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Comparison of Recirculating, Static, and Elutriate Aquatic Sediment Bioassay Procedures

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Aquatic sediments can contain concentrations of contaminants due to cultural influences that are higher than background water column levels and these contaminants could disrupt natural biological communities (Adams *et al.* 1992). Acute sediment toxicity tests have become more common as a means of measuring potential harm to biological communities (Burton 1991). They have been proposed as sentinels of potential harm and are also becoming important in determining the need for sediment quality criteria (Chapman 1986). Testing procedures have included numerous techniques such as static tests, flow-through tests, recirculating tests, and elutriate tests (Burton 1991; Ingersoll and Nelson 1990; Mac *et al.* 1990; Malueg *et al.* 1984a; Nebeker *et al.* 1984).

The purpose of this paper is to compare results of tests using recirculating, static and elutriate techniques for measuring the toxicity of various sediments to the cladoceran *Daphnia magna. Daphnia,* although not sediment-dwelling organisms, have shown their usefulness in such tests (ASTM 1994; Malueg *et al.* 1983; Nebeker *et al.* 1984). Recirculating test data from Green Bay and Columbia Slough and static and elutriate test data from these two locations in addition to Torch Lake, Phillips Chain of Lakes System, Little Grizzly Creek System and the Keweenaw Penninsula are newly reported here. Complete mortality and sediment chemistry data from the other recirculating, static and elutriate tests in this paper may be found in Malueg *et al.* (1984a; 1984b) and Chapman *et al.* (1986).

MATERIALS AND METHODS

The recirculating tests were conducted in an Anderson-Prater type apparatus which consisted basically of a rectangular glass chamber placed over two 4-L jars (Malueg *et al.* 1983). A 5-cm layer of sediment was placed in the chamber and overlain with water. Water was continuously aerated and recirculated with an air-lift pump between the sediment chamber and jars. After a day of equilibration, five *Hexagenia* nymphs were placed in the sediment for 10 d. At the same time, 20 *Daphnia* (< 24 hr old) were placed in a 90-mL screened glass cup in the

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water path above the sediment surface. Two *Daphnia* tests were conducted, the first during the initial 48 hr and the second during the last 48 hr of the 10-d period. Tests were run in an environmental room held at 20 ± 1°C and a 16:8 1ight:dark cycle.

In the static tests 200 mL of sediment were added to 1-L glass beakers (Nebeker *et al.* 1984); water (800 ml) was gently poured over the sediment. The beakers were then placed in a water bath at 19.5 ± 0.5°C and 16-hr light photoperiod. Dissolved oxygen was maintained near saturation in the test beakers by slowly bubbling air through glass-tipped plastic air lines extending 2 cm below the water surface. The beakers were covered to minimize evaporation. Fifteen *Daphnia* (£ 24 hr old) were introduced into each test beaker the day after sediment and water were added for all static tests except for Columbia Slough where 10 daphnids per beaker were added. *Daphnia* were exposed during the first and last 48-hr intervals of a 10-d test period and were removed from the beakers with a pipette. No daphnids were exposed from Day 3 through Day 7.

Water for the elutriate tests was prepared by shaking one part wet sediment with four parts of water (volume/volume) in a 1.9-L glass jug at about 100 oscillationslmin for 30 min (Nebeker et al. 1984). The mixture was then allowed to settle for approximately 18 hr. The supernatant was siphoned off and centrifuged at 10,000 rpm (16, 300 G) for 15 min at about 10 to 15° C. Two-hundred milliliters of centrifugate were poured into each of three replicate 250-mL beakers for each sample site. The beakers were placed on a laboratory bench at a temperature of 19.5 ± 0.5°C and a 16 hr photoperiod. Air was supplied by glass-tipped plastic lines which were submerged about 1 cm below the water surface. Beakers were uncovered. Ten *Daphnia* (1.24 hr old) were introduced into each replicate test beaker and exposed for 48 hr.

