RECOVERY AND TRANSPORT OF HEAVY METALS BY *SPARTINA ALTERNIFLORA* FROM DREDGING SPOILS

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ABSTRACT

The capacity of smooth cordgrass (*Spartina alterniflora* Loisel.), a dominant grass of Atlantic tidal salt marshes, to transport heavy metal from sediments to estuarine waters was studied. The DTPA extractable heavy metal content of marsh sediments from upper Narragansett Bay was correlated with the metal content of *S. alterniflora* growing in those sediments. The plant Zinc content correlated well with the extractable zinc levels in the sediments. The plant content of cadmium, copper and nickel was best correlated with the sediment content of other metals. Plant cadmium was particularly well correlated with sediment zinc.

The mobility of cadmium and zinc within *S. alterniflora* was determined in solution cultured plants grown in 0 and 15 ppt NaCl. Salinity reduced the root absorption rate of cadmium and zinc by almost fifty percent. Translocation of zinc from roots to shoots was reduced by salinity while cadmium transport was not affected. Both metals were phloem mobile translocating from leaves to roots and other sink regions. The presence of these metals on leaf surfaces following root absorption, indicated elimination from leaves via salt glands. These findings demonstrate that heavy metal absorption and transport occurs in *S. alterniflora* which can serve as a vector of heavy metals from tidal marsh sediments to estuarine waters.

PROJECT OBJECTIVES

The viability of tidal salt marsh construction as an environmentally sound means of dredging spoil disposal may hinge upon the capability of marsh vegetation to transport heavy metals from contaminated sediments to estuarine waterways. Field and laboratory studies were employed to establish the relationship between sediment metal content and the metal burden in aerial plant parts. The capacity of the common marsh grass _Spartina_ _alterniflora_ to absorb metals and translocate them to leaves was also investigated. Specific objectives of this study included:

1. Determining the plant available metal content of marsh sediments from several sites along upper Narragansett Bay.
2. Correlating the metal content of plants with the available metal loads of sediments in which the plants were growing.
3. Formulating predictive equations by which the metal content of vegetation can be calculated based upon the extractable metal content of the sediment.
4. Determining the rate of metal absorption by plant roots and the impact of salinity on that rate.
5. Measuring the mobility of metals within _S._ _alterniflora_ plants following absorption by roots or leaves.

Data generated from this study will be utilized to evaluate the environmental impacts of proposed marsh construction (dredge disposal) projects in Rhode Island marine waterways.

INTRODUCTION

The disposal of dredging materials has become a major problem in the management of coastal waterways. Increasing costs and environmental concerns have restricted ocean dumping of these materials, forcing the search for alternative disposal methods. Prominent among the alternatives being considered is the establishment of salt marshes on tidal banks created with dredged spoil. Such marsh areas would not only permanently stabilize spoil banks but also provide an energy source for estuarine life and, in many cases, replace marsh acreage lost to land reclamation and harbor development. This approach to dredge disposal appears to offer an environmentally acceptable and reasonably priced solution to the disposal problem at least for moderate sized dredging operations.

However, when the dredging spoil contains heavy metals, disposal via tidal marsh construction presents problems. The grasses utilized to stabilize spoil beds could absorb and concentrate metals from contaminated spoil transporting them to above ground shoots which can enter the food chain of detrital feeders and ultimately contaminate human food sources. Industrial coastal states, which for many years have experienced heavy metal discharges into estuarine waters, are most affected by this problem. The disposal dilemma is aggravated by the fact that such contaminated spoils are usually not suitable for ocean dumping or land disposal and even marsh construction on heavy metal contaminated spoils may negatively affect the quality of estuarine waters.

The research reported here attempts to quantify the heavy metal recovery capacity of Spartina alterniflora, the grass most commonly used for stabilizing dredged materials. Emphasis has been placed on cadmium and zinc because of their common occurrence in estuarine benthic sediments. Some studies have considered copper because of its high level in most Narragansett Bay sediments (Seavey and Pratt 1979) and nickel which is widely used in the electroplating industry. S. alterniflora cultured hydroponically has been used to measure heavy metal uptake and translocation under various salinity conditions. By relating these findings to heavy metal recovery by S. alterniflora grown in selected sediments under similated marsh conditions, we have initiated an information base for evaluating the magnitude of the heavy metal recovery problem from spoil based marshes and have furnished some of the analytical tools necessary for predicting the potential heavy metal inputs into estuarine waters.

