasbora daniconius* exposed to sublethal concentration of 0.05 mg/l of mercury as HgCl₂ from 96 h static bioassay. 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*. 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 (PbNO₃) and mercuric nitrate (HgNO₃) on the activity of lipids in the liver and kidney of *Heteropneustes fossilis*. Ribero *et al.* (1996) further studied the lethal effects of inorganic mercury on cells and tissues of *Trichomycterus brasiliensis*. McCrary and Heagler (1997) have studied the use of simultaneous multiple species acute toxicity to mosquito fish *Gambusia affinis* to compare the relative sensitivities of aquatic organisms to mercury. Viarengo *et al.* (1997) investigated the *in vitro* effects of Cu²⁺, Hg²⁺ and CH₃Hg⁺ on the fish liver microsomal EROD activity. Devlin and Clary (1998) have studied the *in vitro* toxicity of methyl mercury to fathead minnow cells. Further, Sivaramakrishna and Radhakrishnaiah (1998) reported the impact of sublethal concentration of mercury on nitrogen metabolism in a freshwater fish, *Cyprinus carpio*. Mat-Jais and Mohamed (2000) studied the *in vitro* inhibition of acetylcholinesterase activity in *C. straitus* brain tissue by mercury; Hg, Cd, Pb, Ni and Zn; and the role of extra cellular calcium. Gupta and Kumar (2006) have observed several histopathological lesions in the tissues of *C. mrigala* fingerlings exposed to a sublethal concentration of mercury.

Khadiga *et al.* (2002) reported that the water concentrations 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. Bogdan *et al.* (2002) investigated the effect of mercury on the lipid composition of liver and muscles in the perch, *Perca fluviatilis*.

The metal's toxicity and their accumulation in the aquatic animals and fishes may be enhanced by change in temperature. Vernberg *et al.* (1973) revealed that mercury increased the mortality of *Uca pugilator* larvae at high and low temperature salinity extremes. 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.). Ribeiro *et al.* (1996) further studies the lethal effects of inorganic mercury on cells and 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. 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 animals is significantly changed with the change in water hardness. Pickering and Henderson (1966) recorded variations in LC50's in softwater and hardwater 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 uptake from water in *Oncorhynchus kisutch*. 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. Pascoe *et al.* (1986) demonstrated toxicity tests with rainbow trout and confirmed that cadmium is less toxic in hard water (96 h LC50-2.6 mg Cd/l) than soft water (96 h LC50-1.3 mg Cd/l).

3. MATERIALS AND METHODS

Bioassay Method

The widely applicable basic routine static bioassay method, constitutes the simplest procedure. It is suitable for the detection and evaluation of short-term 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 the surface absorption of oxygen from the diluent generally provide an adequate amount of dissolved oxygen for the zooplankton during the test period (APHA, 1989; Mayer and Hamelink, 1977).

Physico-Chemical Characteristics of Water

The dechlorinated soft and hard waters were used at different temperatures for the evaluation of copper and mercury toxicity separately and in combination. The experimental water was also analysed for physical and chemical determinations such as pH, electrical conductivity, dissolved oxygen, total alkalinity, free carbon-di-oxide, nitrates (as $\text{NO}_3\text{-N}$) and phosphates (as $\text{PO}_4\text{-P}$). The pH and electrical conductivity values of the water were determined by using electronic pH meter and conductivity meter respectively. The desired temperature has been maintained using submersible glass heater equipped with thermostat. The dissolved oxygen was measured by the unmodified basic Winkler titrimetric method. The other chemical measurements such as alkalinity, total hardness etc. were analysed as per the standard methods of American Public Health Association (APHA) for the examination of water and waste water (1989).

Toxicant Solution

To commence with the assays for copper and mercury toxicity, a common stock solutions for each elements was prepared by dissolving appropriate amount of reagent grade salts ($\text{CuSO}_4 \cdot 5 \text{H}_2\text{O}$ and HgSO_4) in one litre of deionized diluent water. The diluent water used for bioassay test was acidified by slowly adding 2 to 5 drops

of 50% HCl for complete dissolution of crystals. The series of different concentrations of copper (as miligram/ litre) and mercury (as microgram/ litre) were prepared by adding the stock solution into the measured diluent water with the help of pipette. The series of concentrations of copper and mercury were based on the progressive bisection of intervals on a logarithmic scales (APHA, 1989: Table 3.1)

Exposure System

Glass beakers of 100ml capacity were used for the evaluation of short-term toxicity (96h) for the zooplankter copepod, *Cyclops* (Photograph-1). The bioassays were conducted with selected water hardness, i.e. 520 and 270 mg/l at temperatures of $30\pm 1^{\circ}\text{C}$ and $18\pm 2^{\circ}\text{C}$ in the months of January & February. The higher temperature, i.e. $30\pm 1^{\circ}\text{C}$ was maintained using a submersible glass heater equipped with thermostat in the glass aquarium. The static bioassays for a temperature of $30\pm 1^{\circ}\text{C}$ were conducted in the said aquarium (Photograph 2).

Culture of copepod, i.e. *Cyclops*

Culture of the *Cyclops* was developed separately in a glass aquarium. The temperature range of $25\text{-}30^{\circ}\text{C}$ was maintained for better culture of *Cyclops* during the coldest months, i.e. December, January & February (Photograph 3). The culture water was initially fertilized with FYM @ 500mg/l for a period of 7 days. After fertilization pure culture of copepod was inoculated in the fertilized water. Culture of copepod was developed within 15 days. The copepod has been identified as *Mesocyclops leuckart*. The mature healthy specimens of equal size of *Cyclops* were selected from the culture for the bioassay study. Before conducting preliminary/full-scale trials, the *Cyclops* were acclimatized in the same experimental conditions for a minimum period of 4 days.

Water Sources

Diluent waters of different hardness used in 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. 520 mg/l was collected from a bore well.

