CHAPTER: I

TOXICITY EVALUATION
INTRODUCTION

Pollution is an undesirable change in the physical chemical or biological characteristics of air, land and water, that may or will be harmful to human life or that of desirable species. Whatever may be due to mode of contamination, polluted environment is less suitable for existing life forms (Menzel, 1977). The water pollution referred to any type of contamination in water.

Since the industrial revolution, the efforts of removing man made pollutants from the natural environment have been unable to keep pace with the increasing amount of waste materials and a growing population that further aggravates the situation. This has often resulted in the transformation of lakes, river, and coastal waters in to sewage deposits, where the natural biological balance is severely upset and in some cases totally disrupted. With the growth of technology two groups of substances in particular have a lasting effect on natural balance in aquatic system.

(i) Nutrients: which promote unrestricted biological growth and in turn, oxygen depletion and (ii) Sparingly degradable (refractory) synthetic chemicals, and other waste substances which often constitute multiple (adverse) effects on the aquatic ecosystems. Experts estimate that industrial and
domestic waste introduce up to a million different pollutants into natural waters. These include substances that are not considered dangerous, although many of them add a disagreeable odour or taste to the water and others significantly upset the ecosystem without being directly harmful to humans. Other groups do, however, have direct and indirect influence on nontarget organisms and can cause serious damage. Substances such as polycyclic aromatics, pesticides, radioactive matter and trace elements (heavy metals) endanger human life as well as other animals.

Aquatic pollution from agricultural chemicals can result from widespread application of highly toxic chemicals in agricultural farm, forest, garden etc. In addition to this the promiscuous dumping of industrial, agricultural and domestic pollutants had its impact on water quality in our major water ways, lakes, ponds, bays and ground water supplies (Kraybill, 1964).

The widespread use of synthetic organic pesticides is causing harmful effects to fishes, shell fishes, and wild life animals. Undesirable effects caused by pesticides to the aquatic organisms and their hazards are elegantly reviewed by many workers (Tarzwell, 1959; Klein, 1960; Woodward, 1960; Sanger, 1964; Cope, 1965; Stickel, 1967; Johnson, 1968; Ciaccio, 1971). The effect of pollutants on organisms may be direct or
indirect and the nature of effect varies but can include structural and functional modification at both the cellular and sub cellular level in a variety of organisms.

The toxic materials contained in the effluent discharge from industrial establishments, municipal wastes and insecticides have direct lethal effects on most of the cultural animal species. Fin-fish and shell-fish mortality has been observed throughout the world (Thomas, 1968). Extensive use of pesticides in agriculture creates pollution problems in environment. In many countries large scale mortality of fish has been reported due to pesticides in water bodies as pollutants. It was examined that about 13% of fish kills in England and Wales and 25% in Scotland in 1967 were due to pesticides (Third Report of the Research Committee on toxic chemicals) Anon (1970).

Many incidences of fish mortality have also been recorded in India, mostly in small ponds and rivers where rains have washed DDT, Endrin & Aldrin spread from field or by washing of spraying equipments (FAO, 1967). Most disastrous effect of pesticide pollution was observed in the river Punarbhava of West Dinajpur (West Bengal, India). The insecticides DDVP, phosphomidon, and parathion sprayed on paddy fields have been washed away due to heavy rains into the adjacent rivers, causing
mass mortality of carps, catfishes and other species of commercially important fish species (Konar, 1975).

In India, pollution problems have noticeably increased after growth of industrialization and increase in population. The marine water pollution is observed in limited area along the coast. But inland waters like rivers, ponds etc. are heavily polluted, because they are used for the discharge of sewage, municipal waste and industrial effluents which contain vast array of inorganic and organic compounds. Many compounds present in sewage and industrial effluents are very toxic to organisms.

Many conservationists have expressed the belief, that the industrial wastes, the wastes from agriculture create a great threat of pollution. The chlorinated hydrocarbons and organophosphorous insecticides were mostly found in agricultural waste as dangerous pollutants.

Based upon the target species the pesticides are classified as insecticides, rodenticides, molluscicides, herbicides, bactericides, fungicides, nematocides, etc. All these pesticides are classified into three general groups depending upon their chemical nature and origin, (1) synthetic organic compounds which include organochlorines, organophosphates and carbonates (2) natural organic compounds, which include,
nicotine, pyrethrine etc. and (3) inorganic compounds which includes mercuric compounds, copper compounds and arsenic compounds and fluorides. The insecticides are classified as organophosphorous insecticides, chlorinated hydrocarbons, synthetic pyrethroids and carbamates. Most of the synthetic insecticides especially organochlorine and organophosphorous insecticides have become increasingly important addition to chemical wastes polluting natural aquatic communities. The need to understand the specific nature of toxicant or any particular pollutant, and the interplay among pollutants for single species and for total ecological system is imperative. Among these pollutants insecticides are considered hazardous because of their ability to kill or immobilize freshwater, estuarine and marine organism at extremely low concentration (Tarzwell, 1963; Cope, 1965; and Eisler, 1969).

Pesticides enter aquatic environment through intentional application, aerial drifts or run off from application or accidental release. Some pesticides are applied directly to water to control aquatic weed, algae, unwanted fish, unwanted molluscs, unwanted invertebrates and noxious insects. Agricultural run off from field and grazing lands is considered the major route of pesticide movement into water (Li, 1975). It has been estimated that industrial waste (effluents) from pesticide manufacturing plants is the second greatest source of
pesticides in aquatic environment (Li, 1975). Mississippi fish kill of the early 1960 described as "enormous" by Graham (1970), was due to a pesticide manufacturing plant near Memphis, Tennessee.

Pillai (1986) showed the extent of organochlorine insecticide residues in soil, water, air and rain water in Delhi area. Out of the 50 samples each of soil and earthworms collected from these parts 48 samples showed that the soil and earthworms contained DDT residue. The area near the vicinity of DDT factory showed high levels of DDT residues. A two year survey of the Yamuna river in Delhi showed that water contained an average of $0.24 \mu g \ L^{-1}$ and the bottom sediment had $0.24 \ mg \ kg^{-1}$ of total DDT residues. The fishes collected from Yamuna river showed very high bioaccumulation of DDT residues. Several investigators have studied pesticide contamination in marine water of Southern California. Li (1975) concluded that the buildup of DDT in Southern California costal marine organisms could be due to industrial waste discharge rather than to extensive agriculture usage.

The extent of contamination is reviewed in a report by Lunsford (1981), who presents the results of monitoring Kepone in surface and bottom water samples. The toxicity and bioconcentration of Kepone in aquatic species were studied by Bahner et al. (1977), Hansen et al. (1977a,b), Schimmel and
Wilson (1977) and Walsh et al. (1977). Studies on the effect of pesticides on the aquatic organisms, particularly on fishes. A study of Eisler (1970) showed that with some marine fish chlorinated insecticide will be considerably more toxic than a group of organophosphates. Georgakis and Khan (1971) determined the 24h LC50 values of Aldrin, Dieldrin, Hepatocholor and their photisomers for guppies, fathead minnows and blue gills. Wildish et al. (1971) studied the toxic effect of some organophosphorous insecticides to Atlantic salmon. Chambers and Yarborough (1974) investigated parathion and methyl parathion toxicity to mosquito fish. Other workers also have found the toxic effects of various pesticides to fishes (McKim et al., 1974; Shim and Self 1974; Anees 1975; Zitko and Cunningham, 1975; Lone and Javaid, 1976; Brung et al., 1977; Johnson and Finley, 1980; Woodward and Mauck, 1980).

Comparatively little attention has been paid to study the toxicity of pesticides or insecticides to aquatic invertebrates like Crustaceans and molluscs. Stewart et al. (1967) and Butler et al. (1968) studied the acute toxicity for bay mussels Mytilus edulis and pacific oyster Crassostrea nuttallii. Eisler (1970b) studied the effect of 7 organochlorine and 5 organophosphorous insecticides to marine decapod crustaceans and molluscs for 96h exposure. Some workers found the toxic effects to invertebrates (Lowe et al.; 1971; Roberts, 1976; Havlik and Marking, 1987).
Pillai (1977) made an extensive study on DDT toxicity and its residues in human blood, earthworm *Pheretima posthuma* and snail *Viviparous beliciformes* from Delhi area adjoining a DDT factory. Snails accumulated DDT in large amount when exposed to different concentrations while excreted rapidly when transformed to freshwater pond. Agrawal (1978) showed that *Lymnaea accuminata* was more susceptible to pesticides like Aldicarb, Zectra and Carbaryl than *Pila globosa*.

