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Acute Toxicity of Zinc Sulphate ($ZnSO_4.H_2O$) to Guppies (*Poecilia reticulata* P., 1859)

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ABSTRACT

In this study 96 hours LC50 values of zinc sulphate (ZnSO4.H2O); an important toxic industrial pollutant, on Poecilia reticulata were determined. Behavioral changes at different concentrations of zinc sulphate (ZnSO4.H2O) were monitored. The results were evaluated by the Statistical Probit Analysis Method and 96 hours LC50 value for guppies was found to be 30.826 mg/l. The lower and upper confidence limits for the LC50 were 30.387 and 31.280 mg/l, respectively.

Key Words: Acute Toxicity, Zinc Sulphate, Bioassay, Poecilia Reticulata.

1. INTRODUCTION

Heavy metals are important environmental pollutants. Metal contamination of the environment results both from natural sources and industrial activities. Metals in soil and water may enter the food cycle with an additional contribution from air. Further potential sources of human exposure include consumer products and industrial waste as well as the working environment [1]. The aquatic media are contaminated not only from the air but also from the land itself. The major factors contributing to heavy metal contamination are household and industrial waste containing either organic or inorganic matter [2].

There are a number of studies carried out on the toxicity of zinc sulphate. Zinc is an essential trace element for humans taking role in electron transfer in many enzymatic reactions. However, its prolonged and excessive intake may lead to toxic effect such as carcinogenesis, mutagenesis and teratogenesis as a result of its bioaccumulation [3, 4].

Although the toxicity of zinc to several fish species has been documented, the toxicity of this metal is not well known for all aquatic organisms [5]. Alsop et al. [6] examined chronic waterborne zinc exposure and the consequences of zinc acclimation on the gill/zinc interactions of rainbow trout in hard and soft water. They found that all zinc exposed trout had been acclimated to the metal, as seen by an increase of LC_{50} by 2.2 to 3.9 times over that of control fish.

Malik et al. [7] studied the effect of zinc toxicity on biochemical composition of muscle and liver of murrel (*Channa punctatus*). Selected specimens of murrel were exposed to a sub lethal zinc concentration. They reported that the zinc exposure produced marked changes in the chemical composition of liver and muscle tissues. The metabolism of the fish decreased with the time of exposure and there was a decline in the calorific value of lipid, protein, and glycogen in muscle and liver.

Khunyakari et al. [8] investigated toxicity of nickel, copper and zinc in *Poecilia reticulata*. Heavy metal exposure caused increased mucus like secretion over gills, excessive excretion, anorexia and increased fin movement. Copper was found to be the most toxic followed by zinc and nickel.

Bieniarz et al. [9] examined reproduction of fish in conditions disadvantageously altered with the salts of zinc and copper. They report that elevated Zn levels in water affected the reproduction of fish. Khangarot [10] found that *Poecilia reticulata* was particularly resistant to zinc.

Pierson [11] studied effects of chronic zinc exposure on the growth, sexual maturity, reproduction and bioaccumulation of the guppy, *Poecilia reticulata.* The zinc burden increased from 56 to 70 days during the

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onset of pregnancy. The experiments show that uptake of zinc during logarithmic growth and pregnancy indicates that females actively transfer zinc to the embryo. When guppies exposed to 0.607 mg/l zinc for 134 day, the wet weight of females was reduced by % 40.

Uviovo and Beatty [12] investigated effects of chronic exposure to zinc on reproduction in the guppy (*Poecilia reticulata*). They concluded that zinc had no effect on brood time, but the total number of young produced and brood size fell with increasing zinc concentrations (0.36-1.7 g Zn/l); dead young with abnormalities were not included in brood size and live young were significantly smaller in the presence of zinc. According to the results this change in size of young at birth provides a very sensitive parameter of zinc toxicity.

Crandal and Goodnigth [13] examined effects of sub lethal concentrations of several toxicants on growth of the common guppy *Lebistes reticulatus*. They used 2 ppm lead nitrate, 5 ppm hydrated zinc sulphate and 0.5 ppm sodium pentachlorophenate in their experiment. They found that with all three toxicants growth was retorted, mortality was increased and sexual maturity was delayed.