Preliminary or screening tests

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

Full scale tests for toxicity evaluation

After preliminary examination, elaborate experiments were conducted to evaluate the toxicity of both the elements, copper and mercury were measured by testing various concentrations in the range known by preliminary exploratory test. In joint toxicity, test range was established on the basis of concentration applied for evaluating the individual toxicity of each element. The test containers (glass beakers) filled with 100 ml toxicant solution were placed in 3 rows. Each container was labelled with the details of the experiment such as concentration, type of toxicant and combination, replicate number, date and time of the experiment. The acclimatized Cyclops were transferred to these beakers after about 20 minutes of the preparation of test solutions. Ten test zooplankter (Cyclops) were placed in each experimental beaker. Proper controls were run simultaneously. The experiments were continued for a period of 96 h. The number of Cyclops died in each concentration of toxicant solution were observed carefully and recorded at the time intervals of 24, 48, 72 and 96 hours. The dead specimens were removed from the test solution after knowing the exact mortality which was observed by their body movements, either spontaneous or in response to mild mechanical stimulation by prodding the zooplankton with a smooth glass rod.

Parameters applied for reporting the data

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

different concentrations and time intervals (24, 48, 72 and 96 hours) for both metallic ions separately and in combinations by Probit Analysis (Finney, 1971).

2. 95 Per cent Confidence limits: The 95 per cent confidence limits, i.e. lower confidence limits (LCL) and upper confidence limits (UCL) 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 static bioassay carried out at the same time with exactly the same condition.
3. Safe or harmless concentrations: Presumable safe or harmless concentrations of copper and mercury have also been determined from the short-term toxicity test conducted for the Cyclops using the following formula of Hart, Doudoroff and Greenbank (1945):

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

$$\text{Where } S^2 = 24 \text{ hours LC50} / 48 \text{ hours LC50}$$

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

4. Statistical Analysis: The LC50 values for the test specimen, Cyclops were also tested between different water hardness and temperature for coefficient of correlation (r) at 1% and 5% levels of significance (Sendecor and Cochran, 1980).

4. RESULTS

Physico-chemical Characteristics of the Water

Physico-chemical characteristics of the diluent water of different hardness, i.e. 270 and 520 mg/l used for the determination of copper and mercury toxicity (separately and in combination) for a freshwater copepod, *Cyclops* at two temperatures, i.e. 30 ± 1 and $18\pm 2^{\circ}\text{C}$ have been summarized in Table 1. Characteristics of the water revealed that the same are standard one as in natural conditions it did not contain any toxic substances. However, few changes observed in the quality of water at two temperatures were mainly in hardness, total alkalinity, dissolved oxygen and electrical conductivity.

Concentrations Used in Bioassay

The test range of both copper and mercury for static bioassay study were selected on the basis of results obtained in preliminary or screening tests. Variations in the ranges of concentrations were observed with the change in water hardness and temperature. The concentration of copper (as mg/l) and mercury (as $\mu\text{g/l}$) separately and in combination, used for the evaluation of short-terms (96 h) toxicity with different water hardness and temperature for a freshwater copepod, *Cyclops* are presented in Tables 2A and 2B. The concentrations of copper and mercury were based on logarithmic scale as per guide lines suggested by APHA (1989).

Measurement of Acute Toxicity

In the present study, changes in the acute toxicity have been observed mainly due to change in water hardness and temperature. Short-term static bioassays for a freshwater copepod, *Cyclops* have been conducted to evaluate the toxicity of copper and mercury ions (separately and in combination) in relation to selected water hardness and temperature. The per cent mortality of the test *Cyclops* with different concentrations of copper and mercury ions for both water hardness and temperature for the durations of 24, 48, 72 and 96 hours have been summarized in Appendices I-XII.

Median Lethal Concentration (LC50)

Median Lethal Concentration (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 copper and mercury toxicity (separately and in combination) for selected water hardness and temperatures were computed by Probit Analysis (Finney, 1971). The LC50 values of copper and mercury for 24, 48, 72 and 96 hours of exposure for the Cyclops have been summarized in Tables 3-6.

LC50's for Cyclops

Copper

The LC50's of copper for Cyclops for different time intervals of 24, 48, 72 and 96 h have been summarized in Table 3. The LC50's were found to change significantly with change in water hardness and temperature. In comparison to mercury copper was found less toxic to Cyclops. The 24, 48, 72 and 96 h LC50's to Cyclops for copper ions were observed as 1.856, 1.519, 1.333 and 1.155 mg Cu/l respectively with a water hardness of 270 mg/l at a temperature of $30\pm 1^{\circ}\text{C}$. These values were considerably increased at a low temperature of $18\pm 2^{\circ}\text{C}$ with the same water hardness of 270 mg/l. Herein the LC50's of Cyclops for copper ions were recorded as 2.486, 2.131, 1.593 and 1.236 mg Cu/l for 24,48,72 and 96 h respectively.

The LC50's of Cyclops for copper ions were further enhanced with an increase in water hardness. The 24, 48, 72 and 96 h LC₅₀'s of Cyclops for copper with a water hardness of 520 mg/l were noticed as 3.468, 2.741, 2.212 and 1.586 mg Cu/l respectively at a temperature of $30\pm 1^{\circ}\text{C}$. Whereas, the LC50's of Cyclops for copper ions with a water hardness of 520 mg/l at a temperature of $18\pm 2^{\circ}\text{C}$ were recorded higher as compared to LC50's with a water hardness of 520 mg/l at a temperature of $30\pm 1^{\circ}\text{C}$ and water hardness of 270 mg/l at both temperatures, i.e. 30 ± 1 and $18\pm 2^{\circ}\text{C}$.

Herein the LC50's of Cyclops for copper were recorded as 5.745, 4.767, 4.123 and 3.279 mg Cu/l for the time intervals of 24, 48, 72 and 96h respectively.

