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Acute Toxicity of Zinc and Copper Singly and in Combination to the Bluegill (*Lepomis macrochirus*)

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Zinc and copper are common components of aquatic effluents from various industrial and mining sources. The acute toxicity of various compounds of Cu and Zn has been determined for many species of fish using continuous flow as well as static toxicity tests under different chemical, temperature, and temporal regimes (TRAMA 1954, PICKERING & HENDERSON 1966, BALL 1967, PATRICK et al. 1968, BRUNGS et al. 1973, EISLER & HENNEKEY 1977, BENOIT & HOLCOMBE 1978, CAIRNS et al. 1978). Similarly, the toxicity of mixtures of these two, as well as of more complex mixtures of heavy metals, has been studied by many researchers (LLOYD 1961, SPRAGUE 1964, SPRAGUE & RAMSAY 1965, BROWN & DALTON 1970, EISLER & GARDNER 1973, ANDERSON & WEBER 1975, MARKING 1977, MUSKA & WEBER 1977, LEWIS 1978, FINLAYSON & ASHUCKIAN 1979.) While many of these studies report the toxicity of Cu and/or Zn to the bluegill (Lepomis macrochirus), to our knowledge the literature does not mention the acute toxicity of mixtures of these two metals to this species. The present study was undertaken to investigate the acute toxicity of divalent Zn-Cu mixtures to the bluegill.

METHODS

Proportional Diluter. Six, continuous flow 96-h toxicity tests were conducted using a solenoid valve controlled proportional diluter system similar to that described by PELTIER (1978). In this system, six bioassay chambers of 6.4-L capacity received 233 mL of solution every 3 min and provided a flow rate of 4.66 L/h (Fig. 1). This was equivalent to 17.5 chamber volumes during each 24-h period.

The head boxes, proportioning boxes, mixing chambers, and fish chambers were constructed of 0.64-cm plexiglass with methylene chloride fused joints (Fig. 1). Delivery tubes and standpipes were polytetrafluoroethylene, and fittings were glass. The toxicant reservoir was polyethylene.

Toxicant solutions were delivered to the mixing head box by a variable speed peristaltic pump (Fig. 1). The concentration of the toxicant in the bioassay chambers could be controlled between tests by one or a combination of four methods. To change toxicant concentrations between tests, the toxicant concentration could be changed; the diluent or toxicant flow rates could be changed; or

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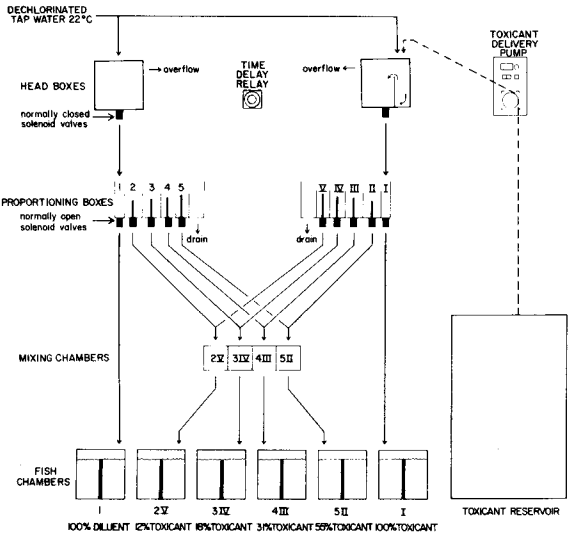

Figure 1. The solenoid controlled, continuous flow, proportional diluter. The time delay relay simultaneously opens the valves on the head boxes and closes those on the proportioning boxes allowing the proportioning boxes to fill with diluent and toxicant solutions. When the level of the liquid in the diluent proportioning box reaches the top in compartment 5, a float valve is tripped and the solenoid valves are returned to their normal state, delivering toxicant and diluent first to the mixing chambers and ultimately to the fish chambers. This restarts the time delay relay, and the cycle is repeated.

the diluent:toxicant ratio could be changed by adjusting the standpipe height in the proportioning boxes (Fig. 1).

