THE IMPACT OF EELGRASS ON HEAVY METAL MOBILIZATION

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Abstract

The Impact of Eelgrass on
Heavy Metal Mobilization

Release of cadmium and manganese radionuclides from eelgrass
leaves and root-rhizomes into seawater and seawater plus $1 \times 10^{-4}M$
disodium ethylenediaminetetraacetate (EDTA) was monitored over
periods of six hours following incubations of 1 to 98 hours.
Flux of both isotopes from tissues is initially rapid, and en-
hanced by the addition of EDTA. The initial release rate is in-
dependent of incubation time, indicating desorption of metals
from exterior tissue surfaces. High initial release rates become
rapidly attenuated. Fitting a power ($y = ax^b$) to the data proved
valuable to inferring uptake capacity of tissues from observed
metal release characteristics. In addition to desorption, and
diffusion from intercellular spaces and cells, and biologically
controlled release could be described with the aid of curve fits.
Manganese apparently is released more slowly during latter phases
of release, pointing to greater biological accumulation potential.

Cadmium and manganese radionuclide uptake by Zostera marina L.
tissues and translocation between root-rhizomes and leaves was
examined. Cadmium concentrations in root-rhizomes increased with
incubation time but appeared to reach saturation levels at 24 hr
of exposure. Translocation of cadmium between root-rhizomes and
leaves was observed to occur in both directions. A greater flux
of cadmium downward was noted and root-rhizomes appear to be a
cadmium sink. Cadmium flux in either direction could be enhanced
by a salt gradient. Cadmium appears to move through eelgrass by
diffusion or mass flow through vascular tissues and apparent free
spaces. Manganese is less mobile but is more readily fixed by
leaves. Manganese mobility is not enhanced by salt gradients.
Incorporation of cadmium into root-rhizomes from labelled anoxic
sediments was not equal to that from labelled anoxic seawater
media. Long-term depuration of cadmium labelled eelgrass trans-
planted to the field is discussed.

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Introduction

Metals are commonly found in estuarine sediments in concentrations higher than in the overlying water column (Gambrell et al., 1976 a, b; Leland et al., 1976; Pulich, 1976). The bioavailability of these metals is partly affected by physico-chemical interactions in the sediment milieu (Gambrell et al., 1976 a, b; Reddy and Patrick, 1977; Khalid et al., 1978). Rooted seagrass, such as Zostera marina L., may affect the balance of these interactions by virtue of its intimate association with sediments through its root-rhizome system. Z. marina roots have been shown to take up phosphate, nitrogen, and carbon, and translocate these compounds to leaves (McRoy and Barsdate, 1970; McRoy et al., 1972; McRoy and Goering, 1974).

Pulich (1976) found that concentrations of iron, manganese, zinc, and copper in leaves and roots of five seagrass species was greater than in sediments. Faraday (1978) and Faraday and Churchill (1979) recently noted that cadmium is taken up by both leaves and root-rhizomes of Z. marina, but that translocation of this metal only occurred downward. Other studies (Gentner, 1977; Mayes et al., 1977; Harding and Whitton, 1978; McIntosh et al., 1978) have shown that bidirectional translocation of metals including cadmium, occurs in rooted aquatic macrophytes.

There are two mechanisms by which rooted plants can absorb solutes from the exterior of the root system into the vascular

1

tissues. Active uptake, or movement against an electrochemical gradient, of ions may occur across cell plasmalemmas of cortical cells in roots via carriers, followed by transport via cytoplasmic cell connections into other cell layers, and eventually into vascular tissues. An alternative mechanism of absorption may be diffusion of ions across cell walls and passive, apoplasm movement in cell wall spaces by diffusion and/or mass flow (Nye and Tinker, 1977). Cell wall spaces consist of macropores between cells, micropores in cell walls and spaces between cell layers. These cell wall spaces are included in the definition of apparent free space (see Dainty, 1969 for discussion) and may account for 10 - 20% of most root volumes (Gauch, 1972). The definition of apparent free space also includes lacunar and vacuolar space. Some apparent free space may not be available for ion movement because it is gas-filled, even in wholly water saturated roots of aquatic plants. Since apparently little of the root volume is free space, much of the cell wall volume must be in rapid equilibrium with external solutes. Uptake, then, may be considered as a sequence or combination of up to five events: transport or diffusion across outer cells in the root cortex; movement in free spaces of the root cortex; uptake into living parts of cells, across cell walls; transport in cytoplasm of cells and release into vascular tissues; and transport in vascular tissues (Nye and Tinker, 1977).

Pickering and Puia (1969) demonstrated that ^{65}Zn-uptake in
Fontinalis antipyretica occurs through at least three successive
phases. The first phase is characterized by a rapid rate of
uptake that lasts no more than twenty minutes. The duration of
this phase decreased with increasing incubation concentration and
was not influenced by temperature or light intensity. Veltrup
(1978) observed a similar phase in excised barley roots, attri-
buting it to adsorptive processes on root surfaces. The second
phase defined by Pickering and Puia is distinguished from the
first by its reduced rate of uptake, lasting approximately ninety
minutes. Light and temperature slightly affected this phase.
They argue that exchange absorption is the driving mechanism,
and that penetration into cytoplasm and cell organelles occurs
in this phase. The third phase was observed to last for several
days and was characterized by a very slow rate of uptake. Tem-
perature and light intensity, among other factors affecting
metabolism, affected this phase and were pointed to as evidence
for the presence of active accumulation into cell vacuoles.
Pickering and Puia (1969) and Vallée-Shealtiel (1962) observed
similar triphasic uptake characters in ^{133}Ba and rubidium
experiments, respectively. Gutknecht (1961) also found that zinc
uptake by the two-cell layered alga Ulva lactuca occurred via
three phases, excepting transport to vascular tissue.

Faraday and Churchill (1979) reported that cadmium uptake, assayed by flame atomic absorption, in Zostera marina L. was concentration dependent. Uptake by root-rhizome portions of plants, incubated 24 hr in two-compartment chambers similar to those described in the current investigation, was proportional to substrate cadmium concentrations (1, 5 and 10 ppm). Maximum uptake was observed at 72 hr for tissues incubated with 1 ppm cadmium. Translocation of cadmium from leaves to root-rhizomes after 72 hr of leaf incubation amounted to 27% of total plant uptake. No cadmium translocation from root-rhizomes to leaves was detected. On the other hand, Mayes et al., (1977) found that significant quantities of cadmium and lead were translocated from roots to leaves of the freshwater macrophyte Elodea canadensis.

This study describes characteristics of ^{109}Cd and ^{54}Mn release from labelled tissues of eelgrass, Zostera marina L. In studying uptake of these two metals from sediments and water, extensive data were collected on time-release of metal to establish minimum rinsing times required to remove material not actively incorporated into tissues. Several investigators have recognized the need for removal of readily exchangeable ions to distinguish genuine metal uptake and characterize uptake kinetics (eg., Pickering and Puia, 1969; Gentner, 1977; Reddy and Patrick, 1977; Veltrup, 1978). Data from these release studies were amenable to

various curve-fitting procedures. A number of equation parameters, especially from power-curve fits, proved quite useful to infer the reverse processes, namely uptake.

The uptake and bidirectional translocation of cadmium and manganese isotopes by intact shoots, as well as uptake by excised root-rhizomes and leaves, of Zostera marina L. was examined by direct methods. A number of environmental effects, including light, salinity, and type of incubation medium (water or sediment), on uptake and translocation are discussed. Experiments on the release of cadmium from plants placed in the field are also described.

Materials and Methods

Plant Sample collection

Plants were collected from three locations in Great South Bay, Long Island, New York (Fig. 1). Eelgrass beds at Location 1 are exposed to high salinities (30-$32^{o/oo}$) due to the proximity of Fire Island Inlet. At Location 2, eelgrass beds are contained within a sheltered cove on the bay side of the barrier beach and are exposed to similar salinities. Beds sampled at Location 3 exist close to shore on the mainland side of the bay and are subject to lower salinities resulting from the isolation of these beds from Fire Island and Moriches Inlets and dilution of the bay water by the outflow from Carmen's River. Water depth at all three locations was 0.7 to 1.3m.

Plant samples were collected at various times throughout the year with a gardening pitchfork. Sediments were washed from clumps of plants as they were removed from the eelgrass bed. Most of the plants collected by this technique experienced little or no damage.

In selecting plants, all that appeared to be immature, senescing or damaged were discarded. In addition, plants that were heavily epiphytized were discarded. Attempts were made to cull the remaining specimens so that the collection would repre-

Figure 1. Map of Great South Bay showing collection sites at Gilgo State Park (1), Sand Island (2), and Bellport Bay (3).

CONNECTICUT

LONG ISLAND SOUND

LONG ISLAND

MORICHES INLET

MORICHES BAY

GREAT SOUTH BAY

SOUTH OYSTER BAY

JONES INLET

N.Y. CITY

NEW JERSEY

N

0 _____ 1 MI.

NASSAU CO.
SUFFOLK CO.

ISLIP

CONKLIN BAYPORT
PT.

OAKDALE

SAYVILLE

BLUE PT.

SMITH PT.

BELLPORT BAY

3-

4

ROBERT MOSES CAUSEWAY

2

POINT OF WOODS

FIRE IS. COAST GUARD STA.

FIRE ISLAND

FIRE IS. INLET
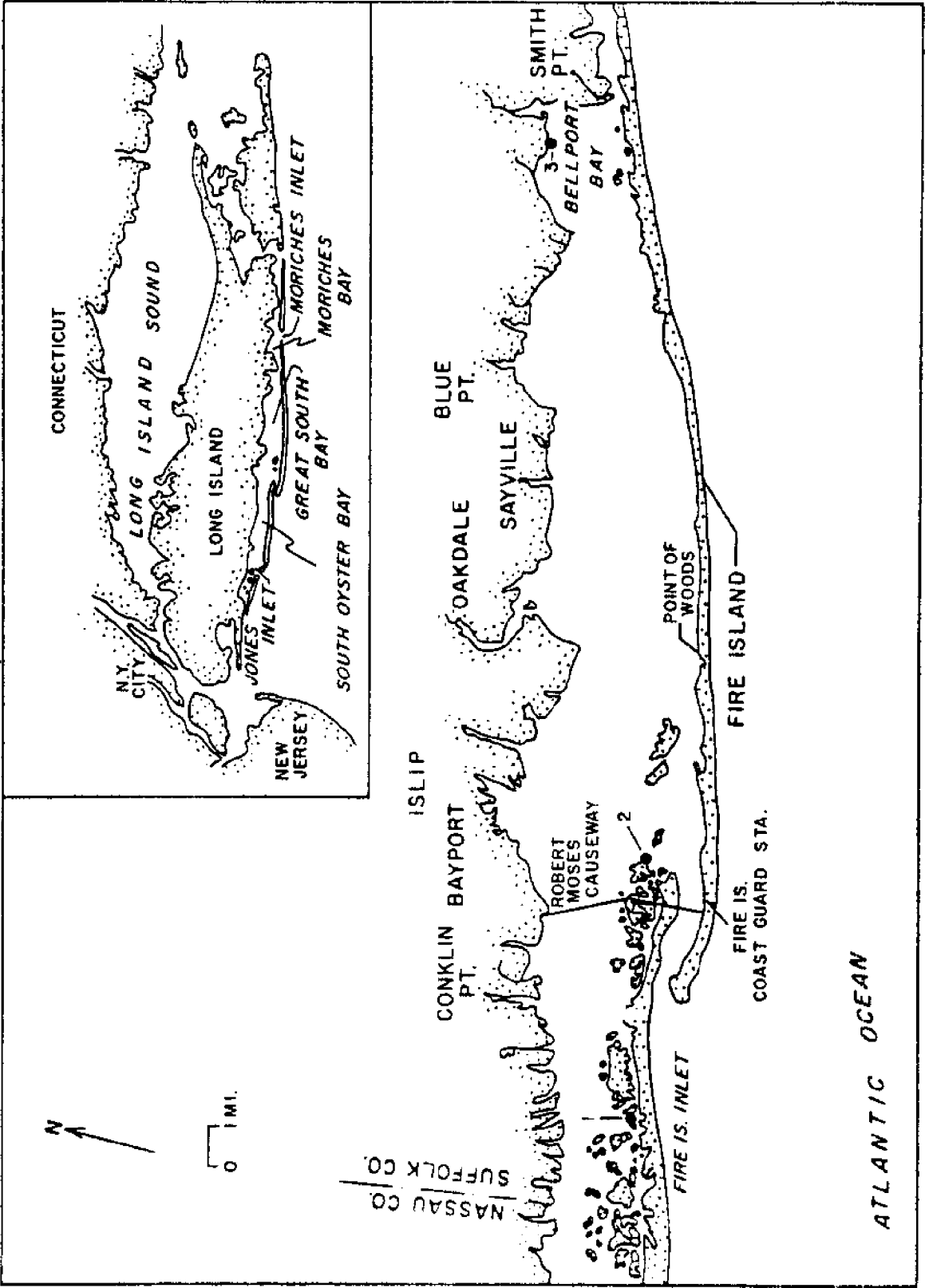
ATLANTIC OCEAN

sent a uniform size class. Up to this point, the eelgrass plants had not been removed from the water. Once culled, the plants were immediately transferred to a large bucket filled with sea water where they either awaited use in field experiments or were transported back to the laboratory. In the lab, plants were transferred to a sea water aquarium. The aquarium contained routinely changed seawater that had been previously collected at the sample site. The temperature of the aquarium was kept approximately 2°C cooler than in situ temperatures. These temperatures helped to maintain the plants in good condition during storage.

