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The Relevance of Rooted Vascular Plants as Indicators of Estuarine Sediment Quality

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Abstract. Toxicity assessments and numerical quality assessment guidelines for estuarine sediments are rarely based on information for aquatic plants. The effect of this lack of information on contaminated sediment toxicity evaluations is largely unknown. For this reason, the toxicities of whole sediments collected from 15 sites in three urbanized Florida bayou-estuaries were determined for the benthic invertebrates *Mysidopsis bahia* and *Ampelisca abdita* and the plants *Scirpus robustus* Pursh (saltmarsh bulrush) and *Spartina alterniflora* Loisel (saltmarsh cordgrass). The results of the bioassays, conducted for 7 to 28 days, were compared for interspecific differences and to effects-based, sediment quality assessment guidelines. A variety of inorganic and organic analytes were detected in the estuarine sediments, and concentrations of as many as 7 analytes exceeded the sediment guidelines at the 15 sampling locations. Toxicity occurred at 2 of the 15 sampling stations based on invertebrate survival. Twelve of the 15 sediments had either a significant stimulatory or inhibitory effect on early seedling growth relative to a reference sediment ($p < 0.05$). The phytoresponse was specific to the location, test species, and plant tissue. There was no consistent trend between the sensitivities of the plants and invertebrates exposed to the sediments collected from the same sites. Of the 12 sediments that significantly affected seedling growth, 10 were not acutely toxic to the invertebrates. Consequently, the plant test species provided information that would have been missing if only animal test species were used. For this reason, the phytotoxicity database needs to be expanded for contaminated sediments to further evaluate interspecific sensitivities and to provide perspective on the environmental relevancy of proposed sediment quality criteria and effects-based assessment guidelines for which this information is usually missing. However, additional test method development and field validation are needed to support this effort, which includes the identification of sensitive plant test species, response parameters, and the chemical and physical sediment factors that influence plant growth.

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Contaminated sediments have been shown to adversely affect benthic invertebrates and influence the environmental condition of other biota (US EPA 1994a, 1996a, 1998). The determination of the sources, causes, magnitude, and spatial extent of sediment contamination in the nations' waters is of high priority since specific information is limited for many coastal areas. Consequently, there is a considerable need for the chemical characterization of sediments collected from geographically diverse areas and the determination of any corresponding biological impact.

Historically, the biological effects of contaminated sediments have been estimated either from chemical concentrations alone (FDER 1994; US EPA 1996a, 1997; Engle and Evans 1997) or in combination with results of animal acute bioassays (Pastorok and Becker 1984; Schlekat *et al.* 1994; Hoffman *et al.* 1994; Zarbock *et al.* 1996; Long *et al.* 1996, 1997). The conclusions of these evaluations, often part of large monitoring programs, are affected by the uncertainties associated with the bioavailability of the reported or estimated contaminant concentrations and the relevance of the results of the few and often different types of bioassays used. It is widely recognized that interacting physical, chemical, and biological factors can effect the bioavailability of sediment contaminants and that differences in the sensitivities of the animal test species used in the bioassays are common (Traunspurger and Drews 1996).

No scientific consensus exists on the suite of test species needed to characterize the toxicity of sediments, although some have been proposed (Van de Guchte 1991; Giesy and Hoke 1990; US EPA 1994b). Most standard test methods designed to determine sediment toxicity recommend animal species (Dillon and Gibson 1990; ASTM 1993; Hill *et al.* 1993; US EPA 1994a; APHA *et al.* 1995). About 30 species of freshwater and 71 species of marine benthic species have been used for this purpose (Traunspurger and Drews 1996), and new species are being constantly evaluated (Lewis and Foss 2000). Of those test species used to date, however, few have been aquatic vascular plants.

Phytoassessment has not been considered in most contaminated sediment evaluations nor in the derivation of sediment quality assessment guidelines and national sediment criteria. This trend exists despite the fact that aquatic plants are in-

cluded in environmental assessments for commercial chemicals and pesticides (US EPA 1985), wastewaters (US EPA 1992a, 1992b), and the derivation of water quality criteria (Stephan *et al.* 1985). This relative lack of consideration is important to recognize since the benthic macroflora is an ecologically important component of the shallow estuarine and near-coastal areas characteristic of the Gulf of Mexico. In these areas, rooted submerged and emergent plants serve as food sources and provide shelter and substrate for many commercially important organisms. Furthermore, they have important structural and functional attributes that relate to energy and nutrient cycling and sedimentation processes. In recent years, the submerged aquatic vegetation has been declining in the Gulf of Mexico, which may be due, in part, to declining sediment quality.

Rooted aquatic plants, in addition to their ecological importance, are desirable test species in sediment bioassays because they are exposed to contaminants in the water column and sediment, and can respond to inhibitory and stimulatory compounds. Bioassay methods specific for vascular plants and sediments are available that are based on measuring root elongation, seed germination, and early seedling growth (Folsom *et al.* 1984; Weber *et al.* 1994; APHA *et al.* 1995; Ramanathan and Burks 1996; Powell *et al.* 1996; ASTM 1997). In addition, tests with algae and phytoplankton have been used to determine the toxicities of sediment elutriates (Birmingham and Colman 1983; Ross *et al.* 1986; Munawar and Munawar 1987; Hall *et al.* 1996; Wildhaber and Schmitt 1996). Despite the availability of these phytoassessment methods, rooted plants have been used infrequently to assess sediment toxicity, and there is insufficient evidence on which to base their value relative to that of the common practice of using animal test species alone. The objective of this study was to develop comparative information on the sensitivities of benthic macroinvertebrates and plant seedlings exposed to several estuarine sediments collected from the same locations.