Water Characteristics. The diluent water was dechlorinated tap water. Except for the first Cu test, temperature was maintained at 22 $\pm$ 1°C. During test 3, a malfunction of the temperature controller resulted in temperatures of 19 $\pm$ 2.5°C. Temperature of the diluent water was monitored continuously.

Dissolved oxygen, conductivity, pH, alkalinity, and total hardness were measured in each of the six bioassay chambers at the start of each toxicant test and once during each subsequent 24-h period for the duration of the test. Consequently, five measurements were taken from each chamber during each test.

Dissolved oxygen (mg/L), conductivity (micromhos/cm), and pH were measured electronically. Alkalinity and total hardness were determined titrimetrically according to methods described by AMERICAN PUBLIC HEALTH ASSOCIATION et al. (1976).

Samples for determination of the toxicant concentration were taken according to the above schedule. These were acidified with concentrated nitric acid, sealed with parafilm, and held until the end of a test before analysis. However, the average of the five samples from each bioassay chamber was used for calculation of LC50 values. The toxicant concentrations, as mg/L of metal ion, were determined by atomic absorption spectrophotometry.

The dissolved oxygen concentration was maintained between 6.6 and 9.5 mg/L in all bioassay chambers throughout the testing periods. Conductivity ranged from 106 to 136 micromhos/cm, pH from 6.8 to 7.5, alkalinity from 23.2 to 32.8 mg/L, and total hardness from 21.2 to 59.2 mg/L.

Test Specimens and Conditions. All test specimens were obtained from the same commercial source. The average standard length of the test fish for all six toxicity tests was 49 mm (range, 32 - 67 mm). The variation in size within any test did not exceed the average for that test $\pm$ 25% (average = $\pm$ 18%).

Fish were acclimated for at least 2 weeks in 450-L holding tanks prior to testing. Water in the holding tanks was from the same source as the diluent water in the proportional diluter. Continuous light was maintained in the holding tanks and the diluter. All fish were free of any visible disease (mortality rate = 0) for at least 2 weeks before testing.

Toxicant Solutions. Toxicant solutions were prepared by dissolving $ZnSO_4 \cdot 7H_2O$ and/or $CuCl_2 \cdot 2H_2O$ in distilled water. The toxicity ratio of Zn to Cu in the tests for joint toxicity was 1:1.

Test Protocol. Ten fish were placed in each of the bioassay chambers at the start of each test. The number of individuals surviving in each chamber was recorded after 1, 2, 4, 8, 12, and 24 h and every 24 h thereafter. Death was determined as the loss of equilibrium. Fish that could not respond to prodding with a blunt rod by righting themselves were considered dead.

Calculations. Probit analysis as described by FINNEY (1971) was performed to determine 96-h LC50s and their 95% confidence limits. A computer program available on SAS76 was used to carry out this analysis (BARR et al. 1976).

Two toxicity tests were run with each toxicant solution. Toxicant concentrations were adjusted slightly for the second test to allow for better resolution at concentrations near the median lethal concentration since probit analyses are more meaningful with mortalities other than 100% or 0%. The results of successive tests with the same toxicants were in fairly close agreement (Table 1). Consequently, the results of tests using the same toxicant were pooled to increase sample size, and the probit analysis was run on the pooled data.

TABLE 1. Results of toxicity tests.