Incubation chambers

Translocation processes between the root-rhizome complex and leaves of eelgrass plants were examined in partitioned chambers that effectively separated these plant portions. A variety of chambers have been built and used with success (McRoy and Barsdate, 1971; Faraday, 1977). The chambers designed for this study, however, differ in that they also permit coring of plants with intact sediments about their roots. The chambers were constructed from plexiglass tubing (Fig. 2). The outer diameter of the upper compartment was equal to the inner diameter of the lower compartment, enabling the two compartments to fit together. A groove was cut into the base of the upper compartment was fitted with an O-ring. A plexiglass disc was cut to size and cemented within

Figure 2. Photograph showing two-compartment incubation chamber with plant in place.
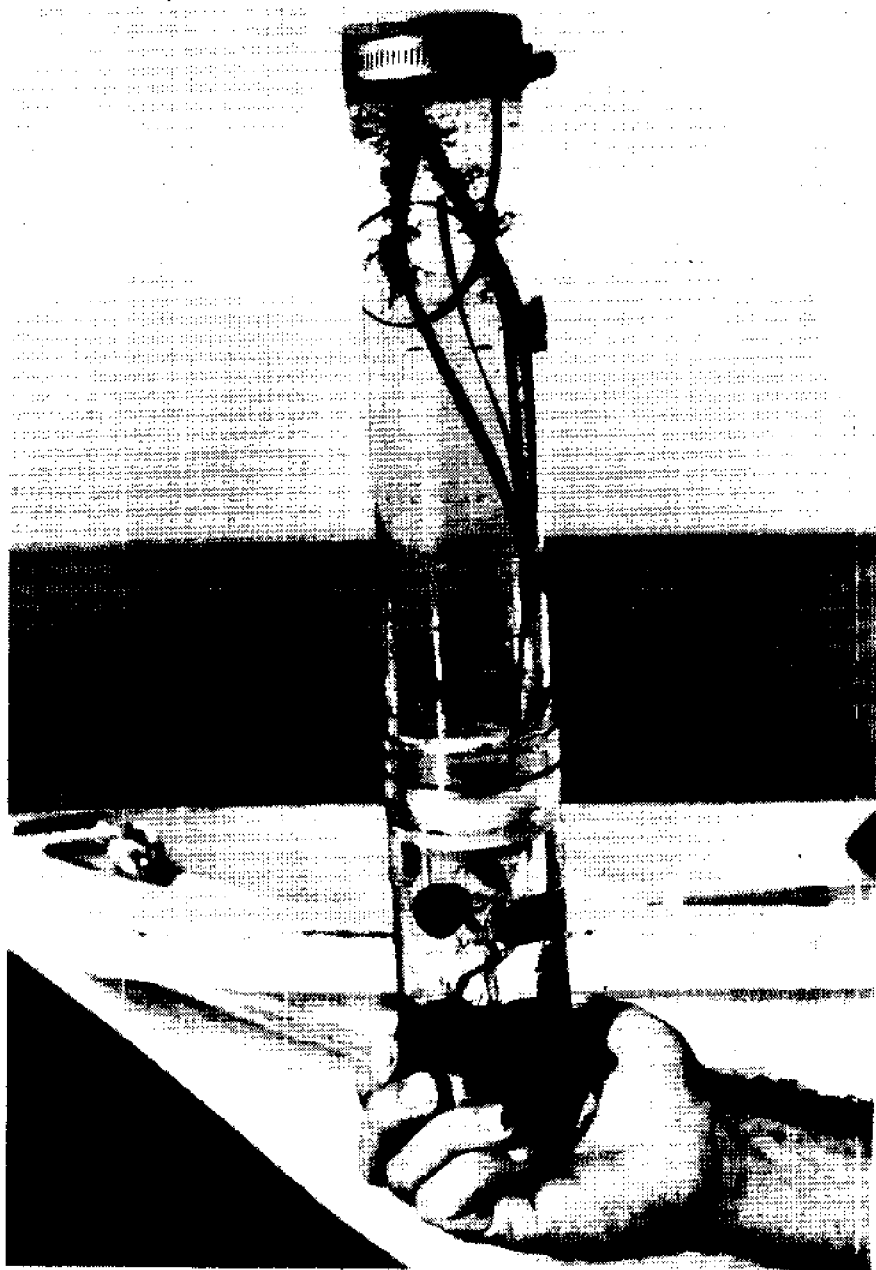
the lower compartment. The disc restricted the extent to which the upper compartment could slide into the lower. A hole was cut into the disc to accommodate a #10 silicone stopper. With the stopper in place, the upper compartment was isolated from the lower compartment. The ends of the incubation chamber were capped with plastic core tube caps that had a bead of silicone sealant placed about the inside, and were clamped. Rubber septums in both the upper and lower compartments permitted injection of incubation solutions and removal of water samples via syringes fit with needles.

Uptake experiments

The uptake of trace metals was assayed by monitoring radioisotope incorporation into various eelgrass tissues. Radioisotopes ^{109}Cd (as $CdCl_2$) and ^{54}Mn (as $MnCl_2$) were chosen because of the relative abundance of the stable counterparts of these isotopes in dredged spoils. Further, one is required in plant nutrition (Mn) and the other is not. These isotopes are gamma emitters.

Two different incubation methods were employed in this study. The first involved the use of sediments in the lower root-rhizome compartment. Initially, experiments were conducted with plants and intact sediments that were obtained by a coring technique to minimize disturbance of the anaerobic sediment

environment and its geochemistry. A relatively isolated plant

(at least 15 cm from neighboring plants) was located on the bay

floor, and a partially sliced disc of silicone rubber sheet

(5mm thick) was placed about the base of the plant. The lower

compartment of one of the chambers was placed carefully over the

plant's leaves until the compartment base was about the silicone

disc, and resting on the bottom. The leaves of the plant were

then gently pulled through the opening in the partitioning disc

and the compartment slowly forced into the sediment until the

silicone disc was in firm contact with the partitioning disc.

At this point, sediments were dug away from the sides of the

compartment by hand. The compartment was removed from the

bottom by wedging a hand beneath the compartment. The bottom

of the chamber was then sealed with a clamped core tube cap.

A fresh bead of silicone sealant about the inside of the core

caps was found to be essential to forming a water tight fit.

Apiezon sealing compound (VWR Scientific, Rochester, N.Y.) was

placed around the plant base to ensure a good seal between

compartments. The upper compartment, with rubber septums re-

moved, was set into place over the leaves and seated. Once the

upper compartment was filled with sea water, the septums were

replaced. One ml aliquots of 5μCi of radionuclide, ^{109}Cd or

^{54}Mn , were injected at various angles and depths into the

sediments contained in the root-rhizome compartment. Incubations

were conducted in the field for periods of 1 to 24 hr to determine root-rhizome uptake over time and subsequent transport to the leaves. An analysis of the radionuclide distribution in the sediment core after incubation was conducted by carefully removing the sediment core and slicing it horizontally into 2 cm sections and radially into 9 sections. This analysis indicated that injection at various angles and depths into the core did not result in uniform distribution of the isotope. Therefore, there was not assurance whether radionuclide was injected near the root-rhizome complex nor how much of the tracer was available to the root-rhizomes. This injection method was abandoned and the following procedure for working with isotopically enriched sediments was applied.

Sediment was collected in buckets and plants were collected using a pitchfork as described earlier. In the lab sediments were mixed in a bucket with approximately $100\mu Ci$ of radioisotope into a slurry. The sediment was incubated for a one day period, with occasional (once per 6-12 hr) resuspension of the slurry. The sediments were then allowed to go anaerobic for an additional 12 hr.

Individual plants were placed with small amounts of Apiezon sealant into a #10 silicone stopper that had a small hole drilled into the center and a razor slice from the edge of the stopper to the center hole. The stopper and plant were then firmly set

into the partitioning disc of the lower compartment and more
Apiezon sealant was placed about the base of the plant and to
the top of the stopper (after the stopper had been wiped dry).
The upper compartment was filled with sea water, sealed and set
into place. The cap was removed from the lower compartment and
treated sediment gently added to the lower compartment until it
was full. The lower compartment was recapped and clamped as
before.

The second incubation method employed consisted of working
with water in both upper and lower compartments. Individual
plants were set up as in the pre-treated sediment case, but the
lower compartment was filled with 99.97% N_2 (balance CO_2) purged
seawater. Seawater was collected at the sample site or Flax
Pond facilities and approximately 10 l of seawater in a 2m X 6.5 cm
core tube was bubbled with the gas mixture (introduced at the
bottom of the tube) for at least 20 minutes. This resulted in
oxygen levels of less than 0.1 ppm.

Once the plants were set up in the incubation chambers, 1 ml
of $5 \mu Ci$ of a radiolabeled tracer was injected into either the
leaf compartment or the root-rhizome compartment with a syringe
through a rubber septum. The exception to this, of course, is
the case where the sediments were treated before the incubation
and then added to the lower compartment.

Incubations were conducted for periods of 1 to 36 hr using triplicate chambers for every chosen incubation period. At the conclusion of the incubation, a 5 ml water sample was obtained with an uncontaminated syringe from the compartment of each chamber that was not directly exposed to the tracer. These aliquots were immediately placed into individual gamma counting vials and the remaining low activity water was discarded. The chamber halves were separated and the plant portion in the uninjected compartment was carefully cut away from the rest of the plant at the point where the plant emerged from the silicone stopper. The plant was rinsed in seawater for 10 sec and placed in a backwashing beaker (backwashing is discussed in detail later). At this time, all replicates were handled in the same manner, with each plant portion not directly exposed to the isotope placed into a separate backwashing beaker. Next, a 5 ml aliquot of water was removed from the spiked compartment and placed into a counting vial (this was not possible for sediment incubations). Remaining labeled water or sediment was carefully disposed of in radioactive waste containers. The remaining plant material was removed from the chambers, rinsed in seawater for 10 sec and placed into a second backwash beaker. This procedure was again repeated for all replicates.

Several experiments were also conducted to examine uptake and bidirectional transport potential under the influence of

salinity gradients. Seawater was collected from Great South Bay (salinity = 25 o/oo). Three chambers were set-up, as before, with equal salinities in top and bottom compartments (e.g., 25:25). Two other sets of three had seawater diluted with deionized, distilled water added to the top compartments to reduce salt concentrations (e.g., 18.75:25 and 12.5:25). The usual amounts of either isotope (5.0 μCi) were added to top compartments. Here we examined uptake and downward transport of isotope <u>against</u> a potential salt gradient. In another series of experiments either isotope was added to the bottom compartment to examine uptake and upward transport <u>with</u> a potential salt gradient. Reciprocal experiments were conducted in which the salt gradient was reversed, i.e., downward, using similar dilution ratios. These experiments each lasted about 24 hr.

Numerous uptake experiments were also conducted with excised leaf and root-rhizome tissue. These excised samples were directly immersed in 200 ml of radiolabeled cadmium or manganese (2.5 μCi each) solution. After the conclusion of the exposure period (between 0.25 and 98 hr), the samples were individually rinsed and backwashed. Six leaf or root-rhizome samples were incubated for each exposure time.

Plant radionuclide-loss during backwashing

All plants, except where noted, were backwashed. The necessity for backwashing has been discussed by Faraday (1977)

Several aspects of backwashing were examined in this study.

The release of radionuclides from incubated plants during backwashing was principally examined for ^{109}Cd incubated plant samples, although some data for ^{54}Mn incubated plants are presented. The five incubation categories of ^{109}Cd incubated plant samples for which detailed backwash data were collected are:

1) excised root-rhizome sections incubated in beaker experiments and backwashed in unlabeled seawater

2) excised root-rhizome sections incubated in beaker experiments and backwashed with EDTA added to the seawater

3) intact root-rhizome complexes incubated in chambers with the root-rhizomes directly exposed to the incubation solution and backwashed with EDTA added to the seawater

4) excised leaves incubated in beaker experiments and backwashed with EDTA added to the seawater

5) intact leaves incubated in chambers (where only the root-rhizomes were directly exposed to the incubation solution) and backwashed with EDTA added to the seawater.

It is important that the reader observe the distinction between the two categories of leaf incubations. The excised leaf sections

in 4) were directly immersed in the incubation solution. On the other had, exposure of intact leaves in 5) could only have occurred through translocation of radionuclides from the root-rhizome complex to the leaves of the plant.

Backwashing in this study consisted of placing leaf and root-rhizome sections from each chamber or excised tissue material into 200 ml of seawater or 200 ml seawater plus 0.5 ml of 5×10^{-2} M disodium ethylenediaminetetraacetate (EDTA) added (final concentration = 1×10^{-4} M). The backwashing procedure continued for 6 hr, with the backwashing solution being changed at 10, 30, 60, 120, 180, 240, 300, and 360 min. A 5 ml aliquot was removed from the backwash beaker after each interval and placed into counting vials. At the end of the backwash, plants were matted dry. The root-rhizome sections were separated into roots and rhizomes, in most instances, and placed into separate counting vials. When epiphytes were present, they were also placed into a separate gamma counting vial. All radioactive samples were counted in a Searle Model 1185 (NaI) gamma counter. After counting, plants were dried to constant weight at 75°C.

Depuration experiments

In addition to uptake experiments in the laboratory, two field experiments were conducted to examine long-term depuration of cadmium from labelled plants. In each experiment, 250 individual sediment-free shoots of eelgrass were collected in the

field and returned to the laboratory. These plants were culled in the field for uniformity in leaf length (mean of 30 plants = 28.6 cm) and number (3 to 4/plant) and internode length (mean of 30 plants = 4.7 cm) and number (4/rhizome). Only plants with at least 50% of the roots showing visible root caps were used. One batch of 25 was separated and maintained in an aquarium. The remaining plants were wrapped gently in cheesecloth in bunches of approximately 100 plants. These were incubated in plastic buckets containing 10 l seawater and 100 μCi ^{109}Cd for 18 hr under constant light. The next morning, all labelled plants were back-washed for 6 hr with EDTA. A batch of 25 plants was harvested and sectioned into leaf, root, and rhizome tissue to determine initial ^{109}Cd activity, and were designated as Day 0 plants. The remaining 200 plants, plus the 25 unlabelled ones, were taken back to the eelgrass collection site in buckets of seawater. Here, all plants were planted in sandy sediments in a rectangular pattern with 30 cm spacing (see Churchill et al., 1978 for planting techniques). Subsequently, batches of 20 labelled plants were harvested from the field on Days 2, 4, 8, 16, and 32. Harvested plants were carefully cleaned of sediments and separated into leaf, root, and rhizome fractions, which were blotted and put into counting tubes. Numbers of new leaves and internodes were recorded for each plant. Survival (%) of the 25 unlabelled control plants was determined at the end of the experiment.

