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Low-Energy Wastewater Recycling Through Wetland Ecosystems: Copper and Zinc in Wetland Microcosms

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Abstract: The advanced treatment of wastewater in a wetland ecosystem was evaluated using laboratory microcosms to determine heavy metal uptake by plant species including *Typha domingensis, Laguncularia racemosa, Distichlis spicata, Bacopa monnieri, Spartina bakeri,* and *Juncus roemerianus.* Partitioning of the Cu and Zn in the sediment, in the aboveground and belowground portions of the plants, and between species was determined. Microcosms were set up in a growth chamber, and metals were added at a concentration of 5 mg/l in various amounts to give treatment totals of 45 mg, 135 mg, and 225 mg. *Bacopa* took up 74.2% and 46.2% of the Cu and Zn, respectively, in the plant tissue, while accumulation by the other species ranged from 2.6% to 13.3%. Overall, belowground plant tissue accumulated a greater concentration of metals than aboveground tissue with the exception of Zn for the 225 mg treatment. No detrimental effects on the vegetation from the high concentrations of metals applied were observed after 63 days. Therefore, the marsh ecosystem is capable of assimilating heavy metals.

INTRODUCTION

Wetlands, which serve as a link between both terrestrial and aquatic systems, represent important ecosystems with numerous beneficial natural functions. Wetland sediments provide a cleansing action that removes nutrients, toxic materials, and particulate matter from overlying water. This research focuses on the flux of selected heavy metals in the water, sediments, and vegetation of the marsh ecosystem.

FRESHWATER WETLANDS AND WILDLIFE, 1989, CONF-8603101, DOE Symposium Series No. 61, R. R. Sharitz and J. W. Gibbons (Eds.), USDOE Office of Scientific and Technical Information, Oak Ridge, Tennessee.

Municipal wastewater may contain trace metals (EPA, 1983) that cause problems with their potential toxicity and tendency to accumulate in the food chain. Sediments of coastal marshes and bays are anoxic and will act as sinks for metals, accumulating Mn, Zn, and Fe, immobilized in the sediment matrix. A reduction in mean effluent metal concentration (Fe, Mn, Cd, Cu, Ni, and Zn) of between 15% and 32% occurred in experimental marsh raceways (Windom, 1977). In a study by Best et al. (1982), Cu, Cd, Mn, and Zn added to a forested wetland decreased by 80% to 90% within 40 m of the point of discharge.

Previous studies have shown that heavy metals entering cypress wetlands receiving treated sewage were immobilized by organic peat sediments with complexation by dissolved organic matter functioning as a competing process (Tuschall and Brezonik, 1983). Associations of the metals include the formation of complexes with organic material and coprecipitation with hydrous iron and manganese oxides or sulfides. Humic materials can complex heavy metals to an appreciable extent, thereby changing the toxicity, solubility, and eventually the fate of the heavy metals in the environment. This was shown in a microcosm study by Best et al. (1982), where adsorption by the organic peat sediment was the controlling mechanism of Cd, Cu, Mn, and Zn removal from the water column. An important mechanism that helps regulate solution concentrations of metal ions which could be toxic is the precipitation of certain metal sulfides in flooded soils and sediment. Insoluble and relatively stable sulfides of Fe, Mn, Zn, Cu, and Hg may form in a flooded soil or sediment where reducing conditions are intense and sulfide is present (Engler and Patrick, 1975).

Metals are absorbed by roots of *Spartina alterniflora* and become incorporated into the aboveground tissues (Banus et al., 1975). Dunstan et al. (1975) showed that the concentration of Cd and Cu in *Spartina alterniflora* did not directly correlate with the concentration of these metals in the sediment and suggested that the plants were well buffered against increasing levels of these metals since only a small portion of each metal accumulated in the plant-available fraction of the sediment. Water hyacinths *(Eichhornia crassipes)* receiving treated sewage have been shown to accumulate heavy metals with leaves and stems containing high levels of Cr, Cu, Fe, Hg, Mn, Ni, and Zn (Dinges, 1978). A summary of data on the uptake of various heavy metals by plants in widely varying environments showed freshwater marsh plants accumulated 0.3 to 30 kg/ha of Zn and 0.04 to 15.9 kg/ha of Cu (Stanley Consultants, 1977).