Test number	Tank number	average concentration (mg/L) Zn^{++}	average concentration (mg/L) Cu^{++}	# Surviving (of 10)	LC50 (mg/L)	95% confidence limits
1 (Zinc)	1 (Control)	0.02	-	10	3.6	3.0 - 4.6
	2V	2.56	-	9		
	3IV	3.64	-	5		
	4III	6.22	-	0		
	5II	10.90	-	0		
	I	23.24	-	0		
2 (Zinc)	1 (Control)	0.07	-	10	3.0	-[a]
	2V	1.24	-	7		
	3IV	2.12	-	7		
	4III	3.54	-	8		
	5II	5.70	-	1		
	I	9.94	-	0		
3 (Copper)	1 (Control)	-	0.04	10	1.1[b]	-
	2V	-	0.33	10		
	3IV	-	0.47	10		
	4III	-	0.83	10		
	5II	-	1.40	0		
	I	-	2.42	0		
4 (Copper)	1 (Control)	-	0.00	10	0.9	0.7 - 1.2
	2V	-	0.49	9		
	3IV	-	0.73	7		
	4III	-	1.29	3		
	5II	-	2.26	0		
	I	-	4.00	0		
5 (Mix)	1 (Control)	0.03	0.00	10	Zn=1.2[b]	-
	2V	0.39	0.11	10	Cu=0.3	
	3IV	0.57	0.16	10		
	4III	1.18	0.30	5		
	5II	1.80	0.49	0		
	I	3.14	0.92	0		
6 (Mix)	1 (Control)	0.04	0.01	10	Zn=1.9	1.4 - 2.9
	2V	0.43	0.13	10	Cu=0.5	0.4 - 0.8
	3IV	0.62	0.19	9		
	4III	1.20	0.35	6[c]		
	5II	1.96	0.53	8		
	I	3.68	0.98	0		

[a]Critical limits not calculated.

[b]There must be at least two responses that are less than 100% and greater than 0% for probit calculations. LC50 estimated from plot on log-probit paper.

[c]Only nine fish at start.

Joint Toxicity. Since organisms may be more sensitive to some toxicants than to others, a method of equating the toxicity of the various components of a mixture was needed. SPRAGUE (1973) recommended that the concentration of a toxicant in a mixture be reported as a proportion of the LC50 determined singly. In this method, the sum of the ratios for all components of a toxicant mixture is determined. If this total is 1.0, the mixture is considered additive. A total less than 1.0 is considered synergistic, while one greater than 1.0 is considered antagonistic. MARKING (1977) used this concept as a basis for the development of a linear additive index in which values equalling 0 indicate additive toxicity, positive values indicate synergistic mixtures, and

negative values indicate antagonistic mixtures. Marking's additive index (MAI) has the advantage of providing a method for establishing critical limits for values that are near 0.

MAI can be calculated using the equation

$$\frac{A_m}{A_i} + \frac{B_m}{B_i} = S$$

where A_m = LC50 for Zn in mixture, A_i = LC50 for Zn individually, B_m = LC50 for Cu in mixture, B_i = LC50 for Cu individually, and S = the sum of the biological effects. Then

$$MAI = (1/S) - 1.0 \text{ if } S \leq 1.0 \text{ and}$$

$$MAI = 1.0 - S \text{ if } S \geq 1.0.$$

The significance of the deviation from 0 can be determined by substituting values from the 95% confidence intervals for the LC50 values in the formula for MAI to establish a range for MAI. If the range includes 0, the deviation is not considered significant. The values that yield the greatest deviation from MAI are used to establish the range.

RESULTS

Survival of fish and toxicant concentrations are indicated in Table 1. The LC50 value calculated from the combined tests for Zn was 3.2 mg/L with 95% confidence limits (C.L.) of 2.1 - 4.6 mg/L. Combining the results from tests 3 and 4 produced an LC50 of 1.0 mg/L for Cu (C.L. = 0.85 - 1.2 mg/L). Tests 5 and 6 (Zn and Cu) gave an LC50 of 1.4 mg/L for Zn (C.L. = 1.0 - 2.0 mg/L) and one of 0.4 mg/L for Cu (C.L. = 0.3 - 0.6 mg/L). The MAI was determined to be +0.218 (C.L. = -0.64 and +1.30).

