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ACUTE TOXICITIES OF COPPER SULPHATE, ZINC SULPHATE AND LEAD NITRATE TO *LIZA PARSIA* (HAMILTON-BUCHANAN)

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ABSTRACT

Acute toxicity tests through static bioassays were conducted exposing *Liza parsia* separately to copper sulphate, zinc sulphate and lead nitrate in the laboratory. The 96 hr LC50 values found were 85.6, 60.3 and 103.5 ppm for copper sulphate, zinc sulphate and lead nitrate respectively. The 96 hr LC50s calculated for elementary copper, zinc and lead were 21.8, 13.7 and 64.7 ppm respectively. Based on 12 hr LC50 the copper was found more toxic than zinc and lead. In 24, 48, 72 and 96 hr LC50s the zinc was found more toxic to the experimental animals than copper and lead. The animals were experimented in salinity 9.8 ± 0.76‰, pH 7.19 ± 0.12, temperature 28.0 ± 1.5°C and total hardness 2956.0 ± 142.2 ppm.

INTRODUCTION

HEAVY metals (Mn, Ni, Cr, Zn, As, Cd, Pb, Fe and Cu) and their salts constitute the widely distributed group of highly toxic and long - retained substances (Metelev *et al.*, 1983). It is essential to understand the effects of heavy metals on our coastal exploitable fishery resources as also cultivable fishes and prawns for better management, monitoring and formulation of policies. Classical approaches to biomonitoring have included acute bioassay which takes death as the end-point of the test. Since 1950s the acute toxicity testing has become the 'workhorse' in monitoring pollution effects (Buikema *et al.*, 1982). According to Koeman *et al.* (1978) and Genjatulin (1990) the toxicological hazards measured by bioassay procedures are more realistic than those predicted from the results of chemical analyses and the available information on the toxicity of the compounds detected.

Waldichuk (1974) stated that, to measure the acute toxicity the standard bioassays are normally conducted for 48 - and 96-hr period. According to Mohapatra and Noble (1991) the LC50 values can be used to find out the 'Safe levels' of toxicants in the water medium. In most of the predictions, the 'application factor' (0.1 - 0.0001) is actually used along with LC50 values (NAS/NAE, 1973). An application factor ranging from 0.030 to 0.099 was calculated for zinc, conducting bioassay on Guppy *Poecilia reticulata* (Pierson, 1981). The Environmental Protection Agency (EPA) guidelines indicated that lead concentrations in both marine and fresh water systems should not exceed 1% of the 96 hr LC50 for sensitive species (Laws, 1981).

Dehadrai (1990) stated that the copper, zinc and lead are the toxic metals in marine environment. They were selected as the toxicants for the present study.

According to Buikema *et al.* (1982), fish has been more commonly used as test organism, because they are considered to be the best understood organism in the aquatic environment and perceived as most valuable by the majority of scientists. Among mullets, *Liza parsia* along with *Mugil cephalus*, has gained considerable importance in fish culture, because of its resistance to environmental changes and easy availability. It was, therefore, selected for this study.

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MATERIAL AND METHODS

Liza parsia of 75.0 - 105.0 mm TL sizes and 15.0 - 30.0 g weight were collected live by cast nets from brackishwater canals of Puduvypeen area in Cochin. They were acclimatized to laboratory condition for about one week by maintaining in salinity 9.8 ± 0.76 ‰, pH 7.19 ± 0.12 , temperature 28.0 ± 1.5°C and total hardness 2956.0 ± 142.2 ppm.

To avoid fungal attack, the medium was treated with 11mg of malachite green per 100 litres of water (Mohapatra, 1989; Mohapatra and Noble, 1991). The fishes were fed once a day with pellet feed and the faecal matter alongwith other wastes were daily siphoned out.

Laboratory reagents such as copper sulphate ($CuSO_4$ $5H_2O$), zinc sulphate ($ZnSO_4$ $7H_2O$) and lead nitrate ($Pb(NO_3)_2$) were used for the preparation of stock solutions individually.