Use of the STP-4 wastewater treatment plant and polishing pond at Kennedy Space Center has expanded beyond original design capacity. An economical alternative to expansion of current facilities is to allow a natural system, such as a marsh, to provide partial tertiary treatment of the existing secondary effluent. A pilot-scale research program was initiated in an impounded oligohaline marsh at Kennedy Space Center to develop preliminary data on wetland efficiency at water quality enhancement (Owens-Mion, 1986). In addition to a field study focused on the flux of nutrients and selected heavy metals in the water, sediments, and vegetation of the marsh ecosystem, a laboratory microcosm experiment was conducted to determine heavy metal uptake by the dominant plant species and to look at the partitioning of Cu and Zn between components of the microcosms.

METHODS

Twelve microcosms, each in a 50.8 × 38.1 × 22.9 cm plastic container, were set up in a growth chamber. The experimental design consisted of three control microcosms and three metals treatment concentrations with three microcosms per treatment. Equal amounts of sediment were dug out of the experimental marsh near the field study site and put directly into the microcosm containers to retain the flooded condition. Equal numbers of plants from the same location in the marsh as the sediment were transplanted into the microcosm containers. Biomass and size of the plants varied between microcosms through the experiment due to new growth and senescence. The microcosms were kept saturated with marsh water to simulate the conditions of the experimental marsh. To prevent excessive accumulation of salts, deionized (DI) water was substituted for marsh water before the addition of Cu and Zn began. Plants chosen for the microcosms were dominant species in the test marsh. They included: *Laguncularia racemosa, Typha domingensis, Distichlis spicata, Bacopa monnieri, Spartina bakeri,* and *Juncus roemerianus.* The transplanted microcosms were allowed to stabilize for 3 months. During experimentation, a solution containing 5 mg/l Cu and Zn was added to the microcosms with DI water to maintain a depth of 2.54 cm over the sediment to simulate the loading rate in the field. The metals solution and the DI water were added in the following manner:

Group A (control)	DI water added 5 times per week
Group B	Solution containing Cu and Zn added 1 time and DI water 4 times per week
Group C	Solution containing Cu and Zn added 3 times and DI water 2 times per week
Group D	Solution containing Cu and Zn added 5 times per week

The metals solution was sprinkled over the microcosms for even application. When the treatments began, all 12 microcosms had 2.54 cm of surface water. Surface water was sampled periodically during the study in plastic bottles, acidified to a pH of less than 2.0 with HNO_3, filtered through Whatman #2 qualitative filter paper, and analyzed for Cu and Zn. After 9 weeks of treatment, aboveground and belowground vegetation were harvested and sediment core samples collected. The sediment attached to the roots was allowed to dry and was then removed by shaking the roots. Plants and sediments were analyzed for Cu and Zn. The digestion procedure used for total metals in plant tissue was dry ashing in a muffle furnace. The ash was dissolved in 6 *M* HCl (Wolfe, 1962). Sediment samples were digested for total metals on a hot plate with HNO_3 for 12 hours (Baker and Amacher, 1982). Copper and Zn concentrations in sediment and vegetation were determined by atomic absorption spectrophotometry.

Tests to determine differences in heavy metal uptake between plant species, using data from control and highest treatment level microcosms and between aboveground and belowground plant tissue, were performed using student's t-test. Two tests were performed using STAT80: one using a pooled estimate of the variance if the population variances were equal and the other using the separate sampling variances when population variances were unequal (Fullerton, 1985).