The toxic characteristics of zinc depend largely on the physicochemical characteristics of water. The principle factors effecting toxicity are the hardness and the pH of water. Decreasing hardness and the increasing pH increases the lethality of dissolved zinc [14-16].

It has also been established that there are marked differences in zinc sensitivity between various species. For instance the order *Perciformes* was found to be the most resistant and *Clupeiformes* the most sensitive [17]. Salmonoid fishes were found to be highly susceptible to zinc intoxication in soft water and the 96-hr LC_{50} value for the rainbow trout was found to be 0.066 mg/l [16]. However this value goes up to 2.5 to 4.71 mg/l in very hard water [18].

Non-salmonoid fis on the other hand are known to be less sensitive than salmonoids. 96-h LC_{50} for common carp in soft water was found to be 3.12 mg/l [19]. Seven day LC_{50} values of fish species such as *Gobio gobio*, *Abramis brama*, *Rutilus rutilus*, *Cyprius carpio*, *Scardinus erytrophtalmus* and *Perca fluviatilis* were found to range between 8 to 17 mg/l [20].

This study investigates the toxic effects of zinc sulphate on the standard test species, guppy (*Poecilia reticulata*) by the determination of 96-hour LC_{50} values and evaluates behavioral disorders of the guppy exposure to different concentration of the toxicant.

2. MATERIALS AND METHODS

This study was carried out in the Zoology Research Laboratories of Department of Biology, Gazi Faculty of Education, Gazi University. Male adult guppies were obtained from a local fish breeder in Ankara and brought to the laboratory within 30 minutes in plastic bags with sufficient air. The plastic bags were placed into the maintenance aquarium for 30-35 minutes for acclimatization. Then the bags were cut open and the fish were allowed to swim into the aquarium water. Test chambers were glass aquaria of about 25 liter capacity.

The aquaria were aerated with a central system for a period of 48 hours and the fish were exposed to 15 days conditioning period at room temperature. The fish were fed with commercial pelleted food at least once a day during this period. Acclimated fish were not fed 24-h before the start of the tests. Care was taken to keep the mortality rate of fish not more than 5% in the last four days before the experiment was started.

Zinc sulphate (ZnSO4.H2O, Merck) in tap water was used in the static bioassays. The test organisms were subjected to different concentrations of the zinc sulphate (ZnSO4.H2O). For the acute bioassay tests, 10 fish were used per concentration. The containers were not aerated at the dosing time. The amount of zinc sulphate to be added in each aquarium was calculated after the volume of each aquarium was accurately determined.

There was a simultaneous control group together with the actual experiments. The control group was kept in experimental water without adding the zinc sulphate; keeping all other conditions constant. The mortality rate in the control group did not exceed 10% and 90% of the fish looked healthy throughout the experiment.

The experiment was carried out in two series and a total of 220 Poecilia reticulata were used. This species was selected for static bioassays because it can be easily cultured and raised under laboratory conditions through a complete life cycle, and it is one of the standard test species used for laboratory toxicity studies [21, 22].

Water quality parameters (temperature, dissolved oxygen (DO), $CaCO_3$ hardness and pH) using in the aquaria were periodically determined before the bioassay tests. The water temperature was kept 24 ± 10C. Also the experimental medium was aerated in order to keep the amount of oxygen not less than 4 mg/l.

All experiments were carried out for a period of 96 hours. The number of dead fish were counted every 12 hours and removed from the aquaria as soon as possible. The mortality rate was determined at the end of the 96th hour. No food was given to the fish during the experiments. The behavioral changes of the healthy fish and the fish exposed to various doses of zinc sulphate were photographed and evaluated as regard to behavioral anomalies [21].

The experiments were carried out with static acute experimental method. In this method the experimental solution and the samples (i.e. fish) are put in a suitable experimental cell (i.e. aquarium) and kept like that for a certain period. Since the decreased amount of oxygen and increased metabolic waste become a problem in long term experiments, the duration of such experiments are usually kept at 96 hours or less [21]. The bioassay system was as described in standardized methods [22, 23].

