BIOASSAY
INTRODUCTION

Qualitative aspects of toxicology are important because they are fundamental to the safety evaluation process in which, one first determines the toxicologic profile of the substance and then establishes how the chemical can be employed safely to prevent injury (Plaa, 1982). According to Durham (1974), toxicity is the ability of a chemical molecule or compound to produce injury once it reaches susceptible site which is determined by the dosage. Cairns (1984) reported that from a regulatory point of view, toxicity tests are used for three major purposes; they are 1. screening of chemicals and products, 2. establishing limits and 3. monitoring; the author further stated that bioassay test can be used to establish the maximum acceptable concentration of a pollutant in a given environment without deliberate application of the chemical causing any unfavourable biological consequences.

Ramade (1987) in his review stated that many specialists have focused their attention on the problem of bioassay in the monitoring of terrestrial and aquatic ecosystems. The aim of using bioassay in monitoring of environmental pollution is to establish a relationship between toxicity and concentration of a pollutant being studied in the biotope; the toxic effects can be divided into two categories viz., effects that occur very quickly after a brief exposure to a chemical agent (acute) and those that appear only after repetitive exposure to the substances (chronic) (Durham, 1974; Ramade, 1987; Nagel, 1993).
Plaa (1982) described that an acute effect is observed within the first several days of exposure to the agent; in most instances all these reactions are discernible within 1 to 2 weeks after administration; on the other hand chronic toxic properties of a substance may not be demonstrable until after several months of continuous repetitive exposures.

Brown (1980) stated that the ultimate response of an organism to acute exposure is dependent on the amount of toxicant at the critical target sites; the amount of toxicant at the target site is dependent in part, on the dose. The author added that between exposure to the dose and the response, there are four major occurrences viz., absorption, distribution, metabolism and excretion; in this context, metabolism includes both toxification and detoxification processes.

Ramade (1987) explained the characteristic of acute toxicity as the cause of rapid death of contaminated individual or population by provoking the most drastic reaction, or very serious physiological disorder shortly after absorption through the skin or by the lungs or by mouth of a fairly large single dose or repeated dose of a poisonous compound.

Hagen (1959) pointed out the following objectives of acute toxicity tests:-

a. To assess toxicity in non-target species

b. To predict hazard to non-target species

c. To provide information on the mechanism of toxic action.
d. To provide data on which user risk benefit relationships may be assessed and

e. To aid the establishment of exposure levels in studies designed to assess long term effects in experimental toxicology.

Toxicity can be calculated by determining the mortality rate after a fixed time as a function of increasing doses of the toxicant (Ramade, 1987). Different characteristic constants of the toxicant being studied can be determined by using dose mortality tests. The most important constant is the LC50 (median lethal concentration), which is the theoretical value causing 50% mortality in the population being studied. The LC50 value is determined after 24 hours or 48 hours of exposure in tests of acute toxicity and in some cases after 96 hours (Ramade, 1987).

Exposure of animals to sublethal levels of pollutants may inflict stress on the mechanism required for maintaining a healthy physiological state; these changes may result in physiological, biochemical and behavioural processes, which will show a more accurate prediction of acceptable levels of pollutants in the environment (Koeman and Strik, 1975; Waterpaugh and Beitinger, 1985; Usha Rani and Ramamurthi, 1987; Mason, 1996).

Metals are mobilised from soil due to occurrence of acid rain (Haines, 1981; Spry et al., 1981). According to Cronan (1978) of the toxic metals, aluminium appears to be the primary element mobilised by strong acids of meteoric origin from the regions with acidic and base deficient soils.
Aluminium has been recognised as an extremely important toxic metal in many temperate freshwater environments but has received little attention in tropical aquatic ecosystem (Phillips, 1988). In recent years aluminium has come to be regarded as the major factor in the loss of fisheries in soft acid waters (Schofield and Trojnar, 1980; Driscoll, 1984; Oremerod et al., 1988; Dietrich and Schlatter, 1989a; Spry and Weiner, 1991; Cummins, 1994). Studies have revealed that aluminium toxicity depend more on pH and speciation (Sadler and Lynam, 1987; Neville and Campbell, 1988; Exley et al., 1994, 1996; Poleo, 1995). Exley et al. (1991, 1994), Exley and Birchall (1992) and Poleo (1995) have investigated the molecular mechanisms involved in the aluminium toxicity.

