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# LEVEL <sup>12</sup> DREDGED MATERIAL RESEARCH PROGRAM



MISCELLANEOUS PAPER D-78-6

## FIELD BIOASSAY TEST FOR DETECTING CONTAMINANT UPTAKE FROM DREDGED MATERIAL BY MARSH PLANTS

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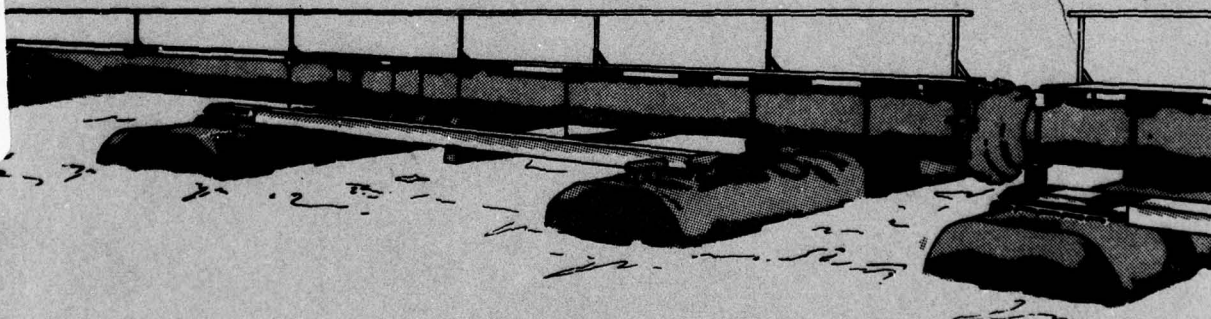
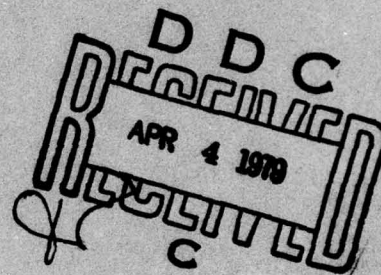
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Final Report

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SUBJECT: Transmittal of Miscellaneous Paper D-78-6

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1. The miscellaneous paper transmitted herewith represents the results of one of the research efforts (work units) of the Corps of Engineers' Dredged Material Research Program (DMRP). This study was conducted under the direction of the Habitat Development Project (HDP) of the DMRP. The HDP had as its main objectives the development of wetland and upland habitats on dredged material and the evaluation of the impact of disposal in shallow water and upland sites.

2. This report, "Field Bioassay Test for Detecting Contaminant Uptake from Dredged Material by Marsh Plants" (Work Unit 4A26), presents an inexpensive and useful method of alerting the user to potential problems of contaminant uptake by marsh plants established on dredged material. Certain procedural refinements are necessary before this method is ready for general field application, and users not intimately familiar with the processes of contaminant mobility are advised to seek qualified assistance.

3. Work Unit 4A26 was one of several research efforts designed by the DMRP to assess the potential for uptake and mobilization of contaminants through habitat development on dredged material. Related work units were 2A05, which provided a state-of-the-art review of nutrient and heavy metal cycling in marsh-estuarine ecosystems; 4A06, which evaluated the effects of Eh, pH, and salinity on the availability of sediment bound metals; 4A11H, which compared the water quality and sediment status of a natural and a man-made marsh in the James River, Virginia; 4A11L, which evaluated the uptake of organohalides from contaminated sediments into plant and animal tissues; 4A15, in which marsh plants were subjected to various concentrations of heavy metals in a hydroponic solution; and 4A15A, in which an extraction procedure was developed to predict heavy metal uptake from dredged material.

JOHN L. CANNON  
Colonel, Corps of Engineers  
Commander and Director



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19. ABSTRACT (Continue on reverse side if necessary and identify by block number) A method designed to evaluate the response of marsh plants grown in bioassay experiment test units was tested in Georgia and Oregon marshes. Indigenous marsh plants were grown on three types of contaminated dredged material and compared with control plants grown on native soils.  Although uptake of contaminants by marsh plants was not definitively demonstrated, the bioassay experiment test unit appears to be an efficient, (Continued)		

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20. ABSTRACT (Continued).

→ relatively inexpensive technique for identifying, prior to dredged material disposal, potential problems concerning contaminant uptake by marsh plants. ↗

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#### AUTHORS' NOTE

The technique herein described, called the bio-assay experiment test (BET), represents an inexpensive and useful method of alerting the user to potential problems of contaminant uptake by marsh plants established on dredged material. Certain procedural refinements are necessary before this method is ready for general field application. Analytical results presented should be interpreted with care because of the preliminary nature of this research and the site-specific design of the BET technique. (P.L.W., J.L.G., C.H.P.)



## PREFACE

The work described in this report was initiated in June 1977 for the U. S. Army Engineer Waterways Experiment Station (WES), under Purchase Order No. DACW39-77-M-3753 as part of the Dredged Material Research Program (DMRP), Habitat Development Project (HDP), with the University of Georgia Marine Institute, Sapelo Island, Georgia. The DMRP was sponsored by the Office, Chief of Engineers, and was assigned to the Environmental Laboratory (EL), WES.

This report was prepared by Paul L. Wolf, John L. Gallagher, and Carlos H. Pennington.

The efforts of Mary Musselman, Thomas Pearson, and Denise Seliskar toward the completion of the project are greatly appreciated.

The study was conducted under the supervision of Dr. C. J. Kirby, Chief, Environmental Resources Division (ERD), and Dr. H. K. Smith, Project Manager, HDP, and under the general supervision of Dr. John Harrison, Chief, EL. Dr. C. H. Pennington monitored the study for the HDP.

Director of WES during preparation of this report was COL J. L. Cannon, CE. Technical Director was Mr. F. R. Brown.

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FIELD BIOASSAY TEST FOR DETECTING CONTAMINANT UPTAKE  
FROM DREDGED MATERIAL BY MARSH PLANTS

PART I: INTRODUCTION

Approximately 290 million cubic meters of sediment is dredged each year in the United States (Khalid et al., 1977). The creation of dredged material islands adjacent to dredging sites and transportation of sediments to land are two of the methods employed for disposal of these sediments. In the Southeastern United States, disposal of sediments often occurs on valuable marshlands.

The U. S. Army Corps of Engineers has developed techniques to vegetate dredged material disposal sites. Among the benefits derived from vegetating these sites are the stabilization of the material, particularly on dredged material islands adjacent to waterways, and the creation of habitats for birds, small mammals, and terrestrial invertebrates (Reimold et al., 1978). In addition, the creation of more marshland may benefit estuarine productivity. The importance of the marshland system to the estuary has been well documented by Odum (1961), Odum and de la Cruz (1967), Sweet (1971), Gosselink et al. (1974), and Reimold (1974). It should be kept in mind that a balance of open water, mudflat, and marshland should be sought.

