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EVALUATION OF BIOASSAY ORGANISMS FOR FRESHWATER SEDIMENT TOXICITY TESTING

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INTRODUCTION

Sediment Criteria Project

The Washington Department of Ecology (Ecology) is developing criteria for contaminated freshwater sediments. As part of that effort, Ecology is currently evaluating various bioassays to determine which are most suitable for establishing criteria or guidelines.

The Washington Sediment Management Standards (WAC 173-204), adopted in the spring of 1991, establish a narrative standard for sediment quality statewide. The rule also contains chemical and biological criteria for sediments in Puget Sound, and a reserved section for the development of freshwater sediment criteria.

This report is an element of the freshwater sediment criteria development project being done by Ecology's Environmental Investigations and Laboratory Services Program (EILS) under contract to Ecology's Sediment Management Unit (SMU).

Note: This report is a reference document only. The publication of this information in no way implies that any numbers or methods contained herein are currently endorsed or recommended by Ecology for use in regulatory or permitting decisions.

Bioassays

Laboratory-based bioassays have been used to determine the effects of sediment contaminants on living organisms. Organisms with statistically significant responses to known concentrations of contaminants can indicate the likelihood of biological impacts in a contaminated environment. Results of these tests can be used to make regulatory decisions about permissible contaminant concentrations and exposure limits to sensitive organisms. However, conclusions from laboratory tests must be applied with caution and factors such as ecological relevance, effects of other contaminants, and species tolerance must be considered.

To evaluate different bioassay organisms and techniques, we conducted several bioassays at four highly contaminated sites in Washington State, in Steilacoom Lake, Lake Union, Lake Washington, and Lake Roosevelt. The studies in which these bioassays were applied were conducted for several programs within the Department of Ecology. With the help of many collaborators we were able to conduct up to 11 different bioassays on samples from depositional areas in these four freshwater lakes. This paper reports:

- the results of these bioassays;
- a review of the contaminant concentrations in the sediment samples used in these bioassays;
- the apparent effectiveness and sensitivity of each of these bioassays; and
- our recommendations regarding the most appropriate bioassays for evaluating freshwater sediments in Washington.

METHODS

Bioassay Organisms

Daphnia magna

Daphnia magna, a common "water flea", is predominantly a water column organism. However, it may spend an extensive amount of time feeding on the sediment surface ingesting particles down to 0.5 µm (Burton, 1991a). The organism is used in effluent studies, and the procedure has been adapted for sediment toxicity tests. The acute procedure used here was the 48-hour survival test, while the chronic procedure was the three brood, seven-day reproduction test. The bioassays were conducted according to American Society for Testing and Materials (ASTM) E1383-90, Draft Annex X4 Methods, *Daphnia* and *Ceriodaphnia* sp. (American Society for Testing and Materials, 1990).

Ceriodaphnia dubia

Ceriodaphnia dubia, a water flea closely related to *Daphnia magna* and with similar feeding habits, is also used in effluent testing and has been adapted for sediment work. It appears to be slightly more sensitive than *D. magna* and has a faster reproductive rate after birth (Burton, 1991a). As with *Daphnia*, the acute procedure used here was the 48-hour survival test, while the chronic procedure was the three brood, seven-day reproduction test. The bioassays were conducted according to ASTM E1383-90, Draft Annex X4 Methods, *Daphnia* and *Ceriodaphnia* sp. (American Society for Testing and Materials, 1990).

Hyalella azteca

Hyalella azteca, an amphipod, spends time both in the water column and feeding at the sediment surface (Nebeker and Miller, 1988). It is frequently used to determine freshwater sediment toxicity. The procedure used was the 14-day survival test, conducted according to ASTM E1383-90 (American Society for Testing and Materials, 1990).

Chironomus tentans

Chironomus tentans, a true fly larvae, (order Diptera) is a benthic organism frequently used for sediment toxicity tests because of its burrowing characteristics. Wentsel *et al.* (1978) demonstrated its capability of developing a degree of resistance to metal pollution. A review of the procedures, which consisted of ten-day survival (acute) and percent weight reduction (chronic) tests, can be found in Henry (1991a,b).

Hexagenia limbata

Hexagenia limbata, a burrowing mayfly nymph, (Order Ephemeroptera) is a benthic dweller used in sediment toxicity tests. It buries itself in sediment, unlike most of the other test organisms. One problem with *Hexagenia* is that it is unavailable during May and June and cannot be raised in the laboratory. Also, contaminant sensitivity may vary depending on the age of the organism being used (Sylvia Morse, Personal Communication). Procedures, which consisted of a ten-day survival test, can be found in Henry (1991a,b).

Ostracods

Ostracods (Order Ostracoda) have recently begun to be used in contaminated sediment bioassays. The justification for their use is based on the fact that many species spend part of their time burrowing, exposing them to contaminants through both direct contact and ingestion. There are as yet no established protocols for the ostracod bioassay. Tests performed on the Steilacoom Lake, Black Lake, and Quendall/Baxter sediments were conducted following methods found in Woodward *et al.* (In Draft).

Microtox®

Microtox® bioassays measure light output from a bioluminescent marine bacteria, *Photobacterium phosphoreum*, to indicate possible inhibition caused by contaminants. The basis of the test is that toxic components can inhibit enzymatic processes and cause a decrease in light intensity.

Results are reported as Effective Concentration 50% (EC_{50}). An EC_{50} is the percent of test material that, when mixed with a control, reduces the light output by 50%. However, other systems, such as the Washington State Sediment Management Standards and Puget Sound Dredged Disposal Analysis (PSDDA), disregard EC_{50} measurements and use a 20% light reduction from reference as the endpoint. Due to differences in analytical methods as well as a low number of replicates, a Microtox® EC50 response below 100%, in this study only, is defined to indicate toxicity. While not a statistically significant response, this approach does help us to determine if Microtox® can be used to detect toxicity in freshwater sediments. EC_{50} values greater than 100 (>100) generally indicate very low toxicity.

For these tests, Manchester Environmental Laboratory followed a modification of the Microtox® procedure used by the Puget Sound Estuary Program (1987). Another researcher involved in this project, Dr. Mary Henry of the University of Minnesota, used the 100% Assay Procedure (Microbics, Inc., 1989). Microtox® was originally designed for marine water samples, but has also been adapted to freshwater sediment studies using pore water and elutriate solutions. Data are usually reported based on a five minute and a 15 minute test.

Indigenous Microbial Enzyme Activities

Indigenous microbial enzyme activities, which may act as early-warning indicators of chronic toxicity, measure the ability of indigenous sediment bacteria to metabolize a nitrophenol-linked enzyme substrate. Results are usually reported in micrograms of nitrophenol metabolized per gram of sediment, dry weight. The four enzyme systems tested were: alkaline phosphatase (APA); dehydrogenase, or electron transport system activity (DHA); b-galactosidase (GAL); and b-glucosidase (GLU). Microbial enzyme activity is expected to be inversely correlated with contaminant levels. The test procedures are reviewed in Burton (1991b). Despite consistent results from one site, the ecological significance of microbial enzyme data remains unclear.

Other Potential Bioassay Organisms

An ideal organism for bioassays in the Pacific Northwest would be an indigenous benthic dweller with a high degree of sensitivity to the types and concentrations of sediment contaminants normally found in the Northwest. It would be easily and inexpensively

cultured, be readily available throughout the year, have excellent reliability, and be found throughout the depositional environment so that bioassay and benthic data could be correlated.

While this ideal organism has not yet been identified, R.W. Plotnikoff of the Washington State Department of Ecology suggests several possibilities. *Hexagenia* spp. can be found in limited distribution west of the Cascade Mountains in lakes north of Everett, WA. *Clistoronia magnifica* has also been found near sea level west of the Cascades. Both organisms are sensitive to sediment-bound toxicants in lakes and streams. *C. magnifica* is easier to rear in laboratory populations. He also suggests a third organism, *Sialis* sp., an alderfly. Further consideration of these organisms will depend on the ability to find reliable field collection sites as well as the results of sensitivity tests (Plotnikoff, 1991).

Plants, including the alga *Selenastrum capricornutum* and the duckweed *Lemna minor*, are used in sediment toxicity studies (Burton, 1991a). However, these two are essentially water-column organisms, and may require a transfer of contaminant into the water column before responding to sediment concentrations. Walsh *et al.* (1991) have used the freshwater marsh plant *Echinochloa crusgalli* in toxicity tests of both water and sediment. Two sets of parameters observed were seed germination and early growth, and survival and growth. The phototoxic effects of some test effluents were significant, similar to those seen with certain invertebrates (see Additional Factors Affecting Bioassays, below). We are investigating the addition of this or similar plants to our list of test organisms.

Additional Factors Affecting Bioassays

Ultraviolet Light Enhanced PAH Toxicity

Burton (1991c) reports significant increases in PAH toxicity to *Ceriodaphnia dubia* and *Daphnia pulex* when the organisms are exposed to ultraviolet light. This increased toxicity, which occurs following PAH uptake by the organisms through ingestion, adsorption, or other means, was demonstrated by Spacie (1991) who conducted *Ceriodaphnia* bioassays under ultraviolet lamps. Therefore, PAHs such as anthracene and benzo(a)pyrene may be much more toxic to aquatic organisms *in situ* from natural sunlight than in a laboratory bioassay under standard lighting. While the toxic products themselves have not yet been identified, Dr. Spacie believes that a highly reactive oxygen radical, capable of bursting cell walls, could be responsible. The use of ultraviolet lamps may become necessary in future laboratory bioassays to fully determine the magnitude of PAH toxicity.

