

Effect of extended storage time on the toxicity of sediment-associated cadmium on midge larvae (*Chironomus tentans*)

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The effect of the duration of spiked sediment storage on cadmium toxicity was studied. Sediment samples were spiked with cadmium to obtain concentrations of 0.6, 16.0, 29.0 and 53.0 $\mu\text{g Cd per g sediment}$ (dry weight). The spiked sediment was then stored in sealed plastic containers at 4 °C in the dark. Sediment bioassays, using *Chironomus tentans*, were conducted immediately and at periodic intervals for up to 4 months. Though the levels of cadmium in the bulk sediment samples from different stored periods were not significantly different, different toxicity levels to *C. tentans* were observed. The toxicity was significantly different between subsequent storage times. There was a significant decrease in the bioaccumulation factor (BAF) values with extended storage times, indicating a reduction in the bioavailability of cadmium. This study suggests that the storage of spiked sediment used in sediment toxicity study can influence the results.

Keywords: *Chironomus tentans*; sediment toxicity; sediment storage time; sediment-associated cadmium.

Introduction

The influence of storage times or ageing on sediment-associated contaminants is an important factor in the regulation and bioassessment of contaminated sediment (Forstner, 1987). A reduction in the bioavailability of sediment-associated contaminants has been observed with extended storage times (Brannon *et al.*, 1980; Swartz *et al.*, 1985; Nebeker *et al.*, 1986; Anderson *et al.*, 1987; Stemmer *et al.*, 1990; Landrum *et al.*, 1990, 1992). Because a reduction in the bioavailability would result in a decrease of bioaccumulable contaminants, the influence of storage is reflected in the potential toxic effects and biological responses, e.g. growth and bioaccumulation. To determine the influence of storage time on the toxicity of sediment-associated cadmium, larvae of *Chironomus tentans* were exposed to four batches of spiked sediment having different storage times. The end-points used in this study for detecting the bioavailability reduction were mortality, body burden and growth.

Materials and methods

Test system

The test apparatus was a single-pass, flow-through system which is a modification of the designs of Prater and Anderson (1977a,b) and LeBlanc and Surprenant (1985).

The test unit was made of flint glass and was assembled with silicone sealant (Fig. 1). Each unit had two compartments: a water exposure chamber and a water and sediment chamber. The dual chamber design permitted the simultaneous exposure of benthic and pelagic organisms without interference between them (Sae-Ma, 1993). The volume of each compartment was approximately 3.5 l. Tygon™ tubing was used to supply dilution water and air to the system.

Dilution water was supplied to the system by gravity flow from a constant level head tank. Valves were used to control the volume of water entering each bioassay unit. The flow was adjusted to 20 ml min⁻¹, which ensured eight complete turnovers of the test water during a 24 h period (3 h retention).

Test species

The test species were third-instar *Chironomus tentans* (midges) larvae from the stock cultures of the Water Quality Laboratory, School of Public Health, University of Michigan. *Chironomus tentans* were raised in the rearing chamber as described in Meier and Torres (1978) and fed twice a week with a mixed diet of *Ankistrodesmus* sp. and a suspension of ground trout chow and Tetra Fin™ fish flakes. The culture was kept at 21 ± 1 °C, with 16:8 h light:dark photoperiod.

Sediment and water analysis

Standard methodologies (Walkley and Black, 1934; American Society for Testing and Materials, 1950; Lambe, 1955;