One of the major potential problems associated with marsh development is the movement of contaminants from dredged materials. If the contaminants are absorbed and translocated to the aerial portions of plants, they may be passed along the marsh food web either through the detritus food chain or directly through grazing by herbivorous invertebrates, waterfowl, and small mammals. The extent to which uptake into the aerial portion occurs probably depends on the type of dredged material, the environment associated with various elevations in the intertidal zone, the nature of the contaminant, and the species of plants present. Carefully controlled quantitative laboratory tests designed to measure heavy metals accumulation in marsh plants grown on dredged

materials have been conducted by Lee et al. (1976). There is, however, a need for a field method that can be used to evaluate potential contaminant uptake under specific complex sets of real world environmental conditions. This information is needed for sites where contaminated material is proposed as a substrate for creating a marsh.

The purpose of this study was to test a technique designed to be used by District Engineers to evaluate the response of indigenous plant species grown in contaminated dredged material prior to making final disposal plans. The technique involves the use of a bioassay experiment test (BET) unit first described by Gallagher et al. (1977). The unit can be filled with dredged material and planted with sprigs in the laboratory, transported to the field, implanted on site, and removed for evaluation at a later date.

The method is not intended as a quantitative procedure for estimating the flux of contaminants through the soil-plant complex. Its purpose is to alert the District Engineer that the contaminant is being mobilized and that further consideration should be given before the project continues.

## PART II: METHOD OF CONDUCTING THE ASSAY

### Construction of Bioassay Experiment Test (BET) Units

Each bioassay experiment test (BET) unit consists of a plastic bucket (24 cm deep  $\times$  25 cm diam) with 120 holes (6 mm diam) drilled into the sides and bottom (Figure 1). A slot (12 cm wide  $\times$  5 cm deep) is cut into each bucket slightly below the lip to allow free flow of water in and out of the unit at the marsh surface level. At the same time the rim reduces the accretion of adjacent marsh sediment within the BET unit.

### Site-Specific Design of the Assay

Each site will require a slightly different design depending on the variability of the dredged material within the project, the elevation

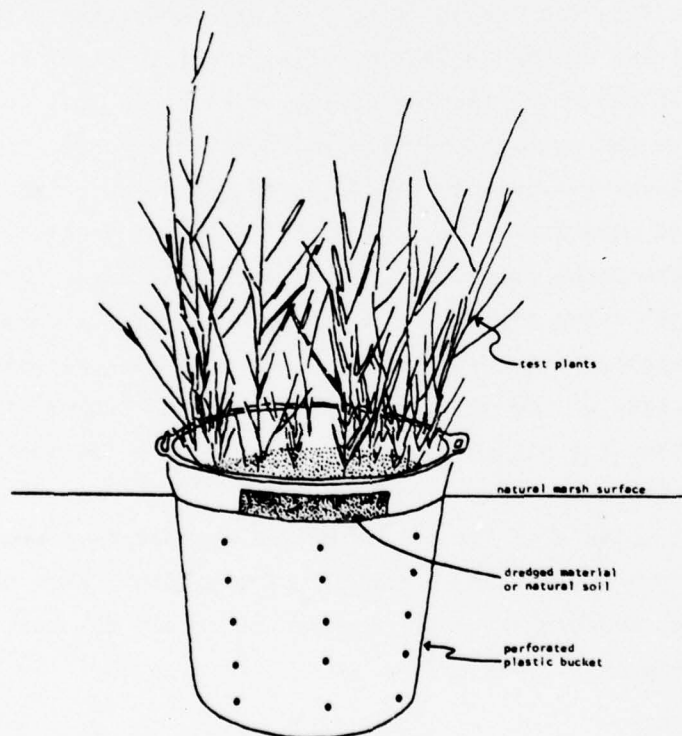


Figure 1. Bioassay experiment test (BET) unit



range of the deposition and the variety of species to be planted. If the project is small with only one plant species, a uniform dredged material, and level final project elevation, a single test site would suffice. Most projects will be more complex and two or more sites will be needed. The BET units are deployed in a random fashion within an area of the same intertidal elevation as the projected project elevation. Five to ten experimental and control units will be an adequate set size to provide information for most situations. At least one such set should be added for each major shift in sediment type, plant species, or elevation change.

#### Establishing the Site

Each control BET unit should be filled with soil from the marsh closest to the project site. The experimental units are filled with dredged material from the project site. It may be necessary to allow a period of dewatering of the dredged material prior to placing the units in the field. If the proportion of solids to water is low, the test will not simulate the actual condition which would be experienced in the proposed project where a period of dewatering would occur prior to planting. The dredged material in the BET units is therefore allowed to consolidate to the point when planting would be feasible. Sprigs of the species to be tested are dug from the adjacent marsh, washed in water from the adjacent aquatic system, and planted in the units. Although the density of the planting depends on the species, it is generally true that the higher density plantings give the most material for analysis at the end of the study.

The planted units are carried to the field where they are placed in a previously determined random pattern at the site. Each unit is then implanted at a depth where the dredged material, BET unit slot, and adjacent sediment surface are all at the same level.

#### Incubation Period

The optimum time for starting the study is the beginning of the

growing season when the initiation and elongation of new shoots is maximum for many species. After the plants become established, the aerial parts of the plants are harvested and discarded. Following a period of regrowth which is long enough to produce sufficient material for analysis, the units are harvested. Only the regrowth is used for analysis.

#### Sample Preparation and Analysis

Both dredged material and control BET unit plant shoots should be harvested and the soils sampled. The laboratory doing the analysis for the possible contaminants should be consulted for directions to be used in preparing the samples for analysis. Usually this will involve a series of washings, drying (either air or oven), and grinding. Great care should be taken to assure that cross sample contamination and sediment contamination of the plant tissue do not occur.

### PART III: INTERPRETATION OF THE RESULTS

Statistical analysis of the data should be designed to aid the investigator in answering the question: Do the plants growing in the dredged material convey the contaminant from the sediment into the plants and thus potentially into the grazing and detrital food webs? If the assay indicates the plants are taking up the contaminant under the field conditions, the feasibility of using the dredged material to create a marsh under the test conditions should be examined closely. Further testing including laboratory studies of the nature conducted by Lee et al. (1976) may be appropriate before a positive decision can be made.

The BET technique is thus designed as an inexpensive screening procedure to alert the District Engineer to potential problems in using contaminated dredged material for marsh creation.



## PART IV: TEST OF THE METHOD

### Introduction

As an aid in refining the protocol described in Part II, 96 BET units were constructed and deployed in a variety of marshes in Georgia and Oregon. The testing was extensive in plant and soil types as well as geographical range rather than intensive since it was desired to see the range of problems likely to be encountered.