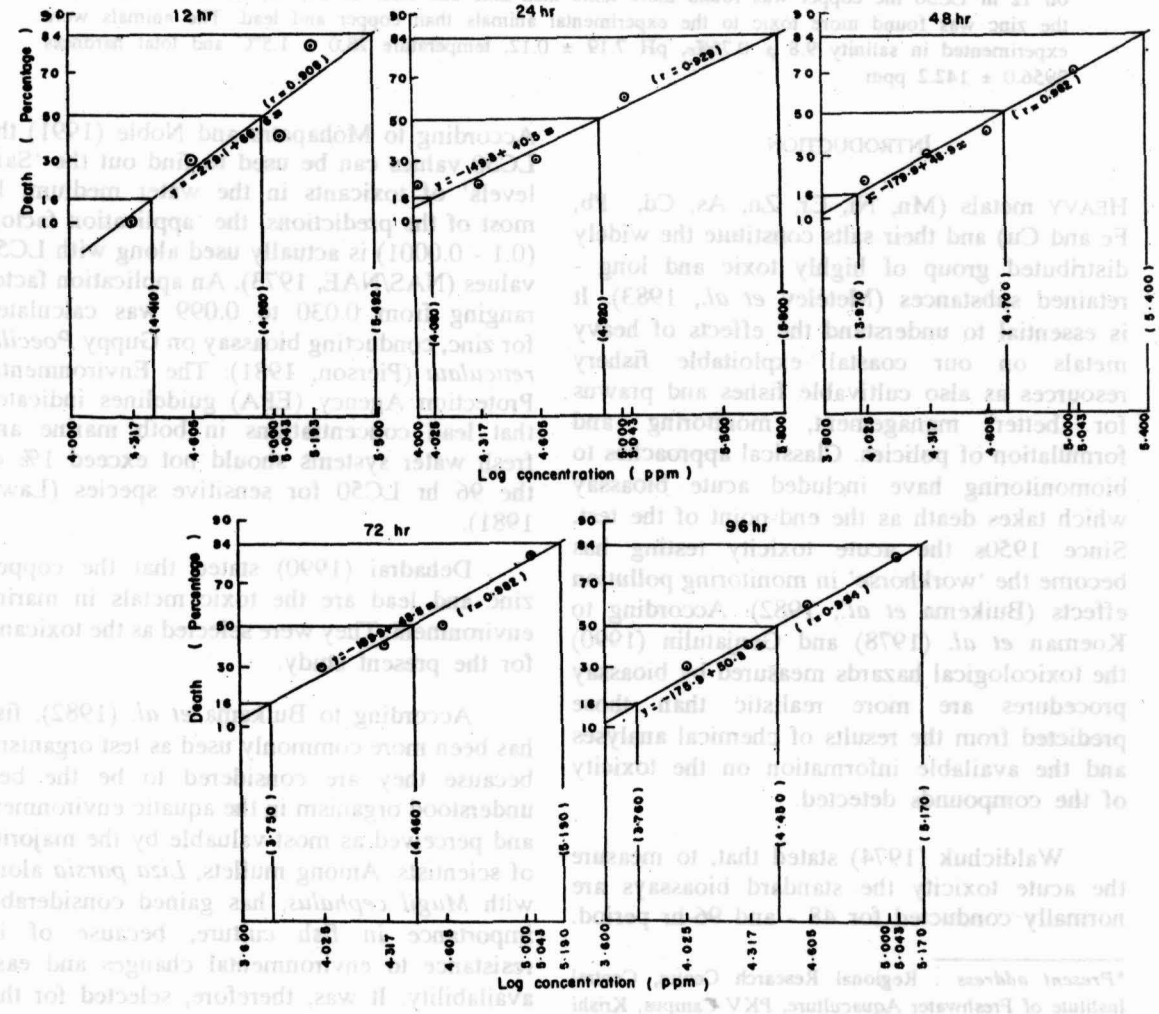

FIG. 1. Response curve of copper sulphate at 12, 24, 48, 72 and 96 hours.