RESULTS

Copper and Zn uptake by aboveground and belowground portions of the microcosm vegetation from the control and group D are illustrated in Figs. 1 through 4. Of the Cu in the microcosm plant tissue, *Bacopa* took up 74.2% (averaged over all three treatment levels), while accumulation by the other species ranged from 2.6% to 8.8%. *Bacopa* incorporated 46.2% of the Zn contained in the microcosm vegetation. Other species took up between 8.2% and 13.3%. A student's t-test on group D microcosms revealed *Bacopa* belowground tissue accumulated significantly greater amounts of Cu than *Juncus* belowground tissue. Likewise, *Juncus* roots took up significantly more Cu than *Spartina* roots. The differences in Zn uptake between *Bacopa* roots and *Spartina* roots were also statistically significant (Table 1).

Differences in heavy metal uptake between aboveground and belowground plant tissue were tested using t-tests. *Bacopa* roots accumulated significantly greater amounts of Cu than aboveground portions of the plant in microcosm treatment groups B and D. Aboveground *Distichlis* took up significantly more Cu than the roots in treatment group D. Significantly higher concentrations of Zn were found in belowground tissue of *Bacopa* in group B. *Spartina* roots accumulated significantly more Zn than the aboveground portions in groups

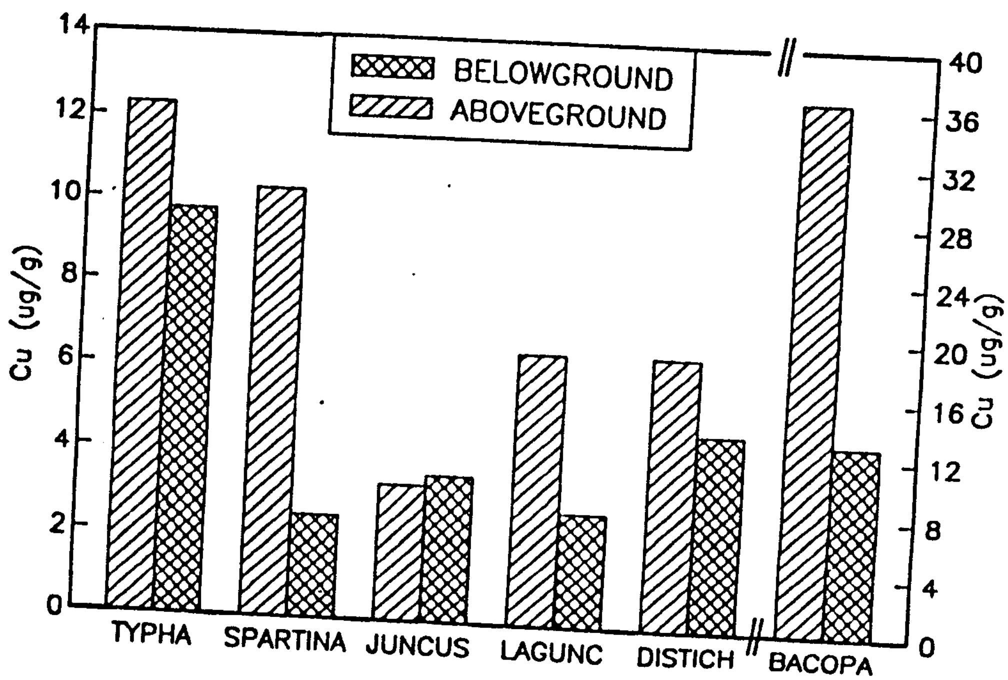

Fig. 1 Copper concentrations (μg/g) in aboveground and belowground portions of microcosm vegetation (control).