DISCUSSION

Differential sensitivity to toxicants makes interspecific comparisons difficult, even if test conditions are similar. The chemical composition and temperature of diluent water can have a definite effect on the toxicity of heavy metals (MOUNT 1965, CAIRNS et al. 1978). However, temperature has been shown to have relatively little effect on the toxicity of Zn or Cu to the bluegill within the range of 13 - 28°C (CAIRNS et al. 1975). Total hardness, on the other hand, has a definite effect on the toxicity of these metals to this species (CAIRNS & SCHEIER 1957a, PICKERING & HENDERSON 1966) and is usually cited as one of the most important chemical factors affecting heavy metal toxicity.

The acute toxicity of Zn to bluegills under conditions similar to those in our study (temperature 19 - 23°C, 26 mg/L total hardness) has been reported by others. All the studies used either $ZnSO_4$ or $ZnCl_2$. PICKERING & HENDERSON (1966) reported

96-h LC50 values of 4.9 (C.L. = 3.6 - 6.3), 5.4 (C.L. = 3.8 - 7.0), 5.5 (C.L. = 4.3 - 6.9), and 5.8 mg/L (C.L. = 4.6 - 7.5) in soft water at 25°C. CAIRNS & SCHEIER (1957a) reported values between 2.9 and 3.8 mg/L in soft water at 18°C and 1.9 - 3.6 mg/L at 30°C. The work of CAIRNS & SCHEIER (1957b) resulted in the highest reported 96-h median lethal concentration (8.0 mg/L) for these conditions. Additionally a 24-h LC50 of 8.9 mg/L in soft water at 30°C was reported by CAIRNS et al. (1978). The 24-h LC50 for Zn was calculated from the data of the present study to be 6.8 mg/L (C.L. = 5.9 - 8.0).

The acute toxicity of Cu to bluegills under similar conditions has also been reported using $CuSO_4$ or $CuCl_2$ as a source. PICKERING & HENDERSON (1966) reported a 96-h LC50 of 0.7 mg/L (C.L. = 0.5 - 0.9) at 25°C. TRAMA (1954) reported a value of 0.7 mg/L at 20°C; PATRICK et al. (1968) reported one of 1.3 mg/L at 18°C; and BENOIT (1975) reported one of 1.1 mg/L at 20°C.

Although the acute toxicity of Zn-Cu mixtures to bluegills has not been reported, several reports exist which deal with the toxicity of such mixtures to other species, primarily salmonids. SPRAGUE (1964) reported synergistic effects for this mixture using the Atlantic Salmon (Salmo salar). SPRAGUE & RAMSAY (1965) worked with the same species and found that at lower concentrations this mixture displayed additive toxicity, while at higher concentrations the toxicities were synergistic. LLOYD (1961) obtained similar results with rainbow trout (S. gairdneri). In soft water at low concentrations the joint toxicity was additive, and at high concentrations synergistic results were obtained. When Lloyd repeated his tests in hard water, the joint toxicity was additive at all concentrations. SELLERS et al. (1975) noted possible synergistic effects while studying the effects of a Zn-Cu mixture on the ventilatory responses of rainbow trout.

Other workers have studied the acute joint toxicity of Zn and Cu to various other freshwater fishes. LEWIS (1976) reported that toxicity was synergistic to a cyprinid (Agosia chrysogaster) in hard water. ANDERSON & WEBER (1975) reported that the acute toxicity of Zn-Cu mixtures were supra-additive (synergistic) to male guppies, Poecilia reticulata (Poeciliidae), in water of intermediate hardness (125 mg/L).

No absolute median lethal concentration for either Zn or Cu can be obtained, even when "similar" conditions are observed. Factors such as genetic variability, specimen size, behavior, type of toxicity test, chemical variability, the method of measuring the toxicant concentration, and the method of determining the LC50 could all contribute to the variability found in the literature. The 96-h median lethal concentrations for Zn and Cu of 3.2 and 1.0 mg/L were well within the limits of the various values reported by others.

The value of the MAI calculated from the results of this study was +0.218 with a range from -0.64 to +1.30. Since 0 is

contained within these limits, the toxicity of Zn-Cu mixtures to bluegills was additive under the conditions of these tests.

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