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Effects of Calcium Addition as $Ca(NO_3)_2$ on Zinc Toxicity to Fathead Minnows, *Pimephales promelas*, Rafinesque

Robert D. Judy, Jr.[1] and Patrick H. Davies[2]

[1]*Department of Biological Sciences, North Texas State University, Denton, Tex. 76203, and*
[2]*Colorado Division of Wildlife, Fishery Research Center, P. O. Box 2287, Ft. Collins, Colo. 80522*

Zinc enters aquatic ecosystems as a by-product of many industrial and mining processes (USEPA 1976, VAN LOON & BEAMISH 1977). Zinc toxicity has been determined in many freshwater organisms (CAIRNS et al. 1976, PATRICK et al. 1968). Since Zn^{++} toxicity is greatly affected by pH, alkalinity, total hardness and temperature (MOUNT 1966), maximum acceptable toxicant concentration (MATC) values have been established by EPA as 0.01 *LC50 for the receiving water in question, as opposed to a fixed numerical value (USEPA 1976).

Previous investigators have used "natural" soft and hard waters for both static and flow through bioassays. The actual antagonism of Ca^{++} and Zn^{++} toxicity has received little research attention. Although a variety of values exist for Zn^{++} toxicity related to pH, total hardness and alkalinity (USEPA 1976), controlled research with a standard "synthetic" water has not been conducted (CAIRNS 1969).

Calcium is a naturally-occurring element in the aquatic system contributing to hardness and alkalinity (COLE 1975, WETZEL 1975). Variations in water chemistry primarily result from differing levels of Ca^{++} and Mg^{++} ions. The role Ca^{++} plays in inhibition of Zn^{++} toxicity was examined in this research.

MATERIALS AND METHODS

Fathead minnows, Pimephales promelas, 1-2 g weight and 5.0-6.5 cm total length, were used as the test organism (ALDEMAN & SMITH 1976). Standard acute bioassay procedures were employed (SPRAGUE 1973, STANDARD METHODS 1971) except that each test tank was aerated.

Three separate experiments were conducted. Water chemistry parameters (Ca^{++}, Zn^{++}, Mg^{++}, alkalinity, dissolved oxygen, and pH) were measured at the beginning and end of each experiment. Ca^{++}, Zn^{++}, and Mg^{++} levels were monitored using a Perkin-Elmer 303 Flame Atomic Absorption Spectrophotometer (PE 303 FAAS). Alkalinity was measured by standard titration technique, and dissolved

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oxygen (D.O.) was monitored using the Azide modification of the Winkler titration (STANDARD METHODS 1971). Determinations of pH were made with a Corning Model 12 Research pH meter.

Zinc was added as $ZnSO_4 \cdot 7HOH$ to all test tanks except controls using a non-acidified 1000 mg/L Zn^{++} stock solution. Calcium was added as $Ca(NO_3)_2$ in experiments 2 and 3 using a 10,000 mg/L Ca^{++} stock solution. Fish were fed sulfa marizine feed for 10 days while acclimating for 2 weeks at 20 ± 1^{o}C. Fish were not fed the 2 days immediately prior to testing. Fish were randomly selected from a stock group and placed into 3-L test jars with aeration. Dead fish were removed at 1, 2, 4, 8, 12, and every additional 12 h for a total of 96 h. Mortality data were plotted as percent survival at 96 h for all experiments.

Part 1. Zn^{++} was added at 20.0, 10.0, 5.0, 2.0, 1.0, 0.5, and 0.0 mg/L. Mortality data were plotted on log-probit paper as mean percent dead at 96 h and the LC50 was calculated by the Litchfield-Wilcoxin Nomograph Analysis (LITCHFIELD & WILCOXIN 1949).

Part 2. The second experiment focused on the broad affects of Ca^{++} as $Ca(NO_3)_2$ to nominal 6.0 mg/L Zn^{++} solutions. A Zn^{++} level greater than the determined LC50 value was used to insure significant mortality. Nominal solutions of 40 (soft tap), 100, 150, 200, 250, and 300 mg/L Ca^{++} were made. Zinc was added at a constant 6.0 mg/L (nominal) level to all but the control tanks. Group A tanks had a control with filtered, soft, tap water. Group B tanks had a control with the hightest level of Ca^{++} ion used to monitor any mortality resulting from the presence of high nitrate.

Part 3. The third experiment was designed to determine the definitive Ca^{++} levels needed to reduce mortality. Each of the seven test tanks had varying levels of Ca^{++} as before, except at closer intervals. Calcium was added in 25 mg/L increments. Again Group A had the soft, tap water control and Group B had the high Ca^{++} control. Zn^{++} and Ca^{++} levels were monitored daily. Mean mortality values for all tests were calculated. Student's t-test was used to analyze the data.