In the present study, the concentration - response relationship was studied by evaluating the median lethal concentration of aluminium to *Cyprinus carpio* var. *communis*, where the response is mortality of fish.
MATERIAL AND METHODS

Essential criteria for choosing test species for biotests should be stenococious species because they are more vulnerable to any modification of the environment than are euryococious species (Ramade, 1987). According to McDonald et al. (1989), the external surface of aquatic animals are structurally and physiologically delicate than comparable to liquid exposed surfaces in terrestrial animals. Aquatic animals have remarkable capacity of extracting and concentrating certain chemicals from water via., respiration (Sharma, 1985). Hence, among aquatic test species, fish have been widely used as representative of toxicity studies for their ecological and economic importance, availability (Stebbing et al., 1980), recreational value (EIFAC, 1969) and higher position in trophic level in the aquatic food chain (Forstner and Wittmann, 1983). Turing (1947) pointed out that fish are a very useful barometer of the real state of purity of water. By considering the above points, the right species should be chosen as a model, so that the observed results can be transferred to other species and the laboratory results can be extrapolated to field conditions (Nagel, 1993).

In order to extrapolate meaningful, relevant and ecologically significant results from aquatic toxicity tests not only appropriate tests but also appropriate organisms should be used; several criteria should be considered in selecting organisms for toxicity testing (Rand and Petrocelli, 1985).
1. Since sensitivities vary among species, species representing a broad range of sensitivities should be used whenever possible.

2. Widely available and abundant species should be considered.

3. Wherever possible, species should be studied which are indigenous to or representative of the ecosystem that may receive the impact.

4. Species that are recreationally, commercially and ecologically important should be included.

5. Species that are amenable to routine maintenance in the laboratory and techniques should be available for culturing and rearing them in the laboratory so that chronic toxicity tests can be conducted.

6. If there is adequate background information on a species (i.e., its physiology, genetics and behaviour) the data from a test may be more easily interpreted.

FISH MATERIAL

The scale carp, *Cyprinus carpio* var. *communis* (Linnaeus), an exotic fish belonging to the family Cyprinidae were selected for the present investigation based on the following criteria.

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

2. It is sensitive to environmental factors under consideration.
3. Readily available throughout the year in abundant quantity, commercially important and distributed throughout the world.


5. Voraciously omnivorous, efficiently converts the food ingested into flesh, grows very fast, and prone to artificial feeds.

6. Tolerant to wide fluctuations of temperature and resistant to disease.

7. Hardy nature for handling and transport.

8. Suitable animal for bioassay testing.

**RECRUITMENT OF FISH FOR EXPERIMENT**

Healthy specimens of *Cyprinus carpio* var. *communis*, were selected and transported to laboratory in polythene bags containing oxygenated water from Tamil Nadu Fisheries Development Corporation Limited, Aliyar Fish Farm, Aliyar, Tamil Nadu, India. Fish of the same age and size from the same broodstock were collected.

Fish stock were maintained in a clean disinfected large rectangular tank previously cleaned with water and disinfected with potassium permanganate. They were acclimatized to laboratory conditions for a fortnight, before being used for experiments. Media were changed frequently to avoid fungal growth and contamination by metabolites. These stock were well aerated with aerators. During the acclimatization period, fish stocks were fed *ad libitum* with groundnut oil cake and rice bran powder in the ratio of 1:2. Feeding was stopped two days prior to
the commencement of experiments to keep the experimental animals more or less in the same state of metabolic requirement. During acclimatization, the fish stock was maintained at natural photoperiod and ambient temperature. From this stock fish with an average length of 6-7 cm and weighing 4-5 g were segregated and transferred to clean rectangular glass aquarium tanks (75 x 35 x 37 cm) of 150 L water capacity.