Results

Factors affecting liberation of metals

The release of metals from eelgrass tissues into backwashing solutions is characterized by an initially high liberation rate during the first 30 min, followed by decreased rates thereafter. As a typical example, release of ^{109}Cd from excised root-rhizomes incubated for 1 hr is shown in Fig. 3. The activity (DPM) released is expressed as a cumulative-% of the total accumulated during incubation, prior to backwashing. The activity released into seawater and seawater plus EDTA was compared for root-rhizomes incubated for periods up to 24 hours. Differences in the amount of ^{109}Cd released were tested for significance using a pure Model 1, 2-way ANOVA (Sokal and Rohlf, 1969). Results of this analysis are shown in Table 1.

First, EDTA inclusion significantly increases (P < .001) the loss of material accumulated by root-rhizomes during incubation. The additional loss resulting from EDTA presence is independent of the length of incubation, as is evident from the nonsignificant (P >.01) interaction term. Further, the additional loss can be shown to occur only during the early stages (see Fig. 3) of backwashing. EDTA appears to remove isotope material that has adsorbed onto the surface of root-rhizomes, and possibly onto adhering particles.

A second factor determining total loss of accumulated cadmium to backwashing is length of incubation. Table 1 indicates that

Figure 3. Histogram of cumulative % ^{109}Cd lost by excised
root-rhizomes incubated for 1 hr and backwashed with
1 X 10^{-4} M EDTA in seawater (unshaded) and with straight
seawater (shàded). Each bar portion represents the mean
of three backwashed plant samples.

TIME (hr)

CUMULATIVE % ACTIVITY RELEASED
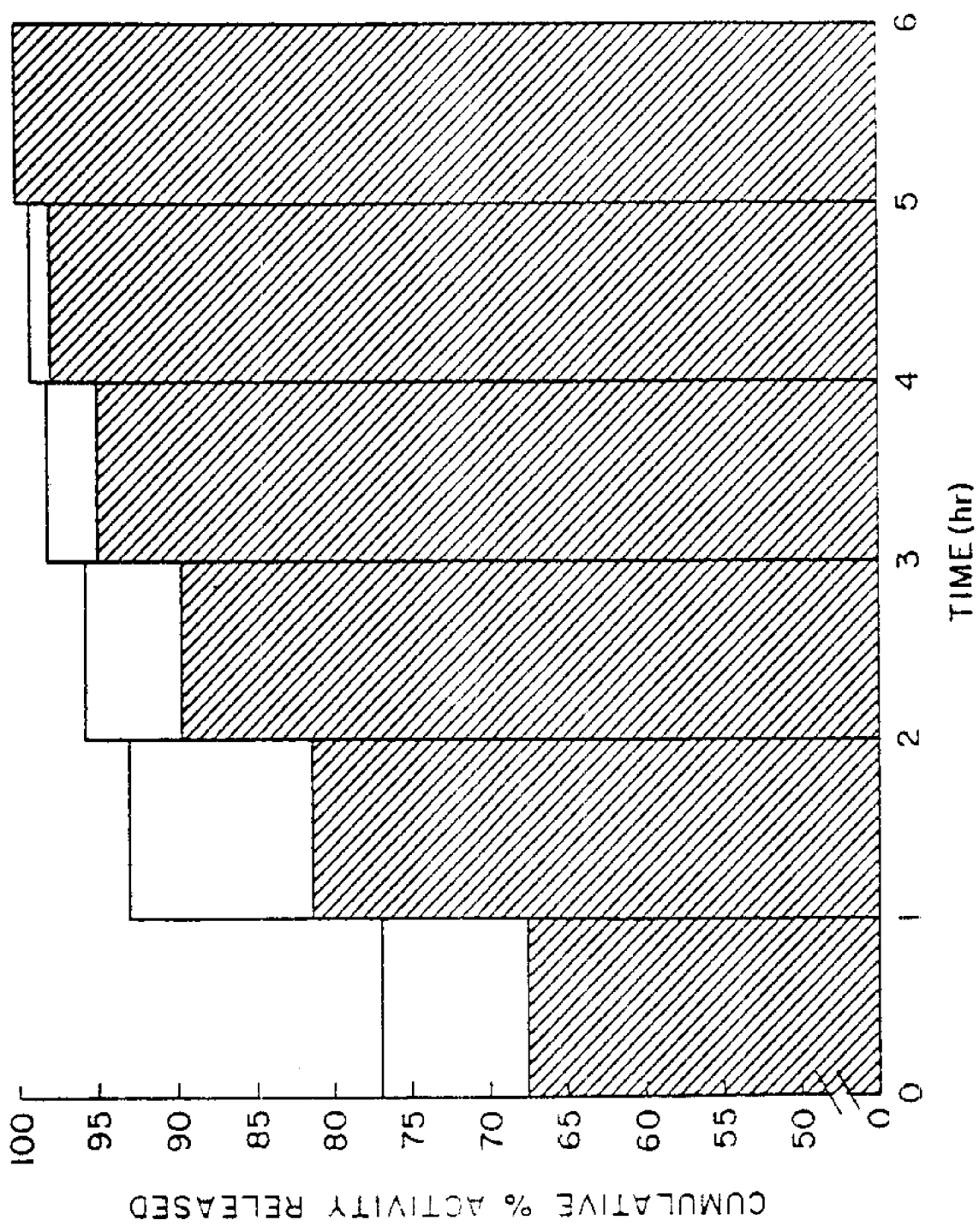
Table 1. 2-way table analyzing the effects of EDTA in backwashes and
incubation time on %-release of 109Cd from eelgrass root-rhizomes.
Data were arcsine transformed.

Source of Variation	df	SS	MS	F_s
EDTA presence	1	0.4412	0.4412	48.208 ***
Incubation length	6	1.5387	0.2564	28.016 ***
Interaction	6	0.0274	4.569×10^{-3}	<1 NS
Error	26	0.2563	9.152×10^{-3}	
Total	41	2.2636		

*** p < .001
NS p > .01

total loss significantly decreases (P < .001) with increased
incubation time. This pattern holds true for both seawater and
seawater plus EDTA backwashes, and may be observed for both excised
and whole-plant incubations.

The third factor affecting loss of cadmium is type of tissue,
and how each was incubated. Excised root-rhizomes experienced a
94.1% (incubated 1 hr) and 37.5% (incubated 24 hr) total loss in
EDTA backwashes. On the other hand, root-rhizome tissues incubated
attached to leaves in chambers lost only 71.9 and 9.9%, respec-
tively. Root-rhizomes of whole plants appear to have a greater
affinity for cadmium than excised tissues. In contrast, excised
leaves incubated in beakers released 72.8 and 11.7% while leaves
from chamber plants released 99.9 and 75.0% of the cadmium. It
should be noted that leaves from chamber plants were not directly
exposed to isotope material, and could only have acquired cadmium
by translocation from the root-rhizome complex. Nonetheless, it
is evident that leaves obtaining cadmium via the latter route
have a lower affinity for cadmium than those exposed directly.

Fitting curves to backwash data

Figure 4 illustrates the curvilinear nature of metal loss
from eelgrass tissues, in this case root-rhizomes incubated with
^{109}Cd in beakers. With the aid of the histogram, it can be seen
that the initial loss rate is high. The rate of loss decreases
with time, approaching a constant value. Replicate values of

Figure 4. Curvilinear loss rate of ^{109}Cd-incubated excised root-rhizomes (13hr) backwashed with 1 x 10^{-4} M EDTA in seawater. Each backwash interval is represented by six plant samples. Histogram shows time course of % loss of total lost by tissue during 6 hr.

% of total DPM released by plant

TIME (min)

DPM released · mg⁻¹ · min⁻¹
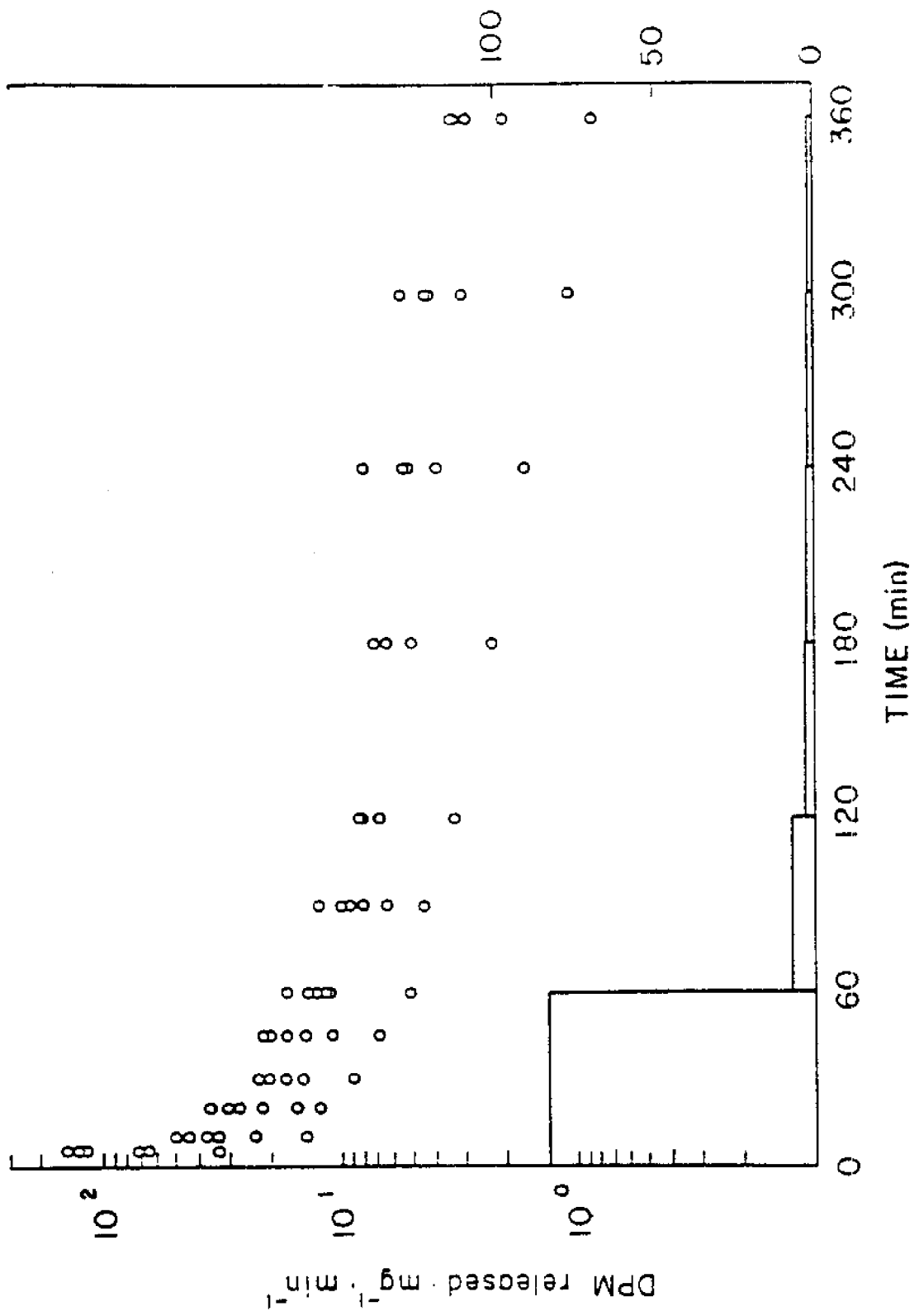
y (DPM released $.mg^{-1} .min^{-1}$) for x's equal to 180, 240, 300, and 360 min were regressed by least-squares technique, resulting in an equation $y = 9.86 - 0.019x$. Regression ANOVA analysis indicated that the slope significantly differs from zero ($P < .01$). Therefore, the observed loss rate after 180 min continues to decrease, albeit slowly. A similar pattern can be observed for manganese treated plants (Figure 5), however, the resulting linear regression for the last four points ($y = 1.86 - 0.002x$) had a slope not significantly different from zero ($P > .01$). This indicates that manganese loss rates after 180 min are constant and low. Further testing showed that the slopes for manganese and cadmium were significantly different ($P < .01$).

A number of curvilinear functions were fit to the backwash data. Power curve ($y = ax^b$) fits consistently yielded the highest coefficients of determination (r^2). Accordingly, power curves were fit to each of the backwash data sets. An example of such an extrinsic fit to root-rhizome data from whole plants incubated with cadmium for 8 hr is shown in Fig. 6. The goodness-of-fit for this example (r^2) is 0.95, ie., 95% of the variation from the mean is explained by the curve. Typically, r^2 values ranged from 0.89 to 0.99, with few exceptions.