RESULTS

Part 1. Mean Zn^{++} levels measured are given in Table 1. Water quality characteristics during the experiment were: alkalinity--44 ± 10 mg/L, hardness--45 ± 7 mg/L, pH--7.0 ± 7.4, D.O.--6.0 ± 0.0 mg/L, temperature 21 ± 1^{o}C, and specific conductance 160 ± 10 μmhos/cm.

TABLE 1

Analysis of Zn^{++} and Water Chemistry in Acute Bioassay of *P. promelas*

Zn^{++} Levels -- PE 303 FAAS

Nominal	9/16/77 Group A	9/16/77 Group B	9/20/77 Group A	9/20/77 Group B	$\bar{X}$ 96 h	% Mortality 96 h $\bar{X}$
20.0 mg/L	18.0	18.2	18.0	17.6	18.1	100.0
10.0 "	8.4	8.2	7.2	6.6	8.3	100.0
5.0 "	4.2	3.6	2.6	2.4	3.2	40.0
2.0 "	1.7	1.7	1.5	1.5	1.6	20.0
1.0 "	1.0	1.0	1.0	1.0	1.0	5.0
0.5 "	0.4	0.4	0.4	0.4	0.4	0.0
0.0 "	0.0	0.0	0.0	0.0	0.0	0.0

A 96 h LC50 of 3.1 mg/L Zn^{++} was calculated by the Litchfield-Wilcoxin Nomograph Analysis. Confidence limits on the LC50 value extended from 4.3 mg/L to 2.2 mg/L. The confidence limits of the slope of 1.715 were 2.164 and 1.358. The toxicity of Zn^{++} to *P. promelas* was significant ($p \leq 0.05$). Initial mortalities did not occur before 8 h. Total length of dead fish ranged from 5.0 - 6.5 cm with a mean value of 5.86 cm.

Part 2. Alkalinity increased during this experiment, probably due to addition of CO_2 by aeration of the test tanks, and pH values were lower than those in Part 1. Dissolved oxygen remained at 6.0 mg/L at a water temperature of 20.5 $\pm$ 1°C. Addition of Ca^{++} as $Ca(NO_3)_2$ to constant Zn^{++} solutions inhibited Zn^{++} toxicity from 65% in soft tap water to 0.0% in 117.5 mg/L Ca^{++} (Fig. 1). Zinc levels decreased slightly over the 96 h period. (Table 2). Total hardness determinations (EDTA titration) were approximately double the added concentration of Ca^{++} due to what appears to be interference by NO_3^-. Consequently total hardness levels are not reported and Ca^{++} and Mg^{++} levels were determined by flame AAS.

TABLE 2

Chemical Parameters after Initial Ca^{++} Addition in Acute Bioassay of *P. promelas*.

Zn^{++} Concentration mg/L -- PE 303 FAAS

	Nominal	96 h $\bar{X}$ Group A	96 h $\bar{X}$ Group B
I	0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
II	6.0	3.9 $\pm$ 0.3	4.1 $\pm$ 0.2

III	6.0	4.4 ± 0.2	4.3 ± 0.3
IV	6.0	4.9 ± 0.3	4.6 ± 0.8
V	6.0	4.9 ± 0.2	5.0 ± 0.3
VI	6.0	5.0 ± 0.0	5.0 ± 0.0
VII	6.0	5.1 ± 0.0	5.0 ± 0.2

Ca^{++} Concentration mg/L -- PE 303 FAAS

	Nominal		96 h $\bar{X}$ of	96 h $\bar{X}$
	Group A	Group B	both groups	Mortality
I	10.0	300.0	Controls	0.0
II	40.0	40.0	9.8 ± 1.1	65.0
III	100.0	100.0	70.0 ± 7.1	25.0
IV	150.0	150.0	117.5 ± 10.6	0.0
V	200.0	200.0	165.0 ± 7.1	0.0
VI	250.0	250.0	215.0 ± 21.2	0.0
VII	300.0	300.0	247.5 ± 10.6	0.0

Mg^{++} levels identical for all test tanks at 2.8 mg/L.

