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Storage Duration and Temperature and the Acute Toxicities of Estuarine Sediments to *Mysidopsis bahia* and *Leptocheirus plumulosus*

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Many statutory needs for sediment quality assessment exist (USEPA 1996). A variety of sediment toxicity tests have been used to support the development of sediment quality guidelines and to determine the benthic impacts of dredging activities and point and non-point source toxics. Although several sediment test methods have been standardized and some widely used (USEPA/COE 1991, ASTM 1993A, USEPA 1994, APHA et al. 1995), the optimal conditions for storage duration and temperature of sediments before use in toxicity tests are still being investigated. Current recommendations for storage are usually for 2 weeks or less at a temperature between 1 and 7°C (ASTM 1993B). These guidelines are based on the physical structure of sediments as it relates to the availability of nutrients and contaminants during storage (Thomson et al. 1980, MacDonald and McLaughlin 1982).

The reported trends in the scientific literature describing the effects of storage duration and temperature on sediment toxicity are not consistent (for example, Malueg et al. 1986, Schuytema et al. 1989, Carr and Chapman 1995). This inconsistency indicates a need for further research. The objective of this baseline study was to provide additional background information for this important issue using estuarine sediments collected from two urban-impacted estuaries and a reference location.

MATERIALS AND METHODS

Sampling Locations and Storage - The whole sediments were collected during 1995 from three sampling areas near Pensacola, FL (Fig. 1). Two of the sediments were collected from estuarine areas adjacent to Pensacola Bay. One sediment was collected from a location in Bayou Texar and the second from Bayou Grande. The chemical condition of these bayous is impacted by the extensive urbanization in their watersheds. The third sediment was collected from Perdido Bay, located approximately 5 miles west of Pensacola, and served as a reference.

Sediments were collected using a petite PONAR grab sampler. Two replicate samples of sediment were collected at each site, combined and homogenized, placed in 5 gal polyethylene containers, sealed, placed on ice, and returned to the lab for

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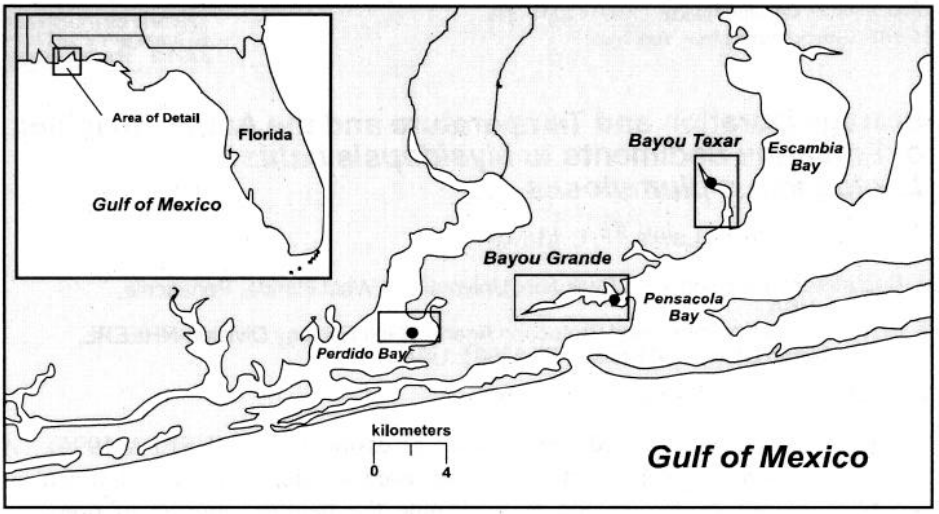

Figure 1. Location of sampling areas.

storage. The samples were split and stored in the dark at three temperatures (-18 °C, 4°C and 24°C) for 2, 4, 16, 32, 64 and 128 days. Sediments to be frozen were stored in separate zip-lock bags. Prior to use in a toxicity test, each sediment sample was re-homogenized without decanting the overlying water. The sediments were chemically-characterized following USEPA (1986) procedures. Salinity and pH were determined using portable instrumentation.

