INTRODUCTION

Water pollution has focused the attention of both the scientific community and the public on environmental problems. Not only does water pollution affect the health and welfare of people and organisms, but it also damages vegetation and properties Sehgal and Saxena, (1986).

According to (Mason et al; 2000), there are five major types of toxic pollutants known to man:

i) Metals arising from industrial processes and some agricultural applications (lead, copper, nickel and zinc etc.)

ii) Organic compounds, originating from industrial, agricultural and some domestic sources (herbicides, PCB’s, organochloride pesticides, chlorinated aliphatic hydrocarbons, organometallic compounds and phenols)

iii) Gases (ammonia and chlorine)

iv) Anions (cyanides, fluorides, sulphides and sulphites)

v) Acids and alkalis

Metals with an atomic number greater than 20 are considered as heavy metals. Introduction of metals into aquatic systems are due to both natural causes and human activities.
Natural sources of metals to aquatic systems include weathering of soils and rock, and volcanic eruptions. Sources through human activities include mining, processing, and use of metals and substances that contain metals. Some metals, such as manganese, iron, copper, and zinc are essential to living beings in small quantities and hence are called micronutrients or essential elements/metals. They are required usually in trace quantities and hence are known as essential trace elements/metals. On the other hand, metals such as mercury, cadmium, and lead are not required even in very small quantities by any organisms and hence are called non-essential elements/metals. However, virtually all metals, whether they are essential or not, are toxic to aquatic organism if exposure levels are sufficiently high.

Metals are introduced into the environment by a wide range of natural and anthropogenic sources (Wepener, *et al.*; 2000A) and with anthropogenic being either domestic or industrial (Biney *et al.*; 1994). Heavy metals are often present at elevated concentrations in aquatic ecosystems, due to A) rapid growth in population (Biney *et al.*; 1994) Seymore, (1994), B) The increase in industrialization (Biney *et al.* 1994, Pelgrom *et al.*, 1994), C) The increase of
urbanization and socio-economic activities, exploration and exploitation of natural resources, D) Extension of irrigation and other modern agricultural practices and as well as the E) lack of environmental regulations (Biney et al; 1994). Consequently aquatic organisms are exposed to the elevated levels of metals (Pelgrom et al; 1995) levels not previously encountered Nussey, (1998), posing a great threat to aquatic organisms in particular, and to the whole ecosystem in general (Zou and Bu 1994, Zou, 1997).

Heavy metals pose a great threat to aquatic organisms not only because they are highly toxic in low quantities but also because of their potential characteristic of combining with biological molecules. Metals like mercury, cadmium, and lead all show a great affinity for sulfhydryl (-SH) groups and exert toxic effects largely by combining with such groups on proteins. This result in disruption of enzyme mediated processes in the cells Laws, (1981). Another quality of these metals is that they rapidly adsorb to particulate materials such as detritus, and suspended, sediments, and particle like organisms such as plankton. When these particles are ingested by other organisms, metals get transferred. And in the process the whole food chain may get the metals transferred in a series. Benthic organisms are the most likely
candidates to be affected by metals in sediments because the benthic environment is the ultimate respiratory of particulate materials that wash into aquatic systems. Invariably sediments near open drain outfalls contain high metal concentrations because of discharges from municipal and industrial wastes. One of the most significant effects of metallic pollution is that aquatic organisms can adsorb and accumulate concentrations in their tissues. For example, there may be up to 15 times as much mercury present in fish as in algae. (Ajmal et al; 1983) have reported elevated level of Cd, CO, Cu, Cr, Fe, Mn, Ni, Pb, and Zn in the fish and submerged plants from the Ganges river. There are many reports on toxic effects of certain metallic compounds. (Banerjee and Banerjee, 1988, Kohli et al, 1988, Joseph, 1989; and Sinha et al, 1992).

Increasing urbanization, rapid industrialization and development in agricultural technology has led to the use of innumerable synthetic chemicals and productions of hazardous waste. Although the green revolution throughout the world has successfully met the challenge of hunger, the use of thousands of chemicals as fertilizers, insecticides, fungicides, herbicides, rhodenticides and other biocides has posed serious problems of environmental pollution leading threat to the human beings,
wildlife and important biotic components of the food chain. The modern style of living and ever-increasing needs of mankind has led to rapid industrial development as a result thousands of water-based industries have come up during last three-four decades. The industries like pulp and paper, textile, tanneries, distilleries, electroplating, fertilizer, food and dairy products, thermal and nuclear power plants and other plastic and allied products has been another serious problem of environmental pollution.