Materials and Methods

Study Area and Sediment Collection

Sediments were collected during June and September 1995 from 15 stations located in Bayous Texar, Chico, and Grande, which are located near Pensacola, Florida (Figure 1). The condition of these adjacent residential bayous has been impacted by the extensive urbanization and industrialization in their watersheds (Lewis *et al.* 2000a). Sediments were collected using a Ponar sampler (volume 2.1 L) to an approximate depth of 13 cm. Replicate samples were collected from each station, homogenized, and split for chemical and toxicological analysis. The sediments were stored at 4°C for 5 to 28 days before use in the bioassays.

Chemical Analysis

Sediments collected during June and September were analyzed for chemical quality. Particle size distribution was determined following standard methods (APHA *et al.* 1995). Concentrations of those trace metals, organochloride pesticides, PCBs (polychlorinated biphenyls), and PAHs (polycyclic aromatic hydrocarbons) listed in Table 1 were determined following U.S. EPA techniques (US EPA 1997) and are reported in terms of dry weight. Samples for trace metal analysis (chromium, copper, cadmium, lead, nickel and zinc) were digested in nitric acid using a microwave oven. Samples were then analyzed using a Jarrell-Ash Atom Comp Series 800 Intracoupled Plasma Spectrophotometer (Fisher Scientific Co., Franklin, MA). The method detection limits (MDLs) for the metals ranged from 0.7 to 4.0 μg/g dry weight.

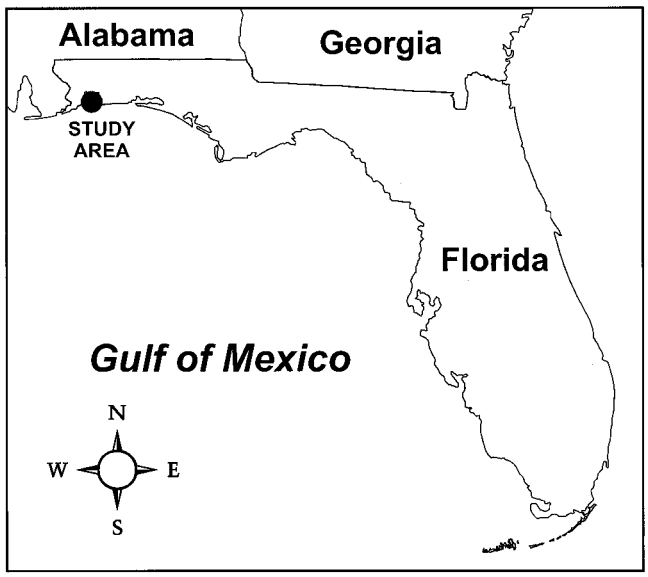

Fig. 1. Location of the three tidal bayous near Pensacola, (Escambia County) Florida, from which the sediments were collected

The 15 sediment samples were analyzed for chlorinated pesticides (14 compounds), PCBs (12 congeners), and PAHs (13 compounds). The samples were solvent extracted (acetone/acetonitrile) for 30 min and the elutriates were analyzed using a HP-5890 Series II gas chromatograph equipped with an electron capture detector (Hewlett Packard Corp., Palo Alto, CA). The analysis used multilevel calibration and internal standards for peak identification and quantification. The method detection limits were 1.0 ng/g dry weight (pesticides and PCBs) and 0.2 ng/g dry weight (PAHs). Standards, blanks, and spiked surrogates were used in all analytical determinations.

The concentrations of the organic and inorganic contaminants in the sediments were compared to sediment quality assessment guidelines (SQAGS) proposed for Florida coastal waters (MacDonald *et al.* 1996). These guidelines are intended as informal benchmarks to help assess the biological significance of sediment chemical quality (Long and MacDonald 1998). The specific guideline values used in the comparison were the threshold effects level (TEL) and the probable effects level (PEL). A thorough description of these terms, including their appropriate use, has been published (FDER 1994; MacDonald *et al.* 1996). Concentrations exceeding the TEL but which are less than the PEL represent concentrations that occasionally are associated with adverse effects, whereas those exceeding the PEL represent concentrations that are frequently associated with benthic impacts.

The test waters overlying the sediments during the bioassays were analyzed for several parameters daily. Concentrations of dissolved oxygen (mg/L), the pH, and temperature (°C) were analyzed using portable instrumentation (Hydrolab Corp., Austin, TX). Salinity was determined using a refractometer (Leica Co., Buffalo, NY).