METHODS

Field Sampling

Sediment samples were collected from ten sites along upper Narragansett Bay during the summers of 1980, 1981 and 1982. The data from the 1980 collections are presented in Table 1. Sediments represented several textural classes ranging from coarse sand or gravel to fine silty materials. These were sampled to a depth of 8-10 cm usually at the base of S. alterniflora plants. All collections were made at low tide when access to tidal flats and sample recovery was not hampered by standing water. Sediment samples were stored in closed plastic containers during transport to the laboratory.

At the time of sediment sampling, five to ten S. alterniflora plants were removed intact from the sites of sediment collection. Excess sediment was dislodged in estuarine water, shoots were separated from roots, and both were transferred to cloth bags for transfer to the laboratory.

Sediment Analysis

Prior to analysis, sediments were oven dried at 65°C and ground to pass a 2 mm screen. Three 10 g replicates from each site were extracted using the method of Lindsey and Norvell (1974). This involved shaking with 20 ml of DTPA (Diethylenetriaminepentaacetic Acid) solution adjusted to pH 7.0 for two hours. The extracts were analyzed for Cd and Zn using a Perkin-Elmer 5000 atomic absorption spectrophotometer. All controls and standards were prepared with DTPA extracting solution.

Plant Analysis

Field collected plant materials were thoroughly cleaned with distilled water prior to oven drying at 65°C. When leaves were coated with sediment, they were wiped with tissue soaked in 80% ethanol followed by distilled water washing. Oven dried plant tissues were ground in a Wiley mill to pass a 20 mesh screen. Duplicate one g samples were ashed for five hours at 315°C in a muffle furnace and the ash dissolved in 5 ml of 2N HCl. The filtered ash solutions were assayed for Cd, Zn, Cu, Pb, and Ni using atomic absorption spectrophotometry.

In a controlled experiment, sediments collected from two sites were maintained under wet reducing conditions prior to being transferred into 10 cm plastic pots. Each 10 cm pot was placed within a 15 cm pot lined with a plastic bag. The space between the two pots was filled with a quartz sand and perlite mixture and saturated with sea water. In this way, the sediment in the inner pot was maintained in a water-logged marsh-like condition. One *S. alterniflora* tiller was planted into each sediment filled pot, and maintained in a growth chamber under 16 hour, 25°C, 50% R.H. days and 8 hour, 15°C, 70% R.H. nights. After two months the plants and sediments were assayed for Cd, Cu, Ni and Zn using methods employed for field samples.

Statistical Analysis and Equation Development

All computations were performed by the statistical analysis system (SAS) (Helwig and Council, 1979). Duncan's multiple range test was used to separate the mean metal concentrations of plant and sediment samples. Coefficients of determination (R^2) were generated and predictive equations developed by linear and stepwise multiple regression of the metal concentrations in both field and growth chamber cultured plants (dependent variable) and their substrate extractable metals (independent variable).

Metal Absorption Rates and Translocation

Single *S. alterniflora* culms, collected from an unpolluted salt marsh in Jerusalem, R.I., were hydroponically cultured in opaque 300 ml flasks containing aerated nutrient solution designed for C_4 plants (Chevalier and Schrader) 1977. Plants were maintained in a growth chamber under the same conditions described for sediment cultured plants. Seven days prior to an experiment, nutrient solutions were made 0 or 15 ppt NaCl and either 1 ppm Cd or 100 ppm Zn. At the initiation of an experiment all plants, roots and shoots, were rinsed with distilled water. For root absorption studies .5 µCi of ^{115m}Cd or ^{65}Zn were introduced via fresh nutrient solution containing the same metal and salinity as the pretreatment solution. One ml aliquots of each solution were taken for radioassay. For leaf treated plants, 2.5 µCi of ^{115m}Cd or ^{65}Zn were applied to a fully expanded leaf of plants cultured in 1 ppm Cd or 100 ppm Zn respectively. The radioisotope was applied in solution containing a surfactant (X-77) to insure wetting the leaf surface. Leaf applications were confined within lanolin paste dikes placed at right angles to the axis of the leaf blades.

After five days, plants were harvested and nutrient solutions, after returned to original volume, were sampled for radioassay. Leaves of individual plants were washed with 15 ml distilled water and the wash assayed for radioactivity and metal content. The plants were freeze dried, after which half were prepared for gross radioautography while the remaining half were subdivided into roots, lower shoot, upper shoot, and treated leaf when appropriate, ground in a Wiley mill, and radioassayed in a planchet counter. Methods used were those described by Lytle and Hull (1980).