The range-finding bioassays were conducted as per APHA-AWWA-WPCF (1976) with 10 experimental organisms exposed to each concentrations in log scale such as 1, 10, 100 and 1000 ppm for selection of experimental concentrations for static bioassay. Based on the Table of APHA-AWWA-WPCF (1976), the concentrations between 56 and 180 ppm for copper sulphate and zinc sulphate and between 75 and 210 ppm for lead nitrate were selected for experimentation.

Conversion of log value (ppm) to observed value (ppm)

Hours	LC	Log value	Observed Value
12	LC84	5.492	242.7
	LC50	4.950	141.2
	LC16	4.410	82.3
24	LC84	5.800	330.3
	LC50	4.920	137.0
	LC16	4.080	59.1
48	LC84	5.400	221.0
	LC50	4.700	109.9
	LC16	3.970	53.0
72	LC84	5.190	179.5
	LC50	4.460	86.5
	LC16	3.750	42.5
96	LC84	5.170	175.9
	LC50	4.450	85.6
	LC16	3.760	42.9

Static bioassay methods of Reish and Oshida (1987) were used in the entire experiments. Each bioassay consisting of a series of 5 experimental concentrations and a control were used. Ten specimens of *L. parsia* were experimented in each concentration — of metals and controls. Precautions were taken to avoid contamination of the controls. Test animals were not fed during the experiment. The percentage of mortality at the end of every 12, 24, 48, 72 and 96 hr was accounted.

The data obtained from the experiments were processed by 'Probit analysis' (Reish and Oshida, 1987) for determination of median lethal concentration (LC50). The percentage mortality *vs.* log concentrations were plotted in probability papers or shortly 'Probit paper' and the 'Response curves' were obtained by fitting the best fits (with correlation coefficient 'r') to the points (Mohapatra, 1989).

The values for LC16, LC50 and LC84 were obtained from the response curves. The slope function, 95% confidence limit and 95% fiducial limits (upper and lower) were calculated using the following formulae (Reish and Oshida, 1987):

$$\text{Slope (S)} = \frac{\frac{LC84}{LC50} + \frac{LC50}{LC16}}{2}$$

$$95\% \text{ confidence Limit } (f_{LC50}) = S \frac{2.77}{\sqrt{N}}$$

Where N = Total number of organisms tested at those exposed concentrations whose expected results were between 16% and 84%, and 2.77 is a constant.

95% fiducial limits are :

$$\text{Upper} = LC50 \times f_{LC50}$$

$$\text{Lower} = \frac{LC50}{f_{LC50}}$$

The lethal concentrations for each toxicants were plotted against time in hours in 'Nomograph paper' to get 'Toxicity curves' and the corresponding 95% fiducial limits were shown for each LC50 values on graph paper.

The level of availability of copper, zinc and lead from its compound form *viz.* $CuSO_4$ $5H_2O$, $ZnSO_4$ $7H_2O$ and $Pb(NO_3)_2$ when the organisms died in the experimental tanks were calculated using the formula of Reish and Oshida (1987) :

$$\text{Grams of compound containing 1.0g of element} = \frac{\text{Molecular weight of compound}}{\text{Molecular weight of element}}$$

i.e. 1g of $Cu\ SO_4\ 5H_2O$, $Zn\ SO_4\ 7H_2O$ and $Pb(NO_3)_2$ contain 0.2545, 0.2274 and 0.6256 g of copper, zinc and lead respectively.