Mercury

LC50 values of mercury for Cyclops for different time intervals of 24, 48, 72 and 96h have been summarized in Table 4. The LC50's showed that Cyclops is more susceptible to mercury ions as compared to that of copper ions. 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 Cyclops were recorded as 33.092, 21.033, 11.856 and 9.702 $\mu\text{g Hg/l}$ respectively with a water hardness of 270 mg/l at a temperature of $30\pm 1^{\circ}\text{C}$. The LC50 values were considerably increased with the same water hardness of 270 mg/l and at a low temperature of $18\pm 2^{\circ}\text{C}$. The LC50 values of mercury were found as 42.319, 36.913, 32.119 and 27.165 $\mu\text{g Hg/l}$ for 24, 48, 72 and 96 h exposure respectively. The LC50's of mercury for Cyclops also increased significantly as in case of copper with the increase in water hardness. The 24, 48, 72 and 96h LC50's of mercury for Cyclops with a water hardness of 520 mg/l at a temperature of $30\pm 1^{\circ}\text{C}$ were noticed as 62.343, 52.469, 42.600 and 32.788 $\mu\text{g Hg/l}$.

The LC50's of mercury for Cyclops with water hardness of 520 mg/l at a temperature of $18\pm 2^{\circ}\text{C}$ were further increased in comparison to LC50's with water hardness of 520 mg/l at a temperature of $30\pm 1^{\circ}\text{C}$ and with water hardness of 270 mg/l at both the temperatures, i.e. 30 ± 1 and $18\pm 2^{\circ}\text{C}$. The 24, 48, 72 and 96 h values of mercury for Cyclops were recorded as 79.498, 70.359, 62.696 and 54.710 $\mu\text{g Hg/l}$ respectively with water hardness of 520 mg/l at a temperature of $18\pm 2^{\circ}\text{C}$.

Copper in combination with mercury

The LC50's of copper in combination with mercury for Cyclops for different time intervals of 24, 48, 72 and 96 h have been presented in Table 5. The LC50's were found to change significantly with the change in water hardness and temperature and also in combination with mercury ions. The 24, 48, 72 and 96 h LC50's for copper ions in combination with mercury were observed as 1.242, 1.155, 1.059 and 0.965 mg Cu/l respectively with a water hardness of 270 mg/l at a temperature of $30\pm 1^{\circ}\text{C}$. Further such values were slightly increased at a low temperature of $18\pm 2^{\circ}\text{C}$

with the same water hardness, i.e. 270 mg/l. Herein the LC50's of Cyclops for copper ions in combination with mercury were recorded as 1.638, 1.462, 1.288 and 1.163 mg Cu/l for 24, 48, 72 and 96h respectively.

The LC50's of Cyclops for copper ions in combination with mercury ions were also further enhanced with an increase in water hardness. The 24, 48, 72 and 96 h LC50's of Cyclops for copper in combination with mercury with a water hardness of 520 mg/l were noticed as 1.855, 1.710, 1.589 and 1.456 mg Cu/l respectively at a temperature of $30\pm 1^{\circ}\text{C}$. Whereas, the LC50's of Cyclops for copper ions in combination with a water hardness of 520 mg/l at a temperature of $18\pm 2^{\circ}\text{C}$ were recorded highest as 3.409, 3.000, 2.523 and 2.136 mg Cu/l for the time intervals of 24, 48, 72 and 96 h respectively.

Mercury in combination with copper

The LC50's of mercury in combination with copper ions for Cyclops for different time intervals of 24, 48, 72 and 96 h have been summarized in Table 6. The Cyclops were found very susceptible to mercury ions as revealed from lower LC50 values. It is interesting to note that LC50's were significantly changed with the change in water hardness and temperature. The 24, 48, 72 and 96 h LC50's to Cyclops for mercury ions in combination with copper ions were observed as 25.877, 24.050, 22.055 and 20.063 $\mu\text{g Hg/l}$ respectively with a water hardness of 270 mg/l at a temperature of $30\pm 1^{\circ}\text{C}$. These LC50 values at a lower temperature of $18\pm 2^{\circ}\text{C}$ and water hardness of 270 mg/l were recorded higher as compared to LC50's at $30\pm 1^{\circ}\text{C}$. Herein the LC50's of Cyclops for mercury in combination with copper were recorded as 29.313, 26.428, 23.729 and 21.840 $\mu\text{g Hg/l}$ for 24, 48, 72 and 96h respectively.

The LC50's of Cyclops for mercury in combination with copper were further enhanced with an increase in water hardness. The 24,48, 72 and 96h LC50's of mercury in combination with copper for Cyclops with a water hardness of 520 mg/ l were noticed as 37.706, 34.976, 32.658 and 30.085 $\mu\text{g Hg/l}$ respectively at a temperature of $30\pm 1^{\circ}\text{C}$. The LC50's of mercury in combination with copper for Cyclops with a water hardness of 520 mg/l at a temperature of $18\pm 2^{\circ}\text{C}$ were recorded highest as compared to LC50's with a water hardness of 520 mg/l at a temperature of

30±1⁰C and water hardness of 270 mg/l at both temperature, i.e. 30±1 and 18±2⁰C. Herein the LC50's of mercury in combination with copper ions for Cyclops recorded as 50.031, 46.342, 41.539 and 37.359 µg Hg/l for the time intervals of 24, 48, 72 and 96h respectively.

95 Per cent confidence limits & their ratios

The 95 percent confidence limits, i.e. lower confidence limit (LCL) and upper confidence limit (UCL) for LC50 values were also estimated for different time intervals of 24, 48, 72 and 96 hours (Tables 7-10). 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.

Copper

The ratios of confidence limits, i.e. upper and lower confidence limits for median lethal concentration of copper ions for a freshwater copepod, Cyclops ranged in between 1.918-2.130; 1.574-2.309, 1.457-1.999 and 1.595-2.384 for 24, 48, 72, and 96 h respectively for both the water hardness and temperature (Table 7), signify the better performance of the bioassay test. However, slightly higher ratio of confidence limits i.e. 3.068 may be due to variation in sensitivity of Cyclops during 24 h exposure.