Power curve plots of root-rhizome data from intact plants incubated for 2, 8, and 24 hr are shown in Fig. 7. Changes in metal loss as a function of incubation time are more easily dis-

Figure 5. Curvilinear loss rate of ^{54}Mn-incubated excised root-rhizomes (13 hr) backwashed with 1 x 10^{-4} M EDTA in seawater. Each backwash interval is represented by six plant samples. Histogram shows time course of % loss of total lost by tissue during 6 hr.
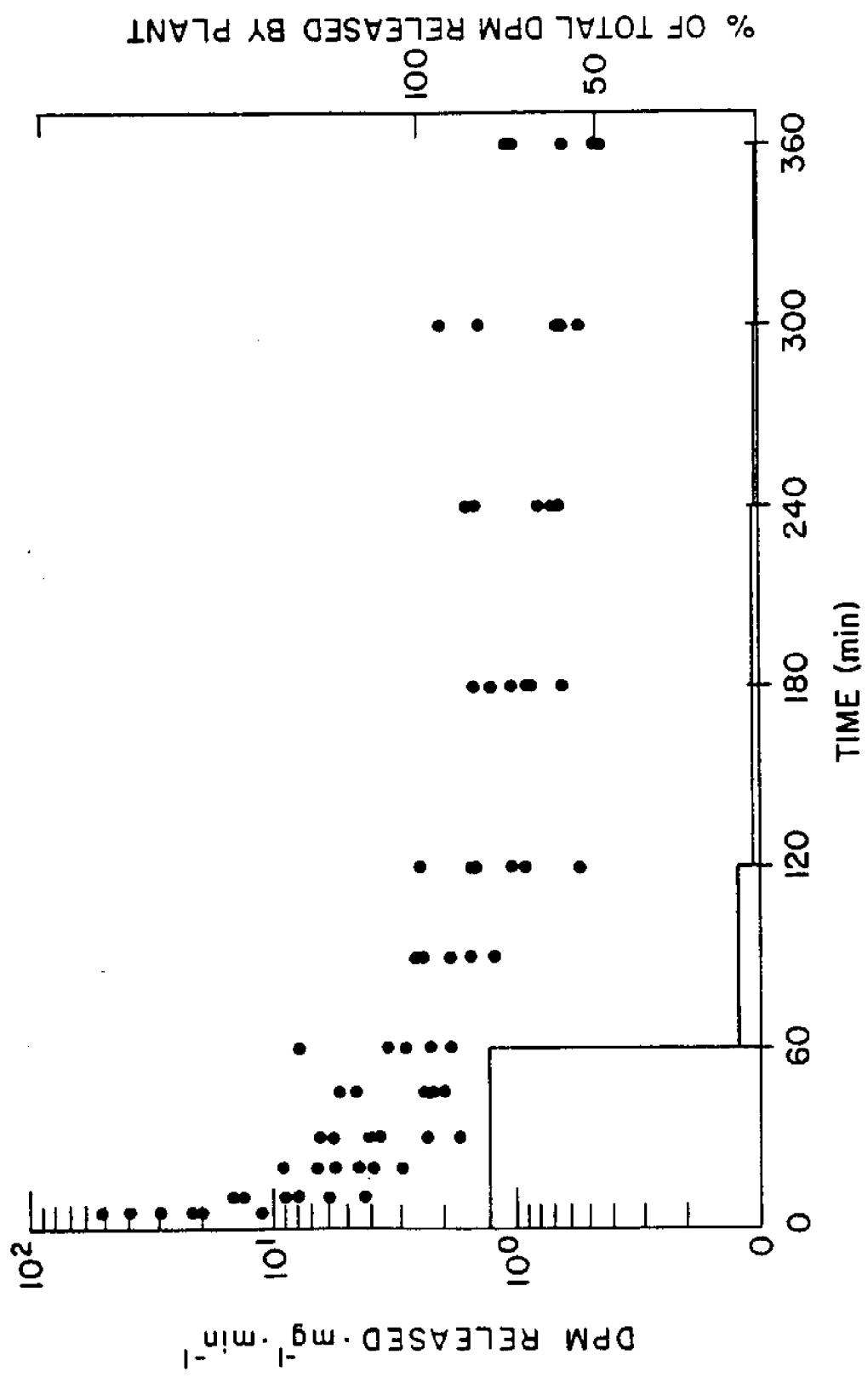
Figure 6. Power curve $(y = ax^b)$ fits to backwash data
(seawater plus 1 x 10^{-4} M EDTA) from whole-plant root-
rhizomes incubated with ^{109}Cd for 8 hr in chambers. Each
backwash interval is represented by three plant samples.
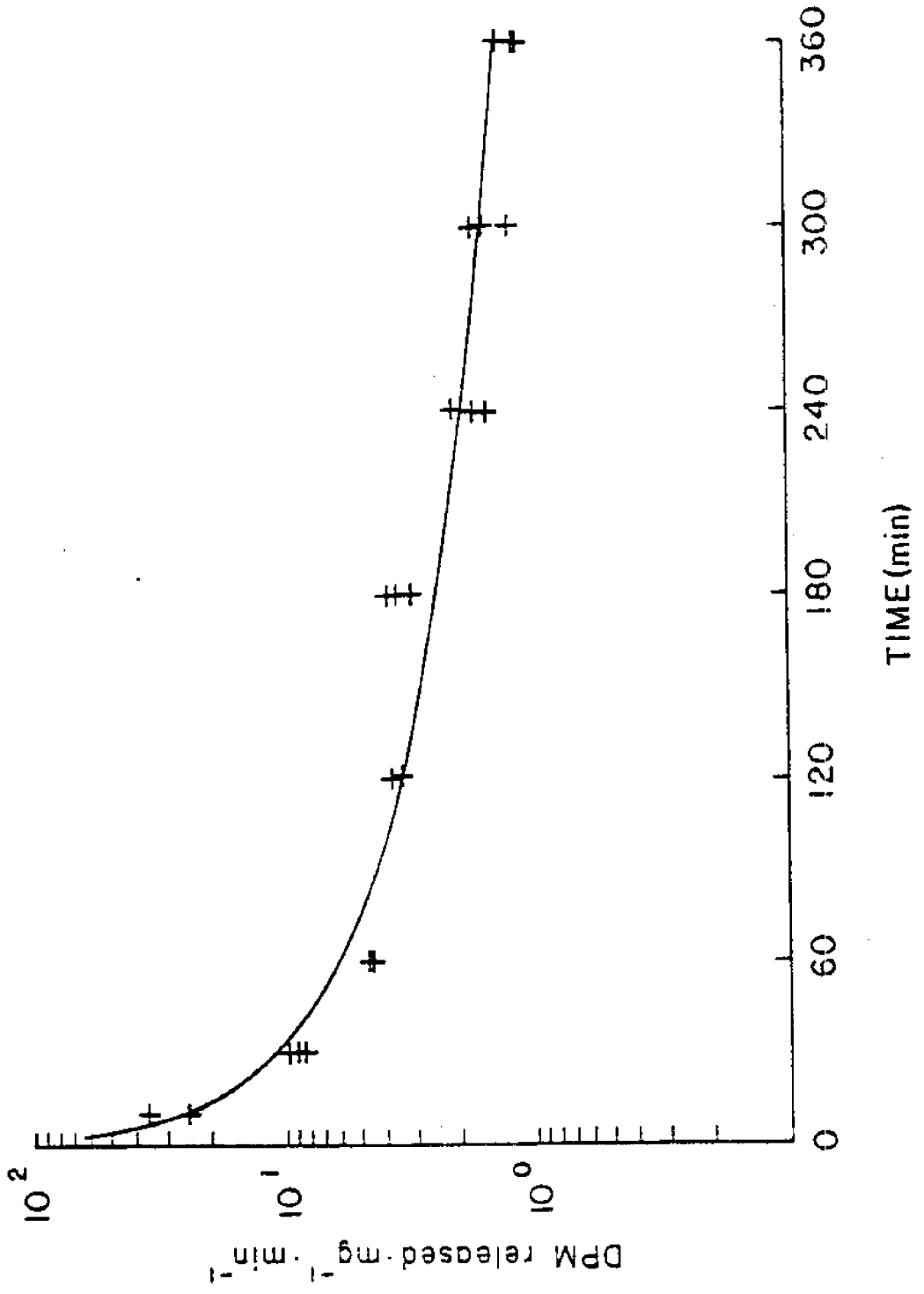
Figure 7. Power curve $(y = ax^b)$ fits to backwash data (seawater plus 1×10^{-4} M EDTA) from whole-plant root-rhizomes as a function of incubation time. (2, 8 and 24 hr) Backwash samples were obtained at 10, 30, 60, 120, 180, 240, 300 and 360 min from triplicate plant samples.

TIME (min)

DPM released · mg⁻¹ · min⁻¹
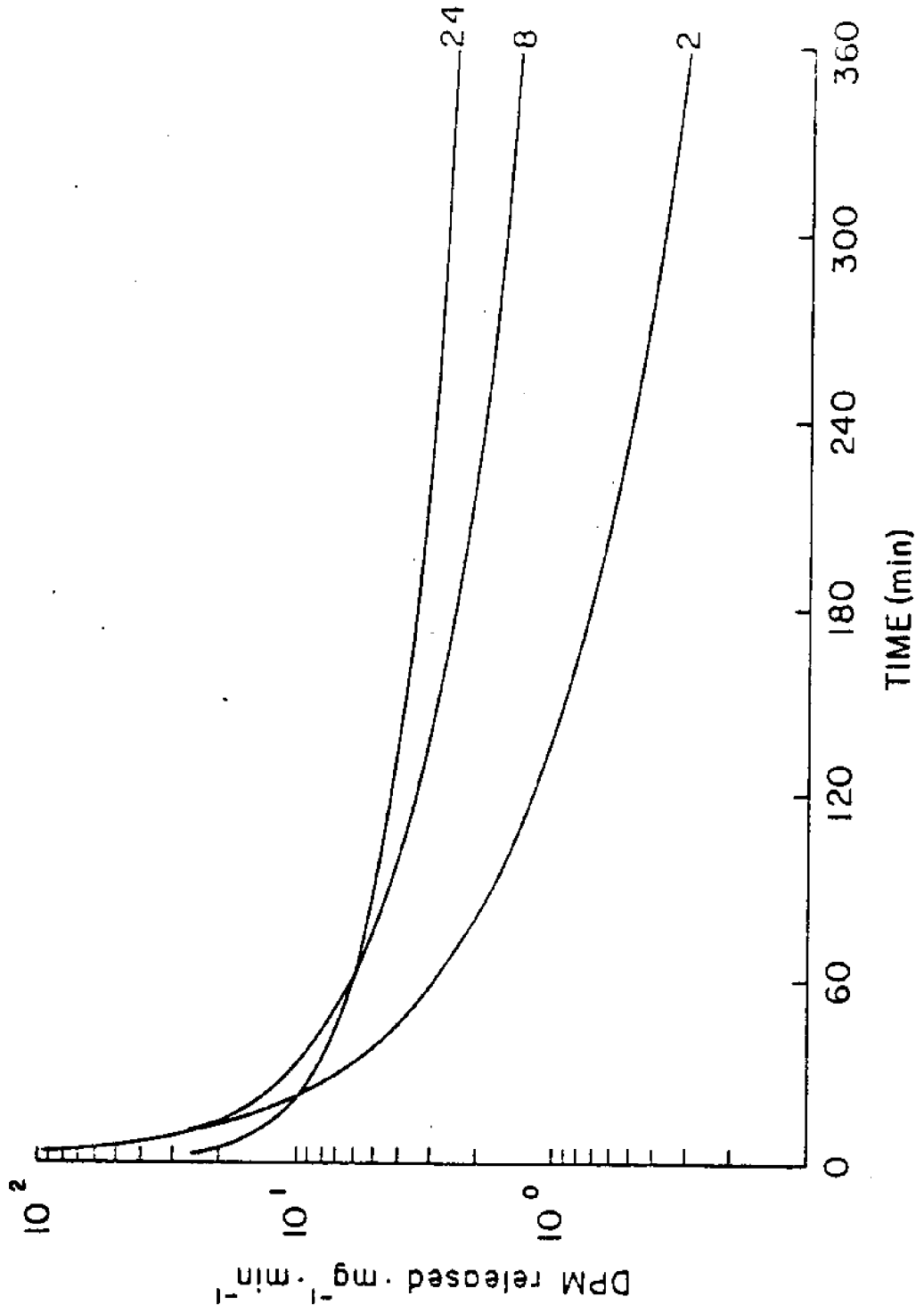
cerned with this type of plot. Power curve constants were useful in exposing trends in plant responses to varying incubation times. Table 2 indicates the relationship of coefficient a and exponent b to incubation time. Note that b increases and a decreases with increased incubation time.

Linear regressions of b with incubation time for excised root-rhizomes exposed to cadmium and backwashed in seawater and seawater plus EDTA, and whole-plant root-rhizomes backwashed only with seawater plus EDTA, are plotted in Fig. 8a. Several points of interest can be noted. First, for any observed incubation time, the values of b were greater for excised tissues backwashed without EDTA than for those backwashed with EDTA. Second, b values were always greater for whole-plant root-rhizomes than for excised ones. Next, the rates for increase of b (slopes) were significantly greater ($P < .01$) for root-rhizomes backwashed with EDTA than the rate for tissues not backwashed with EDTA. Finally, the rate of increase of b for intact root-rhizomes backwashed with EDTA is equal to the rate for excised tissue backwashed with EDTA ($P > .01$).

Linear regressions of ln a with incubation time for root-rhizomes exposed to cadmium are shown in Fig. 8b. Coefficient a values are inversely proportional to exponential b values. For any given incubation time, a was always smallest for intact root-rhizomes, and less for excised tissue backwashed without EDTA

Table 2. Relationships of power curve coefficient a and exponent b to cadmium incubation time in eelgrass root-rhizomes from whole plants backwashed with EDTA

Incubation time (hr)	a	b
2	462	-1.24
4	161	-0.86
8	182	-0.83
16	89	-0.77
24	47	-0.50

Figure 8. Linear regression of exponent $\underline{b}$ (a) and natural
log of coefficient $\underline{a}$ (b) from power curve fits for A) excised
root-rhizomes backwashed without EDTA, and B) whole-plant
root-rhizomes and C) excised root-rhizomes backwashed with
1×10^{-4} M EDTA, as a function of incubation time with ^{109}Cd.
Goodness of fit (r^2) in (a) are 0.77, 0.80 and 0.82, and in
(b) 0.87, 0.92 and 0.78 for A, B, and C, respectively.

