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Toxicity of Nickel, Copper, Zinc and Aluminum Mixtures to the White Sucker (*Catostomus commersoni*)

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The initial purpose of this study was to investigate several water quality parameters as possible causes of a fish kill which occurred in an eastern freshwater creek in 1978. The dead fish were found just downstream of a point where the natural creek received the confluence of two industrial effluent streams. One of the effluent streams originated from a holding pond containing elevated metal levels, and it was believed that a malfunction in the pH adjustment unit was responsible for a temporary drop in pH, leading to increased solubility of metals leaving the pond. The other effluent stream was more alkaline, and the white precipitate found near the dead fish suggested that the metals had formed a hydroxide precipitate when the two streams combined. An examination of many potentially causative factors, both physical and chemical, revealed that the only unusual condition present at the time of the kill was the presence of elevated metal levels in the creek sediments and dead fish tissues.

Table 1 presents data on elevated metals found in the effluents during the week preceding the kill. The dead fish were found

TABLE 1

Water chemistry analyses at two sampling sites near the fish kill

Sample Location	Sample Date	pH	Ni	Cu	Zn	Al
Station #1 (Holding Pond Discharge)	Day 1*	8.3	0.2	<0.02	0.02	<1
	Day 2	6.5	2.1	0.03	0.18	<1
	Day 3	7.4	0.5	<0.02	0.02	<1
	Day 4	6.3	1.2	0.04	0.27	<1
	Day 5	4.7	2.4	0.47	0.76	14
	Day 8	6.5	1.1	<0.02	0.06	<1
Station #2 (Confluence of the two effluent streams)	Days 1-8**	--	0.3	0.09	0.20	15

* Daily composites; ** Weekly composite

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on the morning of day 8, and the low pH levels recorded at Station #1 on day 5 indicate that the kill was caused by conditions occurring on day 5 or soon afterwards. Station #2 was actually the closer of the two sampling stations to the location of the kill, but short-term conditions there may have been more extreme than data in Table 1 indicate, since the water sample from Station #2 was a weekly composite.

A review of the literature data on metal toxicity did not conclusively demonstrate that any one of the four metals shown in Table 1 need have been singly responsible for acute lethality to fish. Data on aluminum are very scarce but the metal may be considered much less toxic to most aquatic species than the other three metals. The highest levels of nickel, copper, and zinc were found during day 5 at Station #1; most of data in the literature suggest that 2.4 mg/l of nickel or 0.76 mg/l of zinc would not be acutely lethal under normal circumstances (REHWOLDT et al. 1971). The value of 0.47 mg/l for copper does overlap with ranges of some reported copper LC_{50} values for fish (GECKLER et al. 1976), but this in itself is not conclusive information since pH and hardness interactions can significantly reduce metal toxicity (CHAPMAN and MCCRADY 1977). Regarding the important remaining issue of mixed metal toxicity, there is not a great deal of information available in the literature but the existing data demonstrate a synergistic interaction among nickel, copper, and zinc (SPRAGUE and RAMSEY 1965). However, varying experimental methods of different investigators do not allow clear comparisons of their results to the levels and circumstances found at our site at the time of the kill incident.

MATERIALS AND METHODS

Two bioassay experiments were performed in order to determine whether the metal/pH mixtures found at the two sampling stations (Table 1) could explain acute mortality to fish. The nominal concentrations used in Experiment 1 were based on those found at Station #1 on day 5, reflecting the most biologically severe conditions found at that station during the week. The nominal concentrations used in Experiment 2 were based on those found in the weekly composite taken at Station #2. Table 2 shows the pH levels and nominal metal concentrations used in the two experiments, each of which consisted of six treatments (A-F). Each treatment in Experiment 1 corresponds to the same-lettered treatment in Experiment 2 in the sense that the same pH range and metal combination was used in both cases, although the actual concentrations of those added metals were different.

White suckers (Catostomus commersoni), an indigenous species, were obtained from a local commercial fish hatchery and were held in 200 gal. tanks at 19 ± 2 C. Holding and test water were the same to avoid any acclimation problems. A mixture of well water and reverse osmosis water yielding a hardness of 225 ppm as $CaCO_3$ was used as dilution water. Test fish weighed an average of 3.9 g and had an average length of 8.4 cm.