Mercury

The ratios of confidence limits, i.e. upper and lower confidence limit for median lethal concentration of mercury ions for Cyclops have been summarized in Table 8. These values ranged between 1.445-2.449, 1.373-1.719, 1.370-1.641 and 1.518-1.947 for 24, 48, 72 and 96 h respectively with both the water hardness and temperature also signify the betterness of the bioassay test.

However higher ratios of confidence limits, i.e. 4.619, 4.660, 6.339 and 4.028 indicated sensitivity variation of Cyclops to mercury ions during 24, 48, 72 and 96 h of exposure particularly with water hardness of 270 mg/l at a temperature of 30±1⁰C.

Copper in combination with mercury

The ratios of confidence limits, i.e. upper and lower confidence limits for median lethal concentrations of copper in combination with mercury for Cyclops ranged between 1.473-1.896, 1.334-1.597, 1.268-1.639 and 1.322-1.715 for the time intervals of 24, 48, 72 and 96 h respectively with both the water hardness and temperature (Table 9). The ratios of confidence limits were observed to be highly significant.

Mercury in combination with copper

The significant ratios of confidence limits, i.e. upper and lower confidence limits for median lethal concentration of mercury in combination with copper for Cyclops ranged between 1.427-1.589, 1.305-1.335, 1.248-1.397 and 1.329-1.489 for 24, 48, 72 and 96 h respectively with both the water hardness and temperature (Table 10).

Presumable safe or harmless concentration

The presumable safe or harmless concentrations of copper and mercury separately and in combination for the zooplankter, i.e. Cyclops were also determined at both the water hardness and temperature using the formula of Hart *et al.* (1945) (Table 11). The safe concentration for Cyclops ranged between 0.373 - 1.186 mg/l for copper, 4.011 -18.695 µg/l for mercury separately. Whereas, these values ranged between 0.322 - 0.792 mg/l for copper in combination with mercury ions and 6.712 - 12.885 µg/l for mercury in combination with copper ions.

Safe Dischargeable Concentrations

The safe dischargeable concentrations for copper and mercury separately and in combination ranged between 1.037-1.125 mg/l and 1.037-1.254 µg/l, respectively (Table 11).

Coefficient of Correlation (r)

The LC50 values of copper and mercury separately and in combination for the zooplankter, Cyclops were also tested for coefficient of correlation (r) at 1% level of significance (Appendices XIII-XVI). Significant positive correlation coefficient (r) indicate uniform behaviour of test zooplankter, Cyclops at different water hardness and temperatures.

5. DISCUSSION

In the present study short-term (96 h) toxicity of copper and mercury ions (separately and in combination) in relation to selected water hardness and temperature has been evaluated for a freshwater copepod, i.e. Cyclops. Results of the toxicity tests were expressed as median lethal concentration (LC 50), 95% confidence limits and their ratios and presumable safe and dischargeable concentration. 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 conclusion have been made:

1. The wide range of toxicant concentrations tested for copper and mercury alone were found for the Cyclops.
2. Whereas range of toxicant concentrations for copper and mercury in combination were observed narrow for the Cyclops.
3. In general, mercury ions were found so much toxic to Cyclops as compared to copper ions.
4. However, significant differences in LC50 values with the change in water hardness and temperature for both copper and mercury were observed for the Cyclops.
5. Significant variations were also noticed in presumable safe or harmless and dischargeable concentrations for both copper and mercury whether tested separately or in combination for the Cyclops.

The LC50 values of metallic ions revealed that it is relatively more toxic in water of low hardness at higher temperature as compared to that of water of high hardness and at both the temperatures. Biesinger and Christensen (1972) have evaluated toxicities of various metals to the cladoceran zooplankton *Daphnia magna* on the basis of a 48 h LC50, a 3 weeks LC50 and a 16 per cent decrease in number of young borns, i.e. reproductive impairment. Similar results have been recorded in the present study for median lethal concentration of copper and mercury for Cyclops. The 96 h LC50 of mercury with water hardness of 520 mg/l

at a temperature of $18\pm 2^{\circ}\text{C}$ for Cyclops has been recorded maximum as $54.710\ \mu\text{g Hg/l}$. This 96 h LC50 value of mercury for Cyclops is higher than those reported for *Rasbora daniconius* (LC50- $0.08\ \mu\text{g Hg/l}$; Gupta and Rajbanshi, 1995), *Notemigonus crysoleuces* and *Gambusia affinis* (LC50 – 16.75 and $52.62\ \mu\text{g Hg /l}$ respectively; McCrary and Heagler, 1997). However, 96 h LC50 value of copper with water hardness of $270\ \text{mg/l}$ at a temperature of $30\pm 1^{\circ}\text{C}$ for Cyclops has been found minimum, i.e. $1.155\ \text{mg Cu/l}$. Similar trend of 96 h LC50 values of copper for *Heteropneustes fossilis* has been recorded by Gupta and Rajbanshi (1991).

Studies on the effects of zinc, copper and cadmium in combination have shown that there is more than just an additive effect of toxicity (Eisler and Gardner, 1973; Wong *et al.*, 1977; Gupta *et al.*, 1994).

Lalande and Alloul (1984) studied acute toxicity of cadmium, copper, mercury and zinc to *Tropocyclops prasinus* and observed that metals when mixed together had a pronounced synergistic action than the individual action of these metals. On the other hand, Attar and Malay (1982) and Vareille and Martin (1982) have found that metals acting together show a reduced toxicity. These authors (*op. cit.*) also explained that this could be attributed to a form of antagonism related to competition for common uptake sites in organisms. The present results are also in agreement with the above findings, as the median lethal concentrations for different time intervals for copper and mercury when tested in combination considerably decreased for the zooplankter, Cyclops. Differences in the behavior of metals in mixtures on aquatic organisms have also been reported in a review of the literature (European Inland Fisheries Advisory Commission, 1980). This review showed that the same metal might be additive, more than additive or less than additive depending upon species, combination of metals or water quality.

