

EFFECT OF METAL MIXTURE (Cu, Zn, Pb, and Ni) ON CADMIUM PARTITIONING IN LITTORAL SEDIMENTS AND ITS ACCUMULATION BY THE FRESHWATER MACROPHYTE *ERIOCAÛLON SEPTANGULÀRE*

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Abstract—The effect of a metal mixture (Cu, Zn, Pb, and Ni) on Cd fractionation in sediment and its accumulation by the freshwater macrophyte *Eriocaulon septangulare* was examined in an in situ experiment in the littoral zone at the Experimental Lakes Area, northwestern Ontario, Canada. Fresh sediment was spiked with Cd alone and together with the metal mixture at three concentration levels. Macrophytes were planted in the spiked sediment and placed at a water depth of 0.5 m. The distribution of Cd among sediment fractions (easily reducible [ER], reducible [R-ER], and organic [ORG]), pore water, and macrophytes was determined every 2 weeks for 10 weeks. Small differences among treatment levels in the recovery of Cd from the geochemical fractions were observed after 2 and 8 weeks but not after 10 weeks. At the highest concentration of the metal mixture, Cd repartitioned from the ER fraction onto the R-ER fraction after 2 weeks in situ. After 10 weeks, Cd was accumulated by the shoots (<2.6 µg/g dry weight) and roots (<45 µg/g dry weight) of *E. septangulare* and had not reached steady state. Significantly higher Cd concentrations were found in the shoots of plants in the treatment with Cd alone and the treatment with the highest concentration of the metal mixture than in treatments with intermediate levels of the mixture. Partitioning of Cd among geochemical fractions in sediment alone did not explain differences in tissue Cd concentrations related to treatment level.

Keywords—Cadmium Mixtures Freshwater macrophyte Simultaneous extraction Bioaccumulation

INTRODUCTION

The distribution and toxicity of Cd in the aquatic environment are affected by pH, hardness, alkalinity, sulfate, and concentration of organic matter [1–4]. Attempts have been made to take these factors into account when water- and sediment-quality guidelines for Cd have been developed [5,6]. Nevertheless, trace metals may also affect the distribution and toxicity of Cd [7–9], and their influence is not generally considered in guidelines. This influence may be of critical importance because Cd is released into the environment primarily in mixtures with other trace metals through nonferrous metal mining, smelting and refining processes, industrial effluents, and domestic sewage [10–12].

The study of the toxicity of metal mixtures has focused on aqueous exposures under controlled laboratory conditions [8,13–16]. Studies of this kind are helpful for understanding the effect of metal interactions on uptake and toxicity, but ecologically relevant information is needed on how metals interact to affect their distribution and bioavailability in the natural environment, particularly in sediments. This study is one of the first attempts to examine effects of metal mixtures in a field situation that represents the environments to be protected by regulatory criteria.

Sediments can be both a sink and a source of trace metals in aquatic ecosystems. Assessing the potential risks that sediment-based metals pose to aquatic organisms living both in, or on, sediment depends on their bioavailability [17]. Chemical extraction techniques that selectively partition metals into geo-

chemical fractions have been useful in improving our understanding of metal bioavailability [4,18,19]. The distribution of metals among geochemical fractions in sediment depends on the relative affinity of metals for each fraction, the solubility product (K_{sp}) of mineral phases containing metals in each fraction, and the concentrations of all metals present [7]. The latter effect was examined in this study.

Submerged aquatic macrophytes have recently been examined as potential bioindicators of trace metal contamination in the aquatic environment [20,21]. Isoetids are rooted macrophytes that possess an isoetoid growth form characterized by a short stem and a rosette of stiff leaves [22]. Isoetids possess characteristics that make them potential biological indicator species; for example, they are widespread throughout eastern North America [23,24], abundant [25], easy to collect and identify unequivocally, and they concentrate metals [23]. Their predominantly sediment-based nutrition makes them useful organisms for studying the availability of contaminants from the sediment [26], and recent studies have examined the relationship between metal concentrations in isoetids and total metal concentrations in sediment [27,28].