### Methods and Materials

Experiments using the BET units were conducted in Georgia and Oregon. Tests were conducted at each site using four species of marsh plants grown on contaminated dredged materials from three locations. The dredged materials were obtained from: (a) Broad Lake, Yazoo County, Mississippi, suspected to be contaminated with chlorinated pesticides; (b) the Hudson River, approximately 17 km south of Fort Edward Dam, New York, containing polychlorinated biphenyls (PCB's); and (c) Cedar Creek, Bridgeport Harbor, Connecticut, contaminated with heavy metals. Interstitial water salinity of the dredged material from Cedar Creek was 30 percent, while water collected from Broad Lake and the Hudson River was essentially nonsaline (<3 percent). The dredged materials were loaded into new 208-l drums that were steam-cleaned immediately prior to being filled and shipped to each site.

Upon arrival at each site, the dredged materials were transferred to the BET units described earlier in this report (Figure 1).

Dredged materials were stored in the BET units to allow for drainage and evaporation of water prior to the implantation of the units into the marsh. The Hudson River and Broad Lake sediments required 3-4 days for dewatering. The dredged material from Cedar Creek, which had the least solids per volume, required at least seven days of dewatering before the substrate was dense enough to support the plants. BET units containing the dredged materials were placed in the marsh

during August 1977. Each bucket was positioned in a marsh excavation at a depth where the surface of the material within the bucket was at the same level as the natural marsh surface (Figure 1).

Distichlis spicata (saltgrass) and Salicornia virginica (woody glasswort) were used for study in Georgia and Oregon since both are native to the two areas. In Georgia, Spartina alterniflora (smooth cordgrass) and Spartina patens (saltmeadow cordgrass) were the other two species included. Carex lyngbyei (Lyngbye's sedge), which appears to be the western ecological analog of east coast S. alterniflora, was selected for Oregon. Deschampsia cespitosa (tufted hairgrass) occupies a position high in the intertidal zone in Oregon marshes similar to that occupied by S. patens along the Atlantic Coast and was chosen as the last species to be studied. In Georgia, the buckets were implanted in a marsh on the eastern side of Sapelo Island near Cabretta Island. The buckets to be planted with C. lyngbyei were incubated in a marsh in Siletz Bay, Oregon. All other Oregon BET units were placed in stands of the appropriate plants in marshes bordering Netarts Bay.

A randomized block design was employed for the experiments (Figures 2 and 3). Treatments consisted of four plant species grown in replicates of three on four types of substrate (three contaminated and one control) at both sites for a total of 96 experimental units. Control units consisted of natural marsh substrate obtained from the area of BET unit introduction. All units were placed in the area of natural occurrence for each plant species being tested.

Sprigs used for implantation into the BET units were collected in natural marsh areas in both states. After removal from the marsh, sprigs were washed in seawater (30 percent salinity) to remove any substrate from the root systems. When sprigs could not be introduced into BET units on the day of their removal from the marsh, they were stored in seawater at 5°C. Storage time never exceeded three days. Plants were not introduced until the dredged material had approached a consistency similar to that of the natural substrate. More time was needed for drying the dredged material from Cedar Creek because of the high water content of this sediment. In Georgia, plant sprigs were