Leptocheirus plumulosus is an infaunal amphipod (family Aoridae) that has a wide distribution from Cape Cod, Massachusetts, to northern Florida (Bousefield 1973). They have been used successfully in 10- and 28-day whole sediment toxicity tests, (DeWitt et al. 1989, Schlekat et al. 1992, McGee et al. 1993). *L. plumulosus* was cultured in two 21 L plastic aquaria receiving flow-through natural seawater. Uncontaminated press-sieved reference sediment was placed in the culture aquaria to a depth of 3 cm. Cultures were fed 2 L of the alga, *Phaeodactylum tricornutum,* and 0.5 g of a mixture of dry fish food, shrimp maturation feed and dried wheat grass leaves three times weekly. On test days, a small amount of sediment containing amphipods was siphoned from the main culture to supply the test organisms.

The second test species was the epibenthic, *Mysidopsis bahia,* which like *L. plumulosus,* has been recommended for sediment toxicity tests (ASTM 1990). Mysids were cultured in filtered seawater at 21 ± 1 ppt salinity and at 24 ± 1 °C. They were fed daily *Artemia salina* nauplii collected 48h post-hydration of cysts. To obtain the test organisms, gravid females were separated from the culture on a light table and placed in a glass beaker overnight. Juvenile mysids were isolated the following day and newly hatched mysids were placed in aerated culture containers until use.

A total of 63 whole sediment-toxicity tests (solid phase) were conducted with each sediment sample and test species. The 10-d static toxicity tests using *Leptocheirus plumulosus* followed the ASTM guideline (1993A). ASTM (1990) and USEPA/COE (1991) procedures were used for the mysid exposures. Mysid toxicity tests were conducted using 600 mL beakers containing 120 mL of press-sieved (1 mm mesh screen) sediment and 400 mL of seawater. Aliquots of 180 mL of sediment and 800 mL of seawater were added to 1-L beakers for the toxicity tests using *Leptocheirus.* The seawater used in the test was salinity controlled at 21 ± 1 ppt and maintained at 24 ± 1 °C. A baffle was used to minimize disturbance of the sediment bed when adding the overlying seawater. The sediment and water were allowed to equilibrate for 24 hrs prior to addition of the test species. The test waters were aerated(two bubbles/second), and dissolved oxygen, temperature, salinity, and pH were measured in all test chambers prior to the addition of the test animals and daily thereafter in one replicate.

Juvenile *L. plumulosus* (2-3 mm) were removed from cultures using a 0.5-mm screen sieve the day of test initiation. Five animals were randomly added to each 1-L beaker after water-quality parameters had been measured. Amphipods were not fed during the experiment. Five *M. bahia* (4- to 7-day old) were pipetted individually into 10 mL glass beakers, the contents of which were added randomly to each of the 600 mL test chambers. Each beaker received approximately 50 *Artemia* nauplii/mysid per day during the test. A photoperiod of 16:8h light:dark was used in all tests.

Twenty sediment dilution toxicity tests were conducted with the Bayou Texar sediment and mysids to more specifically determine the effects of storage conditions. Seven-day tests were conducted with sediment stored at the three test temperatures for 0, 8, 16, 32, and 64 days before use. The Bayou Texar sediment was diluted using the press-sieved reference sediment collected from Perdido Bay. The dilutions were based on a volume-to-volume ratio consisting of 20%, 30%, 40%, 50%, 60% and 100% bayou sediment. Each dilution consisted of 5l of sediment. The prepared portions of the test sediments (reference plus contaminated sediment) were homogenized and distributed to 1-L Nalgene containers for storage at 4 ° C and 24 ° C and in zip-lock bags for storage at - 18 °C.

Results of the toxicity tests are reported as either mean percent mortality or as volume percent for the LC50 values. A two-way ANOVA was used to analyze the influence of locational effects, temperature and storage times on sediment toxicity. The effects on survival were compared over time for each storage temperature by using a one-way ANOVA. Percent survival was arcsine-transformed to meet the assumption of homogeneity of variance. Tukeys HSD test was used to calculate means separation. The level of statistical significance was P = 0.05. Probit analysis was used to derive the LC50 value and 95% confidence limits where possible.