**TOXICANT**

**Metal**

According to Howells *et al.* (1990) aluminium is an important metal with the potential to replace zinc and tin in various end user industries; aluminium chloride is used as a pigment in the petroleum and organic chemical industries as a catalyst; aluminium sulphate is used in the pulp and paper industries and in treatment of potable water supplies where it acts as a coagulant.

Aluminium sulphate (anhydrous form) used for the present investigation was obtained from New India Chemical Enterprises, Kochi-682 024, India. The physical and chemical properties of aluminium sulphate (No.A11429) are as follows:

**Physical properties**

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. Formula</td>
<td>$\text{Al}_2(\text{SO}_4)_3 \cdot 16\text{H}_2\text{O}$</td>
</tr>
<tr>
<td>b. Molecular weight</td>
<td>342.15</td>
</tr>
<tr>
<td>c. Colour</td>
<td>White</td>
</tr>
<tr>
<td>d. Purity</td>
<td>97% as $\text{Al}_2(\text{SO}_4)_3$</td>
</tr>
</tbody>
</table>
Chemical properties

According to Driscoll and Schecher (1988), aluminium in solution is amphoteric and can form both organic and inorganic complexes, tending to polymerize; it is a group III element found in solution only in the trivalent state; although it is metallic, it exhibits marked covalent tendencies and thus forming relatively stable complexes with a variety of inorganic and organic materials.

WATER QUALITY

Tap water free from chlorine was used for the present study. The hydrobiological features such as temperature, pH, dissolved oxygen, total alkalinity, salinity and total hardness, were estimated for each set of experiment as these factors have a significant influence on the biodegradability and toxicity of pollutants.

ANALYSIS OF WATER CHEMISTRY

Water temperature, pH, alkalinity, hardness, calcium and dissolved oxygen were monitored daily both in control and experimental tanks. Water temperature was measured by a thermometer. pH values were determined by a pen type pH meter (pH Scan 1, Eutech Cybernetics PTE Ltd, Singapore). Dissolved oxygen content was estimated by Winkler's method using starch indicator. Total alkalinity was determined using methyl orange as an indicator. Total hardness was estimated using Erichrome Black - T indicator and calcium level was determined using murexide indicator. Other parameters such as salinity, fluorine, silicate and dissolved organic carbon were estimated weekly. The above physico-chemical analysis of water used in the present experiments were carried out as per APHA et al.
The analytical data for a single set of experiments for the above parameters are given in Table 1 as the value varied negligibly for the waters used for the other sets of experiments.

Table 1. Physico-chemical features of water used for the present investigation

<table>
<thead>
<tr>
<th>Physico-chemical features</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature</td>
<td>25.0±1.0 °C</td>
</tr>
<tr>
<td>pH</td>
<td>7.5 ± 1 units</td>
</tr>
<tr>
<td>Dissolved oxygen</td>
<td>7.1 ± 0.02 mg/L</td>
</tr>
<tr>
<td>Total alkalinity</td>
<td>35.0 ± 5 mg/L</td>
</tr>
<tr>
<td>Salinity</td>
<td>0.21 ± 0.1 ppt</td>
</tr>
<tr>
<td>Total hardness</td>
<td>18.0 ± 0.5 mg/L</td>
</tr>
<tr>
<td>Calcium</td>
<td>4.23 ± 0.5 mg/L</td>
</tr>
<tr>
<td>Fluoride</td>
<td>Nil</td>
</tr>
<tr>
<td>Silicate</td>
<td>0.001 ppm</td>
</tr>
<tr>
<td>Dissolved organic carbon</td>
<td>Nil</td>
</tr>
</tbody>
</table>

Values are means ± S.E. of five individual observations

BIOASSAY

Of the two types of bioassay experiments viz., continuous flow (Mount and Warner, 1965) and static (APHA, 1971), the static bioassay method was chosen considering the limitations of the laboratory facilities.