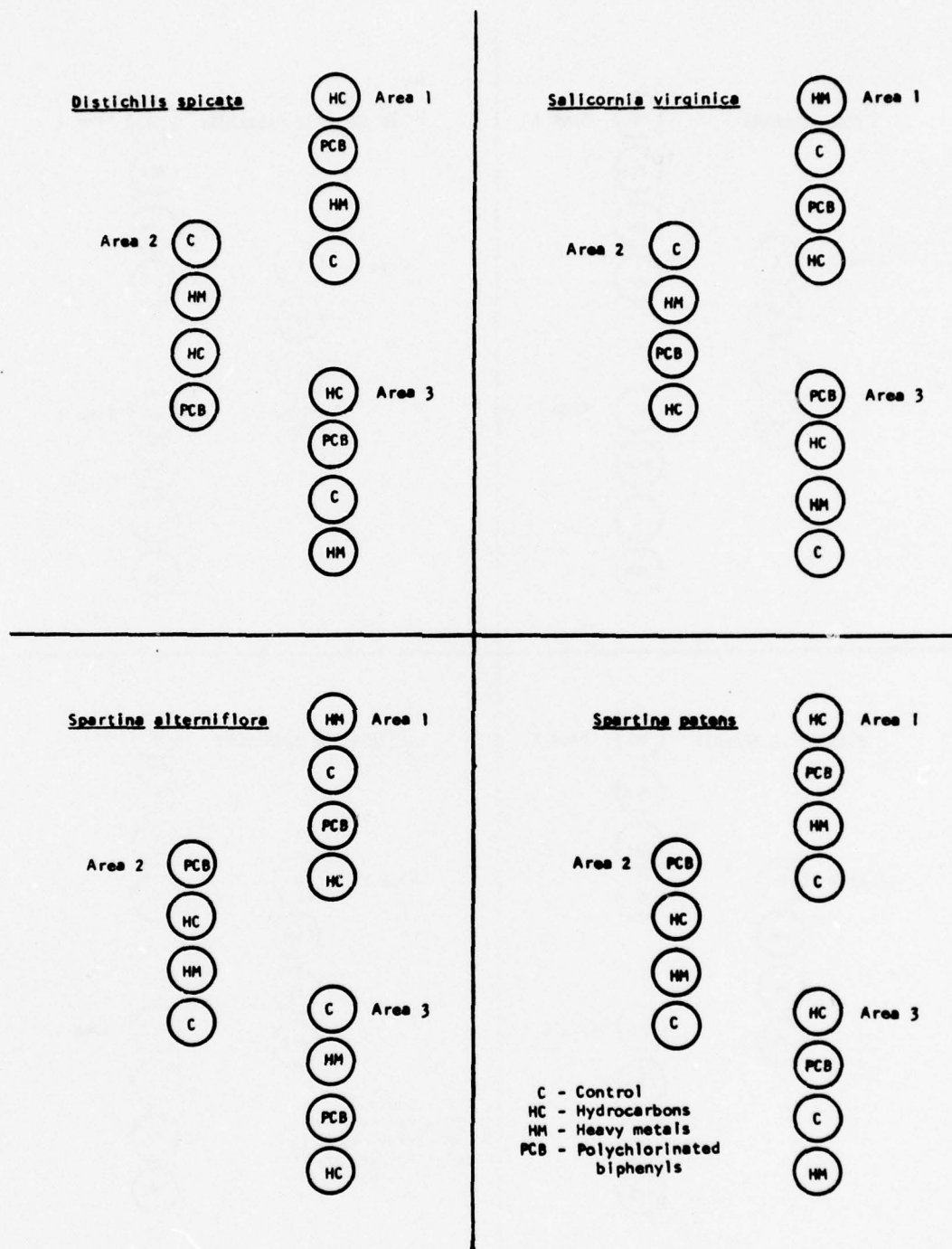


Figure 2. Experimental design of BET units in Georgia



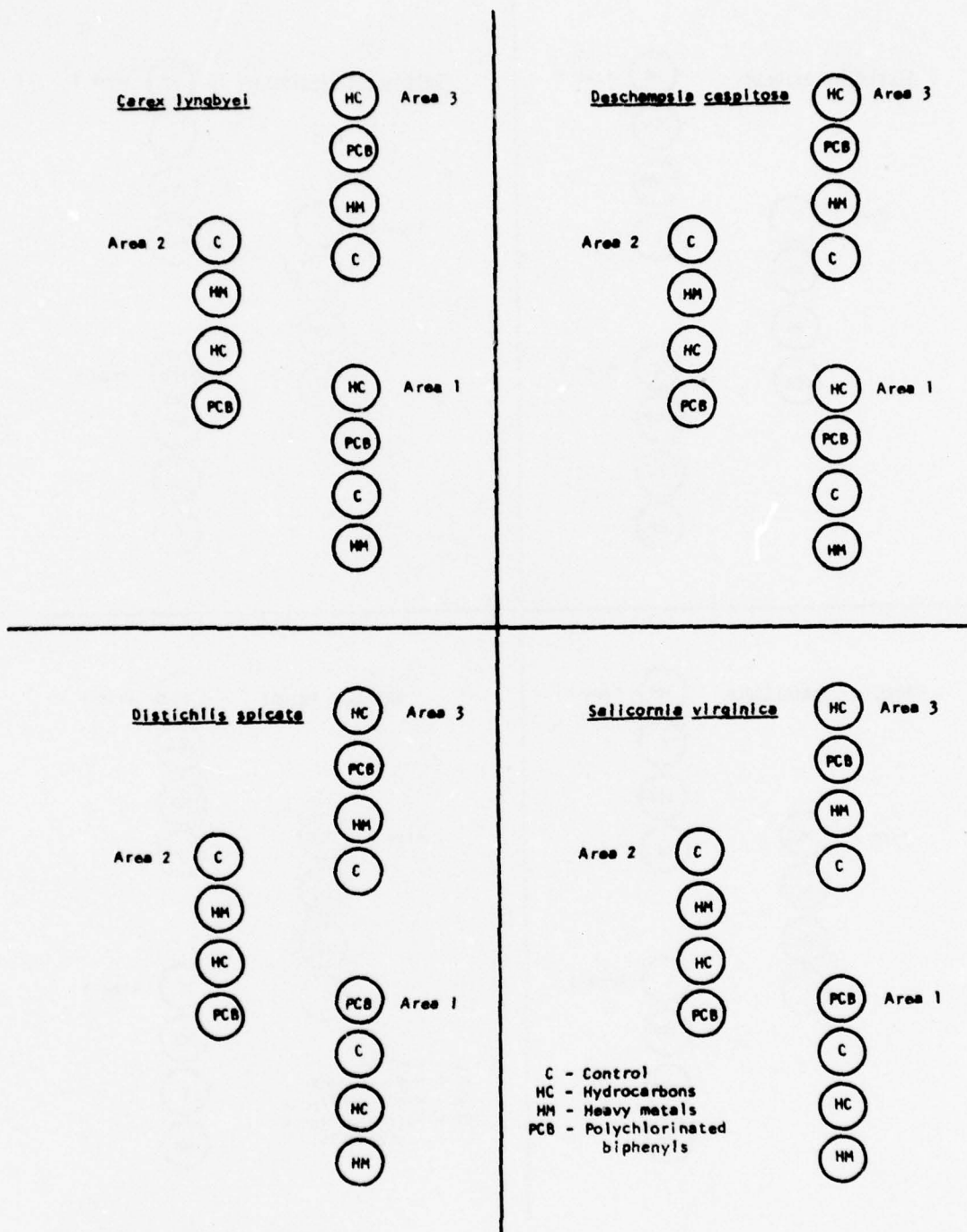


Figure 3. Experimental design of BET units in Oregon

implanted between 26 August and 7 September 1977 and from 12 through 25 August 1977 in Oregon. The average number of stems/unit implanted for each species in Georgia was 135 for D. spicata, 126 for S. virginica, 26 for S. alterniflora, and 101 for S. patens. In Oregon, buckets were planted with as many stems as possible and actual stem counts were not recorded.

Approximately 3-4 days following the introduction of plants, the BET units were checked to observe sediment levels and plant survival. In those units where the dredged material had settled below the slot, dredged material was added to establish the previous levels. Sprigs which appeared to be dead were replaced with freshly dug, washed sprigs.

Plant samples were collected from each BET unit at the end of two weeks and eight weeks. Plants were massed on a balance in the field to insure that a sufficient quantity (at least 20 g wet weight) of material was available for chemical analyses. Only live plant stems were harvested for chemical analyses. However, some dead material was associated with plants removed from the heavy metals substrate. In some instances the regrowth after the first harvest was not sufficient to allow the collection of 20 g wet weight.

Approximately 850 cc of each type of dredged material and substrate from each of the four natural marsh areas at each site were collected at the beginning of the experiment. At the end of eight weeks, pooled sediment samples representing each type of dredged material (280 cc from each unit) and control units for each species were also collected. The handling of dredged materials was done with rubber gloves, and one individual harvested all materials from each type of dredged material to minimize the possibility of cross contamination. All samples were stored at 5°C prior to processing.

Plants for heavy metals analysis were first rinsed in glass distilled water. The leaf blades were cut from plant stems. Each leaf and stem was then wiped three times with a Kim wipe moistened with distilled water. The plant material was then wiped in a similar fashion with a Kim wipe moistened with methanol. Following this procedure, the plant material was again washed with distilled water followed by a

washing with 1 N hydrochloric acid and, finally, another washing with distilled water. Plant materials for pesticide and PCB analyses were washed three times in distilled water only. The methanol and hydrochloric washings were eliminated.

The washed plant material for heavy metal analysis was oven-dried at 70°C until constant weights were obtained. Samples for chlorinated hydrocarbon and PCB analysis were air-dried. Dried plant material was ground in a stainless steel Wiley mill (#40 screen) and stored in glass bottles. Approximately 10 g (dry weight) of powder/unit of pesticide and PCB materials and 2 g (dry weight) of powder/unit of heavy metals materials were processed for chemical analyses.

Plant and sediment samples were shipped from the two sites to the Analytical Laboratory Group (ALG) of the Environmental Laboratory (EL) at the Waterways Experiment Station (WES) for analyses. All samples were stored at 4°C until analyses could be made.