Table 1. List of compounds analyzed in the sediments which were used for the invertebrate and plant bioassays. Chemical abstract service registry (CAS) numbers presented

Chlorinated Pesticides	PAHs	PCB Congeners	Trace Metals
● HCB (118-74-1)	● Naphthalene (91-20-3)	● C-8 (34883-43-7)	● Cadmium (7440-43-9)
● Lindane (53-39-9)	● Acenaphthylene (208-96-8)	● C-18 (37630-65-2)	● Chromium (7440-47-3)
● HEP (76-44-8)	● Fluorene (86-73-7)	● C-28 (7012-37-5)	● Copper (7440-50-8)
● Aldrin (309-00-2)	● Phenanthrene (85-01-8)	● C-44 (41464-39-5)	● Nickel (7440-02-0)
● HEP-epoxide (1024-57-3)	● Anthracene (120-12-7)	● C-52 (35693-99-3)	● Lead (7439-92-1)
● o, p-DDE (3424-826)	● Fluoranthene (206-44-0)	● C-66 (32598-10-0)	● Zinc (7440-66-6)
● a-Chlordane (57-74-9)	● Pyrene (129-00-0)	● C-105 (32598-14-4)	
● t-Nonachlor (39765-30-5)	● Benzo(a)anthracene (56-55-3)	● C-118 (31508-00-6)	
● Dieldrin (60-57-1)	● Chrysene (218-01-9)	● C-128 (38380-07-3)	
● p, p-DDE (72-55-9)	● Benzo(b)fluoranthrine (205-99-2)	● C-138 (35065-28-2)	
● o, p-DDD (53-19-0)	● Benzo(k)fluoranthrine (207-08-9)	● C-153 (35065-27-1)	
● p, p-DDD (72-54-8)	● Benzo(a)pyrene(50-32-8)	● C-170 (35065-30-5)	
● o, p-DDT (789-02-6)	● Indeno-1,2,3-pyrene (193-39-5)	● C-180 (35065-29-3)	
● p, p-DDT (50-29-3)	● Dibenzo(a,h)anthracene (53-70-3)	● C-187 (52663-68-0)	
● Dieldrin (60-57-1)	● Benzo(g,h,i)perylene (191-24-2)	● C-195 (52663-78-2)	
● Endosulfan I (959-98-8)		● C-206 (70186-72-9)	
● Endrin (72-20-8)		● C-209 (2051-24-3)	
● Endosulfan II (33213-65-9)			
● Endrin aldehyde (7421-93-4)			
● Endosulfan sulfate (1031-07-8)			
● Endrin ketone (53494-70-5)			

Whole Sediment Toxicity: Benthic Invertebrates

Whole sediment acute bioassays were conducted with the epibenthic invertebrate *Mysidopsis bahia* and the infaunal amphipod *Ampelisca abdita*. Both species are recommended for use in sediment bioassays (ASTM 1993; US EPA 1994b). The test methodologies followed standardized procedures (ASTM 1993; US EPA 1994b; US EPA 1996b) and several experimental conditions are summarized in Table 2. The static bioassays with mysids were conducted with the same sediments as those used in the phytotoxicity tests which were collected in June. *A. abdita*, in a supplemental study, were exposed to sediments collected during September from the same locations as those used in the mysid and plant bioassays. The bioassays were of 4 days (mysids) and 10 days (*Ampelisca*) duration after which survival was determined.

A reference sediment was included in the bioassays. This sediment was collected from Perdido Bay, Florida, and its nontoxic nature has been confirmed in numerous occasions at the U.S. EPA Gulf Ecology Division Laboratory (Gulf Breeze, FL). Survival of *M. bahia* and *A. abdita* after exposure to this sediment has averaged 95.3% (±1 standard deviation = 4.7; range = 83–100) and 96.2% (±5.1; range = 86–100), respectively, in 15 and 11 previously conducted bioassays.

Table 2. Summary of the experimental conditions for the whole sediment bioassays conducted with the two estuarine invertebrate test species

	Test Species	
Experimental Conditions	*Mysidopsis bahia*	*Ampelisca abdita*
Temperature (°C)	20	20
Light quality	Ambient	Ambient
Light intensity (fc)	50–100	50–100
Photoperiod	16L:8D	16L:8D
Test vessel volume (L)	1.9	1
Volume of sediment (ml)	200	200
Test water volume (ml)	800	750
Replicates	3	5
Organisms/replicate	10	20
Salinity (ppt)	20	30
Feeding	daily (*Artemia*)	none
Aeration	Constant bubbling	Constant bubbling
Duration of test (days)	4	10
Endpoint	Death	Death

Whole Sediment Toxicity: Vascular Plants

Two rooted emergent hydrophytes were used as test species *M. Spartina alterniflora* Loisel (cordgrass) and *Scirpus robustus* Pursh (saltmarsh bulrush) (Figure 2). *S. alterniflora* is a major primary producer and energy source in Gulf coast estuaries and is also a dominant plant in salt marshes along the Atlantic coast (Valiela *et al.* 1978). This species has value in shore erosion control and its rhizomes and seeds are consumed by wetland mammals and waterfowl. It is considered a saltwater index plant (Folsom *et al.* 1984). *S. robustus* (Family Cyperacea) is a well-known source of carbohydrate-rich corns to waterfowl (Drifmeyer and Redd 1981; Kantrud 1996). It grows primarily in estuarine intertidal emergent wetlands. The salinity tolerance of *S. alterniflora* is estimated to be 0–35 ppt and 0–39 ppt for *S. robustus* (Environmental Concern Inc., 1996; Kantrud 1996).

Seedlings of the two species were exposed to whole sediments collected from the 15 sampling stations. The seedlings were grown either from seeds (*S. alterniflora*) obtained from a commercial source (Environmental Concern, St. Michaels, MD) or field-collected from mature plants (*S. robustus*). The seeds were stored until use at 4°C and for *S. alterniflora*, in 4 ppt sea water. Procedures for sediment preparation, germination, culture, and exposure followed those reported by *Walsh et al.* (1990, 1991) and Weber *et al.* (1994) and are briefly described below.

