INTRODUCTION

Heavy metals have become major environmental pollutants today and at higher concentrations, they may be lethal to aquatic organisms (Schroeder, 1973; Rubin, 1974). According to Moore and Ramamoorthy (1984), there has been rapid and continuous increase in the worldwide production and use of cadmium since 1925. The above authors further stated that cadmium, even in minute concentrations of ppb, is lethal to freshwater organisms, particularly fishes.

Bioassay studies are accepted as standard methods for assessing the toxicity of a chemical (APHA, 1974). Sprague (1973) defined acute toxicity as the stimulus severe enough to bring about response speedily enough within four days for fish. Acute toxicity is measured as median lethal concentration ($LC_{50}$), which kills 50 per cent of the test organisms (Sprague, 1969; APHA, 1974). The median lethal toxicity study is considered very significant in formulating water quality criteria (NAS, 1972). Acute toxicity is the result of single or repeated or continuous exposure within 24 h period, but chronic toxicity is the result of repeated or continuous exposure over a long period of time (Doull et al., 1980).

Ferrando and Andreu (1991a) are of the opinion that static acute toxicity test provides rapid and reproducible concentration-response curves for estimating toxic effects of chemicals on aquatic organisms; these tests
provide a data base for determining relative toxicity to a variety of species. The above authors further stated that pH, salinity and hardness are some important factors of the medium that influence the toxicity of a compound.

Shaw et al. (1990) reported that evaluation of LC$_{50}$ value, i.e., the concentration of the substance which kills 50 per cent of the organism, is valuable as they are quick and useful for asserting harmful effects of a pollutant. Toxicity tests for determining the effects of heavy metals on aquatic organism have traditionally been carried out under controlled laboratory conditions (Ramirez et al., 1989).

Wetzel (1975) and Cole (1979) reported that hardness of water is due to the presence of bivalent metal cations like calcium and magnesium. Several studies have shown that increasing water hardness reduces heavy metal toxicity to fish (Howarth and Sprague, 1978; Waiwood and Beamish, 1978; Alabaster and Lloyd, 1982; Bradley and Sprague, 1985; Pascoe et al., 1986). Calcium appears to be the principal modifier of heavy metal toxicity (Davey, 1976; Judy and Davies, 1979; Calamari et al., 1980). Carroll et al. (1979), Calamari et al. (1980) and Wright et al. (1985) have suggested that calcium acts directly on the organism, thus decreasing heavy metal toxicity by a biological mechanism. Competition for binding sites on the gill surface between calcium and divalent metal ions have
been proposed to influence metal uptake and toxicity to fish (Pagenkopf, 1983; Hunn, 1985). Rodgers and Beamish (1983) and Part et al. (1985) noted that calcium dependent changes in gill permeability, causing a decrease in metal uptake as calcium concentration increases, may be a mechanism of protection.

Aquatic ecosystem is known to be highly sensitive to the residual toxicity of heavy metals as compared to the terrestrial ecosystem; as fish represent the highest trophic level in the aquatic food chain, persistent heavy metal residues and other toxins of similar nature accumulate to a maximum concentration in their body when compared to other organisms in aquatic environment (Pundir and Saxena, 1992). Metelev et al. (1983) reported that fish have been valued for many years as excellent indicators of water quality.

Keeping in view the above information, it is proposed to study to assess the median lethal concentration of cadmium nitrate to *Cyprinus carpio* and the impact of water calcium hardness on the metal toxicity.
MATERIAL AND METHODS

The exotic scale carp, *Cyprinus carpio* var. *communis* is now extensively cultivated in India and in the Far-East; it is omnivorous and under traditional methods of management, it makes near optimal use of naturally occurring pond food; when additional feed is provided, the carp can utilize a wide range of by-products; hence, it has been called the swimming pig.