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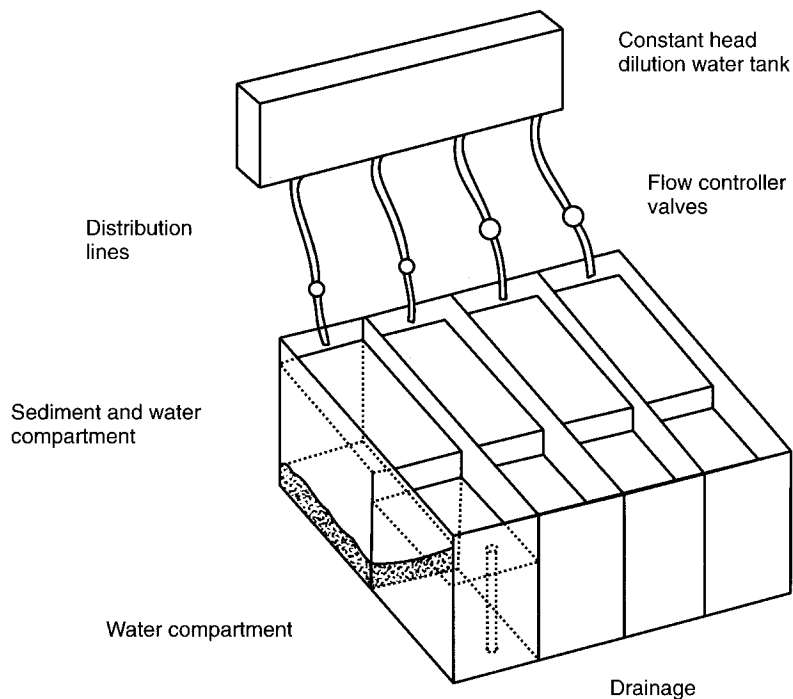


Fig. 1. The continuous flow sediment bioassays apparatus. Each unit consists of a water and sediment chamber and a water exposure chamber. The test species can be placed in either of the chambers.

Bohn *et al.*, 1979; Kezdi, 1980; Katz and Jenniss, 1983) were used to analyse the sediment samples for the following parameters: the pH, moisture content, particle size distribution, cation exchange capacity (CEC) and percent organic carbon content.

The water quality was monitored every other day for dissolved oxygen (DO), pH, specific conductance, temperature, total suspended solids and total volatile solids. All of the water and sediment samples were analysed in triplicate.

Triplicate samples were taken for the cadmium determination. The cadmium concentration, as total, dissolved and particle bound, was measured by atomic absorption spectrophotometry (a Jarrell-Ash 850 equipped with deuterium arc lamp background correction) using the flame (air: acetylene) aspiration method (American Public Health Association, American Water Works Association and Water Pollution Control Federation, 1981; Katz and Jenniss, 1983). Quality control of the cadmium analysis was carried out by analysing blanks and standard solutions.

Spiking procedures

A 1000 mg l⁻¹ cadmium chloride (CdCl₂ 8H₂O) solution was used as a stock solution. Nominal concentrations of 0, 10, 25 and 50 mg Cd per kg sediment were made by soaking 16 kg of air dry sediment in the cadmium chloride solution prepared from the requisite volume of stock solution for each concentration. The control sediment was prepared using dilution water instead of the cadmium

chloride solution. The sediment slurry was mixed for 30 min using an electric drill equipped with a stainless steel blade. The spiked sediments were stored in sealed plastic containers at 4 °C in the dark until used. The actual cadmium concentrations in the test sediments were measured before each experiment.

Experimental design

Toxicity tests were conducted using cadmium-spiked sediments after 1, 31, 79 and 120 days storage periods. Thus, four sets of experiments were completed. Each test consisted of two replicates each of a control and three cadmium concentrations.

After the dilution water was added to the test vessels, 900 g of spiked sediment was placed in each sediment chamber using a stainless steel spatula to form a 5 cm deep layer. The flow of the dilution water was stopped for 12 h to allow settling of the suspended materials. After 12 h, the flow was resumed and adjusted to ensure a uniform flow to all test units.

Fifty *C. tentans* were randomly transferred into each sediment chamber. Midges that showed no movement into the sediments after 30 min were replaced. The test was begun immediately after all of the test organisms were distributed into the units (approximately 30 min). The flow rate was checked daily to ensure a uniform flow of dilution water, which varied less than 10%, to each unit.

The midges were assumed to feed on organic matter and bacterial cells in the sediments and were not fed additional

food. The experiments were conducted at 22.0 ± 2.0 °C and 16:8 h light:dark photoperiod. The sediment chambers were not aerated to minimize sediment resuspension. However, the level of dissolved oxygen in the sediment chambers was monitored.

