Aquatic Insects as Biological Monitors of Heavy Metal Pollution

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Heavy metal fish-kills pose a complex set of problems for the investigating biologist and the agency charged with jurisdiction. Heavy metal fish-kills are most often caused by acute exposure to a high concentration of metal over a short time period. First visual signs of a fish-kill (dead fish) are usually evident many hours or days after the pollutant has passed and is diluted beyond detection. In the case of a deliberate spill by unscrupulous industry, the dump is usually timed to coincide with a weekend or holiday when enforcement personnel are off guard or on vacation. Fixing blame in such a case may be an impossible task for even the most competent investigator.

NEHRING (1973)* felt aquatic insects used as biological monitors of heavy metal fish-kills must fulfill three prerequisites: 1) the insects should be more tolerant of the heavy metal than the fish in question; 2) the insects must concentrate the toxic metal in relative proportion to the metal content of the water; and 3) the insects must concentrate the metal pollutant by some predictable factor over a short time period. Fulfilling these three requirements aquatic insects should prove useful as biological monitors of heavy metal pollution.

The purpose of the study was to: 1) examine the toxicity of heavy metals (copper, lead, zinc, and silver) in two species of aquatic insects; 2) compare metal toxicities between fish and these aquatic insects, and 3) evaluate aquatic insects as biological monitors of heavy metal pollution.

METHODS AND MATERIALS

The test aquatic insects were a mayfly, (Ephemeroptera) Ephemerella grandis, and a stonefly (Plecoptera) Pteronarcys californica. The test apparatus, a proportional flow through diluter (MOUNT and BRUNGS 1967), provided five concentrations and one control (each 8 liters) and delivered two liters of toxicant every 90 seconds, giving a turnover of toxicant every six minutes (SPRAGUE 1969). Stock solutions of toxicant lead, zinc, copper, and silver were prepared from reagent grade $Pb(NO_3)_2$, $ZnSO_4 \cdot 7\ H_2O$, $CuSO_4 \cdot 5H_2O$, and $AgNO_3$, respectively.

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A common water supply for both holding tank and test apparatus allowed continuous acclimation of the naiads, eliminating the need for lengthy acclimation periods prior to testing. Ten stonefly naiads were used per concentration. The effects of size, age, and sex were minimized by measuring the naiads to insure the same mean body length in each concentration. The same sex ratios were used between concentrations within a test. For the mayfly bioassays, 50 naiads were used per concentration. Due to the very small size of the mayfly (less than 0.01 g dry weight) pooled samples of several specimens were used in many mayfly metal analyses.

After test initiation, each concentration was checked five times daily for deaths. Dead naiads were removed, rinsed in distilled water, and dried to a constant weight. Specimens were then weighed, inserted in a digestion vial, digested and analyzed. The digestion procedure (ADRIAN 1971) was modified as described by NEHRING (1973). Insect digestions were analyzed by flame atomic absorption spectrophotometry.

Water quality analyses, according to Standard Methods (1971), were run at test initiation, midway through the test, and at test termination. Dissolved oxygen varied from 7 - 12 mg/l between tests but not more than 0.5 mg/l between concentrations within a test. Temperature within a test varied less than 0.5 C between concentrations, but varied from 3 - 9 C between bioassays. Conductivity varied from 130 - 340 umhos/cm both between and within tests due primarily to the metal concentration being tested. With the exception of one copper bioassay (pH = 6.3) all bioassay pH values were 7.0 to 7.2. Alkalinity and hardness, measured as mg/l $CaCO_3$, varied from 30 mg/l to 70 mg/l between tests, but not more than 10 mg/l within a test. Test water samples were analyzed for metal content daily by flame atomic absorption spectrophotometry as described previously (NEHRING *ibid.*)

RESULTS AND DISCUSSION

GOETTL, *et al.* (1971), MOUNT and STEPHAN (1969), and MCKIM and BENOIT (1971) tested the effects of copper on various species of fish. GOETTL, *et al*, (*ibid.*, 1972) tested the effects of lead on rainbow trout. SPRAGUE (1964), BRUNGS (1969), and GOETTL, *et al.* (*ibid.*) tested the effects of zinc on various species of fish. GOETTL, *et al.* (1974) tested the effects of silver on rainbow trout. Comparison of the TL_{50} values for lead, zinc, copper, and silver to fish (found by the above investigators) with the TL_{50} values of these metals to the aquatic insects (Table 1) reveal aquatic insects to be more tolerant of all heavy metals tested with the exception of silver. The mayfly was less tolerant of silver than rainbow trout.