RESULTS AND DISCUSSION

Metal concentrations and several physical characteristics of the test sediments collected from the two bayous appear in Table 1. There was no attempt to correlate the presence or absence of toxicity with a particular metal. However, it can be seen that several potentially toxic metals such as copper and zinc were greater in the Bayou Texar sediment. The only noticeable change in physical appearance of the stored sediments was for those frozen. These sediments clumped and were difficult to sieve. Also, the interstitial or pore water from the Bayou Texar frozen sample was consistently turbid and had a yellow tint.

Table 1. Chemical and physical quality of the bayou sediments. Values for metals are in µg/g dry weight. ND = not determined, BD = below method detection limit.

Parameter	Bayou Texar	Bayou Grande	Parameter	Bayou Texar	Bayou Grande
Arsenic	3.1	0.2	AVS[1]	53	1.3
Cadmium	3.4	0.5	TVS(%)[2]	25	0.7
Chromium	51.0	14.0			
Copper	542.0	4.2	% sand	21	84
Mercury	BD	BD	% clay & silt	67	17
Lead	172.0	7.1	% <5 mm clay	64	17
Selenium	BD	BD	% <2 mm clay	61	17
Zinc	1111.0	9.8	% silt	18	0
Nickel	18.0	0.5			

[1]AVS - acid volatile substances (µg/g dry weight)
[2]TVS - total volatile solids

The pH of the Bayou Texar sediment was the most variable and decreased with lower storage temperature and with longer storage times. The pH of this sediment stored 128 days was usually less one unit or more than that stored for 2 days. For example, after 2 hours the pH was 7.2 units relative to 5.3 units after 128 d. The pH of frozen Bayou Texar sediment stored for comparable periods was consistently lower than that stored at room temperature (24°C). The pH range of sediment stored at -18°C for 128 days was 5.1 to 5.5 units relative to 5.3 to 6.2 units for sediments stored at 24°C. This pH trend was not evident for the other sediment samples for which the pH values were equivalent. The pH of the reference sediment (Perdido Bay) remained very stable in the upper pH 7 to lower pH 8 range regardless of storage conditions. Similarly, there was no consistent trend in pH values for the Bayou Grande sediment, which remained in the upper pH 7 range. Salinity of the Perdido Bay and Bayou Grande sediments was stable and independent of storage conditions. In contrast, salinity of the Bayou Texar sediment decreased with increasing storage time by as much as 1 to 2 ppt.

Control survival during the study was within acceptable limits and averaged 95.2 ± 1.6% for mysids and 98.1 ± 0.9% for amphipods. The initial acute toxicities of the three test sediments prior to storage were different. The Bayou Texar sediment was the most toxic and mortality was 100% for both test species. In contrast, mortality was 10% or less for the other sediments. The different storage durations and storage temperatures had no statistically significant effect (P = 0.05) on the acute toxicities of the undiluted whole sediments in most cases. The Perdido Bay sediment was not acutely toxic; survival exceeded 90% for all the storage conditions. The Bayou Texar sediment remained very toxic for the 128-day storage duration while the Bayou Grande sediments remained relatively non-toxic when stored using the same conditions (Table 2). There were only a few exceptions to this trend. The Bayou Texar sediment stored at 24°C remained toxic to mysids, but the sediment sample stored for 128 days was significantly less toxic to *Leptocheirus* (50% survival). The frozen Bayou Grande sediment was significantly toxic to mysids in tests conducted with samples stored 4, 8 and 16 days, however; samples stored 32, 64 and 128 days were less toxic.

Table 2. Percent survival of *Leptocheirus plumulosus* (L) and *Mysidopsis bahia* (M) in 10-day whole sediment toxicity tests.

Collection Location	Storage Duration(d)	Storage Temperature (°C)					
		-18		4		24	
Bayou Texar		L	M	L	M	L	M
	2	0	0	0	0	0	0
	4	0	0	0	0	0	0
	8	0	0	0	0	0	0
	16	0	0	0	0	0	0
	32	0	0	0	0	0	0
	64	0	0	0	0	0	0
	128	10	10	0	0	50*	0
Bayou Grande		L	M	L	M	L	M
	2	100	90	100	100	100	100
	4	100	70*	90	100	90	100
	8	100	50*	90	100	100	90
	16	100	70*	100	100	100	70
	32	100	100	100	100	100	100
	64	90	90	100	90	100	70
	128	100	100	100	90	100	100

* Significantly different (p > 0.05).