(a)

(b)

INCUBATION TIME (hr)
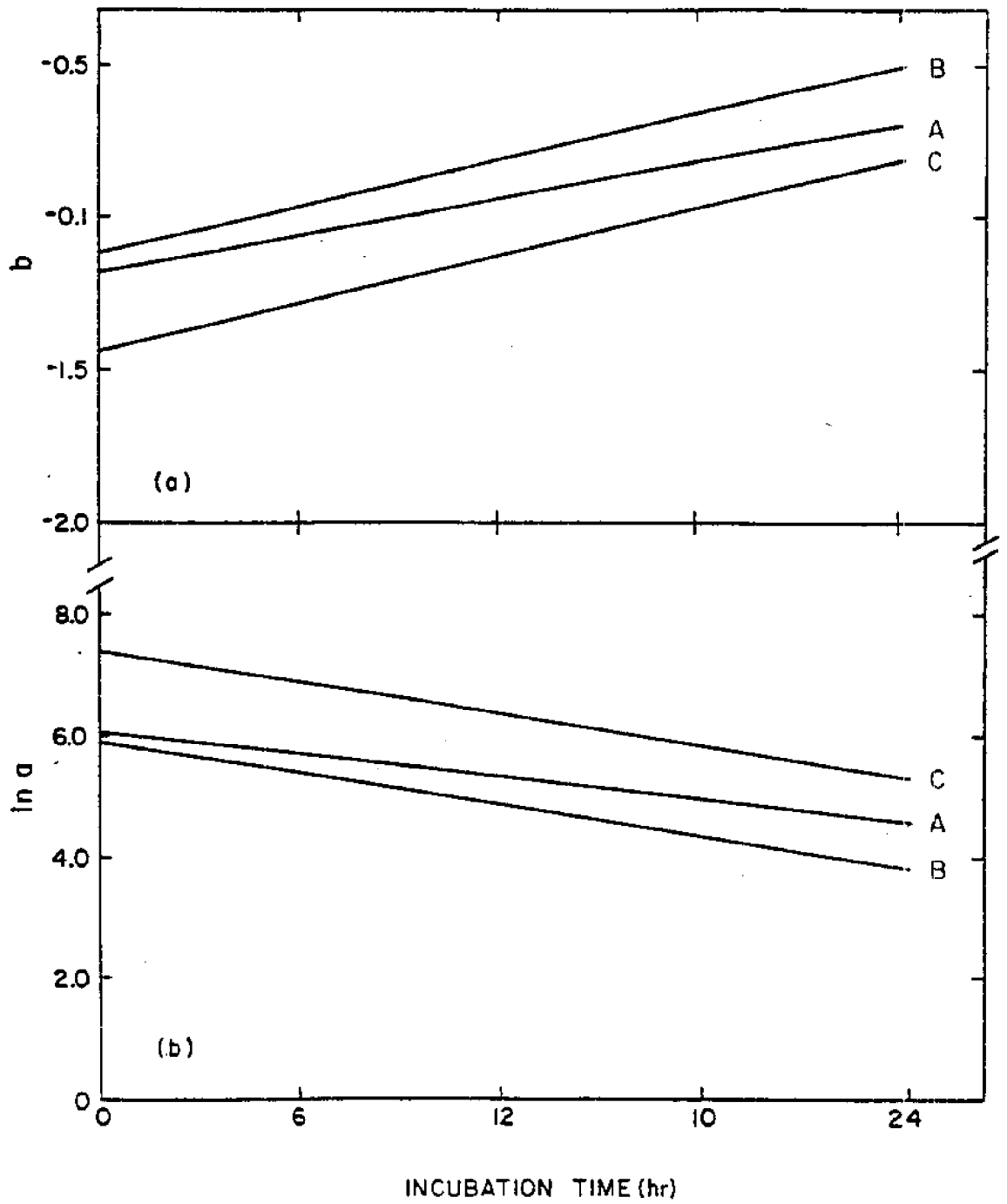
than for backwashed with EDTA. Both intact and excised tissue
backwashed with EDTA exhibited equal rates of decrease in $\underline{a}$.
This rate of decrease was greater than that observed with tissues
not backwashed with EDTA.

Power curve fits to manganese backwash data resulted in r^2
values similar to those for cadmium. The values of $\underline{a}$ and $\underline{b}$
resulting from fits to manganese and cadmium treated excised
root-rhizomes were compared by a t-test of means (Sokal and Rohlf,
1969) for 13.5 hr incubations. Values of $\underline{b}$ did not differ sig-
nificantly (P > .01), while $\underline{a}$ for cadmium treated plants was sig-
nificantly greater (P < .01). Initial rates of release during
backwashing ($DPM.mg^{-1}.min^{-1}$ during the first hr) were similarly
compared, showing that loss rates for cadmium were greater than
for manganese (P < .01). Sufficient backwash data were not ob-
tained to allow statistical comparison with leaves indirectly
exposed to manganese and cadmium, however, the data suggest that
there was no difference in leaf-loss of these metals.

Excised tissue uptake

In one experiment excised tissues consisting of living and
dead root-rhizomes and living leaves were incubated in beakers
for up to 98 hr with ^{54}Mn, and not backwashed. The data for each
tissue-type was fit by least squares linear regression of metal
uptake against incubation time (Sokal and Rohlf, 1969). Table 3
shows the regression equations, coefficients of determination

Table 3. Regression statistics and concentration ratios (CR) of manganese uptake by excised living root-rhizomes (LRR), dead root-rhizomes (DRR) and living leaves (LL) with incubation time. Note: these tissues were not backwashed; incubation concentration = 1.67 X 10^{-12} mg/ml.

TYPE	REGRESSION LINE	"r²"	MEAN CONCENTRATION AT 98 HR (mg.g dry wt^{-1})	CR (98 hr)
LL	lny = -17.24 + 0.74lnx	.80	6.67 X 10^{-7}	4.0 X 10^5
LRR	lny = -17.27 + 0.67lnx	.83	1.00 X 10^{-6}	6.0 X 10^5
DRR	lny = -16.92 + 0.53lnx	.74	4.14 X 10^{-7}	2.5 X 10^5

(r^2), and mean manganese concentrations (mg.g dry wt^{-1}) and concentration ratio (CR). The CR was obtained by dividing the metal concentration in plant tissues (mg.g dry wt^{-1}) by the concentration in the incubation solution (mg.ml^{-1}). Note the rapid uptake rate (slope) of manganese in these unbackwashed tissues. The slopes of the regression lines were tested for equality and not found to be significantly different (P > .05). Note also that the data were $\underline{\ln}$ transformed before testing significance. A non-parametric comparison of mean metal concentrations at 98 hr (Sokal and Rohlf, 1969) indicated that all three tissue types were similar (P > .05) in manganese content. The three CR's were high (2.5 X 10^5 to 6.0 X 10^5), demonstrating an apparently great affinity for manganese. No differences in CR's could be statistically attributed to life or death.

Two groups of excised root-rhizomes were incubated in beakers with ^{109}Cd for up to 24 hr. One group was backwashed with EDTA, while the second group was not. The mean CR's for cadmium after 24 hr were 6.2 X 10^3 and 9.9 X 10^3 for tissues backwashed with and without EDTA, respectively.

All of the plant data presented hereafter are for tissues backwashed exclusively with EDTA.

Uptake of ^{54}Mn by excised root-rhizomes and leaves over various periods of time up to 8 hr is compared in Table 4. Uptake rates (slopes of regression lines) for leaves were signifi-

Table 4. Linear regressions of ^{54}Mn uptake by excised root-rhizomes and leaves as a function of incubation time. Note: "r2" = coefficient of determination; CR = concentration ratio; manganese concentration in incubation solution = 2.3 X 10-12 mg/ml.

TISSUE	SAMPLE NO.	EQUATION	r^2	CR (8 hr)
Leaves	5	$y = 2.62 \times 10^{-9} + 3.45 \times 10^{-9}(x)$	.99	2.0×10^4
Root-rhizomes	5	$y = 1.73 \times 10^{-9} + 2.78 \times 10^{-9}(x)$	.90	1.0×10^4

cantly greater (P < .01) than for root-rhizomes. The mean CR's for leaves and root-rhizomes after 8 hr were 2.0×10^{-4} and 1.0×10^4, respectively. These CR values for backwashed manganese treated tissues cannot be compared to cadmium backwashed tissues because of large incubation time differences.

Concentration ratios for ^{54}Mn and ^{109}Cd treated root-rhizomes were compared at 13.5 hr of incubation (Table 5) and were not found to be significantly different (P > .01) in a t-test of means (Sokal and Rohlf, 1969). This indicates that the proportion of uptake of these two isotopes into root-rhizomes was equal at 13.5 hr.

Uptake by whole-plants from seawater

Uptake and translocation of manganese and cadmium from root-rhizomes to leaves was examined in three chamber experiments. Uptake as a function of time for root-rhizomes exposed to ^{109}Cd is shown in Figure 9. Translocation to leaves of these same plants is shown in Figure 10. Uptake into root-rhizomes increased during the first 24 hr and appeared to saturate thereafter. Translocation of cadmium to leaves increased for at least the first 48 hr of incubation and appeared to continue thereafter at a reduced rate. The CR's for root-rhizomes and leaves after 93 hr were 4.1×10^5 and 2.0×10^3, respectively.

Manganese uptake by whole plants was characteristically variable, especially during short incubation periods. Uptake

Table 5. Manganese and cadmium concentration ratios after 13.5 hr of incubation of excised root-rhizomes

METAL	SAMPLE NO.	INCUBATION CONCENTRATION ($mg \cdot ml^{-1}$)	MEAN TISSUE CONCENTRATION ($mg \cdot g\ dry\ wt^{-1}$)	CR
Mn	6	3.22×10^{-9}	3.28×10^{-7}	1.0×10^{5}
Cd	6	3.83×10^{-6}	8.60×10^{-4}	2.3×10^{5}

Figure 9. Cadmium uptake by eelgrass root-rhizomes with time in three chamber experiments. Cadmium concentrations in root-rhizome compartment water were: 3.4×10^{-9} mg.ml^{-1} for + and X; 2.7×10^{-9} mg.ml^{-1} for *. Bold symbols indicate two or more points. Each sampling time is represented by three plants.

Figure 10. Cadmium in eelgrass leaves translocated from root-rhizomes with time in the same three experiments as in Figure 9. Bold symbols indicate two points. Symbols correspond to the same plants as in Figure 9.
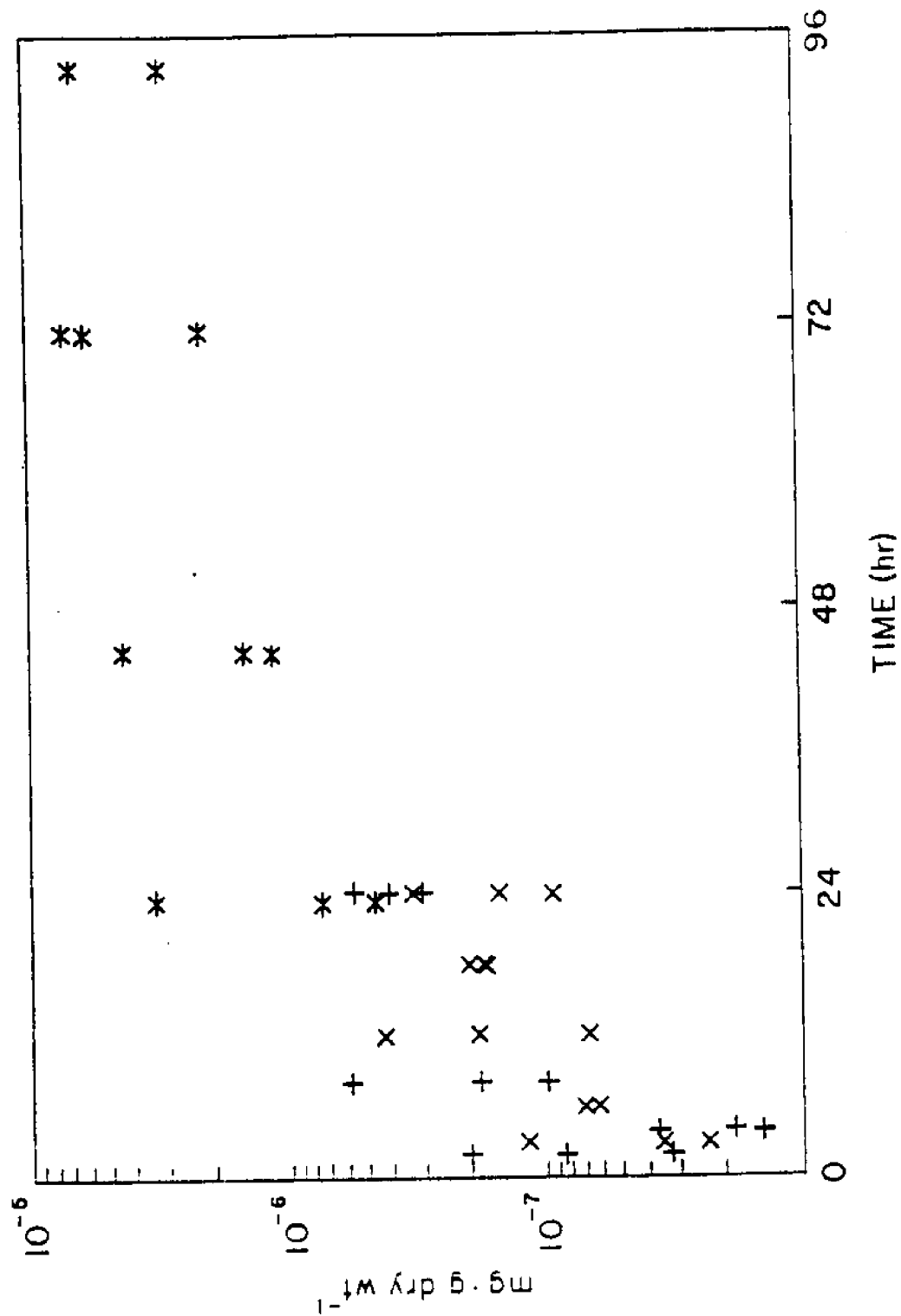
by root-rhizomes from two experiments is shown in Figure 11.
Manganese concentrations appear to be initially high, followed
by a gradual decrease with increased incubation time. This
apparent decrease is accompanied by an increase in leaf manganese
content (Figure 12). Unlike cadmium content in leaves as a
function of time, the rate of manganese translocation to leaves
does not appear to be attenuated during 90 hr incubations.
Perhaps the most interesting difference between eelgrass tissue
uptake of cadmium and manganese is that the concentration ratio
for manganese in leaves (CR = 8.6 X 10^4) was greater than in
root-rhizomes (CR = 4.6 X 10^3). This is opposite to the pattern
for cadmium.

