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# SAFE ZINC AND COPPER LEVELS FROM THE SPRING CREEK DRAINAGE FOR STEELHEAD TROUT IN THE UPPER SACRAMENTO RIVER, CALIFORNIA [1]

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**Acid mine discharges in the Spring Creek drainage, collecting behind the Spring Creek Diversion Dam, contribute large quantities of toxic zinc and copper to the upper Sacramento River. Ratios of copper-to-zinc for the Spring Creek Diversion Dam discharge into the Sacramento River fluctuate between 1:2 during storm periods and 1:8 during dry periods. Source control and treatment of the waste for copper removal will result in copper-to-zinc ratios of 1:12 or less in the future. Since the flows into the river of both the waste and the "dilution" water from Shasta Lake can be controlled, dilution factors which will provide for the complete protection of salmonids in the Sacramento River need to be determined and implemented. To determine these factors, long-term (60-day) and short-term (96-hr) bioassays with the waste were conducted on steelhead trout (*Salmo gairdneri*) eggs, alevins, and swim-up fry.**

**The bioassays indicated that eggs were more resistant than alevins and fry to solutions containing both zinc and copper while solutions containing only zinc affected all stages equally. At the lower copper-to-zinc waste ratio of 1:12, copper was absent in solution at partially toxic levels and toxicity was attributable to zinc alone, while at the higher waste ratio of 1:4, copper was present in sufficient quantities to increase the toxicity of the waste. The incipient lethal levels (10% mortality) for the period from eggs-to-fry were .12 mg/l Zn at the lower waste ratio, and .10 mg/l Zn and .011 mg/l Cu at the higher waste ratio. The incipient lethal level of control fry, those which had not been previously exposed to the waste, was .03 mg/l Zn. The presence of aluminum and iron in the waste apparently did not affect the toxicity of either zinc or copper. "Safe" levels of zinc and copper for steelhead trout are below .03 mg/l and .01 mg/l, respectively.**

## INTRODUCTION

Acid mine waste from the Spring Creek drainage (Figure 1) has caused numerous kills of anadromous and resident salmonid fishes in the upper Sacramento River between Keswick Reservoir and Cottonwood Creek, California (Prokopovich 1965; Calif. Dept. Fish and Game, Region 1, unpublished data; Nordstrom 1977). Of the anadromous salmonid fishes which have been affected, steelhead trout and chinook salmon, *Oncorhynchus tshawytscha*, are particularly important because of their recreational value; chinook salmon are also commercially valuable.

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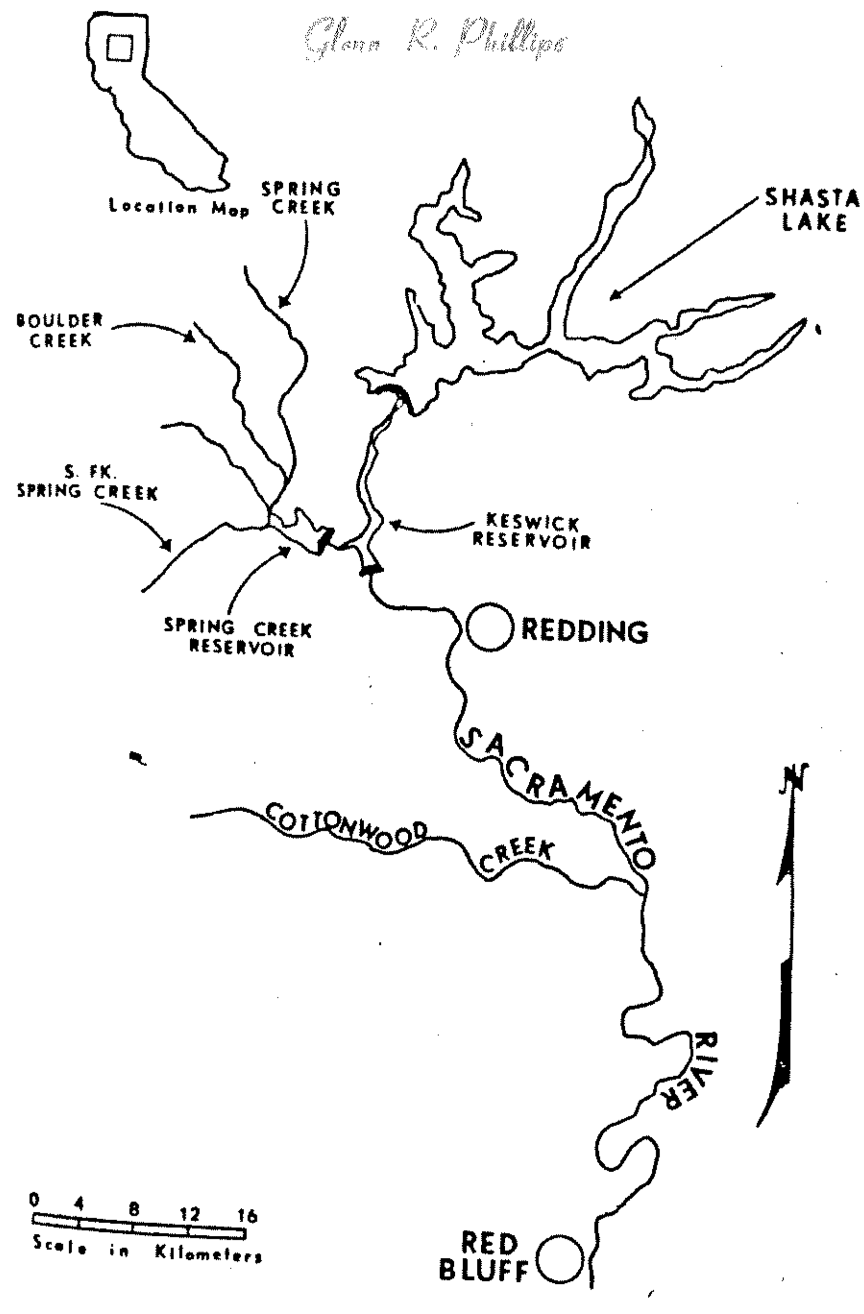


FIGURE 1. Location of Spring Creek drainage, Upper Sacramento River basin, California.

The acid mine discharge originates from both abandoned and operating mines, waste dumps, and naturally exposed sulfide mineral deposits. The waste contains large quantities of zinc, copper, aluminum, iron, and other heavy metals in smaller concentrations. Of the four most abundant metals, zinc and copper are extremely toxic to salmonids.

Although the toxicities of zinc (Colorado Game, Fish, and Parks 1971; Nehring and Goettl 1974; Solbe 1974; Zitko and Carson 1977), copper (Sprague and Ramsey 1965; Hazel and Meith 1970; McKim and Benoit 1971), and their combinations (Lloyd 1961; Sprague 1964; Sprague, Elson, and Saunders 1965; Thomsen, Hazel, and Meith 1970) have been well investigated and summarized (Chapman 1973; Finlayson and Rectenwald 1978), none have previously identified the additive effects of zinc and copper and other metals from an acid mine waste on eggs, alevins, and swim-up fry of steelhead trout or chinook salmon. The toxicities of aluminum (Freeman and Everhart 1971; Freeman 1973) and iron (Davis 1970; Sykora, Smith, and Synak 1972; Becker and Keller 1973) have been investigated but not in conjunction with other toxic metals such as zinc and copper. It is the dissolved fractions of these metals, ions in solution, which produce the toxic reactions in fish. Metals in solution are in equilibrium with their oxide and hydroxide precipitates or colloids, and this ionic balance is typically dependent on pH and water hardness.

