EVALUATION OF THE EFFECTS OF STORAGE TIME ON THE TOXICITY OF SEDIMENTS

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ABSTRACT

With the need to collect large numbers of sediment samples and the limited number of laboratory personnel it would be beneficial to be able to store samples longer than the currently suggested 14 day period. Using *D. magna* and *C. tentans* bioassays, sediments from six locations in the North American Great Lakes were evaluated to see if length of storage at 4°C had an effect on toxicity over a 112 day period. No statistical difference was observed over this time period.

INTRODUCTION

A number of assays have been developed to assess the toxicity of sediments (Giesy & Hoke 1989). While a number of these assays are fairly simple, time is required to prepare the sediments and conduct the assays. To be effective in directing remediation of toxic sediments or disposal of dredged materials, the toxic potencies of a large number of samples need to be assessed. It is also more efficient to collect a large number of samples, simultaneously from a single location or during a sampling cruise and store the samples for subsequent analyses. Currently, samples are collected and assays conducted on fresh samples, generally within 14 days of collection. This is done to minimize any changes that may occur in the sediment during storage. Samples have generally been stored, unfrozen, at 4°C to avoid chemical or physical changes in the sediments. Recently, Carr et al. (1989) found that for some assays of sediment pore water from some locations, storage, freezing and thawing od pore water had no effect on the results of subsequent toxicity tests. However, it has long been known that freezing and thawing may change the availability of toxicants from sediments and their toxicity to benthic invertebrates (Schuytema 1989).

The present study was conducted to determine the effects of storage time on the toxicity of sediments from several locations in the Laurentian Great Lakes as measured in laboratory bioassays. We tested the null hypothesis that there was no change in toxicity of sediments to *D. magna* and *C. tentans* as a function of time, up to 112 d of storage.

Samples were collected by the U.S. Army Corps of Engineers, St. Paul, MN (U.S.A.C.O.E.) from six locations: Duluth Harbor, MN, 21st Street Channel, St. Joseph, MI #1, St. Joseph, MI #2, Rouge River, MI, Saginaw Bay, MI, Sebewaing, MI (Reference). Samples were shipped to the Michigan State University Aquatic Toxicology Laboratory in E. Lansing, MI by Thermo Analytical Inc. on May 25, 1989.

Samples from six locations were mechanically homogenized under an argon atmosphere and stored in plastic buckets at 4°C. When subsamples were taken for use in bioassays the contents of each bucket were re-homogenized again without decanting the overlying water.

MATERIALS AND METHODS

D. magna:

Five hundred milliliters of sediment were placed in 4 L polycarbonate containers with 2.5 L of well water at 22°C and allowed to settle for 24 hr. Each jar was aerated with forced air delivered through a glass pipet inserted to 4 cm below the surface. During exposures, chambers were maintained at 22°C under a photoperiod of 16L:8D at an intensity of 300 lux. The acute (48hr) and chronic (10d) effects of sediments on survival and reproduction of *D. magna* were determined by placing 20, 5-d old *D. magna* in each of two replicate test vessels per location. *D. magna* were fed *S. capricornutum* at a rate of 7.0×10^8 cells/day. After 48 hr the adults were counted and at the end of each test (10 d) the number of surviving adults and total number of neonates produced were enumerated.

C. tentans:

The effects of sediments on survivorship and dry weight of larvae of the midge *C. tentans* were determined by placing 25 12-d old larvae in a 3 L polycarbonate container with 100 g dry weight equivalent of sediment (not dried) and 2 L of well water. The sediment and water were added and allowed to settle at 22°C for 3 d before adding the larvae. Two replicates per locaton were conducted simultaneously so that a total of 50 organisms were tested for each holding period. Test chambers were covered with plastic and aerated with forced air introduced through a glass pipet inserted 4 cm below the water surface. Test containers were maintained in an incubation chamber at 20°C under a photoperiod of 16L:8D. After 14 d the test was terminated, the sediment was washed through a 0.8 mm screen and the larvae, pupae and adults (exuvia) were collected. Pupae and larvae were dried for 24 h at 100°C and weighed.

The relative sensitivity of *D. magna* and *C. tentans* were monitored by exposure to 1-octanol as a reference toxicant (Table 1).

Table 1. Toxicity of 1-octanol to *C. tentans* and *D. magna*, as determined by the moving average method (span of 2).