RESULTS AND DISCUSSION

Metal Content of Sediments and Plants: Field Study

The ten sites selected for sediment analysis exhibited wide variation in their content of Cd and Zn (Table 1). Generally sediments high in extractable Zn were also high in Cd, and coarse textured sediments normally contained less extractable metal. The Edgewood site was a notable exception to this rule. Those sites in the proximity of industrial discharge were highest in metals while the more remote locations, e.g. Prudence Island, were much less affected.

Spartina alterniflora plants growing in some of the above mentioned sediments also demonstrated wide variation in metal content (Table 2). Generally roots were the most heavily contaminated plant organs. Leaves and aerial stems also contained substantial metal but generally much less than the roots. Cadmium in the upper Pawtuxet Cove site was a notable exception to this rule. Rhizomes exhibited substantial variability in metal content. Zinc was often more concentrated in rhizome tissue than it was in aerial shoots. Although care was taken to remove all sediment from root and rhizome samples, some undoubtedly remained and may have inflated the metal content of those tissues. Except for plants from the upper Pawtuxet Cove site, Pb was least mobile of the metals studied being barely detected in most shoot and rhizome samples. By comparison, Zn exhibited greatest mobility. The relatively high Zn levels in most sediments may have contributed to greater transport but Zn is generally recognized as being among the most mobile divalent cations in plants.

A good relationship between sediment Zn content and that recovered in roots and shoots of *S. alterniflora* was confirmed by the stepwise regression analysis (Table 3). Extractable sediment Zn and shoot Zn were highly correlated ($R^2 = 0.91$). The variation in root Zn content was better explained if the sediment Cd levels were included in the model. Rhizomes exhibited a poor relationship between sediment and tissue Zn levels even when Cd and the Zn:Cd ratio were considered.

The shoot content of Cd was best correlated with the sediment content of extractable Zn (Table 4). The inclusion of sediment Zn levels also improved the model's predictive value of root Cd content. Again sediment metal content proved to be a poor indicator of rhizome metal load. The best three variable equation for predicting the shoot Cd concentration was:

$$\text{Shoot Cd} = 0.005 \text{ DTPA Zn} - 0.004 \text{ DTPA Zn:Cd} - 1.34 \text{ DTPA Cd} \qquad + 1.27.$$

In this equation, 98% of the variation in shoot Cd can be attributed to the three sediment variables at the highly significant 0.0001 level.

Sediment - Plant Metal Interactions: Growth Chamber Study

The sediment extractable metal content of six replicate samples after two months of supporting plant growth proved to be unexpectedly variable (Table 5).

Although all sediments in the six pots for each location were drawn from a common field sample, significant differences in extractable Cd, Zn, and Ni were detected. This supports the high degree of marsh sediment variability reported by Lee et. al. (1978).

Plant tissue analyses of S. alterniflora culms growing in each pot were related via a step-wise regression analysis with extractable sediment content (Table 6). As with the field study, shoot Cd content was best correlated with sediment Zn levels. All tissue levels of Zn were best related to sediment Zn while root and shoot Ni levels were positively influenced by sediment Cu and negatively correlated to sediment Cd. Tissue Cu was poorly predicted by the extractable metals assayed in this study.

It appears that the metal content of S. alterniflora shoots is related to the available metals in the marsh sediment but in a complex fashion. Zinc was the only metal that consistently exhibited good corlelation between plant and sediment content. Cadmium and Ni in plant tissues were often best related to other metals in the sediment but good predictive equations could be written. Plant Cu content apparently was most influenced by sediment factors not measured in this study.

Heavy Metal Absorption and Distribution

The absorption rates of Cd and Zn by S. alterniflora roots as measured by radioisotope loss from nutrient solutions over a five day period, showed some inhibition by 15 ppt NaCl especially for Zn (Table 7). Salt inhibited Zn absorption almost 50% and translocation from roots to shoots approximately 65%. The much greater absorption rate of Zn over Cd was undoubtedly related to the 100X excess of solution Zn over Cd. The high absorption rates of Zn suggest that the uptake process is strongly related to the external ion concentration and probably less by the number or turnover rate of carrier sites in root cell plasma membranes. A preliminary study suggested that Cd absorption was related to solution Cd concentration as a hyperbolic function (Table 8). This indication of saturation absorption kinetics provides support for the idea that Cd entry into root cells is limited by a specific ion carrier. This is inconsistent with our findings that high sediment Zn levels stimulate Cd absorption. An alternative explanation would place the ion interaction at binding sites within the sediment and not carrier sites on cell membranes.