After a 21 day exposure period, the test organisms were removed and kept in the dilution water for 4 h for gut clearance. The organisms for each concentration were analysed for their tissue cadmium concentrations. The percent survival and biomass were recorded.

Statistical analysis

The data were analysed following the procedures presented in Gad and Weil (1986) and Neter *et al.* (1985) using SYSTAT™ (Wilkinson, 1989). The cadmium concentrations of the sediments of different storage times were compared using Tukey multiple comparisons. Probit analysis (US Environmental Protection Agency, 1989) was used to determine the cadmium LC₅₀ and LD₅₀ in the midges. Because the toxicity is directly related to the chemical dose at the receptor (Landrum *et al.*, 1992), the response used to calculate the LD₅₀ is the proportion of the total mortality in the replicates and the cadmium concentrations in the midges instead of those in the sediments.

Analysis of variance (ANOVA) was used to test the

significant effect of the storage times on the toxicity in exposed midges. Pearson's correlation coefficients were used to determine the relationships between the storage times and percent survival of exposed midges.

To reveal the significant influence of storage time on the cadmium bioavailability, the *t*-test was used to compare the bioaccumulation factor (BAF) values between the tests with sediments from varying storage times. All of the differences were considered statistically significant at $p \leq 0.05$.

Results and discussion

The results of the sediment physicochemical properties and water quality parameters are presented in Tables 1 and 2, respectively. The bulk concentrations of the sediments with varying storage times are presented in Table 3. The percent survival, biomass and tissue cadmium concentrations of *C. tentans* after the exposures and the BAF values are given in Table 3 and Fig. 2.

The extended storage times significantly ($p = 0.001$) influenced the toxicity of the sediment-associated cadmium. A positive relationship ($r^2 = 0.371$) between the times and percent survival indicates a toxicity reduction resulting from extended storage times (Fig. 2I).

Table 1. Physical and chemical characteristics of the test sediments used in the continuous flow toxicity study with *C. tentans*

Parameter	Mean (SE)
Cation exchange capacity (meq per 100 g dry sediment)	36.60 (1.2)
Moisture content (%)	57.18 (2.3)
Particle size (%)	
<2 µm (clay)	34.00 (1.6)
2–6 µm (fine silt)	18.30 (0.7)
6–20 µm (medium silt)	19.70 (0.2)
20–60 µm (coarse silt)	27.00 (0.2)
>60 µm (sand)	1.00 (0.01)
pH	7.41 (0.01)
Organic carbon (%)	1.27 (0.01)

Note: Soil type is silty-clay loam

Table 2. Water quality parameters measured during the continuous flow toxicity study

Parameter	Storage time (days)			
	1	31	79	120
Conductivity (µmhos cm ⁻¹)	825.0 ± 95	780.0 ± 90	910.0 ± 66	811.0 ± 73
pH	7.8 ± 0.2	7.7 ± 0.1	7.7 ± 0.2	7.8 ± 0.1
Temperature (°C)	21.0 ± 1.0	21.0 ± 1.0	21.0 ± 1.5	21.0 ± 1.0
Total hardness (mg CaCO ₃ l ⁻¹)	143.0 ± 12	159.0 ± 15	162.0 ± 15	155.0 ± 13
Total suspended solids (mg l ⁻¹)	0.6 ± 0.1	0.4 ± 0.6	0.6 ± 0.2	0.5 ± 0.2
Volatile suspended solids (mg l ⁻¹)	0.2 ± 0.04	0.1 ± 0.03	0.2 ± 0.04	0.1 ± 0.02

Table 3. Sediment cadmium concentrations (μg per g dry weight) from bulk chemical analysis of sediments having different storage times of 1, 31, 79 and 120 days

