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Influence of storage time on toxicity of freshwater sediments to benthic macroinvertebrates

David L. DeFoe, Gerald T. Ankley*

US Environmental Protection Agency, National Health and Environmental Effects Research Laboratory, Mid-Continent Ecology Division, 6201 Congdon Boulevard, Duluth, MN 55804, USA

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Abstract

Guidance concerning recommended storage times for sediments to be used in toxicity tests generally has not been based upon systematically collected experimental data. The objective of this study was to better define the effects of storage time on toxicity of a series of freshwater sediments. Sixteen sediments with varying types of contaminants were collected, homogenized and stored at 4°C in 1 liter aliquots, which were periodically tested for toxicity to the amphipod *Hyalella azteca* and the midge *Chironomus tentans* after storage times of up to 101 weeks. The sediments ranged from non-toxic to extremely toxic (100% mortality) in 10-day assays, with several of the samples displaying an intermediate degree of toxicity (e.g. partial mortality, reduced growth). Biological responses in most of the samples did not vary with time relative to their statistical relationship to control values; samples identified initially as toxic (or non-toxic) tended to remain toxic (or non-toxic) regardless of when they were tested. The variations that were observed in biological responses over time generally were not systematic; that is, there were no apparent trends in samples becoming more (or less) toxic in the 10-day assays. This suggests that the source of at least some of the temporal changes in toxicity were due to inherent biological variability of the assays used to assess the sediments, rather than the effects of storage. In *C. tentans* tests with the least toxic sediments, among-replicate variability tended to be greater in initial assays than in tests with samples that had been stored for some period of time. This may have been due to the presence of indigenous competitive or predatory organisms that did not survive during prolonged storage.

Keywords: Sediment; Storage; Benthic invertebrates; Survival; Growth

1. Introduction

Biological assays historically have been utilized to assess the potential toxicity of sediment-associated contaminants (Nebeker et al., 1984; Giesy and Hoke, 1989; Burton, 1991), and the recent development of standard methods for performing sediment toxicity tests likely will increase the frequency of their use (Environment Canada, 1994a, b; US Environmental Protection Agency, 1994a, b; American Society for Testing and Materials, 1995). Although these standard methods explicitly describe organism collection and/or culture, physical test systems, assay conditions and test interpretation, relatively little specific guidance has been provided concerning sediment collection and manipulation prior to testing. To a large degree, study objectives dictate factors related to collection (e.g. the use of core versus grab samples; West et al., 1994), but considerable uncertainty exists as to appropriate guidance regarding sample manipulations after collection. One of the foremost questions in this area is related to different aspects of sample storage. Currently it is recommended that sediments utilized for toxicity tests be stored under cool (4°C) conditions, in inert, sealed containers with minimal headspace (American Society for Testing and Materials, 1994; US Environmental Protection Agency, 1994a; Environment Canada, 1996). These types of recommendations, although not necessarily based upon specific biological data, are generally agreed upon under the assumption that alterations to the geochemical nature of the sediment should be minimized (e.g. Ho and Lane, 1973; Thomson et al., 1980).

* Corresponding author.

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In keeping with this concept, it is often recommended that samples be tested 'as soon as possible' after collection (US Environmental Protection Agency/Army Corp of Engineers, 1991, 1994; US Environmental Protection Agency, 1994a, b; American Society for Testing and Materials, 1995). The somewhat subjective nature of this guidance has led to attempts to dictate specific time periods for sediment holding. These recommendations range from two weeks or less to less than eight weeks (Chapman, 1988; US Environmental Protection Agency/Army Corps of Engineers, 1991, 1994; Environment Canada, 1996). The differences in these recommendations reflect, in part, the fact that they have not been based upon comprehensive evaluations of potential variations in sediment toxicity over time.

There have been several reports of the effects of storage time on sediment toxicity (Malueg et al., 1986; Landrum, 1989; Stemmer et al., 1990; Othoudt et al., 1991; Tatem et al., 1991; Dave, 1992; Dillon et al., 1994; Becker and Ginn, 1995; Moore et al., 1995; Redmond et al., 1996). The results of these studies vary from almost no change in toxicity over time, to extreme toxicity variations within relatively short time periods. However, using data from most of these studies as a basis for standard recommendations for storage time is problematic in terms of one or more experimental design issues. These include: (a) limited sample size (number of sediments), (b) utilization of non-standardized toxicity tests/endpoints, (c) use of spiked samples (i.e. possible lack of equilibrium conditions at test initiation), (d) lack of a range of responses (toxicity) or contaminant type(s), and/or (e) lack of systematic testing (i.e. tests on a given schedule for an extended period of time).