In this study the acute toxic effect of zinc sulphate on the standard test species, guppy (Poecilia reticulata Pallas, 1859) was determined by the use of Finney's Probit Analysis LC50 Determination Method [24]. The computer analyses were carried on with LC50 1.00 software developed by E.P.A. [25]. The data were also evaluated according to 'Behrens–Karber method' by the use of the following formula [26].

$$LC_{50} = LC_{100} - \frac{ab + ... + ab}{n}$$

In the above mentioned formula, LC50 and LC100 indicate the lethal doses for the 50% and 100% of the samples. Value "a" gives the difference between the two consecutive doses, "b" the arithmetic mean of the mortality caused by two consecutive doses, and "n" the number of samples in each group.

3. RESULTS

Table 1 shows the temperature, hardness, dissolved oxygen (DO) and pH of the aquaria in zinc sulphate toxicity tests. Table 2 shows the relation between the zinc sulphate concentration and the mortality rate of *Poecilia reticulata*, Pallas, 1859. The results obtained from acute static 96-hour toxicity experiments of zinc sulphate for guppy and estimated LC_{50} values with confidence limits are listed in Table 3.

Table 1. The temperature, hardness, oxygen content (DO) and pH of the aquaria in zinc sulphate toxicity tests.

Concentration (mg/l)	Temperature (°C)	pH	Hardness (mg CaCO3/l)	Dissolved oxygen (DO) (mg/l)
Control group	24.0	7,83	225	6,5
28.5	23.9	7.80	205	5.7
29.0	24.2	7.85	225	6.0
29.5	24.1	7.85	240	6.4
30.0	24.3	7.70	215	6.0
30.5	25.0	7.88	235	5.8
31.0	24.3	7.80	220	6.4
31.5	24.5	7.56	215	5.8
32.0	25.0	7.79	225	5.6
32.5	24.7	7.85	200	5.2
33.0	24.6	7.31	215	6.2

Table 2. The relation between the zinc sulphate concentration and the mortality rate of *Poecilia reticulata*, Pallas, 1859.

Concentration (mg/l)	Number of exposed fish	Number of dead fish	Death in the bioassay	Expected death	Estimated death
28.5	10	0	0.0000	0.0000	0.0484
29.0	10	2	0.2000	0.2000	0.0981
29.5	10	2	0.2000	0.2000	0.1760
30.0	10	3	0.3000	0.3000	0.2826
30.5	10	3	0.3000	0.3000	0.4109
31.0	10	5	0.5000	0.5000	0.5474
31.5	10	7	0.7000	0.7000	0.6764
32.0	10	8	0.8000	0.8000	0.7855
32.5	10	8	0.8000	0.8000	0.8685
33.0	10	10	1.0000	1.0000	0.9254

Chi - Square Heterogeneity (calculated) = 3.653
Chi - Square Heterogeneity calculated)
(tabular value at 0.05 level = 15.507
Mu = 1.488920
Sigma = 0.020518

Parameter	Estimate	Std. Err.	95% Confidence Limits
Intercept	-67.564964	12.555664	(-92.174065, -42.955864)
Slope	48.736649	8.434407	(32.205208, 65.268089)

Theoretical Spontaneous Response Rate = 0.0000

Table 3. Estimated LC values and confidence limits.

Point	Concentration (mg/l)	95 % Confidence Limits	
		Lower	Upper
LC 1.00	27.618	26.051	28.460
LC 5.00	28.521	27.327	29.180
LC 10.00	29.015	28.022	29.581
LC 15.00	29.353	28.495	29.862
LC 50.00	30.826	30.387	31.280
LC 85.00	32.373	31.812	33.375
LC 90.00	32.750	32.113	33.939
LC 95.00	33.317	32.553	34.804
LC 99.00	34.407	33.375	36.509