Water bodies serves as the ultimate sink for all sorts of chemicals used for any purpose and all sorts of hazardous wastes produced by industries. Some of these directly find their way to aquatic ecosystems, while other reaching indirectly. Some of the toxicants are biodegradable and being converted into nontoxic substances over a period of time depending on self-purification capacity of water body, while some of like heavy metals are either non-degradable or slowly degradable resulting in accumulation and bio-concentration within the ecosystem and its biotic components.

Toxicity testing is essential component of water pollution evaluation and the study of changes in physico-chemical parameters only does not help much in the assessment of effect of pollution in aquatic biota, Trazwell, (1971). The history of toxicity
testing dates back to the time of Aristotle (over 2000 years ago) when he observed the effect of seawater on freshwater animals. Toxicology arose as the formal discipline in early 1980’s and since then man has been serious of knowing the adverse effects of chemicals and drugs on human beings. After 1940’s interest was generated towards the effects of chemical and wastes to non-human organisms like fish Bukiema et al, (1982). The toxicologists have demonstrated and advocated the utility of experimental toxicity testing of industrial waste and other toxicants to fish or predicting potential damage to the aquatic fauna of water bodies.

The toxicity tests are useful for providing answers to a variety of questions and purposes.

- Suitability of environmental conditions for aquatic life.
- Optimum concentrations of environmental factors for aquatic life.
- Toxicity of waste to a species.
- Relative toxicity of toxicants to a tests species.
- Relative sensitivity of different species to a toxicant.
- Effectiveness of water treatment methods.
- Permissible discharge rates of effluents.
- Sublethal effects of toxicants on a stage or complete life cycle of species.

- Short-term effects of episodic skills of toxicants to aquatic fauna.

Selection of the toxicity tests is the most important aspect of toxicology. Toxicity tests are classified according to: (a) duration short term, intermediate and/or long term, (b) methods of adding tests solutions static, re-circulation, renewal or flow through, and (c) purpose effluent quality monitoring, relative toxicity, relative sensitivity, test, odor, growth rate etc. (APHA, 1985).

The fact that the metal ions have a biological significance is contradictory to the classical concept that inorganic chemistry is restricted to nonliving chemical systems, whereas the living world falls within the realm of organobiochemistry. Modern research has led to a broader understanding of the inextricability of overlapping concepts in the field of applied chemistry, such as occur in nature, and stresses the need to diverge from artificial compartmentation. It has been borne out by experimental evidence that the role of heavy metal ions of living systems follows the
pattern of natural availability and abundance of the same metals occurring in nature.

An element is essential when: (1) it is consistently determined to be present in all healthy living tissues within a zoological family, whereby tissue concentrations from species to species should not vary by a wide range, (2) deficiency symptoms are noted with depletion or removal, which disappear when the elements are returned to the tissue, (3) the deficiency symptoms should be attributed to a distinct biochemical defect on the molecular level.

Among these, short-term tests are useful for routine monitoring for exploratory test and for estimating effluent discharge. These tests determine LC$_{50}$ and are quick estimate of toxicity, assessment of relative toxicity of different toxicants and assessment of a toxicant to different species. These tests are also useful for estimating toxicant concentration to be used in intermediate and long-term test. The intermediate test is conducted when a toxicity test is dealt with long life cycle organism or longer life cycle stage, which require additional time for determination of LC$_{50}$.
The toxicity of heavy metals lies in the capacity of their selective effect on enzymes of nerve tissue – cholinesterase (ChE), which led to excessive accumulation of acetylcholine in the organism (Pan and Datta, 1998, Quistad and Casida, 2000, Fulton and Key, 2001). Other enzymes like esterase, protease and peroxides are also inhibited by organophosphate compounds and slightly enhance the activity of catalyze.

The aquatic environment in recent times is witnessing an unprecedented in pour of various kinds of biocides in alarming quantities and sources of such release are too numerous to be mentioned. The common toxic metals found in industrial effluents are cadmium, chromium, nickel, lead, copper, zinc etc. are non essential elements and do not require by the animal. Therefore any accumulation of these metals is a burden on the organism and is likely to prove as a source of toxicity. Gupta and Chakrabarti, (1994) reported toxicity of zinc to *Notopterus notopterus* and *Punctius japonicus*. Zyadan and Abdel, (2000) studied the toxicity and bioaccumulation of copper, zinc and cadmium in fishes. (Villar et al; 2000) determined lethal concentration of copper in the neurotropical fish, *Cnesterodon decemmaculatus*. Contaminations of the aquatic environment with the heavy metals are matter of
concern because these heavy metals can enter the food chain as result of bioaccumulation and can cause serious health problems in human (Frieberg et al., 1973, Piscator 1980).