The objective of this study was to assess changes in sediment toxicity with storage time. To attempt to avoid the limitations associated with previous studies conducted on this topic, we tested a relatively large number of field-collected sediments, with varying toxicity and contaminant profiles, using a pre-set 'core' testing schedule. The sediments were assayed using standard methods to evaluate growth and/or survival of the freshwater invertebrates *Hyalella azteca* and *Chironomus tentans*.

2. Materials and methods

Surficial sediments were collected, over the course of 14 months, from 16 sites with varying degrees of documented or suspected contamination (Table 1). Included in the sample set were those contaminated predominantly by metals, such as copper (Keweenaw Waterway; Ankley et al., 1993a) and zinc (Turkey Creek; Ankley et al., 1996), the pesticide DDT and its metabolites (Indian Creek; Hoke et al., 1994; West et al., 1994) and oil/grease/creosote and associated polycyclic aromatic hydrocarbons (Hog Island Inlet, Grand Calumet River, Little Scioto River; Schubauer-Berigan and Ankley, 1991; Ankley et al., 1994; Ireland et al., 1996).The control (reference) sediment used for all tests was from West Bearskin Lake, Minnesota (Ankley et al., 1993b).

Upon receipt at the laboratory, test sediments were immediately homogenized and divided into separate 1 liter polyethylene containers with minimal headspace. For each experiment, a separate container was utilized; this avoided possible bias associated with repeated

Table 1
Test sediment locations, codes, and suspect contaminants

Location	Code	Contaminant(s)	Reference
Hog Island Inlet, Superior, WI	HI	Oil, PAH[a]	Ankley et al. (1994)
Holland Harbor, Holland, MI	HH	Unknown	—
Rouge River 1, Detroit, MI	R1	Oil, PAH	R. Powers, EPA, pers. comm.
Rouge River 2, Detroit, MI	R2	Creosote	R. Powers, EPA, pers. comm.
Raisin River, Monroe, MI	RR	PCB[b]	E. Lancaster, EPA, pers. comm.
Ashtabula Harbor, Ashtabula, OH	AH	Coal	S. Pickard, ACOE, pers. comm.
Des Plaines River, Chicago, IL	DP	Ammonia	J. Arthur, EPA, pers. comm.
Grand Calumet River, Chicago, IL	GC	Ammonia, metals, PAH	Schubauer-Berigan and Ankley (1991)
Keweenaw Waterway, Houghton, MI	KW	Copper	Ankley et al. (1993a)
Indian Creek, Huntsville, AL	IC	DDT, DDD, DDE	West et al. (1994); Hoke et al. (1994)
Stryker Embayment, Duluth, MN	SE	PAH	Schubauer-Berigan and Crane (1996)
Little Scioto River, Marion County, OH	LS	Creosote, PAH	Ireland et al. (1996)
Langley Pond, Langley, SC	LP	Metals	W. Peltier, EPA, pers. comm.
Turkey Creek, Joplin, MO	TC	Zinc	Ankley et al. (1996)
Fairborn, Fairborn, OH	FO	Ammonia	G. Burton, Wright State, pers. comm.
Erie Pier, Duluth, MN (confined disposal facility)	EP	Various	John Safstrom, ACOE, pers. comm.
West Bearskin Lake, Grand Marais, MN	WB	None	Ankley et al. (1993b)

[a] PAH, polycyclic aromatic hydrocarbons.
[b] PCB, polychlorinated biphenyls.

homogenization and subsampling of a 'master' sample. Sediments were stored in the dark in a walk-in cooler, with a continuous temperature recorder, at 4 ± 1°C. The West Bearskin Lake sediment was treated in a manner analogous to the test sediments, except that the 1 litre aliquots came from a homogenized single sample that already had been stored for several months. Based upon approximately 10 years of experience and literally hundreds of assays with the West Bearskin sediment, we felt that the relative constancy of test results obtained with this sediment made it a suitable baseline reference for the storage experiment.