C. tentans
Goodness of fit (G = 0.006)
LC_{50} = 46.78 mg/L
95% confidence interval = 40.36 - 54.30
D. magna
Goodness of fit (G = 0.058)
LC_{50} = 33.56 mg/L
95% confidence interval = 28.02 - 38.64

Experimental Design and Statistical Analysis

The experimental design was a 7 x 5 completely crossed design with two replications. There were six (6) locations, one of which was designated as a reference, and a control (washed silicon sand). There were five holding times, 7, 14, 28, 56 and 112 d. The responses measured were survival of adult *D. magna* after 48 h exposure and total number of neonates produced in 10 d (productivity; DMPROD) and survivorship and growth of *C. tentans* (CTWT). Relative values were also reported by ratioing the response at a particular holding time--location combination to the reference location at the same holding time.

The responses of organisms were examined statistically with two-way ANOVA followed by Tukey's HSD multiple range test for locational effects and for holding times. Levels of significance were set at 0.05 for type I error.

RESULTS

Acute toxicity to *D. magna*:

The toxicity of sediments varied among the six locations and the effects of holding time were location-specific. The acute toxicity of the sediments demonstrated few patterns relative to holding time (Figs. 1-3). In general, the sediments from the St. Joseph location #1 and the Rouge River were the most toxic (Figs. 2-3). Although there were significant location and holding time differences (Table 3), there were no trends in acute toxicity to *D. magna* as a function of holding time, other than the fact that survival was generally

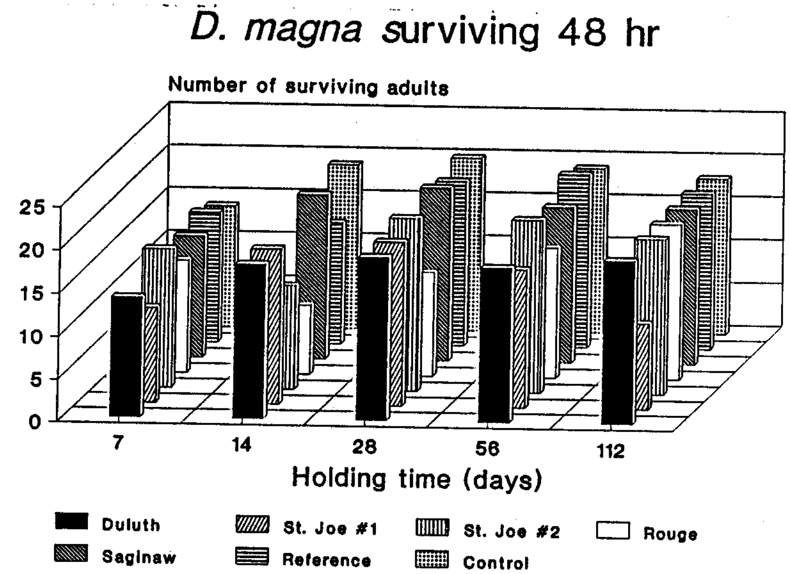

Figure 1. Histograms of number of the original 20 adult *D. magna* surviving to 48 h as a function of holding time and location. There were two reps per location (40 adults total)

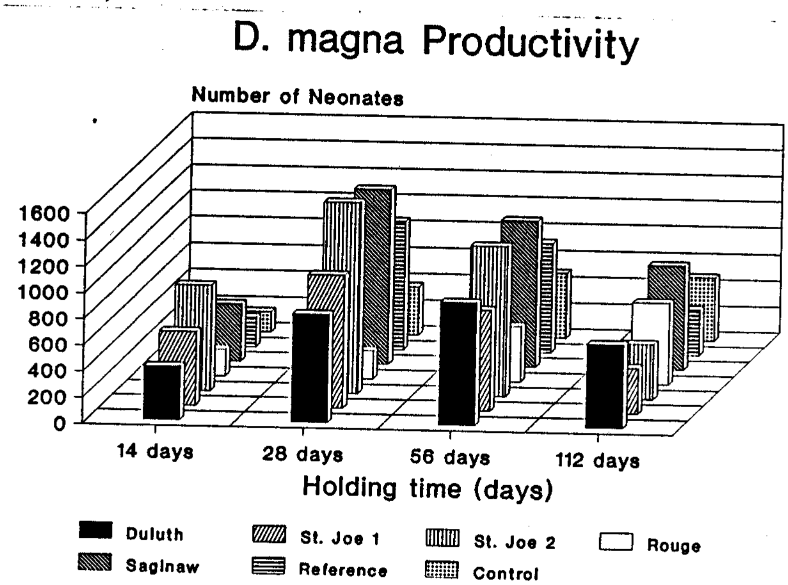

Figure 2. Histogram of total *D. magna* productivity as a function of holding time by location.

lowest after 7 d of holding time (Fig 1). Because the survivorship in the control treatment varied as a function of time, the number of surviving adults was normalized to the survivorship in the Sebewaing reference. However, this did not alter the conclusions. When