Copper to Acid Volatile Sulfide Ratio (Cu/AVS)

The acid volatile sulfide (AVS) theory states that copper and certain other metals will be bound by sulfides present in sediment. Only when the molar ratio of copper to AVS (Cu/AVS) exceeds one will the free form of the metal (Cu^{2+}), considered the primary toxic species, become biologically available. According to theory, as the ratio increases, so does the toxicity of the sediment (DiToro *et al.*, 1990). However, data presented here, as well as work done by others (Ankley *et al.*, In Draft), indicate that results from field and lab studies are not consistent with theory. Determination of the true role of Cu/AVS in sediment toxicity requires further investigation.

Sampling

Sediment Sampling Procedures

Sampling procedures generally followed Puget Sound Protocols (Puget Sound Estuary Program, 1987). Sediment was retrieved with either a Ponar or Van Veen grab sampler. Both samplers were made of stainless steel. The top two centimeters of sediment not contacting the side walls of the sampler were spooned into a stainless steel bucket, homogenized, and transferred to certified contaminant-free containers. Samples were kept at 4°C until analyzed. All equipment had been cleaned prior to sampling using standard techniques. Refer to the individual reports for specifics regarding each project.

Sediment Sampling Locations

Steilacoom and Black Lakes:

Steilacoom Lake is an artificial impoundment covering about 320 acres in a residential area of Pierce County. Precipitate from copper sulfate algicide treatments has resulted in the accumulation of this metal in lake sediments reaching levels of at least 1100 mg/kg dry weight (Bennett and Cubbage, In Prep., a). Steilacoom Lake was chosen for the sediment criteria project because significant bioassay responses could be largely linked to copper, which is the predominant identified contaminant in the lake.

Sediment sampling and testing was done in two phases. During Phase I, sediments from 12 sites throughout the lake were collected. Sediments were submitted for analysis of total recoverable copper using nitric acid digestion (Puget Sound Estuary Program, 1987). Sediments were also submitted for bioassays using the amphipod *Hyalella azteca*, and for Cu/AVS analysis. Cu/AVS values were calculated from data supplied by EPA Duluth, which also performed the *Hyalella* bioassays (Ankley *et al.*, In Draft).

In Phase II, three sites were selected for more extensive sampling and testing based on the sediment copper concentrations and Cu/AVS ratios found in Phase I. Phase II samples were analyzed for total recoverable metals (arsenic, cadmium, chromium, copper, lead, mercury, nickel, and zinc) and were tested using a suite of bioassays (*Daphnia magna, Ceriodaphnia dubia, Hyalella azteca, Chironomus tentans, Hexagenia limbata,* ostracods, Microtox®, and indigenous microbial enzyme activity). Analyses were also performed for total recoverable pore water copper (centrifuged, unfiltered), since toxic effects have been traced to this parameter. Black Lake, a glacial remnant located in Thurston County, was the source of reference sediment.

Quendall/Baxter (Lake Washington):

The Quendall/Baxter site, on the eastern shore of Lake Washington near Renton, was the location of at least two significant creosote spills, both leading to high levels of contamination in the underlying sediments. Three separate studies were conducted at this site. For Quendall/Baxter Phase I, Norton (1991) investigated 18 sites, although sediment from only four sites was used for *Daphnia magna* and *Hyalella azteca* bioassays. Bennett and Cubbage (In Prep., b) sampled four sites in Quendall/Baxter Phase II and tested a suite of bioassays. For Quendall/Baxter Phase III, Norton (In Prep.) tested sediment from three sites using *H. azteca* and Microtox®.

Lake Union:

Lake Union, a lake located near downtown Seattle and surrounded by industrial and commercial businesses, was the site for two sediment studies. Lake Union Phase I, conducted by Yake *et al.* (1986) used one sediment sample from a creosote contaminated area near Gas Works Park and one bioassay organism, *H. azteca*. The reference sediment was from Chester Morse Lake, a reservoir located in a watershed protected as Seattle's drinking water source. Both Lake Union and Chester Morse Lake are in the Cedar River drainage system.

Lake Union Phase II, conducted by Cubbage (In Draft), collected sediments from 21 sites throughout Lake Union and conducted bioassays for *D. magna, H. azteca,* and Microtox® on eight sites. Reference sediment was obtained from adjacent Lake Washington. Land use near the reference site is largely residential.

Lake Roosevelt:

Lake Roosevelt is a large reservoir on the Columbia River near the Canadian border. Sediments in the lake are contaminated by a range of heavy metals discharged from a large lead and zinc smelter in British Columbia. Johnson *et al.* (1990) analyzed Lake Roosevelt sediments for metals and conducted bioassays using *D. magna, H. azteca,* and Microtox®. The primary contaminants of concern at this site were initially arsenic, cadmium, copper, lead, mercury, and zinc. Dioxins and furans were the object of a later study (Johnson, Personal Communication).

Bioassay Evaluation Criteria

In reviewing evaluation criteria for marine sediment bioassays, Pastorok and Becker (1989) provide an extensive discussion of seven bioassays (with a total of 14 endpoints) evaluated against the following 11 criteria: dose response, sensitivity, statistical power, cost-effectiveness, ecological relevance, ease of use, availability of test organisms, endpoint reliability, relationship to indigenous biota, holding constraints, and stage of protocol development. This evaluation benefitted from a large volume of data on marine toxicity tests with Puget Sound sediments and bioassay organisms. These data allowed the generation and comparison of statistical analyses for the various bioassays, some of which used multiple endpoints. The authors were able to come to definitive conclusions and make recommendations regarding the use of these bioassays for future studies.

The overall concordance among the bioassays was low, probably because different contaminants cause differential responses among organisms with various bioassay endpoints. The authors suggested that, since the various tests did not appear to be inherently interchangeable, a battery of tests should be used for sediment toxicity testing in Puget Sound. Use of the battery of tests approach has been recommended by other researchers in aquatic toxicology (Burton *et al.*, 1987; Dutka *et al.*, 1988).

The criteria evaluated in this report were selected from those in the document cited above that were most relevant to this project. The criteria determined to be of primary importance are reliability and ecological relevance. Those of secondary importance are cost, availability, ease, and time. These criteria are discussed below. The criteria selected for this study were chosen because they were relevant, and because adequate data were available for evaluation.

Other criteria, such as sensitivity and dose responsiveness, were not included at this time because they could not be quantified from available data.

Reliability

Reliability is the most important criterion for evaluating these bioassays. Reliability requires that a bioassay be both sensitive in detecting sediment contamination, and that it show a statistically significant, repeatable, direct response to sediment contaminant concentrations. Within reason, no other criteria should take precedence over reliability. [Note: the definition of reliability used in this report differs from the definition used previously in documents associated with the development and application of the Apparent Effects Threshold Method in the Washington Sediment Management Standards.]

Ecological Relevance

Ecological relevance is defined here as the degree to which the results of a laboratory bioassay on collected sediment give useful information about the biological effects of contaminants in that sediment under field conditions. Tests with low ecological relevance, though they might be consistent and sensitive, will have limited utility in predicting the response of organisms and communities that live in or attempt to colonize the sediments *in situ*. Although this criterion is presently qualitative, it may in the future be defined in a way that can be tested and quantified.

Cost

The cost for bioassays used in the present study ranges from $150 per sample for Microtox® to $840 per sample for *Daphnia*. While this is a considerable spread, the relative cost of a few, fairly expensive bioassays compared to the cost of an entire project including sediment characterization, planning, data interpretation, and report preparation, may be small.

Availability

Most of the bioassays in this group are readily available. Some, such as *Daphnia magna* or various microbial tests, are available in kit form. Others will undoubtedly become more available as they grow in popularity. *Hexagenia* is a special case since it is a seasonal organism, unavailable during May and June. This lack of availability may be inconvenient to projects wishing to use the organism. In general, however, availability is not a significant problem for most of these bioassays.

Ease

Ease is sometimes quoted as a criteria (Pastorok and Becker, 1989). Ease is a function of the complexity of the test, the care with which it has been devised, and the skill and training of the operator. For a bioassay procedure to be commercially feasible, each step is carefully considered and modified to make it as simple and consistent as possible. Most commercially available bioassays can be easily and consistently carried out by qualified lab personnel.

Time

Most bioassays require from one to 14 days of testing time to complete. Since the chemical analyses that usually accompany bioassays may have a turnaround time varying from weeks to months, the time for the bioassays themselves are not a major factor. Experience has shown that proper planning and scheduling by the samplers, as well as the lab conducting the bioassays, are the most important factors in receiving timely results. In cases where significant commitments to expensive items such as equipment and manpower have been made, however, time may be of the essence and bioassays should be chosen accordingly.

RESULTS

This section contains a summary of results from a number of site specific sediment studies conducted at contaminated sites throughout Washington State by the Toxics Section of EILS.