Torch Lake, a 1,077 ha lake in Houghton County on the Keweenaw Peninsula of Michigan, historically has been contaminated with copper mining tailings (Wright *et al.* 1973). The Torch Lake sediment was collected from a single site with a Ponar grab sampler in 1982 (Malueg *et al.* 1984a). The Keweenaw Waterway, which bisects the Keweenaw Peninsula of Lake Superior, Michigan, has also received extensive deposits of copper tailings. Sediment samples were collected in 1982 by Ponar grab at five stations at 700-m intervals in mid-channel at each end of the waterway (Malueg *et al.* 1984b). Samples from the Little Grizzly Creek system, Plumas County, California, were collected in 1982 at 10 sites by scraping surface sediment into jars (Malueg *et al.* 1984a). Most of the sites had been contaminated by copper tailings. Samples from the Phillips Chain of Lakes system, Price County, Wisconsin, were collected in 1982 with a Ponar grab from six sites (Malueg *et al.* 1984a). Historically, some of the sites have received waste water containing elevated heavy metal concentrations as well as treated domestic sewage. Sediment from the Green Bay, Wisconsin, area was collected in 1982 from seven sites in the Fox River and Green Bay. The Fox River sediments were collected from the mouth to 4.2 km upstream. The Green Bay

samples were collected from 1.9 to 6 km north and east of the mouth of the Fox River. Green Bay sediments in general were classified by the U.S. EPA as moderately to heavily polluted to a point 14.5 km lakeward from the mouth of the Fox River (Sullivan and Delfino 1982). Sediment was collected from three sites from Columbia Slough, Portland, Oregon, in 1983 by an Ekman grab. The slough had been impacted by many years of industrial organic discharges, natural organic deposition, and poor flushing (DEQ State of Oregon 1972). Sediments from Toronto and Toledo harbors were collected with a box corer in 1983 as part of a bioassessment study involving several laboratories (Chapman *et al.* 1986). The sediments tested were received as pooled composites from four sites in each harbor. Control sediments for all the tests, with the exception of the Toronto and Toledo harbors recirculating tests and all the Columbia Slough sites, were obtained from Soap Creek Pond No. 7, located 12 km north of Corvallis, OR, in a semi-restricted rural area with no known sources of pollution. Control sediments for the exceptions was obtained from Porter Lake, a small rural oxbow lake 13 km south of Corvallis Oregon.

Water for all tests was obtained from a well near the EPA's Willamette Research Station in Corvallis, OR. This water typically has a median pH of 6.8, mean total hardness of 34 mg/L as $CaCO_3$ and a mean total alkalinity of 31 mg/L as $CaCO_3$ (Samuelson 1976). Trace metal concentrations were below or near detection limits.

The test organisms, the cladoceran *Daphnia magna*, were ≤ 24 hr old at the initiation of the toxicity tests. They were reared and handled according to procedures outlined by Nebeker *et al.* (1984) and Cairns *et al.* (1984). Control mortality was generally less than 10% (Table 1). Higher values were accepted in the case of the controls for Green Bay Recirculating I (13 %) and Static II (18 %) tests because of the similarity and relationships between the test results for these two methods.

Three replicates of sediment were run in each toxicity test. A Chi-square analysis which compares the homogeneity of means of binomial populations was used to test for significant ($p = 0.05$) differences between pooled control and pooled test survival. A Chi-square value of ≥ 3.84 was assumed to indicate mortality due to more than chance alone and caused by toxic material in the sediment. The frequency of similar results (regardless of whether there was or was not a significant difference between test and control sediments) between recirculating, static, and elutriate tests was determined. The number of times these tests resulted in similar conclusions was then divided by the total number of tests within any one comparison set. For instance, in Table 2 there was a total of 11 comparisons between the initial and final recirculating daphnid tests. Out of these 11 comparisons, 9 resulted in similar conclusions; the frequency of similarity between these tests was thus 9/11 or 0.82. The ratio of toxic to non-toxic sediments within the number of similar comparisons was also determined. For instance, in the previous example, the ratio of toxic to non-toxic sediments was

Table 1. Pooled mortality (dead/total) of *Daphnia magna* exposed to various sediments in three types of sediment tests.