*Cyprinus carpio* var. *communis* was chosen for the present investigation for the following reasons:

1. The family Cyprinidae is well represented amongst the piscine inhabitants of freshwaters and estuaries of India.

2. This species has the greatest advantage of breeding in confined waters and rapidly multiplies without much effort.

3. It is a commercially important fish.

4. Easily available and adaptable to the laboratory conditions.

5. Tolerant to a wide range of temperature and salinity.
6. Hardy nature for handling and transport.

7. Quicker growth rate.

RECRUITMENT OF FISH FOR THE EXPERIMENT

Specimens of *Cyprinus carpio* were procured from TAMIL NADU FISHERIES DEVELOPMENT CORPORATION LIMITED, ALIYAR FISH FARM, TAMIL NADU, INDIA. They were transported in polythene bags containing aerated water and acclimatized in the laboratory for about 2 weeks by stocking them in a large rectangular cement tank (4'x 8'x 3'), previously washed with potassium permanganate (to prevent fungal infection).

During acclimatization, the fish were fed with rice bran and groundnut oil cake *ad libitum*. Water in the tank was renewed daily to ensure sufficient oxygen supply to the fish.

SELECTION OF FISH

Prior to the commencement of experiments, a suitable number of healthy fish were transferred and maintained in small glass tanks. Fish with an average length of 8.0 cm and an average weight of 6.5 g were
selected for the experimental work. Fish belonging to both the sex were used. Feeding was stopped two days before the commencement of experiment to keep the experimental animals more or less in the same state of metabolic requirement.

**WATER CHEMISTRY**

Physico-chemical parameters of water have a considerable influence on the toxicity and accumulation of metals in aquatic organisms (Lloyd, 1965; Tabata, 1969a). Hence, in the present study, tap water free from chlorine was used to prepare different concentrations of heavy metals. As the variation in the hydrobiological values of water used for different sets of experiments was negligible, the values for one set is given in Table 1.

**ANALYTICAL TECHNIQUES FOR WATER CHEMISTRY**

Temperature was determined by using a thermometer and pH value by a pH meter (pH Scan 1, Eutech Cybernetics, Singapore). The salinity of the water was estimated by Mohr’s method according to Saxena (1987) and Strickland and Parsons (1965), using 0.1595 N silver nitrate and potassium chromate as an indicator. Dissolved oxygen was determined by Winkler’s method (Strickland and Parsons, 1965). Total hardness was measured using ammonia buffer solution and Erichrome black-T as an indicator and total alkalinity was determined by using methyl-orange as an indicator (Saxena, 1987). Calcium was estimated according to the method of Saxena (1987) using murexide as an indicator.
BIOASSAY

Of the two types of bioassay methods viz., continuous flow (Mount and Warner, 1965) and static (APHA, 1971), the static bioassay method was chosen considering the limitations of the laboratory facilities. According to Sprague (1971, 1973), static bioassay test with toxin dilutions of known accuracy is superior to continuous flow test of uncertain concentrations.

TOXICANT

Cadmium nitrate (Analytical grade) was obtained from Loba Chemie, Bombay, India. The physical and chemical properties of cadmium nitrate are as follows:

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Formula</td>
<td>Cd(NO₃)₂·4H₂O</td>
</tr>
<tr>
<td>Molecular weight</td>
<td>308.48 g/mol</td>
</tr>
<tr>
<td>Colour</td>
<td>White crystal</td>
</tr>
<tr>
<td>Melting point</td>
<td>59.4°C</td>
</tr>
<tr>
<td>Solubility in cold water</td>
<td>215 g/100cc</td>
</tr>
</tbody>
</table>

DETERMINATION OF MEDIAN LETHAL CONCENTRATION

Preliminary toxicity tests were carried out to find the median lethal tolerance limit of fish to cadmium nitrate for 24 h. For determining LC₅₀ concentration, separate circular plastic tubs of 12 litre capacity were used. 10 fish were introduced into each tub with 10 L tapwater which already received different concentrations of [appropriate quantity of stock solution was made up to 1L] cadmium nitrate (3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5 ppm). One gram cadmium nitrate was transferred to one litre standard flask. Water was added up to the mark and the salt was dissolved well. From this stock solution, appropriate quantity was taken and made up to one litre to get the desired concentration of metal solution.
A control tub with 10 litres of water and 10 fish was also maintained.