Sediments were tested within 1 day of receipt (day 'zero'), which corresponded to 1–3 days after initial collection, after 2 weeks, after 6–8 weeks, and after 18–22 weeks of storage. All samples also were tested after longer more variable storage periods, in some cases up to 101 weeks. Ten-day toxicity tests with *H. azteca* and *C. tentans* were conducted using standard methods (US Environmental Protection Agency, 1994a). Tests were initiated with 100 ml of sediment and 10 organisms, either 10–18-day old *H. azteca* or 9–10-day old (third instar) *C. tentans*. Test animals were fed daily; chambers containing *C. tentans* received 1.0 ml of a Tetrafin slurry, while *H. azteca* received 1.5 ml of a yeast-Cerophyll-trout chow mixture (US Environmental Protection Agency, 1994a). All control sediments and most test sediments were assayed using four replicates; however, on occasion, data consisted of duplicates for the most toxic sediments. Both species were tested at 23 ± 1°C in an automated system that provided two renewals of overlying Lake Superior water per day (Benoit et al., 1993). General characteristics of the incoming lake water were: dissolved oxygen (DO), 6.8 to 7.7 mg l^{-1}; pH, 7.4 to 7.9; hardness, 44–47 mg liter^{-1} as $CaCO_3$; and alkalinity, 45 to 46 mg liter^{-1} as $CaCO_3$. Temperature, pH and DO were measured periodically, five to eight times over the course of the 10-day tests. Alkalinity, hardness and conductivity were measured at least once between test days 4 and 7. All these parameters remained within acceptable ranges (US Environmental Protection Agency, 1994a). At test termination, surviving organisms were sieved from the sediments, enumerated, and the *C. tentans* dried for 14–20 h at 90–100°C for determination of final dry wt.

For each sample (location), data on growth and/or survival of *H. azteca* and *C. tentans* tested after various sediment storage time intervals were compiled and reviewed prior to analysis. Results were not used in the analyses if they did not meet the performance-based criteria for test acceptance (US Environmental Protection Agency, 1994a). Specifically, *H. azteca* data were excluded from the analysis if survival of the amphipod from the corresponding test with West Bearskin sediment was less than 80%. Similarly, if *C. tentans* survival was less than 70%, or final dry wt was less than 0.6 mg organism^{-1} in the West Bearskin sediment, midge data for the corresponding test sediment were excluded from the analysis (US Environmental Protection Agency, 1994a). Although within acceptable limits, there was variation in performance of the two test organisms in the control sediment [Fig. 1(a)–(c)]. Based upon past experience, the source of this variation likely was due to slight differences in quality (e.g. size) of organisms originating from the culture unit. To help normalize the data for this effect, test results were expressed as a percentage of control values prior to statistical analysis and graphical presentation.

For any given test sediment, there are two aspects of variation as a function of storage time. There is variation relative to concurrently tested controls, and