Average exposure levels and corresponding average accumulation levels for copper, lead, silver, and zinc are presented in Tables 2 through 5 below. The proportionality of the exposure level and the average accumulation level is quite recognizable. Comparison of the average exposure levels and average accumulation levels within each test (Tables 2 - 5) reveal the level of accumulation in the insect is a factor of 100 or greater than the level of exposure. This greatly simplifies analysis procedures when detection of heavy metal residues below 1 mg/l become difficult.

In each test the average level of exposure was paired with the corresponding average accumulation level in the insect. These data were subjected to linear regression analysis. The correlation coefficients in seven of the 14 bioassays were 0.97 or greater (Table 6).

TABLE 1 Insect TL_{50} Values (14 day) for Copper, Lead, Silver, Zinc.

Test Metal	Test Insect	TL_{50} Values (mg/l)
Copper	mayfly	0.18 - 0.20
Copper	stonefly	10.1 - 13.9
Lead	mayfly	3.5
Lead	stonefly	greater than 19.2
Silver	mayfly	less than 0.001
Silver	stonefly	0.004 - 0.009
Zinc	mayfly	greater than 9.2
Zinc	stonefly	greater than 13.9

TABLE 2 Copper Bioassays, Average Exposure vs. Average Accumulation

Mayfly		Stonefly	
Exposure (mg/l)	Accumulation (ug/g)	Exposure (mg/l)	Accumulation (ug/g)
10.0	9125	12.2	2540
4.82	5787	10.4	2096
2.51	3882	8.13	1767
1.22	1933	6.47	1199
0.63	1240	----	----
0.00	94.7 [1]	0.00	122.3 [1]

TABLE 3 Lead Bioassays, Average Exposure vs. Average Accumulation

Mayfly		Stonefly	
Exposure (mg/l)	Accumulation (ug/g)	Exposure (mg/l)	Accumulation (ug/g)
9.24	104,700	19.2	8172
4.90	73,200	7.44	2249
2.34	31,780	4.43	1666
1.32	14,560	1.96	736.6
0.69	5,702	1.08	716.7
0.00	126.6 [2]	0.00	8.18 [2]

[1] Natural background copper and zinc levels.
[2] Due to holding tank contamination by lead base paint chips.

TABLE 4 Silver Bioassays, Average Exposure vs. Average Accumulation

Mayfly		Stonefly	
Exposure (mg/l)	Accumulation (ug/g)	Exposure (mg/l)	Accumulation (ug/g)
0.75	65.31	0.738	53.28
0.40	36.65	0.399	30.76
0.23	47.97	0.217	22.95
0.12	28.73	0.105	13.62
0.06	25.32	0.050	9.13
0.00	0.00	0.000	3.97 [3]

TABLE 5 Zinc Bioassays, Average Exposure vs. Average Accumulation

Mayfly		Stonefly	
Exposure (mg/l)	Accumulation (ug/g)	Exposure (mg/l)	Accumulation (ug/g)
9.20	2361	13.6	561.2
4.32	2381	5.54	497.1
2.29	2187	2.83	415.7
1.04	2029	1.61	507.7
0.60	1794	0.77	439.4
0.00	1116 [1]	0.00	357.2 [1]

TABLE 6 Bioassay Parameters and Correlation Coefficients (r).