	10/14	10/17
Alkalinity $\bar{X}$ ± SD	30.1 ± 2.3	47.6 ± 3.4
pH $\bar{X}$ ± SD	----------	7.5 ± 0.2

Part 3. Definitive inhibition of Zn^{++} toxicity by Ca^{++} addition was shown as a graded response of mortality in all test tanks. Toxicity of Zn^{++} to *P. promelas* decreased from 100% mortality in filtered, soft, tap water to 0.0% in 141.3 mg/L Ca^{++} (Fig. 2). Zn^{++} concentrations decreased slightly as before. Mortality was greatest in the lowest Zn^{++} solutions. Calcium levels dropped slightly. Alkalinity did not change, and pH values increased slightly during the test. Dissolved oxygen was 6.0 mg/L at a temperature of 20.5 ± 1 ^{o}C (Table 3). There was no size selected mortality in any of these tests.

TABLE 3

Definitive Chemical Parameters After Addition of Ca^{++} in Acute Bioassay of *P. promelas*

Zn^{++} Concentrations mg/L -- PE 303 FAAS

	Nominal		96 h $\bar{X}$	
	Group A	Group B	Group A	Group B
I	0.0	0.0	0.0 ± 0.0	0.0 ± 0.0
II	6.0	6.0	4.6 ± 0.6	4.6 ± 0.6
III	6.0	6.0	4.6 ± 0.5	4.6 ± 0.6

IV	6.0	6.0	4.9 ± 0.3	4.7 ± 0.6
V	6.0	6.0	4.9 ± 0.3	4.7 ± 0.6
VI	6.0	6.0	5.1 ± 0.1	4.8 ± 0.6
VII	6.0	6.0	5.0 ± 0.3	4.8 ± 0.5

Ca^{++} Concentrations mg/L -- PE 303 FAAS

	Nominal		96 h $\bar{X}$		96 h $\bar{X}$ Mortality %
	Group A	Group B	Group A	Group B	
I	12.9	139.0	11.0 ± 2.6	137.0 ± 2.8	0.0
II	12.9	13.0	11.0 ± 2.6	11.3 ± 2.5	100.0
III	39.0	40.0	35.5 ± 5.0	36.3 ± 5.3	60.0
IV	63.5	64.9	59.3 ± 6.0	61.5 ± 4.9	35.0
V	90.0	92.5	87.5 ± 3.5	89.3 ± 4.6	10.0
VI	114.0	115.0	111.0 ± 4.2	112.5 ± 3.5	2.5
VII	145.0	145.0	140.0 ± 7.1	142.5 ± 3.5	0.0

Mg^{++} concentrations identical in all tanks at 2.2 mg/L.

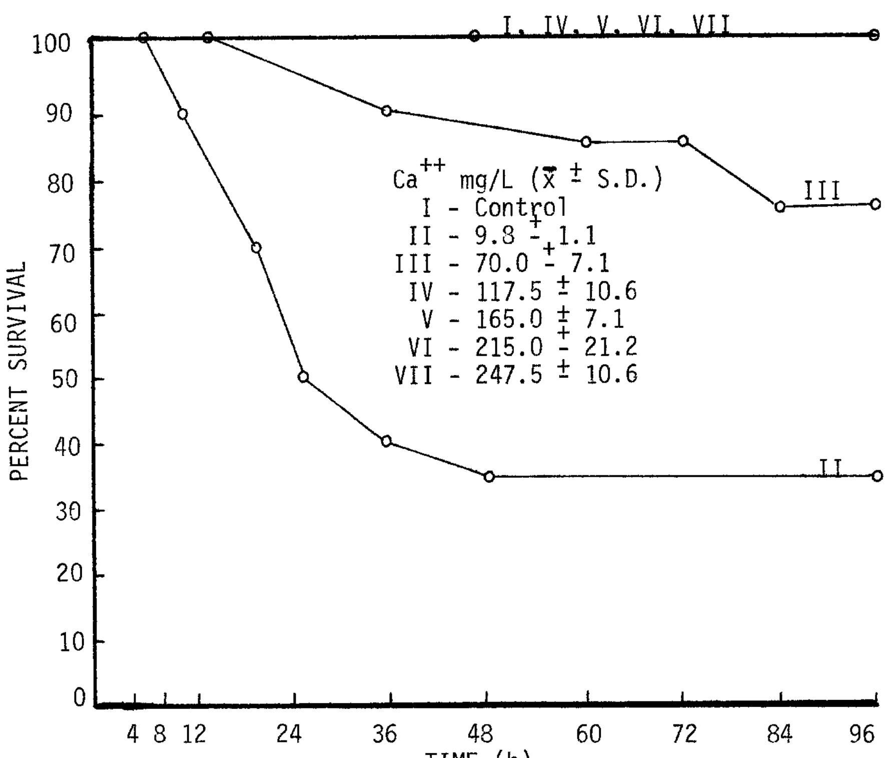

FIG. 1. Mean percent survival in Groups A and B showing broad range inhibition of Zn^{++} toxicity to *P. promelas* by Ca^{++}.