Problems with this acid mine discharge are not new. The waste polluted the Sacramento River prior to the construction of Shasta Dam in 1944 and Keswick Dam in 1950. However, when Sacramento River streamflow was uncontrolled, flood flows coincided with those of the tributaries, thereby diluting Spring Creek runoff and probably diluting the waste to levels tolerable to fish (U.S. Fish and Wildlife Service 1959). However, more devastating problems arose after Shasta Dam was completed because, while "dilution" water in the upper Sacramento River Basin was stored in Lake Shasta during the heavy rains, the runoff from Spring Creek was left to enter the Sacramento River unobstructed. Hence, the toxic runoff from Spring Creek contributed a greater percentage of the total Sacramento River flow.

Since 1963 the waste has been collected in Spring Creek Reservoir and metered into Keswick Reservoir on the Sacramento River. The flow of the waste through the Spring Creek Diversion Dam is determined by the amount of "dilution" water available (Nordstrom 1977), the "dilution" water originating from Shasta Lake. This water quality management program has been based on the "tolerable" dilution factors calculated by Lewis (1963). He formulated two separate release schedules, based on two separate dilution factors using time-of-year and copper concentrations in the waste as discharge criteria. One release schedule was calculated for May through December, and the other for January through April. Lewis (1963) developed these factors from toxicity data obtained by exposing juvenile rainbow trout (*Salmo gairdneri*) and chinook salmon to the waste during 96-hr static bioassays. Although the release schedules have provided a margin of protection for salmonids in the Sacramento River, complete protection has probably not been provided because of questions not considered in the design of the bioassays and the problems this created in the interpretation of the results. These problems are:

1) juvenile fish were used in the bioassays; however, it has been demonstrated that younger and more sensitive life cycle stages of salmonids exist;
2) the bioassays did not consider periods of exposure beyond 96 hr, although greater periods of exposure undoubtedly exist in the river;
3) copper was the only toxic element considered in the bioassays; however, it has been recently demonstrated that zinc may be the only toxic element remaining in solution below Keswick Dam after the dilution of the waste to partially toxic levels (D. Wilson, Associate Water Quality Biologist, Calif. Dept. Fish and Game, pers. commun.);
4) static bioassays were used which did not consider the loss of copper and zinc from solution with time; therefore, the tests were not representative of a continuous discharge;
5) total copper, not dissolved copper, was measured as the toxic substance in the bioassays; however, it has been demonstrated that it is the dissolved fractions of total copper and zinc which produce the toxic reactions in fish;
6) the release schedules were based on the time-of-year runoff and total copper concentrations occurring in the waste, not on dissolved copper and zinc concentrations occurring in the waste or the river.

The quality of the acid mine waste has also changed since the development of the release schedules. Much of the copper contained in the waste is now removed by several copper cementation plants located throughout the drainage (Nordstrom 1977). All metals, with the exception of copper, remain in the same ratio to each other even though their concentrations decrease during storm periods and increase during dry periods. Copper concentrations, however, increase in ratio to the other metals during storm periods, and hence, the copper-to-zinc ratios of the waste normally fluctuate between 1:2 and 1:12 for wet and dry seasons, respectively (D. Wilson, pers. commun.).

The objective of our study was to gather accurate and relevant information which would answer the questions not considered by Lewis (1963) and those created by the recent developments in the drainage (Nordstrom 1977). This information is necessary to permit the calculation of new release schedules which should provide for complete protection of salmonids below Keswick Dam. The general approach of our study was to expose newly fertilized eggs of steelhead trout to different concentrations of two copper-to-zinc ratio (1:4 and 1:12) wastes and to raise the individuals to the swim-up fry stage in the same concentrations. We also raised cohorts of the above individuals to swim-up fry in waste-free water for use in bioassay testing at the swim-up fry stage. This was done to determine if prior exposure of the fry affects their sensitivity to the waste.

## MATERIALS AND METHODS

The acid mine waste used in the study was obtained from Boulder Creek, a tributary to Spring Creek (Figure 1). Most of the heavy metal pollutant load, with the exception of copper, originates from Boulder Creek (Nordstrom 1977). Because of the large quantity of iron (ca. 1%) present in the waste (Table 1), which precipitates out as ferric hydroxide and ferric oxide (collectively known as rust) upon dilution, we diluted the waste to 25% of original strength with distilled water and then readjusted the copper and zinc concentrations using reagent grade copper sulfate ($CuSO_4 \cdot 5H_2O$) and zinc sulfate ($ZnSO_4 \cdot 7H_2O$). The iron content was reduced by 75% because the original iron concentration would coat and smother the developing eggs with a heavy rust precipitate.

Preliminary investigations at the Department's Water Pollution Control Laboratory (WPCL) revealed an antagonistic relationship between aluminum and zinc (Finlayson, unpublished data, 1977). For example, the 96-hr LC50 of a zinc sulfate stock solution to juvenile rainbow trout was .14 mg/l Zn. No acute toxicity to rainbow trout could be found with aluminum sulfate at concentrations below 100 mg/l A1. However, when zinc and aluminum were present together at a ratio of 1:2, the 96-hr LC50 of zinc sulfate was increased to .52 mg/l Zn. The polymerization of aluminum hydroxide into large colloidal particles occurs at near-neutral (6.0–8.0) pH (Hem 1968), and this process was witnessed during the preliminary tests. The incorporation of zinc ions into the aluminum hydroxide colloids would effectively remove zinc from solution and could explain the antagonistic effects of aluminum on zinc toxicity. Since Spring Creek waste generally contains zinc and aluminum at a ratio of 1:1.5 (D. Wilson, pers. commun.), we also added reagent grade aluminum sulfate [$A1_2(SO_4)_3 \cdot 16H_20$] back to the waste.

**TABLE 1. Chemical Composition of Boulder Creek Waste and Adjusted Stock Toxicants Used in Bioassays (Ratios as copper : zinc : aluminum)**

| | Boulder Creek waste | Adjusted Boulder Creek wastes 1:4:6 Stock toxicant | Adjusted Boulder Creek wastes 1:12:18 Stock toxicant |
|---|---|---|---|
| Copper (mg/l) | 18.6 | 387. (5) * | 120 (5) |
| Zinc (mg/l) | 1490. | 1650. (5) | 1560. (5) |
| Aluminum (mg/l) | 1487. | 2720. (5) | 2410. (5) |
| Iron (mg/l) | 9490. | 2940. (4) | 2690. (4) |

* Sample sizes in parenthesis.