Uptake under the influence of salt gradients

Root-rhizomes of intact plants in chambers were incubated
for about 21 hr with cadmium and manganese, and the top chamber
seawater concentration was diluted such that a potential upward
salt flux existed. This experiment was designed to test whether
an upward salt flux enhanced upward (acropetal) transport of
metal. Cadmium acropetal transport (Table 6) appears enhanced
by a salt gradient, as noted by increased CR's. Even though
isotope was added to the root-rhizomes, leaves had somewhat
higher concentrations than root-rhizomes. Small quantities of
cadmium were apparently released to the water bathing the leaves.

Figure 11. Manganese uptake in eelgrass root-rhizomes with time in two chamber experiments. Manganese concentration in root-rhizome compartment water was 1.4 X 10^{-12} mg.ml^{-1} for both + and X. Bold symbols indicate two points.
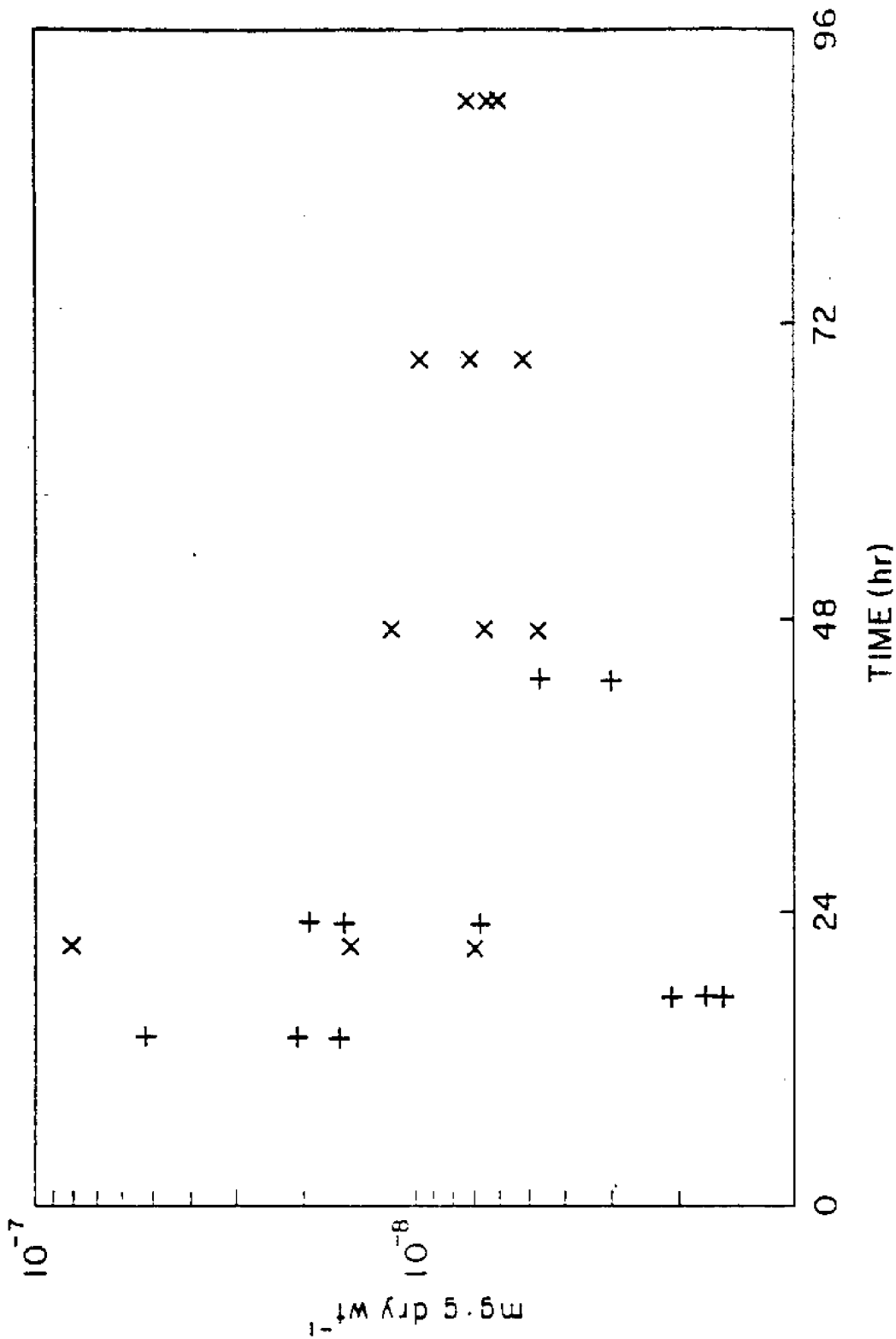
Figure 12. Manganese in eelgrass leaves translocated from root-rhizomes with time in the same two experiments as in Figure 11. Bold symbols indicate two points. Symbols correspond to the same plants as in Figure 11.
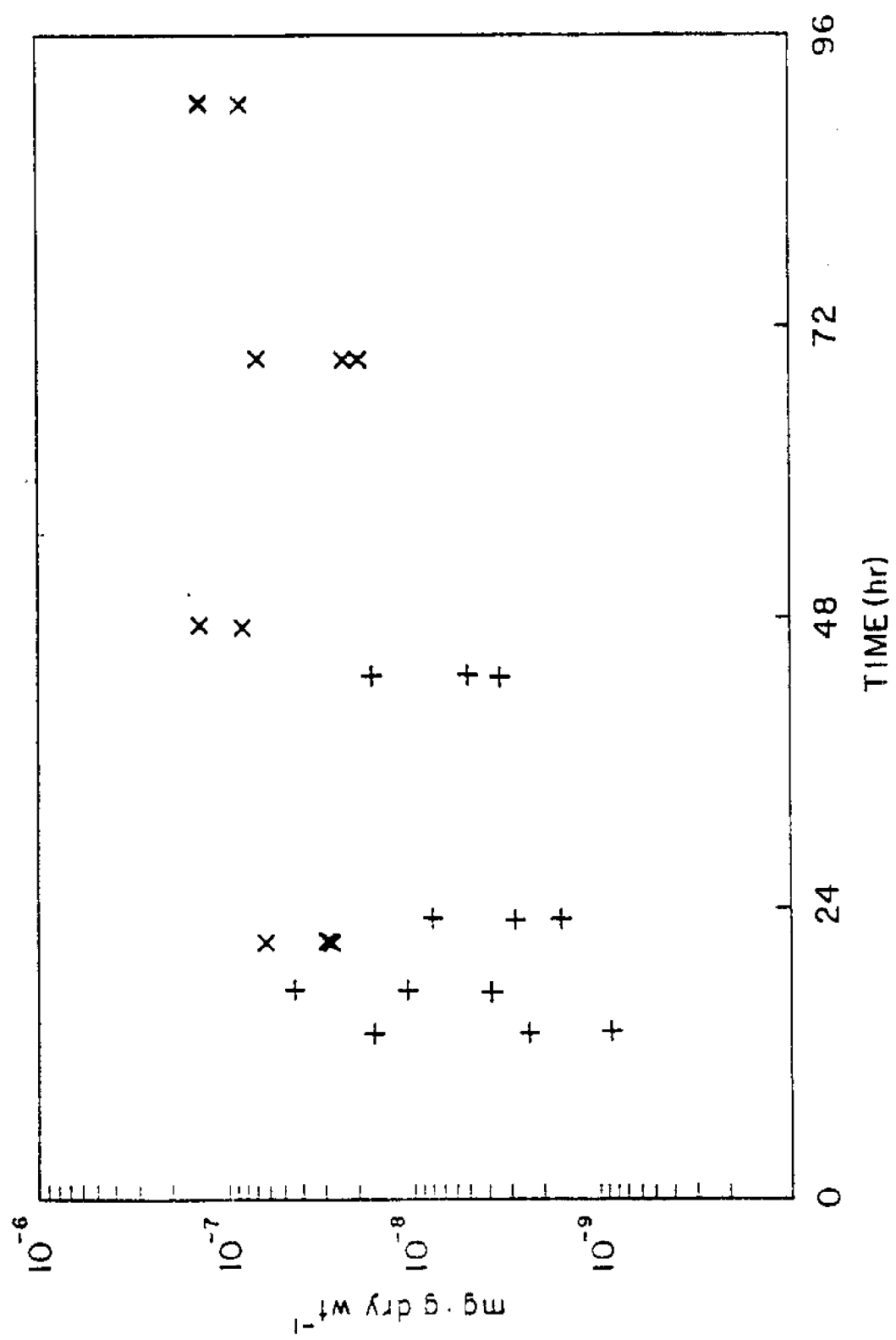
Table 6. Means of whole-plant uptake and upward transport of ^{109}Cd and ^{54}Mn of eelgrass in chambers with an upward salt gradient. Three plants were incubated with each metal at each salinity gradient for 21 hr. Solute concentrations of ^{109}Cd and ^{54}Mn in the root-rhizome compartments were 2.7 X 10^{-9} and 1.4 X 10^{-12} mg.ml^{-1}, respectively. Release of metal by leaves (leakage) and concentration ratios (CR) are given. Note: ND = not detected above background activity.

SALINITY (o/oo) RATIO (TOP : BOTTOM)	LEAKAGE (mg.ml^{-1})	LEAVES (mg.g^{-1})	CR	ROOT-RHIZOMES (mg.g^{-1})	CR
Cd					
27:27	2.1 X 10^{-10}	3.9 X 10^{-4}	1.4 X 10^{5}	1.1 X 10^{-4}	4.2 X 10^{4}
20:27	7.2 X 10^{-10}	8.7 X 10^{-4}	3.2 X 10^{5}	2.6 X 10^{-4}	9.8 X 10^{4}
14:27	6.1 X 10^{-10}	6.2 X 10^{-4}	2.3 X 10^{5}	2.2 X 10^{-4}	8.2 X 10^{4}
Mn					
27:27	ND	2.1 X 10^{-10}	1.5 X 10^{2}	5.8 X 10^{-8}	4.0 X 10^{4}
20:27	ND	8.6 X 10^{-11}	6.1 X 10^{1}	8.7 X 10^{-8}	6.1 X 10^{4}
14:27	ND	2.2 X 10^{-10}	1.6 X 10^{2}	8.6 X 10^{-8}	6.0 X 10^{4}

Very little manganese was transported from root-rhizomes to leaves, and there was no salt gradient effect on transport.

Acropetal transport potential of cadmium and manganese under the influence of a downward salt gradient was also examined (Table 7). Cadmium acropetal transport against a salt gradient is considerably less than with a gradient (see Table 6), as evidenced by differences in CR's. No appreciable quantities of manganese were transported to leaves, and less manganese was incorporated into root-rhizomes than in the previous experiment. No other trends could be attributed statistically to a downward salt gradient.

Transport of cadmium and manganese from leaves to root-rhizomes (basipetal) under the influence of salt gradients was also examined. The data in Table 8 indicate the extent of metal uptake and transport against an upward salt gradient. Both metals were accumulated by leaves, however, the concentration ratios (CR) for manganese were several orders of magnitude greater than those for cadmium. The concentration of manganese in leaves is apparently equal to cadmium concentrations, despite the difference in incubation solute concentrations. Basipetal transport of both metals was observed, but transport of manganese was greater than that of cadmium, as evidenced by the CR's in root-rhizomes (relative to the concentration of metal in the

Table 7. Means of whole-plant uptake and upward transport of ^{109}Cd and ^{54}Mn in eelgrass in chambers with a downward salt gradient. Three plants were incubated with each metal at each salinity gradient for about 21 hr. Solute concentrations of ^{109}Cd and ^{54}Mn in the root-rhizome compartments were 2.7 X 10^{-9} and 1.4 X 10^{-12} mg.ml^{-1}, respectively. Release of metal by leaves (leakage) and concentration ratios (CR) are given. Note: ND = not detected above background activity.

| SALINITY (o/oo) RATIO (TOP : BOTTOM)	LEAKAGE (mg.ml^{-1})	LEAVES (mg.g^{-1})	CR	ROOT-RHIZOMES (mg.g^{-1})	CR
Cd					
19:19	1.2 X 10^{-8}	3.2 X 10^{-6}	1.2 X 10^3	1.6 X 10^{-3}	5.9 X 10^5
19:15	1.6 X 10^{-8}	2.6 X 10^{-6}	1.0 X 10^3	6.6 X 10^{-4}	2.4 X 10^5
19:10	8.5 X 10^{-9}	1.3 X 10^{-6}	4.8 X 10^2	8.9 X 10^{-4}	3.3 X 10^5
Mn					
19:19	ND	1.3 X 10^{-11}	0.9 X 10^1	5.2 X 10^{-9}	3.7 X 10^3
19:15	ND	6.9 X 10^{-11}	4.9 X 10^1	9.2 X 10^{-9}	6.6 X 10^3
19:10	ND	3.7 X 10^{-11}	2.6 X 10^1	9.3 X 10^{-9}	6.6 X 10^3

Table 8. Means of whole-plant uptake and downward transport of ^{109}Cd and ^{54}Mn in eelgrass in chambers with an upward salt gradient. Three plants were incubated with each metal at each salinity gradient for about 32 hr. Solute concentrations of ^{109}Cd and ^{54}Mn in the leaf compartments were 1×10^{-9} and 4.3×10^{-12} mg.ml^{-1}, respectively. Release of metals by root-rhizomes (leakage) and concentration ratios (CR) are given. Note: ND = not detected above background activity.