The biological accumulation of metals by the aquatic organisms poses a serious problem to human populations. Bioaccumulation is the ability of an organism to concentrate an element or the compound from food and water to a level higher than that of its environment Menjer and Nelson, (1980). Thus, bioaccumulation studies are important in the estimation of potential environmental harm. Pierson (1981) studied effects of chronic zinc exposure on the growth, sexual maturity, reproduction and bioaccumulation in the guppy, Poecilia reticulata. Gupta and Sharma, (1994) reported on bioaccumulation of zinc in Cirrhinus mrigala fingerlings during shorter static bioassay. Athikesavan and Vincent (2000) studied toxic effects of nikel and zinc and bioaccumulation in Hypophthalmichthys molitrix.

The aquatic environment with its water quality is considered as the main factor that affects the state of health and disease in both man and animal. The recent increased use of chemicals an industry and agriculture represent the dangers of chemical pollution. The most important heavy metals that
contaminate water bodies are Zn, Ca, Pb, Cd, Hg and Cr. Increasing levels of these metals in water bodies become toxic to the biota.

Heavy metal compounds are found in many of the materials and processes of regional industrial activities and to a lesser extent agricultural activities (UNEP 2000). Lead, cadmium, and chromium, which were used for anti-algae and fungi-fouling paints for marine craft and structures. More toxic, and of documented environmental degradation in the Pacific, is the use of organotins, such as TBT in anti-fouling paints. The composition of waste has changed with the biodegradable proportion of the waste decreasing, accompanied by an increase in non-biodegradable waste such as nicad and lithium batteries, waste oil, and food and drink cans. These contribute to heavy metal problems in the fresh and marine water environment.

Several attempts have been made to develop biological markers or bioindicators of heavy metal contamination of aquatic ecosystem. Fish have been used for many years to indicate whether water is clean or polluted. Fish are excellent bioindicators of heavy metal contamination. The freshwater fish *R. daniconius* has been used as the bioindicator of heavy metal
pollution in order to investigate acute toxicity of zinc, lead, nickel, singly and in combination. The proposed investigation on impact of interactions of metals on acute toxicity to *R. daniconius* would reveal the antagonism and synergism of metals in relation to acute toxicity.
MATERIALS AND METHODS

Fishes of average size *R. daniconius* were regularly collected from ‘Van’ river 10 km away from Parli-vaijnath, Dist.Beed (Marathwada region) Maharashtra state. Experiments related to toxicity evaluation was carried during June 2002 to September 2002. All physicochemical parameters of the water used to perform toxicity evaluation were maintained as per the record available for the ‘Van’ river from the municipal council Parli-Vaijanath. *R. daniconius* is sensitive to aquatic disorders and easily maintainable in the laboratory. The *R. daniconius* mainly feeds on insects, crustaceans and filamentous algae and other suitable organisms found very close to the surface of water. The fishes have oblique cleft of mouth, which help to capture various insects, larvae and other floating organisms.

The test fishes were brought to the laboratory without any mechanical injury. The fishes were acclimatized in well-aerated water, the physiochemical characters of the aged test water were (average values) temperature: 27°C-28°C; conductivity: 0.72 m MHO; dissolved oxygen 6.2mg/l (5.8-6.5 mg/l.); total hardness (as CaCO₃): 72ppm(70-90ppm); total alkalinity: 26.4 ppm (22.6-28.2ppm); total acidity: 6.4ppm (6.0-7.0ppm); and pH 7.8(7.6-7.9)
for two weeks before being used for tests. During the period of acclimatization the water was changed for every 24 hours, and the fishes were fed thrice a week on fresh pieces of flesh of fish. Feeding was stopped 24 hours before the toxicity tests. The used water was clear and dechlorinated which was used to maintain the fishes as well as for the tests concentrations. The aging of the water is necessary before it is used for maintaining the fishes as it helps to stabilize its composition and moreover so as to eliminate residual chlorine which is otherwise considered highly toxic to fishes. The fishes were maintained in sufficiently large aquaria so as to avoid overcrowding. During acclimatization, the care was taken that one-litre or more water volume should be available per gram body weight of the fish in the aquaria. The fishes were exposed to diffused day light during the daytime, where the daily photoperiod was about 10-12 hrs. All the necessary care was taken to keep the aquaria tanks away from various mechanical or visual disturbances. The stockfishes in which the mortality exceed 5% the complete batch was discharged. The large glass containers contain 25 liters were used as test chamber. Artificial aeration and feeding during the toxicity test was avoided. Pilot experiments were conducted to find out the range of the toxicity of the particular toxicant. The chosen range of concentration was such that it
resulted in 0 to 100% mortality. The fishes used, were washed with very light KmnO₄ solution before they were transferred from the acclimatization aquarium to the experimental container one by one with the help of a small hand net. Similarly controlled groups of fishes were also maintained with 0 toxicant concentration under similar conditions.