Anderson and Weber (1975) suggested that discrepancies found between additive action and more than additive action of metals in organisms may be due to water quality characteristics, such as water hardness that may alter metallic forms. They (*op. cit.*) also noted that more than additive responses were demonstrated in tests with copper and zinc using soft water and that additive responses were observed in exposures with hard water. In the present study LC50 values for Cyclops were observed to change with the change in water hardness and temperature. Tabata (1969) and Black *et al.* (1975) also opined that hardness has a strong influence on the toxicity of heavy metals, i.e. increasing hardness increases the value of median effective concentration or median lethal concentration to aquatic organisms. Naddy *et al.* (2003) have examined whether the sensitivity of *Ceriodaphnia dubia* to copper toxicity was influenced by the hardness of the water in which they were reared or in which

they were exposed and also found that organisms cultured in very hard water were 1.5 fold less sensitive to copper as compared to moderately hard water. However, the hardness of the exposure water had a greater (2.5 fold) effect on copper median effective concentration (EC50s). Further, Carvalho *et al.* (2004) have studied the hepatic metallothionein in a teleost fish, *Prochilodus scrofa* exposed to copper at pH 4.5 and pH 8 and observed that the pH of the water is the determining factor in copper toxicity. Also, Long *et al.* (2004) characterized the acute toxicity of copper to *Daphnia magna* in waters with low hardness and low pH. The 48 h total copper median lethal concentrations (LC50's) was related to water hardness over a hardness range of 8 to 51 mg/l as CaCO₃. Although toxicological difference existed between water hardness of 7 and 20 mg/l as CaCO₃, differences in pH (range 5.5-8.5) did not influence acute copper toxicity.

Munawar *et al.* (1985) opined that predatory cyclopoid copepods are far less sensitive to metals than filter feeding. These authors (*op. cit.*) also suggested that cladocera especially *Daphnia magna* is the most sensitive standard crustacean species. Whereas, Moolenaar and Kenga (1979) opined that aquatic animals especially *Daphnia* and other zooplankton are more sensitive indicators of toxic effects than plants and fishes. The present results also agreed with the above findings as the low LC 50's of mercury were recorded for Cyclops. The bioassays which were conducted with a diluent water of 270 mg/l hardness as CaCO₃ at a temperature of 30±1°C revealed that mercury ions were highly toxic for the test zooplankton, i.e. Cyclops. The present results also agreed with the findings of 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: Piekering and Gast, 1972) held the view that cadmium was more acutely toxic at a higher pH. Voyer (1975) emphasized that resistance of mummichog, *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 the present investigation range for presumable safe or harmless and dischargeable concentrations have also been determined for copper and mercury separately and in combination for their safe use in the waters for the test species, i.e. Cyclops. The values obtained are interesting and significant since they are not constant when copper and mercury

tested separately and in combination. 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 species of fishes. The 24 and 48 h LC50 values varied greatly for the calculation of presumable safe or harmless concentration. The variation was much greater when metallic ions were used in combination. Probably, some of the variation may be due to the chemical reaction between metallic salts and diluent water.

The results of the present investigation suggest that further study is needed to determine the long-term extensive effects of these toxic metallic ions on survival, growth and reproduction of this copepod also in relation to different water quality interactions such as temperature, pH, dissolved oxygen, hardness and macro and micronutrients etc. The investigation is also needed to assess the impact of stresses caused due to loading of these metallic ions in the aquatic ecosystem on the metabolism and biodiversity of zooplankton. The studies on biomonitoring and biomagnification are also required particularly for different trophic levels of those aquatic ecosystems, which are affected due to copper and mercury pollution in different ways.

Table 3.1: Guide to the selection of test concentrations based on a logarithmic scale*

Test concentrations in mg/l, mg/l or per cent

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)

Table 1: Physico-chemical characteristics of the selected diluent water of different hardness used in short-term toxicity tests for both copper and mercury separately and in combination for a fresh water copepod, Cyclops.

CharaCteriStiCs	Averages values			
Water temperature (⁰C)	30±1	30±1	18±2	18±2
Dissolved oxygen (mg/l)	5.5	5	7	6.5
pH	7.6	7.7	7.2	7.4
Free carbon dioxide (mg/l)	Nil	Nil	Nil	Nil
Total alkalinity (mg/l)	180	120	180	210
Total hardness (mg/l)	270	520	270	520
Electrical conductivity (μ mhos/cm)	820	800	825	820
Nitrates NO₃-N (mg/l)	0.60	0.65	0.51	0.60
Phosphates PO₄- P (mg/l)	0.05	0.04	0.05	0.04

Table 2A: Concentrations of copper (as mg/l) and mercury ($\mu\text{g/l}$) separately used in full scale 96 h static bioassays with water hardness of 270 and 520 mg/l and at temperatures of 30 ± 1 and $18\pm 2^\circ\text{C}$ for a freshwater copepod, Cyclops.

Temperature & water hardness	$30\pm 1^\circ\text{C}$		$18\pm 2^\circ\text{C}$	
	270 mg/l	520 mg/l	270 mg/l	520 mg/l
Heavy Metals				
Copper	2.4	4.9	3.2	7.5
(as mg/l)	1.8	3.2	2.4	5.6
	1.35	2.1	1.8	4.2
	1.00	1.35	1.35	3.2
	0.87	0.87	1.00	2.4
	0.00 (control)	0.00 (control)	0.00 (control)	0.00 (control)
Mercury	49	75	56	87
(as $\mu\text{g/l}$)	32	56	42	75
	18	42	32	65

	10	32	24	56
	3.2	24	18	49
	00 (control)	00 (control)	00 (control)	00 (control)

Table 2B: Concentrations of copper (as mg/l) and mercury ($\mu\text{g/l}$) in combination used in full scale 96 h static bioassays with water hardness of 270 and 520 mg/l and at temperatures of 30 ± 1 and $18\pm 2^{\circ}\text{C}$ for a freshwater copepod, Cyclops.