The mortality/survival in the experimental and control tubs was recorded after 24 h. The concentration at which 50% kill of fish occurred after 24 h treatment was taken as the median lethal concentration (LC$_{50}$) for 24 h. The median lethal concentration was arrived after conducting five preliminary toxicity tests. LC$_{50}$ concentration for 24 h was calculated by the probit analysis method of Finney (1978). By employing Chi-square test, the homogeneity of the population used in the present study was verified according to Busvine (1971).

OBSERVATION OF MORTALITY

Dead fish from the experimental tubs were removed immediately. Death was indicated by failure of the fish to respond to gentle prodding with a glass rod and cessation of opercular movement.

CALCULATION OF REGRESSION LINE

Critical concentrations or susceptibility of an organism to any toxicant can be estimated with sufficient accuracy from a probit/log concentration graph (Busvine, 1971). The two variables are plotted on a plain paper, or the data (percentage mortality and concentration) can be plotted on a logarithmic probability paper. A straight line is fitted by eye and the critical concentration determined
by inspection according to Busvine (1971). Values determined graphically are often remarkably close to calculated results, but they give no precise information on limits of accuracy.

**OBSERVATIONS ON THE INFLUENCE OF WATER HARDNESS ON CADMIUM TOXICITY**

The toxicity of metals are influenced or modified by pH, alkalinity and hardness of water (Stiff, 1971a; Butler, 1978; Ferrando and Andreu, 1991a). Pascoe et al. (1986) observed that the toxicity of some heavy metals to freshwater fish is reduced in hard water when compared with soft water. So it was planned to study the influence of water hardness on cadmium toxicity in relation to the survival of fish *Cyprinus carpio* exposed to LC$_{50}$ (24 h) concentration (5.2 ppm).

Normal tap water was used as a source of soft water. Hard water with hardness 50, 100, 150, 200 and 250 mg/L as CaCO$_3$ was prepared by adding appropriate quantity of calcium nitrate. Ca(NO$_3$)$_2$ is readily soluble and has previously been used for artificial hardening of water (Judy and Davies, 1979; Pascoe et al., 1986). Moreover animals can tolerate varying amounts of nitrate (Raju and Rao, 1983). Double distilled water was used for reducing the hardness if hardness was more than the desired level.
As there is no literature on the impact of water hardness on cadmium toxicity to *Cyprinidae carpio* in Indian waters we have tried with the broad range trials followed by narrow range to find out optimum water hardness. Narrow range trials were carried out to arrive at the optimum water hardness because broad range trials do not give the exact value.

**Broad range trials**

Six tubs were taken. One tub received 10 litres of normal tap water and the rest received hard water ranging in hardness from 50 to 250 (50, 100, 150, 200, 250) mg/L as CaCO$_3$. A common control was maintained. Except the control tub, all others received LC$_{50}$ (24 h) concentration of cadmium nitrate. To all the seven tubs 10 fish were introduced. The survival/mortality of fish were observed for 96 hours.

**Narrow range trials**

Seven tubs were taken, out of which one tub received 10 litres of normal tap water whereas, the others received hard water ranging in hardness from 150 to 200 (150, 160, 170, 180, 190 and 200) mg/L as CaCO$_3$. A common control was maintained. Except for the control tub all others received LC$_{50}$ (24 h) concentration of cadmium nitrate. Ten fish were introduced in each tub. The survival/mortality of fish were observed for 96 hours.

**Preparation of stock solution**

One gram cadmium nitrate was transferred to one litre standard flask. Water was added upto the mark and the salt was dissolved well. From this stock solution, appropriate quantity was taken and made upto one litre to get the desired concentration of metal solution.
RESULTS

Environmental factors may markedly modify the acute toxic effects of pollutants (Mason, 1981). Zitko and Carson (1976) and Pascoe et al. (1986) suggested that hydrobiological features of water like dissolved oxygen (DO), biochemical oxygen demand (BOD), pH, temperature, salinity and hardness have profound influence on the nature, biodegradation and availability of toxicants in aquatic environment.