Several pyrethroids are now used in agricultural fields as pesticides. Among these *cypermethrin* is widely used as insecticide for crop protection. The outstanding potency of synthetic pyrethroids is to kill insects and their relatively low mammalian toxicity (Miyamoto 1976; Elliott 1977). There is worldwide recognition of these compounds as efficient pest control agents (Padopoulou-Mourkidas, 1983). Consequently the environmental occurrence of synthetic pyrethroid became worldwide (Casida, 1980).

All pyrethroids so far used act on the nervous system of target animal. They are not mutagenic or carcinogenic. Any marginal effects on reproduction may be due to a secondary response to their primary action on the nervous system. Pyrethroids produced lesions in the sciatic and posterior tibial nerves of the experimental rats. It is also possible that heterogeneity of sodium channels may be caused in the nerves
(Honriager, 1982). Pyrethrroids are not readily metabolised in insects, as it occurs in mammalian body, hence they are more toxic in insects.

The monocrotophos is an organophosphorus pesticide and used for control of insect pest. Monocrotophos is most toxic to vertebrates and invertebrates and it was non persistent. It exerts its toxic action by inhibiting certain important enzymes of the nervous system, cholinesterases. It causes obstacle in the transmission of nerve impulses at synaptic connection causing rapid twitching of voluntary muscles, finally leading to paralysis.

Toxicity of organophosphate insecticides has been evaluated for Indian fishes (Arora et al., 1971a; Annes, 1975). Bookhout and Monroe (1975) investigated the effect of malathion on the development of crabs. Toxicity of sumethion to the freshwater field crab was studied by Bhagyalakshmi and Ramamurthi (1980). Shukla and Mishra (1981) have tested the toxicity of six organophosphorous insecticides on the nymphs of fly, Bcarthythermis contaminata.

The intrusion of metal contaminants into the aquatic system has various sources, like smelting processes and fuel combustion via atmospheric fallout, effluents and dumping activities, from runoff of terrestrial systems, and land application of sewage
materials and leaching of garbage. The amount of heavy metal discharged as industrial and agricultural wastes and sewage effluents have promoted concern their effects on freshwater aquatic life. The increased every day discharge of heavy metals in urban environment disturbs the equilibrium state of the aquatic communities.

The trace metals are of biological interest because of their role as micronutrients and some metals act as toxins (Cu, Hg, Ag, and Cd). Some metals such as Cu and Zn can act either as stimulatory or inhibitory mode depending on their level of availability.

The trace metals are not usually eliminated in aquatic system by natural processes, but due to increased anthropogenic inputs of these metals into aquatic system from highly sophisticated man-made industries, the trace metals can exist in a variety of different chemicals forms in natural waters including free ions, inorganic complexes, organic complexes and particulate matter.

Most of the toxic metals such as Hg, Cd, Ar, Cu and others tend to accumulate in bottom sediments from which they may be released by various processes of remobilization where they affect aquatic life.
Recent investigations with copper (Sunda and Guillard, 1976; Andrew et al., 1977; Anderson and Morel, 1978; Waiwood and Beamish, 1978) have demonstrated that the toxicity and bioavailability of trace metals is highly dependent on their chemical form. These investigations have shown that biological response (i.e. toxicity and accumulation) to dissolved trace metals is the function of free metal ion concentration which is determined not only by the total dissolved concentration but also by the extent of metal complex to both organic and inorganic legends. Most of the inorganic copper compounds are used as fungicides. The mixture of copper sulphate and hydrated lime (Bordeaux, mixture) is used to control fungi. The copper sulphate is used as Molluscicide to control terrestrial and aquatic molluscs.

Copper is an essential metal in a number of enzymes. Excessive intake of copper results in its accumulation in the body tissue. The heavy metal content of aquatic animals originates from two routes of intake, free ions of metal are taken up directly through epithelium of skin, gills and alimentary canal, while others being accumulated in food organisms, are incorporated by nutrition (Salanki et al., 1982). Several workers have agreed that the uptake of metals from food is the most important route in the environment (Preston, 1971; Pentreath, 1973; Schulz—Baldes, 1974; Cunningham and Tripp, 1975 and Phillips, 1976).
Some molluscs have tremendous capacity to accumulate significant amount of \( \text{Cu}^{++} \) ions even at low concentration of Cu (Zamuda and Sunda, 1982; Zarogian and Johnson, 1983). The total flux of copper to atmosphere is approximately 75,000 metric tons per year. Approximately 17,000 metric tons of solid copper wastes are deposited annually into the oceans (Nriagu 1979c). The presence of copper in ecosphere has attracted considerable attention and recently has been subject to intense research.

The concentrations of free \( \text{Cu}^{++} \) ions are toxic to number of aquatic organisms (Evans, 1980; Albrecht et al., 1981) including phytoplankton (Sunda and Lewis 1978), bacteria (Sunda and Gillespie, 1979).

The invertebrates inhabiting polluted freshwater generally carry residues of 5-200 mg per Kilo gram dry weight of soft tissue. Manly and George (1977) reported in the mussel *Anodonta anatinus* found in the river Thames (U.K.) that the copper level was 21-103 mg per kilogram soft tissue.

Some species including the gastropods *Helix aspersa* and *Littorina littorea* show a relatively even distribution of copper throughout their tissue (Coughtrey and Martin, 1976; Ireland and Wootton, 1977).
Copper is usually more toxic to freshwater fish than any other heavy metals except mercury. The copper is much less toxic to marine fish due to high complexing capacity of salt water (Eisler and Gardner, 1973).

Mercury is considered a non essential but highly toxic element for living organism. Even at low concentration mercury and its compounds create potential hazards due to enrichment in the food chain. The discovery that consumption of high concentration of mercury compounds accumulated in fish and shell fish have evoked disastrous end effects in nutritional food chain (Goldwater, 1971). After unravelling the case of mysterious 'Minamata Illness' society suddenly became aware an existence of toxic metals in the environment.

Inorganic mercurial fungicides are most toxic fungicides. Hg²⁺ ions are toxic to all forms of life. The input of mercury in environment creates serious problem (Kendall, 1977 and Glickstein, 1978).

Mass scale human poisoning and subsequent tragic events of Minamata in Japan (Kurland et al., 1960) have drawn wide attention to mercurial compounds and their effects. Mercury has been reported in certain costal environments (Gardner, 1975).
Toxicity of mercury to marine animals has been extensively studied by some workers (Gorner and Sparrow 1956; Calabrese et al. 1973).

Metal pollution is now not limited to marine habitat but it has spread into inland freshwater. Recently, with the deterioration of water quality of Husainsagar Lake (India), contamination of lake water with heavy metals has been reported by Prahalad (1987).

The mercurial compounds are highly toxic to aquatic animals in comparison to other heavy metals (Bryan, 1971). Acute toxicity of \( \text{HgCl}_2 \) to marine and freshwater invertebrates depends on species (Rehwoldt, et al., 1973). The toxicity of \( \text{Hg} \) was studied in some species of fishes by a few workers (Akiyama, 1970; Wobeser 1975; and Dhanekar et al., 1985).

The present study on the toxicity evaluation of the pesticides (Cypermethrin and Monocrotophos) and heavy metals (Copper sulphate and mercury chloride) was carried out on the freshwater snail, *Thiara tuberculata* which is commonly found in the freshwater bodies, especially the Kham river near Aurangabad.
MATERIAL AND METHODS

Medium sized snails *Thiara tuberculata* were collected from the Kham river at Aurangabad and brought to the laboratory. The snails were cleaned and kept in plastic troughs containing dechlorinated tap water for 2 - 3 days to acclimatize to the laboratory conditions. Overcrowding was avoided by keeping a small number of snails in different troughs. Water from the plastic troughs was changed after every twelve hours and the pH of the water maintained during the experiment was 7 - 8. The medium sized active acclimatized snails were selected for evaluation of toxicity. Series of static bioassay were conducted under laboratory conditions. Twenty snails were exposed to 10 - 20 different concentrations of pesticides, cypermethrin and monocrotophos and heavy metals, copper sulphate and mercuric chloride. Each group was kept in plastic trough containing four litre polluted water of definite concentrations. After an interval of 12 hours polluted water in troughs were replaced by the same concentrations of pesticides and 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 hours. Each experiment was repeated thrice to obtain constant results.
Acute toxicity tests were carried out under static conditions up to 96 hours. The data collected was then analysed statistically by means of the Probit methods on transforming the toxicity curve (% mortality versus concentration) into regression lines (mortality in probits/log concentration) (Finney, 1951), which allow the average median lethal concentration or LC$_{50}$ value to be calculated for 24, 48, 72 and 96 hours. To count the mortality, the snails were examined individually and those who have lost the ability to close the operculum and showed no sign of the foot movement on touching with a needle were taken as dead.