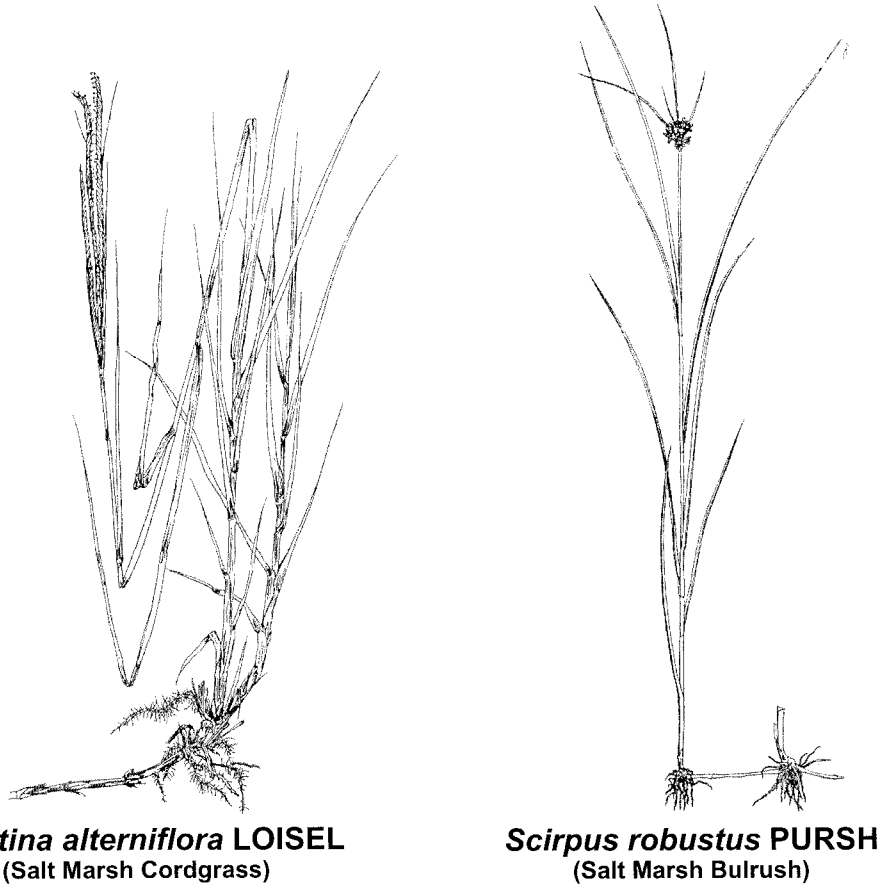

Fig. 2. The rooted vascular plants exposed to whole sediments for 21 to 28 days. Figures from Mason (1957) and Tobe (1998). For a more detailed physical description and their ecological value, see Thunhorst (1993); Kantrud (1996); and Tobe (1998)

Culture Technique. Prior to germination, seeds of *S. alterniflora* were treated with a 20% solution of commercial bleach (active ingredient 5.25% sodium hypochlorite) for 20 min. Seeds of *S. robustus* were scarified with concentrated sulfuric acid for 50 min. Germination was initiated by placing 25–50 seeds of each species in petri plates containing deionized water for 7–10 days. Seeds were incubated under an 8 h light: 16 h dark photoperiod (lighted from 6 AM to 8 PM). Diurnal incubation temperatures were 18°C dark:35°C light. The resultant seedlings were transplanted and cultured in fine sand (Mystic White No. 90; New England Silica, Inc., South Windsor, CT) for 7–10 days, and maintained in a growth chamber under a photo period of 16 h light (6 AM to 10 PM)/8 h darkness (10 PM to 6 AM) at a day/night temperature of 25/22 ± 1°C. Intensity of the fluorescent lights was between 200 to 225 $\mu E\ m^{-1}s^{-2}$ as measured at the surface of the growth chamber bench. The seedlings were watered twice with Hoagland's nutrient solution (Hoagland and Arnon 1950) during the culture period.

Sediment Preparation. The sediment samples were removed from storage 24–48 h prior to use and homogenized with a stainless steel spatula. Interstitial water was collected by centrifugation (6,000 *g*) and analyzed for pH (Beckman Model 12 pH meter, Beckman Corp., New York, NY) and salinity (refractometer, Leica Corp., Buffalo, NY). The homogenized sediments were added to Styrofoam test containers, 7.5 cm diameter × 5.5 cm high, that had openings in the bottom for drainage. The test chambers were placed in plastic containers that were used to maintain a constant supply of water to each sediment/plant system.

Test Method. Two seedlings of the same species were transplanted to each test chamber containing the whole undiluted sediments. Seedlings of similar size (5.0 cm) and age (20 days) were used. The tests were conducted in triplicate for each of the 15 bayou sediment samples and also for the reference sediment. Twenty-two milliliters of Hoagland's nutrient solution were added three times weekly during the 21-day (*S. robustus*) and 28-day (*S. alterniflora*) tests. Daily observations were made for foliar symptoms and seedling survival. At test termination, the seedlings were removed from the sediments. Shoots and roots were cut from the caryopses and separately dried at 105°C to a constant weight (to nearest 0.1 mg) to determine postexposure biomass. An increase or decrease in biomass was considered a negative response. In addition to the biomass, the number of rhizomes (horizontal stems or stolons) were counted for each plant.