Plant and sediment samples were processed for chemical analysis following guidelines of APHA et al. (1976). Digest solutions were analyzed for aluminum (Al), chromium (Cr), copper (Cu), iron (Fe), manganese (Mn), nickel (Ni), and zinc (Zn) by flame atomic absorption and emission (Perkin Elmer - Model 306 or 503 Atomic Absorption Spectrophotometer and Spectrometrics Argon Plasma Emission Spectrometer). Total water-soluble and exchangeable cadmium (Cd) and lead (Pb) were measured by flameless atomic absorption (Perkin Elmer - Model 2100 HGA graphite furnace). Arsenic (As) concentrations were determined with a Zeeman-Shift Atomic Absorption Spectrophotometer.

The chlorinated hydrocarbons were extracted from plant and sediment samples following guidelines established by the EPA (1974 and 1975). Analysis of plant and sediment extracts was conducted with a gas chromatograph (Perkin Elmer Model 990 GC) equipped with an electron capture detector. Pesticides included in the analysis were aldrin, dieldrin, endrin, endosulfan, heptachlor, heptachlorepoxyde, lindane, o,p' - DDT, p,p' - DDT, p,p' - DDD, and p,p' - DDE. The PCB's measured were Arochlor isomers 1242, 1248, 1254, and 1260.

Logarithmic transformation was performed on plant and sediment



data obtained from the BET units used for the heavy metal studies. Analysis of variance was used to test for differences among sediments and Duncan's New Multiple Range Test was used to separate means. A Student's T-test was applied to plant data. Significance is expressed at the 0.05 probability level (Steele and Torrie, 1960). A Student's T-test was used to evaluate differences between control and experimental BET units in the PCB tests.

### Results and Discussion

Plant survival. In Georgia the aerial parts of all plant species on each type of dredged material appeared to be dead or dying two weeks after transplanting into the BET units. At eight weeks, however, new sprigs and live stems were present in the control, chlorinated pesticide, and PCB units. All plant material in the heavy metals units appeared to be dead or dying. In June 1978, no plant material, either live or dead, was present in the heavy metals units for any plant species tested (Figure 4a). The live stems for the control, pesticide, and PCB units were approximately one half the densities for the same units at the time of planting (Figure 4b).

D. spicata, a species tolerant to a variety of conditions, appeared to be surviving well in all three dredged materials by 1 September 1977 in Oregon. S. virginica, at the other extreme, showed poor survival. In Oregon, S. virginica usually grows on coarsely textured, highly saline soils. Two of the three dredged materials used in this study were finely textured with low interstitial water salinities, and hence poor growth might be expected. The water flooding the marsh where these BET units were incubated was nearly marine (32 percent), and interstitial water salinities rose rapidly. Salicornia virginica was replanted after four weeks, and growth on the then more saline media was much better. Deschampsia cespitosa and C. lyngbyei exhibited growth responses intermediate to those of the first two species mentioned. In June 1978, only S. virginica was surviving on the heavy metals material, and even there the growth was poor. The other plant species were growing well in all other units.



a. Heavy metal substrate originally planted with Salicornia virginica



b. PCB substrate planted with Spartina patens

Figure 4. BET units in Georgia in June 1978

It is difficult to conclude whether the failure to achieve significant growth in the heavy metals materials was related to the texture of the dredged material or to the concentrations of various metals in these sediments. In a previous study, where five species of marsh plants were grown on three types of dredged materials in a greenhouse, Gallagher et al. (1977) found that D. spicata grew moderately well on dredged material of a similar consistency to the heavy metals material used in this study. Thus, the extremely high levels of potentially toxic materials in the heavy metals sediments could account for the very poor survival of plants grown in these materials. Other workers have observed high mortality or stunted growth in plants grown on highly concentrated heavy metals sediments (Dunstan et al., 1975).

Contaminant uptake. The dredged materials obtained from Broad Lake, Mississippi, were suspected to contain high concentrations of pesticides but were found to be virtually free of contamination. Total pesticide concentrations of Broad Lake sediments shipped to Oregon were  $< 0.010 \mu\text{g/g}$ . The only pesticide found to be above detectable limits in Broad Lake sediments shipped to Georgia was dieldrin, at a concentration of  $0.150 \mu\text{g/g}$ . Pesticide concentrations were  $< 0.010 \mu\text{g/g}$  in natural substrates used in the control BET units at each site.

Concentrations of chlorinated pesticides at the end of eight weeks in experimental and control plants were less than detectable limits for all species at both sites with the exception of two units in Georgia where low levels of heptachlor were identified.

Natural marsh soils from each site were essentially free of PCB's at the time of planting ( $< 0.100 \mu\text{g/g}$ ) and the end of eight weeks ( $< 0.200 \mu\text{g/g}$ ). At the same time, PCB's in dredged material from the Hudson River exhibited a rather high concentration (Table 1).

The data indicate that some PCB uptake may have occurred in D. spicata in Oregon and S. alterniflora in Georgia. A Student's T-test indicated the PCB concentrations in S. alterniflora grown on the contaminated dredged material were significantly higher (0.01) than those growing on natural soils. It is clear that there was a higher concentration of PCB's in the D. spicata grown on the contaminated dredged



Table 1

PCB Concentrations ( $\mu\text{g/g}$ ) in Dredged Material from Hudson River, New York, Natural Marsh  
Soils from Georgia and Oregon, and Mean PCB Concentration ( $\bar{X} \pm 1$  Standard  
Error) in Four Species of Marsh Plants from Georgia and Oregon

Location and Species	Sample				
	Initial Natural Marsh Soil	8 Week's Sampling		Plants*	
		Marsh Soil		Control	Experimental
Georgia.					
<i>Distichlis spicata</i>	<0.10	<0.200	627	0.90 $\pm$ 0.62	0.91 $\pm$ 0.21
<i>Salicornia virginica</i>	<0.10	<0.200	794	1.07 $\pm$ 0.66	1.00 $\pm$ 0.45
<i>Spartina alterniflora</i>	<0.10	<0.200	238	0.27 $\pm$ 0.07	2.04 $\pm$ 0.43
<i>Spartina patens</i>	<0.10	<0.200	389	0.47 $\pm$ 0.09	0.56 $\pm$ 0.11
Oregon					
<i>Deschampsia cespitosa</i>	<0.10	<0.200	1000	<1.00 $\pm$ 0	0.015 $\pm$ 0.003
<i>Distichlis spicata</i>	<0.10	<0.200	1570	<1.00 $\pm$ 0	2.475 $\pm$ 0.202
<i>Carex lyngbyei</i>	<0.10	<0.200	940	<1.00 $\pm$ 0	0.490 $\pm$ 0.360
<i>Salicornia virginica</i>	<0.10	<0.200	959	<1.00 $\pm$ 0	0.015 $\pm$ 0.006
PCB concentration in dredged material from Hudson River, New York					
Georgia					
Oregon					

\* N = 3, for each species and each treatment

material although the control values were known only to be  $< 1.00 \mu\text{g/g}$  dry weight of tissue. The presence of PCB's in some of the control plants may have been caused by contamination during the sampling process or analysis, even though precautions were taken to prevent this from occurring. In a few instances the BET would have raised questions about the plants acting as vectors for the transfer of PCB compounds from the sediments to the estuarine food web.