**Calculation of Percent Mortality:**

To count the natural Mortality caused in control animals, they were treated exactly in the same way as the experimental animals. Abbotts formula (1925) was employed. This formula gives the percentage of the original number of animals dead by the treatment.

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

Where \( P \) = corrected mortality

\( O_m \) = observed mortality

\( C_m \) = Control mortality (all % S)
It was observed that there was no mortality in control group of snails. The mortality data obtained in experimental animals for each dose was calculated by Finney’s formula

\[ p = \frac{r}{n} \times 100 \]

Where \( p \) = percent mortality
\( r \) = mortality observed
\( n \) = number of animals exposed in a batch.

The mortality data thus obtained was put into Probit/Log concentration transformation so as to plot Probit regression lines. These regression lines were plotted for the purpose of calculating the required concentration of pesticides and heavy metals to produce 50% mortality and 10% mortality. The standard error of the log LC\(_{50}\) (variance \( 'v' \) of the calculated log LC\(_{50}\)) and \( \chi^2 \) (Chi square) value and fiducial limits to pesticides and heavy metal pollutants were calculated from regression equation. The lethal dose and safe concentration of pollutant was calculated from the above data.

**Calculation of Regression line:**

To plot a well studied straight line between log concentration and probit kill, the method described by Finney (1951) as simplified by Busvine (1971) was followed. To trace a regression equation and to plot a regression line the detailed steps carried are given below:
The regression equation and line was calculated for the snail *Thiara tuberculata* when exposed for 24, 48, 72 and 96 hours to pesticides cypermethrin and monocrotophos and heavy metals, CuSO$_4$ and HgCl$_2$.

1. In the first column of the table serial numbers of troughs were entered.

2. In column No. II the concentration of the pollutant in ppm was entered.

3. In the IIIrd column, headed 'x' the log of respective concentration to base 10 was entered.

4. In the IVth column, headed 'n' the number of animals taken in a batch was noted.

5. In column No. V, observed mortality for 24, 48, 72 and 96 hours was recorded.

6. The percent mortality (p) entered in column VI was calculated by formula $P = \frac{x}{n} \times 100$

   If the mortality occurred in the control set of animals then by using the Abbotts formula the corrected mortality was calculated & entered in the VIth column

   $$P = \frac{Cm - Cm}{100 - Cm}$$
7. The empirical probit value was read from Table 1 (Transformation of percentages to probits) from Finney's book, and recorded in column No. VII.

8. The empirical probits were plotted against 'x' (log conc. of pollutant). The provisional straight line was drawn to suit the maximum points, judging its position by eye (Fig. 1).

9. The expected probit (Y) values were read from the provisional line of the graph for values of 'x' and tabulated in column VIII with two places of decimals.

10. From the column C = oo of table II in Finney's book, the weighing coefficient (w) for Y was read and entered in column No. IX.

11. Each weighing coefficient (w) was multiplied by 'n' (number of snails exposed per batch) from column II and then product W were listed in column X.

12. From table No. IV (Finney's book) the working probit (y) value was read corresponding to each 'Y' and 'p' and listed in column XI.

13. Now for each line, the value of W and 'x' and W and 'y' were multiplied and the products Wx and Wy listed in column XII and XIII.

14. The products of column, X, XII and XIII were summed up at the foot of each representative column and given the abbreviation Sw, Swx, Swy respectively.
15. In column, XIV, XV and XVI the products 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 products of column XIV, XV and XVI are \( Swx^2 \), \( Swy^2 \) and \( Swxy \) respectively and they were entered at the foot of each column.

16. Then the values for \( \bar{x} \) and \( \bar{y} \) were calculated by using the following equations.

\[
\bar{x} = \frac{Swx}{SW} \quad \quad \bar{y} = \frac{Swy}{SW}
\]

17. Now the value of the estimated regression co-efficient 'b' was found out by the following equation:

\[
b = \frac{Swxy - \bar{x}Swy}{Swx^2 - \bar{x}Swx}
\]

18. The regression equation may now be written down as

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

19. From the regression equation value of Y corresponding to the original values of 'x' were calculated and entered in column XVII as improved expected probit \( y' \). These values of improved expected probit \( Y' \) did not differ by more than 0.2 as compared to the expected probits \( Y \) in column VIII, in any case the fit of line may be considered adequate. However, if there is no discrepancy the value of Y were taken as improved expected probit \( Y' \) and the whole cycle of calculation from VIII was repeated.
20. The regression line was then plotted between log concentration (x) and improved expected probit Y'.

Calculation of LC_{10} and LC_{50}:

For the calculation of LC_{10} and LC_{50} of the pollutants from regression equation, Y = 3.7184 and Y = 5 (values from Finney's table No.1) were kept to calculate X values. Antilogs of the X values are the LC_{10} and LC_{50} of the pollutants in ppm. The LC_{10} and LC_{50} values for 24, 48, 72 and 96 hours were calculated for the pollutants cypermethrin, monocrotophos, CuSO_{4} and HgCl_{2}.

Calculation of Accuracy of the LC_{50}:

The variance 'V' of the calculated log LC_{50} was calculated by the expression:

\[ V = \frac{1}{b^2} \left( \frac{1}{SW} + \frac{(m - \bar{x})^2}{SW \cdot x^2 - (\bar{SW}x)^2} \right) \]

Where \( V \) = Variance (the standard error of LC_{50})

Calculation of chi-square (\( \chi^2 \)) values:

The value of \( \chi^2 \) (Chisquare) was calculated to test the homogeneity of the data. This is given by the expression

\[ \chi^2 = (Swy^2 - \bar{y} Swy) b (Swxy - \bar{x} Swy) \]
The values of $X^2$ was compared with the table of the statistic for $n-2$ degrees of freedom (where 'n' is the number of experiments) should this value be higher than the figure of $X^2$ for the 5% level. There is indication of heterogeneity.

Calculation of Fiducial limits:

The fiducial limits $m_1$ and $m_2$ with 95% confidence were calculated from the variance ($V$) by the following formula:

$$m_1 = m - 1.96 \sqrt{V} \quad \text{and}$$

$$m_2 = m + 1.96 \sqrt{V}$$

Where $m =$ calculated log $LC_{50}$ value

$V =$ Variance (Standard error of $LC_{50}$ )

Calculation of Lethal dose:

The lethal dose was calculated due to its importance from agricultural point of view. The lethal dose was calculated by the following formula:

Lethal dose = $LC_{50}$ value x time of exposure.

Calculation of safe concentration:

Hart et al. (1945) have proposed a formula for calculation of safe concentration of toxicants for animals.
\[ C = \frac{48 \text{ hrs TIM} \times 0.3}{S^2} \]

Where as \( C = \) Safe concentration and

\[ S = \frac{24 \text{ hrs TIM}}{48 \text{ hrs TIM}} \]

Where TIM = Median tolerance limit, or known as \( LC_{50} \) value, which is the concentration at which 50\% of the test snails were killed in particular time period.
OBSERVATIONS AND RESULTS

Acute toxicity tests were carried out in the laboratory upto 96 hours duration for two pesticides, cypermethrin and monocrotophos and two heavy metals, CuSO$_4$ and HgCl$_2$. The LC$_{10}$ and LC$_{50}$ values were calculated for 24, 48, 72 and 96 hours by the method described by Finney (1951) and simplified by Busvine (1971) as in table (1). The results obtained after toxicity evaluation of pollutants to *Thiara tuberculata* are summarised in tables 5, 6, and 7.