Statistical Analysis

All statistical analyses were performed using commercially available software (SAS Institute Inc. 1989). The data usually met assumptions of normality (Shapiro-Wilks test for normality) and equality of variances (Bartletts test for homogeneity of variance). In some cases a square root transformation was required to correct for lack of normality. One-way analysis of variance (ANOVA) was conducted to determine if seedling biomass (roots and shoots) was significantly different. Duncan's *post hoc* test was performed to determine if differences occurred between the reference and bayou sediments. The probability level determining significance was $\alpha = 0.05$.

Results

Sediment Chemical Quality

The chemical quality of the sediments collected during June and September was similar. The differences in concentrations

Table 3. Chemical quality and particle size distribution of the sediments collected during June and used in the bioassays with mysids and the vascular plants

Bayou	Sampling Station	Cadmium	Chromium	Copper	Nickel	Lead	Zinc	Total Trace Metals	Total Pesticides[1]	Total PAHs[2]	Total PCBs[3]	Particle Size (%)	
												Sand	Silt/Clay
Texar	1	BD	26.5	25.9	6.7	70.0	179.0	308.1	16.2	9,961.1	39.2	7	93
	2	0.8	48.2	45.0	12.9	96.0	325.0	527.9	25.6	1,876.0	17.9	12	88
	3	1.4	30.8	88.6	8.7	145.0	548.0	822.5	60.9	3,490.9	36.6	4	96
	4	2.7	32.7	338.0	11.2	125.0	833.0	1342.6	91.0	22,122.2	43.5	2	98
Chico	1	BD	5.9	22.6	2.2	31.0	154.0	215.7	29.5	8,890.5	39.7	75	25
	2	BD	21.7	95.8	10.6	37.0	301.0	466.1	43.5	5,474.8	49.2	48	52
	3	1.3	34.4	172.0	16.3	79.0	753.0	1056.0	53.4	14,813.3	150.2	7	93
	4	0.8	25.3	78.0	9.0	62.0	405.0	580.1	43.0	3,948.2	55.5	29	71
	5	1.6	37.9	103.0	21.8	283.0	510.0	957.3	ND	ND	169.7	ND	ND
Grande	1	10.5	397.0	56.0	8.0	141.0	152.0	764.5	23.2	3,630.9	91.5	29	71
	2	BD	5.0	2.6	BD	24.0	12.8	44.4	10.9	7,057.6	4.4	ND	ND
	3	3.7	178.0	40.2	11.9	130.0	150.0	513.8	4.6	1,014.7	75.4	88	12
	4	2.0	57.5	17.7	6.4	55.0	84.0	222.6	27.1	4,303.9	57.9	ND	ND
	5	2.7	156.0	24.8	12.8	71.0	122.0	389.3	4.8	580.2	37.4	14	86
	6	BD	25.6	8.7	3.3	17.0	35.1	89.7	3.7	228.0	5.9	43	57
MDL		0.7	1.2	0.7	1.5	4.0	0.7	—	—	—	—	—	—

Values in μg/g dry weight for metals and ng/g dry weight for other analytes
BD = Below method detection limit (MDL). ND = not determined
[1] Cumulative total for 14 compounds
[2] Cumulative total for 13 compounds
[3] Cumulative total for 12 congeners

of specific analytes at the same stations were usually ±20%. Therefore, for the interspecific sensitivity comparisons, the chemistry results for the sediment collections were considered equivalent.

The concentrations of specific trace metals and the cumulative total concentrations of trace metals and the organic contaminants for the June sediment collection are shown in Table 3. There was considerable spatial variation in chemical quality within and among the bayous. Differences in concentrations of trace metals, chlorinated pesticides, PAHs, and PCBs among stations located within the same bayou were as great as 10-fold. Copper, lead, and zinc were the greater contaminants in Bayous Texar and Chico and zinc, chromium, and lead predominated in Bayou Grande. The total metal concentrations averaged for all stations within each of the three bayous ranged between 337.4 μg/g (±1 standard deviation = 274.4) and 750.4 μg/g (±447.8).

The total concentrations of pesticides and PAHs averaged for all stations were similar in Bayous Texar and Chico and were greater by a factor of 2 to 3 than those in Bayou Grande (Table 3). For example, the average total PAH concentrations in Bayous Texar and Chico were 8,575.0 (±9,969.2) ng/g and 8,282.0 (±4,820.6) ng/g respectively, compared to 2,802.4 (±2,676.3) ng/g in Bayou Grande. The more frequently detected pesticides were DDT and the associated metabolites. The maximum total concentrations of trace metals, pesticides, and PAHs occurred for the same station in Bayou Texar and were 1,342.6 μg/g, 91.0 ng/g, and 22,122.2 ng/g, respectively.

Total PCB concentrations in Bayou Chico sediment were approximately four times greater (103.1 ± 85.6 ng/g) than in Bayou Texar (28.1 ± 10.1 ng/g) and approximately two times higher than in Bayou Grande (41.9 ± 29.4 ng/g). The maximum total PCB concentration of 169.7 ng/g occurred in a Bayou Chico sediment sample.

The sediments used in the bioassays usually were dominated by silts and clays (Table 3). Two stations in Bayous Chico and Grande were sand-dominated. The silt/clay content in the sediments ranged from 12% to 98% and 2% to 88% sand. Pore water salinities, on average, ranged from 18 to 24 ppt for the 15 bayou sediments used in the bioassays with *S. robustus* relative to 23 ppt for the reference sediment. The average pore water salinities for the 15 sediments to which *S. alterniflora* were exposed ranged from 17 to 22 ppt. The reference sediment had a mean salinity of 21 ppt.