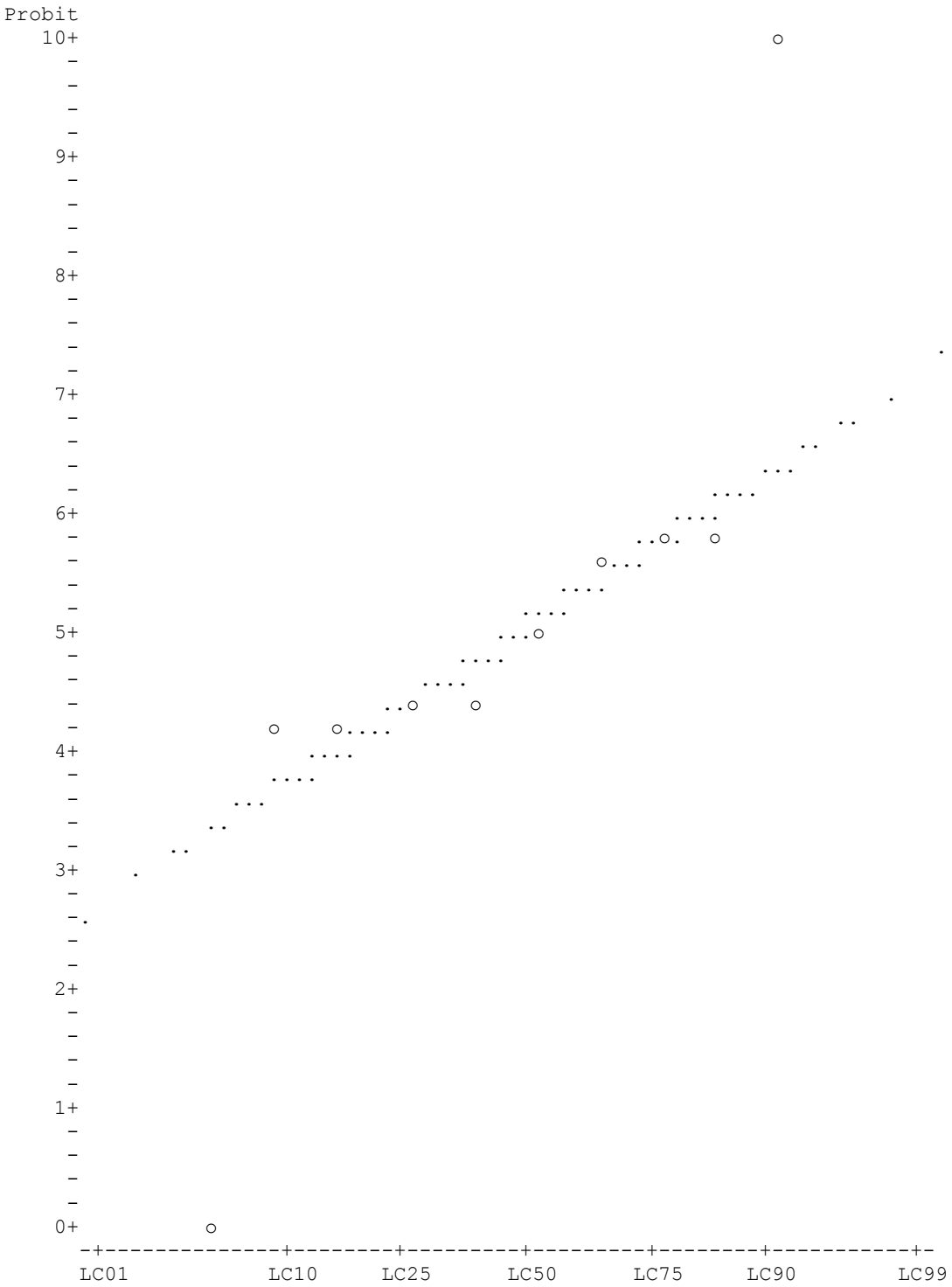

Figure 1. Plot of adjusted probits and predicted regression line.

The mean LC50 value of zinc sulphate on guppy individuals was found to be 30.826 mg/l by the use of E.P.A computer program based on Finney's Probit Analysis Method. This value was estimated to be 30.9 mg/l with the Behrens-Karber's method. Figure 1 shows the plot of Finney's adjusted probits and LC50 results.