Two copper-to-zinc-to-aluminum ratio wastes were tested in both chronic (60-day) and short-term (96-hr) bioassays. In one waste, the ratio was adjusted to 1:4:6, and in the other waste, the ratio was adjusted to 1:12:18. The concentrations of zinc and aluminum were held constant at approximately 1600 and 2400 mg/1, respectively, and copper concentrations were adjusted by adding copper back to the waste at the appropriate ratio. During the bioassays, the waste stock solutions were replenished every 14 days, and each new solution was analyzed for copper, zinc, aluminum, and iron as a quality control measure (Table 1).

The wastes were diluted in a geometric series (100, 56, 32, 18, 10%) and continually delivered to the developing eggs, alevins, and fry using modified Mount and Brungs (1967) type plexiglass proportional diluters (Figure 2) designed and constructed by us at WPCL. Water from the American River, a tributary to the Sacramento River was filtered and used for dilution. Since the American and Sacramento rivers are of similar pH and water hardness, similar dissolved fractions of the metals would also be expected in the Sacramento River and in our bioassay waste concentrations. Immediately prior to entry into the proportional diluters, the concentrated wastes were prediluted to .06 and .1% of original strength for the 1:4:6 ratio and 1:12:18 ratio wastes, respectively. These initial dilutions constituted the 100% concentrations used in the geometric series of dilutions. Predilution was accomplished using a peristaltic pump and adjustable flow meter to pump into the plexiglass predilution chamber along with a metered supply of dilution water (Figure 2). After mixing in the predilution chamber, the waste then flowed through a plexiglass filtering chamber



packed with spun glass for removal of residual ferric oxide and hydroxide precipitate. The spun glass was replaced weekly. After filtration, the prediluted waste then flowed into the toxicant cells of the proportional diluters.

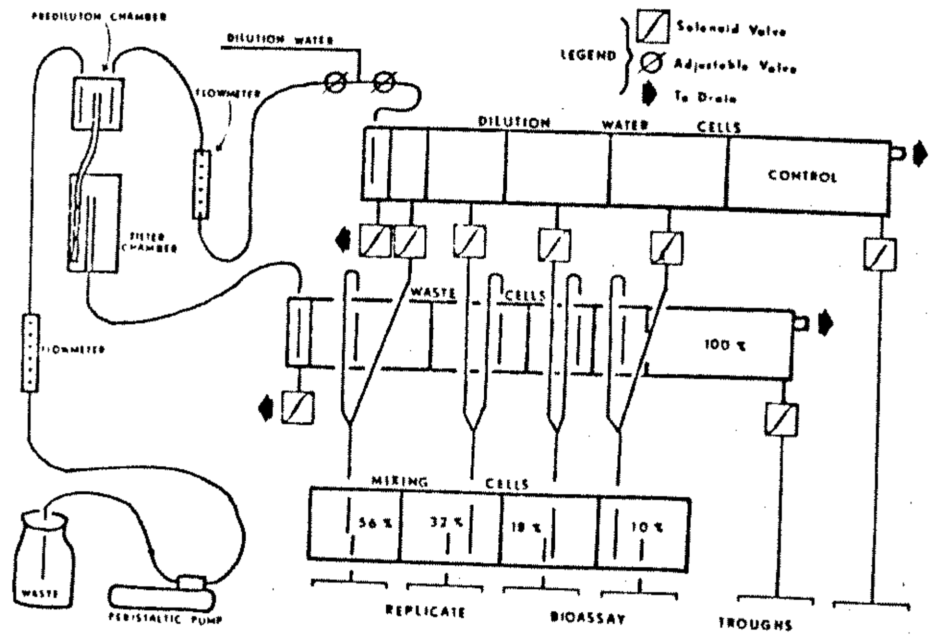


FIGURE 2. Schematic diagram of waste predilution system and modified Mount and Brungs type proportional diluter.

The waste concentrations and control dilution water from the proportional diluters were divided in two and supplied to replicate plexiglass bioassay troughs. The troughs were 92-cm long by 13-cm wide, with the water level adjusted to a depth of 10 cm by a standpipe. The troughs were subdivided into five compartments by alternating baffles which allowed for the complete circulation of the diluted wastes over the developing eggs, alevins, and fry. The fertilized eggs were incubated in egg baskets, three baskets per trough. The egg baskets were perforated polyethylene plastic jars, 6.5-cm in diameter and 9-cm high. The troughs had a volume of approximately 12 liters, and approximately 500 ml of the diluted waste was supplied to each trough every 7 minutes. The volume of each trough was replenished every 2.8 hours.

Continual-flow bioassays using standard methods (Peltier 1977) were carried out at WPCL with freshly fertilized steelhead trout eggs and the resulting alevins and swim-up fry. All concentrations were tested in replicate. The eggs were obtained by dry spawning three female and two male adult fish on 10 February 1978 at the Department's Nimbus Hatchery on the American River.

Following dry spawning, the eggs were segregated and placed in the different concentrations of the diluted wastes and allowed to water-harden. The "controls" were water-hardened in filtered American River water. Water-hardening took place in 2-liter glass beakers and lasted 3 hours. After water-hardening, 50 eggs were hand counted and carefully floated into each egg basket (150 eggs per trough or 300 eggs per concentration). "Control" eggs which were not used in the chronic bioassay were deposited in several, square, 10-liter, overflowing plexiglass aquaria, placed in the dark with a supply of flowing water, and allowed to hatch and develop into swim-up fry. These "control" swim-up fry were used later in short-term bioassays with the same equipment and concentrations that were used in the chronic bioassays.

The 60-day chronic bioassays began on 10 February 1978 with fertilized steelhead eggs and ended on 11 April 1978 when individuals were at the swim-up fry stage. The 96-hour acute bioassays began with the additional "control" swim-up fry on 24 April and ended on 28 April 1978.

During the bioassays, water samples were collected twice weekly from the troughs for analyses of both total and dissolved copper, zinc, aluminum, and iron. Concentrations of these metals were determined by atomic absorption analysis (American Public Health Association 1975). Samples for dissolved metals were collected by filtration through Gelman ® type A-E glass-fiber filters. All samples were preserved with nitric acid and hydrochloric acid. Dissolved oxygen, pH, and temperature were determined in each trough three times a week, whereas water hardness was determined only once a week.

Deaths were not recorded in the chronic bioassays until the embryos left the "tender" stage and entered the "eyed" stage. Once the "eyed" stage was entered, dead embryos could be safely removed from the egg baskets. The dead were recorded and removed three times a week. Additionally, the ferric oxide and hydroxide precipitate which formed in the bioassay troughs was carefully suctioned away with a turkey baster to avoid disturbing the developing eggs.

Mortality estimates were calculated from the following equations:

(1) $M_e = (1 - S_{xe}/S_{ce})\ 100$

where $M_e$ is mortality of eggs, $S_{xe}$ is the survival of eggs from fertilization to hatch in concentration X, and $S_{ce}$ is the survival of control eggs from fertilization to hatch in waste-free water.

(2) $M_a = (1 - S_{xa}/S_{ca})\ 100$

where $M_a$ is mortality of alevins, $S_{xa}$ is the survival of newly hatched alevins to swim-up fry in concentration X, and $S_{ca}$ is the survival of newly hatched control alevins to swim-up fry in waste-free water.