Table 1 gives the data on the hydrobiological characteristics of water used for the present bioassay study. The water quality characteristics of the test water were: temperature 25.0±2.0°C; pH 7.2±0.1; salinity 0.6±0.1 ppt; dissolved oxygen 6.2±0.02 mg/L; total hardness 18.0±0.5 mg/L; alkalinity 175.0±5 mg/L; and calcium 4.0±0.5 mg/L.

Acute cadmium toxicity in fish caused erratic movement, surfacing, gulping of air, increased opercular movement, turning upside down, and excess mucous secretion. At the end of 24 h treatment, 50 per cent of experimental fish died while the other 50 per cent of fish survived. There was no mortality in the control tubs.

Table 2 and Fig.1 present the data on the log concentration/probit regression of the fish Cyprinus carpio when treated with different concentrations of cadmium.
Table 1. Hydrobiological features of the water used for the present investigation

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Hydrobiological Features</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Temperature</td>
<td>25.0 ± 2.0°C</td>
</tr>
<tr>
<td>2.</td>
<td>pH</td>
<td>7.2 ± 0.1</td>
</tr>
<tr>
<td>3.</td>
<td>Salinity</td>
<td>0.6 ± 0.01 ppt</td>
</tr>
<tr>
<td>4.</td>
<td>Dissolved oxygen</td>
<td>6.2 ± 0.02 mg/L</td>
</tr>
<tr>
<td>5.</td>
<td>Total hardness</td>
<td>18.0 ± 0.5 mg/L</td>
</tr>
<tr>
<td>6.</td>
<td>Alkalinity</td>
<td>175.0 (170-180) mg/L</td>
</tr>
<tr>
<td>7.</td>
<td>Calcium</td>
<td>4.0 ± 0.5 mg/L</td>
</tr>
</tbody>
</table>

Values are mean ± S.E. of five individual observations.
Table 2. Calculation of log concentration/probit regression line for experiments in which the fingerlings of *Cyprinus carpio* var. *communis* were exposed to different concentrations of cadmium nitrate.

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Conc. of Cd(NO₃)₂ in ppm</th>
<th>No. of fish used</th>
<th>% Dead</th>
<th>Log Conc.</th>
<th>Empirical probit</th>
<th>Expected probit</th>
<th>Working probit</th>
<th>Coefficient of variance</th>
<th>Weight</th>
<th>WX</th>
<th>WY</th>
<th>WX²</th>
<th>WY²</th>
<th>WX*WY</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.0</td>
<td>10</td>
<td>28</td>
<td>0.6021</td>
<td>4.42</td>
<td>4.345</td>
<td>4.4260</td>
<td>0.532</td>
<td>5.32</td>
<td>3.2032</td>
<td>23.5463</td>
<td>1.9286</td>
<td>104.2159</td>
<td>14.1774</td>
</tr>
<tr>
<td>2</td>
<td>4.5</td>
<td>10</td>
<td>36</td>
<td>0.6532</td>
<td>4.64</td>
<td>4.640</td>
<td>4.6392</td>
<td>0.601</td>
<td>6.01</td>
<td>3.9257</td>
<td>27.8816</td>
<td>2.5643</td>
<td>129.3483</td>
<td>18.2121</td>
</tr>
<tr>
<td>3</td>
<td>5.0</td>
<td>10</td>
<td>48</td>
<td>0.6990</td>
<td>4.95</td>
<td>4.905</td>
<td>4.9496</td>
<td>0.634</td>
<td>6.34</td>
<td>4.4317</td>
<td>31.3805</td>
<td>3.0978</td>
<td>155.3209</td>
<td>21.9351</td>
</tr>
<tr>
<td>4</td>
<td>5.5</td>
<td>10</td>
<td>56</td>
<td>0.7404</td>
<td>5.15</td>
<td>5.140</td>
<td>5.1512</td>
<td>0.634</td>
<td>6.34</td>
<td>4.6941</td>
<td>32.6586</td>
<td>3.4755</td>
<td>168.2310</td>
<td>24.1802</td>
</tr>
<tr>
<td>5</td>
<td>6.0</td>
<td>10</td>
<td>64</td>
<td>0.7782</td>
<td>5.36</td>
<td>5.360</td>
<td>5.4208</td>
<td>0.616</td>
<td>6.16</td>
<td>4.7937</td>
<td>33.3921</td>
<td>3.7305</td>
<td>181.0119</td>
<td>25.9857</td>
</tr>
</tbody>
</table>