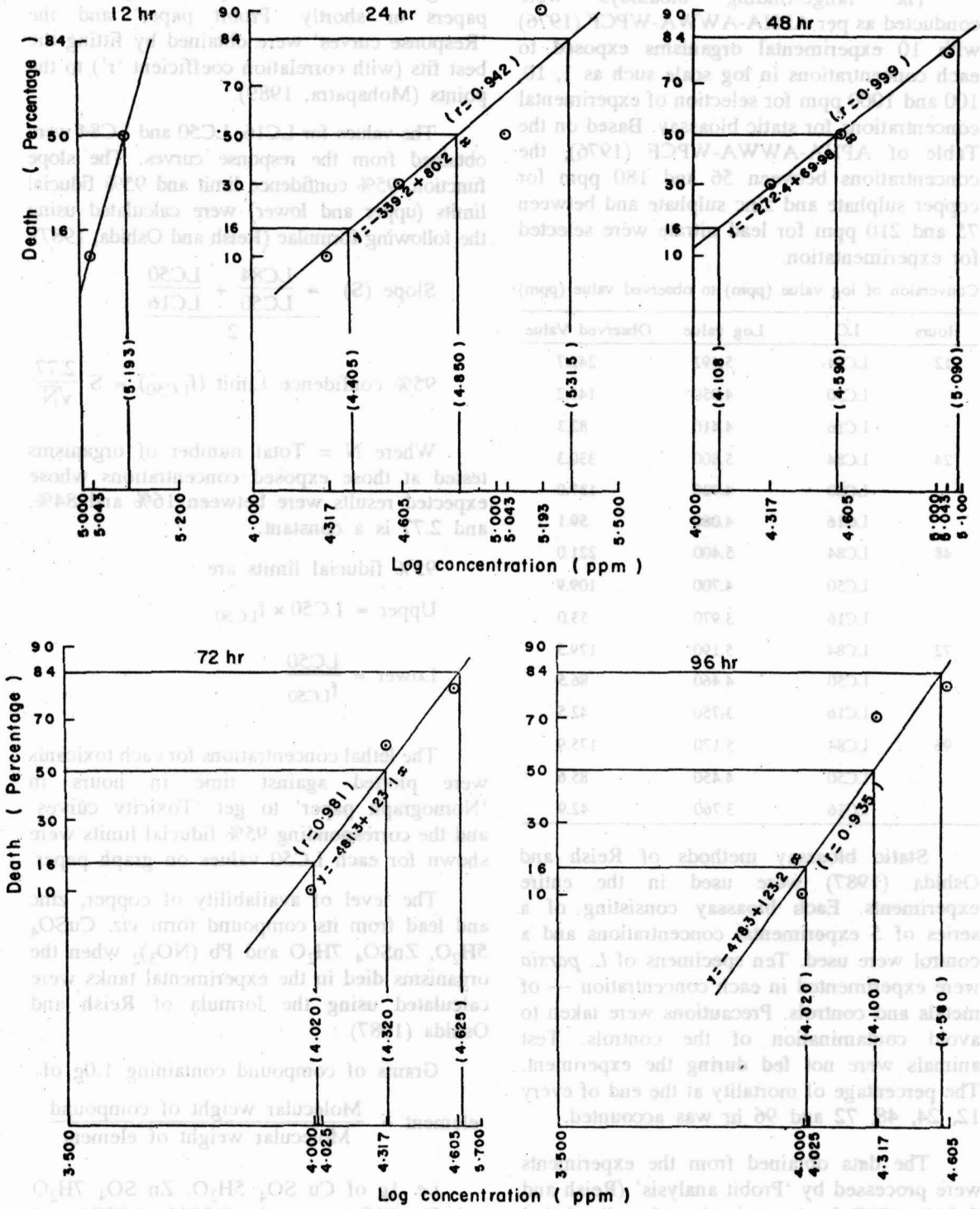

FIG. 2. Response curve of zinc sulphate at 12, 24, 48, 72 and 96 hours.