Each sediment project is named and, if there is more than one project per location, sequentially numbered. Only sites that were a source of bioassay results for sediments are listed in the tables. For these sites, data are also given on the concentrations of chemicals considered to be likely sources of potential toxicity. All acute bioassays use mortality as the endpoint, although the values given for each test are percent survival. Survival rates that are significantly lower than those for controls are determined using Dunnett's test with $p<0.05$ and are shown outlined in the appropriate tables.

Microtox® results are given as Effective Concentrations 50%, (EC_{50}), or the ratio of sediment that gives a 50% light reduction (see test description above). Usually, the more contaminated the sediment, the lower the percent of sediment needed to reduce the light output to the 50% level.

Sediment metal and total PAH concentrations that equal or exceed the Ontario Provincial Sediment Quality Guidelines are shaded in the corresponding table for each project. These sediment guidelines have been established by the Ontario Ministry of the Environment (Canada) for metals and assorted organics in freshwater sediments (Persaud *et al.*, 1991). For some contaminants, three levels of exposure have been established and the likely results of such exposure on benthic organisms determined. These are:

1) No-Effect Level: No toxic effects have been observed on aquatic organisms.
2) Lowest-Effect Level: This level of sediment contamination can be tolerated by most benthic organism.
3) Severe-Effect Level: Pronounced disturbance of sediment-dwelling organisms can be expected that would be detrimental to the majority of benthic species.

For metals and total PAH, only lowest-effect and severe-effect levels have been established. A summary of additional sediment guidelines can be found in FSEDCRIT (Bennett and Cubbage, 1991).

Sediment Sampling Results

Steilacoom and Black Lakes

Contaminant concentrations and results for Steilacoom Lake and Black Lake bioassays (*Daphnia magna, Ceriodaphnia dubia, Hyalella azteca, Chironomus tentans, Hexagenia limbata,* and Microtox®), are given in Table 1A. Indigenous microbial enzyme activity data are given in Table 1B and graphed in Figures 1 and 2.

All sediments from Steilacoom Lake exceeded the Provincial Sediment Quality Guidelines, severe-effect level for copper set at 110 mg/kg dry weight. The Black Lake sediment slightly exceeded the lowest-effect level of 16 mg/kg dry weight.

Steilacoom Lake Phase I found copper concentrations ranging from 183 to 1100 mg/kg dry weight. Cu/AVS levels ranged from 0.3 to 59. *Hyalella azteca,* the only bioassay tested using Phase I Steilacoom Lake sediments, showed no significant toxicity with any sediment (Ankley *et al.,* In Draft).

Phase II sediment sampling sites were chosen primarily to test the relationship of sediment toxicity to Cu/AVS ratios. The sites chosen, S2-4, S2-11, and S2-12, had ratios of 6.5, 26.9, and 0.6, respectively. Theory predicted an increase in toxicity with an increasing ratio. However, significant bioassay response by *Hyalella azteca* and *Hexagenia limbata* occurred only at site 12 which had the lowest Cu/AVS ratio. Total recoverable sediment pore water copper concentrations, with a maximum of 441 µg/l at site 12, are consistent with these responses. Indigenous microbial enzyme activities correlate well with total recoverable sediment pore water copper concentrations, marginally well with total recoverable dry weight concentrations of sediment copper, and not at all with Cu/AVS at these three sites. The pore water copper was not identified or quantified by individual ionic states or other chemical speciation. None of the bioassay results corresponded with Cu/AVS values. The most likely reason for this lack of correlation is that copper was bound by sediment components in addition to AVS. It was therefore not biologically available in the toxic, free (Cu^{2+}) form.

Black Lake sediment, with negligible copper levels, showed significant toxicity to *Daphnia magna, Ceriodaphnia dubia, Hyalella azteca,* and response by microbial enzymes. The Microtox® test also showed evidence of toxicity. Chronic tests with both *D. magna* and *C. dubia* showed a decline in production of young when compared with Steilacoom sediments. When Black Lake sediment was vigorously aerated for one hour, *C. dubia* survival increased from 30% to 100%, and production of young increased nearly five times (see Appendix A). Since there are no known toxins in Black Lake, it is presumed that either a naturally occurring volatile compound or a reducing agent, such as H_2S, was responsible for the toxicity (Burton 1991b).

Quendall/Baxter (Lake Washington)

Refer to Table 2 for analytical and bioassay results.

Four Quendall/Baxter sites exceeded the Provincial Sediment Quality Guidelines, severe-effect level of 11,000 mg PAH/kg organic carbon for total PAH. All other sites exceeded the lowest-effect level of 2 mg/kg dry weight.

Table 1A. Steilacoom Lake Phase II results – bioassays listed as percent survival.

Steilacoom/	Total Recoverable Copper											
Black Lake	Sediment	Pore Water		*D. magna*		*C. dubia*	*H. azteca*	*C. tentans*	*H. limbata*	Microtox		Microbial
Sample	mg/kg dry	ug/l	Cu/AVS	(acute)		(acute)	(acute)	(chronic/acute)	(acute)	EC50		Enzymes
S2-4	396	161	6.5	90	97	90	80	6, 15	90	>100	>100	Table 1B
S2-11	839	240	26.9	100	95	90	82	17, 14	100	a	a	"
S2-12	892	441	0.6	100	98	100	70	14, 15	50	a	a	"
B2-2 (Ref.)	24	11	-	60	97	30	67	8, 15	100	73	63	"
Control	-	-	-	100	100	100	90	0, 14	90	b	b	"

Note 1: Steilacoom Lake Phase I bioassays for Hyalella azteca were negative at all sites. Sediment copper range: 183-1100 mg/kg dry.

Note 2: Black Lake sediment was chronically toxic to Daphnia magna and Ceriodaphnia dubia, determined by reduced production of young (Appendix A).

Note 3: Daphnia magna (acute) data from Wright State University and Manchester Environmental Laboratory, respectively.

Note 4: Chironomus tentans - Chronic: Percent weight reduction. Acute: number of survivors out of 15 replicates.

EC50 - Ratio of sediment which gives a 50% light reduction.

Microtox (Manchester Lab)- EC50 values at 5 and 15 minutes, (a) - negative gammas indicating low toxicity, (b) - no EC50 calculable from data. Univ. of Minnesota Microtox data showed no toxicity at any site.

Indicates survival significantly lower than control; in the case of Microtox, EC50 less than 100%.

Exceeds Provincial Sediment Quality Guidelines, lowest-effect level for copper, 16 mg/kg dry weight.

Exceeds Provincial Sediment Quality Guidelines, severe-effect level for copper, 110 mg/kg dry weight.

Table 1B. Steilacoom Lake Phase II – microbial enzyme activities.

Steilacoom/	Total Recoverable Copper		Relative Enzyme Activity			
Black Lake	Sediment	Pore Water	(ug product/g dry wt. sed./time)			
Sample	mg/kg dry	ug/l	APA	DHA	GAL	GLU
S2-4	396	161	109	52	80	113
S2-11	839	240	69	25	49	62
S2-12	892	441	52	6	19	18
B2-2 (Ref.)	24	11	98	30	55	66
Control	-	-	ND	ND	ND	ND

Note 1: Analytical results are total metals.

APA = alkaline phosphatases; DHA = dehydrogenases (or electron transport system activity); GAL = b-galactosidase; GLU = b-glucosidase.

ND = Analysis Not Done.

Exceeds Provincial Sediment Quality Guidelines, lowest-effect level for copper, 16 mg/kg dry weight.

Exceeds Provincial Sediment Quality Guidelines, severe-effect level for copper, 110 mg/kg dry weight.