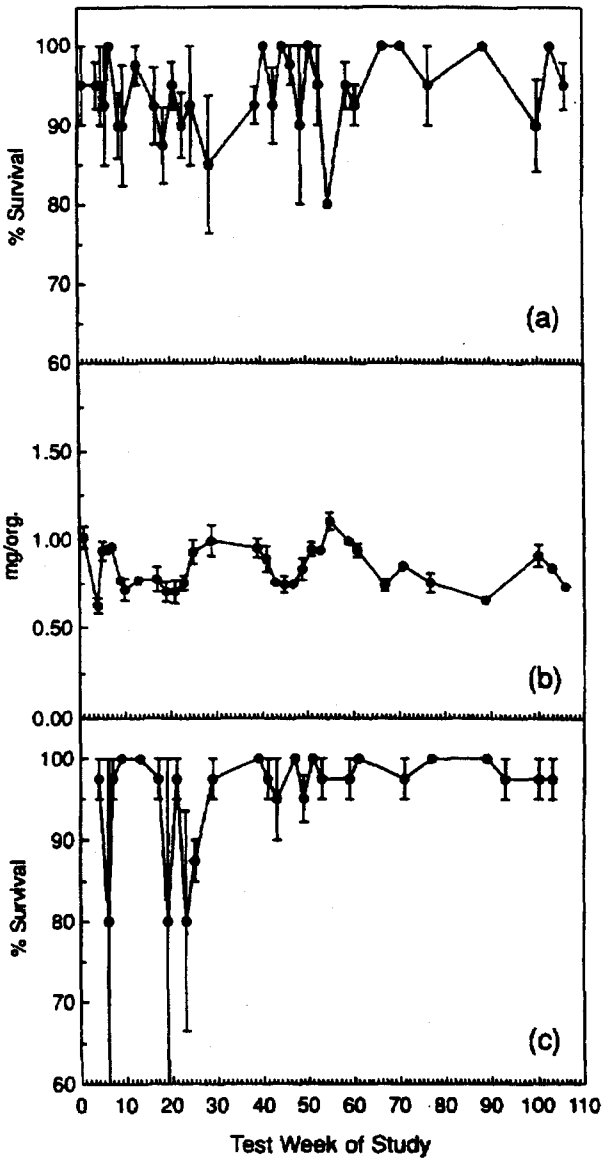

Fig. 1. Performance over time in West Bearskin control sediment for: (a) *Chironomus tentans* survival, (b) *C. tentans* growth and (c) *Hyalella azteca* survival. Error bars indicate the SD associated with the mean values ($n = 4$).

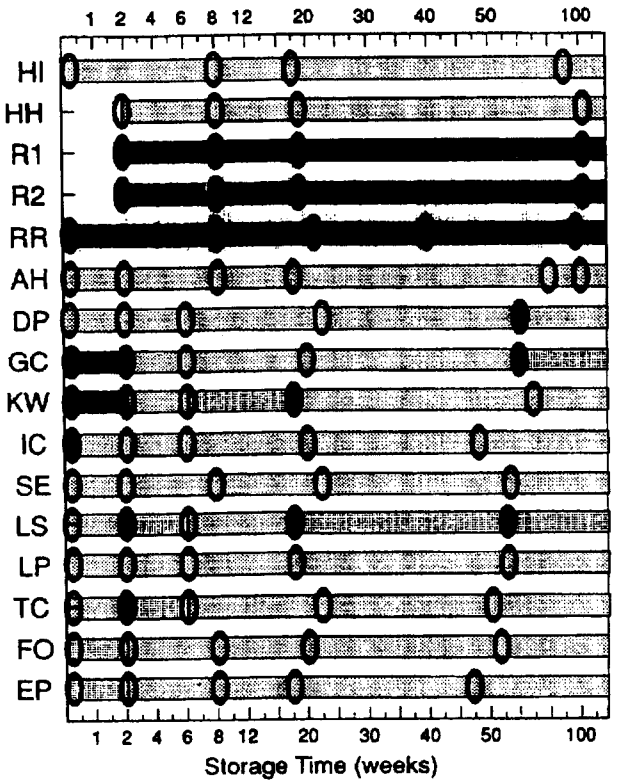

Fig. 2. Survival results for *Chironomus tentans* exposed to test sediments. Open and closed (filled) ovals indicate times when sediments were tested; open ovals indicate no difference from the concurrent control, while closed ovals indicate a significant ($p \leq 0.05$) reduction from control performance. Differences in hatching/shading indicate organism response relative to an overall assessment of variability in tests with the control sediment. Light shading, stippling, diamonds and dark shading indicate, respectively, that test data were 0–2, 2–4, 4–6 and >6 standard deviations lower than the overall mean control value.