Temperature & water hardness Heavy Metals	$30\pm 1^{\circ}\text{C}$		$18\pm 2^{\circ}\text{C}$	
	270 mg/l	520 mg/l	270 mg/l	520 mg/l
Cu : Hg	1.35 :28	2.10 :42	1.80 :32	4.20 :56
	1.15 :24	1.80 :37	1.55 :28	3.20 :49
	1.00 :21	1.55 :32	1.35 :24	2.40 :42
	0.87 :18	1.35 :28	1.15 :21	2.10 :37
	0.75 :15.5	1.15 :24	0.87 :18	1.80 :32
	0.00:00	0.00:00	0.00:00	0.00:00

Table 3: Medium lethal concentrations (LC50's)* of copper (mg/l) for 24, 48, 72 and 96 h exposure to Cyclops with selected water hardness and temperature.

Water hardness	Water hardness 270 mg/l				Water hardness 520 mg/l			
	24h	48h	72h	96h	24h	48h	72h	96h
Temperature (°C)								
30±1	1.856	1.519	1.333	1.555	3.468	2.741	2.212	1.586
18 ± 2	2.486	2.131	1.593	1.236	5.745	4.767	4.123	3.279

*based on Probit analysis

Table 4: Medium lethal concentrations (LC50's)* of mercury ($\mu\text{g/l}$) for 24, 48, 72 and 96 h exposure to Cyclops with selected water hardness and temperature.

Water hardness	Water hardness 270 mg/l				Water hardness 520 mg/l			
	24h	48h	72h	96h	24h	48h	72h	96h
Temperature ($^{\circ}\text{C}$)								
30\pm1	33.092	21.033	11.856	9.702	62.343	52.469	42.600	32.788
18 \pm 2	42.319	36.913	32.119	27.165	79.498	70.359	62.696	54.710

*based on Probit analysis

Table 5: Medium lethal concentrations (LC50's)* of copper (mg/l) in combination with mercury for 24, 48, 72 and 96 h exposure to Cyclops with selected water hardness and temperature.

Water hardness	Water hardness 270 mg/l				Water hardness 520 mg/l			
	24h	48h	72h	96h	24h	48h	72h	96h
Temperature (°C)								
30±1	1.242	1.155	1.059	0.965	1.855	1.710	1.589	1.456
18 ± 2	1.638	1.462	1.288	1.163	3.409	3.000	2.523	2.136

*based on Probit analysis

Table 6: Medium lethal concentrations (LC50's)* of mercury ($\mu\text{g/l}$) in combination with copper for 24, 48, 72 and 96 h exposure to Cyclops with selected water hardness and temperature.

Water hardness	<i>Water hardness 270 mg/l</i>				<i>Water hardness 520 mg/l</i>			
	24h	48h	72h	96h	24h	48h	72h	96h
Temperature ($^{\circ}\text{C}$)								
30\pm1	25.877	24.050	22.055	20.063	37.706	34.976	32.658	30.085
18\pm2	29.313	26.428	23.729	21.840	50.031	46.342	41.539	37.359

*based on Probit analysis

Table 7: 95 per cent confidence limits and their ratios for 24, 48, 72 and 96 h LC50's of COPPER for a freshwater copepod, Cyclops.

Duration	24 h			48 h			72 h			96 h		
	UCL	LCL	R	UCL	LCL	R	UCL	LCL	R	UCL	LCL	R
Water hardness (Temperature °C)												
270 (30±1)	3.149	1.478	2.130	1.955	1.242	1.574	1.683	1.039	1.619	1.453	0.776	1.872
270 (18±2)	3.848	2.006	1.918	2.771	1.747	1.586	1.894	1.300	1.457	1.464	0.918	1.595
520 (30±1)	7.556	2.463	3.068	4.524	1.959	2.309	3.145	1.573	1.999	2.229	0.935	2.384
520 (18±2)	9.546	4.559	2.094	6.750	3.686	1.831	5.215	3.161	1.649	4.113	2.112	1.946

UCL- Upper confidence limit; LCL-Lower confidence limit; R- confidence Ratio (UCL/LCL)

Table 8: 95 per cent confidence limits and their ratios for 24, 48, 72 and 96 h LC50's of MERCURY for a freshwater copepod, Cyclops.

Durations	24 h			48 h			72 h			96 h		
	UCL	LCL	R	UCL	LCL	R	UCL	LCL	R	<i>UCL</i>	LCL	R
Water hardness (Temperature °C)												
270 (30±1)	95.634	20.703	4.619	51.362	11.020	4.660	23.583	3.720	6.339	16.232	4.029	4.028
270 (18±2)	77.770	32.860	2.367	50.547	29.389	1.719	40.139	25.443	1.577	33.056	20.628	1.602
520 (30±1)	120.236	49.081	2.449	73.832	42.363	1.743	54.363	33.125	1.641	41.130	21.122	1.947
520 (18±2)	103.008	71.293	1.445	85.155	62.039	1.373	71.947	52.480	1.370	61.735	40.650	1.518

UCL- Upper confidence limit; LCL-Lower confidence limit; R- confidence Ratio (UCL/LCL)

Table 9: 95 per cent confidence limits and their ratios for 24, 48, 72 and 96 h LC50's of COPPER IN COMBINATION WITH MERCURY for a freshwater copepod, Cyclops.

Durations	24 h			48 h			72 h			96 h		
	UCL	LCL	R	UCL	LCL	R	UCL	LCL	R	UCL	LCL	<i>R</i>
Water hardness (Temperature °C)												
270 (30±1)	1.737	1.100	1.579	1.388	1.040	1.334	1.245	0.937	1.328	1.095	0.828	1.322
270 (18±2)	2.363	1.434	1.647	1.748	1.285	1.360	1.496	1.078	1.387	1.359	0.870	1.562
520 (30±1)	2.429	1.649	1.473	2.033	1.524	1.334	1.793	1.413	1.268	1.708	1.114	1.533
520 (18±1)	5.368	2.831	1.896	4.011	2.511	1.597	3.234	1.910	1.693	2.546	1.484	1.715

UCL- Upper confidence limit; LCL-Lower confidence limit; R- confidence Ratio (UCL/LCL)

Table 10: 95 per cent confidence limits and their ratios for 24, 48, 72 and 96 h LC50's of MERCURY IN COMBINATION WITH COPPER for a freshwater copepod, Cyclops.