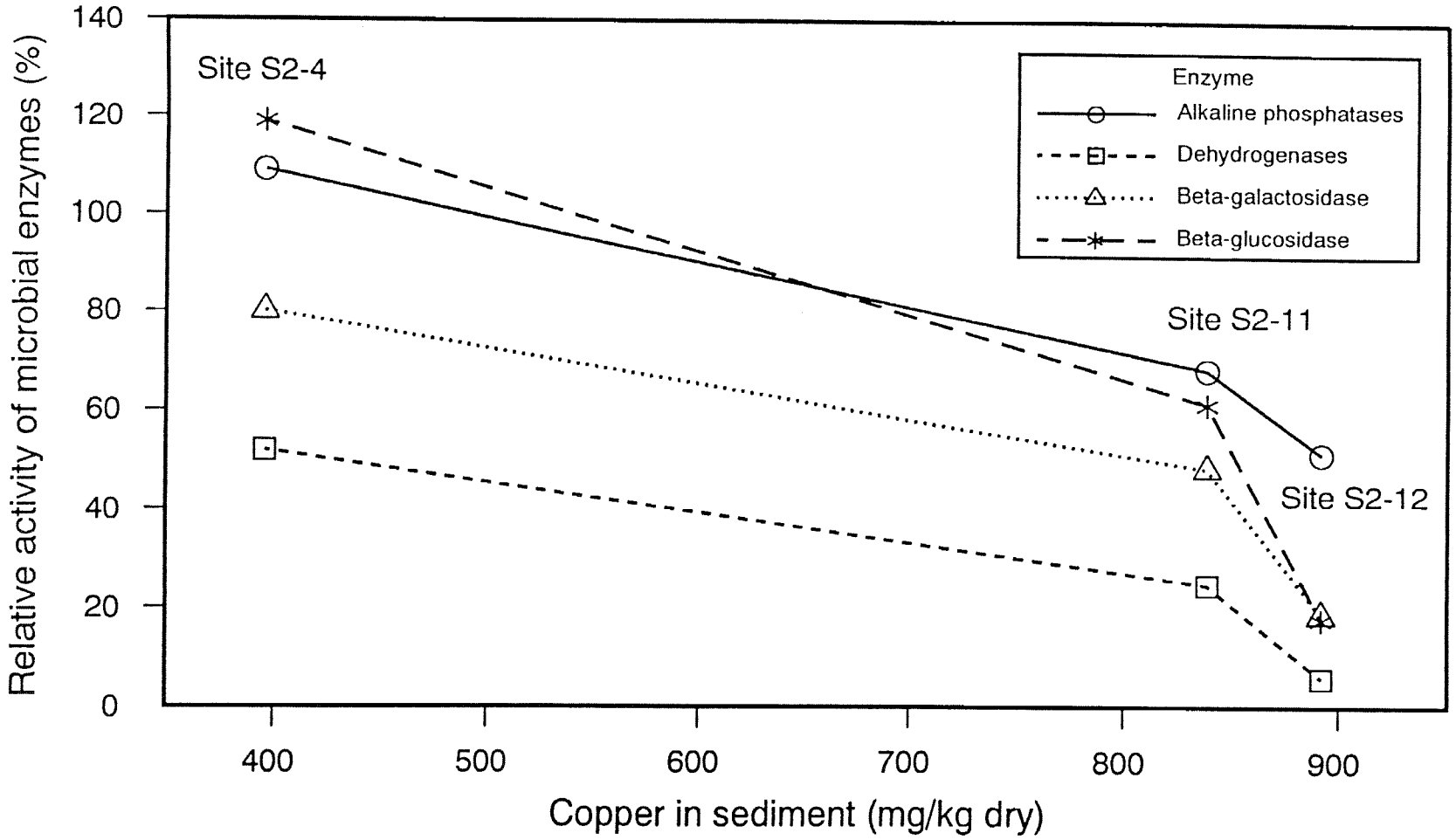

Figure 1. Bacterial enzyme activity vs. sediment copper concentrations.

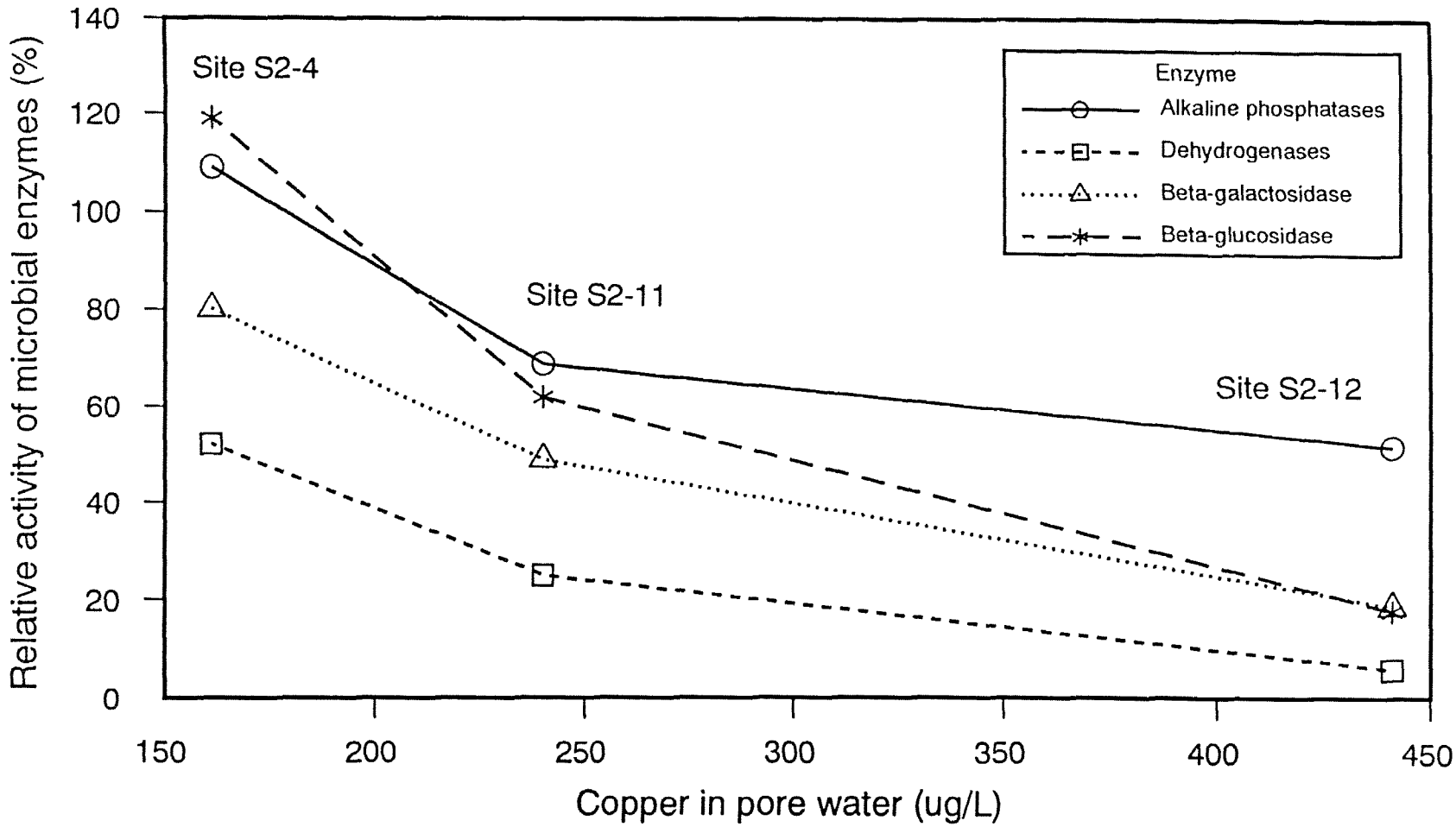

Figure 2. Bacterial enzyme activity vs. pore water copper concentrations.

Table 2. Quendall/Baxter I, II, III results – bioassays listed as percent survival.

Quendall/ Baxter Sample	Sediment LPAH, mg/kg Dry	Sediment LPAH, mg/kg TOC	Sediment HPAH, mg/kg Dry	Sediment HPAH, mg/kg TOC	*D. magna* (acute)	*C. dubia* (acute)	*H. azteca* (acute)	% Wt. Loss *C. tentans* (chronic)	*C. tentans* (acute)	*H. limbata* (acute)	Microtox** EC50		Microtox[2] EC50	
QB1-2	0.4	6	4	70	94	.	88	.	.	.	.	.	.	.
QB1-13	160	2,200	720	9,700	98	.	48	.	.	.	.	.	.	.
QB1-15	3.2	13	25	100	97	.	88	.	.	.	.	.	.	.
QB1-1 (Ref.)	0.6	15	7	190	98	.	70	.	.	.	.	.	.	.
QB2-5	2.2	128	27	1,600	95	90	75	50	53	100	19	21	84	77
QB2-12	16	730	112	5,100	85	90	75	8	73	80	30	29	63	62
QB2-13	55	4,200	290	22,300	77	70	32	23	80	90	66	77	38	43
QB2-3 (Ref.)	0.6	30	6.4	320	90	80	80	23	80	100	25	21	100	100
Control	-	-	-	-	80	90	92	0	80	85 avg.	a	a	.	.
QB3-B1	5,750	70,000	3,500	42,000	.	.	0	.	.	.	1.0	0.9	.	.
QB3-B2	20,000	200,000	13,000	130,000	.	.	0	.	.	.	0.1	0.3	.	.
QB3-R (Ref.)	0.25	4	3.3	58	.	.	50	.	.	.	33	24	.	.
Control	-	-	-	-	.	.	92	.	.	.	.	.	.	.

Dry = mg PAH/kg dry weight.

TOC = mg PAH/kg total organic carbon.

(.) No bioassay conducted.

Ceriodaphnia dubia (chronic) - no significant decrease in production of young.

EC50 - Ratio of sediment which gives a 50% light reduction.

**Microtox - Manchester Environmental Laboratory. EC50 values at 5 and 15 minutes. (a) stands for negative gammas, indicating low toxicity.

[2]Microtox - University of Minnesota. EC50 values at 5 and 15 minutes.

Indicates survival significantly lower than control; in the case of Microtox, EC50 less than 100%.

Exceeds Provincial Sediment Quality Guidelines, lowest-effect level for total PAH, 2 mg/kg dry weight.

Exceeds Provincial Sediment Quality Guidelines, severe-effect level for total PAH, 11,000 mg/kg organic carbon.

Hyalella azteca bioassays and two of the Microtox® bioassays showed apparent toxic responses correlating to high PAH levels and consistent with increasing contamination.

A significantly depressed *Hyalella* survival rate of 48% at site QB1-13, (11,900 mg PAH/kg organic carbon) corresponds well with the Provincial Sediment Quality Guidelines, severe-effect level of 11,000 mg PAH/kg organic carbon. Significant bioassay responses occurred at sites QB2-13, QB3-B1, and QB3-B2, all with PAH levels exceeding the Provincial Sediment Quality Guidelines. The 50% survival, a significant toxic response, at reference site QB3-QBR is without explanation.