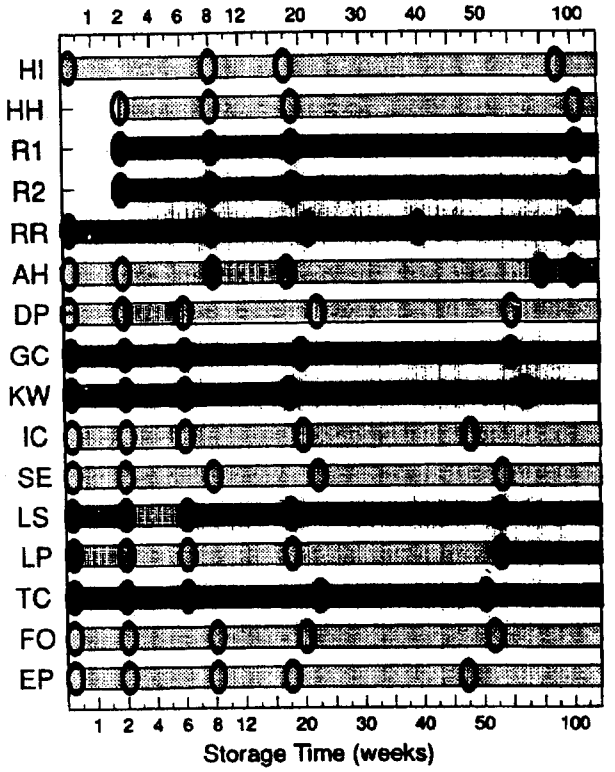

Fig. 3. Growth (weight) results for *Chironomus tentans* exposed to test sediments. Open and closed (filled) ovals indicate times when sediments were tested; open ovals indicate no difference from the concurrent control, while closed ovals indicate a significant ($p \leq 0.05$) reduction from control performance. Differences in hatching/shading indicate organism response relative to an overall assessment of variability in tests with the control sediment. Light shading, stippling, diamonds and dark shading indicate, respectively, that test data were 0–2, 2–4, 4–6 and >6 standard deviations lower than the overall mean control value.

within-sample variation over time. From a regulatory perspective, the most important consideration in terms of test sediment variation is whether results remain consistent with regard to interpretation relative to the control(s). That is, even if a sediment varies somewhat over time, this is of limited significance in current decision-making frameworks (e.g. US Environmental Protection Agency/Army Corps of Engineers, 1991, 1994; US Environmental Protection Agency, 1994a), which focus more on the presence/absence than degree of toxicity. To examine this statistically, we utilized one-tailed *t*-tests (i.e. determination of whether test values were less than the corresponding West Bearskin control) to assess relationships at any given storage period. Differences were considered significant at $\alpha = 0.05$. Our assessment of the second aspect of variations in a test sediment over time, relative to itself, was more qualitative in nature. In this case, we used data from all tests with the control sediment [Fig. 1(a)–(c)] to determine endpoint-specific estimates of variability. We then categorized each test sediment at each time period as to whether it was within 0 to 2, 2 to 4, 4 to 6 or >6 standard deviations lower than the overall control mean for the endpoint under consideration (Figs. 2–4).

3. Results

In all, more than 175 tests were conducted as part of this study. Figs. 2–4 summarize the results and statistical analyses for *C. tentans* survival and growth, and *H. azteca* survival, respectively. Storage time would not have affected statistical interpretation of the presence/absence of toxicity in terms of *C. tentans* survival in 10 of 16 (*ca* 60%) of the test sediments. Three of the sediments (R1, R2, RR) always caused significantly lower *C. tentans* survival than concurrent controls, and survival of the midge in seven of the 16 test sediments (HI, HH, AH, SE, LP, FO, EP) was not significantly lower than corresponding control values at any test

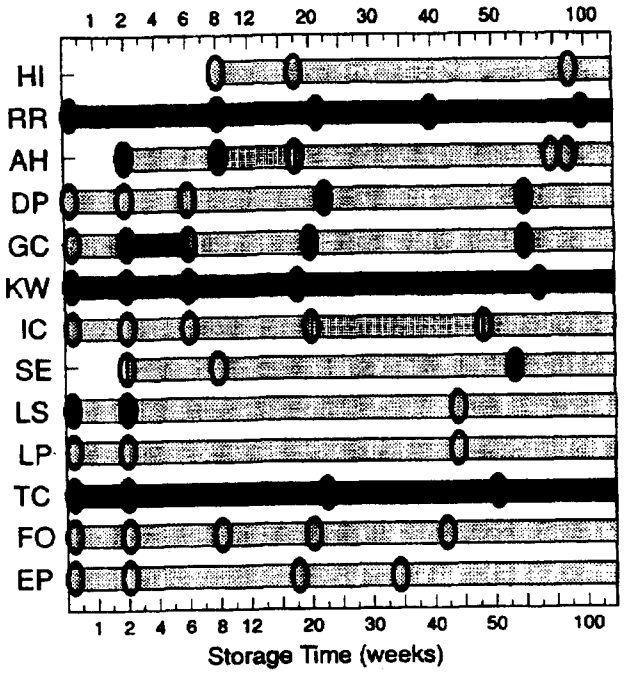

Fig. 4. Survival results for *Hyalella azteca* exposed to test sediments. Open and closed (filled) ovals indicate times when sediments were tested; open ovals indicate no difference from the concurrent control, while closed ovals indicate a significant ($p \leq 0.05$) reduction from control performance. Differences in hatching/shading indicate organism response relative to an overall assessment of variability in tests with the control sediment. Light shading, stippling, diamonds and dark shading indicate, respectively, that test data were 0–2, 2–4, 4–6 and >6 standard deviations than the overall mean control.