(3) $M_t = (1 - S_{xt}/S_{ct})\ 100$

where $M_t$ is mortality for the period of fertilization to swim-up fry, $S_{xt}$ is the survival from fertilization to swim-up fry in concentration X, and $S_{ct}$ is the survival from fertilization to swim-up fry in waste-free water.

(4) $M_f = (1 - S_{xf}/S_{cf})\ 100$

where $M_f$ is mortality of "control" swim-up fry, $S_{xf}$ is the survival of the "control" swim-up fry in concentration X during the 96-hr exposure, and $S_{cf}$ is the continued survival of the "control" swim-up fry during the 96-hr exposure in waste-free water.

Standard log-probit (concentration-mortality) analysis (APHA 1975) was used to calculate the $LC50_e$, $LC50_a$, $LC50_t$, and $LC50_f$ (concentration producing 50% mortality) and the $ILL_e$, $ILL_a$, $ILL_t$, and $ILL_f$ (incipient lethal level; concentration producing 10% mortality) for the wastes. Heavy metal concentrations at the LC50's and ILL's were extrapolated from known metal concentrations above and below these levels.

# RESULTS

## 60-Day Chronic Bioassay

The bioassay test concentrations had a pH range of 6.3 to 7.5. Water hardness of the test concentrations varied from 34 to 65 mg/l $CaCO_3$. Water temperatures during the bioassays were between 9 and 10 C and dissolved oxygen was near saturation at all times.

The concentrations of total metals present in the 1:4:6 ratio and 1:12:18 ratio waste bioassays increased with increased waste concentrations (Appendices 1 and 2, respectively). Dissolved zinc concentrations in the 1:4:6 ratio waste bioassay ranged from 1.15 to <.01 mg/l for .067% waste and control, respectively; dissolved copper concentrations ranged from .15 to <.01 mg/l for the same concentrations (Appendix 1). Dissolved aluminum ranged from .07 to <.01 mg/l with the highest concentrations occurring in .021 and .012% wastes and the lowest concentrations occurring in .037 and .067% wastes and control. Dissolved iron ranged from .80 to .15 mg/l for the .067% waste and control, respectively. The dissolved concentrations of zinc, copper, and iron averaged 86.2, 42.1, and 40.7% of total concentrations, respectively.

Dissolved zinc concentrations in the 1:12:18 ratio waste bioassay ranged from 1.25 to <.01 mg/l for .100% waste and control, respectively; dissolved copper concentrations ranged from .042 to <.01 mg/l for the same concentrations (Appendix 2). Dissolved aluminum ranged from .18 to <.01 mg/l with the highest concentrations occurring in .032% waste and the lowest occurring in .056 and .100% wastes and control. Dissolved iron ranged from 1.12 to .14 mg/l for .100% waste and control, respectively. The dissolved concentrations of zinc, copper, and iron averaged 75.0, 30.3, and 39.6% of total concentrations, respectively.

Estimates of $M_e$, $M_a$, and $M_t$ increased with increased concentration in both the 1:4:6 ratio (Table 2) and 1:12:18 ratio (Table 3) wastes. Complete mortality (100%) occurred in the highest waste concentrations tested while minimal mortality (8.2 to 18.2%) occurred in the lowest waste concentrations tested. Survival of the control eggs-to-fry ranged from 88.0 to 94.7%.

The $LC50_e$ and $LC50_a$ of the 1:4:6 ratio waste were .017 and .010% waste, respectively (Table 4). Zinc and copper concentrations at these two levels were .30 mg/l Zn and .035 mg/l Cu, and .19 mg/l Zn and .021 mg/l Cu, respectively. Eggs were more resistant to zinc and copper concentrations than were alevins in this ratio waste. Similar differences between the sensitivities of eggs ($ILL_e$) and alevins ($ILL_a$) to zinc and copper occurred at the incipient lethal level. The $LC50_t$ and $ILL_t$ were .009 and .006% waste, respectively, and resembled the $LC50_a$ and $ILL_a$, thereby suggesting that the developmental period from alevins-to-fry was more sensitive to copper and zinc concentrations than the developmental period from eggs-to-hatch.

The $LC50_e$ and $LC50_a$ of the 1:12:18 ratio waste were .035 and .042% waste, respectively, with zinc concentrations of .42 and .52 mg/l, respectively (Table 4). Dissolved copper was absent at these levels. Eggs were more sensitive at the LC50 level to zinc than were fry in this ratio waste. However, the $ILL_e$ and $ILL_a$ were .018 and .010% waste, respectively, with zinc concentrations of .23 and .14 mg/l, respectively. Even though fry were more resistant to zinc than eggs at the LC50 level, the opposite was true at the ILL. The $LC50_t$ and $ILL_t$ were .032 and .010% of waste, respectively. The $LC50_t$ resembled the $LC50_e$ but the $ILL_t$ resembled the $ILL_a$ thereby suggesting that zinc concentrations, in the absence of [illegible]

TABLE 2. Mortality Estimates for Steelhead Eggs Raised to Swim-Up Fry Stage in Five Concentrations of the 1:4:6 Copper-to-Zinc-to-Aluminum Ratio Waste

| Concentration of waste | | Percent mortality: Eggs[b] ($M_e$) | Alevins[c] ($M_a$) | Total[d] ($M_t$) |
|---|---|---|---|---|
| .067% | (1)[a] | 100 | - | 100 |
| | (2) | 100 | - | 100 |
| .037% | (1) | 98.6 | 100 | 100 |
| | (2) | 98.6 | 100 | 100 |
| .021% | (1) | 66.2 | 100 | 100 |
| | (2) | 63.2 | 100 | 100 |
| .012% | (1) | 24.6 | 69.9 | 77.3 |
| | (2) | 23.6 | 80.6 | 85.2 |
| .007% | (1) | 2.2 | 6.6 | 8.3 |
| | (2) | 6.7 | 12.4 | 18.2 |

[a] Numbers in parentheses indicate replicates.
[b] Survival of control eggs ranged from 92.0 to 98.7%.
[c] Survival of control alevins ranged from 95.7 to 97.3%.
[d] Total survival of controls to swim-up fry stage ranged from 88.0 to 94.7%.

TABLE 3. Mortality Estimates for Steelhead Eggs Raised to Swim-Up Fry Stage in Five Concentrations of the 1:12:18 Copper-to-Zinc-to-Aluminum Ratio Waste

| Concentration of waste | | Percent mortality: Eggs[b] ($M_e$) | Alevins[c] ($M_a$) | Total[d] ($M_t$) |
|---|---|---|---|---|
| .100% | (1)[e] | 100 | - | 100 |
| | (2) | 100 | - | 100 |
| .056% | (1) | 94.4 | 100 | 100 |
| | (2) | 97.3 | 100 | 100 |
| .032% | (1) | 25.0 | 33.9 | 50.4 |
| | (2) | 18.4 | 38.8 | 51.8 |
| .018% | (1) | 2.8 | 3.9 | 6.7 |
| | (2) | 17.6 | 19.9 | 41.0 |
| .010% | (1)[e] | 51.8 | 38.6 | 90.4 |
| | (2) | 2.0 | 9.5 | 10.0 |

[a] Numbers in parentheses indicate replicates.
[b] Survival of control eggs ranged from 94.0 to 98.7%.
[c] Survival of control fry ranged from 93.9 to 96.1%.
[d] Total survival of controls to swim-up fry stage ranged from 90.0 to 92.7%.
[e] This aquaria suffered an extreme outbreak of fungus and was not used in the calculation of bioassay statistics.