The obtained regression equations to pesticides, cypermethrin and monocrotophos and heavy metals, CuSO$_4$ and HgCl$_2$ for 24, 48, 72 and 96 hours are listed in tables (5 and 6). The LC$_{10}$ and LC$_{50}$ values for pesticides and heavy metal pollutants are summarized in table (7). The LC$_{10}$ values for 24, 48, 72 and 96 hours exposure to cypermethrin are 8.5980, 5.6506, 2.4900 and 1.3880 ppm respectively. The LC$_{10}$ values of monocrotophos to 24, 48, 72 and 96 hours are 68.9286, 49.5222, 29.8744 and 17.3940 ppm respectively. The copper sulphate has LC$_{10}$ values 9.0011, 7.0876, 5.1605 and 2.2387 ppm for 24, 48, 72 and 96 hours treatment respectively. The mercuric chloride LC$_{10}$ values for 24, 48, 72 and 96 hours are 5.7852, 3.5476, 2.1034 and 1.3174 ppm respectively. The copper
sulphate $L_{C_{10}}$ values are two times more than $L_{C_{10}}$ values of mercuric chloride and the $L_{C_{10}}$ values of monocrotophos are about eight times more than cypermethrin.

The $L_{C_{50}}$ values for 24, 48, 72 and 96 hours exposure to cypermethrin are 10.2046, 8.1507, 4.6344, and 2.8209 ppm respectively. The $L_{C_{50}}$ values for monocrotophos are 106.5123, 84.6642, 62.4453 and 47.3914 ppm for 24, 48, 72 and 96 hours respectively. The $L_{C_{50}}$ values of monocrotophos are near about 10 times more than cypermethrin. The $L_{C_{50}}$ values for copper sulphate when exposed to 24, 48, 72 and 96 hours are 12.7732, 10.7398, 8.6876 and 5.4512 ppm respectively. The $L_{C_{50}}$ values for mercuric chloride are 7.6553, 5.3716, 3.8242 and 2.3642 ppm for 24, 48, 72 and 96 hours respectively. The $L_{C_{50}}$ values of copper sulphate are two times more than $L_{C_{50}}$ values of mercuric chloride. The $L_{C_{50}}$ values of cypermethrin are near about two times more than $L_{C_{50}}$ values of mercuric chloride. The $L_{C_{50}}$ values of mercuric chloride are very low in comparison to copper sulphate, cypermethrin and monocrotophos. The $L_{C_{50}}$ values of monocrotophos are very high in comparison to other pollutants.

The heavy metal compounds were more highly toxic to freshwater snail, *Thiara tuberculata* than pesticides. The $HgCl_2$ is more toxic than other pollutants.
The calculated accuracy for the Log LC$_{50}$ values are summarised in the table 5 and 6 under the columns 'Variance' 'V'. The standard error (Accuracy or variance) for 24, 48, 72 and 96 hours Log LC$_{50}$ values of cypermethrin are 0.0001175, 0.0002230, 0.0006622, and 0.0015170 respectively. The variance for the 24, 48, 72 and 96 hours log LC$_{50}$ values of monocrotophos are 0.0001165, 0.0001722, 0.0003277 and 0.0005907 respectively. The variance 0.0001501, 0.0002061, 0.0003191 and 0.0009494 are for the 24, 48, 72 and 96 hours log LC$_{50}$ values of copper sulphate respectively. The accuracy of log LC$_{50}$ values of mercuric chloride at 24, 48, 72 and 96 hours are 0.00009525, 0.0002053, 0.0004171, and 0.0006578 respectively.

The Chi-square ($\chi^2$) values are summarised in the tables (5 and 6) under the column ($\chi^2$). These values were used to test the homogenity of data.

The fiducial limits for log LC$_{50}$ values are summarized in the table (5 and 6) under the column fiducial limit $m_1$ and $m_2$. The 95% confidence of LC$_{50}$ values (fiducial limit) to pesticide and heavy metal pollutants are $m_1$ (minimum limit) and $m_2$ (maximum limit). The minimum and maximum fiducial limits for 24, 48, 72 and 96 hours log LC$_{50}$ values of copper sulphate are 1.08228 to 1.13031; 1.00285 to 1.05914; 0.90388 to 0.97391 and 0.67610 to 0.79689 respectively. The minimum and maximum fiducial limits for 95% confidence to 24, 48, 72 and 96 hours
log LC₅₀ values of mercuric chloride are 0.86477 to 0.90302; 0.68201 to 0.73818; 0.54246 to 0.62253 and 0.32332 to 0.42387 respectively. Minimum and maximum fiducial limits for cypermethrin are 1.02814 to 1.07065; 0.88192 to 0.94047, 0.61556 to 0.71643 and 0.37405 to 0.52674 for 24, 48, 72 and 96 hours log LC₅₀ values respectively. The fiducial limits for monocrotophos are 2.0062 to 2.0485; 1.9019 to 1.9534; 1.7600 to 1.8309 and 1.6280 to 1.7233 for 24, 48, 72 and 96 hours log LC₅₀ values respectively.

The safe concentration of the pesticides, cypermethrin and monocrotophos and heavy metals, copper sulphate and mercuric chloride was calculated and cited under the column of safe concentration 'C' in the tables (5 and 6).

The safe concentration for cypermethrin is 1.2939 ppm. The safe concentration for monocrotophos is 16.0480 ppm. The pesticide monocrotophos is less toxic to Thiara in comparison to cypermethrin. The safe concentrations of copper sulphate and mercuric chloride are 2.2777 ppm and 0.7934 ppm respectively. The mercuric chloride is most toxic among the above pollutants, and the monocrotophos is least toxic among the above pollutants.

Lethal doses for the pesticides and heavy metals are entered in the tables 5 and 6 under the column 'lethal dose'. For the immediate 100% mortality of the snail, the lethal dose
was calculated. The lethal dose for the pesticide cypermethrin at 24, 48, 72 and 96 hours exposure are 244.91, 391.23, 333.67 and 270.80 ppm respectively. The lethal doses for the pesticide monocrotophos are 2556.29, 4063.8, 4496.06 and 4549.57 ppm for 24, 48, 72 and 96 hours exposure respectively. The heavy metal copper sulphate's lethal doses for 100% mortality of the snail Thiara tuberculata are 306.55, 515.51, 625.50 and 523.31 ppm to 24, 48, 72 and 96 hours exposure respectively. The mercuric chloride lethal doses for 24, 48, 72 and 96 hours exposure are 183.72, 257.83, 275.34 and 226.96 ppm respectively.

For the 100% immediate mortality, the snail requires highest lethal dose of the pesticide monocrotophos and minimum lethal dose of mercuric chloride. Thus mercuric chloride is most toxic among the pollutants, copper sulphate, cypermethrin and monocrotophos. The order of toxicity in decreasing manner is $\text{HgCl}_2 > \text{CuSO}_4 > \text{Cypermethrin} > \text{Monocrotophos}$. 
Table 1

Calculation of Regression equation for LC$_{10}$ and LC$_{50}$ of *Thiaia tuberculata* exposed to Cypermethrin.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Conc of Pesticide mg/litre base 10</th>
<th>Log of cone to exposed 10</th>
<th>No of animals per 48 hrs 'r'</th>
<th>Mortality</th>
<th>Percentage</th>
<th>Probit</th>
<th>Expected probit 'y'</th>
<th>Weight coefficient</th>
<th>Weight 'w'</th>
<th>Working Probit 'w'</th>
<th>Weight 'y'</th>
<th>$w^2$</th>
<th>$y^2$</th>
<th>$w y$</th>
<th>Improved Expected Probit 'y'</th>
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\[
\begin{align*}
SW &= 69.0904 \\
S\times x &= 62.9109 \\
S\times y &= 345.0047 \\
S\times x^2 &= 57.9356 \\
S\times y^2 &= 1765.6935 \\
S\times xy &= 319.4051 \\
\end{align*}
\]

i) \( x = (S\times x / Sw) = (662.9109 / 69.0904) = 9.1015 \)

ii) \( y = (S\times y / Sw) = (345.0047 / 69.0904) = 4.9933 \)

iii) \( b = (S\times xy - x S\times y) / (S\times x^2 - x S\times x) = (319.4051 - 0.9105 \times 345.0047) / (57.9356 - 0.9105 \times 62.9109) \)

\[= (5.2784 / 0.6533) = 8.0549 \]

iv) \( y = y + b(x - \bar{x}) = 4.9933 + 8.0549 (x - 9.1015) \)

\[y = 8.0549x - 2.3404 \]
Table 2
Calculation of regression equation for LC_{10} and LC_{50} of *Thiara tuberculata* exposed to monocrotophos