Radiotracer distribution patterns within S. alterniflora plants following root or leaf exposure to ^{115m}Cd or ^{65}Zn indicated marked symplastic translocation (Table 9). Within five days, the roots contained 35% of the leaf applied Cd and about 20% of leaf applied Zn. Salinized culture solutions appeared to have no effect on Cd distribution within S. Alterniflora while Zn translocation from roots to shoots was somewhat inhibited. This agrees with the lower Zn content found in shoots of plants cultured in nutrient solution containing 15 ppt NaCl (Table 7). The rate of Zn translocation from leaves to roots appeared to be facilitated by culture in a salinized medium (Table 9). Because transport was measured over a five day interval, the greater Zn levels in the roots following leaf exposure might reflect reduced circulation within the plant due to inpaired apoplastic return of Zn from roots to leaves. Thus, ^{65}Zn accumulation in roots regardless of the site of application might be a manifestation of a common response to salinity i.e. reduced acropetal translocation.

When leaves were washed following five days of culture in ^{115m}Cd or ^{65}Zn labeled nutrient solution, the wash solution contained radioactivity (Table 10).

This was especially true of Cd treated plants growing in 15 ppt NaCl enriched solution. Zinc was recovered on leaf surfaces independent of the culture solution salinity. Because _S. alterniflora_ leaves contain numerous salt glands, it is highly likely that divalent metal ions are excreted along with salt via the salt glands to the leaf surface. This elimination of metal ions might explain why the leaf metal content often is much less than that of roots (Table 1). This also suggests that _S. alterniflora_ can extract heavy metals from marsh sediments and transport them to estuarine waters in quantities greater than that indicated by shoot metal concentrations.

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Table 1. Metal Concentration of Ten Marsh Sediments from upper Narragansett Bay, R.I. (Summer 1980)

Site	Metal Concentration		Texture Grade
	Cd	Zn	
Pawtuxet Cove (L)	0.48 d*	213.3 d	mc**
Pawtuxet Cove (U)	1.65 b	1603.3 a	f
Pawtuxet Cove Marina	0.51 d	232.7 d	f
Edgewood Y.C.	0.10 e	48.0 e	mc
Edgewood	1.25 c	356.0 c	c
Port of Providence	0.06 e	15.0 ef	c
I-195 Bridge	0.11 e	26.7 ef	c
Blackstone Park	2.14 a	507.0 b	f
Prudence Island (E)	0.03 e	3.3 f	mc
Prudence Island (W)	0.02 e	3.7 f	c

* Means within a column followed by the same letter are not significantly different p = 0.05.

** Texture grades: c = coarse, mc = medium coarse, f = fine.

Table 2. Metal concentration in *Spartina alterniflora* Collected at Five Sites in Upper Narragansett Bay, R.I. (Summer 1980).

Site	Plant Part	Metal Concentration			
		Cd	Cu	Pb	Zn
			ppm		
Pawtuxet Cove (U)	Root	2.0	174	288	175
	Rhizome	0.0	15	20	67
	Shoot	3.2	38	45	114
Pawtuxet Cove (L)	Root	3.5	31	74	135
	Rhizome	1.0	11	9	60
	Shoot	0.0	3	0	19
Edgewood Y.C.	Root	1.0	26	96	88
	Rhizome	0.0	4	0	18
	Shoot	0.2	4	0	18
Seekonk River	Root	6.0	82	106	153
	Rhizome	1.0	10	4	39
	Shoot	0.0	4	10	30
Prudence Island	Root	1.4	17	21	82
	Rhizome	0.5	6	1	55
	Shoot	0.1	4	0	22

Table 3. Regression Summary between Zn Content of Field Collected S. alterniflora and Sediment Metal Concentration

Plant Part	Variable	R^2	Significance
Root	Zn	0.66	0.0001
	Zn:Cd	0.77	0.0001
	Zn,Cd,Zn:Cd	0.79	0.0001
Rhizome	Zn:Cd	0.18	0.0808
	Zn + Cd	0.48	0.0070
	Zn,Cd,Zn:Cd	0.49	0.0223
Shoot	Zn	0.91	0.0001
	Zn + Cd	0.97	0.0001
	Zn,Cd,Zn:Cd	0.99	0.0001

Table 4. Regression summary between Cd content of S. *Alterniflora* and sediment metal concentration

Plant Part	Variable	R^2	Significance
Root	Cd	0.34	0.0113
	Cd + Zn	0.50	0.0055
	Cd,Zn,Zn:Cd	0.55	0.0086
Rhizome	Zn	0.21	0.0537
	Cd + Zn	0.32	0.0572
	Cd,Zn,Zn:Cd	0.46	0.0324
Shoot	Zn	0.71	0.0001
	Cd + Zn	0.91	0.0001
	Cd,Zn,Zn:Cd	0.98	0.0001