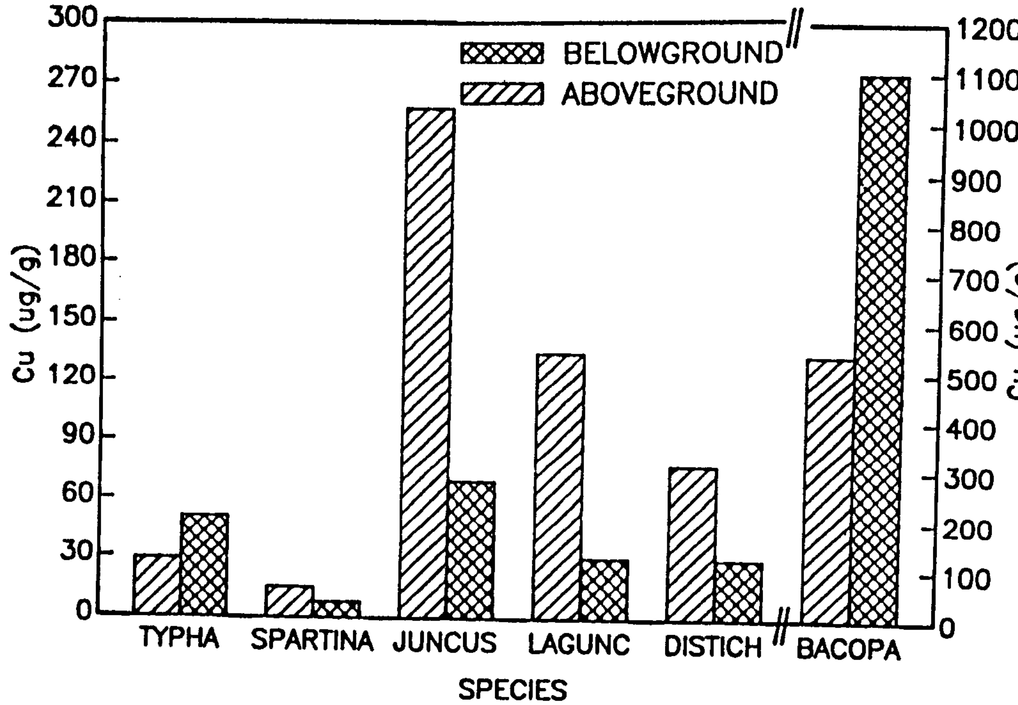

Fig. 2 Copper concentrations (μg/g) in aboveground and belowground portions of microcosm vegetation (treatment D).

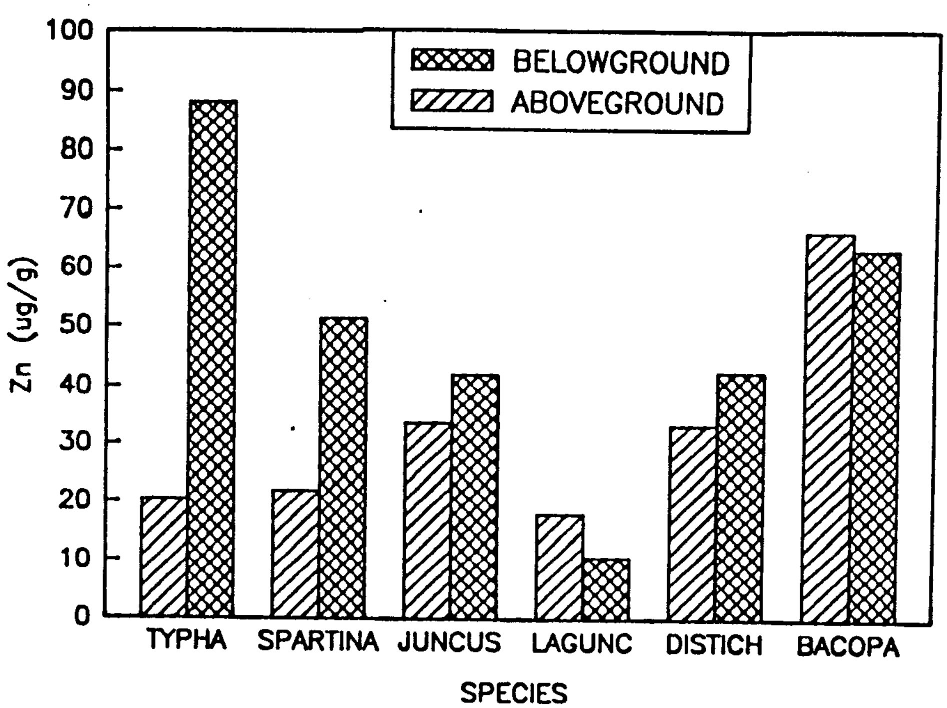

Fig. 3 Zinc concentrations (μg/g) in aboveground and belowground portions of microcosm vegetation (control).