Due to the almost “all or none” results of the whole sediment toxicity tests, dilution toxicity tests were conducted to provide some additional insight on the effect of the storage conditions using the toxic Bayou Texar sediment. In these 7-day studies, toxicity increased (LC50 values decreased) with increasing storage time at each of

the three storage temperatures (Table 3). The LC50 values ranged from an initial 44% and decreased to a low of 3%. The increase in acute toxicity, as measured by the decrease in LC50 values, was obvious after the first 8 days of storage. The average decrease in LC50 values for this time period was 40(SD = ±13)%.

The effects of storage time and temperature on the toxicity of several sediments have been reported in the scientific literature. Some results suggest that sediment toxicity is variable over time (Malueg et al. 1986, Schuytema et al. 1989, Stemmer et al. 1990, Carr and Chapman 1995). A study by Michnowsky et al. (1982) found that concentrations of eight extractable metals from freshwater sediment were not stable after one week of storage and generally increased with storage duration. A similar study by Thomson et al. (1980) showed a shift in four sediment-bound metals of refrigerated estuarine sediments within 15 days. However, some of these studies evaluated laboratory-spiked sediments, and the results may not apply to "naturally" metal-contaminated sediment such as that collected from Bayou Texar.

Table 3. LC50 values for mysids (*Mysidopsis bahia*) after exposure to five concentrations of Bayou Texar sediment diluted with the reference sediment.

Storage Time (d)	LC50[1]	CI		LC50	CI		LC50	CI	
0	44	38	52	44	38	52	44	38	52
8	17*	4	25	28*	19	35	24*	13	31
16	11*	---	—	20*	10	27	19*	9	25
32	10*	---	—	11*	---	—	9*	0.5	16
64	3*	---	—	9*	—	—	14*	6	21

'LC50 values based on pooled mortality data from two replicates after a 7-day exposure. Values represent LC50 value (% sediment) and associated 95% confidence interval. * Significantly different. ($p > 0.05$)

The general recommendation within the scientific community is not to freeze sediments intended for toxicity testing (USEPA/COE 1991, ASTM 1993A, B, USEPA 1994). This is supported by the results of this study. Freezing sediments has been shown to change the availability of toxicants to benthic invertebrates (Schuytema et al. 1989) and cause flocculation of inorganic and organic material (Thompson et al. 1980). Toxicity values for frozen stored sediment and *Daphnia magna* were shown to be unreliable when compared with toxicity estimates for sediments held at 4°C (Malueg et al. 1986). In contrast to these findings, Chien et al. (1990) found that nitrite was not effected by freeze storage, but ammonia and nitrate were. Freeze storage of estuarine sediments was found to have minimal

impact on the concentrations of dissolved inorganic phosphate and silicate (MacDonald and McLaughlin 1982) and on toxicity (Carr et al. 1989, Dillon et al. 1994). The toxicities of several sediments stored at approximately 25 °C, have been reported (Thompson et al. 1980, Michnowsky 1982, Dillon et al. 1994). Room temperature was the least desirable storage condition in these studies, due to a greater fluctuation in the test results relative to those for sediments stored at other temperatures.

Some recent studies indicate sediments can be stored longer than current recommendations and yield acceptable results. Tatem (1988) showed two contaminated marine sediments retained their toxicity for seven months. Othoudt et al. (1991) evaluated six sediments from the Great Lakes area over 112 days and found no statistical difference in toxicity. Likewise, it was reported that sediments could be held longer than 4 weeks without any significant influence on toxicity and bioaccumulation data (Tatem et al. 1991). Dillon et al. (1994) concluded that the test results for an estuarine sediment remained fairly consistent over 20 weeks and that toxicity tests on sediments held longer than 6 weeks "might be acceptable and possibly environmentally conservative." Finally, the survival and growth of the marine polychaete, *Nereis arenaceodentata*, using sediment collected from San Francisco Bay and stored at 4°C was not significantly altered over 2 years (Moore and Dillon 1994).