## 96-Hr Acute Bioassay

The bioassay test concentrations had a pH range of 6.5 to 7.6. Water hardness of test concentrations ranged from 25 to 60 mg/l $CaCO_3$. Water temperatures during the bioassays were between 10 and 11C, and dissolved oxygen was near saturation at all times.

The concentrations of total metals present in the 1:4:6 ratio and 1:12:18 ratio waste bioassays increased with increased waste concentrations (Appendices 3 and 4, respectively). Dissolved zinc concentrations in the 1:4:6 ratio waste bioassay ranged from 1.21 to < .01 mg/l for .067% waste and control, respectively; dissolved copper concentrations ranged from .14 to < .01 mg/l for the same concentrations (Appendix 3). Dissolved aluminum was consistently <.01 mg/l for all waste concentrations and control; dissolved iron ranged from .56 to

TABLE 4. Summary of Statistics for 60-Day Bioassays on 1:4:6 and 1:12:18 Copper-to-Zinc-to-Aluminum Ratios of Acid Mine Wastes. Statistics represent means based on replicates.

| | Eggs-to-hatch: $LC50_e$ 1:4:6 | $LC50_e$ 1:12:18 | $ILL_e$ 1:4:6 | $ILL_e$ 1:12:18 | Hatch-to-swim-up fry: $LC50_a$ 1:4:6 | $LC50_a$ 1:12:18 | $ILL_a$ 1:4:6 | $ILL_a$ 1:12:18 | Total: $LC50_t$ 1:4:6 | $LC50_t$ 1:12:18 | $ILL_t$ 1:4:6 | $ILL_t$ 1:12:18 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Concentration of waste (%) | .017 | .035 | .009 | .018 | .010 | .042 | .007 | .010 | .009 | .032 | .006 | .010 |
| Total zinc (mg/l) | .35 | .61 | .20 | .29 | .22 | .72 | .17 | .18 | .20 | .56 | .14 | .18 |
| Dissolved zinc (mg/l) | .30 | .43 | .17 | .23 | .19 | .52 | .14 | .14 | .17 | .39 | .12 | .14 |
| Total copper (mg/l) | .073 | .039 | .043 | .019 | .046 | .050 | .036 | < .01 | .043 | .035 | .033 | < .01 |
| Dissolved copper (mg/l) | .035 | < .01 | .019 | < .01 | .021 | < .01 | .014 | < .01 | .019 | < .01 | .012 | < .01 |

.11 mg/l for .067% waste and control, respectively. The dissolved concentrations of zinc, copper, and iron averaged 81.5, 42.3, and 46.2% of total concentrations, respectively.

Dissolved zinc concentrations in the 1:12:18 ratio waste bioassay ranged from 1.18 to <.01 mg/l for .100% waste and control, respectively; dissolved copper concentrations ranged from .042 to <.01 mg/l for the same concentrations. Dissolved aluminum was consistently <.01 mg/l for all waste concentrations and control; dissolved iron ranged from .57 to .08 mg/l for .100% waste and control, respectively. The dissolved concentrations of zinc, copper, and iron averaged 74.5, 37.1, and 43.1% of total concentrations, respectively.

Estimates of $M_f$ increased with increased waste concentrations of both the 1:4:6 ratio and 1:12:18 ratio wastes (Table 5). Complete mortality (100%) occurred at the three highest concentrations tested in both ratio wastes. Survival of the controls ranged from 98 to 100%.

**TABLE 5.** Mortality estimates for Steelhead Swim-up Fry Exposed for 96-hr to Five Concentrations of 1:4:6 and 1:12:18 Copper-to-Zinc-to-Aluminum Ratio Wastes

| 1:4:6 | | | 1:12:18 | | |
|---|---|---|---|---|---|
| Concentration of waste[a] | | Percent[b] mortality ($M_f$) | Concentration of waste[a] | | Percent[c] mortality ($M_f$) |
| .067% | (1)[a] | 100 | .100% | (1) | 100 |
| | (2) | 100 | | (2) | 100 |
| .037% | (1) | 100 | .056% | (1) | 100 |
| | (2) | 100 | | (2) | 100 |
| .021% | (1) | 100 | .032% | (1) | 100 |
| | (2) | 100 | | (2) | 100 |
| .012% | (1) | 91 | .018% | (1) | 57 |
| | (2) | 96 | | (2) | 64 |
| .007% | (1) | 95 | .010% | (1) | 42 |
| | (2) | 96 | | (2) | 42 |

[a] Numbers in parentheses indicate replicates.
[b] Survival of controls was 100%.
[c] Survival of controls was 98%.

Estimates of $M_f$ in all 1:4:6 ratio waste concentrations were greater than 91% (Table 5) which precluded the calculation of a $LC50_f$ or an $ILL_f$ (Table 6). The mean $M_f$ in the .007% waste, which was the lowest concentration tested, was 95.5%, and zinc and copper at this level were .14 and .012 mg/l, respectively. The $LC50_f$ of the 1:12:18 ratio waste was .013% waste; zinc and copper at this level were .18 and <.01 mg/l, respectively (Table 6). The $ILL_f$ was .003% waste, with zinc and copper at .03 and <.01 mg/l, respectively.

**TABLE 6.** Summary of Statistics for 96-hr Bioassays on 1:4:6 and 1:12:18 Copper-to-Zinc-to-Aluminum Ratios of Acid Mine Wastes. Statistics represent means based on replicates.

| | 60-Day swim-up fry | | | |
|---|---|---|---|---|
| | $LC50_f$ | | $ILL_f$ | |
| | 1:4:6 | 1:12:18 | 1:4:6 | 1:12:18 |
| Concentration of waste (%) | <.007 | .013 | - | .003 |
| Total zinc (mg/l) | <.22 | .32 | - | .06 |
| Dissolved zinc (mg/l) | <.14 | .18 | - | .03 |
| Total copper (mg/l) | <.034 | .016 | - | .010 |
| Dissolved copper (mg/l) | <.012 | <.01 | - | <.01 |

## DISCUSSION

Bioassays were conducted on two copper-to-zinc-to-aluminum ratio acid mine wastes. Both 60-day chronic and 96-hr acute exposures were investigated with fertilized steelhead eggs to the swim-up fry stage, and swim-up fry, respectively. The toxicity data demonstrated that swim-up fry not previously exposed to the waste were more sensitive to the waste than swim-up fry which had been previously exposed to the waste. The incipient lethal level of the 1:12:18 ratio waste during the 60-day exposure was .14 mg/l Zn, while that during the 96-hr exposure was .03 mg/l Zn. The LC50 of the 1:4:6 ratio waste during the 60-day exposure was .17 mg/l Zn and .019 mg/l Cu, while that during the 96-hr exposure was <.14 mg/l Zn and <.012 mg/l Cu. This indicates that acclimation to zinc and copper concentrations in the waste was occurring during embryonic and alevin development.