	SALINITY (o/oo) RATIO (TOP : BOTTOM)	LEAKAGE (mg.ml^{-1})	LEAVES (mg.g^{-1})	CR	ROOT-RHIZOMES (mg.g^{-1})	CR
Cd	25:25	1.6×10^{-7}	2.6×10^{-7}	2.6×10^{2}	2.8×10^{-8}	2.8×10^{1}
	19:25	3.8×10^{-8}	2.7×10^{-7}	2.7×10^{2}	4.8×10^{-8}	4.8×10^{1}
	13:25	1.7×10^{-8}	8.6×10^{-8}	8.6×10^{1}	2.6×10^{-9}	0.3×10^{1}
Mn	25:25	ND	10.0×10^{-7}	2.3×10^{6}	8.9×10^{-11}	2.1×10^{2}
	19:25	ND	7.2×10^{-7}	1.7×10^{6}	3.0×10^{-10}	7.0×10^{2}
	13:25	ND	6.4×10^{-7}	1.5×10^{6}	4.1×10^{-10}	9.5×10^{2}

water of the leaf compartment). The decreasing CR's of manganese with increased upward salt gradients were not significant. Cadmium is apparently also released from the root-rhizome complex, but manganese is not.

In the final salt gradient experiment, basipetal transport with a downward salt gradient was examined (Table 9). Cadmium concentration ratios in leaves (CR = 9.4 X 10^3 to 1.2 X 10^4) were considerably lower than in root-rhizomes (CR = 5.8 X 10^4 to 1.8 X 10^5). The increase in CR in root-rhizomes with increased salt-gradients is statistically significant (P > .01) indicating enhanced basipetal transport with greater salt gradients. Cadmium was also detected in root-rhizome water, indicating release (leakage) by these tissues. In fact, the concentration of cadmium in this bottom chamber water was equal to the concentration of the initial leaf chamber incubation water. Manganese incorporated into leaves was not transported downward in any quantity. None appeared in the water bathing root-rhizomes.

Uptake by whole-plants from sediments

Uptake and acropetal transport of cadmium and manganese from sediments was examined in four experiments. Three experiments with ^{109}Cd inoculated sediments (Table 10) indicate that upward translocation of this metal occurred. In Experiment 1, plants were incubated under constant light for 3 da. Leaves

Table 9. Means of whole-plant uptake and downward transport of ^{109}Cd and ^{54}Mn in eelgrass in chambers with a downward salt gradient. Three plants were incubated with each metal at each salinity gradient for 27 (Cd) and 37 (Mn) hr. Solute concentrations of ^{109}Cd and ^{54}Mn in leaf compartments were 1×10^{-9} and 2.2×10^{-3} $mg \cdot ml^{-1}$, respectively. Release of metals by root-rhizomes (leakage) and concentration ratios (CR) are given. Note: ND = not detected above background activity.

	SALINITY (o/oo) RATIO (TOP : BOTTOM)	LEAKAGE (mg·ml⁻¹)	LEAVES (mg·g⁻¹)	CR	ROOT-RHIZOMES (mg·g⁻¹)	CR
Cd	25:25	1.9×10^{-7}	1.1×10^{-5}	1.1×10^{4}	5.8×10^{-5}	5.8×10^{4}
	25:19	1.0×10^{-6}	9.4×10^{-6}	9.0×10^{3}	1.1×10^{-4}	1.1×10^{5}
	25:13	1.0×10^{-6}	1.2×10^{-5}	1.2×10^{4}	1.8×10^{-4}	1.8×10^{5}
Mn	25:25	ND	5.2×10^{-7}	2.4×10^{6}	8.2×10^{-11}	3.7×10^{2}
	25:19	ND	3.6×10^{-7}	1.7×10^{6}	2.1×10^{-11}	9.5×10^{1}
	25:13	ND	4.2×10^{-7}	1.9×10^{6}	4.7×10^{-12}	2.1×10^{1}

Table 10. Cadmium uptake in eelgrass from sediments in chambers. Root-rhizomes of whole-plants were exposed to ^{109}Cd enriched sediments for 3 days in Experiment 1 and 4 days in Experiment 2 and 3. Experiments 1 and 2 were conducted in constant light, while Experiment 3 was in constant darkness. Concentration of cadmium in Experiment 1 = 3.04 X 10^{-7} mg.g wet sediment^{-1}, while that in Experiments 2 and 3 = 2.53 X 10^{-7} mg.g wet sediment^{-1}. Note: CR = concentration ratio.

	TISSUE TYPE	SAMPLE NO.	TISSUE CONCENTRATION (mg.g^{-1})	% OF TOTAL IN PLANT	CR
Exp. 1	Leaves	9	3.0 X 10^{-7}	9.4	1.0
	Roots	9	9.1 X 10^{-6}	83.1	30.0
	Rhizomes	9	3.6 X 10^{-7}	7.4	1.2
	Root-rhizomes	9	3.1 X 10^{-6}		10.1
Exp. 2	Leaves	6	5.1 X 10^{-7}	17.3	2.0
	Roots	6	6.7 X 10^{-6}	66.4	26.5
	Rhizomes	6	5.1 X 10^{-7}	14.2	2.0
	Root-rhizomes	6	2.0 X 10^{-6}		8.0
Exp. 3	Leaves	3	2.9 X 10^{-7}	8.6	1.1
	Roots	3	6.0 X 10^{-6}	78.7	23.7
	Rhizomes	3	4.8 X 10^{-7}	12.8	1.9
	Root-rhizomes	3	2.3 X 10^{-6}		9.2

(CR = 1.0) had cadmium concentrations similar to those in rhizome sections (CR = 1.2) (P >.05). Roots had the highest cadmium content (CR = 30.0), which represented 83.1% of the total taken up by plants from the sediments. In Experiments 2 and 3, six plants were incubated in constant light and three in total darkness for 4 da. Using a 1-way ANOVA and a SNK-aposteriori test procedure (Sokal and Rohlf, 1969), the light-incubated leaves were shown to have higher cadmium concentrations than dark-incubated leaves (P < .01). In Experiment 1 leaves and rhizomes had equal cadmium concentrations, whereas this is not the case in dark-incubated plants (P < .01).

Manganese uptake and basipetal transport was measured in a single chamber experiment (Table 11). The mean concentration of manganese in leaves, roots, and rhizomes was determined to be equal (P >.01) using a non-parametric comparison of means (Sokal and Rohlf, 1969). This is in contrast to the previous cadmium experiments, where roots had significantly higher concentrations. The largest proportion of manganese accumulated by eelgrass was associated with the leaves and rhizomes, even though all tissues had equal concentrations. This is because roots represent less off the total plant biomass. The CR's for leaves were greater for manganese treated plants than for ones treated with cadmium.

Table 11. Manganese uptake in eelgrass from sediments in chambers. Root-rhizomes of whole-plants were exposed to ^{54}Mn enriched sediments for 3 da. under constant light. Concentration of manganese in sediments was 6.4×10^{-10} mg.g wet sediment^{-1}. Note: CR = concentration ratio

TISSUE TYPE	SAMPLE NO.	TISSUE CONCENTRATION (mg.g^{-1})	% OF TOTAL IN PLANT	CR
Leaves	9	4.1×10^{-9}	47.4	6.4
Roots	9	4.2×10^{-9}	13.8	6.5
Rhizomes	9	2.2×10^{-9}	38.8	3.4

Long-term depuration of cadmium

Depuration studies were conducted with two groups of intact plants. During the backwash procedure, one group of plants to be placed in the field was accidently exposed to temperatures of 35°C for several hours due to a failure in a cooling system. These plants were planted in the field regardless, and are hereafter referred to as "stressed plants." The stressed plants suffered a high mortality in the field. No plants survived beyond Day 16 of the experiment. During the first week, most had lost all their leaves, and root-rhizomes were located by excavation of the plot. New leaf growth was not observed in any of these plants. Old leaves exhibited a loss of total cadmium activity ($P < .01$) when compared to average total cadmium activity in leaves of control plants brought back to the laboratory on Day 0 (Table 12). Cadmium activity on a weight basis ($CPM.mg^{-1}$) in the remaining leaves was significantly lower ($P < .01$) on Day 16. The losses in total cadmium can be explained by loss of leaf biomass, while loss in activity on a weight basis can only be explained by leaching of metal from decaying leaves. During the same period, the root-rhizome complex did not exhibit significant ($P > .05$) losses in either total cadmium or cadmium concentration. When rhizomes were tested separately, analysis indicated that rhizomes lost a significant amount of total cadmium and activity on a weight basis ($P < .01$). All tests for

Table 12. Depuration of ^{109}Cd from stressed eelgrass shoots 16 days after replaning in the field. Day 1 plant statistics represent initial levels. All data are means of 25 plants. Means for roots and rhizomes separately do not equal means for root-rhizomes since the latter were recalculated from original data on a per plant basis. CPM = counts per min. ^{109}Cd activity corrected for background. Total CPM equals average cadmium activity per plant tissue

TISSUE	DAY 0			DAY 16		
	AVE. DRY WT (mg)	TOTAL CPM	CPM.mg^{-1}	AVE. DRY WT	TOTAL CPM	CPM.mg^{-1}
Leaves	95.7	9456	97.1	22.3	926	41.5
Roots	17.8	88104	4949.7	20.8	69389	3336.0
Rhizomes	28.1	2738	97.4	33.3	2273	66.5
Root-rhizomes	47.2	91605	1940.8	48.3	71890	1488.4

significance were conducted with $\log_{10}$ transformed CPM data.

The second depuration experiment was conducted with healthy plants that showed evidence of growth in the field during the 32 day experiment. Approximately 40% of the single shoots planted were lost, mostly due to inundation by moving sand waves. Data in Table 13 shows the changes in cadmium activity in plant tissues. New growth of rhizome internodes (now 5.5 per shoot; average of 20 plants) and leaves was noted on Day 17. New leaf growth was proportional to new rhizome growth, ie., an average increase of 1.5 internodes per plant corresponded to an average leaf number increase of 1.5. New roots were first detected on Day 32. Leaves experienced significant (P < .001) decreases in both total leaf activity and activity on a weight basis. Most of this loss could be accounted for by loss of old leaves. No old leaves were found on Day 32. The remaining loss could partly be attributed to new growth, or a dilution effect. New leaf material also contained cadmium by Day 17, however much of this activity (on a weight basis) appeared diluted by Day 32. These new leaves must have derived cadmium from the root-rhizome complex. Both old roots and rhizomes lost significant (P < .001) total cadmium activity. Almost all of the activity lost by old rhizomes could be found in new rhizomes. Cadmium on a weight basis was equal in old and new rhizomes. Roots appear to have lost most of their activity during the first 17 days. Differences between Days 17 and 32 were not significant.

Table 13. Depuration of ^{109}Cd from healthy eelgrass shoots replanted in the field, 17 and 32 days after incubation. Day 0 plant statistics represent initial levels. All data are means of 20 plants, except for Day 32, where n = 7. Means for roots and rhizomes separately do not equal means of root-rhizomes since the latter were recalculated from original data on a per plant basis. CPM = counts per min. ^{109}Cd activity for background. Total CPM equals average cadmium activity per plant tissue

TISSUE		DAY 0			DAY 17			DAY 32		
		AVE. DRY WT. (mg)	TOTAL CPM	CPM.MG⁻¹	AVE. DRY WT. (mg)	TOTAL CPM	CPM.MG⁻¹	AVE. DRY WT. (mg)	TOTAL CPM	CPM.MG⁻¹
Leaves	(old)	137.0	111900	958.7	178.0	35031	194.0	---	---	---
	(new)	---	---	---	50.7	9071	119.9	139.5	1275	12.5
Roots	(old)	26.3	93907	4098.2	36.7	17292	641.0	37.2	16019	465.6
	(new)	---	---	---	---	---	---	17.6	233	17.7
Rhizomes	(old)	50.4	7089	176.3	52.3	4922	95.2	49.5	3664	72.2
	(new)	---	---	---	19.2	1815	99.6	17.1	745	40.9
Root-rhizomes		76.7	101543	1568.5	89.0	22440	316.3	121.4	19606	161.5

Extensive rinsing (backwashing) of labelled tissues with EDTA, as described in this study, resulted in a greater loss of metal than without EDTA inclusion. Most of this additional loss occurs during the first hour, and EDTA inclusion affected total loss throughout backwashing in a consistent fashion. Since the resulting increased loss is consistent, ie., independent of incubation time, one can conclude that whatever the source of material that EDTA is removing, it must have accumulated within one hour (the shortest incubation time observed). If this initial process is desorption, then one would expect that the concentration of EDTA used would successfully chelate material loosely bound to outside root surfaces. Desorption processes are indicated by the data in Fig. 3, where the additive effect of EDTA was exhibited only during early backwash stages. The reverse process, adsorption, must occur within the same time span. The interpretation of this initial phase of metal accumulation as being an adsorptive process is consistent with views expressed by Veltrup (1978), and others.

Stewart and Schulz-Baldes (1976) and Faraday (1978) observed decreases in plant metal concentrations during extensive backwashing. Faraday, working with $CdCl_2$ uptake in Zostera marina, attributed this loss to removal of material that had entered apparent free space within the plant through simple diffusion.

He did not use any additives in backwashes to allow differentia-
tion between diffusive and desorptive loss. Faraday also noted
a rapid rate of initial loss and that this rate was rapidly at-
tenuated as the backwash continued. Noting that there were no
further detectable changes in plant cadmium concentrations after
2 hr, Faraday concluded that backwashing for 2 hr was necessary
and sufficient to remove non-biologically accumulated metal.
The present study, however, monitored the appearance of highly
detectable isotope material in the backwash rather than detecting
cadmium with atomic absorption spectrometry analysis of plant
tissues sacrificed after each backwash interval. Our results
show that cadmium release continues beyond this point at reduced
rates, although probably by different mechanisms.

The loss of cadmium and manganese from labelled tissues was
observed to be similar during the first two hours of backwashing.
On the other hand, the rate of manganese loss after three hours
was constant, whereas it was not for cadmium. These data suggest
that the rates of cadmium and manganese loss during latter back-
wash stages are controlled by different mechansims. In fact,
very little manganese is lost after 3 hr, suggesting that the
material still in the plant is biologically fixed, and quite
rapidly at that.