Two of the three Microtox® bioassays showed direct correspondence between apparent toxicity and increasing PAH levels. These two were the Microtox® (University of Minnesota) analyses performed on the QB2 samples, and the Microtox® (Manchester Environmental Laboratory) analyses performed on the QB3 samples. However, apparent toxicity with the Microtox® (Manchester Environmental Laboratory) analyses for the QB2 sediments corresponded inversely to increasing PAH levels (and therefore correspond inversely to the University of Minnesota data). The source of these differences is not known. The QB3-QBR reference sediment also showed an apparent toxic effect, the source of toxicity being undetermined. *Ceriodaphnia dubia* at site QB2-13, the most heavily contaminated of the four sites in the series with 24,100 mg PAH/kg organic carbon, had a 70% survival rate. This value was not a significant depression from the control because of high data variance. Similar variability occurred with the chronic three brood production test (Burton, 1991c). *Chironomus tentans* had only a chronic response (50% weight loss) at one of the lesser contaminated of the four sites in the series (QB2-5, with about 1,600 mg PAH/kg organic carbon). No other bioassays gave significant responses with Quendall/Baxter sediments. Since PAH toxicity can be increased following uptake by aquatic organisms and exposure to ultraviolet light, *in situ* toxicity may actually be greater than indicated from laboratory bioassays.

Lake Union

Table 3 gives the analytical results and bioassay data for the two Lake Union studies. All sediments except those from sites 19 and 22 exceeded the Provincial Sediment Quality Guidelines lowest-effect level of 2 mg/kg dry weight for total PAH. No sediment exceeded the severe-effect level of 11,000 mg PAH/kg organic carbon. *Hyalella azteca* showed significant mortality when exposed to sediments from the Lake Union Phase I sampling trip with a level of 9,700 mg total PAH/kg organic carbon.

For Lake Union II bioassays, *Daphnia magna* showed significant response to PAH at site 11, the second most contaminated (4,500 mg PAH/kg organic carbon). *Hyalella azteca* responded significantly at site 9, the most contaminated (6,500 mg PAH/kg organic carbon), as well as at site 11. *H. azteca* responses to total PAH, LPAH, and HPAH are further explained in the Bioassay Evaluations section. Microtox® responded at all but the Lake Washington reference site, although a principal components analysis (Cubbage, In Draft) could not determine whether the effect was from metals, PAH, or both.

Lake Roosevelt

Refer to Table 4 for analytical results and bioassay data. Both *Daphnia magna* and *Hyalella azteca* showed significant mortality when exposed to sediments from Castle Rock and

Table 3. Lake Union I and II results – bioassays listed as percent survival.

Sediment Sample/Site	Sediment LPAH, mg/kg Dry	Sediment LPAH, mg/kg TOC	Sediment HPAH, mg/kg Dry	Sediment HPAH, mg/kg TOC	Sediment Total Metals, mg/kg Dry Weight As	Cd	Cr	Cu	Pb	Hg	Ni	Zn	*D. magna* (acute)	*H. azteca* (acute)	Microtox EC50	
Gas Works Park	702	2,000	2,710	7,700	ND	2.0	20.0	156	300	0.17	88.3	320	.	5	.	.
Chester Morse (Ref.)	U	U	U	U	ND	0.5	10.0	160	14	0.20	9.8	84	.	92	.	.
8	14	114	121	1,000	149	1.7 J	83.0	526	715	2.0	63.9	904	96	88	34	25
9	264	2,300	511	4,400	20 U	0.5 U	19.2	68	124	1.4	56.8	286	88	8	9	10
11	176	1,000	627	3,500	1,150	0.5 U	51.2	213	470	2.9	133.0	470	18	0	84	57
15	1.5	29	20	390	61	0.7 J	75.7	382	678	2.0	61.9	562	94	76	67	56
18	1	17	16	260	49	2.3 J	56.9	338	719	1.7	52.3	689	98	82	35	42
19	U	U	0.7	4	20 U	0.7 J	33.8	77	93	0.46	39.2	136	100	80	15	14
20	0.9	9	5	54	20 U	1.0 J	36.8	62	223	0.52	33.7	193	98	74	64	60
21	0.4	4	3	28	20 U	0.6 J	27.4	30	91	0.16	26.8	84	100	90	37	35
22 Lk. Wash. (Ref.)	U	U	0.1	18	20 U	0.5 U	19.7	11	26	0.04	22.6	40	92	84	>100	>100
Control	-	-	-	-	-	-	-	-	-	-	-	-	94	94	.	.

Dry = mg PAH/kg dry weight.

TOC = mg PAH/kg total organic carbon.

ND = Analysis Not Done.

(.) No bioassay conducted.

U = Analyzed for but not detected.

J = Estimated value; not accurate.

EC50 - Ratio of sediment which gives a 50% light reduction.

Microtox - EC50 values at 5 and 15 minutes.

Indicates survival significantly lower than control; in the case of Microtox, EC50 less than 100%.

PAH Guideline

Exceeds Provincial Sediment Quality Guidelines, lowest-effect level for total PAH, 2 mg/kg dry weight.

Metals Guidelines

Exceeds Provincial Sediment Quality Guidelines, lowest-effect level for this metal, mg/kg dry weight.

Exceeds Provincial Sediment Quality Guidelines, severe-effect level for this metal, mg/kg dry weight.

Table 4. Lake Roosevelt results – bioassays listed as percent survival.

Lk. Roosevelt	Sediment Total Metals, mg/kg Dry Weight							*D. magna*	*H. azteca*	Microtox
Sample	As	Cd	Cu	Pb	Mn	Hg	Zn	(acute)	(acute)	EC50
Little Dalles	16.4	5.3	964	452	1,610	0.46	4870	100	88	>100
French Pt. Rocks	12.0	12.0	151	528	569	1.66	515	100	90	77
Castle Rock	16.7	5.7	61	236	1,800	0.56	188	73	80	>100
Swawilla Basin	12.6	6.9	49	165	2,000	0.13	242	92	70	>100
Spokane River Arm	14.8	11.0	45	125	1,300	0.11	671	99	86	57
Sanpoil River Arm (Ref.)	6.2	2.7	38	32	613	0.05	142	96	90	>100
Control	-	-	-	-	-	-	-	99	96	.

EC50 - Ratio of sediment which gives a 50% light reduction, 15 minute test only.

(.) No bioassay conducted.

Indicates survival significantly lower than control; in the case of Microtox, EC50 less than 100%.

Exceeds Provincial Sediment Quality Guidelines, lowest-effect level, mg/kg dry weight.

Exceeds Provincial Sediment Quality Guidelines, severe-effect level, mg/kg dry weight.

Swanilla Basin. Microtox® showed apparent toxic effects with sediments from French Point Rocks and Spokane River Arm. No specific contaminant could be correlated to any of these results (Johnson, 1991). Sediment metals' concentrations that exceed the lowest-effect and the severe-effect levels established by the Provincial Sediment Quality Guidelines are also given in the table.

Although many of the metals' concentrations exceed the Provincial Sediment Quality Guidelines, there may be a logical explanation for the comparative lack of bioassay response. A primary source of contamination is the discharge of 250 to 400 metric tons of slag daily from the Cominco Smelter. The metals bound in slag are usually resistant to mobilization and less bioavailable than metals from other sources. However, under the acidic conditions (Ph 2.0) of fish and invertebrate digestive tracts, leaching can become a significant source of metals (Ministry of the Environment, 1979).

Project Summary Listing

Table 5 lists each project, the major contaminant(s), the number of sites providing sediment for bioassays, and whether or not at least one bioassay of each type listed from that project gave statistically significant results or, in the case of Microtox®, had an EC_{50} of less than 100%.

Bioassay Evaluations

Table 6 shows cost, testing time, availability, and problems of the evaluated bioassays. Recommendations for use are then made based on test results and the most important criteria, reliability. Evaluations are discussed below:

Daphnia magna

Daphnia magna is predominantly a water column organism. Toxicity might only be indicated if sufficient contamination has transferred from the sediment into the water column, depending on how much particulate matter the organism has ingested. Apparently, neither transfer nor ingestion occurred in sufficient amounts with either the Steilacoom or Quendall/Baxter sediments to show toxic effects. Both significant mortality and chronic toxicity (reduced production of young, see Appendix A) from Black Lake sediment was probably the result of either a volatile compound or a reducing agent. There were no significant hits at any Quendall/Baxter site, and only one at Lake Union. While there were two hits at Lake Roosevelt, the cause of toxicity is not apparent. *D. magna* appears to have low utility for freshwater sediment studies, especially where the contaminants are either in low concentration or are not easily transferred to the water column.

Ceriodaphnia dubia

Ceriodaphnia dubia, like *Daphnia magna*, resides primarily in the water column and thus may not be affected by contaminants sorbed onto sediment. It showed significant toxicity only with the Black Lake sediment. The use of this organism for sediment toxicity testing is equivocal.

Table 5. Summary of freshwater sediment bioassays – Sediment Criteria Project.