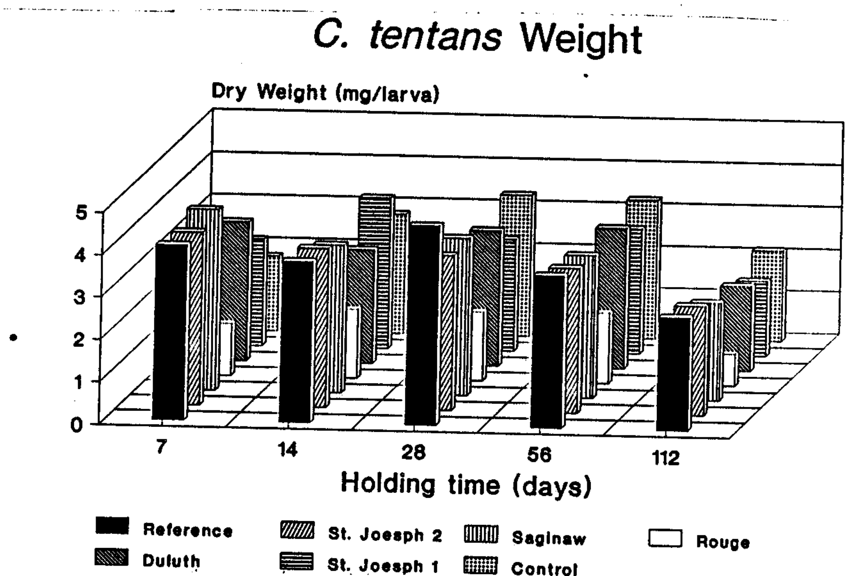

Figure 3. Histogram of *C. tentans* weight as a function of location by holding time.

this is done only the productivity over Rouge River sediment and control sand exhibited negative relative productivities. However, the variability was great enough that the six lesser productivity values were not statistically different from one another (Table 3).

D. magna Productivity

There were no obvious trends in relative productivity of adult *D. magna* as a function of holding time, however there was greater relative productivity in three locations after 14 days of holding time (Fig. 2). When productivity was examined as a function of holding time, even though there were statistically significant differences among holding times, there was no apparent trend (Fig. 2 and Table 4). The least production was observed after 14 d holding time. However, the production after 14 d was not statistically different from that after the longest holding time (Table 4). Therefore, we conclude that there was no significant trend due to holding time on sediment toxicity to *D. magna*.

Significant variation in the total productivity of *D. magna* was abserved over the Sebewaing reference sediment at different holding times, therfore, the relative productivity for each location was calculated as a percentage of that over the Sebewaing reference sediment (Tables 3 and 4). When this was done, the productivity over Rouge River sediment and control sand exhibited negative relative productivities. However, the variability was great enough that the six lesser productivity values were not statistically different from one another (Table 3).

Weight Gain of *C. tentans*

There were significant effects of sediment type and holding time on the growth of *C. tentans*. The effect on weight gain of *C. tentans* was of greater significance in the Rouge River sediment than in any other location (Table 5). The greatest weight gain of *C. tentans* was observed in the Sebewaing reference sediment (Table 5).

When the effect of holding time on *C. tentans* weight was investigated there was no trend due to holding time (Fig 3 and Table 6). The least weight was observed after 112 d of holding, while the best growth was after 28 d of holding.

When *C. tentans* weight in each location was examined, relative to the control (Sebewaing) the pattern of responses changes completely (Table 6). Still there is no trend due to holding time discernable, however it can be seen that the average weight of the *C. tentans* grown on sediments from the five locations, relative to the reference, were negative after all holding times (Table 6).

Table 2. Results of two-way analyses of variance.

	Probability of > F		
SOURCE	Location	Holding Time	Interaction
DM48HR	*****	*****	N.S.
DMPROD	***	***	N.S.
CTWT	*****	*****	**

Probabilities of exceeding F by chance denoted by asterices as below.

$P < 0.0001$ = *****
$0.0001 > P < 0.001$ = ****
$0.001 > P < 0.01$ = ***
$0.01 > P < 0.05$ = **
$0.05 > P < 0.10$ = *
$P > 0.10$ = N.S.
DM48HR = Survival of *D. magna* adults after 48 hr.
DMPROD = Productivity of *D. magna* after 10 d.
CTWT = Mass of *C. tentans* larvae after 14 d.

Table 3. Results of Tukey's HSD multiple range tests among locations for *D. magna* productivity and relative productivity. Probability of type I error = 0.05, 4 holding times, 7 locations, 2 replications. Total corrected degrees of freedom = 55. Mean responses across time are given for each location. Locations underscored by the same line are not statistically different from one another. MSD= the minimum difference to be significant at the 0.05 level of probability.