Conversion of log value (ppm) to observed value (ppm)

Hours	LC	Log value	Observed Value
12	LC50	5.193	180.0
24	LC84	5.315	203.4
	LC50	4.850	127.7
	LC16	4.405	81.9
48	LC84	5.090	162.4
	LC50	4.590	98.5
	LC16	4.108	60.8
72	LC84	4.625	102.0
	LC50	4.320	75.2
	LC16	4.020	55.7
96	LC84	4.580	97.5
	LC50	4.100	60.3
	LC16	4.020	55.7

AF value for the calculation of safe levels of heavy metals, 96 hr. LC50 values of heavy metals can be multiplied by 0.0001. Accordingly, the 96 hr. LC50 values for $CuSO_4$, $ZnSO_4$ and $Pb(NO_3)_2$ are given in Tables 2, 4 and 6 respectively. The results of bioassay expressed in terms of LC50 values for 12, 24, 48, 72 and 96 hours obtained in probit analysis on probit papers (Fig. 1) are given in Table 2. The results of acute toxicity studies expressed in terms of LC50 values of elementary copper, (calculated from the acute toxicity results for compound form) for 12, 24, 48, 72 and 96 hours are given in Table 7. The LC50 values showed gradual decrease with increase in time.

TABLE 1. Mortality rate of *L. parsia* in different concentrations of copper sulphate during acute toxicity studies (Animals exposed in each concentration are 10)

Exposure period (hrs)	Concentration (ppm)									
	56		75		100		155		180	
	Nos	%	Nos	%	Nos	%	Nos	%	Nos	%
12	—	—	—	10	3	30	4	40	8	80
24	2	20	2	20	3	30	6	60	10	100
48	2	20	3	30	4	40	7	70	10	100
72	3	30	4	40	5	50	8	80	10	100
96	3	30	4	40	6	60	8	80	10	100

TABLE 2. Acute toxicity of copper sulphate on *L. parsia*

Exposure Period (hrs.)	LC50 (ppm)	95% Fiducial limits (ppm)		LC 16 (ppm)	LC 84 (ppm)	Slope function	95% confidence limit
		Upper	Lower				
12	141.2	185.6	107.4	82.3	242.7	1.718	1.315
24	137.0	199.7	94.0	59.1	330.3	2.365	1.458
48	109.9	150.3	80.4	53.0	221.0	2.044	1.368
72	86.5	118.6	63.1	42.5	179.5	2.055	1.371
96	85.6	116.6	62.8	42.9	175.9	2.025	1.362

RESULTS AND DISCUSSION

Copper sulphate

The mortality rate (in percentage) of *L. parsia* in different concentrations such as 56, 75, 100, 135 and 180 ppm and in different exposure periods of 12, 24, 48, 72 and 96 hours is given in Table 1. In the absence of

The 95% fiducial limits, LC16, LC84, slope function and 95% confidence limit of each response curve for different exposure periods in hours are also given in Table 2. The 12, 24, 48, 72 and 96 hr LC50 values with its 95% fiducial limits were plotted on log-log paper to get the toxicity curve (Fig. 4).