However, although we planned to test the sensitivities of waste-exposed and waste-unexposed fry, the swim-up fry used in the 96-hr tests were further developed (free-swimming) than the swim-up fry at the end of the 60-day test (not free-swimming). Hence, we probably were not testing identical life cycle stages. Chapman (Dr. Gary Chapman, Western Fish Toxicology Station, National Environmental Research Center, Corvallis, Oregon, pers. commun.) found salmonid swim-up fry to be the most sensitive life cycle stage to heavy metals. Hence, our data may be demonstrating differences between the sensitivities of two life cycle stages as well as an acclimation to heavy metals.

When soluble copper was present in the diluted waste (1:4:6 ratio waste) the eggs were more resistant to both copper and zinc concentrations than were the resulting alevins and fry. Hazel and Meith (1970), testing a stock solution of copper sulfate, found similar differences between the sensitivities of chinook salmon eggs and fry to copper. Conversely, differences between the sensitivities of eggs and fry to zinc were not apparent when copper was absent in the diluted waste (1:12:18 ratio waste). Hence, although copper determinately affects fry, zinc appears to equally affect the eggs and fry. Regardless, our data and that of Hazel and Meith (1970) and Chapman (pers. commun.) show that swim-up fry are as sensitive or more sensitive than eggs to zinc and copper concentrations.

The LC50's of zinc (in the absence of copper) for steelhead fry ranged from .18 to .39 mg/l and the ILL's ranged from .03 to .14 mg/l. Thomsen et al. (1970), testing a stock solution of zinc sulfate to juvenile steelhead stock from Nimbus Hatchery, found the 96-hr LC50 of zinc to be .12 mg/l. Our range of zinc ILL's is in excellent agreement with that of Thomsen et al. (1970), who found the 96-hr ILL of zinc to juvenile steelhead to be .06 mg/l. It is surprising that our data agree as well as they do since the toxicity of zinc to rainbow trout has been reported to vary considerably (.24 to .83 mg/l) at the 96-hr LC50 level (Colorado Game, Fish, and Parks 1971). Based on our estimated ILL's and that of Thomsen et al. (1970), concentrations of zinc (in the absence of copper) which will completely protect steelhead trout fry are below .03 mg/l. The U.S. Environmental Protection Agency (1976) recommends a "safe" concentration of zinc at .01 times the 96-hr LC50 for the species in question. Using this criterion and our LC50 estimates for zinc, "safe" concentrations of zinc (in the absence of copper) for steelhead fry are between .0018 and .0039 mg/l.

The LC50's of copper and zinc for steelhead fry ranged between <.14 mg/l

Zn and < .012 mg/l Cu, and .17 mg/l Zn, and .018 mg/l Cu. The ILL estimates during the 60-day test were .12 mg/l Zn and .012 mg/l Cu. No ILL could be determined for the 1:4:6 ratio waste in the 96-hr test because of the high mortality (mean of 95.5%) which occurred in the lowest waste concentration (.007%) tested. For juvenile steelhead exposed to stock solutions containing both zinc and copper sulfates, Thomsen et al. (1970) found the 96-hr LC50 to be .16 mg/l Zn and .034 mg/l Cu, and the ILL to be .10 mg/l Zn and .01 mg/l Cu. Based on our estimated ILL's and that of Thomsen et al. (1970), concentrations of zinc and copper which will completely protect steehlead trout fry are below .03 mg/l and .01 mg/l, respectively. The U.S. EPA (1976) recommends a "safe" concentration of copper at .1 times the 96-hr LC50 for the species in question. Using this criterion, the one given for zinc above, and [redacted]

Our zinc and copper toxicity data agree well with that of Thomsen et al. (1970) who tested toxicant stock solution containing only zinc and copper, not an actual acid mine waste. The Spring Creek acid mine waste contains other metals, including aluminum and iron, which could be toxic. Freeman and Everhart (1971) found the threshold of acute toxicity around 1.5 mg/l soluble aluminum. The U.S. EPA (1976) reports that soluble concentrations of iron below 1.0 mg/l are "safe" for all freshwater aquatic life and Davis (1970) states that iron is probably not directly harmful to fish except it may become a nuisance when precipitated. Svkova et al. (1970) found that increased turbidity caused by an iron precipitate makes fish food items harder to see and therefore, decreases fish growth. Hence, we must dismiss both aluminum and iron toxicity in our bioassays since the highest soluble concentrations measured for the two metals in all the tests were .18 mg/l and 1.12 mg/l, respectively. Although we expected aluminum to act antagonistically on zinc toxicity, we must dismiss this hypothesis also because of the low zinc LC50's and ILL's found. Perhaps iron, rather than zinc, co-precipitates with aluminum in the acid mine waste. The incorporation of zinc into aluminum polymers may occur only when these two elements are present.

Our data demonstrated that both iron and aluminum rapidly precipitate out of solution with dilution and hence, increase in pH. Dissolved iron concentrations are quite low (ca. 0.3 mg/l) in Keswick Reservoir, although the iron concentrations of the Spring Creek Diversion Dam discharge average 400 mg/l (D. Wilson, pers. commun.). Low concentrations of dissolved aluminum would also be expected in Keswick Reservoir. Hence, even though large concentrations of iron and aluminum occur in the Spring Creek waste, dilution and precipitation cause the dissolved concentrations of the two metals to be so low that they do not exist in toxic concentrations. The presence of iron and aluminum in the waste apparently does not affect the toxicity of zinc and copper since our data agree reasonably well with those of Thomsen et al. (1970).

The release schedules calculated by Lewis (1963) allow total copper concentations in the Sacramento River below Keswick Dam to reach .025 and .045 mg/l for the periods of May through December, and January through April, respectively. Our data show dissolved copper averaged 38.2% of the total copper concentration. Thus, the criteria established by Lewis (1963) allow dissolved copper concentrations to reach .010 mg/l during May through December, and .017 mg/l during January through April. We found steelhead fry mortality occurring at dissolved copper concentrations less than .01 mg/l. Hence, the release schedules calculated by Lewis (1963) do not provide for complete protection from copper toxicity.