Test Metal	Test Insect	Range of Exposure (metal in mg/l)	Correlation Coefficient
Copper	Stonefly	0.74 - 13.9	0.986
Copper	Stonefly	5.51 - 18.5	0.901
Copper	Stonefly	6.47 - 12.2	0.994
Copper	Mayfly	0.63 - 10.0	0.982
Copper	Mayfly	0.08 - 1.06	0.974
Lead	Stonefly	1.08 - 19.2	0.991
Lead	Mayfly	0.69 - 9.24	0.985
Silver	Stonefly	0.05 - 0.74	0.996
Silver	Stonefly	0.004-0.067	0.909
Silver	Stonefly	0.006-0.104	0.830
Silver	Mayfly	0.06 - 0.75	0.893
Silver	Mayfly	0.01 - 0.15	0.666
Zinc	Stonefly	0.77 - 13.6	0.779
Zinc	Mayfly	0.60 - 9.20	0.694

The correlation coefficients (Table 6) indicate aquatic insects accumulate heavy metals in relative proportion to the metal concentration in the water. With the exception of silver, the aquatic insects tested were more tolerant of lead, zinc, copper and silver

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[3] Contamination from unknown source

than fish tested under similar water qualities. Thus, aquatic insects satisfy two of the three prerequisites cited earlier. The last prerequisite was that aquatic insects must concentrate heavy metals by some predictable factor over a short time period.

This predictable factor, termed the " concentration factor" , is determined by dividing the average level of exposure into the average level of metal accumulation in the insect. Considering the stonefly copper bioassay (Table 2), each level of accumulation divided by the corresponding level of exposure yields concentration factors (control not included) ranging from 185 to 217. The average concentration factor (202.6) is divided into the average levels of metal accumulation in another bioassay where the same metal and aquatic insect were used, giving estimated levels of exposure. Similar operations were performed on seven bioassays listed in Table 6.

The concentration factor is very effective in estimating the average level of exposure to lead, copper, and silver (Table 7). In 19 of 28 instances the concentration factor estimated the actual level of exposure with an accuracy of 80% or better. In 10 of 28 instances the concentration factor estimated the actual level of exposure with an accuracy of 90% or greater.

TABLE 7 Effectiveness of Concentration Factors in Estimation of Average Levels of Exposure to Lead, Copper and Silver.

Percent Accuracy	Frequency
50 - 59%	1/28
60 - 69%	3/28
70 - 79%	5/28
80 - 89%	9/28
90 - 99%	10/28

Thus, aquatic insects as tested here do concentrate heavy metals by some predictable, reproducible factor. But the truly critical test comes when laboratory results and techniques are tested under field conditions.

To test the concentration factor hypothesis, we selected Willow Creek, a tributary of the Rio Grande near Creede, Colorado. This stream is naturally polluted with 1 - 2 mg zinc/liter. (GOETTL, et al. ibid.) A laboratory concentration factor (1950) was calculated from the fourth concentration (1.04 mg/l) of the mayfly zinc bioassay (Table 5).

Several hundred mayfly naiads (Ephemerella grandis) were put in a livebox in Willow Creek. These mayflies were cropped off at 24 hour intervals starting 49 hours after test initiation and continuing until 404 hours of exposure. The mayfly mean zinc accumulation was divided by the zinc concentration factor of 1950 to derive an estimated zinc exposure level. During the test, actual levels of exposure to zinc were monitored on a 24 hour basis, enabling a comparison of estimated exposure levels for zinc (calculated with the concentration factor) and actual zinc exposure levels (Figure 1). The two lines in Figure 1 are virtually parallel. The concentration factor hypothesis appears valid under field conditions, at least under the conditions of this test.