Table	Site	Contaminant	No. Sites	*D. magna* (acute)	*C. dubia* (acute)	*H. azteca* (acute)	*C. tentans* (chronic)	*C. tentans* (acute)	*H. limbata* (acute)	Microtox EC50	Microbial Enzymes
1A	Steilacoom I	Copper	12	.	.	–	.	.	.	.	.
1A, 1B	Steilacoom II	Copper	3	–	–	+	–	–	+	–	+
1A, 1B	Black Lake	Ref: Steilacoom II	1	+	+	+	–	–	–	+	+
2	Quendall/Baxter I	PAH	4	–	.	+	.	.	.	.	.
2	Quendall/Baxter II	PAH	4	–	–	+	+	–	–	+	.
2	Quendall/Baxter III	PAH	3	.	.	+	.	.	.	+	.
3	Lake Union I (GWP)	PAH, Metals	1	.	.	+	.	.	.	.	.
3	Chester Morse Lk.	Ref: Lake Union I	1	.	.	–	.	.	.	.	.
3	Lake Union II	PAH, Metals	8	+	.	+	.	.	.	+	.
3	Lake Washington	Ref: Lake Union II	1	–	.	–	.	.	.	–	.
4	Lake Roosevelt	Metals	6	+	.	+	.	.	.	+	.

(+) Significant effect (for Microtox, EC50 less than 100%). (–) No significant effect. (.) No bioassay conducted.
EC50 – Ratio of sediment which gives a 50% light reduction.

Table 6. Bioassay evaluations.

Bioassay	Reliability	Ecological Relevance	Cost per Analysis*	Organism/Test Available?	Ease	Testing Time**	Problems	Recommended?
Daphnia magna	Low	Low	$150 - 840	Yes	Moderate	2	No Response	No
Ceriodaphnia dubia	Low	Low	$150 - 750	Yes	Moderate	7	No Response	No
Hyalella azteca	High	High	$150 - 720	Yes	Moderate	14	Insensitivity?	Yes
Chironomus tentans	Low	Low	$600	Yes	Moderate	10	Inconsistent	No
Hexagenia limbata	Moderate	High	$600	July–April	Moderate	10	Seasonal	Yes
Microtox	Moderate	Moderate	$150 - 390	Yes	High	1	Interpretation	Yes
Microbial Enzymes	Moderate	Moderate	$150	Indigenous	High	1	Applicability	No

* Approximate prices, subject to change.

** Bioassay time only (days).

Hyalella azteca

Hyalella azteca was the only bioassay organism tested at all project areas. In several instances maximum organism mortality coincided with maximum sediment contamination, which indicates a dose-response relationship. Mortality also correlated with total recoverable pore water copper concentrations in the Steilacoom Lake Phase II sediments. The continued use of *Hyalella* in sediment bioassays is strongly recommended.

Hyalella's comparatively consistent performance may appear to be an artifact of its frequent use compared to that of some other organisms,(i.e., *Hyalella* was used more often). However, this bioassay, in any single study, was the most consistent in showing dose-response mortality. There may be other tests as consistent and suitable as *Hyalella*, but the tests examined in this review did not perform as well as *Hyalella*.

Hyalella showed consistent responses to PAH in sediment. Figure 3 shows the percent survival of *Hyalella* in bioassays from the Quendall/Baxter (Lake Washington) sites and the Lake Union sites. These tests represent four sampling trips and 16 different sampling sites. A dilution series of five dilutions from one site in which sediments from a highly contaminated site in Lake Union were mixed with sediments from a clean site are also shown. For more information, see Yake *et al.*, 1986. The results show a sharp decrease in survival as the sediment PAH concentration goes above about 4,000 mg/kg organic carbon. From this analysis, the Provincial Sediment Quality Guideline appears to not fully protect biota from acute effects of total PAH. This finding is surprising because the Provincial Sediment Quality Guidelines are derived from statistical analysis of indigenous biota in the screening level concentration approach (Neff *et al.*, 1986). This apparently greater sensitivity of *Hyalella* has at least three possible explanations. The SLC relies on local organisms that may have become inured to surrounding contamination, thus the SLC may have an inherent upward bias. Another explanation is that some lethal contaminant may be unmeasured but covarying with total PAH concentration in the Lake Union and Lake Washington sites examined in this study. A third possible explanation may be that total PAH offers an imprecise measure of potentially toxic compounds.

Figure 4 compares bioassay results on LPAH (2 and 3 ring PAH compounds) and HPAH (4, 5, and 6 ring compounds). The LPAH bioassay shows a clear demarcation between significant and non-significant mortality at approximately 1000 ppm OC. The relationship between mortality and HPAH is more equivocal with both significant and non-significant mortality occurring between 2000 and 6000 ppm OC. This stronger relationship to LPAH may reflect the stronger acute toxicity of LPAH compared to HPAH. These *Hyalella* responses to PAH are the most consistent response of any of the bioassay organisms tested and give a clear indication of the PAH concentrations that could be expected to cause biological harm.

Chironomus tentans

Chironomus tentans gave significant chronic response at only one Quendall/Baxter site. It showed no significant response with Steilacoom Lake sediments, although this result is not surprising considering the organism's demonstrated potential for tolerance to metals (Wentsel *et al.*, 1978). These results do not currently justify its continued use.

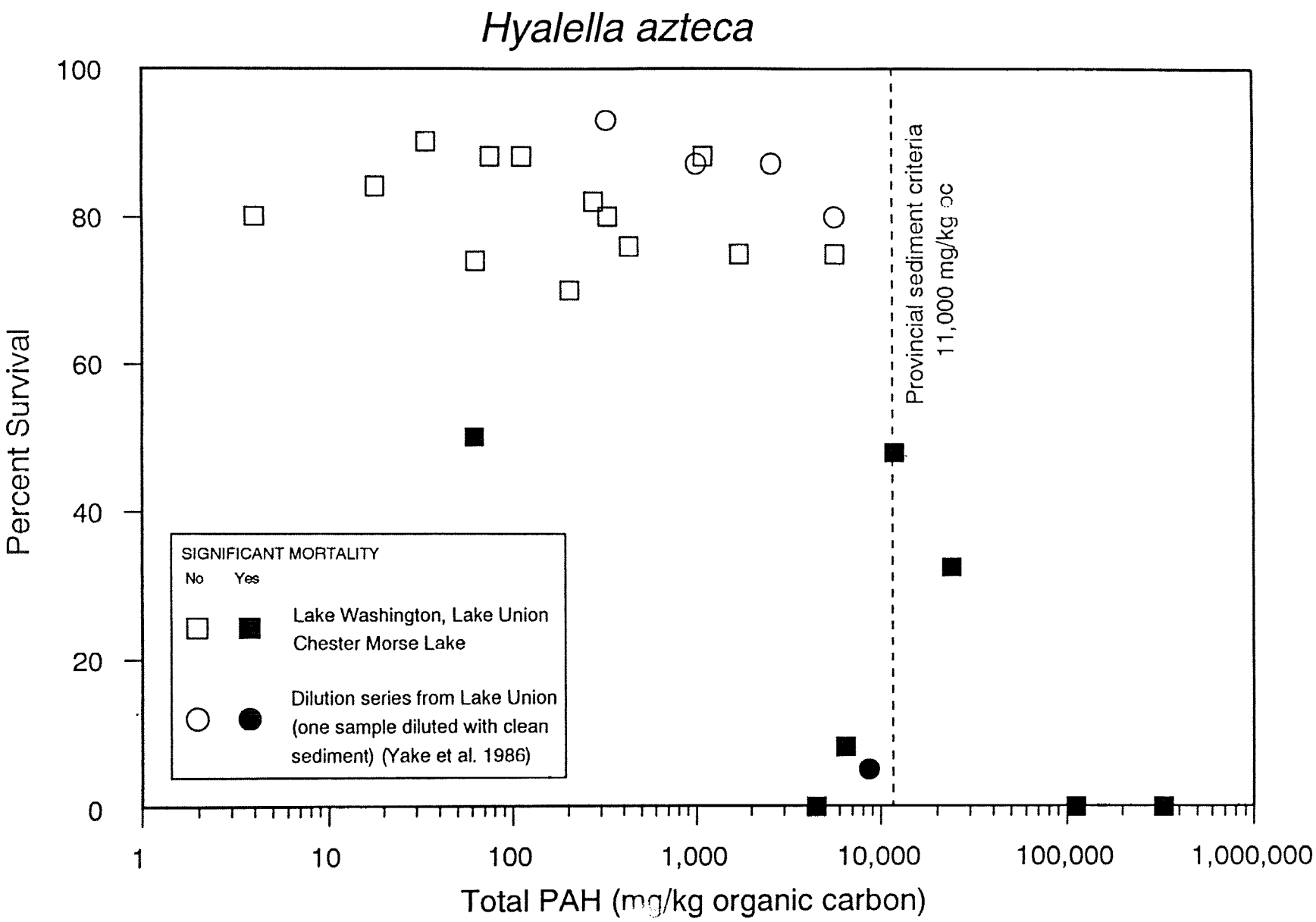

Figure 3. Hyalella azteca survival vs. total PAH concentration in sediment. Mortality significance from comparison to control with Dunnett's test at $p<0.05$.