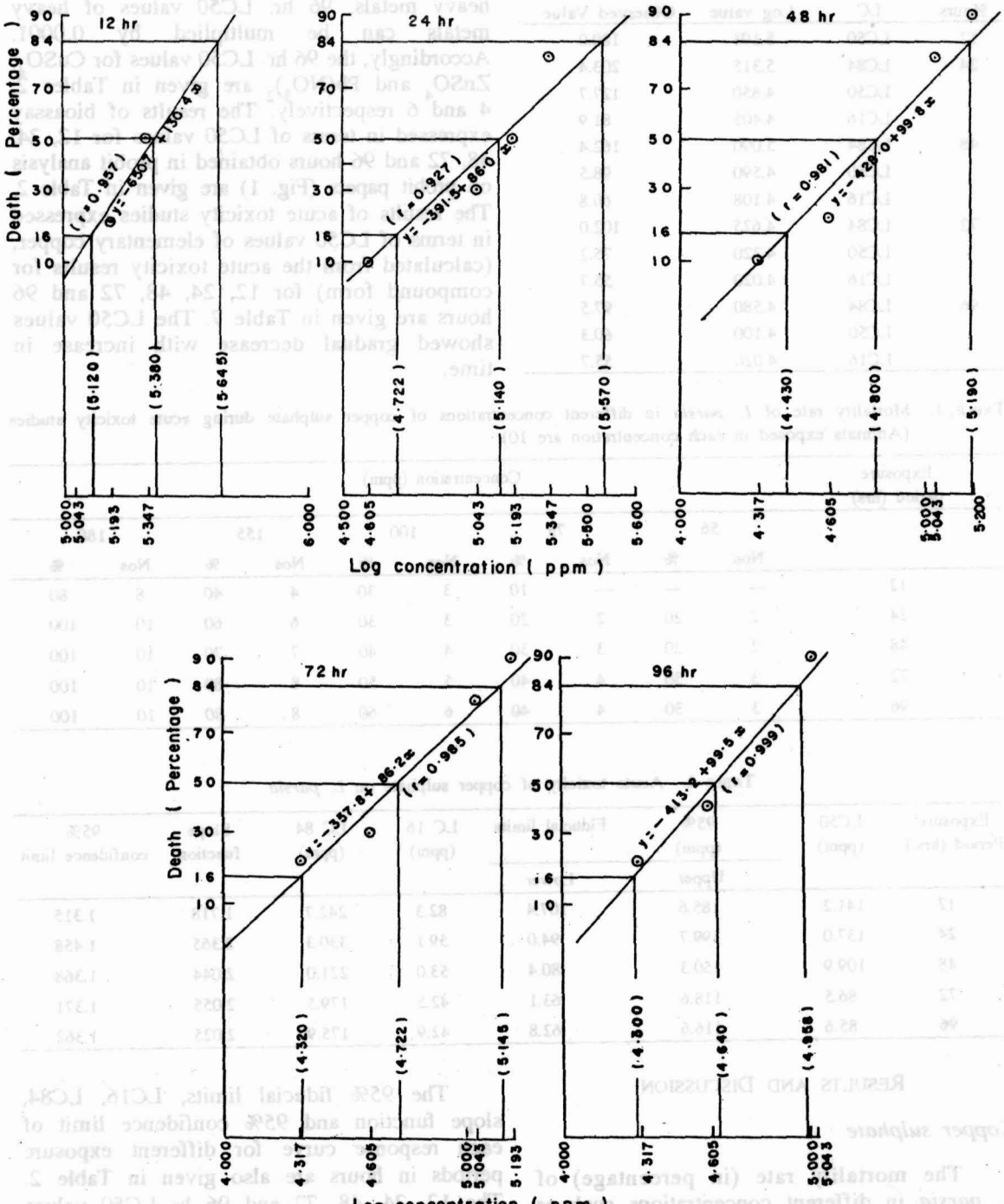

FIG. 3. Response curve of lead nitrate at 12, 24, 48, 72 and 96 hours.

Conversion of log value (ppm) to observed value (ppm)

Hours	LC	Log value	Observed Value
12	LC84	5.645	282.9
	LC50	5.380	217.0
	LC16	5.120	163.7
24	LC84	5.570	262.4
	LC50	5.140	170.7
	LC16	4.722	112.4
48	LC84	5.190	179.5
	LC50	4.800	121.5
	LC16	4.430	83.9
72	LC84	5.145	171.6
	LC50	4.722	112.4
	LC16	4.320	75.2
96	LC84	4.958	142.3
	LC50	4.640	103.5
	LC16	4.300	73.7

48, 72 and 96 hours obtained in probit analysis on probit papers (Fig. 2) along with 95% fiducial limits, LC16, LC84, slope function and 95% confidence limits are given in Table 4. The LC50 values of elementary zinc 12, 24, 48, 72 and 96 hours are given in Table 7.