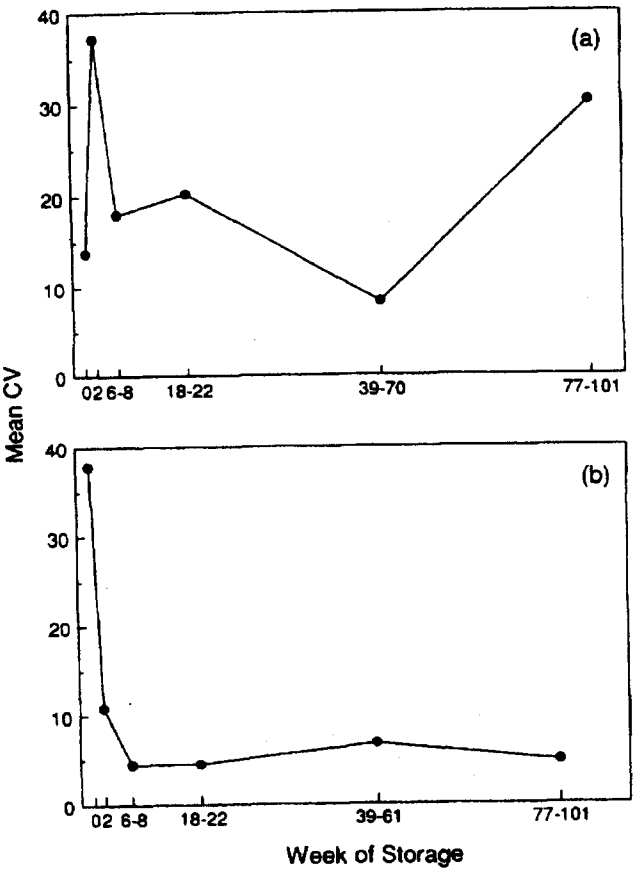

Fig. 5. Mean coefficient of variation (%) over time for *Chironomus tentans* survival in: (a) nine most toxic, and (b) seven least toxic sediments.

period (Fig. 2). With the exception of the initial tests with FO and EP, survival data for these latter seven sediments were within 0 to 2 standard deviations of the overall control values. Two of the remaining six sediments (DP, IC) consistently exhibited *C. tentans* survival that was within 0 to 2 standard deviations of the overall control value (Fig. 2), but each had one instance where the test results were significantly lower than the corresponding controls. If one considers these two data points as within the realm of statistical chance, and not a true indication of toxicity, then *C. tentans* survival, relative to controls, in 12 of 16 (ca 75%) of the samples did not change appreciably over time. The remaining four sediments (GC, KW, LS, TC) exhibited slight to moderate toxicity, with *C. tentans* survival in the range of 2 to 4 standard deviations lower than the overall control value. Although *C. tentans* survival in these sediments fluctuated over time, there were no apparent trends in these fluctuations. For example, the Little Scioto River (LS) sediment caused statistically significant decreases in survival in the second, fourth and fifth, but not in the first and third test periods (Fig. 2).

Growth (dry wt) of *C. tentans* in the test sediments generally was not affected by storage time (Fig. 3). In all, data interpretation for 14 of the 16 samples (ca 90%) would have been similar irrespective of when they were tested. Growth in seven of the 16 samples was always significantly lower than corresponding control values. In four of these sediments (R1, R2, RR, KW) final dry wt of surviving organisms was always >6 standard variations lower than the overall mean control value, while in the other three samples (GC, LS, TC) dry wt varied between <6 and 2 standard deviations of the overall control value (Fig. 3). *Chironomus tentans* growth in seven of the nine remaining samples (HI, HH, DP, IC, SE, FO, EP) was never significantly lower than corresponding West Bearskin controls. The final two sediments (AH, LP) would be classified as having slight to moderate effects on growth, and fluctuated over time in terms of significance relative to controls; however, there were no apparent trends in these fluctuations (Fig. 3).