<table>
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<th>Sr. No.</th>
<th>Conc. of Pesticide (mg/litre)</th>
<th>Log of cone to base 10</th>
<th>No of animals exposed per 48 hrs.</th>
<th>Mortality % (p=100r/n)</th>
<th>Probit 'r'</th>
<th>Empirical Probit 'x'</th>
<th>Expected Probit 'y'</th>
<th>Weighing Coefficient 'w'</th>
<th>Working Probit 'w'</th>
<th>( W_y )</th>
<th>( W_x )</th>
<th>( W_{x^2} )</th>
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<th>( W_{xy} )</th>
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</table>

\[
SW = 95.1246, \quad S_w = 183.7721, \quad S_{wy} = 476.6995, \quad S_{y} = 355.7712, \quad S_{x} = 2455.0225, \quad y' = 930.6012
\]

1) \( \bar{x} = \frac{(S_{wx} / Sw)}{(183.7721 / 95.1246)} = 1.9319 \)

2) \( \bar{y} = \frac{(S_{wy} / Sw)}{(476.6995 / 95.1246)} = 5.0323 \)

3) \( b = \frac{(S_{wxy} - xS_{wy})}{(S_{wx}^2 - xS_{wx})} = \frac{(930.6012 - 1.9319 \times 476.6995)}{(355.7712 - 1.9319 \times 183.7721)} = \frac{5.8017}{0.7419} = 7.8200 \)

4) \( y = \bar{y} + b(\bar{x} - \bar{x}) = 5.0323 + 7.8200 (x - 1.9319) \)

\[ y = 7.8200x - 10.0751 \]
Table 3

Calculation of Regression equation for LC$_{10}$ and LC$_{50}$ of *Thiara tuberculata* exposed to heavy metal CuSO$_4$

<table>
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<th>Sr. No.</th>
<th>Conc of CuSO$_4$ (mg/litre)</th>
<th>Log of cone to base 10</th>
<th>No of animals exposed</th>
<th>Mortality per 48 hrs 'r'</th>
<th>Percentage mortality 'p' = (100r/n) %</th>
<th>Empirical Probit 'Y'</th>
<th>Expected Probit 'Y'</th>
<th>Weighing coefficient 'w'</th>
<th>Working Probit 'w'</th>
<th>Working Probit 'w' $^2$</th>
<th>Weighing coefficient 'w' $^2$</th>
<th>Improved Probit 'y'</th>
<th>Expected Probit 'y'</th>
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\[
\begin{align*}
&S = 96.5872 \\
&S_k = 100.1746 \\
&S_y = 487.0529 \\
&S_{k^2} = 104.806 \\
&S_{y^2} = 2502.7008 \\
&S_{ky} = 511.6193
\end{align*}
\]

i) \( x = \frac{(S_{wx} / Sw)}{(100.1746 / 96.5872)} = 1.0371 \)

ii) \( y = \frac{(S_{wy} / Sw)}{(487.0529 / 96.5872)} = 5.0426 \)

iii) \( b = \frac{(S_{wy} - xS_{wy})}{(Sw^2 - xSw)} = \frac{(511.6193 - 1.0371 \times 487.0529)}{(104.806 - 1.0371 \times 100.1746)} = 6.4978 / 0.915 = 7.1003 \)

iv) \( y = y + b(x-x) = 5.0426 + 7.1003 (x-1.0371) \)

\[
y = 7.1003x - 2.3211
\]
## Table - 4

Calculation of Regression equation for LD10 and LD50 of *Thiaira tuberculata* exposed to heavy metal HgCl₂

<table>
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<th>Conc of HgCl₂ mg/litre (ppm)</th>
<th>Log of cone to base 10</th>
<th>No of animals exposed</th>
<th>Mortality per 48 hrs 'r'</th>
<th>Percentage mortality p = (100r/n)%</th>
<th>Probit 'x'</th>
<th>Empirical Probit 'y'</th>
<th>Expected Probit 'w'</th>
<th>Weight 'w'</th>
<th>Working Probit 'wx'</th>
<th>( \frac{w^2}{2} )</th>
<th>Weight 'wy'</th>
<th>( \frac{y^2}{2} )</th>
<th>( Wxy )</th>
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<td>5.5244</td>
<td>5.50</td>
<td>0.58099</td>
<td>11.6198</td>
<td>5.524  9.4457</td>
<td>64.1877</td>
<td>7.6784</td>
<td>354.5732</td>
<td>52.1782</td>
<td>5.5889</td>
</tr>
<tr>
<td>8</td>
<td>7.0</td>
<td>0.8450</td>
<td>20</td>
<td>16</td>
<td>80</td>
<td>5.8416</td>
<td>5.7</td>
<td>0.53159</td>
<td>10.6318</td>
<td>5.824  8.9838</td>
<td>62.0259</td>
<td>7.5913</td>
<td>361.8592</td>
<td>52.4119</td>
<td>5.8172</td>
</tr>
<tr>
<td>9</td>
<td>7.5</td>
<td>0.8750</td>
<td>20</td>
<td>18</td>
<td>90</td>
<td>6.2816</td>
<td>5.9</td>
<td>0.47144</td>
<td>9.4288</td>
<td>6.216  8.2502</td>
<td>58.6094</td>
<td>7.2189</td>
<td>364.3161</td>
<td>51.2832</td>
<td>6.0307</td>
</tr>
</tbody>
</table>

\[
\begin{align*}
\text{SW} &= 96.5872 \\
\text{SKx} &= 71.0996 \\
\text{SKy} &= 487.0529 \\
\text{SKx}^2 &= 53.2448 \\
\text{SKy}^2 &= 2502.7008 \\
\text{SKxy} &= 365.0044
\end{align*}
\]

\[ i) \quad \bar{x} = (\text{Swx} / \text{Sw}) = (71.0996 / 96.5872) = 0.7361 \]

\[ ii) \quad \bar{y} = (\text{Swy} / \text{Sw}) = (487.0529 / 96.5872) = 5.0426 \]

\[ iii) \quad b = (\text{Swy} - \bar{x}\text{Swy}) / (\text{Sx}^2 - \bar{x}\text{Sx}) = (365.0049 - 0.7361 \times 487.0529) / (53.2448 - 0.7361 \times 71.0996) \]

\[ = 7.1136 \]

\[ iv) \quad y = y + b(x-\bar{x}) = 5.0426 + 7.1136 (x-0.7361) \]

\[ y = 7.1136x - 0.1937 \]
Fig. 1: Regression and provisional line for *Thiara tuberculata* exposed to cypermethrin, showing $LC_{10}$ $LC_{50}$ values in log concentration at different durations.

(a) 24 hours (b) 48 hours.
Fig. 2: Regression and provisional lines for *Thiara tuberculata* exposed to cypermethrin, showing $LC_{10}$ and $LC_{50}$ values in log concentration at different durations
(c) 72 hours (d) 96 hours
Fig. 3: Regression and provisional lines for *Thiara tuberculata* exposed to Monocrotrophos, showing \( LC_{10} \) and \( LC_{50} \) values in log concentration at different durations:

(a) 24 hours  (b) 48 hours
Fig. 4: Regression and provisional lines for *Thiaratuberculata* exposed to Monocrotophos, showing LC10 and LC50 values in log concentration at different durations.

(c) 72 hours  (d) 96 hours
Fig. 5: Regression and provisional lines for *Thiara tuberculata* exposed to copper sulphate, showing $LC_{10}$ and $LC_{50}$ values in log concentration at different durations.

(a) 24 hours  (b) 48 hours
FIG. 5

(a) 24 HOUR

Regression Line
Provisional Line

Empirical Probit

Log Conc.

(b) 48 HOUR

Regression Line
Provisional Line

Empirical Probit

Log Conc.
Fig. 6: Regression and provisional lines for *Thiara tuberculata* exposed to copper sulphate, showing $LC_{10}$ and $LC_{50}$ values in log concentration different durations.

(c) 72 hours  (d) 96 hours
Fig. 7: Regression and provisional lines for *Thiara tuberculata* exposed to mercuric chloride, showing LC$_{10}$ and LC$_{50}$ values in log concentration at different durations.