Acute toxicity tests were conducted over 96 hrs. The experimental troughs containing 5 litres dechlorinated water were used to keep the animals. Stock solution of the toxicants were prepared in double glass – distilled water and added to the test medium to get the desired concentrations of heavy metals. After every 12 hours the polluted water was changed by the fresh solution of the same concentration of heavy metals. The resulting mortality was noted in the range of 10 to 90% for each concentration for the duration of 24, 48, 72 and 96 hrs. Each experiment was repeated thrice to obtain constant results. The same experiment was obtained constant results on the glass surface as well as simulation of muddy surface in the glass tub, show similar trend of toxicity nickel < zinc < lead, however the lethal toxicity of these was reduce on muddy surface.
The data collected was analyzed statically by means of probit method on transforming toxicity curve (% mortality Vs. concentration), which allows the average median lethal concentration of \( LC_{50} \) to be calculated for 24, 48, 72 and 96 hrs. Dead fishes were counted individually. The criterion for death was the failure of the animal to respond to the pricking of its foot with a needle.

**CALCULATION OF PERCENT MORTALITY**

Abbott’s formula (1925) was used for getting the exact mortality, which could be obtained by subtracting the natural mortality in control group from experimental group

\[
P = \frac{O \text{m} - C \text{m}}{100 - C \text{m}} \times 100
\]

Where,

\( P \) = Corrected mortality

\( O \text{m} \) = observed Mortality

\( C \text{m} \) = controlled mortality

It was observed that there was no mortality in control group of fishes. The mortality data obtained in experimental set of fishes for each dose was calculated by Finney’s formula.
P = \frac{r}{n} \times 100

Where,

P = \textbf{Percentage mortality}

r = \text{Mortality observed}

n = \text{No. of animals in batch}

The mortality data thus obtained was put into probit/log concentration transformation so as to plot probit regression lines. The regression line calculated the 50 % mortality and 10 % mortality causing concentrations of heavy metals. The standard error of the log\(_{50}\) (variance ‘V’ of the calculated log LC\(_{50}\)) and X\(^2\) (Chi-square) value and fiducial limits to heavy metal pollutants were calculated from regression equation. The lethal dose and safe concentration of pollutant were calculated.
CALCULATION OF REGRESSION LINE

The method described by Finney (1951) was applied for drawing well-studied strength line between log concentration verses probit kill. Following steps were carried out plot regression line and regression equation.

Different concentration of heavy metals was used. The fishes were exposed for 24, 48, 72, 96 and 24 hrs. at muddy surface and regression line, regression equation were calculated.

In column No. I of the table serial numbers of troughs was entered.

In column No. II of the table concentration of the pollutants in PPM were noted.

In the III column, headed “X” the log of respective concentration to base $_{10}$ was entered.

In the IV column, headed “n” the number of animal taken for each batch was noted.

In the V column the observed mortality in respective concentration for 24, 48, 72, 96 and 24 hrs. at muddy surface was recorded.
In column No VI contains the present mortality (p) by formula \( p = 100 \frac{r}{n} \), if mortality in control group of animals occur then use Abbott’s formula:

\[
P = \frac{O_m - C_m}{100 - C_m} \times 100
\]

The empirical probit values were read from Table No.1 for transformation of percentage to probit from statistical analysis of Finney and recorded on column No. VII.

Provisional straight line was drawn by judging maximum point which plotted Empirical probit verses log concentration \((10)\).

Column No. VIII consist of expected probit \((y)\) values read from provisional line of the graph.

Column No. IX contains weighing coefficient \((w)\) from the column \(c = 100\) of Table II in Finney’s book. For \(w-y\) was read and entered in the column.

In the column No. X consists of weighing coefficient \((w)\) multiplied by \(n\) (number of animal exposed for each batch) and product was \(w\).
Column No. XI contains the working probit (y) values which was read from Table No. IV Finney`s book by corresponding y and p.

Column No. XII and XIII contain product of Wx and Wy respectively.

Column No. X, XI and XII were summed up at the foot of each respective column and abbreviated as Sw, Swx, and Swy respectively.

Column No. XIV, XV and XVI the product of W multiplied by $X^2$, w multiplied by $Y^2$ and W multiplied by x and y were entered respectively. The summations of the product of column XIV, XV and XVI and $Swx^2$, $Swy^2$ and $Swxy$ respectively they were entered at foot of each column.