Using the waste dilution factors calculated by Lewis (1963), we were able to estimate the concentrations of dissolved zinc occurring in the Sacramento River below Keswick Dam as a result of the scheduled releases from Spring Creek Reservoir. In our calculations, we assumed the total copper and zinc concentrations of the undiluted Spring Creek Reservoir discharge to vary between 2 and 10 mg/l Cu, and 10 and 90 mg/l Zn (D. Wilson, pers. commun.), and the dissolved concentrations of zinc, equivalent to the mean found in our tests, were 80.7% of total zinc concentrations. For May through December, the waste would have to be diluted between .25 and 1.2% of original strength to attain the .025 mg/l total copper criterion in the Sacramento River; these dilutions would allow between .02 and .91 mg/l dissolved zinc, respectively, in the Sacramento River. Likewise for the period of January through April, the waste would have to be diluted between .45 and 2.2% of original strength to attain the .045 mg/l total copper criterion in the Sacramento River. These dilutions would allow between .04 and 1.60 mg/l dissolved zinc, respectively, in the Sacramento River. We found the ILL of swim-up fry to be .03 mg/l Zn, which is in the middle of the range of minimal zinc concentrations (.02–.04 mg/l) allowed by these calculations. Even if our calculations depict the worst-case situation of the zinc pollution in the Sacramento River, it is evident that complete protection of steelhead fry from zinc toxicity has not been provided by the release schedules of Lewis (1963).

With respect to "safe" levels of zinc and copper in the Sacramento River, concentrations of both metals must be kept below .03 and .01 mg/l, respectively in order to afford protection of steelhead populations below Keswick Dam. Allowable levels of zinc and copper in excess of these should only be considered as "interim". The release schedules from the Spring Creek Reservoir should be calculated so that, after dilution in Keswick Reservoir, "safe" levels of zinc and copper are released from Keswick Dam into the Sacramento River. It should be noted that .03 mg/l zinc and .01 mg/l copper are near the current practical detection limits of most acceptable analytical techniques, and therefore, actual "safe" levels below these cannot be determined or detected accurately with the present technology.

Today, with even less copper present in the outflow of Spring Creek than was present 15 years ago, the old release schedules provide for even less protection from zinc toxicity. This is because of lower concentrations of copper present in the Spring Creek waste which permits less dilution, and hence higher zinc concentrations occur in the Sacramento River. We have shown that copper and zinc have the same order of toxicity, but zinc concentrations are typically present in the waste at one order of magnitude greater than that of copper. Additionally, the dissolved fraction of zinc is between two and three times greater than that of copper. New release schedules need to be formulated using the "safe" levels of both zinc and copper as criteria but with the most attention given to zinc. Additionally, concentrations of zinc and copper need to be measured in both the waste and in the river if new waste release schedules are to be accurately implemented.

Studies need to be conducted with chinook salmon eggs and fry to extend our knowledge of zinc and copper toxicity to salmonids in the Sacramento River. Chinook are more abundant than steelhead in the main stem of the Sacramento River below Keswick Dam. Moreover, because the Spring Creek Diversion Dam presently overflows during heavy precipitation, additional studies need to be carried out with salmonid fry to identify the effects of high waste concentrations of short duration.

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**APPENDIX 1—Concentrations of Zinc, Copper, Aluminum, and Iron in Bioassay Aquaria During 60-day Bioassay on 1:4:6 Copper-to-Zinc-to-Aluminum Ratio Waste**

| *Concentration of waste* | | *Zn (mg/l)* | *Cu (mg/l)* | *Al (mg/l)* | *Fe (mg/l)* |
|---|---|---|---|---|---|
| | Total (1) [a] | 1.41 (.42) [b] | .34 (.12) | 1.64 (1.04) | 2.70 (1.70) |
| .067% | Dissolved | 1.14 (.10) | .15 (.048) | <.01 | .80 (.32) |
| | Total (2) | 1.35 (.15) | .42 (.20) | 1.73 (.92) | 3.21 (1.03) |
| | Dissolved | 1.15 (.10) | .15 (.042) | <.01 | .67 (.30) |
| | Total (1) | .68 (.21) | .14 (.072) | .84 (.60) | 1.50 (.33) |
| .037% | Dissolved | 63 (.03) | .059 (.009) | <.01 | .25 (.47) |
| | Total (2) | .66 (.19) | .14 (.069) | .80 (.61) | 1.43 (.31) |
| | Dissolved | .61 (.03) | .052 (.018) | <.01 | .36 (.07) |
| | Total (1) | .42 (.07) | .10 (.042) | .49 (.31) | 1.11 (.30) |
| .021% | Dissolved | .37 (.02) | .043 (.012) | .07 (.13) | .37 (.12) |
| | Total (2) | .42 (.09) | .091 (.053) | .50 (.35) | 1.11 (.26) |
| | Dissolved | .36 (.02) | .042 (.011) | .03 (.07) | 35 (.10) |
| | Total (1) | .26 (.05) | .053 (.024) | .24 (.23) | .65 (.15) |
| .012% | Dissolved | .22 (.02) | .028 (.009) | .07 (.07) | .37 (.04) |
| | Total (2) | .25 (.05) | .052 (.018) | .23 (.24) | .68 (.17) |
| | Dissolved | .22 (.01) | .024 (.009) | .04 (.09) | .36 (.06) |
| | Total (1) | .16 (.04) | .042 (.012) | .13 (.18) | .49 (.16) |
| .007% | Dissolved | .13 (.01) | .010 (.010) | .04 (.09) | .28 (.06) |
| | Total (2) | .16 (.03) | .031 (.018) | .11 (.16) | .48 (.16) |
| | Dissolved | .13 (.01) | .016 (.010) | .04 (.09) | .29 (.04) |
| | Total (1) | <.01 | <.01 | .01 (.04) | .18 (.09) |
| Control | Dissolved | <.01 | <.01 | <.01 | .17 (.04) |
| | Total (2) | <.01 | <.01 | .01 (.04) | .16 (.10) |
| | Dissolved | <.01 | <.01 | <.01 | .15 (.05) |

[a] Numbers in parentheses indicate replicates.
[b] Numbers in parentheses represent standard deviations.



**APPENDIX 2—Concentrations of Zinc, Copper, Aluminum, and Iron in Bioassay Aquaria During 60-day Bioassay on 1:12:18 Copper-to-Zinc-to-Aluminum Ratio Waste**

| *Concentration of waste* | | *Zn (mg/l)* | *Cu (mg/l)* | *Al (mg/l)* | *Fe (mg/l)* |
|---|---|---|---|---|---|
| | Total (1) [a] | 1.58 (.43) [b] | .15 (.11) | 2.30 (1.11) | 4.39 (3.12) |
| .100% | Dissolved | 1.25 (.23) | .042 (.018) | <.01 | 82 (.52) |
| | Total (2) | 1.56 (.46) | .13 (.06) | 2.46 (1.09) | 3.99 (2.37) |
| | Dissolved | 1.21 (.17) | .043 (.021) | <.01 | 1.12 (.52) |
| | Total (1) | .93 (.22) | .074 (.04) | 1.56 (1.01) | 2.16 (1.41) |
| .056% | Dissolved | .69 (.09) | .012 (.007) | <.01 | .30 (.14) |
| | Total (2) | .93 (.25) | .080 (.06) | 1.43 (.88) | 2.45 (1.55) |
| | Dissolved | .70 (.10) | .014 (.006) | <.01 | .32 (.14) |
| | Total (1) | .53 (.16) | .034 (.03) | .81 (.38) | 1.28 (.80) |
| .032% | Dissolved | .39 (.06) | <.01 | .11 (.19) | .32 (.12) |
| | Total (2) | .58 (.13) | .042 (.03) | 1.05 (.66) | 1.88 (1.27) |
| | Dissolved | .39 (.06) | <.01 | .18 (.15) | .32 (.12) |
| | Total (1) | .29 (.10) | .012 (.01) | .38 (.26) | .69 (.48) |
| .018% | Dissolved | .23 (.04) | <.01 | .06 (.12) | .33 (.10) |
| | Total (2) | .29 (.11) | .014 (.01) | .38 (.29) | .78 (.43) |
| | Dissolved | .23 (.04) | <.01 | .08 (.13) | .33 (.10) |
| | Total (1) | .18 (.07) | <.01 | .20 (.21) | .51 (.32) |
| .010% | Dissolved | .14 (.02) | <.01 | .04 (.07) | .29 (.08) |
| | Total (2) | .18 (.07) | <.01 | .19 (.17) | .62 (.43) |
| | Dissolved | .14 (.02) | <.01 | .03 (.06) | .27 (.08) |
| | Total (1) | <.01 | <.01 | .01 (.02) | .15 (.10) |
| Control | Dissolved | <.01 | <.01 | <.01 | .14 (.04) |
| | Total (2) | <.01 | <.01 | .01 (.02) | .17 (.06) |
| | Dissolved | <.01 | <.01 | <.01 | .12 (.08) |