The present work examined the effect of a metal mixture on Cd partitioning in littoral sediment and on its accumulation by an isoetid macrophyte in a field experiment. We hypothesized that a mixture of metals (Cu, Zn, Pb, and Ni) experimentally added with Cd to sediment would affect the distribution of Cd among geochemical fractions compared to what would be expected if Cd were added alone to the sediment. Furthermore, we anticipated that differences in the partitioning of Cd in the sediment with or without the metal mixture would affect the availability of Cd for uptake by macrophytes, as judged by differences in Cd concentrations in plant tissues.

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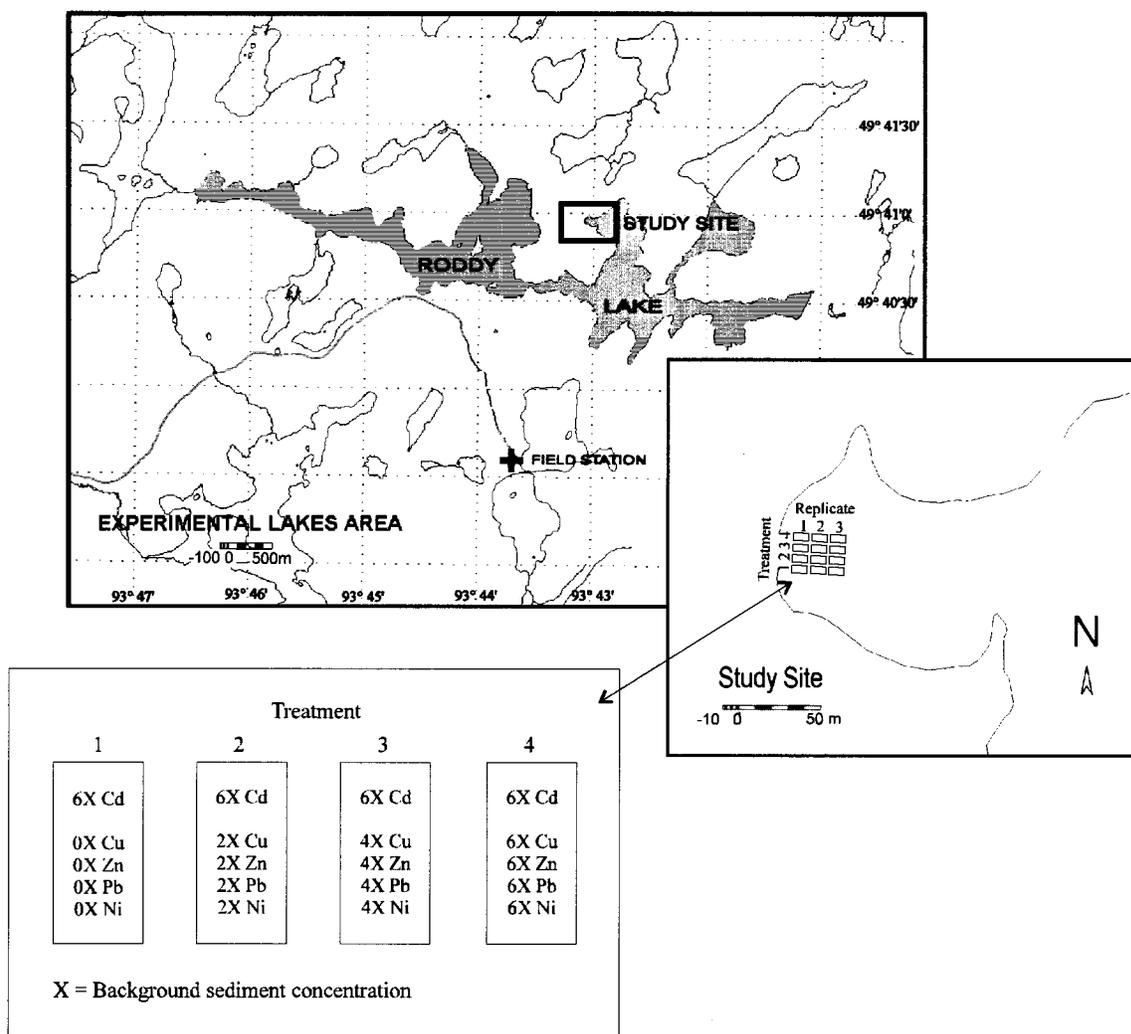


Fig. 1. Location of study site in Roddy Lake at the Experimental Lakes Area. Location of plant trays and the experimental design are also shown.