Heavy metals concentrations in soils from the Georgia BET units are listed in Table 2. Concentrations of Cr, Cu, Fe, Pb, Mn, Ni, and Zn in Bridgeport Harbor dredged material prior to and 8 weeks after planting were significantly greater than levels in native substrates for the same time intervals (Table 3). Al, As, and Cd were not different when the same comparisons were made. Levels of Mn in the initial dredged material were significantly greater than concentrations after eight weeks. Concentrations of other metals included in analyses did not differ. This indicates that some leaching of Mn from the BET units may have occurred during experimentation since Mn concentrations did not increase in any of the plants tested. Native substrates did not differ when initial metal concentrations were compared with levels after eight weeks.

Similar trends were observed in heavy metals concentrations from Oregon soils (Table 4). Levels of Cr, Cu, Mn, Ni, Pb, and Zn in the dredged material prior to planting were significantly greater than concentrations in native substrates prior to and eight weeks after planting (Table 3). Elements not different for the same comparisons were Al, As, Cd, and Fe. There were also no differences in metal concentrations in Bridgeport Harbor materials either before or after planting. After eight weeks, the dredged material contained significantly less Al and significantly greater concentrations of Cr, Cu, Pb, Mn, Ni, and Zn than native substrates either initially or at the end of experimentation. Levels of Cr in native substrate were significantly greater than concentrations after eight weeks. Other elements were not different.

Although data on heavy metals concentrations in natural marsh soils

Table 2

Concentrations of Heavy Metals (mg/kg) in Dredged Material from Bridgeport, Conn.,  
and in Marsh Soils from Sapelo Island, Ga.

Sample	Al	As	Cd	Cr	Cu	Fe	Pb	Mn	Ni	Zn
Dredged material Bridgeport, Conn.	32,700	5.40	42.00	3360	4280	33,400	599	450	412	3260
<u>Distichlis spicata</u>										
Initial natural marsh	9020	0.95	<0.010	12.3	6.85	6930	6.17	294	0.95	7.31
8 week's sampling C	11,400	1.20	<5.00	18.5	7.10	7260	10.30	256	<5.00	15.30
8 week's sampling E	15,000	4.85	49.30	3160.0	3840.0	28,000	595.00	326	422.00	2520.00
<u>Salicornia virginica</u>										
Initial natural marsh	15,000	2.70	<0.010	21.9	7.49	10,200	11.50	241	3.50	18.70
8 week's sampling C	13,700	2.96	<5.00	28.8	8.20	10,800	6.83	272	6.51	25.90
8 week's sampling E	20,600	4.56	35.00	2890.0	3540.00	27,500	500.00	306	373.00	2250.00
<u>Spartina alterniflora</u>										
Initial natural marsh	18,400	4.13	<0.010	27.6	4.32	13,600	10.40	248	9.40	26.00
8 week's sampling C	19,800	5.14	<5.00	42.4	21.10	19,000	16.80	242	10.60	45.40
8 week's sampling E	38,400	3.52	28.50	2280.0	2760.00	27,500	583.00	347	269.00	2200.00
<u>Spartina patens</u>										
Initial natural marsh	5820	1.00	<0.010	12.2	5.14	5370	7.56	278	0.504	5.75
8 week's sampling C	5050	1.10	<5.00	13.5	7.50	4310	4.98	208	<5.00	9.60
8 week's sampling E	16,700	5.27	49.40	3820.0	4510.00	36,200	658.00	347	542.00	3070.00

C = Control units, natural marsh soils at 8 weeks  
E = Experimental units, heavy metals dredged material at 8 weeks



Table 3  
Summary of Multiple Comparisons for Heavy Metal Concentrations  
in Sediment Samples from Georgia and Oregon

Location and Treatments*	Al	As	Cd	Cr	Cu	Fe	Mn	Ni	Pb	Zn
Georgia										
T1 vs T2	ND	ND	ND	ND	ND	ND	>	ND	ND	ND
T1 vs T3	ND	ND	ND	>	>	>	>	>	>	>
T1 vs T4	ND	ND	ND	>	>	>	>	>	>	>
T2 vs T3	ND	ND	ND	>	>	>	>	>	>	>
T2 vs T4	ND	ND	ND	>	>	>	>	>	>	>
T3 vs T4	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Oregon										
T1 vs T2	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
T1 vs T3	ND	ND	ND	>	>	ND	>	>	>	>
T1 vs T4	ND	ND	ND	>	>	ND	>	>	>	>
T2 vs T3	<	ND	ND	>	>	ND	>	>	>	>
T2 vs T4	<	ND	ND	>	>	ND	>	>	>	>
T3 vs T4	ND	ND	ND	>	ND	ND	ND	ND	ND	ND

\* T1: Dredged material prior to planting  
T2: Dredged material eight weeks after planting  
T3: Native substrate prior to planting  
T4: Native substrate eight weeks after planting  
ND: Not statistically different  
> : Concentration of variables in treatment group  $T_i$  is  $T_j$  significantly larger than the concentration of variables in treatment group  $T_j$  where  $i < j$   
< : Concentration of variables in treatment group  $T_i$  is  $T_j$  significantly smaller than the concentration of variables in treatment group  $T_j$  where  $i < j$

Table 4

Concentrations of Heavy Metals (mg/kg) in Dredged Material from  
Bridgeport, Conn., and in Marsh Soils from Oregon