Values are mean of five individual observations.

\[
\bar{X} = 0.6977 \\
\bar{Y} = 4.9340 \\
b = 5.6774 \\
\text{Variance} \ V = 0.0011 \\
\chi^2 = 0.0719
\]

Fiducial limits: At 5% level

- Lower limit \( (m_1) \) = 4.477
- Upper limit \( (m_2) \) = 6.039

Regression equation \( (Y) \) = 0.9729 + 5.6774 \( x \)
Fig. 1. Log concentration of cadmium nitrate Vs. per cent mortality of *Cyprinus carpio* var. *communis* and determination of 24 h LC$_{50}$ value calculated from Table 2.
**FIG. 1**

**Concentration (ppm)**

![Graph showing concentration vs. mortality with empirical probit and concentration values.]

- **Empirical probit (y)**
- **Concentration (ppm)**
- **Log concentration (x)**

Provisional Line

Calculated Line

$y = 0.9729 \times 5.6774x$

Per cent mortality

Log concentration (x)
nitrate for 24 h. The median lethal concentration of cadmium nitrate to fish for 24 h was 5.2 ppm. The Chi-square \((X^2)\) (Busvine, 1971) test on the toxicity data revealed that fish population used for experiments was homogeneous.

Influence of water hardness on cadmium toxicity to \textit{Cyprinus carpio} in broad ranges is given in Table 3 and Fig. 2. In experiment with \(\text{LC}_{50}\) (24 h) concentration of cadmium, when water hardness was increased from 18.0 to 50.0 mg/L, the survival of fish was 80 per cent after 24 h, indicating the inhibition of cadmium toxicity by calcium. When water hardness was elevated to 100 mg/L and 150 mg/L, there was no fish mortality up to 48 h indicating 100 per cent survival. However, the per cent survival of fish in the above experiments showed a declining trend when the exposure period was extended. In water with a hardness of 200 mg/L and 250 mg/L, the fish exhibited 100 per cent survival up to 96 h treatment. The above data may indicate that there is a direct relationship between the level of water hardness and per cent survival of fish exposed to cadmium toxicity.

In acute toxicity study (24 h, 48 h), the per cent survival of fish was 100 per cent when the water hardness was increased by 10 mg/L from 150 mg/L to 200 mg/L as \(\text{CaCO}_3\) in a narrow range (Table 4 and Fig. 3). In 150 mg/L, 160 mg/L and 170 mg/L hardness treatments, fish showed mortality upon extended period of exposure. However, 100 per cent survival of fish was noticed in treatments with 180
Table 3. Per cent survival of fish, *Cyprinus carpio* var. *communis* from studies with LC\textsubscript{50} (24 h) concentration of cadmium nitrate in different water hardness (Broad range trials)

<table>
<thead>
<tr>
<th>Period of exposure (h)</th>
<th>Control</th>
<th>LC\textsubscript{50} (24 h) conc. soft water</th>
<th>LC\textsubscript{50} (24 h) conc. + hardwater (mg/L as CaCO\textsubscript{3})</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Percent survival</td>
<td>Percent survival</td>
<td>50</td>
</tr>
<tr>
<td>24</td>
<td>100</td>
<td>50</td>
<td>80</td>
</tr>
<tr>
<td>48</td>
<td>100</td>
<td></td>
<td>70</td>
</tr>
<tr>
<td>72</td>
<td>100</td>
<td></td>
<td>64</td>
</tr>
<tr>
<td>96</td>
<td>100</td>
<td></td>
<td>58</td>
</tr>
</tbody>
</table>