(a) 24 hours (b) 48 hours
Fig. 8: Regression and provisional lines for *Thiara tuberculata* exposed to Mercuric chloride, showing LC$_{10}$ and LC$_{50}$ values in log concentration at different durations.

(c) 72 hours  (d) 96 hours
Fig. 9: Comparison of $LC_{10}$, $LC_{50}$ and safe concentration at different exposure periods.

(a) Cypermethrin
(b) Monocrotophos
Fig. 10: Comparison of $\text{LC}_{10}$, $\text{LC}_{50}$ and safe concentration at different exposure periods.

(a) Mercuric chloride
(b) Copper sulphate
Fig. 11: Comparison of $L_{C_{10}}$, $L_{C_{50}}$ values of pollutants, cypermethrin, Monocrotophos, copper sulphate and mercuric chloride at different exposure periods.

(a) 24 hours
(b) 48 hours.
Fig. 1Z: Comparison of LC$_{10}$ and LC$_{50}$ values of pollutants cypermethrin, monocrotophos, copper sulphate and mercuric chloride at different exposure period.

(c) 72 hours
(d) 96 hours.
Table - 5

Relative toxicity of pesticide pollutants against the adult snail *T. tuberculata*.

<table>
<thead>
<tr>
<th>Name of Pesticide</th>
<th>time of exposure</th>
<th>Regression Equation ( Y = \bar{Y} + b \times (x-x) )</th>
<th>LC(_{50}) value ( \text{ppm} )</th>
<th>Variance ( V )</th>
<th>( \chi^2 ) value</th>
<th>Fiducial limits ( m_1 ) ( m_2 )</th>
<th>Lethal dose ( \text{ppm} )</th>
<th>Safe concentration ( \text{ppm} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cypemethrin</td>
<td>24</td>
<td>( Y = 11.1523 x - 6.7034 )</td>
<td>11.2046</td>
<td>0.00011755</td>
<td>0.4438</td>
<td>1.02814</td>
<td>1.07065</td>
<td>244.91</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>( Y = 8.0549 x - 2.3404 )</td>
<td>8.1507</td>
<td>0.00022309</td>
<td>0.39623</td>
<td>0.88192</td>
<td>0.94047</td>
<td>391.23</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>( Y = 4.7509 x - 1.8359 )</td>
<td>4.6344</td>
<td>0.000666220</td>
<td>1.40958</td>
<td>0.61556</td>
<td>0.71643</td>
<td>333.67</td>
</tr>
<tr>
<td></td>
<td>96</td>
<td>( Y = 4.1611 x + 3.1255 )</td>
<td>2.8209</td>
<td>0.00151700</td>
<td>0.53602</td>
<td>0.37405</td>
<td>0.52674</td>
<td>270.80</td>
</tr>
<tr>
<td>Monocrotophos</td>
<td>24</td>
<td>( Y = 9.6393 x - 14.5429 )</td>
<td>106.5</td>
<td>0.0001165</td>
<td>1.08204</td>
<td>2.00624</td>
<td>2.04855</td>
<td>2556.2</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>( Y = 7.8200 x - 10.0751 )</td>
<td>84.6</td>
<td>0.0001722</td>
<td>0.69421</td>
<td>1.90197</td>
<td>1.95342</td>
<td>4063.8</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>( Y = 5.6630 x - 5.2133 )</td>
<td>62.4</td>
<td>0.0003277</td>
<td>0.90619</td>
<td>1.76001</td>
<td>1.83098</td>
<td>4496.0</td>
</tr>
<tr>
<td></td>
<td>96</td>
<td>( Y = 4.1649 x - 2.0127 )</td>
<td>47.3</td>
<td>0.0005907</td>
<td>1.11585</td>
<td>1.62806</td>
<td>1.72333</td>
<td>4549.5</td>
</tr>
</tbody>
</table>

*bc a*
Table - 6

Relative toxicity of heavy metal pollutants against the adult snail *Thiara tuberculata*.

<table>
<thead>
<tr>
<th>Name of Heavy metal</th>
<th>Time of exposure Hrs.</th>
<th>Regression equation ( Y = y + b (x - X) )</th>
<th>( LC_{50} ) value ppm</th>
<th>Variance ( V )</th>
<th>( \chi^2 ) value</th>
<th>Fiducial limits ( m_1 ) ppm</th>
<th>( m_2 ) ppm</th>
<th>Lethal dose ppm</th>
<th>Safe concentration ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>CuSO₄</td>
<td>24</td>
<td>( Y = 8.4337 x - 4.3304 )</td>
<td>12.7732</td>
<td>0.0001501</td>
<td>0.56216</td>
<td>1.08228</td>
<td>1.13031</td>
<td>306.55</td>
<td></td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>( Y = 7.1003 x - 2.3211 )</td>
<td>10.7398</td>
<td>0.0002061</td>
<td>0.55906</td>
<td>1.00285</td>
<td>1.05914</td>
<td>515.51</td>
<td></td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>( Y = 5.6668 x - 0.3206 )</td>
<td>8.6876</td>
<td>0.0003191</td>
<td>0.69893</td>
<td>0.90388</td>
<td>0.97391</td>
<td>625.50</td>
<td>2.2777</td>
</tr>
<tr>
<td></td>
<td>96</td>
<td>( Y = 3.4012 x + 2.495 )</td>
<td>5.4512</td>
<td>0.0009494</td>
<td>2.03728</td>
<td>0.67610</td>
<td>0.79689</td>
<td>523.31</td>
<td></td>
</tr>
<tr>
<td>HgCl₂</td>
<td>24</td>
<td>( Y = 10.5356 x - 4.3131 )</td>
<td>7.6553</td>
<td>0.0000952</td>
<td>0.81799</td>
<td>0.86477</td>
<td>0.90302</td>
<td>183.72</td>
<td></td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>( Y = 7.1136 x - 0.1937 )</td>
<td>5.3716</td>
<td>0.0002053</td>
<td>0.55785</td>
<td>0.68201</td>
<td>0.73318</td>
<td>257.83</td>
<td></td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>( Y = 4.9368 x + 2.1241 )</td>
<td>3.8242</td>
<td>0.0004171</td>
<td>0.58204</td>
<td>0.54236</td>
<td>0.62253</td>
<td>275.34</td>
<td>0.7934</td>
</tr>
<tr>
<td></td>
<td>96</td>
<td>( Y = 5.0467 x + 3.1141 )</td>
<td>2.3642</td>
<td>0.0006578</td>
<td>1.10064</td>
<td>0.32332</td>
<td>0.42387</td>
<td>226.96</td>
<td></td>
</tr>
<tr>
<td>No.</td>
<td>Name of the pollutant</td>
<td>Safe concentration 'C' ppm</td>
<td>LC&lt;sub&gt;10&lt;/sub&gt; value in ppm</td>
<td>LC&lt;sub&gt;50&lt;/sub&gt; value in ppm</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-----</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>24 hrs</td>
<td>48 hrs</td>
<td>72 hrs</td>
<td>96 hrs</td>
<td>24 hrs</td>
<td>48 hrs</td>
<td>72 hrs</td>
</tr>
<tr>
<td>2.</td>
<td>Monocrotophos</td>
<td>16.0480</td>
<td>68.9286</td>
<td>49.5222</td>
<td>29.8744</td>
<td>17.3940</td>
<td>106.5123</td>
<td>84.6642</td>
<td>62.4453</td>
</tr>
<tr>
<td>4.</td>
<td>HgCl&lt;sub&gt;2&lt;/sub&gt;</td>
<td>0.7934</td>
<td>5.7852</td>
<td>3.5476</td>
<td>2.1034</td>
<td>1.3174</td>
<td>7.6553</td>
<td>5.3716</td>
<td>3.8242</td>
</tr>
</tbody>
</table>
DISCUSSION

Pollution problems are caused by release of sewage, municipal waste, industrial effluents, organic domestic wastes and pesticides in aquatic medium. The indiscriminate use of pesticides has resulted in contamination of freshwater ecosystem, causing hazards to several non-target organisms, like fishes, mussels, (Matsumura et al., 1972). The toxic materials contained in the effluents discharged from industrial establishment, municipal wastes and insecticides have a direct lethal effect on most of the organisms used for culture. Such mortality of different species of fin-fish and shell-fish has been observed throughout the world (Thomas, 1968). The mass mortality of commercial fishes has been reported by Konar (1975) due to washing of organophosphorous insecticides by heavy rains in adjacent aquatic resources. Various animals exhibit varying degrees of susceptibility to variety of pollutants. The physical, chemical and biological components of environment play an important role in the manifestation of biological responses to pollutants (Moor, 1969). The toxicity of particular pollutants depends on many factors such as animal weight (Pickering et al., 1968), time of exposure, temperature (Macek et al., 1969), pH and hardness of water. The evaluation of LC$_{50}$ concentration of pollutants is an important step before carrying further studies on physiological changes in animals.
Some heavy metals and pesticides have enormous value in agricultural field to control agricultural pests and diseases. The use of these chemicals in field increased along evergrowing population. On the other side, pesticides and heavy metals have become environmental contaminants. Increasing public awareness of environmental issues coupled with major disasters involving toxic chemicals has highlighted the need to identify and regulate toxic substances.