MATERIALS AND METHODS

Experimental site

The site of the in situ exposure experiment was a shallow, protected bay in Roddy Lake (93°43'W, 49°41'N), which is located at the Experimental Lakes Area (ELA), northwestern Ontario, Canada (Fig. 1). The ELA is a research preserve located 52 km southeast of Kenora, Ontario, Canada, on the southwestern part of the Precambrian Shield [29]. Water chemistry values of Roddy Lake are shown in Table 1.

Experimental design

The experiment consisted of four treatment levels with three replicates ($n = 3$) per treatment, for a total of 12 experimental units (Fig. 1). Treatment replicates were arranged, nonrandomly, on the bottom sediment as shown in Figure 1. The experiment was designed to raise Cd sediment concentrations six times above background levels in all treatments. Treatment levels 2, 3, and 4 also received a mixture of Cu, Zn, Pb, and Ni to raise sediment metal concentrations two, four, and six times respectively above background levels (Table 2). Background metal concentrations were based on 0–4 cm deep sediment samples collected at the experimental site in Roddy Lake in October 1992. The target metal concentrations in sediments

Table 1. Surface water chemistry values of Roddy Lake and Lake 104^a

Parameter ^b	Roddy Lake	Lake 104
NH ₄ -N, g/L	9.7 ± 4.0	9
DIC, μmol/L	143 ± 8	120
DOC, μmol/L	413 ± 23	940
Na ⁺ , mg/L	1.00 ± 0.05	0.98
K ⁺ , mg/L	0.43 ± 0.07	0.44
Ca ²⁺ , mg/L	2.41 ± 0.12	2.53
Mg ²⁺ , mg/L	0.68 ± 0.02	0.68
Fe, mg/L	0.02 ± 0.01	0.14
Mn, mg/L	0.01 ± 0.0	0.01
Cl ⁻ , mg/L	0.37 ± 0.02	0.24
SO ₄ ²⁻ , mg/L	3.23 ± 0.09	1.64
Alkalinity, μeq/L	134 ± 4.7	123
pH	6.74–7.27	6.59
O ₂ , mg/L	9.15 ± 0.41	8.7

^a Values for Roddy Lake are mean ± SD of six samples from June 8 to October 15, 1992. Values for Lake 104 are based on a single sample taken on September 15, 1992.

^b DIC = dissolved inorganic carbon; DOC = dissolved organic carbon.

Table 2. Background, target, one-day postspike, and 10-week sediment metal concentrations in the four treatments^a

Treatment	Metal concn. ($\mu\text{g/g}$ dry wt.)				
	Cd	Cu	Zn	Pb	Ni
Background level	0.15 ± 0.01	0.54 ± 0.02	5.33 ± 0.67	1.57 ± 0.09	1.86 ± 0.10
Target level					
1	0.90 (6 \times)	(0 \times)	(0 \times)	(0 \times)	(0 \times)
2	0.90 (6 \times)	1.08 (2 \times)	10.7 (2 \times)	3.14 (2 \times)	3.72 (2 \times)
3	0.90 (6 \times)	2.16 (4 \times)	21.4 (4 \times)	6.28 (4 \times)	7.44 (4 \times)
4	0.90 (6 \times)	3.24 (6 \times)	32.0 (6 \times)	9.42 (6 \times)	11.2 (6 \times)
Postspike					
1	0.95 ± 0.03	0.54 ± 0.01	5.99 ± 0.50	1.35 ± 0.07	1.03 ± 0.06
2	0.96 ± 0.09	0.75 ± 0.05	10.1 ± 1.01	3.59 ± 0.21	1.46 ± 0.14
3	1.01 ± 0.03	1.58 ± 0.02	16.3 ± 0.30	7.31 ± 0.11	4.50 ± 0.24
4	1.01 ± 0.05	2.37 ± 0.06	22.6 ± 1.03	11.1 ± 0.23	4.57 ± 0.55
10 wk					
1	0.73 ± 0.21	0.86 ± 0.07	5.64 ± 0.15	1.70 ± 0.02	1.67 ± 0.11
2	0.75 ± 0.04	1.11 ± 0.06	9.13 ± 0.36	3.09 ± 0.18	1.92 ± 0.28
3	0.75 ± 0.04	1.88 ± 0.01	13.7 ± 0.35	5.93 ± 0.04	4.23 ± 0.16
4	0.79 ± 0.03	2.76 ± 0.19	20.5 ± 0.90	9.73 ± 0.36	3.86 ± 0.04

^a Values are mean \pm SE ($n = 3$) acid-extractable metal concentrations. Values in parentheses are factors by which the sediments were to be increased over background levels.

were at the lower range of those observed in areas receiving atmospheric deposition from mining and smelting processes [30].