Duration Water hardness (Temperature °C)	24 h			48 h			72 h			96 h		
	UCL	LCL	R	UCL	LCL	R	UCL	LCL	R	UCL	LCL	R
270 (30±1)	36.429	22.919	1.589	28.932	21.666	1.335	25.978	19.486	1.333	22.830	17.172	1.329
270 (18±2)	40.755	25.994	1.567	31.179	23.645	1.318	27.034	20.473	1.320	24.897	17.036	1.461
520 (30±1)	48.260	33.801	1.427	40.995	31.407	1.305	36.529	29.267	1.248	34.927	23.442	1.489
520 (18±2)	64.432	44.761	1.439	54.549	41.557	1.313	48.519	34.716	1.397	41.785	30.070	1.389

UCL- Upper confidence limit; LCL-Lower confidence limit; R- confidence Ratio (UCL/LCL)

Table 11: Presumable safe or harmless concentrations (C)* and safe dischargeable concentrations (S)* of copper (mg/l) and mercury (µg/l) separately and in combination for a freshwater copepod, Cyclops with different water hardness and temperatures.

Factors	Temperature 30±1 ⁰ C				Temperature 18±2 ⁰ C			
	Water hardness (270 mg/l)		Water hardness (520 mg/l)		Water hardness (270 mg/l)		Water hardness (520 mg/l)	
	C	S	C	S	C	S	C	S
Copper (as mg/l)	0.373	1.105	0.650	1.125	0.548	1.079	1.186	1.097
Mercury (as µg/l)	4.011	1.254	13.25	1.090	9.66	1.070	18.695	1.063
Copper (as mg/l)	0.322	1.037	0.473	1.042	0.391	1.058	0.792	1.066
(in combination with mercury)	6.712	1.037	9.733	1.038	7.149	1.053	12.885	1.038
Mercury (as µg/l)								
(in combination with copper)								

*C = 48 hours LC50 x 0.3/S²

S² = 24 hours LC50 / 48 hours LC50

Where, C= harmless conc., S = safe dischargeable conc.

5. DISCUSSION

In the present study short-term (96 h) toxicity of copper and mercury ions (separately and in combination) in relation to selected water hardness and temperature has been evaluated for a freshwater copepod, i.e. Cyclops. Results of the toxicity tests were expressed as median lethal concentration (LC 50), 95% confidence limits and their ratios and presumable safe and dischargeable concentration. 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 conclusion have been made:

6. The wide range of toxicant concentrations tested for copper and mercury alone were found for the Cyclops.
7. Whereas range of toxicant concentrations for copper and mercury in combination were observed narrow for the Cyclops.
8. In general, mercury ions were found so much toxic to Cyclops as compared to copper ions.
9. However, significant differences in LC50 values with the change in water hardness and temperature for both copper and mercury were observed for the Cyclops.
10. Significant variations were also noticed in presumable safe or harmless and dischargeable concentrations for both copper and mercury whether tested separately or in combination for the Cyclops.

The LC50 values of metallic ions revealed that it is relatively more toxic in water of low hardness at higher temperature as compared to that of water of high hardness and at both the temperatures. Biesinger and Christensen (1972) have evaluated toxicities of various metals to the cladoceran zooplankton *Daphnia magna* on the basis of a 48 h LC50, a 3 weeks LC50 and a 16 per cent decrease in number of young borns, i.e. reproductive impairment. Similar results have been recorded in the present study for median lethal concentration of copper and mercury for Cyclops. The 96 h LC50 of mercury with water hardness of 520 mg/l

at a temperature of $18\pm 2^{\circ}\text{C}$ for Cyclops has been recorded maximum as $54.710\ \mu\text{g Hg/l}$. This 96 h LC50 value of mercury for Cyclops is higher than those reported for *Rasbora daniconius* (LC50- $0.08\ \mu\text{g Hg/l}$; Gupta and Rajbanshi, 1995), *Notemigonus crysoleuces* and *Gambusia affinis* (LC50 – 16.75 and $52.62\ \mu\text{g Hg /l}$ respectively; McCrary and Heagler, 1997). However, 96 h LC50 value of copper with water hardness of $270\ \text{mg/l}$ at a temperature of $30\pm 1^{\circ}\text{C}$ for Cyclops has been found minimum, i.e. $1.155\ \text{mg Cu/l}$. Similar trend of 96 h LC50 values of copper for *Heteropneustes fossilis* has been recorded by Gupta and Rajbanshi (1991).

Studies on the effects of zinc, copper and cadmium in combination have shown that there is more than just an additive effect of toxicity (Eisler and Gardner, 1973; Wong *et al.*, 1977; Gupta *et al.*, 1994).

Lalande and Alloul (1984) studied acute toxicity of cadmium, copper, mercury and zinc to *Tropocyclops prasinus* and observed that metals when mixed together had a pronounced synergistic action than the individual action of these metals. On the other hand, Attar and Malay (1982) and Vareille and Martin (1982) have found that metals acting together show a reduced toxicity. These authors (*op. cit.*) also explained that this could be attributed to a form of antagonism related to competition for common uptake sites in organisms. The present results are also in agreement with the above findings, as the median lethal concentrations for different time intervals for copper and mercury when tested in combination considerably decreased for the zooplankter, Cyclops. Differences in the behavior of metals in mixtures on aquatic organisms have also been reported in a review of the literature (European Inland Fisheries Advisory Commission, 1980). This review showed that the same metal might be additive, more than additive or less than additive depending upon species, combination of metals or water quality.