Hyalella azteca survival was not assessed over time for three of the test sediments (HH, R1, R2). Three of the 13 remaining sediments (RR, KW, TC) exhibited significant toxicity to the amphipod irrespective of when they were tested (Fig. 4). Five more of the samples (HI, IC, LP, FO, EP) did not cause statistically significant decreases in survival at any test period; except for one

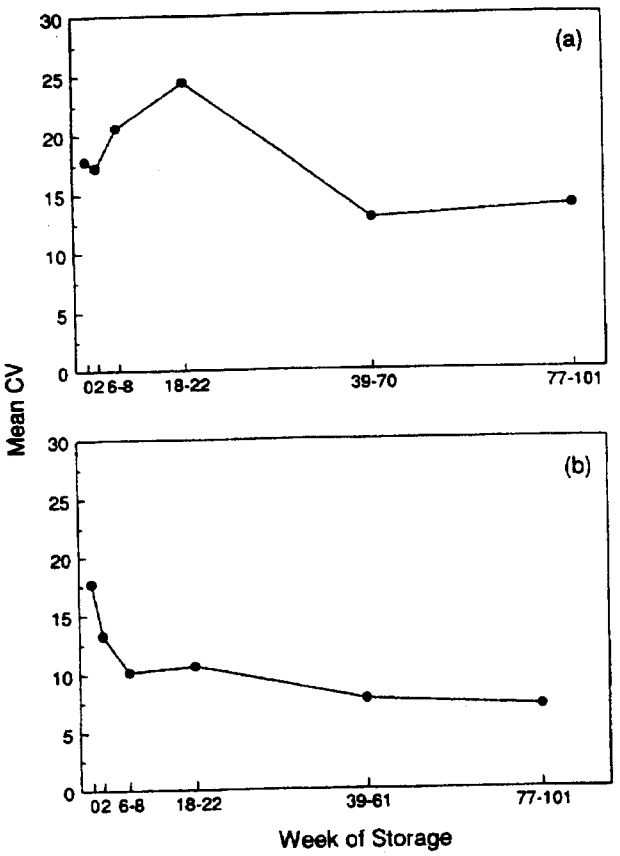

Fig. 6. Mean coefficient of variation (%) over time for *Chironomus tentans* growth in: (a) nine most toxic, and (b) seven least toxic sediments.

test with Indian Creek (IC), *H. azteca* survival remained within 0 to 2 standard deviations of the overall mean control value (Fig. 4). Thus, for 8 of the 13 test sediments (ca 60%), storage time would not have affected statistical interpretation as to the presence/absence of toxicity. Three of the remaining five sediments (DP, SE, LS) exhibited one or more instance of significant decreases in amphipod survival over time; however, data for tests with these sediments were within 0 to 2 standard deviations of the overall control value for *H. azteca* survival. In fact, two of the samples (DP, SE) consistently had greater than 90% survival of *H. azteca*. If these three samples, which did not change categorically, are included with those that did not vary statistically, then 11 of the 13 samples (ca 85%) remained relatively stable over time. The final two samples (AH, GC) fluctuated over time both statistically and in terms of their categorization relative to controls; these fluctuations were relatively random in nature (Fig. 4).

An interesting observation emerged during the *C. tentans* tests with the various sediments. If the test sediments were qualitatively grouped as to their performance relative to the West Bearskin sediment, it was possible to generate two sets of samples. In terms of *C. tentans* growth, nine would be identified, at least occasionally, as toxic (i.e. R1, R2, RR, AH, GC, KW, LS, LP, TC; Fig. 3), while growth in the remaining seven was comparable to (or exceeded) that of animals in the West Bearskin sediment. Variability in test results (i.e. among replicates), expressed as the mean coefficient of variation, for the most toxic samples showed no apparent trend over time [Figs. 5(a) and 6(a)]. However, for the seven non-toxic samples, this variability decreased after 2–4 weeks of storage, i.e. the initial tests were more variable than assays conducted after some period of time [Figs. 5(b) and 6(b)]. In terms of survival, this early variability often was characterized by relatively good performance in three of the four test replicates, with the fourth replicate exhibiting, for example, no recovery of test organisms.

4. Discussion

Under the conditions examined in this study, it does not appear that storage of most sediment samples for moderate periods of time would greatly compromise the results of toxicity tests with benthic macroinvertebrates. Using a wide array of sediments, we found that storage time often did not alter statistical and/or categorical interpretation of test data for the samples. For example, in the case of *C. tentans* growth (dry wt), 90% of the samples did not differ statistically over time relative to the controls. *Chironomus tentans* and *H. azteca* survival were somewhat more variable with respect to this interpretation; depending upon the criteria used to judge whether a sample changed (i.e. statistical versus categorical), 60 to 85% of the test sediments did not vary appreciably over time. Perhaps of most significance was that, irrespective of the endpoint or sediment under consideration, changes that did occur generally were not systematic, i.e. there were no consistent trends in changes in toxicity in the 10-day tests. Alterations in the performance of the animals appeared random in nature, suggesting that the observed variations likely were related as much to the test organisms (i.e. inherent biological variability) as to systematic changes in the stored sediments. One observation supporting this interpretation was the fact that the most variation over time typically was observed in moderately to slightly toxic samples. Others have observed that these types of 'grey' samples typically exhibit more variability in test responses than relatively toxic or reasonably clean sediments (e.g. Becker and Ginn, 1995; Burton et al., 1996).