Sandy, littoral sediment (top 4 cm, from <2 m water depth) collected from the experimental site in Roddy Lake on July 8, 1995, was mixed in a 10-L cement mixer and separated into four homogeneous batches, each approx. 16 kg wet weight. Each batch was then returned to the mixer and spiked according to one of the four treatment levels on July 10 (Table 2). Metals were added to the sediments as salts ($\text{CdCl}_2 \cdot 2.5 \text{H}_2\text{O}$, $\text{CuCl}_2 \cdot 2 \text{H}_2\text{O}$, ZnCl_2 , $\text{Pb}(\text{NO}_3)_2$, and $\text{NiCl}_2 \cdot 6 \text{H}_2\text{O}$, all American Chemical Society grade; Fisher Scientific, Fair Lawn, NJ, USA) dissolved in 250 ml of deionized distilled water, and sediments were mixed for 5 min. Spiked sediment at each treatment level was divided into three replicate batches and placed in acid-washed polyethylene pails, which were stored on the shore and allowed to equilibrate for 3 d. On July 11, 1 d after the metal spike, postspike samples were taken from each replicate batch for analysis. Individual batches of sediment were then placed in 30-cm-wide \times 60-cm-long \times 5-cm-deep plastic potting trays before plants were added.

Nearby ELA Lake 104 (93°50'W, 49°41'N) was the source of the macrophyte *Eriocaulon septangulare*, which was planted in the spiked sediment. *Eriocaulon septangulare* has been successfully transplanted in other experiments at the ELA [31]. Lake 104 is a brown-water lake with a higher dissolved organic carbon level and slightly lower pH than Roddy (Table 1), but transferring plants from Lake 104 to Roddy did not expose them to an appreciable change of water chemistry. On July 11, plants were collected from Lake 104 from water depths of less than 2 m, placed in coolers, and transported to the field camp, where they were stored at lake temperature for 2 d. The plants were agitated in lake water to remove loosely attached sediment and debris and then planted in the trays of spiked sediment. On July 13 (time 0 for the introduction of the plants), the trays were placed on the bottom of Roddy Lake in 0.5 m of water and sampled after 2 (July 27), 4 (August 9), 6 (August 23), 8 (September 7), and 10 (September 26) weeks. The length of exposure was estimated as sufficient for metals to diffuse

throughout the pore water (using Fick's law diffusion coefficient, $2 \times 10^{-5} \text{ cm}^2/\text{s}$).

Sampling of pore water and sediment

Sediment pore water and lake water just above the sediment-water interface were monitored throughout the experiment using in situ samplers or "pore-water peepers" with a 0.2- μm Gelman HT-100 polysulfone membrane (Gelman, Ann Arbor, MI, USA), similar to those described by Hesslein [32]. The peepers consisted of polyethylene sheets (1.3 cm thick) into which compartments (12 rows of two parallel compartments 1 cm apart, 1 cm vertical resolution, ~ 2.5 ml volume each) had been placed. The peeper covers were composed of Lexan plastic sheets (2 mm thick) held in place by stainless-steel screws. Peepers were acid washed with 10% HNO_3 and rinsed with deionized, distilled water. Before installation, peepers were filled with deionized, distilled water and deoxygenated by bubbling with nitrogen for 12 h in deionized, distilled water. Peepers were inserted vertically into two randomly selected trays in each treatment level ($n = 2$) and were sampled and replaced every 2 weeks. Peepers extended 3 cm below and 9 cm above the sediment-water interface. Pore waters were sampled for metal analysis by piercing the membrane of each compartment with an Eppendorf pipette fitted with an acid-washed tip. The pore water was then transferred to an acid-washed polypropylene tube and acidified to 0.1% HNO_3 . The two compartments in each row were combined into one sample to provide sufficient sample volume for analysis. Ambient pH of the pore water at different depths was measured in the field. Immediately upon removal of the peepers from the sediment, a small volume of pore water ($\sim 100 \mu\text{l}$) was extracted from each compartment and injected into a vial. The pH was measured using a portable Orion pH meter (model 610) fitted with the rapid-response Orion pHuture sure-flow probe.