Storage time (days)	Concentration (μg per g dry weight)				
	Sediment	Tissue	BAF value	% survival	Biomass
1	0.605 \pm 0.004	0.263 \pm 0.002	0.435 \pm 0.003	69.0 \pm 1.0	11.11 \pm 0.30
1	19.684 \pm 0.215	32.355 \pm 0.297	1.644 \pm 0.015	45.0 \pm 1.0	12.34 \pm 0.22
1	28.084 \pm 0.826	41.910 \pm 0.326	1.492 \pm 0.012	35.0 \pm 1.0	10.70 \pm 0.74
1	51.804 \pm 0.153	118.026 \pm 2.159	2.278 \pm 0.042	16.0 \pm 4.0	10.52 \pm 0.001
31	0.629 \pm 0.012	0.263 \pm 0.002	0.418 \pm 0.003	60.0 \pm 0.01	13.51 \pm 0.76
31	18.327 \pm 0.448	33.069 \pm 0.210	1.804 \pm 0.012	50.5 \pm 1.5	15.89 \pm 1.65
31	29.100 \pm 0.585	40.570 \pm 0.330	1.394 \pm 0.012	40.0 \pm 2.0	11.05 \pm 0.10
31	58.603 \pm 0.256	141.024 \pm 0.045	2.406 \pm 0.001	19.0 \pm 1.0	9.23 \pm 2.28
79	0.620 \pm 0.013	0.103 \pm 0.001	0.167 \pm 0.002	64.0 \pm 4.0	12.66 \pm 2.06
79	14.578 \pm 0.481	14.984 \pm 0.065	1.028 \pm 0.006	53.0 \pm 2.0	14.67 \pm 1.02
79	29.182 \pm 0.816	18.001 \pm 0.059	0.617 \pm 0.002	51.0 \pm 1.0	11.56 \pm 0.43
79	50.523 \pm 0.299	115.460 \pm 0.062	2.285 \pm 0.001	34.5 \pm 2.5	10.58 \pm 0.84
120	0.610 \pm 0.027	0.197 \pm 0.003	0.324 \pm 0.005	71.0 \pm 3.0	11.75 \pm 1.69
120	13.046 \pm 0.862	21.343 \pm 0.239	1.636 \pm 0.018	62.0 \pm 2.0	12.75 \pm 0.13
120	29.231 \pm 0.656	25.446 \pm 0.324	0.871 \pm 0.011	50.0 \pm 2.0	10.48 \pm 0.32
120	50.616 \pm 0.473	127.253 \pm 0.487	2.514 \pm 0.010	40.0 \pm 2.0	10.13 \pm 0.06

The percent survival, biomass (mg wet weight per midge), tissue cadmium concentrations (μg per g dry weight) and BAF values of *C. tentans* after the exposures. The results are reported as the means and standard errors of the means.

Significant decreases in the BAF values were observed between the experiments with the extended storage and the freshly spiked sediments (Table 4). The decrease in the BAF values is likely due to the shift of cadmium partitioning from the reversible pool into resistant sites within the sediment structure after extended storage times (Karickhoff and Morris, 1985). This change in sorption would result in the reduction of the cadmium bioavailability and toxicity (Stemmer *et al.*, 1990). To confirm the result on the cadmium bioavailability and toxicity reduction, the LC_{50} values (Table 5) for the tests with sediments of 1, 31, 79 and 120 days storage times were compared. The increase in the LC_{50} values, i.e. 28.0, 40.5, 63.4 and 62.7 μg per g dry weight, indicates the toxicity reduction in aged sediment.

Further, the LD_{50} values (Table 5) for the tests with sediments of varying storage times, i.e. 47.3, 80.0, 146.7 and 183.9 μg per g dry weight, were compared. The LD_{50} values of the stored sediments were greater than those of freshly dosed sediments. Because the LD_{50} values were calculated based on the concentrations in the midges, which represented the available portion of cadmium in the sediments, the increase in the LD_{50} values indicates that the cadmium accumulated from aged sediments was in a less toxic form. Thus, not only was the overall bioavailability changed but the accumulated cadmium was in a different chemical form as the storage time increased.

While changes in the toxicity were found with storage, chemical analysis showed no significant difference in the cadmium concentrations between the freshly dosed and stored sediments (Table 4). This result suggests that although

cadmium could be recovered from the sediments it was not readily available to the organisms (Green *et al.*, 1993).