We believe that the data from this study could prove useful in terms of developing guidance for sample storage times for sediments to be used in toxicity tests. To generate this data set, we tested a relatively large set of field-collected sediments with varying toxicity and contaminant profiles, using standard protocols and a

comprehensive pre-set testing schedule. There have been previous attempts to assess the effects of storage time on sediment toxicity. Many of these studies, however, had one or more design shortcomings that contributed to uncertainty in terms of utilizing their results to develop standard guidance for sediment storage times. For example, some utilized contaminant-spiked samples that were not allowed to fully equilibrate before initial assays were conducted (e.g. Landrum, 1989; Stemmer et al., 1990) which generally is not reflective of sediments collected from a field setting. Other studies used only a limited number of samples to make conclusions about acceptable storage times (e.g. Tatem et al., 1991; Becker and Ginn, 1995). Yet other sediment storage studies utilized samples with only a limited range of contaminants or toxicity; for example, Moore et al. (1995) reported the results of a storage study that featured a relatively large number of field-collected sediments, only one of which exhibited toxicity, on one test date.

Although this study was more comprehensive than many previous studies, our results and conclusions do not differ from those of several others who have assessed the effects of storage time on sediment toxicity. For example, Moore et al. (1995) reported that, with one exception, eight non-toxic sediments remained non-toxic for up 740 days of storage at 4°C, which is analogous to our observations with non-toxic samples. Redmond et al. (1996) reported that the toxicity of moderately-toxic estuarine sediments to *Ampelisca abdita* did not change after 7 weeks of storage at 4°C. Similarly, the effects of 10 oil-contaminated marine sediments, of varying toxicity, on *Rhepoxynius abronius* survival did not change significantly after storage at 4°C for up to 52 weeks (W. Stubblefield, ENSR, pers. comm.). And, even in some studies where storage time was reported to influence toxicity, there were no trends in these changes. For example, Othoudt et al. (1991) reported that the toxicity of six sediments to *Daphnia magna* and *C. tentans* varied significantly over a 112-day storage period at 4°C, but the observed variations were random. This is similar to our observations, especially with samples exhibiting slight to moderate toxicity.

The fact that the *C. tentans* survival and growth in the relatively non-toxic sediments tended to be more variable in initial than in later tests was an interesting observation from our study. The cause(s) of this is uncertain but, based on several lines of indirect evidence, seems likely to be related to biological as opposed to chemical 'activity' of the test sediments. For example, Reynoldson et al. (1994) reported that indigenous organisms present in test sediments could affect test results from the standpoint of competition for resources; this typically was manifested in decreased growth of the test species of concern. Another plausible biological influence that could have contributed to test variability would be predatory organisms. In a non-toxic sediment, the presence of even a small number of predatory animals would cause results similar to those we occasionally observed, that is, an absence of organisms in one of four replicates, with good survival in the other three. As sediment storage time increases, the likelihood that either competing or predatory organisms will survive decreases, thus potentially contributing to lower test variability. In several sediments we did observe a decrease in numbers of indigenous organisms with increased storage time (data not shown). The hypothesis that the elevated early test variability was due to biological attributes also is supported by the fact that the trend in decreasing variability was not seen in the toxic sediments where few, if any, indigenous organisms were present.

In summary, our data indicate that storage of sediment samples for moderate periods of time often would not alter interpretation as to the presence/absence of toxicity. Variations that were observed in biological responses were relatively random in nature, and did not appear to be the result of systematic trends related to changes in stored sediments. This conclusion is important for long-term studies, such as toxicity identification evaluations or bioaccumulation tests, which can require weeks to months of work with archived samples to fully define the nature of a sediment. Our data also are significant for those areas of the country where effective collection of sediment samples for testing is limited to a few months of the year. While it may continue to be preferable to test samples as soon as possible after collection, our data suggest that invalidation of toxicity data derived from sediments that have been stored for longer than some preset period of time is not technically warranted.

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