Sediments in the trays were sampled with a 5-cm-internal-diameter Plexiglas® coring tube. One core per tray was collected for a total of three replicate sediment samples per treatment level. Because the plant's root system extended from the top to the bottom of the tray, the whole core (~ 4 –5 cm) was

Table 3. Simultaneous extraction procedure

Fraction	Extraction solution and conditions
Acid extractable	3:1 HCl/HNO ₃ digest for 1 h at 80°C
Easily reducible	Hydroxylamine (NH ₂ OH·HCl), 1 M in 0.01 HNO ₃ , for 0.5 h
Reducible	Hydroxylamine (NH ₂ OH·HCl), 0.04 M in 25% acetic acid, for 6 h at 90°C
Alkaline extracted	NH ₄ OH, 1 M, for 1 wk

placed in a prewashed centrifuge tube, filled with lake water, and frozen within 2 h of collection. Before analysis, sediments were thawed and centrifuged at 3,000 rpm for 30 min to remove excess overlying lake water. The redox potentials of several samples were measured using a Radiometer® calomel half-cell (model K401) and platinum electrode (model P101). At the time of analysis, sediments had a redox potential (Eh) range of +248 to +400 mV, falling within that reported for undisturbed sediments in the field colonized by isoetids (~+300–+600 mV [33]).

Sampling of plants

Individual trays were divided into five quadrants to provide plant material for five sample times. One quadrant was chosen randomly at each sampling, and all plants were removed. Upon removal from the sediment, plants were placed in clean Whirlpak® bags, filled with Roddy Lake water, and transported to the field laboratory, where they were stored at the current lake epilimnetic temperature until they were processed within 24 h. Plants were carefully cleaned in lake water using acid-washed forceps to remove debris and dead or dying root and shoot material; only healthy plant material was analyzed for metals. Normally, plant material from a single developmental stage is compared to control the effects of biological variability on metal uptake, but this is not possible with *E. septanguläre*, which lacks a clear demarcation between life stages.

Because many macrophyte species cause precipitation of metals on their root surface by the release of ligands and by oxygenating the area surrounding the root, steps were taken to distinguish between intracellular and extracellular metals at all sample times. To remove extracellular metals, half of the plants (intact) were washed for 2 min in a titanium (III) chloride solution, then rinsed for 10 s in deionized, distilled water [34]. Titanium (III) chloride stabilized at pH 7 by citrate and ethylenediamine tetra-acetic acid has been shown to rapidly reduce iron hydroxides with low cellular toxicity [35]. The remaining plants were rinsed for 10 s in deionized, distilled water to provide a measure of total metal concentrations for comparisons between adsorbed and intracellular metals. The concentration of adsorbed metal was obtained by subtracting the metal concentration in titanium (III) chloride-washed plants from the total metal concentration in H₂O-washed plants. After being washed, plants were separated into roots and shoots and freeze-dried for metal analysis. Roots were separated from the rest of the plant just below the stem (root-shoot transition zone) and the shoots just above. The stem was not included in the analysis because its morphologic characteristics made it difficult to clean. Dry-weight/wet-weight ratios were determined on a separate set of plants ($n = 3$ plants per replicate tray) by weighing separated roots and shoots, drying them in an oven for 24 h at 60°C, cooling them in a dessicator, and weighing them again. Dry matter was calculated as follows:

$$\% \text{ Dry Matter} = \frac{\text{Dry Weight (g)}}{\text{Fresh Weight (g)}} \times 100$$

Dry matter in plants consists of cellular components from the cell wall and within the cytosol [36]. Changes in dry matter were used here as a basic assessment of the condition of the transplanted *E. septanguläre*.