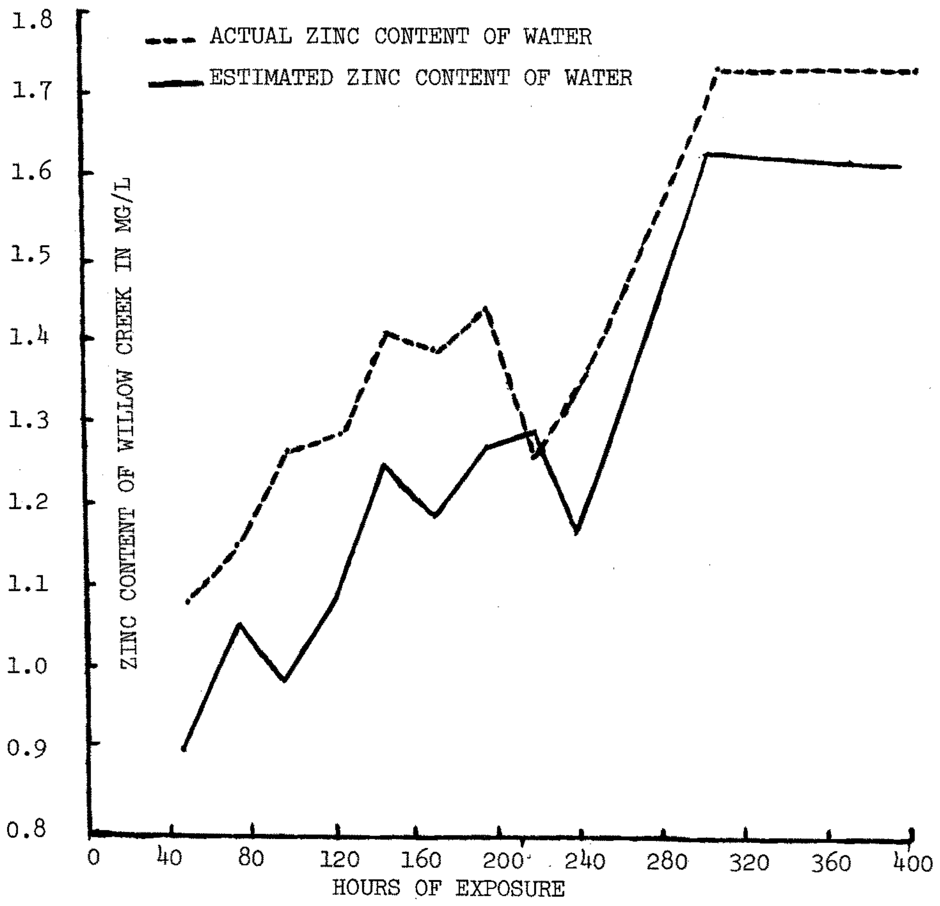

FIGURE 1. Actual vs. Estimated levels of zinc exposure to mayflies in Willow Creek.

In 1971 another field test was made. Above the confluence with Willow Creek the Rio Grande averaged 0.3 - 0.6 mg zinc/l over a two year period. Willow Creek, in contrast averaged 1 - 2 mg zinc/l over the same time period (GOETTL, _et al._, _ibid._). Approximately 1/4 mile below the confluence of these streams an island lies in the middle of the river. Due to a minimum of turbulence and laminar flow of the water, little mixing occurs until one mile downstream from the island. Since Willow Creek enters the river from the north, the water flowing around the north side of the island should have a higher metal content. Although zinc is the main pollutant, Willow Creek also has higher levels of copper and lead than the Rio Grande. Cast exoskeletons of stonefly naiads collected from the north side of the island contained mean accumulations of lead, zinc, and copper of 1117, 1260, and 38.78 ug metal/gram tissue, respectively. Stonefly exoskeletons from the south side of the island contained mean accumulations of lead, zinc, and copper of 854, 941, and 28.07 ug metal/gram tissue, respectively. A t-test analysis of these pairs of data revealed statistically significant p values of 0.10, 0.05, and 0.05 for lead, zinc, and copper, respectively (NEHRING 1973).

Under the conditions of these tests, aquatic insects appear to be excellent biological monitors of heavy metal pollution. They are more tolerant of metals than fish, they accumulate metals in relative proportion to the metal concentration in the water, and they concentrate the metal by some predictable, reproducible factor. As such they present the investigating biologist on heavy metal fish-kills with a most useful tool. Aquatic insects are always on site monitoring the heavy metal content of the water, regardless of the presence or absence of the investigating biologist. With some preliminary work regarding a particular species of insect and heavy metal in question, the investigating biologist may be able to determine the metal content of the water " after the fact" , even though the current has long since swept away the much needed water sample with the metal causing the fish-kill.

ABSTRACT

A mayfly, Ephemerella grandis, and a stonefly, Pteronarcys californica, were exposed to lead, zinc, copper, and silver to determine the acute metal toxicities. The insects tested were found to be more tolerant of the heavy metals than most fish. They concentrated the metals in relative proportion to the occurrence of the metals in the stream by some predictable, reproducible factor. These data, together with field tests, indicate aquatic insects may serve as effective biological monitors of heavy metal pollution where fish-kills are involved.

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