TABLE 2

Design of treatments used in two bioassay experiments

Experiment	Treatment	pH*	Metal Mixtures	Nominal Concentrations (mg/l) (respectively)
1	A	N	Control	0
	B	N	Ni, Cu, Zn, Al	2.4, 0.47, 0.76, 14
	C	N	Ni, Cu, Zn	2.4, 0.47, 0.76
	D	L	Control	0
	E	L	Ni, Cu, Zn, Al	2.4, 0.47, 0.76, 14
	F	L	Ni, Cu, Zn	2.4, 0.47, 0.76
2	A	N	Control	0
	B	N	Ni, Cu, Zn, Al	0.3, 0.09, 0.2, 15
	C	N	Ni, Cu, Zn	0.3, 0.09, 0.2
	D	L	Control	0
	E	L	Ni, Cu, Zn, Al	0.3, 0.09, 0.2, 15
	F	L	Ni, Cu, Zn	0.3, 0.09, 0.2

* N = normal pH (range 6.6-7.7); L = low pH (range 4.5-5.0)

In Experiment 1, triplicate tanks were used for each of the six treatments. Each tank consisted of a glass aquarium containing 4 gallons of test solution. Ten fish were randomly assigned to each tank, with a total of 30 fish per treatment. The test was performed under normal room lighting (approx. 70 ft c) with a photoperiod of 16 hrs, at a temperature of 20 $\pm$ 1 C. The low pH levels in treatments D, E, and F were obtained by adding nitric acid to the test solutions. Mortality was recorded at 24 and 48 hours.

In Experiment 2, methods were similar to those used in Experiment 1 except that mortality was observed at 24, 48, and 96 hours. Also, due to a limitation in the number of white suckers available, seven fish were used per tank with two replicate tanks comprising each treatment, for a total of 14 fish per treatment.

Physical parameters such as dissolved oxygen, pH, and water temperature were measured at the start of the test and every 24 hours. In order to compare nominal to measured concentrations of metals in solutions, water samples were taken from each test tank at the beginning and end of the test and analyzed for Cu, Ni, and Zn using atomic absorption spectrophotometry. Equipment needed for Al analysis was not available.

RESULTS

Measured metal concentrations were in close agreement with the desired nominal concentrations, except for copper levels in

Experiment 1 which were approximately 50% of nominal concentrations. This may have been caused by a rapid coagulation/precipitation phenomenon.

Table 3 and Figures 1 and 2 summarize results of the two bioassay experiments. A simple linear model of the form Y=bX was fitted to the percent mortality data for each treatment as a function of time (hours) elapsed. The regression slopes (b values) are the values given in the last column, so the more toxic the treatment, the greater the slope. The standard error of the slope is also given where enough variability existed in the replications of the response data to allow its calculation.

TABLE 3

Results of fish bioassays with varying metal contents and pH levels

Experiment	Treatment	pH*	Metal Mixtures	Slope of Mortality Line**
1	A	N	Control	0.0
	B	N	Ni, Cu, Zn + Al	2.111 ± 0.093
	C	N	Ni, Cu, Zn	≥ 4.167
	D	L	Control	0.0
	E	L	Ni, Cu, Zn + Al	≥ 4.167
	F	L	Ni, Cu, Zn	≥ 4.167
2	A	N	Control	0.0
	B	N	Ni, Cu, Zn + Al	0.0
	C	N	Ni, Cu, Zn	0.085 ± 0.037
	D	L	Control	0.0
	E	L	Ni, Cu, Zn + Al	0.269 ± 0.054
	F	L	Ni, Cu, Zn	1.020 ± 0.089

* N=normal range; L=low range

** Slope=b from the regression equation Y=bX, where X=time elapsed (hrs) and Y=the observed cumulative percent mortality

In Experiment 1, treatments C, E, and F produced 100% mortality at the end of 24 hours in all replicates, so the regression slopes were simply estimated as 100/24 = 4.167. If the earliest time by which all the fish were dead occurred sooner than 24 hours, the dotted line in Figure 1 would actually ascend more steeply than shown. Using 4.167 as a minimum slope, t-tests showed that treatments C, E, and F were significantly more toxic than treatment B ($p \leq .0001$), which was in turn significantly more toxic than the two control treatments A and D.