Pollutants at very low concentrations might be perceived by organisms' sensory system. If the stimulant is recognized harmful, avoidance may follow. In motile organisms, this could lead to escape. In those forms with little or no mobility such as fresh water mussels and gastropods, avoidance may take the form of reducing exposure of external body surfaces through mucus production, withdrawal of body into shell, or closure of siphons. When exposure to polluted conditions persists, certain biochemical alterations (enzyme activity, alterations in the membrane properties, protein synthesis, lipid synthesis etc.) and responses soon occur. These changes may subsequently affect physiological processes (e.g. osmotic and ionic balance, utilization of energy source etc.) and lead to perceptible alterations in physiological state. If reduced water quality continues as a chronic condition, these alterations in
physiological performance may adversely influence growth and reproductive success of individuals. Reduced productivity of fecundity may also alter community composition and perhaps the function of the entire system.

During the present study of toxicity evaluation of pesticides and heavy metals to *Thiaras tuberculata* it was observed that the *LC*<sub>50</sub> value decreased as the time of exposure increased. The *LC*<sub>50</sub> values of 24, 48, 72 and 96 hours exposure for cypermethrin are 11.2046, 8.1507, 4.6344, and 2.8209 ppm respectively. For monocrotrophos the *LC*<sub>50</sub> values for 24, 48, 72 and 96 hours exposure are 106.51, 84.66, 62.44 and 47.39 ppm respectively. Eisler (1970a) studied the toxicity to marine fish by pesticides. He calculated the *LC*<sub>50</sub> values of 24, 48, 72 and 96 hours for 12 different insecticides to seven species of estuarine fishes.

Anderson et al. (1977) observed that the number of deaths increased with increasing exposure time. Verma et al. (1977) conducted bioassay trials with several pesticides including Fenitrothion to determine *LC*<sub>50</sub> acute toxicity range and relative toxicity for a period of 24, 48, 72 and 96 hours to *Labrochromis rochita*, was 3.92, 4.85, 4.68 and 4.63 ppm, respectively.
Among the two pesticides selected for toxicity evaluation of *Thiara tuberculata* cypermethrin was more toxic than monocrotophos. The LC$_{50}$ value for cypermethrin was (8.1508 ppm) relatively less than LC$_{50}$ value of monocrotophos (84.6642 ppm) for 48 hours exposure. The lethal dose of cypermethrin (391.23 ppm) is relatively smaller than monocrotophos (4063.88 ppm) for 48 hours exposure. The safe concentration of cypermethrin (1.1939 ppm) to *Thiara tuberculata* is aquatic medium is relatively less than monocrotophos (16.04080 ppm) safe concentration, indicating the high toxicity of cypermethrin comparison to monocrotophos.

Considerable amount of work has been done on the determination of toxicity of various pesticides to invertebrates, fishes and vertebrates indicating that the toxicity and the susceptibility vary with the type of pesticide and the test species.

Verma et al. (1980, 1981, 1983) studied the effect of some organophosphorous insecticides (malathion, fenitrothion, dichlorovos, metasystox and dizinon) to fishes (*Mystus vittatus*, *Ophioccephalus punctatus*, *Notopterus notopterus*) and determined the LC$_{50}$ values for 96 h. 96 hours LC$_{50}$ values of dichlorovos were 2.3 and 0.45 mg/lit, respectively for *O. punctatus* and *M. vittatus*. Verma et al., (1982) found LC$_{50}$ for *S. fossilis* using 14 organophosphorous pesticides, which are zolone, ekalux, diazine, rogor, DDVP, Malatex, Sumithion, Ckatin, metosystox, malathion, phosvel, diprex, farmothion, and abate. The
respective LC$_{50}$ values for 96h were 0.083, 1.5, 2.27, 4.57, 6.61, 9.79, 11.81, 12.55, 15.17, 15.0, 22.60, 31.05, 143.10 and 206.60 mg/lit. where as safe concentrations from results were 0.0019, 0.036, 0.0545, 0.1097, 0.1586, 0.2350, 0.3012, 0.2334, 0.3641, 0.3600, 0.5424, 0.7452, 3.4344 and 4.9584 mg/lit. They stated that the safe concentrations calculated for the pesticides for the fishes may help to a great extent in controlling the water pollution by these pesticides.

Gupta et al. (1979) studied the effect of pesticides aldrin (organochlorine) and ethyl parathion (organophosphate) on fishes, and they found aldrin was highly toxic in comparison to ethyl parathion. Kabaeer et al., (1981) studied the toxicity of commercial grade and technical grade malathion to fish Tilapia mossambica, showing 48h LC$_{50}$ values 0.377 ± 0.04 ppm and 5.59 ± 0.09 ppm respectively.

Sharma et al. (1982) studied the effect of rogorg 30% and Kikazin 48% EC to freshwater fish, Channa punctatus and LC$_{50}$ for 96 h was 19.5 and 17.0 ppm, respectively. The workers (Murthy et al., 1983; Sivaprasad Rao et al., 1983; Singh and Sahai, 1984) studied the toxicity of pesticides to fishes.

Toxicity varies with the type of pesticides and the invertebrate test species. Organophosphorous pesticides toxicity was studied in crustaceans and they show varying degree of
toxicity. Butler and Springer (1963) reported that baylex was the most toxic pesticide to crabs and shrimps.

Butler (1966) found that organophosphorous compounds were much less toxic to oysters than chlorinated hydrocarbons.

Eisler (1970) studied the effect of three organophosphorus insecticides (Dioxalthion, methyl parathion and phosdrin) and four organochlorine insecticides (P-P-DDT, Lindane, endrin and dieldrine) to marine molluscs. He found quahog clams (Mercinaria mercinaria) and mud snails (Nassa obsoleta) were more resistant to insecticides during exposure of 96 h than species of marine teleosts and decapod crustaceans including eel, kill fishes, blue head, muller, Silverside, puffer, grass shrimp, and hermit crab. Chatterjee (1975) determined the toxicity of diazinon to fish (Molliensia sphehops) bottom organisms (tubifex) and plankton (cyclops). The LC₀, LC₅₀, and LC₅₀ values for the fish were 0.25, 1.60, and 3.20 ppm, for tubifex 0.25, 3.16, and 4.00 ppm, and for cyclops 0.04, 2.51 and 10.00 ppm respectively. It showed that dizinon was more toxic to fish and bottom organisms than to plankton. While studying the toxicities of different organophosphate pesticides to crab, Oziotelphusa senex senex. Bhagyalaxmi (1981) found that sumithion was more toxic as compared to methyl parathion and malathion. Nagarathnamma and Ramamurthi (1982) studied comparative toxicity
of methyl parathion to different test species of aquatic ecosystem of rice field, including study of the teleost (Cyperinus carpio) the leech Poecilobdella granulosa the pond snail Pila globosa, the freshwater mussel Lamellidens marginalis and the field crab, Oziocelphusa senex senex, their study showed good tolerance in molluscs. This much tolerance might be due to the protective device offered by the shell closing mechanism. Nair and Nair (1982) studied the effect of five organophosphorus pesticides on Alitropus typus and found that folithion was most toxic and dimecron the least. Panwar et al. (1982) studied the effect of three chlorinated hydrocarbons Endrin, r-BHC, DDT) and two organophosphorus insecticides (Ethyl parathion and Malathion) to freshwater gastropod Viviparous bengalensis for 24, 48, 72 and 96 h exposure period and found that endrin was most toxic among chlorinated hydrocarbons and ethyl parathion among the organophosphorus insecticides. Safe concentrations of endrin r-BHC DDT, ethyl parathion and malathion were estimated as 0.0061, 0.01486, 0.0139, 0.6138 and 0.0923 mg/lit respectively. Chaudhari et al. (1988) studied the toxicity of organophosphates pesticides (Cythion 50% EC, Zolone, 35% EC, Rogor 30% EC) to freshwater snails Lymnaea acuminata, Thiara scabra and Thiara lineatus and found that the snails were tolerant to the pesticides. Cythion was the most toxic while zolone was less toxic to the snails.
In the present study the LC$_{50}$ values at 24, 48, 72 and 96 hours were 11.2346, 8.1507, 4.6344 and 2.8209 ppm respectively to cypermethrin; and for pesticide monocrotophos the values were 106.51, 84.66, 62.44 and 47.39 ppm to 24, 48, 72 and 96 hours exposure. The cypermethrin is more toxic than monocrotophos. Cypermethrin is an ideal pyrethroid and therefore widely used for crop protection. It is a broad spectrum insecticide but has relatively low mammalian toxicity. Consequently the environmental occurrence of synthetic pyrethroid became worldwide (Casida, 1980). They are not readily metabolized in insect, as it occurs in mammalian body, hence residues of pyrethroids remain for longer duration in the biosystem of insects causing harmful effects. Cypermethrin acts on nervous system of the target animal causing alterations in sodium channel system of nerves (Honerjager, 1982).