No significant difference in the biomass ($p = 0.127$) was detected between the control and exposed midges (Fig. 2II). This might be attributed to several reasons. First, the changes in the cadmium bioavailability were not large enough to be distinguished by midge growth. Second, the midges used in this study were 'too old', that is the growth rate was too slow. Differences in growth might be detected more readily by starting the test with earlier instars. Third, the estimation of the midge weights was performed on the pooled samples of each replicate not individuals. Growth inhibition determined from mean weight of midges could have possibly been biased by the fact that the organisms were somewhat heavier at the beginning of the exposure.

Day *et al.* (1994) reported lower weight in midges that are reared in high densities than those reared individually. Thus, increasing the number of test organisms in each experimental chamber might result in lower growth and a misinterpretation of the toxic effect. The statistical power could be increased by increasing the number of replicates per treatment or by measuring the weight of individuals.

In addition, Day *et al.* (1994) also observed a lower weight in males than in females due to sexual dimorphism in *Chironomus riparius*, *Chironomus frommeri*, *Chironomus plumosus*, *Chironomus prior* and *Chironomus tardus*. Though, a study was not available for *C. tentans* similar results are anticipated. Thus, weight reduction together with the body length and head capsule width and possibly the ratio of different sexes in test animals should be studied.

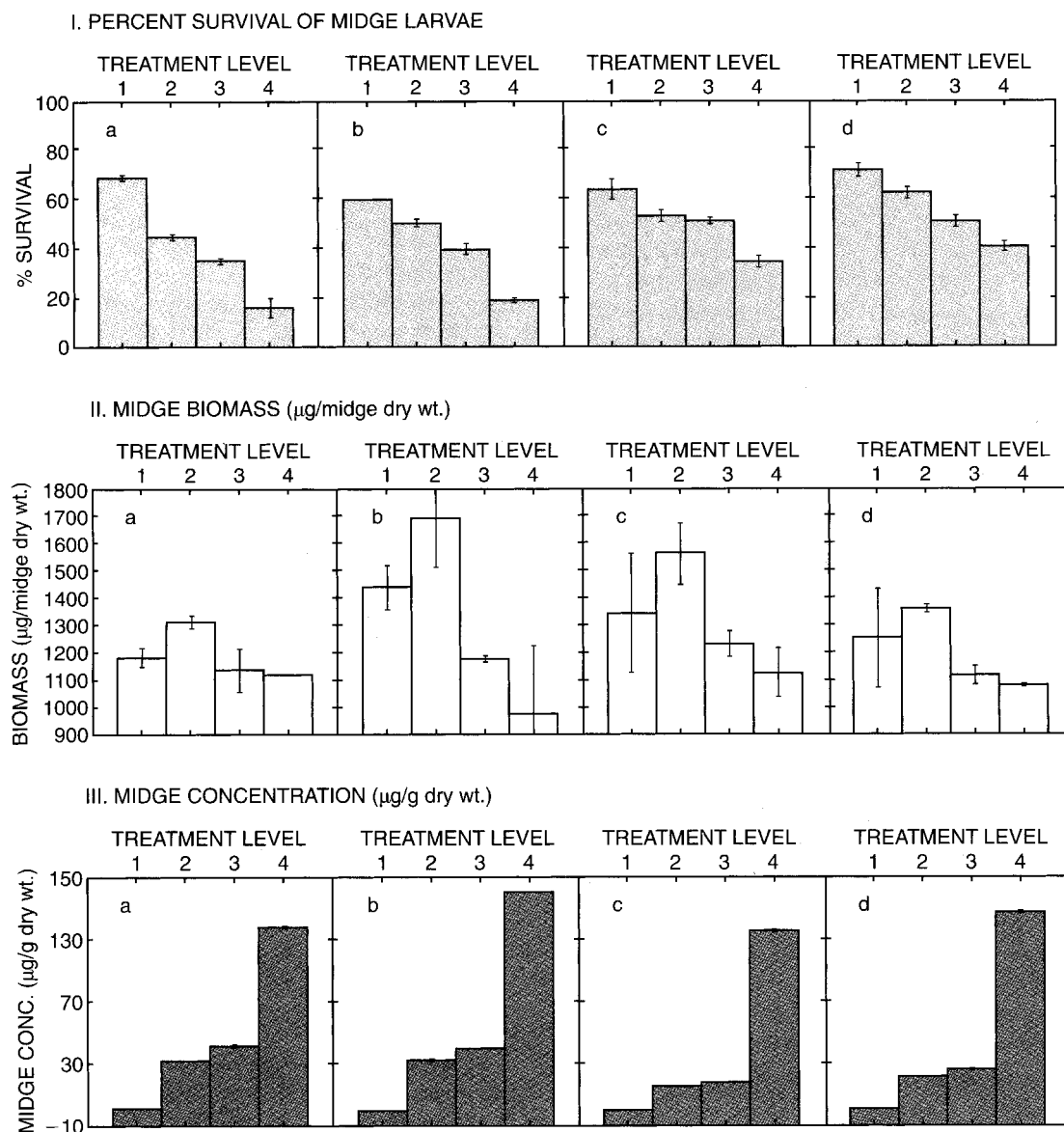


Fig. 2. (I) Percent survival, (II) biomass and (III) tissue cadmium concentration of *C. tentans* after 21 days exposure to cadmium-associated sediments having different storage times of (a) 1, (b) 31, (c) 79 and (d) 120 days with $n = 32$. The error bar represents the standard error of the mean.

Table 4. *t*-test of the BAF values in *C. tentans* after exposure to sediments having different storage times of 1, 31, 79 and 120 days and Tukey's multiple comparisons of the cadmium concentrations in bulk sediments

Comparison paired by storage time (days)	Bioaccumulation <i>p</i> value	Bulk analysis <i>p</i> value
1 versus 31	<0.001	0.999
1 versus 79	0.068	0.999
1 versus 120	0.047	0.998

The *p* value for each comparison is reported.