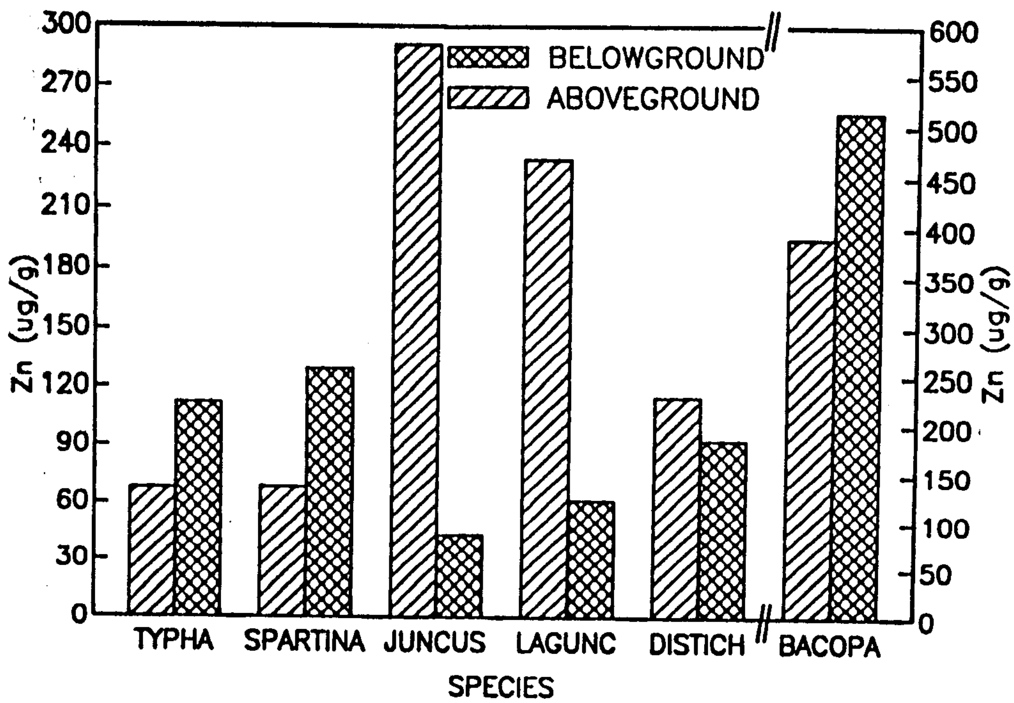

Fig. 4 Zinc concentrations (μg/g) in aboveground and belowground portions of microcosm vegetation (treatment D).

TABLE 1

Microcosm T-Test Summary Table

Parameter	Variables	T-statistic	P-value
Copper	*Bacopa* vs. *Juncus* (belowground)	19.3145	.00133
Copper	*Juncus* vs. *Spartina* (belowground)	15.9890	.00194
Zinc	*Bacopa* vs. *Spartina* (belowground)	5.1448	.00338
	Aboveground vs. belowground		
Copper	*Bacopa* (Treatment B)	4.9727	.01907
Copper	*Bacopa* (Treatment D)	5.0637	.00358
Copper	*Distichlis* (Treatment D)	4.6226	.00493
Zinc	*Bacopa* (Treatment B)	15.1715	.00006
Zinc	*Spartina* (Treatment B)	4.1303	.00725
Zinc	*Spartina* (Treatment C)	2.2645	.04313
Zinc	*Laguncularia* (Treatment B)	2.6634	.02810

B and C. *Laguncularia* aboveground tissue took up significantly more Zn than its roots in group B (Table 1).

Microcosm groups A (control), B, C, and D were given a total of 0 mg, 45 mg, 135 mg, and 225 mg, respectively, of Cu and Zn during the 9-week treatment period. In the treatment groups B, C, and D, 76% to 89% of the Cu and 52% to 67% of the Zn accounted for at the end of the experiment were found in plant tissue. Between 2% and 9% of the metals remained dissolved in the surface water. Percent recovery values and a budget for each of the microcosm groups are presented in Table 2.