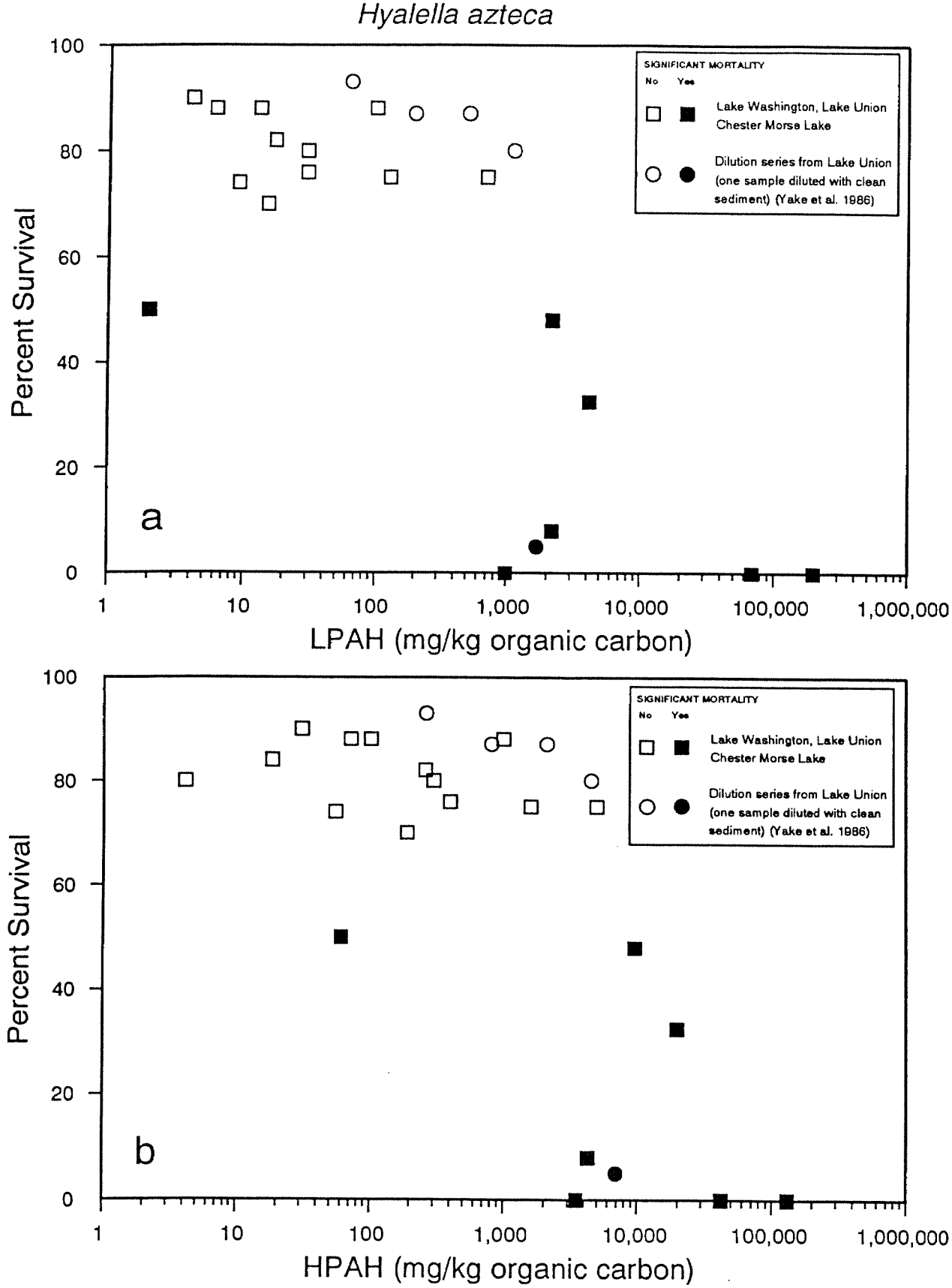

Figure 4. Hyalella azteca survival vs. sediment concentrations of LPAH (a) and HPAH (b). Mortality significance based on comparison with Dunnett's test at $p<0.05$.

Hexagenia limbata

Hexagenia limbata showed significant 50% mortality at the most contaminated Steilacoom site, but no toxicity at another site slightly lower in copper content. When pore water concentrations are considered, the response of *H. limbata* appears more consistent with greater mortality at higher pore water concentrations. Measurement of metals in pore water may provide more consistent results. *H. limbata* showed no significant toxicity with Quendall/Baxter sediments. In spite of seasonal limitations in availability and equivocal results, we recommend further use of this organism until limitations can be definitively resolved.

Ostracods

Ostracod tests were invalid due to high control mortality (Quendall/Baxter) or increased mortality with decreased sediment concentrations (Steilacoom and Black Lakes). A change in experimental procedure might solve this problem (Stewart, Personal Communication).

Microtox®

Microtox® showed no significant response with Steilacoom Lake sediments, although Black Lake sediment did produce a response from an unidentified contaminant. Quendall/Baxter sediments also induced significant effects. However, the two labs that performed the Microtox® tests used different analytical procedures and report contradictory results. All contaminated Lake Union sites showed Microtox® response.

There are several Microtox® test procedures available for determining toxicity in freshwater sediments. We have not yet identified which of the tests is best suited for use in the development of freshwater sediment criteria. It is important that further comparisons be done soon so that the optimum test for sediment toxicity can be identified and recommended. Nevertheless, the fact that the Microtox® tests often indicate toxic conditions, are inexpensive, and are widely used justifies their continued use.

Indigenous Microbial Enzyme Activities

Indigenous microbial enzyme activities, tested only with Steilacoom Lake sediments, showed positive correlation between reduction in microbial activity and increasing levels of both total recoverable sediment copper and total recoverable sediment pore water copper. Since the ecological relevance of microbial enzyme activity analyses still needs to be assessed and evaluated, we are currently not recommending this method for sediment toxicity tests. However, it seems to have considerable potential and we do recommend further investigation and development, with emphasis placed on data interpretation and the correlation of results to known environmental factors.

Summary

The majority of bioassays used to test for toxicity at these sites were based on acute, rather than chronic endpoints. None of the bioassays with chronic endpoints examined so far have shown sufficient consistency to serve as an indicator of sediment toxicity.

Many investigators consider the bioassay organisms *Daphnia magna* and *Ceriodaphnia dubia* to be highly sensitive indicators of sediment toxicity. Our results, however, did not demonstrate this sensitivity. In our opinion, for water-column organisms to demonstrate sediment toxicity, toxic components from the sediment must first migrate into the aqueous phase. Such migrations apparently did not occur in the majority of our tests, rendering these organisms ineffective as toxicity indicators. Our recommendations are made accordingly.

An additional recommendation in dealing with bioassays is to use the battery of tests approach, that is, incorporate a wide variety of tests similar to that used in the Steilacoom Lake study. Since response among different organisms varies depending on the type of contaminant and concentration range, the basis of this approach is to use several bioassay tests and compare results. Out of a battery of properly selected bioassays, it is likely that at least some of them will show a response to significant contamination levels. The bioassays recommended here constitute some of the components of such a battery.

CONCLUSIONS

1. Reliability is the most meaningful criteria to use for the evaluation of bioassay organisms.

2. *Hyalella azteca, Hexagenia limbata,* and Microtox® showed the greatest reliability in demonstrating toxic effects in freshwater sediment bioassays where high concentrations of metals and PAH were the primary contaminants. However, there are several Microtox® test procedures for sediments, and they may produce inconsistent and even contradictory results.

3. Water column dwellers *Daphnia magna* and *Ceriodaphnia dubia* were not found to be adequately sensitive for freshwater sediment bioassays, although they may have application in cases where the contaminant typically becomes dispersed into the water column.

4. *Chironomus tentans* has not proved useful in freshwater sediment bioassays, probably because of a high tolerance to the contaminants examined.

5. Indigenous microbial enzyme activities showed increasing copper toxicity at Steilacoom Lake. While their ecological relevance is currently uncertain, they may be useful as early-warning indicators of chronic sediment toxicity.

6. Total recoverable pore water copper concentrations with Steilacoom Lake Phase II sediments showed reasonable correlation to responses by *Hyalella azteca, Hexagenia limbata,* and microbial enzymes. The actual source of toxicity was not verified.

7. Ratios of copper to acid volatile sulfides did not correspond in the predicted manner to sediment toxicity.

8. Several researchers report that interactions with ultraviolet light can increase PAH toxicity under *in situ* conditions because of the production of phototoxic compounds. The bioassays described in this report may have resulted in lower toxicity than occur in the field.

9. An indigenous, benthic organism of suitable sensitivity could be a useful addition to the current list of acceptable bioassay organisms.

10. The battery of tests approach is useful in determining the range of environmental effects caused by contaminants of different types and concentrations.

RECOMMENDATIONS

1. Continue to evaluate the reliability of these bioassays, and any new bioassays of interest, with regard to the contaminants already tested and other chemicals of concern.

2. At this time, the following organisms are recommended for freshwater sediment bioassays when the sediments are contaminated with metals and PAH: *Hyalella azteca, Hexagenia limbata,* and Microtox®. Continued testing is recommended to further substantiate these findings, including *Hexagenia* tests with porewater. The various Microtox® test procedures currently available should be compared and a single procedure recommended for future use.

3. *Daphnia magna* and *Ceriodaphnia dubia* are not currently recommended for freshwater sediment bioassays except in cases where contaminants may be readily transferred into the water column.