Site		Recirc. I[a]	Recirc. II[b]	Static I[c]	Static II[d]	Elutriate[e]
Torch Lake[f]		58/60	60/60	44/45	45/45	30/30
	Control	1/60	1/60	3/45	1/44	0/30
Keweenaw[g]	11N	22/60	58/60	29/45	1/45	30/30
	12N	42/60	0/60	45/45	24/45	30/30
	15N	3/60	56/60	45/45	45/45	19/30
	Control	0/60	2/60	1/45	1/45	0/30
Grizzly Cr.[f]	G2	60/60	60/60	45/45	45/45	30/30
	G3	60/60	60/60	45/45	45/45	30/30
	G8	60/60	60/60	45/45	45/45	30/30
	Control	0/60	0/60	0/45	0/45	0/30
Phillips[f]	E1	10/60	4/60	33/45	37/45	4/30
	E2	0/60	2/60	5/45	0/45	2/30
	L5	3/60	0/60	3/45	11/45	2/30
	W3	1/60	0/60	2/45	4/45	3/30
	Control	0/60	0/60	0/45	2/45	4/30
Green Bay	1	5/60	7/60	5/45	3/30	14/30
	2	5/60	6/60	3/45	6/46	0/30
	4	10/60	15/60	6/45	4/45	5/30
	Control(1-4)	8/60	1/60	5/45	8/45	1/25
	5	10/60	1/60	-	-	6/30
	6	11/60	2/60	-	-	1/30
	7	17/60	4/60	-	-	18/30
	8	9/60	2/60	-	-	8/30
	Control (5-8)	1/60	0/60	-	-	1/25
Columbia Sl.	1	26/60	3/60	2/30	1/30	-
	2	26/60	0/60	5/30	0/30	-
	3	54/60	1/60	2/30	0/30	-
	Control	9/60[h]	0/60	25/30[h]	0/30	-
Toledo[i]		0/54	1/60	6/45	2/45	2/30
Toronto[i]		49/54	16/60	44/45	0/45	0/31
	Control	0/54	0/60	0/46	0/45	1/30

[a]Initial 48 hr of 10-d recirculating test. [b]Final 48 hr of 10-d recirculating test. [c]Initial 48 hr of 10-d static test. [d]Final 48 hr of 10-d static test. [e]48-hr elutriate test. [f]Recirculating test data from Malueg *et al.* (1984a). [g]Recirculating test data from Malueg *et al.* (1984b). [h]*Chaoborus* predation, Static II controls used in Static I calculations. [i]Data from Chapman *et al.* (1986).

Table 2. Frequency of similar results (significant or non-significant differences between test and control mortality, p = 0.05) between recirculating, static and elutriate sediment tests conducted with sediments contaminated primarily with metals from Torch Lake, Keweenaw Waterway, Little Grizzly Creek System and Phillips Chain of Lakes System.

	Recirc. I[a]	Recirc. II[b]	Static I[c]	Static II[d]
Recirc. II	0.82 (9/11)[e] 6:3[f]	-	-	-
Static I	0.82 (9/11) 7:2	0.73 (8/11) 6:2	-	-
Static II	0.73 (8/11) 6:2	0.73 (8/11) 6:2	0.73 (8/11) 7:1	-
Elutriate[g]	0.82 (9/11) 6:3	0.82 (9/11) 6:3	0.82 (9/11) 7:2	0.73 (8/11) 6:2

[a]Initial 48 hr of 10-d recirculating test. [b]Final 48 hr of 10-d recirculating test. [c]Initial 48 hr of 10-d static test. [d]Final 48 hr of 10-d static test. [e]Frequency of similar results (no. similar results/no. of comparisons). [f]Ratio of toxic to non-toxic sediments within the number of similar comparisons. [g]48-hr elutriate test.

6:3 for the 9 instances of similar conclusions. The ratio of toxic to non-toxic sediments was 1:1 in all instances of dissimilar conclusions.

RESULTS AND DISCUSSION

The frequency of similar test conclusions between all the different tests for sediments contaminated primarily by metals ranged from 0.73 to 0.82, with an average frequency of 0.78 ± 0.05 SD (Table 1). The frequency of similarity was higher for initial and final recirculating tests (0.82) than for initial and final static tests (0.73), probably due to decreased circulation in the beakers. The frequency of similarity between initial recirculating and initial static tests (0.82) was greater than for the frequency between final recirculating and final beaker tests (0.73). Less circulation and lack of particulate resuspension in the beaker could contribute to these results. The static tests did not contain *Hexagenia* which constantly bring particulates into the water column (Malueg *et al.* 1983). These results also suggest that in 10-d tests metal-contaminated sediments do not decrease in toxicity over time as might be expected with predominantly organic contaminated sediments prone to volatilization and potential degradation.