Monocrotophos is an organophosphorus insecticide, highly toxic to vertebrates. It exerts its toxic action by inhibiting certain enzymes of nervous system, e.g. acetylcholinesterase. It causes obstacle in transmission of impulses at synaptic connections causing rapid twitching of voluntary muscle finally leading to paralysis.

In present investigation the snails when exposed to cypermethrin and monocrotophos for toxicity evaluation, showed
the changes in adductor muscles and gradual loss of power to
close the operculum and to withdraw the foot inside the shell.
Similar behaviour was also observed by Pillai (1984) in bivalve
*Indonajia caeruleus*.

Bioaccumulation tendency is one of the most important
biological properties of metals. This tendency of metals causes
some physiological stress in aquatic organisms. The
physiological stress caused by metal pollution either in the
form of increased or decreased metabolism in aquatic
invertebrates can affect several parameters of the animals. The
heavy metal pollution affects the fauna by decreasing growth
rate. The heavy metal pollution in bivalves decreases the
growth rate (Galtsoff, et al., 1974), and exhausts the
biochemical reserves (Bayne and Thomson, 1970).

The present study of toxicity evaluation of heavy metals,
CuSO$_4$ and HgCl$_2$ was conducted on snail, *Thiara tuberculata* and
LC$_{10}$ and LC$_{50}$ values were calculated. The LC$_{50}$ values of
CuSO$_4$ are 12.7732, 10.7398, 8.6876 and 5.4512 ppm at 24, 48, 72
and 96 hours respectively. The LC$_{50}$ values of mercuric chloride
are 7.6553, 5.3716, 3.8242 and 2.3642 ppm to 24, 48, 72 and 96
hours exposure respectively. The safe concentrations for *Thiara*
tuberculata were 2.2777 ppm and 0.7934 ppm for copper sulphate
and mercuric chloride respectively. The lethal doses for 48
hour exposure were 515.51 ppm and 257.83 ppm for CuSO₄ and HgCl₂ respectively. It was found that in *Thiara tuberculata* copper sulphate was relatively less toxic than mercuric chloride. The copper is toxic to animals and plants in aquatic medium and it accumulates in some species. The Cu accumulates in phytoplankton and in bacteria (Sunda and Gillespie, 1979). The bivalves have tremendous capacity to accumulate significant amount of Cu ions, McEneny and George (1977) showed in the mussel *Anodonta anatina* that the copper level was 21 - 103 mg per kilogram soft tissue. Even at low concentration Cu accumulates in bivalves (Zamunda and Sunda, 1982; Zarogian and Johnson, 1983). The copper was found to accumulate in the body tissues of gastropods, *Helix aspersa* and *Littorina littorea* showed accumulation of copper in their tissues (Coughtrey and Martin, 1976; Ireland and Wootton, 1977). Large amount of Cu ions entered in to the body by nutrition.

Increased anthropogenic activities caused a rise in the level of mercury in marine as well as in inland water (Prahalad, 1987). The freshwater molluscs have relatively higher ability to accumulate mercury from the medium than other existing fauna (V-Balogh et al., 1988). Goldwater (1971) studied the mercury in the food chain. He found that the mercury compound was accumulated in shell fish and fishes, forming disastrous effects in food chain. Weis (1977) reported the inorganic mercury in fish.
The mode of action of mercurials is non-selective or non-specific inhibition of enzymes containing especially iron and sulfahydryl sites.

In the study of toxicity of heavy metals to fauna, the mercurial compounds were highly toxic to aquatic animals in comparison to other heavy metals (Bryan, 1971). Many workers studied Mercurial compound toxicity to aquatic animals (Akiyama 1970; Rehwoldt, et al., 1973; Wobeser, 1975) and concluded that the heavy metal, mercury and its compounds were more toxic to aquatic animals.

Klaving et al. (1975) reported acute toxicity levels in 96h where IC$_{50}$ values for HgCl$_2$ toxicity to Fundulus heterocilus were reported as 230 and 2010 ug/lit respectively, in fish collected from spring and summer population. Menezes and Qasim (1983) reported the IC$_{50}$, IC$_{100}$ and sublethal concentration values of HgCl$_2$ to fish S. mossambica at 0.7, 1.5 and 0.01 ppm. Dhanekar et al. (1985) studied acute toxicity of mercury to freshwater fishes, Puntius sophore, Lebistes reticulatus, Sarotherodon mossambicus, Channa punctatus and Heteropeustetes fossilis and found out the IC$_{50}$ values 0.15, 0.25, 0.5, 1.0 and 1.0 ppm respectively. The least effective concentration of HgCl$_2$ was recorded as 0.9 ppm in S. mossambicus, P. sophore (0.022 ppm) L. reticulatus (0.08 ppm), C. punctatus (0.17 ppm) and H. fossilis (0.21 ppm). Nagabhushanam et al. (1986)
studied the acute toxicity of three heavy metals (HgCl$_2$, CuSO$_4$, and ZnSO$_4$) on marine crab, Scylla serrata and performed static bioassay to evaluate the median lethal concentrations for 24, 48, 72 and 96 hours exposure period. The LC$_{50}$ values for 96 hours for HgCl$_2$, CuSO$_4$ and ZnSO$_4$ were recorded as 5.88 ppm, 346.7 ppm and 298.1 ppm respectively, concluding that mercuric chloride is the most toxic heavy metal to crab, Scylla serrata.

Muley and Mane (1988) observed the seasonal variation in toxicity of mercurial salts of the freshwater gastropod Viviparus bengalensis. The toxicity of HgCl$_2$ was more toxic during summer and monsoon than HgSO$_4$.

The present investigation on the toxicity evaluation of Thiar a tuberculata clearly indicates that, any pollutant present in the aquatic environment is harmful to animals as it directly or indirectly affects the individual. The mode of action of these pollutants and the LC$_{50}$ values were different. Among pesticides cypermethrin was relatively more toxic than monocrotophos, and among heavy metals Mercury was more toxic than copper.
SUMMARY

1. The toxicity of two commonly used pesticides (Cypermethrin and Monocrotophos) and two heavy metals (copper sulphate and mercuric chloride) to the medium sized snails *Thiara tuberculata* was assessed by the classic method of Probit.

2. The $LC_{50}$ value, $LC_{10}$ value, variance, fiducial limits, safe concentration and lethal dose of the pesticide and heavy metal pollutants for *T. tuberculata* were calculated.

3. Among pesticides Cypermethrin (synthetic pyrethroid) is more toxic than Monocrotophos (organophosphate) and among heavy metals, $HgCl_2$ is more toxic than $CuSO_4$.

4. Among the four pollutants cypermethrin, monocrotophos, $HgCl_2$ and $CuSO_4$, Mercuric chloride is the most toxic and Monocrotophos is the least toxic.

5. The order of toxicity in decreasing manner is
Mercuric chloride $>$ Copper sulphate $>$ Cypermethrin $>$ Monocrotophos.
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