There were only a few statistical differences in the toxicities for any of the undiluted whole sediments in this study, none of which resulted in any trend for the 128-day storage period. The results of the dilution toxicity tests indicated changes in toxicity which were more related to differences in storage duration than to storage temperature. The trend was for increasing toxicity with increasing age of the test sediment. The maximum differences in LC50 values for sediment stored for the five time periods ranged from 5 (4°C 24°C) to 15 (-18°C). The greater increase in toxicity occurred within the first 8 days of storage, which is a time period within the current recommended storage duration of 2 weeks.

The results of this study confirm the preference of the 4°C storage temperature reported in standard sediment toxicity tests (USEPA/COE 1991, ASTM 1993A,B, USEPA 1994). The maximum difference in toxicity (LC50 values) occurred for those sediments stored at -18°C while the least difference was observed for those sediments held at 4°C. The differences in the LC50 values for the three sediments stored for the same duration but at the different temperatures ranged from one to five-fold.

The current assumption by some that sediment toxicity remains relatively constant over 2 weeks or longer is reinforced by the data presented here, only if the detection oftoxicity of a whole sediment is the objective. This preliminary study demonstrated that the presence or absence of acute toxicity of undiluted whole sediments was consistent over an extended storage time (128 days), and more so when stored at the 4°C. Other researchers have reached similar conclusions (Tatem 1988, Othoudt et

al. 1991, Dillon et al. 1991, Tatem et al. 1991, and Moore and Dillon 1994). In contrast, significant changes in LC50 values were observed for a diluted contaminated sediment; the LC50 values were more conservative as the storage age of the sediment increased. If this calculation is the objective of intended sediment toxicity tests, then, storage time is an important factor to consider. This conclusion reinforces that of Burton and Ingersoll (1994) in that metal-contaminated sediments should be tested as soon as possible since toxicity can be affected by microbial activity, oxidation and varying redox conditions (DiToro et al. 1990, USEPA 1994).

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REFERENCES

American Public Health Association, American Water Works Association, Water Environment Federation (1995) Standard methods for the examination of water and wastewater. 19th Edition American Public Health Association, Washington, DC

American Society for Testing and Materials (1990) Standard guide for conducting life-cycle toxicity tests with saltwater mysids. E 1191-90. ASTM 1990 Annual Book of Standards. Philadelphia, PA

American Society for Testing and Materials (1993A) Guide for conducting 10-day static sediment toxicity test with marine and estuarine amphipods. E1367-92. Standards on Aquatic Toxicology and Hazard Evaluation. Philadelphia, PA

American Society for Testing and Materials (1993B) Standard guide for collection, storage, characterization, and manipulation of sediments for toxicological testing. E 1391-90. ASTM Standards on Aquatic Toxicology and Hazard Evaluation. Philadelphia, PA

Bousefield EL (1973) Shallow water Gammeridean Amphipoda of New England. Cornell University Press, Ithaca, NY, pp. 312

Burton GA Jr, Ingersoll C G (1994) Evaluating the toxicity of sediments. In: The Assessment of contaminated Great Lake sediment. U.S. Environmental Protection Agency Report, Region V, Chicago, IL. EPA/905/B94/002

Carr RS, Williams JW, Fragata CT (1989) Development and evaluation of a novel marine sediment pore water toxicity test with the polychaete *Dinophilus gyrociliatus.* Environ Toxicol Chem 8:533-543

Carr RS, Chapman DC (1995) Comparison of methods for conducting marine and estuarine sediment porewater toxicity test-extraction, storage, and handling techniques. Arch Environ Contam Toxicol 28:69-77

Chien YH, Chen SS, Chen CG (1990) Effects of freeze storage and centrifugal force on inorganic nitrogen content in interstitial water of submerged sediment. J Fish Soc Taiwan 17(3): 199-206

DeWitt TH, Swartz RC, Lamberson JO (1989) Measuring the acute toxicity of estuarine sediments. Environ Toxicol Chem 8: 1035-1048