Toxicant preparation and exposure

Aluminium stock solutions were prepared by dissolving 1 gm of aluminium sulphate (Al₂(SO₄)₃·16 H₂O) in 1 litre of tap water and stored in stoppered acid washed Erlenmeyer flasks. Tests were conducted in previously cleaned, sundried circular plastic tubs of 25 litres capacity. Preliminary toxicity tests were carried out to find out median lethal tolerance limit of fish to aluminium sulphate for 24 hrs. For determining the LC₅₀ concentration, plastic tubs holding 10 litres of water were used.
and appropriate concentrations of aluminium sulphate were mixed to the test water in the tubs. In each tub 10 fish were introduced. A control tub was also maintained with 10 fish in 10 litres of water.

**Determination of median lethal concentration**

At the end of 24 h the survival/mortality of fish in the control and experimental tubs was recorded. The concentration at which 50% mortality of fish occurred after 24 h was taken as the median lethal concentration (LC50) for 24 h. The LC50 concentration for 24 h was calculated by the probit - analysis method of Finney (1978). Dead fish were removed immediately from the experimental tubs. Fish were considered to be dead when they lost their equilibrium, floating belly up, became immobile, cessation of ventilatory and mouth movements and inability to respond to any stimulus.

**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 original data (percentage mortality and concentration) can be plotted on a logarithmic probability paper. A straight line is fitted by eye and the critical concentrations are determined by inspection. Values determined graphically are often remarkably close to calculated results, but they give no precise information on limits of accuracy. Based on the above method, the regression line was calculated in the present study.
RESULT

According to Lloyd (1965) and Tabata (1969), many physico-chemical parameters have a considerable influence on the toxicity and accumulation of metals in organisms. In general, toxicity of heavy metals in water is determined according to the toxicity of the metal itself, the synergistic and antagonistic aspects of the metal and the influence of physico-chemical parameters which determine a metal's availability by activation or deactivation.

Table 1 gives the data on the physico-chemical characteristics of water used for the present toxicity study. Table 2 and Fig. 1 present the data on probit regression / log concentration of fish *Cyprinus carpio* var. *communis* when exposed to various concentrations of aluminium sulphate for 24 h and the same for 96 h is given in Table 3 and Fig. 2. The median lethal concentrations of Al₂(SO₄)₃ for 24 h was 44.90 ppm and for 96 h was 26.42 ppm. The chi-square test of Busvine (1971) on the toxicity data presented in Table 2 and 3 indicates clearly that fish population used for the experiment was homogenous.

Fish exposed to 24 h LC 50 concentration of aluminium sulphate showed a gradual retardation in their movement with extension of time. Profuse mucus production was observed. Towards the end of the experiments fish showed erratic movement, operculum was wide open, and lost equilibrium. Fish were apparently struggling hard for respiration and swam slantingly. Then jerking movement was observed before they died.
Table 2: Calculation of log-concentration / probit regression line for 24 hours experiments in which fish *Cyprinus carpio* var. *communis* were exposed to different concentrations of aluminium sulphate

<table>
<thead>
<tr>
<th>Conc. of $\text{Al}_2(\text{SO}_4)_3$ (in ppm)</th>
<th>No. of fish used</th>
<th>% Mortality</th>
<th>Log conc. $x$</th>
<th>Empirical probit</th>
<th>Expected probit $y$</th>
<th>Working probit $y$</th>
<th>Weight coefficient</th>
<th>Weight $W$</th>
<th>$Wx$</th>
<th>$Wy$</th>
<th>$Wx^2$</th>
<th>$Wy^2$</th>
<th>Wxy</th>
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<tr>
<td>42</td>
<td>10</td>
<td>32</td>
<td>1.6232</td>
<td>4.5323</td>
<td>4.6600</td>
<td>4.5304</td>
<td>0.6010</td>
<td>6.0100</td>
<td>9.7554</td>
<td>27.2277</td>
<td>15.8350</td>
<td>123.3524</td>
<td>44.1960</td>
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<td>44</td>
<td>10</td>
<td>46</td>
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<td>4.9000</td>
<td>4.8992</td>
<td>0.6340</td>
<td>6.3400</td>
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<tr>
<td>46</td>
<td>10</td>
<td>58</td>
<td>1.6628</td>
<td>5.2019</td>
<td>5.1320</td>
<td>5.2016</td>
<td>0.6340</td>
<td>6.3400</td>
<td>10.5422</td>
<td>32.9781</td>
<td>17.5296</td>
<td>171.5391</td>
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<tr>
<td>48</td>
<td>10</td>
<td>64</td>
<td>1.6812</td>
<td>5.3585</td>
<td>5.3580</td>
<td>5.3588</td>
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<td>6.1600</td>
<td>10.3562</td>
<td>32.9979</td>
<td>17.4108</td>
<td>176.7631</td>
<td>55.4760</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>30.4300</td>
<td>50.0132</td>
<td>148.9283</td>
<td>82.2224</td>
<td>732.8414</td>
<td>245.0703</td>
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</table>