In Experiment 2, t-tests similarly performed to compare slopes showed that treatment F was significantly more toxic than treatment E, which in turn was significantly more toxic than treatment C ($p=.05$). However, treatment C was not significantly

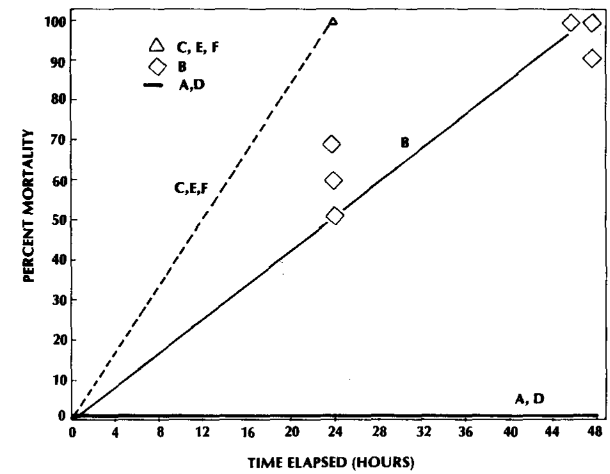

Figure 1. Experiment 1: Fish mortality vs time for six metal/pH treatments. Three replicates for each time (t=0, 24, 48 hrs) for a total of nine points per treatment. Individual points shown only for treatment B (t>0) since the other treatments had all-or-nothing responses.

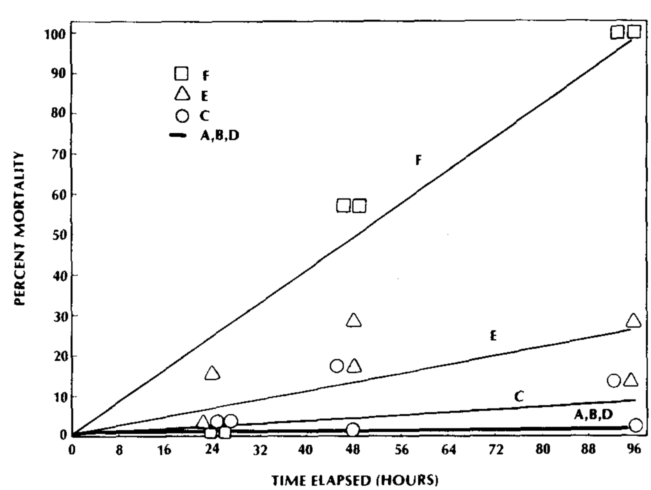

Figure 2. Experiment 2: Fish mortality vs time for six metal/pH treatments. Two replicates for each time (t = 0, 24, 48, 96 hrs) for a total of eight points per treatment. Individual points not shown for t = 0 and for treatments A, B, and D.

more toxic than treatments A, B, and D in which no mortality occurred.

DISCUSSION

A comparison of the results from the two experiments showed that each treatment from Experiment 1 was at least as toxic as the corresponding treatment from Experiment 2 because of the larger metal concentrations used in Experiment 1. Aside from this, there were several ways in which results of the two experiments were in general agreement with each other. In both experiments there was no mortality in the control treatments (in particular, treatment D) showing that low pH by itself was not toxic when no metals were added. Also, in Experiment 1, treatment F (Ni, Cu, and Zn at low pH) was as toxic as, or more toxic than, all the other treatments within that experiment.

A comparison of treatments B & C from both experiments showed that a mitigating influence of aluminum was observed at normal pH levels (compare treatments B and C). In Experiment 1, this was a statistically significant factor, since the metal mixture of nickel, zinc, and copper was significantly less toxic with aluminum than without it. At normal pH levels, aluminum may have had a coagulative effect on the other metals, rendering them less toxic. The main way in which the two experiments' results differed is seen in a comparison of treatments E and F, where the pH was lowered. In the presence of Ni, Cu, and Zn at low pH levels, aluminum exerted a significant mitigating influence in Experiment 2, but not in Experiment 1. The coagulative process may not have been as effective at a lower pH in Experiment 1 since the high metal concentrations used there could have masked any mitigating effects of aluminum.

In conclusion, the literature review performed during this study could only yield inferences as to the possible cause of the fish kill. Copper concentrations in the effluents were close to the acute LC_{50} values reported in the literature, but these types of comparisons are highly speculative since many other factors such as water chemistry, metal synergisms, and dilution become important in determining the toxicity of a particular element. Thus, it is impossible to choose a single element and conclusively establish that it was responsible for causing the kill.

The laboratory experiments did establish that the concentrations of metals in the composite samples from Stations #1 and #2 could explain sudden mortality to certain fish. The concentrations of metals actually experienced by the fish at the time of the kill cannot be established, but if stream levels reached concentrations similar to or greater than those reflected in the Station #2 composite for even a short period of time, acute mortality of fish would be highly probable. If low pH conditions accompanied the elevated metal concentrations, then acute effects could have occurred at even lower metal levels. We can also conclude that aluminum has a significant effect in reducing the mortality rate caused by the metal mixtures. Under severely elevated metal concentrations, however, the mitigating effects of precipitation or coagulation by aluminum could be overridden.

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