Analysis of pore water, sediment, and plant tissue

Metal concentrations (Cd and Fe) in acidified pore waters were measured by flame atomic absorption spectrophotometry (FAAS) and graphite furnace atomic absorption spectrophotometry (GFAAS) using a Varian GTA-95 spectrophotometer (Varian Instruments, Georgetown, ON, Canada). All glassware used in the analysis of metals in pore water, sediments, and plants was washed with concentrated HNO₃ (reagent grade) and rinsed at least three times with deionized, distilled water.

Sediment-bound metals were partitioned into three operationally defined fractions, easily reducible (ER, associated with manganese oxides), reducible (R, associated with manganese and iron oxides), and alkaline extracted (ORG, bound to organic), using the simultaneous extraction method of Bendell-Young et al. [37] (Table 3). Although the ER extraction was expected to remove some of the most reactive amorphous Fe, it is assumed that the majority of the Cd and metal mixture recovered from the ER fraction was associated with the hydroxides of Mn. The fraction of metals associated with most of the iron hydroxides (R-ER) was obtained by subtracting the ER fraction (containing manganese oxides) from the R fraction (containing iron and manganese oxides). Total sediment-bound metals, defined as acid-extractable metals (AE), were also determined in an aqua regia digest (3:1 HCl and HNO₃, 80°C for 1 h). Sediment metal concentrations (Cd, Cu, Zn, Pb, Ni, Fe, and Mn) were measured by FAAS and GFAAS, and standards were made up to volume with appropriate extraction solutions. All metal concentrations in sediment were expressed on a dry-weight basis. A separate aliquot of sediment was used for a dry-weight/wet-weight comparison (dried at 60°C for 24 h). The partitioning of metals in sediments is reported for background, postspike, and 2-, 8-, and 10-week sediments. The coefficient of variation (CV [SE/mean]) of the extraction technique, measured by subsampling ($n = 3$) one core from each treatment level during each extraction, was less than 12%, with few exceptions, for all metal fractions.

Root and shoot tissues were digested with concentrated HNO₃, oxidized with 30% H₂O₂, and analyzed for metals using FAAS and GFAAS or polarized Zeeman Z-8200 with Zeeman background correction (Hitachi Scientific Instruments, Rexdale, ON, Canada). Duplicate samples of National Bureau of Standards reference material 1572 citrus leaves were analyzed with every set of plant samples and were within certified ranges for each metal [38]. Variability in plant metal concentrations among replicate trays was high (CV range, of 4–60% for