4. *Chironomus tentans* is not currently recommended for freshwater sediment bioassays because of a lack of significant response to contaminants at the levels tested. Indigenous microbial enzyme activity analyses are not currently recommended because of questions of interpretability and ecological relevance of the data.

5. Correlate bioassay results to pore water values, especially when benthic (as opposed to water column) organisms are being used.

6. Do not apply the AVS theory until its role in determining sediment toxicity is better defined and characterized. Analyses using AVS should be postponed until further research is done elsewhere.

7. Evaluate the effects of natural and ultraviolet light on the toxicity of creosote-contaminated sediments, and determine their relationships to laboratory conducted bioassay results.

8. Continue to seek out an appropriate indigenous, benthic organism as a sediment toxicity indicator, and develop or apply an appropriate protocol. Suggested organisms include an "indigenous Ephemeropterid", if available, or similar organism.

9. Determine the relationship of indigenous microbial enzyme activity to known contaminant levels, bioassay results, benthic infaunal data, and reference sediments. Research and elucidate the ecological relevance of these findings, and monitor the development of indigenous microbial enzyme activity methods.

10. Select a suite of bioassays according to the battery of tests approach.

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APPENDIX A

Sediment Toxicity Assessment
of
Steilacoom Lake

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February 19, 1991

Jon L. Bennett
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Dear Jon:

Enclosed you'll find a copy of my report for the Steilacoom Lake project. We had some interesting results. The source and agent causing Black Lake sediment toxicity should be investigated. The fact that aeration removed toxicity suggests a volatile or reducing compound is causing the problem. AVS is not an important factor at these sites. The microbial community of Steilacoom Lake is being impacted. This may have a significant effect on the community structure and ecosystem functioning of the lake, now or in the future.

I would like for us to publish this study in Environmental Toxicology and Chemistry, using all the chemical and biological data collected. Let me know your thoughts on this. I doubt I'll have time to take the lead until late summer.

I enjoyed working with you and look forward to future studies.

Sincerely,

G. Allen Burton, Jr., Ph.D.
Associate Professor

Enclosures

xc: Craig Smith

Sediment Toxicity Assessment

of

Steilacoom Lake

by

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Background

The Washington State Department of Ecology designed a study (1) to assess the possible toxicity of Steilacoom Lake sediments which are heavily contaminated with copper. The study design included multiple toxicity assays comprising key trophic levels and chemical and benthic community analyses. Since the U.S. Environmental Protection Agency is considering Acid Volatile Sulfides (AVS) as a sediment criteria normalization tool for metals, this parameter was also measured. The study was coordinated by Jon L. Bennett of the Department of Ecology. Guidance on sample collection and study design was obtained from the collaborating investigators, which included: Allen Burton (WSU), Gary Ankley (USEPA), Art Stewart (Oak Ridge) and Margaret Stinson (State of Washington).

Sample Collection and Processing

Samples were collected, composited and shipped by Department of Ecology personnel. Black Lake (#B2-2) was used as a reference sediment. Three sites were selected on Steilacoom Lake based on historical sediment copper and AVS data, and comprised a gradient of contamination from low to high (#S2-4, S2-11, and S2-12, respectively). Samples were shipped via overnight express, on ice, and were received at WSU cold.

Test Procedures

Methods followed previously published protocols (2-6) and adhered to proper quality assurance procedures as defined by the USEPA (5), the laboratory's standard operating procedures and Quality Assurance Project Plan (7).

Samples were maintained at 4°C until testing. Sediments were mixed for 3 minutes with a hand paddle prior to assay initiation. Chemical and physical

parameters included: alkalinity, hardness, conductivity, pH, temperature, dissolved oxygen, total ammonia, and free copper. All parameters were measured in pore water and overlying waters of the test beakers; except NH_3, Cu, and conductivity were only measured in pore water. Overlying waters were sampled at test termination and returned to the Department of Ecology for further analyses.

Short-term chronic toxicity was determined in whole sediment exposures following draft ASTM methods (4) for cladocerans (see Appendix A). Test species were Daphnia magna and Ceriodaphnia dubia. Briefly, ten replicate 30 ml beakers were used, containing a 1:4 ratio of sediment to water. One neonate (< 24 hr old) was randomly placed in each beaker. Dissolved oxygen, pH, and temperature were monitored daily, before overlying waters were replaced. Alkalinity, hardness and copper were measured at test initiation and termination.

Indigenous microbial enzyme activity was measured as follows. Approximately 1 to 2 ml of cold homogenized sediment was placed in triplicate test tubes containing buffer. Enzyme substrate, for example, *p*-nitrophenyl-B-D-glucoside, was added to the tubes, vortexed, and incubated in the dark at 25°C for 30 min to 2 h. Activity was terminated by placing the tubes on ice or adding 1 to 2 ml acetone, vortexing, and centrifuging (4424 X *g*) for 10 min. The colored reaction product in the supernatant was then measured spectrophotometrically. Substrate was added after activity termination for control tests. Absorbance was converted to µg of product formed using a standard curve and activity defined as product formed per milliliter of gram dry weight of sediment per incubation time. Sediment dry weight determinations were conducted in triplicate with each assay. Microbial activity tests included alkaline phosphatase, dehydrogenase (electron transport activity), β-galactosidase, and β-glucosidase.

Results and Preliminary* Discussion

Summary results are provided in Table 1, including toxicity test responses, free copper and total NH_3 data. Free copper is the toxic form of the metal and was not present at toxic levels. Ammonia concentrations were also low and the below neutral pH reduced unionized ammonia formation.

The sediments from Steilacoom Lake were not toxic to the zooplankton test species; however, indigenous microbial activities were significantly depressed and showed a total copper concentration-response relationship. Sediment S2-12, which apparently had the highest total copper concentrations, had the lowest microbial activity. The sensitivity of sediment microbial communities to toxic contaminants has been demonstrated at every site tested by the P.I., in locations throughout the United States (3). They have been recommended as early warning indicators of ecosystem perturbations by Odum (8) and as criteria tools by others (9). Since these enzyme systems and communities are integral components of key biogeochemical cycling processes (10) and aquatic food webs (11), it is essential that their responses be considered in any ecosystem impact assessment.

The reference sample from Black Lake was toxic to the zooplankton test species. Copper and ammonia concentrations did not appear to be the causative agents. It is of interest that intense aeration of the sediment sample removed the toxicity. This indicates that AVS:metal interactions were not important, as removal of AVS should increase toxicity. It is possible that either a volatile compound or reducing agent (such as H_2S or a heavy metal in a reduced valence state) was lost or oxidized to a less toxic and less available complex. Further study will be required to determine the cause of toxicity.

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* A more complete discussion of the results and their significance may be provided if chemical and biological data from co-investigators is made available in the future.

The toxicity levels observed at Black Lake and Steilacoom Lake were significant to different trophic levels and indicates possible ecosystem degradation. In addition, these results add support to the premise that a multi-trophic level assessment approach is essential to ensure impact detection and to better interpret the significance of sediment contamination.

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Table 1. Data Summary for Lake Steilacoom Study

Assays: Ceriodaphnia dubia and Daphnia magna 3 brood survival/reproduction, indigenous microbial activities (alkaline phosphatases, dehydrogenases, β-galactosidase, β-glucosidase)

Test phase: Whole sediment

Sample date: 26 and 27, November, 1990

Sample stations: B2-2 (488158), S2-4 (488155), S2-11 (488156), S2-12 (488157)

Assay Responses (x ± SD)[a]

Sample	C. dubia Survival	C. dubia Young	D. magna Survival	D. magna Young	Microbial activity[b] APA	DHA	GAL	GLU	Cu++ (ug/l)[e]	NH_3 (total)[e]
Control[c]	100	23.4 (3.8)	100	62.4 (3.1)	NA	NA	NA	NA	0	0
B2-2	30	5.3 (8.6)	60	37.3 (13.1)	97.6 (2.4)	30 (4.2)	54.9 (7.6)	66 (2.6)	1.12	3.02
(Mixed)[d]	100	24.9 (2.9)							8.31	3.26
S2-4	90	18 (4.8)	90	43.5 (7.1)	109.2 (2.9)	52 (6.7)	79.5 (3)	113 (13)	1.76	1.58
(Mixed)	100	16.2 (5.6)							2.52	2.04
S2-11	90	16.9 (4.6)	100	51.7 (7.3)	68.8 (8.2)	24.8 (5)	48.7 (1.2)	61.8 (5.4)	0.36	0.76
(Mixed)	100	22 (4.4)							0.54	1.01
S2-12	100	17.3 (6.1)	100	48.1 (5.9)	51.8 (7.4)	5.8 (1.2)	18.6 (1.8)	17.9 (5.3)	0.39	1.19
(Mixed)	100	19.6 (6.4)							0.51	1.64

[a] Mean young produced and standard deviation (SD) with 10 replicates. Survival is not a mean value, n=10.
[b] APA = alkaline phosphatases; DHA = dehydrogenases (or electron transport system activity) using the tetrazolium salt, INT; GAL = β-galactosidase; and GLU = β-glucosidase.
[c] Moderately hard reconstituted water (used also as a culture water for the test organisms).
[d] Sediments aerated vigorously for 1 hr prior to testing.
[e] Free copper and ammonia measured in interstitial water with ion selective electrodes.