The LC50 values for different exposure periods along with its 95% fiducial limits were plotted on log-log paper to get the toxicity curve (Fig. 4).

Lead nitrate

Table 5 presents the mortality rate of *L. parsia* in different concentrations of lead nitrate such as 75, 100, 155, 180 and 210 ppm and exposure periods such as 12, 24, 48, 72 and

TABLE 3. Mortality rate of *L. parsia* in different concentrations of zinc sulphate during acute toxicity studies (Animals exposed in each concentration are 10)

Exposure period (hrs)	Concentration (ppm)									
	56		75		100		155		180	
	Nos	%	Nos	%	Nos	%	Nos	%	Nos	%
12	—	—	—	—	—	—	1	10	5	50
24	—	—	1	10	3	30	5	50	9	90
48	—	—	3	30	5	50	8	80	10	100
72	1	10	6	60	8	80	10	100	10	100
96	1	10	7	70	8	80	10	100	10	100

TABLE 4. Acute toxicity of zinc sulphate on *L. parsia*

Exposure Period (hrs)	LC50 (ppm)	95% Fiducial limits (ppm)		LC 16 (ppm)	LC 84 (ppm)	Slope function	95% confidence limit
		Upper	Lower				
12	180.0	—	—	—	—	—	—
24	127.7	169.3	96.3	81.9	203.4	1.576	1.325
48	98.5	126.3	76.8	60.8	162.4	1.634	1.282
72	75.2	90.7	62.4	55.7	102.0	1.353	1.206
96	60.3	72.6	50.1	55.7	97.5	2.350	1.204

Zinc sulphate

The mortality rate of *L. parsia* at different hours in different concentrations such as 56, 75, 100, 155 and 180 ppm of zinc sulphate is given in Table 3. The results of bioassay expressed in terms of LC50 values for 12, 24, 96 hours. The results obtained in terms of LC50 values for different periods from probit analysis are given in Table 6. The 95% fiducial limits, LC16, LC84, slope function and 95% confidence limit of each response curve for different exposure periods in hours are also

TABLE 5. Mortality rate of *L. parsia* in different concentrations of lead nitrate during acute toxicity studies (Animals exposed in each concentration are 10)

Exposure period (hrs)	Concentration (ppm)									
	56		75		100		155		180	
	Nos	%	Nos	%	Nos	%	Nos	%	Nos	%
12	—	—	—	—	1	10	2	20	5	50
24	—	—	1	10	3	30	5	50	8	80
48	1	10	2	20	8	80	9	90	10	100
72	2	20	3	30	8	80	9	90	10	100
96	2	20	4	40	9	90	10	100	10	100

TABLE 6. Acute toxicity of lead nitrate on *L. parsia*

Exposure Period (hrs)	LC50 (ppm)	95% Fiducial limits (ppm)		LC 16 (ppm)	LC 84 (ppm)	Slope function	95% confidence limit
		Upper	Lower				
12	217.0	255.3	184.5	163.7	282.9	1.300	1.176
24	170.7	211.5	137.8	112.4	262.4	1.528	1.239
48	121.5	153.8	96.0	83.9	179.5	1.462	1.266
72	112.4	138.5	91.2	75.2	171.6	1.511	1.232
96	103.5	126.9	84.4	73.4	142.3	1.390	1.226

given in Table 6. The LC50 values for elementary lead calculated from its compound forms for 12, 24, 48, 72 and 96 hours are given in Table 7. The 12, 24, 48, 72 and 72 hr LC50 values with its 95% fiducial limits were plotted on log-log paper to get the toxicity curve (Fig. 3). The LC50 values showed gradual decrease with increase in time.