The X and Y were calculated by using the formula:

$$X = \frac{Swx}{Sw},$$

$$Y = \frac{Swy}{Sw}$$

The regression coefficient “b” was found out by the formula:

$$b = \frac{Swxy - X, Sxy}{Swx^2 - X, Swx}$$
The regression equation is written as

\[ Y = y + b (X - x) \]

Column No. XVII consist of improved probit “Y” calculated from regression equation value of Y corresponding to original value of “X” comparable difference between y and y should not be more or less than 0.2.

The regression line was than plotted between log of concentration (x) and improved expected probit (y).

**CALCULATION OF LC\textsubscript{10} AND LC\textsubscript{50} VALUES**

The LC\textsubscript{10} and LC\textsubscript{50} values of pollutant calculated from regression equation, \( y = 3.7184 \) and \( y = 5.000 \). (Values from Finney’s table I) were kept to calculate LC\textsubscript{10} and LC\textsubscript{50} pollutants in ppm for 24, 48, 72, 96, hrs. respectively.

**CALCULATION OF ACCURACY OF THE LC\textsubscript{50}**

The variance ‘V’ of the calculated log LC\textsubscript{50} was calculated by formula where \( V = \text{variance (the standard error of LC}_{50}) \)

**CALCULATION OF CHI – SQUARE ( \( x^2 \))**

The Chi-square was calculated to test homogeneity of the data.
This is given by

\[ X^2 = (Swy^2 - Y.Swy).b (Swxy-X.Swy) \]

The value of \( x^2 \) were compared with the table of the statistics for \( n-2 \) degrees of freedom (where \( n \) is the no. of experiences). This value should be higher than the figure \( x^2 \) for 5\% level. There is an indication of heterogeneity.

**CALCULATION OF FIDUCIAL LIMITS**

Fiducial limits were calculated with 95\% confidence i.e. \( M_1 \) and \( M_2 \) from variance (\( V \)) by the following formula.

\[ M_1 = m-1.96 \sqrt{v} \]
\[ M_2 = m+1.96 \sqrt{v} \]

Where,

\( M = \) calculated log of median lethal values

\( V = \) variance (standard error of \( LC_{50} \))

**CALCULATION OF LETHAL DOSE**

Lethal dose was calculated to it’s importance in agricultural view by the following formula

\[ \text{Lethal dose} = \text{LC}_{50} \text{ value} \times \text{time of exposure} \]
CALCULATION OF SAFE CONCENTRATION

Safe concentration of toxicant was calculated by the formula proposed by Hari et al., (1945).

\[ C = \frac{48\text{hrs. TLM} \times 0.2}{S^2} \]

Where,

\( C = \) Safe concentration

\( S = 24 \text{ Hrs. TLM} / 48 \text{ Hrs. TLM} \)

\( (TLM = \text{median tolerance limit or LC}_{50}) \)
RESULTS

Acute toxicity tests were carried out in the laboratory conditions for 24, 48, 72 and 96 hours duration for three metal and its combination like lead acetate, zinc sulphate, nickel chloride, lead and zinc sulphate, lead acetate and nickel chloride, zinc sulphate and nickel chloride and lead acetate and zinc sulphate and nickel chloride. Acute toxicity tests were conducted by the method described by Finney (1951) and simplified by Busvine (1971). The regression equations were obtained by heavy metals. The results obtained after toxicity evaluation of R. daniconius are cited in (Table 1 to 28 and Figures 1 to 28). The LC_{10} and LC_{50} values for heavy metal were summarized in (Table 29). The LC_{10} of lead acetate for 24, 48, 72 and 96 hours were 3.34, 3.80, 1.99, and 1.90 ppm respectively. The LC_{10} of zinc sulphate for 24, 48, 72, and 96 hrs were 6.64, 5.71, 2.99 and 1.99 ppm respectively. The LC_{10} of nickel chloride for 24, 48, 72 and 96 hrs were 61.07, 18.63, 23.09 and 9.29 ppm respectively. When these heavy metals in combination the LC_{10} values of combination lead acetate and zinc sulphate 24, 48, 72 and 96hrs were 2.49, 3.31, 2.48 and 1.44 ppm respectively. The combination of lead acetate and nickel chloride 24, 48, 72 and 96 hrs were 19.10, 10.45, 8.98 and 5.34 ppm
respectively. The combination of zinc sulphate and nickel chloride for 24, 48, 72 and 96 hrs were 35.83, 26.43, 17.21 and 9.08 ppm respectively. The lead acetate and zinc sulphate, lead acetate and nickel chloride, zinc sulphate and nickel chloride, and lead acetate and zinc sulphate and nickel chloride combination showed LC$_{10}$ for 24, 48, 72 and 96 hrs were 21.81, 19.81, 11.30 and 9.69 ppm respectively.