Values are mean of five individual observations
Fig. 2. Graph showing per cent survival of fish, *Cyprinus carpio* var. *communis* from studies with LC$_{50}$ (24 h) concentration of cadmium nitrate in different water hardness (Broad range trials)
FIG. 2

% SURVIVAL

DURATION IN HOURS

- Control
- LC₅₀(24h) Conc. + Soft water
- LC₅₀(24h) Conc. + 50 mg/l hardness
- LC₅₀(24h) Conc. + 100 mg/l hardness
- LC₅₀(24h) Conc. + 150 mg/l hardness
- LC₅₀(24h) Conc. + 200 mg/l hardness
- LC₅₀(24h) Conc. + 250 mg/l hardness

I, VI and VII

I
II
IV
V
VII
Table 4. Per cent survival of fish, *Cyprinus carpio* var. *communis* from studies with LC$_{50}$ (24 h) concentration of cadmium nitrate in different water hardness (Narrow range trials)

<table>
<thead>
<tr>
<th>Period of exposure (h)</th>
<th>Control</th>
<th>LC$_{50}$ (24 h) conc. + soft water</th>
<th>LC$_{50}$ (24 h) conc. + hardwater</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% Survival</td>
<td>% Survival</td>
<td>Hardness (mg/L as CaCO$_3$)</td>
</tr>
<tr>
<td>24</td>
<td>100</td>
<td>50</td>
<td>100</td>
</tr>
<tr>
<td>48</td>
<td>100</td>
<td></td>
<td>100</td>
</tr>
<tr>
<td>72</td>
<td>100</td>
<td></td>
<td>90</td>
</tr>
<tr>
<td>96</td>
<td>100</td>
<td></td>
<td>84</td>
</tr>
</tbody>
</table>

Values are mean of five individual observations.
Fig. 3. Graph showing per cent survival of fish, *Cyprinus carpio* var. *communis* from studies with LC$_{50}$ (24 h) concentration of cadmium nitrate in different water hardness (Narrow range trials)
FIG. 3

% SURVIVAL

DURATION IN HOURS

- Control
+ || -LC₅₀(24h) Conc. + Soft water
* || -LC₅₀(24h) Conc. + 150 mg/l hardness
* -V -LC₅₀(24h) Conc. + 160 mg/l hardness
* V -LC₅₀(24h) Conc. + 170 mg/l hardness
+ VI -LC₅₀(24h) Conc. + 180 mg/l hardness
* VII -LC₅₀(24h) Conc. + 190 mg/l hardness
* VIII -LC₅₀(24h) Conc. + 200 mg/l hardness

I, VI, VII and VIII
mg/L, 190 mg/L and 200 mg/L up to 96 h. The above data suggest that there is a definite positive correlation between survival of fish and water hardness.
DISCUSSION

Static bioassay tests have been widely used for evaluating the impacts of toxic chemicals on aquatic organisms (Doudoroff et al., 1951). The scientific report of APHA (1974) also accepted bioassay studies as standard methods for assessing the toxicity of a chemical. According to Sprague (1973), the index of toxicity is the median tolerance limits (TLm), which is defined as the concentration at which 50 per cent of the test animals survive or die in a specified time.

When characterizing the toxicity of a specific chemical agent, information is needed not only for single dose (acute) and long-term (chronic) effects, but also for exposures of intermediate durations (Klaassen and Doull, 1980). However, according to Connell (1987), environmental toxicology is principally concerned with the investigation of lethal effects of environmental contaminants and the percentage lethality has a normal distribution in relation to log concentration of toxicant.