Chemical Quality and Sediment Quality Assessment Guidelines

At least one of the SQAGs was exceeded in 14 of the 15 sediments collected during June (Table 4) and in all sediments collected during September. The total number of SQAGs exceeded at the 15 sampling stations ranged from zero to seven (June) and zero to six (September). The total number of PEL guidelines exceeded at each station ranged from zero to four for both sediment collections. Some biological risk was predicted for all sediments but particularly for the nine sediment samples collected during June and September, respectively, where PEL guidelines were exceeded. The mean PEL quotients ranged from 1.1 to 2.1 for both groups of sediments. The quotients represent the measured concentrations of the chemicals in the sediment divided by the corresponding probable effect level guidelines and then averaged for each site. For more detail see Long *et al.* (1998).

Table 4. Comparison of whole sediment toxicity to estuarine invertebrates and plant test species and sediment quality assessment guidelines proposed for Florida

		Benthic Invertebrates		Vascular Plant Seedlings					
				Scirpus robustus		*Spartina alterniflora*		Sediment Quality Assessment Guidelines[1]	
Bayou	Sampling Station	*Mysidopsis bahia*	*Ampelisca abdita*	SW	RW	SW	RW	≥TEL <PEL[2]	≥PEL
Texar	1	100	96	115	167*	53	87	copper, lead, zinc, total PAHs, total PCBs	—[3]
	2	100	97	132	178*	52	86	cadmium, copper, lead, total PAHs	zinc
	3	100	96	75	107	30*	63*	cadmium, copper, total PAHs, total PCBs	lead, zinc
	4	3	6	100	211*	14*	31*	cadmium, total PCBs	copper, lead, zinc, total PAHs
Chico	1	97	84	20*	59	96	137	copper, lead, zinc, total PAHs, total PCBs	—
	2	93	92	45	56	66	139	copper, lead, total PAHs, total PCBs	copper, zinc
	3	100	96	108	181	64	105	cadmium, nickel, lead, total PAHs, total PCBs	zinc
	4	97	96	230*[4]	563*	138*	187*	copper, lead, total PAHs, total PCBs	zinc
	5	97	ND[5]	198*	211	144	217*	cadmium, copper, nickel, total PCBs	zinc, lead
Grande	1	100	13	243*[4]	300*	71	128	copper, zinc, total PAHs, total PCBs	cadmium, chromium, lead
	2	93	94	198*[4]	511*	114	217*	total PAHs	—
	3	100	96	166*	196	32*	70	cadmium, copper, zinc, total PCBs	chromium, lead
	4	100	92	147	96	78	178*	cadmium, chromium, lead, total PCBs, total PAHs	—
	5	97	97	128	115	32*	80	cadmium, chromium, lead, zinc, total PCBs	—
	6	100	93	113	122	74	141	—	—

Values for plants represent mean % shoot (SW) and root (RW) biomass relative to plants exposed to the reference sediment. Values for invertebrate species represent % survival relative to reference sediment

* Significant difference ($p < 0.05$)

Values for Bayou Grande adapted from Lewis *et al.* (2000b)

[1] Compounds that exceed guideline concentrations (MacDonald *et al.* 1996)

[2] TEL = threshold effects level; PEL = probable effects level

[3] No guideline concentrations exceeded

[4] Possibly salinity-influenced

[5] Not determined

Whole Sediment Acute Toxicity

Physicochemical Quality. Dissolved oxygen, pH, salinity, and water temperature varied less than 10% during each bioassay. The dissolved oxygen in the test waters during all bioassays ranged from 73% to 109% saturation; and the pH varied from 7.1 to 8.4 units. Salinity was between 16 and 21 ppt and water temperature ranged from 23.8 to 26.0°C.

Benthic Invertebrates. Sediments collected from 2 of the 15 sampling stations were highly toxic (Table 4). Sediments from Station 4 in Bayou Texar were the most toxic; survival was 3% for mysids and 6% for *Ampelisca*. Toxicity was observed also in Bayou Grande (Station 1) but only to *Ampelisca*; survival was 13% after 10 days of exposure. Survival of the benthic invertebrates after exposure to the remaining sediments exceeded 97% (mysids) and 84% (*Ampelisca*) relative to the reference sediment. Survival in the reference sediment was 100% (mysids) and 99% (*Ampelisca*).

Vascular Plants. The response of the seedlings to the sediments was specific to the site, species and tissue (Table 4; Figures 3 and 4). Seedling survival was 100% in most cases; a sediment collected from Bayou Chico was phytocidal to one seedling. The number of rhizomes ranged between zero and two and no significant differences among the 15 sediments were detected ($p > 0.05$). In contrast, 12 of the 15 sediments had a significant effect on biomass (dry weight), relative to the reference sediment, based on the response of one or both of the test species. Five sediments from Bayou Grande, four in Bayou Texar, and three collected from Bayou Chico had significant effects ($p < 0.05$). *Scirpus* was affected after exposure to nine sediments, eight of which had a significant stimulatory effect relative to the reference sediment. The average increase above that observed for the reference sediment was 167.6% (±1 standard deviation = 151.9). Eight sediments significantly affected either shoot or root biomass of *Spartina* ($p < 0.05$); four were inhibitory and four stimulatory. The biomass increase, relative to the reference sediment for this species, averaged 87.4% (± 32.7) and the biomass reduction, 66.3% (±16.0).