Dillon TM, Moore DW Jarvis AS (1994) The effects of storage temperature and time on sediment toxicity. Arch Environ Contam Toxicol 27:51-53

DiToro DM, Mahony JD, Hansen DJ, Scott KJ, Hicks MB, Mays SM, Redmond MS (1990) Toxicity of cadmium in sediments: The role of acid volatile sulfide. Environ Toxicol Chem 9:1489-1504

MacDonald RW, McLaughlin FA (1982) The effect of storage by freezing on dissolved inorganic phosphate, nitrate and reactive silicate for samples from coastal and estuarine waters. Wat Res 16:95-104

Malueg KW, Schuytema GS, Krawczyk DF (1986) Effects of sample storage on copper-spiked freshwater sediment. Environ Toxicol Chem 5:245-253

McGee BL, Schlekat DF, Reinharz E (1993) Assessing sublethal levels of sediment contamination with the estuarine amphipod, *Leptocheirus plumulosus.* Environ Toxicol Chem 12:577-588

Michnowsky E, Churchland LM, Thompson PA, Whitfield PH (1982) Changes in extractable metal concentrations during storage of surface water samples containing sediments. Wat Res Bull 18:129-132

Moore DW, Dillon TW (1994) Chronic sublethal effects of San Francisco Bay sediments of Nereis (*Neanthes*) arenaceodentata: Effect of storage time on sediment toxicity. Miscellaneous Paper D-94-2, U.S. Army Engineers Waterways Experiment Station, Vicksburg, MS

Othoudt RA, Giesy JP, Grzyb KR, Verbrugge DA, Hoke RA, Drake JB, Anderson D (1991) Evaluation of the effects of storage time on the toxicity of sediments. Chemosphere 22:801-807

Schlekat CE, McGee BL, Reinharz E. (1992) Testing sediment toxicity in Chesapeake Bay with the amphipod *Leptocheirus plumulosis:* An evaluation. Environ Toxicol Chem 11:225-236

Schuytema GS, Nebeker AV, Griffis WL, Miller CE (1989) Effects of freezing on toxicity of sediments contaminated with DDT and endrin. Environ Toxicol Chem 8:883-891

Stemmer BL, Burton GA Jr, Liebfritz FS (1990) Effect of sediment test variables on selenium toxicity to *Daphnia magna.* Environ Toxicol Chem 9:381-389

Tatem H E (1988) Use of *Daphnia magna* and *Mysidopsis almyra* to assess sediment toxicity. Water Quality '88 Seminar Proceedings. R.G. Wiley, ed., U.S. Army Corps of Engineers Committee on Water Quality, Washington, DC, pp. 269-281

Tatem HE, Brandon DL, Lee CR, Jarvis AS, Rhett RG (1991) Effects of storage on sediment toxicity, bioaccumulation potential, and chemistry. Miscellaneous Paper EL-91-2, U.S. Army Engineer Waterways Experiment Station, Vicksburg, MS

Thompson EA, Luoma SN, Cain DJ, Johansson C (1980) The effect of sample storage on the extraction of Cu, Zn, Fe, Mn and organic material from oxidized estuarine sediments. Wat Air Soil Pollut 14:215-233

U.S. Environmental Protection Agency/U.S. Army Corps of Engineers (1991) Evaluation of dredged material proposed for ocean disposal (testing manual). Implementation Manual for Selection 103 of Public Law 92-532 (Marine

Protection, Research, and Sanctuaries Act of 1972, U.S. Army Engineers Waterways Experiment Station, Vicksburg, MS

U.S. Environmental Protection Agency (1986) Test methods for evaluating solid waste, Vol. 1A: Laboratory manual physical/chemical methods. USEPA SW-846, Third Edition, Washington

U.S. Environmental Protection Agency (1994) Methods for assessing the toxicity of sediment-associated contaminants with estuarine and marine amphipods. EPA/600/R/025. Office of Research and Development, Washington, DC

U.S. Environmental Protection Agency (1996) The national sediment quality survey. EPA/823/D/96/002. Office of Science and Technology. Washington, DC