Among all the LC$_{10}$ values of lead acetate compared to zinc sulphate and nickel chloride. The lead acetate is more toxic individually or in combination with zinc sulphate and nickel chloride. R. daniconius is more sensitivity to these metals. Approximately the LC$_{10}$ values for zinc sulphate are two times greater than lead acetate and 30 times greater than nickel chloride.

The LC$_{50}$ of lead acetate for 24, 48, 72 and 96 hrs were 9.72, 8.43, 6.26 and ppm respectively. The LC$_{50}$ of zinc sulphate for 24, 48, 72 and 96 hrs were 14.6, 12.65, 9.38 and 6.26 ppm respectively. And LC$_{50}$ of nickel chloride for 24, 48, 72 and 96 hrs were 82.78, 69.15, 44.86 and 29.22 ppm respectively. When these heavy metals in combination the LC$_{50}$ of lead acetate and zinc sulphate 24, 48, 72 and 96 hrs were 7.82, 6.85, 4.57 and 3.68 ppm respectively. The LC$_{50}$ of lead acetate and nickel chloride for
24, 48, 72 and 96 hrs were 42.92, 32.84, 28.14 and 16.2 ppm respectively. The LC$_{50}$ of zinc sulphate and nickel chloride 24, 48, 72 and 96 hrs were 59.75, 50.53, 40.02 and 30.13 ppm respectively. When these three metals in combination the LC$_{50}$ for lead acetate and zinc sulphate and nickel chloride at 24, 48, 72 and 96 hrs were 37.84, 32.48, 28.09 and 25.21 ppm respectively.

The accuracy calculated for log LC$_{50}$ values are cited in (Table30 and 31). The head variance ‘V’ of log LC$_{50}$ for lead acetate 24, 48, 72 and 96 hrs were 0.001662, 0.002732, 0.005669 and 0.002732 respectively. The variance values of log LC$_{50}$ for zinc sulphate for 24, 48, 72 and 96 hrs were 0.005972, 0.002732, 0.005669 and 0.005669 respectively. The variance value for log LC$_{50}$ of nickel chloride for 24, 48, 72 and 96 hrs were 0.00398, 0.00183, 0.001914 and 0.005972 respectively. The variance value for log LC$_{50}$ to lead acetate and zinc sulphate for 24, 48, 72 and 96 hrs were 0.005669, 0.002276, 0.0816 and 0.005972 respectively. The variance value of LC$_{50}$ of lead acetate and nickel chloride for 24, 48, 72 and 96 hrs were 0.002967, 0.005963, 0.005669 and 0.005605 respectively. The variance value of LC$_{50}$ of zinc sulphate and nickel chloride for 24, 48, 72 and 96 hrs were 0.001129, 0.001816, 0.003082 and 0.006259 respectively. The
variance value of LC$_{50}$ for three metal combination lead acetate and zinc sulphate and nickel chloride 24, 48, 72 and 96 hrs were 0.001387, 0.001055, 0.003735 and 0.003964 respectively.

Chi – square values are summarized in (Table 30 and 31). The calculated values are less than tabulated values, the null hypothesis is accepted. There seems to be good correspondence between calculated and observed values.

Fiducial limits are summarized in (Table 30 and 31.)

Fiducial limits for lead acetate for 24, 48, 72 and 96 hrs were 0.90141 to 1.06121, 0.770041 to 0.974952, 0.509297 to 0.864436 and 0.469011 to 0.673922 ppm respectively. For zinc sulphate for 24, 48, 72 and 96 hrs were 0.866427 to 1.169365, 0.946132 to 1.151043, 0.745388 to 1.040527 and 0.569297 to 0.864436 ppm respectively. Fiducial limits for nickel chloride for 24, 48, 72 and 96 hrs were 1.859548 to 1.937795, 1.497435 to 1.844587, 1.497623 to 1.669117 and 1.167457 to 1.470395 ppm respectively. Fiducial limits for binary combination of heavy metal lead acetate and zinc sulphate for 24, 48, 72 and 96 hrs were 0.666207 to 0.961346, 0.693837 to 0.880869, 0.551311 to 0.708088 and 0.382085 to 0.622585 ppm respectively. Fiducial limits for lead acetate and nickel chloride for 24, 48, 72 and 96 hrs were 1.423647
to 1.637163, 1.21873 to 1.521428, 1.222509 to 1.517648 and 0.290608 to 1.214091 ppm respectively. Fiducial limits for zinc sulphate and nickel chloride for 24, 48, 72 and 96 hrs were 1.677338 to 1.189057, 1.577308 to 1.74434, 1.436567 to 1.654186 and 1.240267 to 1.550395 ppm respectively. Fiducial limits for tertiary combination of heavy metal lead acetate and zinc sulphate and nickel chloride for 24, 48, 72 and 96 hrs were 1.415951 to 1.56192, 1.41588 to 1.543176, 1.196918 to 1.43649 and 1.212957 to 1.459765 ppm respectively.