DMPROD

MSD = 308.08

SJ2	SAG	DUL	SJ1	REF	CON	ROU
961.37	921.50	705.75	674.00	593.87	379.62	367.50
———	———	———	———			
	———	———	———			
		———	———	———		
			———	———	———	———

DMRPROD

MSD = 101.46

SJ2	SAG	DUL	SJ1	REF	ROU	CON
61.88	55.08	18.71	13.49	0.000	-36.08	-38.21
———	———	———	———	———	———	———

CON = Control, Sand
REF = Reference, Sebewaing
ROU = Rouge River
SAG = Saginaw Bay
SJ1 = St. Joseph #1
SJ2 = St. Joseph #2
DUL = Duluth
MSD = Minimum Significant Difference
DMPROD = Total number of neonates at the end of the incubation
DMRPROD = DMPROD for each location normalized to the total number of neonates produced in the reference (Relative DMPROD): $\frac{LOC-REF}{REF} \times 100\%$.

Table 4. Results of Tukey's HSD multiple range tests among holding times (expressed in days) for *D. magna* productivity. Probability of type I error = 0.05, 7 sediment types, 4 holding times, 2 replications total corrected degrees of freedom = 55. Mean number of neonates across locations are given for each holding time. Holding times underscored by the same line are not statistically different from one another. MSD= minimum difference to be significant at the 0.05 level of probability.

DMPROD

MSD = 200.46

28d	56d	112d	14d
884.43	818.79	527.07	400.36
|________	________|		
	|________	________|	
		|________	________|

DMRPROD

MSD = 66.014

14d	112d	56d	28d
77.91	49.76	- 1.98	- 8.25
|________	________|		
	|________	________	________|

See Table 3 for explanatory legend.

Table 5. Results of Tukey's HSD multiple range tests among locations for weight gain of *C. tentans*. Probability of type I error = 0.05, 7 locations, 5 holding times, 2 replications total corrected degrees of freedom = 69. Mean responses across time are given for each location. Locations underscored by the same line are not statistically different from one another. MSD= minimum difference to be significant at the 0.05 level of probability.

CTWT

MSD = 0.5983

REF	SJ2	SAG	DUL	SJ1	CON	ROU
3.773	3.496	3.429	2.893	2.681	2.667	1.374
|________	________	________|			
	|________	________	________|		
			|________	________	________|	
						|________|

CTRWT

MSD = 17.08

REF	SJ2	SAG	DUL	SJ1	CON	ROU
0.000	-6.536	-9.050	-22.955	-28.239	-30.121	-63.786
|________	________	________|			
	|________	________	________|		
		|________	________|		
			|________	________	________|	
						|________|

See Table 3 for explanatory legend.

CONCLUSIONS

Contaminants were found in sediments from all of the locations, including the reference location, however the reference location (Sebewaing, MI) was the least contaminated while the sediment from the Rouge River was the most contaminated (Verbrugge et al 1991). The results of the *D. magna* assay were too variable to demonstrate any effects of holding time on survival or productivity. The only statistically significant effect of location was that sediment from the Rouge River was more toxic than that from any other location. No trends in toxicity to *C. tentans* due to holding time could be demonstrated. The *C. tentans* assay was more sensitive to locational differences than was the *D. magna* test. Weight gain of *C. tentans* was less on sediment from all locations, relative to that on the reference sediment, while the poorest weight gain was observed on sediment from the Rouge River, which was the most contaminated location. It is for this reason that the test design used in this study is neither currently used nor recommend by the MSU Aquatic Toxicology Laboratory. Instead, smaller isolation chambers where the productivity of individual adult *D. magna* and *C. tentans* larvae can be observed individually are recommended.

Table 6. Results of Tukey's HSD multiple range tests among holding times (expressed in days) for dry weight of *C. tentans*. Probability of type I error = 0.05, 5 holding times, 7 Sediment types, 2 replications total corrected degrees of freedom = 69. Mean weight gain across locations are given for each holding time. Holding times underscored by the same line are not statistically different from one another. MSD= minimum difference to be significant at the 0.05 level of probability.

CTWT

MSD = 0.465

28d	14d	56d	7d	112d
3.263	3.103	3.079	3.039	2.026
———	———	———	———	
				———

CTRWT

MSD = 13.275

56d	14d	112d	7d	28d
-14.246	-18.38	-23.846	-27.872	-30.430
	———	———	———	———
———	———	———		

See Table 3 for explanatory legend.

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