Through attempts to develop insight into the response of
eelgrass tissues to backwashing, it was ascertained that a power

curve best approximated the backwash data. The power curve
equation $(y = ax^b)$ can be separated into two components:
a constant a and a power term x^b where x is backwash time in
minutes and b is a constant. The constant a is not representa-
tive of the overall rate of loss, but it does relate to the
magnitude of the predicted loss in early portions of backwashing,
ie., at $x = 1$ minute. The reason for the variation in the in-
fluence that a exerts on the curve is a result of the response of
the power term to time. Perhaps the most important concept that
should be understood about the power term is that at $x = 1$,
the power term is equal to one, regardless of the value of b.
This is the focal point of the power curve, and it indicates that
for $x = 1$, every power curve will predict that $y = a$. The
magnitude of the power term itself focuses about a point where
$b = 0$. For negative values of b, the magnitude of the power term
decreases rapidly for increasing values of x (for all $x > 0$).
Further, the more negative b becomes, the more rapidly the value
of the power term will diverge from unity.

In this study, all initial release rates were shown to be
equal, and this is further manifested by the fact that a and b
are inversely related. It was observed that b was most negative
for tissues incubated for the shortest period of time. Rather
than indicating greater rates of loss during early backwash
stages, it indicates that the rate of loss during later stages

is less than it would have been had b been less negative. A more negative b, therefore, predicts a reduced rate of loss towards the end of the backwash. It is believed that the material released after 3 hr is material that has accumulated within the plant and the magnitude of b is an indicator of the extent of uptake within the plant. Uptake here refers to material in apparent free space and living parts of cells, and, possibly cell cytoplasm. Values of b increased from -1.24 to -0.50 for intact root-rhizomes incubated for 1 to 24 hr, respectively. If b were to increase with time beyond 24 hr, and eventually become zero, the situation $y = a$ would exist throughout the backwash. The rate of loss would then be constant and due to biological processes only because rates of diffusion are negligible with respect to material lost by biological processes.

Linear regression of b with incubation time also demonstrated that the presence of EDTA in backwashes and type of incubation strongly influence observed uptake. Excised root-rhizomes have a more negative b when backwashed with EDTA. It was shown that EDTA-absent backwashes do not adequately remove adsorptive components, the result being that the additional material left in the plant would be assayed as greater uptake. Whole-plant tissues backwashed with EDTA were represented by the largest b values observed.

Values of a from power curve fits are related to the magnitude of the initial rate of loss, which in turn are dependent on

incubation metal concentrations and incubation time. Differences observed in these values from manganese and cadmium treated plants, therefore, may be attributed to differences in the concentration of metal in the incubation medium. For example, excised root-rhizome metal concentrations before backwashing, after 13.5 hr of incubation, were calculated to be 8.60×10^{-4} mg.g^{-1} for cadmium and 3.28×10^{-7} mg.g^{-1} for manganese. These differences are roughly proportional to incubation concentrations (cadmium = 3.83×10^{-9} mg.ml^{-1}; manganese = 3.27×10^{-12} mg.ml^{-1}). Faraday and Churchill (1979) have shown that cadmium uptake (after backwashing) in eelgrass is, in fact, concentration dependent.

The power curve and its constants do not, however, represent realistically the processes through which plants lose metals in backwashes (eg., desorption, diffusion from apparent free spaces, exchange absorption from cells or biologically controlled transport). The reason that these data are fit so well by a power curve may be explained through a simple model of flux. If it is assumed that the plant is semi-infinite with an initial metal concentration of C_o, and that this plant is immersed in seawater (also semi-infinite) with an initial metal concentration of $C = 0$, then the flux of metal out of the plant can be described by:

$$F = \sqrt{\frac{K}{\pi}} \; C_o \; t^{-1/2}$$

where K is a diffusion constant and τ is time of backwashing (Carslaw and Jaeger, 1959). Note that t is to some power.

The flux measured in backwashes was actually the integral of the true flux taken over some time interval (Δt), starting at $t = 0$. The measured flux can be described as:

$$F' = \frac{1}{\Delta t}\int_{t_1}^{t_1 + \Delta t} \sqrt{\frac{K}{\pi}}\ C_0\ t^{-1/2}\ dt$$

$$= C_0\ \sqrt{\frac{K}{\pi}}\ \frac{1}{\Delta t}\ \left[2t^{1/2}\right]_{t_1}^{t_2 = t_1 + \Delta t}$$

$$= 2C_0\sqrt{\frac{K}{\pi}}\ \frac{1}{\Delta t}\ \left[t_2^{1/2} - t_1^{1/2}\right]$$

Since $\Delta t = t_2 - t_1 = (t_2^{1/2} - t_1^{1/2})(t_2^{1/2} + t_1^{1/2})$,

$$F' = \frac{2C_0\ \sqrt{\frac{K}{\pi}}}{(t_2^{1/2} + t_1^{1/2})}$$

The measured flux, therefore, is proportional to C_0 and is a function of sampling time and the sampling interval. Note that the measured flux is still a function of time to some power. The geometry implicit to this model is much too simple to

accurately represent flux from much more complicated plant structures that are not semi-infinite, but it does reveal why measuring flux over discrete intervals will cause the data to fit a power curve. It is also interesting that K actually represents many process constants, ie., each of the release phases, and that the power of t would appear to vary with the assumed plant geometry (Carslow and Jaeger, 1959).

The results of the uptake experiments clearly indicate that manganese and cadmium are accumulated in Zostera marina tissues, and probably by different mechanisms. Manganese appeared to be retained by the tissues exposed to it, with very little loss after adsorptive and diffusive material from external root surfaces and internal apparent free spaces had been removed by backwashing. Manganese is effectively trapped by leaf tissues, with little transport occurring from leaves to root-rhizomes of eelgrass. Manganese might be crossing leaf cell walls and become fixed rapidly in cell cytoplasm and living parts of cells, according to the third phase of uptake described by Pickering and Puia (1969). There does appear to be a distinct acropetal mechanism, ie., transport across cytoplasm connections of cells and release into vascular tissues (see Nye and Tinker, 1977) but a similar mechanism was not observed in basipetal experiments.

Cadmium continued to be released long into the backwash and release would probably continue slowly for quite some time.

Results from uptake experiments indicate that cadmium is more
mobile than manganese. Uptake by root, rhizome, and leaf tissues
was clearly demonstrated, as was bidirectional transport between
these tissues. Faraday and Churchill (1979) found only basipetal
transport of cadmium in eelgrass, using less sensitive techniques.
It appears that acropetal transport occurs, and that the reverse,
basipetal transport, occurs at a faster rate.

Cadmium taken up by Zostera marina is probably transported
across cytoplasm cell connections or into vascular tissues.
Transport in vascular tissues has been characterized by Pickering
and Puia (1969) as a third uptake phase. They also indicate that
this phase is represented by slow processes and that these pro-
cesses are affected by environmental factors, such as light.
We found that rates of transport were greater in the presence of
light, and uptake by leaves especially was greater. There is
reason to expect that some movement could occur through apparent
free space, such as lacunae found throughout the plant. Whether
cadmium moves through vascular tissues or apparent free space by
diffusion, active transport in cytoplasm lining vascular tissues,
or by mass flow is not known. To our knowledge, no mass flow
measurements have been made on eelgrass.

Sculthorpe (1967) indicates that a transient flow of water
and dissolved salts, motivated by root or shoot pressure or by
gas-flow generated during periods of active photosynthesis,

apparently occurs in many aquatic vascular plants. Evidence indicated that a salt gradient established between chamber compartments, and presumably through eelgrass shoots, in some cases enhanced translocation. Acropetal transport of cadmium was enhanced with a positive salt gradient and decreased with a negative gradient. Manganese, as was stated earlier, was not transported upward appreciably during 24 hr experiments and a positive salt gradient had no influence. Basipetal transport of cadmium increases with a more positive salt gradient, but manganese transport was not enhanced, again reflecting the great affinity leaves have for manganese.

Experiments with uptake and acropetal transport from metal-enriched sediments indicated that although acropetal transport was present, it was so on a much smaller scale. Concentration ratios of cadmium in leaves, material which was translocated from root-rhizomes, approximately ranged from 1 to 2 in sediment experiments (with an incubation concentration of 3.04×10^{-7} mg.g wet sediment^{-1}) to 1.4×10^5 in water incubations with cadmium inocula of 2.7×10^{-9} mg.g water^{-1}. Manganese acropteal transport in both types of experiments was also similar to cadmium, ie., greater acropetal transport occurred for water incubations.

Oxygen determinations of the bottom chamber water purged with nitrogen gas indicated that some oxygen was introduced during the filling procedure. However, initial oxygen concentrations

in sealed chambers remained less than 0.1 ppm. In addition, Faraday and Churchill (1979) found that cadmium uptake by root-rhizomes was not influenced by the presence of up to 60% oxygen-saturated water. Sediments were permitted to become anaerobic prior to placement in the chambers. Undoubtedly, some oxygen was also introduced during this filling procedure, but this was probably removed rapidly by biological and chemical oxygen demand. Although both media were anaerobic, there is one substantial difference between them. Anaerobic sediment probably contained sulfides, since they were rich in organic matter. Sulfides are known to make cadmium and, to a lesser extent, manganese less bioavailable (Gambrell et al., 1976 a,b). Cadmium immobilization in anaerobic sediments may account for the observed decrease in all tissue concentrations.

Experiment-to-experiment variability in the amount of metal taken up by eelgrass tissues was noted on several occasions, especially in manganese experiments. This variability might be caused by several factors. The concentrations of cadmium [1 to 4 X 10^{-9} mg.ml^{-1}; = parts per billion (ppb)] and manganese [1 to 4 X 10^{-12} mg.ml^{-1}; = parts per trillion (ppt)] were near, or below, the detection limits of non-isotope material by flame atomic absorption [0.1 ppb (Cd) and 1.0 ppb (Mn)]. The addition of small amounts of radionuclide, especially manganese, to sea-water with potentially and comparatively large, undetectable

stable metal concentrations would result in a dilution effect. ^{109}Cd is routinely supplied with a carrier consisting of stable cadmium (specific activity = 4.1 mCi.mg^{-1}). In the additions of 2.5 or 5.0 µCi, this would result in an addition of stable cadmium such that incubation concentrations of non-isotope cadmium would be 3.05 X 10^{-6} or 6.0 X 10^{-6} mg.ml^{-1}, ie., approximately 1000 X more stable cadmium was added as was ^{109}Cd (1-4 X 10^{-9} mg.ml^{-1}). Because stable cadmium was added, trace (undetectable) amounts of cadmium already present in the incubation seawater would not radically affect total concentrations. On the other hand, ^{54}Mn is routinely supplied carrier-free, with no stable manganese present. We did not add any stable manganese to the small amounts of ^{54}Mn (1 to 4 X 10^{-9} mg.ml^{-1}) used in incubations. Presence of, and temporal variations in, manganese seawater concentrations up to the detection limit (1 ppb) may amount to as much as 1000-fold more stable manganese being present than radionuclide. Therefore, any variations in seawater manganese concentrations would result in a large potential dilution compared to that experienced in cadmium experiments. This may explain why observed manganese uptake, especially during short incubation periods, was more variable than cadmium uptake. Further, many experiments were conducted at different times of the year. Variations in temperatures and differences in the stature of plants may have contributed to experiment-to-experiment variability.

It is well known that temperature affects uptake kinetics of ions in aquatic plants (eg., Pickering and Puia, 1969). Although I selected plants of relatively similar size for each experiment, seasonal differences in tissue structure and density (Sculthorpe, 1967) may also affect uptake.

The depuration experiments yielded some interesting insights into the behavior of cadmium in plants placed in the field. The accidental use of killed (stressed) plants indicated that cadmium initially present in dead root-rhizomes is immobile. No loss to the sediments occurred. In fact, some sediment samples were removed from areas surrounding root-rhizomes and very little radionuclide activity was detected. Since manganese also appeared fixed in tissues exposed to it, little manganese loss to sediments would be expected in a field situation. The implication is that cadmium and manganese in dead root-rhizomes would be effectively trapped in the sediment environment. The second experiment conducted with living plants indicated that acropetal transport of cadmium to newly formed tissues occurred. Most of the cadmium in freshly incubated plants was initially found in the roots. Cadmium in old rhizomes was apparently transferred to new rhizomes and leaves. Very little cadmium was detected in newly formed roots. Further, rhizome cadmium concentrations were similar to those found in leaves, suggesting that these two tissues have similar affinities for cadmium, and may be structurally (and

physiologically) continuous via vascular tissues or lacunae. The fact that little manganese was transported from root-rhizomes to leaves in any of the intact-plant experiments suggests that this metal may not be mobilized from the sediment pool appreciably.

A most interesting point, also related by Faraday and Churchill (1979), is that the downward flux of cadmium in eelgrass is more significant that acropetal flux. Root-rhizome complexes appear to be primarily a sink mechanism for cadmium removal from the overlying water column. Verfaillie (1976) stated that bidirectional transport of cadmium also occurred in aqueously cultured tomato plants. He found, however, that most of the cadmium was confined to the root system, where it was bound to organic compounds resembling metallothionein proteins.

The main mechanism for cadmium mobilization from sediments would appear to be through loss of cadmium bearing leaves, which are contributed to the detrital pool (Harrison and Mann, 1975). Here, leaf detritus is acted upon by the decomposer food chain consisting of bacteria and protozoans (Mann, 1976; Pulich, et al., 1976; Harrison, 1977). Epiphytes and epizoans were removed from some of the depuration plants after they were colonized in the field. Some radioactive cadmium activity was detected in unidentified small red algae and amphipods, and shrimp of the genus Palaemonetes. This could certainly be a more direct means of metal introduction into higher organisms, although the rates

through these organisms are likely to be small. Some cadmium was also leaked directly to the water column from leaves in chamber experiments.

The differences between manganese and cadmium behavior indicate that further studies with a more extensive suite of metals must be conducted before general statements can be made regarding monilization or trapping of metals by marine angiosperms. Studies on rooted aquatic macrophytes (Mayes et al., 1977; Gentner, 1977; Veltrup, 1978; Harding and Whitton, 1978) have found that uptake or mobilization of the same metal differs with plant species, as does the behavior of various metals within the same plant. As Gambrell et al. (1976 a,b) point out, the bioavailability of individual metals is largely dependent on their physicochemical interactions with the sediment milieu.

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