<table>
<thead>
<tr>
<th>$\bar{x}$</th>
<th>$\bar{y}$</th>
<th>$b$</th>
<th>Variance 'V'</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.6435</td>
<td>4.8941</td>
<td>11.878</td>
<td>0.0002</td>
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</table>

$\chi^2 = 0.331$

Fiducial limits at 5% level
Lower limit $m_1 = 42.13$
Upper limit $m_2 = 47.86$
Regression equation $y' = -14.6286 + 11.8775x$

LC$_{50}$ 24 h value = 44.90 ppm
Values are means of five individual observations
Fig. 1. Log concentration of aluminium sulphate Vs per cent mortality of *Cyprinus carpio* var. *communis* and determination of LC 50 24 h value calculated from Table 2.
Concentration (ppm)

Log concentration (X)

Empirical probit (Y)

Mortality (%)

Y = -14.6266 + 11.8775 X

Fig. 1
Table 3: Calculation of log concentration / probit regression line for 96 hours experiments in which fish *Cyprinus carpio* var. communis were exposed to different concentrations of aluminium sulphate

<table>
<thead>
<tr>
<th>Conc. of Al₂(SO₄)₃ (in ppm)</th>
<th>No. of fish used</th>
<th>% Mortality</th>
<th>Log conc. ( x )</th>
<th>Empirical probit</th>
<th>Expected probit ( y )</th>
<th>Working probit ( y )</th>
<th>Weight coefficient</th>
<th>Weight ( W )</th>
<th>Wx</th>
<th>Wy</th>
<th>Wx²</th>
<th>Wy²</th>
<th>Wxy</th>
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<tbody>
<tr>
<td>22</td>
<td>10</td>
<td>32</td>
<td>1342</td>
<td>4.53</td>
<td>4.53</td>
<td>4.528</td>
<td>0.581</td>
<td>5.81</td>
<td>7.7970</td>
<td>26.3076</td>
<td>10.4636</td>
<td>119.1211</td>
<td>36.3049</td>
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<tr>
<td>24</td>
<td>10</td>
<td>40</td>
<td>1.380</td>
<td>4.75</td>
<td>4.77</td>
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<td>8.5008</td>
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<tr>
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<td>10</td>
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<td>4.95</td>
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<td>10</td>
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<td>1.447</td>
<td>5.15</td>
<td>5.14</td>
<td>5.151</td>
<td>0.634</td>
<td>6.34</td>
<td>9.1739</td>
<td>32.6573</td>
<td>13.2747</td>
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<td>30</td>
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<td>1.477</td>
<td>5.36</td>
<td>5.33</td>
<td>5.356</td>
<td>0.616</td>
<td>6.16</td>
<td>9.0983</td>
<td>32.9929</td>
<td>13.4382</td>
<td>176.7102</td>
<td>48.7306</td>
</tr>
</tbody>
</table>

\[
\begin{align*}
\bar{x} & = 1.4130 \\
\bar{y} & = 4.9523 \\
b & = 6.0810 \\
\text{Variance 'V'} & = 0.0009
\end{align*}
\]

\[
\chi^2 = 0.0118
\]

Fiducial limits at 5% level
- Lower limit \( m_1 \) = 23.1100
- Upper limit \( m_2 \) = 30.3528
- Regression equation \( y' \) = -3.6401 + 60.810x

**LC₅₀** 24 h value = 26.42ppm

Values are means of five individual observations
Fig. 2. Log concentration of aluminium sulphate Vs per cent mortality of *Cyprinus carpio* var. *communis* and determination of LC 50 96 h value calculated from Table 3.
DISCUSSION

Bioassay can be defined as use of a living organism to measure the concentration of a substance in water by determining its potency in producing some specific effects in the organism (Alabaster and Lloyd, 1985).