An element is toxic if it damages life functions (growth, reproduction, metabolism, etc.) of an organism. Albergoni and Piccinni (1983) suggested that the mechanism of toxicity may be due to chemical inactivation of enzymes; the more electronegative metals and all the divalent transition metals are more active in this respect. According to the above authors, heavy metals react very
promptly with the amino, imino and sulphydryl groups of proteins. This may find support from the work of Wittmann (1979) who reported that most of the very toxic and relatively available metals are soft acceptors, according to Pearson classification (hard and soft Lewis acids). Soft acceptors (such as Cu\(^+\), Ag\(^+\), Hg\(^{2+}\), CH\(_3\)Hg\(^+\), Cd\(^{2+}\) and others) prefer to bind to soft donors (such as SH\(^-\), S\(^2-\), Alkyl or Arayl-S compounds, CN\(^-\) and others).

Excess mucous secretion under cadmium toxicity in several fish has been recorded (Carpenter, 1927; Ellis, 1937; Wong et al., 1977; Shivaraj and Patil, 1985; Gautam and Lall, 1989). Some metals may also damage cells by acting as antimetabolites or by forming precipitates or chelates with essential metabolites; metals may also affect permeability by interacting with membranes and inhibiting specific transport sites (Albergoni and Piccinni, 1983).

The toxicity data in the present study show that cadmium is toxic to *Cyprinus carpio*. The causo-mechanism of mortality of fish may be due to coagulation film anoxia as observed by Jones (1964) or damage in the respiratory epithelium as seen in *Mystus vittatus* and *Labeo rohita* (Datta and Sinha, 1990) or reduced rate of oxygen consumption as reported by Singh and Singh (1979) in *Mystus vittatus* and Watenpaugh and Beitinger (1985) in fathead minnows, or disturbance in the iono-regulation as shown by Roch and Maly (1979) in rainbow trout,
_Salmo gairdneri_, in addition to the enzyme inhibition theory of Wittmann (1979) and Albergoni and Piccinni (1983).

Small changes in dissolved oxygen and carbon-dioxide content, pH, alkalinity and hardness of the test water alter both the aqueous chemistry of the toxicant and the animals physiological response to that toxicant (Jones, 1939). Much of the early work on the effects of chemical variable on metal toxicity dealt with hardness of the principal hardness cations, calcium and magnesium (Skidmore, 1964). Tabata (1969b), Stiff (1971b) and Pagenkopf _et al._ (1974) have indicated that the bicarbonate alkalinity usually associated with hard waters may also reduce toxicity through formation of non-toxic carbonate complexes or precipitates.

Investigation of toxicity in the presence of added complexing agents indicate that formation of either inorganic or organic complexes greatly reduces metal toxicity. Nishikawa and Tabata (1969) have observed that the reduction in toxicity is related to the stability constants of the metal complexes formed. Depending upon water hardness, particularly in closed system, substantial changes in test condition are known to occur during cadmium bioassay (Pickering and Henderson, 1966; Pickering and Gast, 1972).

Environmental calcium has been shown to help _Morone saxatilis_ and _M. chrysops_ alleviate mortality in striped bass and its white bass.
hybrids (Grizzle et al., 1985; Weirich et al., 1992). It has also been observed that external calcium decreased the toxicity of nitrogenous compounds to sunshine bass (Weirich et al., 1993). Gill and Epple (1992) observed that the toxicity of cadmium to mummichog, Fundulus heteroclitus, reduced with high calcium (200 mg/L as CaCO₃) concentration.

Inhibition of cadmium toxicity to fish in hard water has been recorded in acute toxicity experiments in the present study. This may be due to competition between cadmium and calcium which in many freshwater bodies becomes the major physiologically important cation in the ambient medium (Brown, 1968; Kinkade and Erdman, 1975; Wright, 1980). Pagenkopf (1983) observed protective action of water hardness against metal toxicity to fish due to competition between calcium and metal ions through gill surface. Similar observation has also been made by Part et al. (1985) in Salmonids. Freshwater seems to represent a considerably more hazardous environment than saline water with regard to the cadmium sensitivity of aquatic organisms (Ball, 1967; Cearley and Coleman, 1974). Wright and Frain (1981) suggested that differential mortality in amphipod, Gammarus pulex, to cadmium at different calcium levels may be due to differences in the rate of cadmium accumulation, possibly due to competition between the two metals.