Anderson and Weber (1975) suggested that discrepancies found between additive action and more than additive action of metals in organisms may be due to water quality characteristics, such as water hardness that may alter metallic forms. They (*op. cit.*) also noted that more than additive responses were demonstrated in tests with copper and zinc using soft water and that additive responses were observed in exposures with hard water. In the present study LC50 values for Cyclops were observed to change with the change in water hardness and temperature. Tabata (1969) and Black *et al.* (1975) also opined that hardness has a strong influence on the toxicity of heavy metals, i.e. increasing hardness increases the value of median effective concentration or median lethal concentration to aquatic organisms. Naddy *et al.* (2003) have examined whether the sensitivity of *Ceriodaphnia dubia* to copper toxicity was influenced by the hardness of the water in which they were reared or in which

they were exposed and also found that organisms cultured in very hard water were 1.5 fold less sensitive to copper as compared to moderately hard water. However, the hardness of the exposure water had a greater (2.5 fold) effect on copper median effective concentration (EC50s). Further, Carvalho *et al.* (2004) have studied the hepatic metallothionein in a teleost fish, *Prochilodus scrofa* exposed to copper at pH 4.5 and pH 8 and observed that the pH of the water is the determining factor in copper toxicity. Also, Long *et al.* (2004) characterized the acute toxicity of copper to *Daphnia magna* in waters with low hardness and low pH. The 48 h total copper median lethal concentrations (LC50's) was related to water hardness over a hardness range of 8 to 51 mg/l as CaCO₃. Although toxicological difference existed between water hardness of 7 and 20 mg/l as CaCO₃, differences in pH (range 5.5-8.5) did not influence acute copper toxicity.

Munawar *et al.* (1985) opined that predatory cyclopoid copepods are far less sensitive to metals than filter feeding. These authors (*op. cit.*) also suggested that cladocera especially *Daphnia magna* is the most sensitive standard crustacean species. Whereas, Moolenaar and Kenga (1979) opined that aquatic animals especially *Daphnia* and other zooplankton are more sensitive indicators of toxic effects than plants and fishes. The present results also agreed with the above findings as the low LC 50's of mercury were recorded for Cyclops. The bioassays which were conducted with a diluent water of 270 mg/l hardness as CaCO₃ at a temperature of 30±1°C revealed that mercury ions were highly toxic for the test zooplankton, i.e. Cyclops. The present results also agreed with the findings of 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: Piekering and Gast, 1972) held the view that cadmium was more acutely toxic at a higher pH. Voyer (1975) emphasized that resistance of mummichog, *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.

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The results of the present investigation suggest that further study is needed to determine the long-term extensive effects of these toxic metallic ions on survival, growth and reproduction of this copepod also in relation to different water quality interactions such as temperature, pH, dissolved oxygen, hardness and macro and micronutrients etc. The investigation is also needed to assess the impact of stresses caused due to loading of these metallic ions in the aquatic ecosystem on the metabolism and biodiversity of zooplankton. The studies on biomonitoring and biomagnification are also required particularly for different trophic levels of those aquatic ecosystems, which are affected due to copper and mercury pollution in different ways.

6. SUMMARY

In the present study an attempt has been made to investigate the short-term (96 h) toxicity of copper and mercury ions (separately and in combination) in relation to water hardness and temperature for a freshwater copepod, *Cyclops*, considering various parameters such as median lethal concentration (LC50) for 24, 48, 72 and 96 hours, their confidence limits and ratio and presumable safe or harmless concentrations.

The test range for both copper and mercury static bioassays were selected on the basis of results of the preliminary or screening tests. With the change in water hardness and temperature, variations in the test concentrations were observed. The LC50's for the time intervals of 24, 48, 72 and 96 hours were also found to change significantly with the change in water hardness and temperature. The highest 96 h LC50 values of copper and mercury (separately) for *Cyclops* were noticed as 3.279 mg/l and 54.710 µg/l respectively with water hardness of 520 mg/l at a temperature of $18\pm 2^{\circ}\text{C}$. Whereas the lowest 96 h LC50 values of copper and mercury (separately) for *Cyclops* were recorded as 1.155 mg/l and 9.702 µg/l respectively with water hardness of 270 mg/l at a temperature of $30\pm 1^{\circ}\text{C}$. However, the highest 96 h LC50 values of copper (in combination with mercury) and mercury (in combination with copper) for *Cyclops* were recorded as 2.136 mg/l and 37.359 µg/l respectively with water hardness of 520 mg/l at a temperature of $18\pm 2^{\circ}\text{C}$. Whereas the lowest 96h LC50 values of copper (in combinations with mercury) and mercury (in combination with copper) for *Cyclops* were recorded as 0.965 mg/l and 20.063 µg/l respectively with water hardness of 270 mg/l at a temperature of $30\pm 1^{\circ}\text{C}$. On the basis of LC50 values it is concluded that the test organism *Cyclops* was found most resistant with water hardness of 520 mg/l at a temperature of $18\pm 2^{\circ}\text{C}$ as compared to that of water hardness of 520 mg/l at a temperature of $30\pm 1^{\circ}\text{C}$ and water hardness of 270 mg/l at both the temperatures, i.e. 30 ± 1 and $18\pm 2^{\circ}\text{C}$.

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 (UCL and LCL) recorded for different periods of 24, 48, 72 and 96 h signify the better performance of bioassay tests.

In the present investigation presumable safe or harmless concentrations of copper and mercury ions (separately and in combination) for *Cyclops* were also determined at both water hardness and temperature. The safe concentrations for *Cyclops* ranged between 0.373-1.186 mg/l for copper and 4.011-18.695 µg/l for mercury. Whereas, these values ranged between

0.322-0.792 mg/l for copper in combination with mercury ions and 6.712-12.885 µg/l for mercury in combination with copper ions with water hardness of 270 and 520 mg/l at temperatures of 30 ± 1 and $18\pm 2^{\circ}\text{C}$. The safe dischargeable concentrations for copper and mercury separately and in combination ranged in between 1.037-1.125 mg/l and 1.037-1.25 µg/l, respectively.

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

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