Ferrando and Andreu (1991) reported that static acute toxicity tests provide rapid and reproducible concentration-response curves for estimating toxic effects of chemicals on aquatic organisms which may form a data base for determining relative toxicity to a variety of species.

The median lethal concentration of aluminium sulphate to *Cyprinus carpio* var. *communis* for 24 h in the present investigation was determined as 44.90 ppm and for 96 h 26.42 ppm. Few authors have determined LC50 of aluminium by exposing fishes at neutral pH. Thomsen *et al.* (1988) calculated the LC 50 as 3.80 mg Al/l in softwater and 71.00 mg Al/l in hardwater in 25 day old larvae of *Salmo gairdneri* at pH 7.0. Similarly Birge *et al.* (1980) performed embryo larval bioassay on 11 trace metals of coal waste effluents in embryo of rainbow trout, *Salmo gairdneri*, large mouth bass, *Micropterus salmoides*, and the marbled salamander, *Ambystoma opacum* at pH 7.2-7.8. The LC 50 value of aluminium calculated for trout, bass and salamanders were 0.56, 0.17 and 2.28 ppm, respectively. Burrows (1977) has also given an account of lethal concentration of aluminium compounds to various species of freshwater fishes. The LC50 value of aluminium chloride to rainbow trout, *Salmo gairdneri* under 96 hr was 7.4 and 14.6 ppm at pH 6.5 and 7.5, respectively. (Call *et al.* 1984).
Most metals and their salts are simple inorganic compounds, the toxicity of which is caused by anions, cations or physicochemical properties of the salt; compounds of metals have an adverse effect on the self purification process of a water body; the harmful effect of salts of metals is manifested as forming precipitated insoluble hydroxides of metals deposited on the gills and cause mortality of fish, reducing the pH of water on hydrolysis and specifically imparting toxic effect based on specific action (Metelev et al., 1983).

Aluminium toxicity depends on the species of aluminium present, which is largely dependent on water pH (Burrows, 1977) and the presence of complexing ligands such as fluoride (Driscoll et al., 1980) silicic acid (Birchall et al., 1989) and organic material such as humic acid (Gundersen et al., 1994).

Two theories have been proposed by the previous workers to explain aluminium's toxic mode of action (Sadler and Lynam, 1987; Dietrich and Schlatter, 1989a; Handy and Eddy, 1989). The first one is physical effect, and the second one is surface binding. In the first theory inflammation of gill epithelial cells might have been caused by the precipitation of aluminium hydroxide at the gill surface. In the second one it was postulated that the hydrolysis products of Al viz., Al(OH)\(^{2+}\), Al(OH)\(^{3-}\) may bind to functional groups at the gill epithelium integral to membrane structure and function (Sadler and Lynam, 1987).

Several authors have observed similar symptoms of acute toxicity like inflammation, edema, swelling and in some cases lamellar fusion of the gills (Schofield and Trojnar, 1980; Karlsson-Norrgren et al., 1986a; Jagoe et al., 1987;
Goossemaerts et al., 1988; Tietge et al., 1988). In the present study also, after 24 h exposure significant structural alternations like edema and lamellar fusion of the gills were observed which might have led to mortality of fishes.

Profuse secretion of mucous by fish at low pH on aluminium exposures may clog the inter lamellar spaces in the gill epithelia, leading to reduced water flow over the respiratory surfaces and increased thickness of the diffusion barrier for gases (Westfall, 1945; Plonka and Neff, 1969; Daye and Garside, 1976; Ultsch and Gros, 1979). Youson and Neville (1987) observed aluminium deposit on the gills impairing the gas exchange across the gills resulting in failure of gas exchange causing cellular anoxia, asphyxiation and acute death of fish. However, Exley et al. (1996) showed that aluminium precipitated at the gill surface was not always associated with an acute toxicity rather, the strength of association of aluminium with the gill epithelium occurring through the formation of hydroxy and oxo-bridges with oxygen based groups present in the mucous and gill is the determining factor.