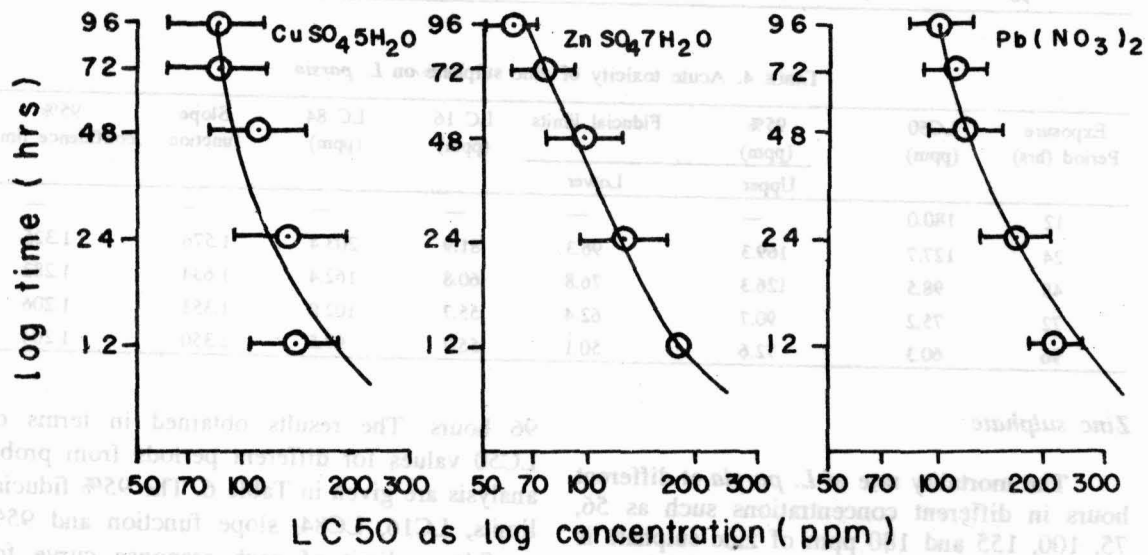

FIG. 4. Acute toxicity curve of copper sulphate, zinc sulphate and lead nitrate.

TABLE 7. Acute toxicity of Cu, Zn and Pb on *L. parsia*

Exposure period (hrs)	LC50 (ppm)		
	Cu	Zn	Pb
12	35.9	40.9	135.8
24	34.9	29.0	106.8
48	28.0	22.4	76.0
72	22.0	17.1	70.3
96	21.8	13.7	64.7

In the present investigation, based on 12 hr LC50, the copper was found to be more toxic than zinc and lead (Table 7). It shows the quickest action of copper on *L. parsia* than other metals. Based on the toxicity data Cu^{2+} was found to be more toxic than Zn^{2+} and Pb^{2+} to fish (Waldichuk, 1974). Conducting bioassays for 12, 24 and 72 hours with *Tilapia nilotica*, Somsiri (1982) reported that the copper is more toxic than zinc.

Based on 24, 48, 72 and 96 hr LC50 the zinc was found to be toxic to *L. parsia* than copper and lead in the present study (Table 7). Similar type of results were also reported for other fishes by various authors. Based on 96 hr LC50s of copper and zinc on Longfin dace *Agosia chrysogaster*, Lewis (1978) reported higher toxic nature of zinc than copper to fish. According to Metelev *et al.* (1983) Rainbow trout can live upto 133 minutes at 18°C in distilled water containing 25 ppm zinc. After that the symptoms of death have appeared. But 143 ppm of copper sulphate did not cause death in trout. It proved the toxicity of zinc over copper. The copper toxicity is generally reduced in the presence of the synthetic chelating agents, sewage effluents and purified humic acid (Winner, 1985). The lead with atomic number 82 and atomic weight 207 found least toxic in the present study. Pundir (1989) reported that the lead is less toxic than zinc in *Nemacheilus bolia*. The toxicity is also controlled by various physico-chemical and biological parameters.

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