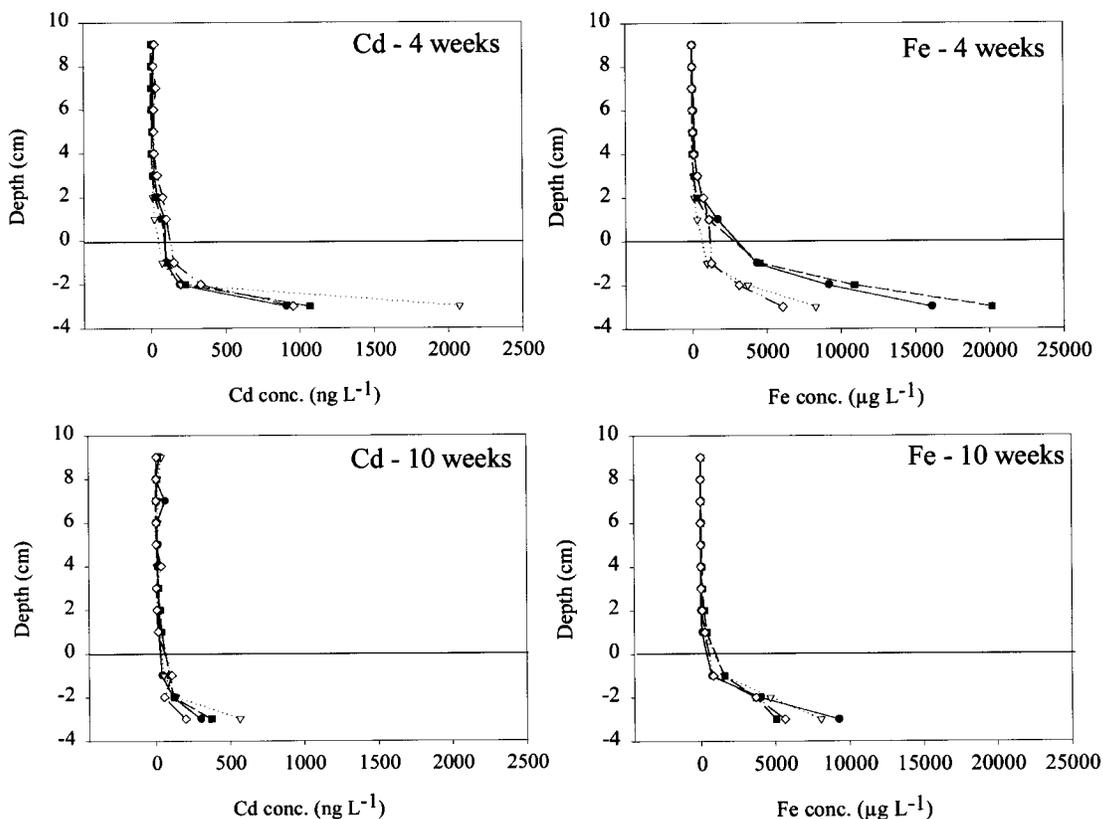


Fig. 2. Pore-water Cd (ng/L) and Fe ($\mu\text{g/L}$) profiles in the spiked sediment and above the sediment–water interface after 4 and 10 weeks in situ. ● = Treatment 1; ▽ = treatment 2; ■ = treatment 3; ◇ = treatment 4. Values are means of two replicate peepers. Note that the sediment–water interface begins at 0 cm depth.

shoots and roots) but similar to that for indigenous plant material (*Nuphar variegatum*) reported by others [30].

Statistical methods

Data were tested for normality using the Shapiro-Wilk statistic, a ratio between the best estimator of the variance and the corrected sum-of-squares estimator of the variance, and log-transformed when required. Statistically significant differences in metal concentrations in sediment fractions and plant parts among treatment levels for each of the sample weeks were determined using SAS® version 6.08 analysis of variance (ANOVA) with treatment level as a factor [39]. Differences in metal concentrations in sediment fractions and plant parts over the whole exposure period were determined using repeated-measures ANOVA with treatment level and time as factors and tested against the replicate tray error within treatment level [39]. Correlations among Cd concentrations extracted from the different sediment fractions were determined using SAS version 6.08 correlation analysis with Pearson correlation coefficients. Statistical significance was accepted at a confidence level of $p = 0.05$.

RESULTS

Postspike sediment metal concentrations and losses of Cd to the overlying water over time

The measured acid-extracted AE metal concentrations were close to those targeted for Cd and Pb (Table 2). Zinc concentrations were approx. 80% of target in treatment levels 3 and 4, and Cu concentrations were approx. 72% of target in all treatment levels. Nickel concentrations in spiked sediments

were the least similar to target values, with actual concentrations only 47% of target.

From the time the trays were introduced into the lake (time 0) until the end of the exposure period (10 weeks), approx. 25% of the Cd was lost to the overlying water from all of the treatment levels (Table 2). In treatment level 1, the greatest losses of Cd occurred within the first 2 weeks, in contrast to the treatment levels with the metal mixture, which had greater losses during the final 2 weeks of the experiment.

Cadmium in pore water

Pore-water Cd concentrations were highest near the bottom of the trays and decreased exponentially upward in the sediment and were near background above the sediment–water interface (Fig. 2). A similar profile was observed for Fe, with concentrations increasing below 2 cm depth (Fig. 2). These profiles suggest that the sediments may have been partially anoxic, in contrast to the measured redox potential of the sediments at the time of analysis. At no time during the exposure were significant differences found in pore water Cd concentrations among treatment levels. Variability in metal concentrations among the replicate peepers for each treatment level was high (mean CV, ~60%). This may have been caused by inadvertent contamination of pore water during sampling of the compartments. For example, small metal-laden particles attached to the outside of the peeper membrane could have been taken up along with the water sample when the tip of the pipette pierced the membrane. Pore-water pH tended to be lower in the sediment than in overlying water, ranging from