Sample	Al	As	Cd	Cr	Cu	Fe	Pb	Mn	Ni	Zn
Dredged material Bridgeport, Conn.	26,100	4.69	44.50	3980	4720	32,400	798	499	468	3390
<i>Carex lyngbyei</i>										
Initial natural marsh	30,800	7.59	0.220	114.0	94.3	43,400	8.09	286	81.2	101.0
8 week's sampling C	33,900	4.36	<5.00	95.7	70.4	46,000	7.73	265	41.1	78.3
8 week's sampling E	13,300	6.70	50.00	3850.0	4400.0	29,100	735.00	358	520.0	2810.0
<i>Deschampsia cespitosa</i>										
Initial natural marsh	37,700	1.98	0.168	98.9	9.1	18,900	8.61	342	25.5	38.7
8 week's sampling C	19,000	1.61	<5.00	90.8	17.3	16,500	3.07	325	14.2	38.1
8 week's sampling E	23,100	5.43	34.20	3540.0	4100.0	32,200	351.00	351	444.0	2510.0
<i>Distichlis spicata</i>										
Initial natural marsh	30,800	5.46	0.113	100.0	76.5	27,000	12.00	207	41.6	75.4
8 week's sampling C	40,700	6.81	<5.00	79.1	29.8	37,100	8.56	260	32.6	71.1
8 week's sampling E	15,600	6.92	38.10	3370.0	3980.0	29,100	539.00	341	441.0	2450.0
<i>Salicornia virginica</i>										
Initial natural marsh	25,300	5.40	0.540	87.4	86.9	33,200	13.10	222	39.7	85.8
8 week's sampling C	29,200	14.50	<5.00	74.9	39.7	64,900	9.83	211	30.6	78.9
8 week's sampling E	21,700	4.84	43.30	3840.0	4480.0	31,800	669.00	423	539.0	3020.0

C = Control units, natural marsh soils at 8 weeks

E = Experimental units, heavy metals dredged material at 8 weeks

are not readily available, some comparisons to previous work can be made. Concentrations of Pb, Cu, and Cd in S. alterniflora substrates in Georgia reported in this study are similar to levels in S. alterniflora sediments bordering the Altamaha River, Georgia (Dunstan et al., 1975). Levels of Mn, Pb, and Zn recorded in this study for the same sediments are much higher than those for S. alterniflora substrates in Virginia marshes (Drifmeyer and Odum, 1975). Banus et al. (1975) reported higher Pb and Zn levels in sediments from S. alterniflora marshes in Massachusetts than those in this report. Concentrations were similar to those found in Georgia soils.

Heavy metals concentrations in plants grown in Georgia BET units are listed in Table 5. Levels of Cd, Cr, Cu, Fe, Ni, Pb, and Zn in S. alterniflora plants are similar to those reported for natural S. alterniflora stands from the Atlantic coast (Banus et al., 1975; Dunstan et al., 1975; Drifmeyer and Odum, 1975; and Lee et al., 1976). Mn levels were lower than those reported by Drifmeyer and Odum (1975). Cd, Cr, Cu, Ni, Pb, and Zn levels in S. patens were comparable to levels reported by Banus et al. (1975), Drifmeyer and Odum (1975), and Lee et al. (1976), while Mn levels were lower than those in S. patens from Virginia. D. spicata levels were also comparable to concentrations of Cd, Cr, Cu, Ni, Pb, and Zn reported by Lee et al. (1976). Data on S. virginica are not available.

Experimental units containing D. spicata, S. virginica, and S. alterniflora plants had significantly higher concentrations of Zn than the control plants (Table 6). Laboratory tests by Lee et al. (1976) and field studies by Banus et al. (1975) and Drifmeyer and Odum (1975) also demonstrated an increase in Zn levels in S. alterniflora grown on media high in Zn content.

Significantly higher levels of Cu were found in experimental S. virginica and S. patens plants than in the controls. Although comparable experimental data for Cu in the above two species are not available, Dunstan et al. (1975) demonstrated a high mortality rate for S. alterniflora seedlings grown on a medium containing 100 µg/kg of Cu.

D. spicata experimental plants had significantly higher



Table 5

Heavy Metal Concentrations ( $\mu\text{g}/\text{kg}$ ) of Control (C) and Experimental (E) Plants  
from Georgia and Oregon at 8 Week's Sampling\*

Species and Location	Al	As	Cd	Cr	Cu
GEORGIA					
<u>Distichlis spicata</u>					
C	411.33 +	62.64	<10.00 +	<10.00 +	<10.00 +
E	223.97 +	115.91	<10.00 +	12.70 +	44.30 +
					9.81
<u>Salicornia virginica</u>					
C	1643.33 +	58.40	<10.00 +	<10.00 +	<10.00 +
E	1446.67 +	139.68	<10.00 +	11.90 +	27.20 +
					2.56
<u>Spartina alterniflora</u>					
C	494.33 +	60.80	<10.00 +	<10.00 +	<10.00 +
E	388.00 +	93.36	<10.00 +	<10.00 +	<10.00 +
					0
<u>Spartina patens</u>					
C	266.67 +	34.84	<10.00 +	<10.00 +	<10.00 +
E	<10.00 +	0	<10.00 +	<10.00 +	22.37 +
					2.32
OREGON					
<u>Carex lyngbyei</u>					
C	108.00 +	33.20	<31.40 +	<31.40 +	<31.40 +
E	181.00 +	10.12	<5.00 +	<5.00 +	11.40 +
					1.23
<u>Deschampsia cespitosa</u>					
C	11.97 +	3.83	<5.00 +	<5.00 +	10.03 +
E	43.23 +	6.82	<5.00 +	<5.00 +	15.23 +
					4.58
<u>Distichlis spicata</u>					
C	108.13 +	9.94	<5.00 +	<5.00 +	7.06 +
E	297.33 +	39.23	<5.00 +	7.81 +	36.30 +
					3.82
<u>Salicornia virginica</u>					
C	68.87 +	12.23	<5.00 +	<5.00 +	7.87 +
E	87.05 +	7.84	<5.00 +	6.67 +	35.30 +
					2.57

(Continued)

\* Mean + 1 Standard Error.

Table 5 (Concluded)