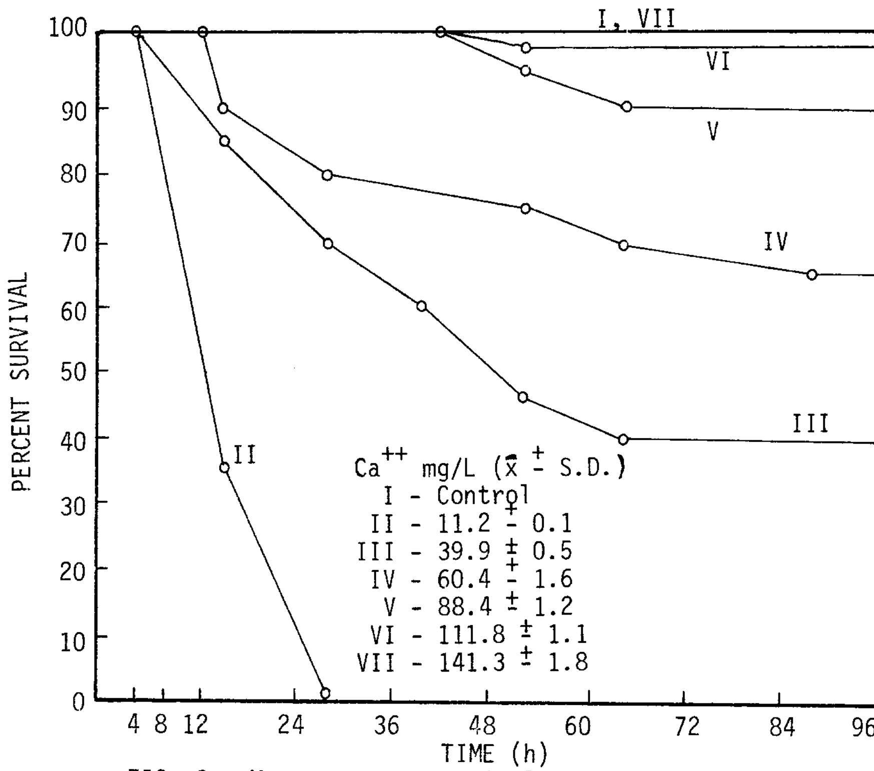

FIG. 2. Mean percent survival in Groups A and B showing definitive inhibition of Zn^{++} toxicity to *P. promelas* by Ca^{++}.

DISCUSSION

The 96 h LC50 of 3.1 mg/L Zn^{++}, significant at 95%, agrees closely with values published by CAIRNS and SCHEIER (1957) for acute 96 h bioassays in soft water. Tests were run in replicate and showed reproducibility as stressed by CAIRNS (1969). The significance of the initial addition of Ca^{++} to equal Zn^{++} solutions is evident in the mortality curves (t-test p = 0.05).

Water chemistry parameters remained fairly constant throughout the tests, in contrast to previous investigations (MOUNT 1966; PICKERING and HENDERSON 1966). Earlier investigators also added Ca^{++} as $CaCO_3$ in analyzing effects of hardness on Zn^{++} toxicity (PATRICK et al. 1968). Alkalinity values did change slightly in Part 2, possibly as a result of aeration, and this may have affected Zn^{++} toxicity. However, in Part 3 the alkalinity remained unchanged. Toxicity differences due to alkalinity changes are not considered important at this time. The 25 mg/L addition of Ca^{++} showed very definite inhibition of Zn^{++} toxicity. Student's t-test showed significance at 95% confidence interval for mean mortalities at all Ca^{++} concentrations.

The possibility of Zn^{++} precipitation was essentially eliminated by addition of Ca^{++} as $Ca(NO_3)_2$. Nitrate ions do not precipitate Zn^{++} out of solution. The two control tanks, Group A with soft, tap water and Group B with highest Ca^{++} and NO_3^- levels, showed no differences in mortality. Zinc toxicity was not increased by NO_3^- concentrations. Flame AAS analysis showed essentially no change in Zn^{++} levels in the test tanks. These experiments demonstrated conclusively that Ca^{++} in solution inhibits the toxicity of Zn^{++} to *P. promelas*. The physiological interactions of Ca^{++} and Zn^{++} have not been investigated, and long-term testing needs to be done on this problem. Water quality criteria for "safe" Zn^{++} levels are affected by the antagonistic influence of Ca^{++} to the toxicity of Zn^{++}.

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