TABLE 2

Total Metals (mg) Budget and Percent Recovery Values for Microcosms

	Group A (0.0 mg added)		Group B (45.0 mg added)		Group C (135.0 mg added)		Group D (225.0 mg added)	
Microcosm	Cu	Zn	Cu	Zn	Cu	Zn	Cu	Zn
Surface water	0.69	0.54	1.93	3.34	7.71	7.89	9.94	16.03
Sediment	7.12	72.39	10.65	72.39	12.77	74.52	12.77	63.87
Aboveground biomass	5.55	14.26	14.35	30.12	21.89	34.50	77.28	85.88
Belowground biomass	3.17	26.09	28.46	50.99	42.66	57.33	98.98	73.31
Total recovered	16.53	113.58	55.39	156.84	85.03	174.24	198.97	239.09
Percent recovery*	-	-	86.36	96.13	50.74	44.93	81.08	55.78

*Percent recovery = [(total recovered in treatment minus total recovered in control)/total added] × 100.

DISCUSSION

Bacopa was responsible for most of the uptake of metals applied. This may cause problems higher up in the food chain with wildlife feeding on *Bacopa*. *Bacopa* roots accumulated more Cu and Zn than aboveground plant tissue. *Bacopa* is a creeping plant with long stems forming mats on wet sand or mud. In the microcosms, *Bacopa* roots were at or above the sediment surface. This partially explains the elevated uptake of Cu and Zn by *Bacopa*. *Juncus* has rhizomes which occur just below the sediment surface. Their location makes the applied heavy metals readily accessible to them. The metals were readily removed from the surface water through plant uptake or immobilization by the sediments. Very little of the added Cu or Zn was retained by the sediments. The sediments in the treatment group D (225 mg of Cu and Zn) retained 1.2 μg/g of Cu and 6.0 μg/g of Zn. The heavy metals applied to the microcosms were apparently in readily available forms for plant uptake.

The microcosm experiment was run for 9 weeks. Calculations were made, using values for Cu and Zn concentrations in domestic wastewater from a table compiled by Best et al. (1982), to determine the years of accumulation microcosm treatment group D represented. The high level of treatment was equivalent to 51.6 years of Cu and 13.8 years of Zn addition at domestic wastewater concentration levels. No detrimental effects on the microcosm vegetation from the high concentration of toxic metals were observed. In fact, the plants seemed to flourish. Plants have internal tolerance mechanisms where metal uptake occurs, but resistance results from the exclusion of metals from sensitive metabolic sites, evolution of metal-tolerant enzymes, or alteration of metabolic pathways (Ernst et al., 1975).

Typha latifolia has been shown to be tolerant of high concentrations of Cu, Ni, Pb, Zn, and Cd (Taylor and Crowder, 1983; McNaughton et al., 1974). Leaf Cu concentrations of *Typha latifolia* grown in solution culture reached 127 μg/g, and toxicity symptoms (reduced leaf elongation and biomass production) appeared at leaf Cu concentrations of approximately 80 μg/g. *Typha latifolia* was tolerant to elevated levels of Zn (5000 μg/g), Pb (435 μg/g), and Cd (73 μg/g) (McNaughton et al., 1974).

CONCLUSIONS

Microcosm experiments were conducted to observe the assimilation of heavy metals by wetland vegetation. The experimental wetland can effectively remove selected heavy metals from secondarily treated municipal wastewater. There were no deterimental effects observed from the addition of heavy metals. Heavy metals were being assimilated by the marsh ecosystem. Vegetation took up the majority of Cu and Zn applied to the microcosms. *Bacopa* was responsible for most of the metals uptake. In all three treatment groups, excluding Zn in group D, belowground plant tissue accumulated more metals than aboveground tissue. Wetland treatment in this system is a viable method for removing Cu and Zn from municipal wastewater.

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