Behavioral changes are the most sensitive indication of potential toxic effects. Optomotor responses are very useful in evaluating of the behavioral changes of fish [27].

The behavioral changes of Poecilia reticulata exposed to various concentrations of zinc sulphate at ppm level are as follows:

Control group: There were no behavioral changes and deaths observed throughout the experiment. The theoretical spontaneous response was zero.

Experiment groups: There were vertical and downward swimming patterns and sudden movements. The motion of fish became extremely slow and they displayed behavioral anomalies such as capsizing in water and loss of balance. Finally the fish sank down to the bottom and became motionless.

4. DISCUSSION AND CONCLUSION

There were two different methods of data evaluation employed for acute the toxicity response. Our results were similar for the two methods tested. Finney's Probit Analysis gave 96- hour LC50 value for the guppy (Poecilia reticulata, Pallas, 1859) exposed to different zinc sulphate concentrations as 30.8 mg/l. This value was estimated to be 30.9 mg/l with Behrens-Karber's method. Control mortality was zero. 95% lower and upper confidence limits for the LC50 values were 30.4 and 31.3 mg/l respectively. The behavioral changes observed in fish were, swimming in imbalanced manner, vertical and downward swimming patterns, capsizing, slowness in motion, sinking down to bottom. These toxic effects increased, as the dose was increased.

Khangarot found that 96-h LC50 value for Poecilia reticulata in hard water (260 mg $CaCO_3$ / l) as 55 mg/l [10]. This result is a little bit higher than ours. The difference may be due to variations in the hardness, pH value and the temperature of water.

Spehar [28], investigated cadmium and zinc toxicity to flagfish, Jardanelle floridae. The 96-hr LC5 values for cadmium and zinc to juvenile flagfish were 2.5 mg/l and 1.5 mg/l respectively.

Williams and Holdway [29], examined that the effects of pulse-exposed cadmium and zinc on embryo hatchability larval development and survival of Australian crimson spotted rainbow fish (Melanotaenia fluviatis). The LC50 values of zinc were found to be 0.51, 0.56 and 1.57 mg/l for 24-h, 3-4-day, and 9-10-day-old larval rainbow fish. In addition, their results show that pulse-exposed metals at moderate concentrations can significantly affect the early the life stages of Melanotaenia fluviatis through decreasing percentage hatch of embryos, inducing spinal deformities and having toxic effects on larvae at relatively low concentrations.

Gomez et al. [30], studied zinc toxicity in the fish Cnesteron decemmaculatus in the Parana River and Rio de La Plata Estuary. They found 24-h LC50 93.2 mg/l for Cnesteron decemmaculatus and contaminant load in the natural waters tested was similar at both sites, with Zn concentration 40 and 44 mg/l respectively.

Herrera et al [31], reported the 96-hr LC50 value of ZnCl2 on Chanos chanos as 25 mg/l. Finlayson and Verrue [32], investigated toxicities of copper, zinc and cadmium mixture to juvenile chinook salmon. They found that median lethal concentrations during 4 days (96-hour LC50 values) were most variable for zinc (39 to 122 mg/l).

Khangarot et al [33], examined toxicity of interaction zinc-nickel, copper-nickel and zinc-nickel-copper to a freshwater teleost, Lebistes reticulatus. The 48-hour median lethal concentrations (LC50) for individual salts were 75 mg/l for Zn^{2+}, 37 mg/l for Ni^{2+} and 2.5 mg/l for Cu^{2+}. Their experiment showed that in the Zn^{2+} – Ni^{2+} mixture, when Ni^{2+} was more in proportion, the toxicity was more than additive. The results indicate that heavy metallic mixtures would pose a greater toxicological danger to fish than the respective individual metals.

Acute and chronic effects of zinc have been widely described for different aquatic organisms and exposure routes. Since this metal is an important constituent in municipal wastes discharged into freshwater and marine, the results obtained in this study clearly reveal the fact that it is necessary to control the use of some heavy metal such as zinc. Further work with toxicity testing methods directly on fish will be very useful in assessing possible ecological risk of heavy metals.

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