[a] Numbers in parentheses indicate replicates.
[b] Numbers in parentheses represent standard deviations.

## APPENDIX 3—Concentrations of Zinc, Copper, Aluminum, and Iron in Bioassay Aquaria During 96-hr Bioassay on 1:4:6 Copper-to-Zinc-to-Aluminum Ratio Waste

| Concentration of waste | | Zn (mg/l) | Cu (mg/l) | Al (mg/l) | Fe (mg/l) |
|---|---|---|---|---|---|
| .067% | Total (1)[a] | 1.25 (.04)[b] | .30 (.03) | 1.05 (.74) | 2.36 (.12) |
| | Dissolved | 1.21 (.04) | .14 (.03) | < .01 | .56 (.14) |
| | Total (2) | 1.25 (.12) | .36 (.09) | 89 (.63) | 2.43 (.29) |
| | Dissolved | 1.16 (.11) | .13 (.04) | < .01 | .33 (.10) |
| .037% | Total (1) | .86 (.20) | .19 (.04) | .79 (.14) | 1.59 (.36) |
| | Dissolved | .78 (.16) | .071 (.02) | < .01 | .35 (.13) |
| | Total (2) | .83 (.18) | .19 (.04) | .74 (.21) | 1.55 (.35) |
| | Dissolved | .73 (.11) | .063 (.02) | < .01 | .24 (.11) |
| .021% | Total (1) | .45 (.01) | .11 (.02) | .44 (.08) | 1.05 (.13) |
| | Dissolved | .39 (.01) | .052 (.01) | < .01 | .45 (.07) |
| | Total (2) | .45 (.02) | .10 (.01) | .40 (.05) | .94 (.08) |
| | Dissolved | .38 (.02) | .053 (.00) | < .01 | .42 (.03) |
| .012% | Total (1) | .34 (.08) | .060 (.01) | .24 (.17) | .70 (.07) |
| | Dissolved | .24 (.00) | .026 (.002) | < .01 | .30 (.03) |
| | Total (2) | .32 (.06) | .063 (.01) | .10 (.14) | .63 (.06) |
| | Dissolved | .24 (.01) | .035 (.001) | < .01 | .33 (.02) |
| .007% | Total (1) | .27 (.12) | .041 (.011) | < .01 | .59 (.06) |
| | Dissolved | .14 (.01) | .012 (.008) | < .01 | .27 (.03) |
| | Total (2) | .18 (.02) | .026 (.001) | < .01 | .43 (.06) |
| | Dissolved | .14 (.00) | .012 (.008) | < .01 | .27 (.05) |
| Control | Total (1) | < .01 | < .01 | < .01 | .19 (.11) |
| | Dissolved | < .01 | < .01 | < .01 | .11 (.04) |
| | Total (2) | < .01 | < .01 | < .01 | .29 (.10) |
| | Dissolved | < .01 | < .01 | < .01 | .11 (.03) |

[a] Numbers in parentheses indicate replicates.
[b] Numbers in parentheses represent standard deviations.

## APPENDIX 4—Concentrations of Zinc, Copper, Aluminum, and Iron in Bioassay Aquaria During 96-hr Bioassay on 1:12:18 Copper-to-Zinc-to-Aluminum Ratio Waste

| Concentration of waste | | Zn (mg/l) | Cu (mg/l) | Al (mg/l) | Fe (mg/l) |
|---|---|---|---|---|---|
| .100% | Total (1)[a] | 1.28 (.53)[b] | .11 (.03) | 1.38 (.46) | 2.45 (.58) |
| | Dissolved | 1.18 (.54) | 042 (.04) | < .01 | .51 (.64) |
| | Total (2) | 1.29 (.52) | .10 (.05) | 1.21 (.72) | 2.29 (.91) |
| | Dissolved | 1.15 (.55) | .043 (.04) | < .01 | .57 (.68) |
| 056% | Total (1) | .76 (.31) | .057 (.031) | .73 (.36) | 1.29 (.48) |
| | Dissolved | 64 (.26) | 013 (.017) | < .01 | 29 (.14) |
| | Total (2) | .75 (.31) | .057 (.031) | .53 (.41) | 1.27 (.46) |
| | Dissolved | .69 (.31) | .021 (.015) | < .01 | .29 (.15) |
| .032% | Total (1) | .44 (.17) | .032 (.028) | .32 (.23) | .93 (.32) |
| | Dissolved | .38 (.16) | .018 (.012) | < .01 | .35 (.16) |
| | Total (2) | .56 (.25) | .039 (.023) | .37 (.28) | 1.05 (.36) |
| | Dissolved | .38 (.16) | .015 (.012) | < .01 | .32 (.17) |
| .018% | Total (1) | .30 (.11) | .024 (.017) | .21 (.15) | .66 (.25) |
| | Dissolved | .23 (.09) | .006 (.008) | < .01 | .28 (.16) |
| | Total (2) | .43 (.25) | .021 (.015) | .21 (.15) | .55 (.17) |
| | Dissolved | .22 (.09) | .006 (.008) | < .01 | .27 (.15) |
| .010% | Total (1) | .25 (.13) | .012 (.008) | < .01 | .35 (.11) |
| | Dissolved | .15 (.06) | < .01 | < .01 | .22 (.09) |
| | Total (2) | .33 (.25) | .012 (.008) | < .01 | .36 (.08) |
| | Dissolved | .15 (.06) | .006 (.008) | < .01 | .19 (.10) |
| Control | Total (1) | < .01 | < .01 | < .01 | .10 (.01) |
| | Dissolved | < .01 | < .01 | < .01 | .09 (.02) |
| | Total (2) | < .01 | < .01 | < .01 | .13 (.02) |
| | Dissolved | < .01 | < .01 | < .01 | .08 (.02) |

[a] Numbers in parentheses indicate replicates.
[b] Numbers in parentheses represent standard deviations.