Species and Location	Fe	Pb	Mn	Ni	Zn
<b>GEORGIA</b>					
<u>Distichlis spicata</u>					
C	1743.00 ± 1268.50	1.17 ± 0.12	65.46 ± 4.31	<10.00 ± 0	23.10 ± 0.61
E	370.00 ± 100.00	5.70 ± 0.83	28.30 ± 1.40	<10.00 ± 0	32.10 ± 3.10
<u>Salicornia virginica</u>					
C	1840.00 ± 85.05	2.33 ± 0.73	60.63 ± 1.02	<10.00 ± 0	33.93 ± 0.18
E	1115.33 ± 502.53	4.03 ± 0.40	60.80 ± 3.32	<10.00 ± 0	40.97 ± 1.38
<u>Spartina alterniflora</u>					
C	468.33 ± 116.14	<1.00 ± 0	37.03 ± 5.83	<10.00 ± 0	10.67 ± 0.44
E	508.67 ± 56.83	3.03 ± 0.94	36.70 ± 4.69	<10.00 ± 0	21.30 ± 1.43
<u>Spartina patens</u>					
C	206.67 ± 64.44	1.76 ± 0.62	66.67 ± 10.36	<10.00 ± 0	20.97 ± 0.32
E	10.00 ± 0	7.47 ± 2.22	68.90 ± 5.85	<10.00 ± 0	34.10 ± 3.95
<b>OREGON</b>					
<u>Carex lyngbyei</u>					
C	202.90 ± 58.66	96.83 ± 73.08	102.43 ± 15.79	<31.40 ± 10.59	35.33 ± 6.94
E	474.67 ± 51.23	2.36 ± 1.41	95.33 ± 11.46	< 7.00 ± 1.00	7.00 ± 1.00
<u>Deschampsia cespitosa</u>					
C	52.57 ± 6.66	2.20 ± 1.02	57.27 ± 1.89	7.80 ± 0.90	23.33 ± 1.89
E	108.87 ± 19.27	10.17 ± 5.43	62.73 ± 4.98	6.53 ± 0.87	9.43 ± 5.43
<u>Distichlis spicata</u>					
C	275.33 ± 33.35	3.17 ± 0.43	22.17 ± 1.07	6.03 ± 0.84	27.73 ± 1.42
E	587.33 ± 68.96	1.93 ± 0.09	26.60 ± 3.01	5.80 ± 0.36	28.20 ± 1.95
<u>Salicornia virginica</u>					
C	424.33 ± 174.04	2.73 ± 0.33	74.33 ± 7.18	<5.00 ± 0	19.90 ± 0.42
E	2368.50 ± 764.89	4.35 ± 1.69	57.90 ± 9.77	5.72 ± 0.29	24.87 ± 6.15

Table 6  
Summary of Student's T-test Comparing Heavy Metal Concentrations  
in Plants from Georgia and Oregon at 8 Week's Sampling

Location and Species	Al	As	Cd	Cr	Cu	Fe	Mn	Ni	Pb	Zn
Georgia										
<u>Distichlis spicata</u>	ND	ND	ND	ND	ND	ND	<	ND	>	>
<u>Salicornia virginica</u>	ND	ND	ND	ND	>	ND	ND	ND	ND	>
<u>Spartina alterniflora</u>	ND	ND	ND	ND	ND	ND	ND	ND	ND	>
<u>Spartina patens</u>	<	ND	ND	ND	>	ND	ND	ND	ND	ND
Oregon										
<u>Carex lyngbyei</u>	ND	ND	ND	ND	ND	>	ND	ND	ND	ND
<u>Deschampsia cespitosa</u>	ND	ND	ND	ND	ND	ND	ND	ND	ND	<
<u>Distichlis spicata</u>	>	ND	ND	ND	>	>	ND	ND	<	ND
<u>Salicornia virginica</u>	ND	ND	ND	ND	>	ND	ND	ND	ND	ND

ND: Not significantly different  
> : Average concentration in plants grown on contaminated substrate was statistically greater than concentration in control plants  
< : Average concentration in plants grown on contaminated substrate was statistically less than concentration in control plants



concentrations of Pb than control plants. Lee et al. (1976) reported an accumulation of Pb in roots of D. spicata grown on a high-Pb medium with little translocation to stems and leaves. Drifmeyer and Odum (1975) demonstrated an increase in Pb levels in S. alterniflora exposed to heavy metals sediments as did Banus et al. (1975). Mn levels were significantly lower in D. spicata experimental plants than in the controls. This decrease cannot be explained although Drifmeyer and Odum (1975) reported lower Mn levels in S. alterniflora grown on heavy metals materials.

Results of heavy metals analyses for Oregon plants are listed in Table 5. Levels of Al, Cu, and Fe were significantly higher in D. spicata experimental plants than in the controls (Table 6). Fe concentrations were also significantly greater in C. lyngbyei experimental plants than in control plants. The increases in Fe levels are somewhat surprising since Fe concentrations in natural marsh substrates and heavy metals dredged material did not differ. Perhaps the environmental conditions in the soil and/or the form and consequently the availability of the iron differed in the two substrates. Experimental S. virginica plants had significantly higher concentrations of Cu than the control plants. D. cespitosa had significantly less Zn than controls, and Pb levels were significantly lower in D. spicata experimental plants than in the controls.

#### Summary and Recommendations

A bioassay experiment test unit for use in testing plants for uptake of potentially toxic materials from contaminant dredged materials was tested in the field. D. spicata, S. virginica, S. patens, and S. alterniflora in Georgia, and C. lyngbyei, D. cespitosa, D. spicata, and S. virginica in Oregon were grown on three types of dredged material containing chlorinated pesticides, PCB's, or heavy metals and compared with control plants grown in native soils.

Uptake of chlorinated pesticides could not be demonstrated because of the low concentrations of contaminants in these sediments. PCB

uptake was discerned in two cases but was not detected in others because (a) either it did not occur, (b) the sample size was too small, or (c) the analytical methods were inadequate. The heavy metals data indicate that, in Georgia, translocation of some ions occurred in the following species: Zn in D. spicata, S. virginica, and S. alterniflora; Cu in S. virginica and S. patens; and Al and Pb in D. spicata. In Oregon, D. spicata grown on heavy metals sediments had higher levels of Al, Cu, and Fe than control plants. Fe concentrations were also higher in C. lyngbyei, and Cu levels were higher in S. virginica experimental than in control plants.

The method of using BET units as a predisposal test for contaminant uptake and plant survival appears to be a workable technique. Advantages of BET units determined by this study include: (a) inexpensive and easy to construct; (b) easy to implant and remove; (c) a large number of plant species can be tested at the same time; and (d) plants can be tested in their natural environment where they are exposed to daily fluctuations in salinity, temperature, tidal flow, and activities of marsh invertebrates. The access port designed into the unit provides an entrance way for marsh vertebrates. The importance of invertebrate activity has been well documented by Wiedemann (1972), Kraeuter and Wolf (1974), Barko et al. (1977), and Edwards and Frey (1977). As a result of these activities the substrate within the buckets acquires characteristics of the associated marsh.

We conclude that the bioassay experiment test unit is an efficient, relatively inexpensive technique for screening for the uptake of contaminants by plants grown on dredged materials. Users are cautioned, however, that additional procedural refinements are necessary prior to general application by field elements.

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Wolf, Paul L

Field bioassay test for detecting contaminant uptake from dredged material by marsh plants / by Paul L. Wolf, John L. Gallagher, Carlos H. Pennington, The University of Georgia Marine Institute, Sapelo Island, Ga. Vicksburg, Miss. : U. S. Waterways Experiment Station ; Springfield, Va. : available from National Technical Information Service, 1978.

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References: p. 31-32.

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(Continued on next card)

Wolf, Paul L

Field bioassay test for detecting contaminant uptake from dredged material by marsh plants ... 1978. (Card 2)

Marine Institute, Sapelo Island. IV. United States. Army. Corps of Engineers. V. Series: United States. Waterways Experiment Station, Vicksburg, Miss. Miscellaneous paper ; D-78-6.

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