There were differences in the response or sensitivity of the two plant tissues. The total number of significant differences observed in this study were almost alike, 11 (shoot biomass) and 12 (root biomass). However, the response of the two tissues exposed to the same sediment was usually not similar

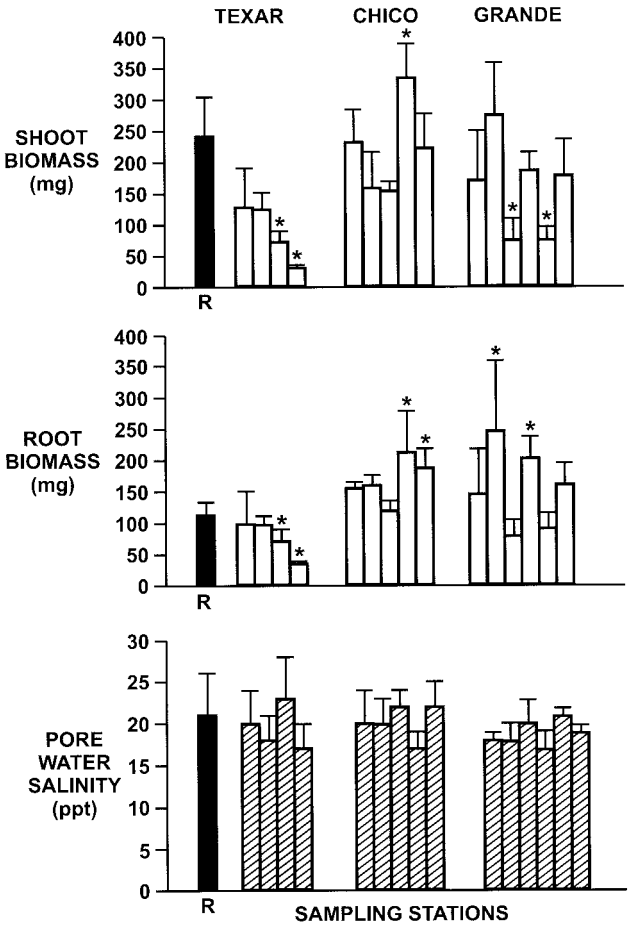

Fig. 3. Comparison of shoot and root biomass (mg) for *Spartina alterniflora* Loisel (cordgrass) after exposure for 28 days to the bayou and reference sediments. Values represent mean (±1 standard deviation) for six seedlings. R = reference sediment. *significant difference ($p < 0.05$). Pore water salinity (ppt) during the bioassays also shown. Data for Bayou Grande from Lewis *et al*. (2000b)

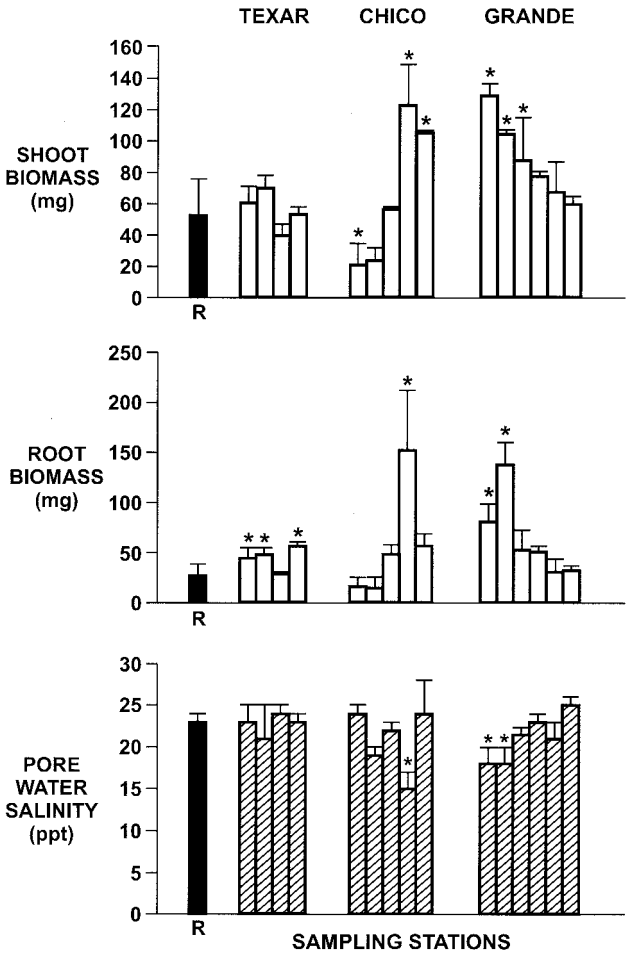

Fig. 4. Comparison of shoot and root biomass (mg) for *Scirpus robustus* Pursh (saltmarsh bulrush) after exposure for 21 days to the bayou and reference sediments. Values represent mean (±1 standard deviation) for six seedlings. R = reference sediment. *Significant difference ($p < 0.05$). Pore water salinity (ppt) during the bioassays also shown. Data for Bayou Grande from Lewis *et al*. (2000b)

(Table 4; Figures 2 and 3). For example, either shoot or root biomass of *S. robustus* was significantly affected after exposure to nine bayou sediments. However, both root and shoot biomass were statistically different from the reference in only three of these sediments. For *Spartina*, shoot biomass was significantly different with five sediments and root biomass was significantly different in six sediments. Both root and shoot biomass were affected at three of the total eight sites where a significant effect occurred.