Table 3. Frequency of similar results (significant or non-significant differences between test and control mortality, p = 0.05) between recirculating, static, and elutriate sediment tests conducted with sediments contaminated primarily with organics from Green Bay, Columbia Slough, Toledo, and Toronto.

	Recirc. I[a]	Recirc. II[b]	Static I[c]	Static II[d]
Recirc. II	0.33 (4/12)[e] 2:2[f]	-	-	-
Static I	0.62 (5/8) 2:3	0.50 (4/8) 1:3	-	-
Static II	0.50 (4/8) 0:4	0.62 (5/8) 0:5	0.62 (5/8) 0:5	-
Elutriate[g]	0.56 (5/9) 2:3	0.56 (5/9) 2:3	0.40 (2/5) 0:2	0.80 (4/5) 0:4

[a]Initial 48 hr of 10-d recirculating test. [b]Final 48 hr of 10-d recirculating test. [c]Initial 48 hr of 10-d static test. [d]Final 48 hr of 10-d static test. [e]Frequency of similar results (no. similar results/no. of comparisons). [f]Ratio of toxic to non-toxic sediments within the number of similar comparisons. [g]48-hr elutriate test.

Similarly, Ingersoll and Nelson (1990) observed decreased survival in the crustacean *Hyalella azteca* exposed to predominantly metal-contaminated sediments in static tests for 29 d as compared to 10 d. The elutriate test comparisons (Table 2) were more similar to those obtained from recirculating tests and initial static tests (0.82) and less similar to comparisons obtained from final beaker tests (0.73), most likely due to decreased circulation and fewer particulates in the beaker tests. The ratio of toxic to non-toxic sediments within the number of similar results ranged from 6:2 to 7:2 and averaged 6.3 : 2:2 (74 % toxic : 26 % non-toxic).

The average frequency of similarity of test conclusions for sediments contaminated primarily by organic pollutants ranged from 0.33 to 0.80, with an average of 0.55 ± 0.1 SD (Table 3). The frequency of similarity was low (0.33) between initial and final recirculating tests, most likely due to volatilization between the initial and final test periods. The initial and final static tests had a higher frequency of similarity (0.62); the decreased circulation in the test chambers would not tend to drive off as much volatiles as in the recirculating tests. Test comparisons between initial recirculating tests and initial static tests (0.62) were the same as the comparisons between the final recirculating and final static tests (0.62). Elutriate frequency of similarity comparisons were more

similar to final static tests (0.80) and less similar to the other tests (0.40 to 0.56). This was in contrast to the predominantly metal-contaminated sediments where the elutriate test comparisons were least similar to the final static tests (Table 2). The ratio of toxic to non-toxic sediments ranged from 0:2 to 2:3 and averaged 0.9 : 3.4 (21% toxic : 79 % non-toxic).

The results suggest that a 48-hr exposure period after a day of sediment water equilibration is sufficient and that longer periods (i.e., 10 d) before daphnids are added can result in less toxicity due to contaminant volatilization. While the results are directly applicable to the number and type of particular sediments and tests used in this study, they suggest that recirculating type tests, static tests, and elutriate tests with *Daphnia* will generally result in similar conclusions regarding toxicity approximately three-quarters of the time in the case of sediments contaminated primarily with metals. Similar conclusions may be reached less often in the case of organic contaminants. The comparison method here, however, represents a simple approach for comparing results from different tests.

Renewal or static solid phase sediment toxicity tests, now part of guidelines for evaluating sediment toxicity (ASTM 1994; USEPA 1994) are generally easier to conduct than are the recirculating and elutriate tests. The static and recirculating tests also allow the animals to have direct access to the sediments. Solid phase tests may also be useful for the evaluations of sediments *in situ*. The recirculating test would be advantageous if more than one organism is to be tested simultaneously; it also provides more water for chemical analysis. The elutriate test may have advantages when there is little testing space available or if interest is primarily in evaluating resuspended sediments or in approximating potential problems associated with contaminant release to the liquid phase across the sediment-water interface. Ankley *et al.* (1991) have suggested, however, that pore water is a better predictor of sediment toxicity than elutriates.

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