Safe concentration of heavy metals lead acetate, zinc sulphate, nickel chloride and lead acetate, zinc sulphate, lead acetate and nickel chloride, zinc sulphate nickel chloride, and Lead acetate and zinc sulphate and nickel chloride are calculated and express in (Table 29, Fig. 29 to 32). The safe concentrations are 1.306, 1.182, 1.165, 1.247, 1.154, 1.197 and 1.114. ppm respectively.

Lethal doses for heavy metals are showed in (Table 30 and 31). The order of toxicity in decreasing manner is lead acetate and zinc sulphate > lead acetate > zinc sulphate > lead acetate and zinc sulphate and nickel chloride > lead acetate and nickel chloride > zinc sulphate and nickel chloride > nickel chloride.
DISCUSSION

The quantitative study of pollutants in aquatic organisms offers an interesting challenge to the researchers. Heavy metals are well known environmental pollutants, they often persist, circulate and eventually accumulate throughout the food chain, thus cause a serious threat to non-target organisms. Akther and Mohan, (1995). Radhakrishnaiah (1988) reported copper concentration in freshwater fish *Labeo rohita*. (Legorburus *et al*; 1988) observed that fish from the more polluted places show higher metal levels. Jaffar *et al*. (1988) observed that, there is a positive correlation between the concentrations of zinc and arsenic in the fish muscle and in water. Tulsi *et al*. (1992) showed significant accumulation of Lead in blood and tissue in the freshwater fish, *Anabas testudineus*. Lead bioaccumulation showed organ-specific distribution with high levels in blood followed by kidney, gill, liver and brain and comparatively lesser amounts in the ovary and muscle. Cuvin, (1994) observed that, concentration of cadmium and mercury in *Oreochromis niloticus* increased with exposure period. Seymore *et al*., (1996) studied bioaccumulation of chromium and nickel in selected tissues of freshwater fish *Barbus marquensis*. They observed that chromium and nickel accumulated
highest in blood, followed by the bile and vertebrae, while skin accumulated the lowest amount. Mason et al., (2000) observed that, concentration of heavy metals is more in detoxifying organs in fresh water in vertebrates and fish.

Tolerance is the ability of the organism to show less response to a specific dose of a toxicant than it showed on a prior occasion from the same dose. It is as if the organism had become partially refractory or had developed an immunity to the effect of the toxicant by virtue of previous exposure. Enhanced tolerance observed in the organism may be as a result of a failure in translocation of the metal ion, such as absorption or distribution, or enhanced termination, that is enhanced excretion or metabolic alteration of the metal in the organism. Such condition would lead to an effective lowering of the metal dose at its site of action in the biological system, thereby resulting in a lesser effect from a specific dose. Another mode of intra metal action also needs mention. Absorption from aqueous medium by organisms involve passive diffusion of the metal probably as a soluble complex, down gradient originated by absorption at the surface, and chelated by constituents of the surface cells, body fluids and internal organs. Tolerance of metals to any organism is determined by the
permeability of various ions. A change in metal oxidation state can alter the permeability of cells to metal ions, a small change in ionic radius of the latter will pre-determine the process as such. Depression in assimilation rate of the toxic metal, copper, and reduction in toxicity could also be happened due to the passive diffusion of the toxic ions along the concentration gradient involving the binding of free copper ions to some carriers were hindered or blocked. The degree of involvement of different mechanisms to explain the observed change in accumulation pattern of metals consequent to acclimation are a matter of speculation.

In the present study the fish showed characteristic change in behavior when transferred to experimental chambers having different metal. The fishes survived rapidly in the experimental media and were trying to jump out of water at short intervals. Later the fishes exhibited restlessness by erratic opercular movement, difficulty in respiration, convulsions and short erratic jerky movements, which is in apparent with the studies of (Lohar et al., and 2000, Mubarak Begam, (1998). The fishes in experimental chambers showed mucous secretion to avoid toxic environment. Where as in present study no such behavioral changes were noticed
in the control fish, which remained active and healthy throughout the experimental period. The fast swimming activity may be due to the irritating effect of the exposed heavy metal where as the excessive secretion is a kind of avoidance by the fish.