Discussion

The sediments in the bayous were contaminated and several effects-based, numerical quality guidelines proposed for Florida coastal areas were exceeded. Nevertheless, acute toxicity to the two invertebrate species was uncommon relative to the more frequent occurrence of phytoinhibitory and phytostimulatory effects on seedlings of two coastal wetland plant species. Significant effects on early seedling growth were observed for 12 of the 15 sediments. Of these 12 sediments, 10 were not acutely toxic to one or both of the benthic invertebrates. For the two sediments that were toxic to at least one of the invertebrates, both stimulated growth of *S. robustus* and there was either no significant effect or an inhibitory effect on *S. alterniflora*.

In addition to the plant-invertebrate sensitivity differences, the response of the two plant species also differed. Of the 12 whole sediments where effects were observed, a consistent inhibitory or stimulatory response of both species was observed for three sediments (Chico Stations 4 and 5, Grande Station 2) based on a change in at least one tissue and a consistent response of both tissues occurred for one sediment (Chico Station 4). Overall, the interspecific response observed in this study (animals versus plant; plant versus plant) reinforces the scientific consensus that the utilization of only a few test species constrains contaminated sediment evaluations that rely solely on bioassay results (Traunspurger and Drews 1996).

The specific reason(s) for the lack of comparability in response of the invertebrates and plants is unknown, but there is

little reason to expect that it would be similar considering their morphological and physiological differences. The few instances in this study where acute toxicity occurred to the benthic animals may have been due to one or a mixture of the detected contaminants, such as those exceeding the guideline values, or to contaminants that were either not analyzed or to those occurring below the MDLs. Scientific understanding concerning the effect levels of phytotoxicants and phytostimulants in sediments is lacking for most rooted vascular plants. The greater biomass observed in this study, other than that possibly due to salinity or some physical substrate factor, could be due to increased concentrations of essential micro-elements and/or to higher macro-nutrient concentrations, particularly phosphorus. Sediment nutrient concentrations were not determined in this baseline study, but results of previous studies have shown relatively high concentrations either in surface water or in sediment in these bayous (Moshiri *et al.* 1978; Wood and Bartel 1994; Lewis *et al.* 2000a). It is important to note also that it was assumed that the use of the supplemental nutrient solution had no differential effect on the results of this study. This assumption, however, will need confirmation, particularly if phytostimulation is found to be a common result of sediment phytotoxicity bioassays such as those conducted here.

Sediment physical and chemical characteristics can affect the survival and condition of invertebrates and rooted plants, as well as the presence of anthropogenic contaminants. The impact of these substrate factors, such as salinity, texture, organic content, and humic acids, are not well understood for most test species used in estuarine sediment bioassays, and an in-depth analysis of their effects was beyond the scope of this study. However, some discussion is warranted for interstitial or pore water salinity, which is a common variable in estuarine sediments.

The pore water salinities in this study were similar in bioassays conducted with the invertebrates and *S. alterniflora* ($p > 0.05$) and any potential effect of salinity was assumed to have been "equal." This same assumption applies to *S. robustus* as well, except for 3 of the 15 sediments for which salinity differed from that of the reference sediment ($p < 0.05$). The salinities of these sediments collected from Bayou Chico (Station 4) and Bayou Grande (Stations 1 and 2) averaged 17.0 ppt (± 1 standard deviation $= 2.4$) compared to 23.0 (± 1.0) ppt for the reference sediment. Consequently, the greater root and shoot biomass in these sediments ($p < 0.05$) may have been due, in part, to lower pore water salinity.

The magnitude of a potential salinity effect in this study is not known. The salinities of the bayou sediments were within the range reported to be suitable for whole sediment bioassays conducted with benthic estuarine invertebrates (range = 17–28 ppt) US EPA 1996b, 1996c). The salinity tolerance range for *S. robustus* and *S. alterniflora* has been reported to be between 0 and 39 ppt based on a combination of laboratory and field observations (Environmental Concern Inc., 1996; Kantrud 1996). Consequently, salinity may have had only a minimal impact, but this issue of understanding the effects of naturally occurring substrate components is important, particularly if the research focus is to identify the impact of anthropogenic contaminants alone.

In summary, the results of this study show the complexity of trying to evaluate the toxicities of sediments using bioassays, particularly if rooted vascular plants are included. Sediment toxicity assessments in relatively small and urbanized estuaries are affected by spatial considerations as well as by the choice of the test species. In this study, early seedling growth of aquatic vascular plants was more commonly affected by sediment contamination than survival of two benthic invertebrates. Therefore it appears that the use of acute animal bioassays alone would have provided a limited perspective on the sediment quality in these common Gulf of Mexico habitats.

Although this study indicates the importance of phytotoxicity data, future efforts will be needed to support their derivation through the additional development, field validation, and application of relevant bioassay techniques. Information on sensitive species, life stages, and response parameters are needed as well as determinations of the effects of sediment nutrient and contaminant concentrations and physical substrate factors on potential test species. The increased availability of these data will result in a better understanding of the interspecific sensitivities of benthic plant and animal test species, and the relevancy of published sediment toxicity surveys where these data are missing. Furthermore, this database would provide a more holistic perspective for proposed national sediment quality criteria and the various numerical sediment quality assessment guidelines currently used.

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