Table 5. Metal Concentrations of Two Narragansett Bay Marsh Sediments after Cultured for Two Months with S. alterniflora

Site	Pot No.	Metal Concentration[+]			
		Cd	Zn	Cu	Ni
		ppm			
Pawtuxet Cove	1	0.90 a[*]	149 a	16 a	5.1 a
	2	1.33 a	200 a	43 a	5.9 a
	3	2.15 a	201 a	56 a	6.6 a
	4	0.83 a	152 a	20 a	5.4 a
	5	0.69 b	124 b	13 a	5.3 a
	6	0.96 a	145 a	18 a	5.4 a
Seekonk River	1	4.23 a	139 a	45 a	8.8 bc
	2	3.82 ab	123 a	41 a	7.7 c
	3	3.74 ab	129 a	42 a	8.1 c
	4	4.23 a	140 a	43 a	9.8 ab
	5	4.39 a	142 a	55 a	10.9 a
	6	2.90 b	111 b	58 a	8.5 c

[*] Values within columns for each site followed by the same letter are not significantly different at $P = 0.05$.

[+] Each value is the mean of three extractions.

Table 6. Regression Summary between Greenhouse Cultured *S. alterniflora* and Sediment Metal Content

Metal	Plant Part	Significant R^2	Variable
Cd	Root	0.65	Cd, Zn:Cd
	Rhizome	0.51*	Ni, Zn:Cd
	Shoot	0.45	Zn
Zn	Root	0.72	Zn
	Rhizome	0.48	Zn
	Shoot	0.63	Zn
Cu	Root	0.50*	Cd, Ni,$^-$ Zn:Cd
	Rhizome	0.44*	Cu, Ni,$^-$ Cd$^-$
	Shoot	0.56*	Cu, Ni, Zn:Cd
Ni	Root	0.83	Cu + Cd$^-$
	Rhizome	0.72	Cd$^-$ + Zn:Cd
	Shoot	0.85	Cu + Cd$^-$

* Regression not significant at the 0.05 level.

$^-$ Negatively correlated with metal content.

Table 7. Metal Uptake Rates and Plant Content of Root Exposed <u>S.</u> alterniflora plants.

Metal	Salinity	Plant No.	Uptake	Metal Content Root	Metal Content Shoot
	ppt		µg/g/day	µg	
Cd	0	1	6.27	36.0	9.6
		2	5.88	30.1	3.5
	15	1	1.58	8.3	1.8
		2	4.97	29.7	4.9
Zn	0	1	260	588	616
		2	294	699	206
	15	1	166	925	145
		2	134	226	112

Table 8. Uptake by *S. alterniflora* Roots at Varying Solution Concentrations

Cd Concentration ppm	Uptake Rate µg/g/day
0.1	0.01
1.0	6.10
10.0	15.80

Table 9. Distribution of Cd and Zn in S. alterniflora Exposed Via Roots or Leaves.

			Metal Content		
				Shoot	
Metal	Organ Exposed	Salinity	Root	Lower	Upper
		ppt		%	
Cd	Leaf[†]	0	35.6 a[*]	44.4 a	20.0 a
		15	36.2 a	45.7 a	18.1 a
	Root[‡]	0	76.5 a	23.5 a	
		15	77.0 a	23.0 a	
Zn	Leaf[†]	0	17.2 b	55.3 a	27.5 a
		15	29.1 a	44.8 b	26.1 b
	Root[‡]	0	54.5 b	45.5 a	
		15	63.0 a	37.0 b	

[*] Values in columns for each metal and exposure method followed by the same letter are not significant p = 0.05.

[†] All leaf exposure means are based on two experiments with three to five replications of salinity treatments in each.

[‡] All root exposure means are based on four experiments with three to five replications of salinity treatments in each.

Table 10. Radioactivity and Metal Concentration of Leaf Wash solutions from Root or Leaf Exposed *S. alterniflora*

Metal	Organ Exposed	Salinity	Radioactivity		Metal Conc. of Leaf Wash
			Plant	Leaf Wash	
		ppt	cpm		ppm
Cd	Root	0	81846	0	0.3
		15	81878	240	0.8
	Leaf	0	6240	5	1.2
		15	4408	88	0.4
Zn	Root	0	2552	174	52.2
		15	3206	252	18.6
	Leaf	0	602	66	21.6
		15	2695	64	6.6

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