Table 5. The cadmium lethal concentration (LC_{50}) and lethal dose (LD_{50}) in *C. tentans* after exposure to sediments having different storage times

Storage time (days)	LC_{50} ($\mu\text{g g}^{-1}$)	LD_{50} ($\mu\text{g g}^{-1}$)
1	28.0 (20.2, 34.8)	47.3 (30.2, 64.9)
31	40.5 (27.8, 53.3)	80.0 (47.9, 120.9)
79	63.4 (38.9, 354.6)	146.7 (54.6, 277.9)
120	62.7 (39.3, 394.6)	183.9 (76.2, 382.5)

The results shown are the LC_{50} and LD_{50} values with the 95% upper and lower limits in parentheses.

Conclusion

The practice of storing dosed sediment raises questions such as under what conditions and how long sediment should be stored to simulate the 'natural' situation. The conditions of the storage environment as well as the time employed can have very significant effects on sediment toxicity. This study proposed that after an extended storage time, cadmium partitioning would change by the movement of cadmium from reversible sites to resistant sites in sediment and the sediment-associated cadmium would become less bioavailable. This statement is confirmed by the results of lower BAF and higher LC₅₀ values in tests with sediments of extended storage. Though the chemistry of cadmium in soil and the mechanisms of its retention are not well understood (Novotny and Chesters, 1981), compositional changes, which occurred during storage, also apparently influenced the cadmium toxicity and was observed as higher LD₅₀ values. After extended storage times of the spiked sediments, cadmium apparently resided in a less toxic form based on the increasing LD₅₀ values. The conclusion that cadmium speciation was responsible for reduced toxicity cannot be confirmed because the chemical forms of cadmium in the sediment samples were not determined.

The lack of a significant effect in the growth inhibition study might be the result of (1) the sensitivity of the analytical procedures employed, (2) the sensitivity of the test organisms (age, stage of life cycle, size, health and history of the organism) and (3) the length of the exposure periods.

Consequently, the storage of contaminated and laboratory-dosed sediments prior to toxicity tests should be further investigated. The influences of the length of storage time and the storage conditions should be evaluated and standardized to eliminate a possible variation in the toxicity testing results.

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