The recent decade has been applied to investigation on acute and sublethal toxicity of heavy metal interaction and their biochemical and physiological impact on indicator species such as fishes. The model fish species used for this type of investigation are rainbow trout, *Onchorhynchus mykiss* and freshwater fish *Tilapia nilotica*. It has been demonstrated that heavy metal get accumulated in various tissues of the *Tilapia nilotica*, Rashid, (2001). Bhilave *et al.*, (2004) observed that, freshwater fish *Cirranus mrigala* and *Cypranus carpio* when exposed to acute concentrations of cadmium and lead, there was more accumulation of heavy metals in liver followed by gill and muscle.

The metal interactions not only influence the acute toxicity but also alter the bioaccumulation patterns of these metals in the exposed fishes. The effects of model combination of seven heavy metals (Cu, Zn, Ni, Cr, Pb, Cd and Mn) on the rainbow trout *Onchorhynchus mykiss* at all stages of development were investigated by Vosyliene *et al.*, (2003). According to parameters
the fish larvae show higher sensitivity to the model metal combination compared to embryos and adult fish. The maximum toxic effect of model combination was observed during hatching period.

The ecotoxicological effect expressed as mortality of four metal ions (Cd, Cu, Zn, Al) and their associations on the star larvae of *C.plumosus* was determined by Fargasova (2001). The effect of individual metals was introduced as acute toxicological effect and expressed as LC$_{50}$ values. On the basis of LC$_{50}$ values the toxicity of metals after 96 hrs treatments was ranked as Cu > Cd > Zn > Al. Similar trend of toxicity was also observed in the present investigation. Among these three metal, Lead proved to be more toxic then zinc and nickel. The toxicity in combination was either increased or decreased because of antagonism or synergisms. Further the study revealed that when in metal pairs in which the original metal is at low concentration shows increased toxicity in combination is the effect of synergism for example in the present investigation the combination of zinc and nickel increased toxicity due to synergism. Similarly, lead showed synergism with zinc and antagonism with nickel. The present investigation reports indicate
certain effect such as antagonism and synergism of metal toxicity for the freshwater fish species *R. daniconius*.

On the other hand, in the present investigation toxicity evaluation of heavy metals lead acetate, zinc sulphate, nickel chloride, lead acetate and zinc sulphate, lead acetate and nickel chloride, zinc sulphate and nickel chloride, and lead acetate and zinc sulphate and nickel chloride was conducted on the freshwater fish *R. daniconius* and LC$_{50}$ values were calculated. The LC$_{50}$ values for 96 hrs exposures were 4.22, 6.26, 29.22, 3.68, 16.20, 30.13 and 25.21 ppm respectively. Lead acetate and zinc sulphate show synergism, as toxicity of combination was more where as the model combination of lead, zinc and nickel showed antagonism, as toxicity was decreased. The nickel had antagonism for both that is lead and zinc. This type of data is very scanty in case of the freshwater fishes where as the toxicity studies performed on non-fish species are in agreement with our findings. In the pattern of sublethal toxicity due to metal combination has been reported in case of juvenile rainbow trout, *Onchorhynchus mykiss* after single and combined exposure to metal and other pollutants by Ait-Aissas *et al.* (2003). Impact of metal interaction on accumulation and elimination of heavy metals have also been documented.
(Allen, 1995; Kargin and Cogun 1999; Cicik et al. 2004). These showed either antagonistic or synergistic effects of metal combination on the freshwater fishes. Rashed (2001A, 2001B) studied (Co, Cu, Cr, Ca, Fe, Mn, Ni, Sr, Pb, Cd and Zn) in different tissues of fish, *Tilapia nilotica* from Nassar Lake to assess both the water pollution with the metals and lethal levels of these metals in the fish. The author has suggested that the metal levels in the fish increased with the increased levels of metals in the lake water and exhibited bioaccumulation of heavy metals from water to body of fish. The present investigation also proposes use of freshwater fish *R. daniconius* species as a model indicator to assess the heavy metal pollution status of the aquatic ecosystems.

The safe concentration for *R. daniconius* to the heavy metals were lead acetate, zinc sulphate, nickel chloride, lead acetate and zinc sulphate, lead acetate and nickel chloride, zinc sulphate and nickel chloride, and lead acetate and zinc sulphate and nickel chloride were 1.247, 1.154, 1.197, 1.14, 1.306, 1.182 and 1.165, ppm. From these results it is clear that lead acetate more toxic either individual or combined form for *R. daniconius* in the present study.