$\text{Al}^{3+}$, a dominant aluminium ion at pH $< 4.5$ is implicated for acute aluminium toxicity in fish (Erichson and Jones, 1939; Neville, 1985; Dalziel et al., 1987; Playle et al., 1989). Exley et al. (1991) stated that the binding of $\text{Al}^{3+}$ at the gill epithelium is a pre-requisite for the acute toxicity.

It is reported that $\text{Al}^{3+}$ is less toxic than any other species of aluminium (Baker and Schofield, 1980; Muniz and Leivestad, 1980a, b; Schofield and Trojnar, 1980; Fivelstad and Leivestad, 1984; Wood and McDonald, 1987; Neville and Campbell, 1988). However, Playle and Wood (1990) stated that $\text{Al}^{3+}$ bound to the gill
surface may undergo deprotonations to form aluminium hydroxide polymers due to changes in the pH of the gill microenvironment and cause toxicity to fish.

Several authors have reported that maximum toxicity occurs at around pH 5.0 to 5.5 where, aluminium hydroxide constitutes the dominant species (Schofield and Trojnar, 1980; Helliwell et al., 1983; Leivestad et al., 1987; Sadler and Lynam, 1987). Subsequently, aluminium polymers were identified as the major cause of acute aluminium toxicity in fish in the pH region 5.0 to 6.0 (Lydersen et al., 1990a, b; Rosseland et al., 1992; Poleo and Muniz, 1993; Witters et al., 1996), contradicting earlier observations attributing toxicity to monomeric species of aluminium hydroxides.

Aluminium polymerization may start as soon as the Al(OH)\(_2^{+}\) starts to yield high molecular species of aluminium (Hem and Robersen, 1967). Zaug (1982) and Playle and Wood (1989, 1990) stated that the gills may act as nucleating surfaces upon which aluminium can polymerize. Lydersen et al. (1991) found that the degree of ongoing polymerization is an important factor in determining acute toxicity of aluminium. Further, the authors reported that fish in contact with a freshly prepared solution of aluminium is exposed to a spectrum of hydrated aluminium species, both monomeric and polymeric, and these solutions are highly toxic than aged solutions.

Recently, Exley et al. (1991) have described that phospholipids, a membrane transport protein, polyanionic mucus consisting negatively charged glycoproteins and sialic acid at the gill surface offer as potential binding sites for aluminium. The stability of binding is dependent on the relative concentrations of
competing cations and anions and intrinsic binding strength of aluminium to gill and mucous ligands where binding may be weak electrostatic or multiple co-ordination to a single aluminium ion (Birchall and Chappell, 1988).

In the present study, addition of LC 50 concentration of aluminium sulphate to the test water of pH 7.5±1, reduced the pH to 4.9±1. Similar drop in pH was observed by Hunter et al. (1980), Hall et al. (1985, 1987), Bielby (1988) and Hall and Hall (1989), while aluminium compounds were discharged into natural water. High concentration of aluminium in combination with low pH have been shown to cause mortality of freshwater fish in both field and laboratory studies (McCahon et al., 1989; Howells et al., 1990; Cummins, 1994; Atland and Barlaup, 1995). A similar drop in pH associated with elevated soluble aluminium concentration might have resulted in the mortality of fish of *Cyprinus carpio* var. *communis* in the present study.

Further, at this pH, the predominant species of aluminium present in the solution may be monomeric aluminium hydroxide, which might have started forming high molecular polymeric species. Growth of aluminium polymers on the gill surface and increased mucous secretion might have caused severe clogging of the interlamellar spaces leading to acute hypoxia and mortality of *Cyprinus carpio* var. *communis* in the present study supporting the observations of Zaug (1982), Playle and Wood (1989, 1990) and Lydersen et al. (1991) on the mechanism of acute toxicity in fish.