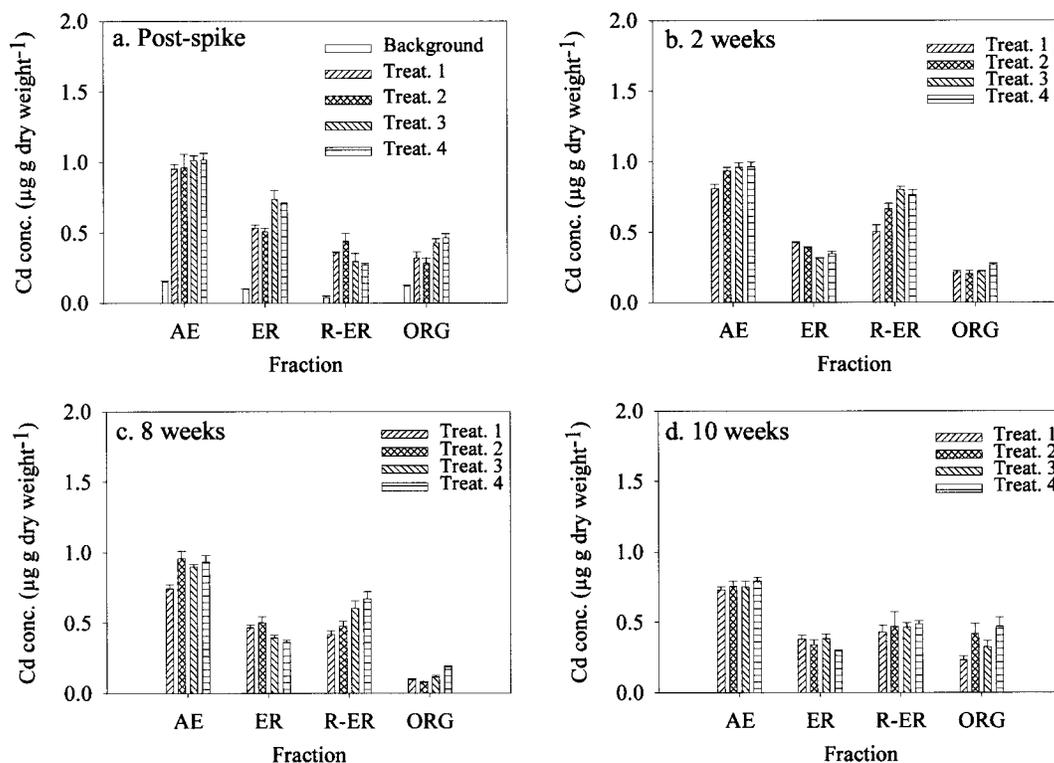


Fig. 3. Cadmium concentration ($\mu\text{g/g}$) in each of the geochemical fractions: acid-extractable (AE), easily reducible (ER), reducible (R-ER), and alkaline-extracted (ORG). Shown are background and postspike samples (a) and samples at 2 weeks (b), 8 weeks (c), and 10 weeks (d). Values are mean \pm SE ($n = 3$).

approx. 6.2 to 6.7 below the sediment to approx. 6.7 to 7.5 above the sediment–water interface at 4 weeks.

Partitioning of Cd in the sediment

The total concentrations and the partitioning of Cd and the other metals in background samples, postspike sediment, and after 2, 8, and 10 weeks are shown in Figure 3. The amount of Cd in the AE fraction ($0.15 \mu\text{g/g}$ dry weight) was slightly higher than that predicted (i.e., $0.085 \mu\text{g/g}$ dry weight) using the regression equation for Cd in littoral sediments and %loss on ignition developed by Stephenson and Mackie [40] but was within the range they found for central Ontario littoral sediments (0.01 – $2.51 \mu\text{g/g}$ dry weight). The largest proportion of Cd in background sediment from Roddy Lake, relative to AE Cd, was associated with the ORG fraction ($\sim 80\%$), followed by the ER ($\sim 60\%$) and R-ER ($\sim 25\%$) fractions (Figs. 3a and 4a). Note that when the concentrations of Cd in each fraction are added together, the total amount exceeds that in the AE fraction (average recoveries for Cd, $\sim 145\%$). Several possible explanations exist for the excess Cd recovered by the simultaneous extraction procedure. The simultaneous extraction procedure is done on individual aliquots of sediment, which may have varied somewhat in total Cd concentrations. When the Cd concentrations in individual fractions are summed and reported as a proportion of total Cd in the treatment level, the error due to variable sediment Cd concentrations is magnified. A significant positive correlation ($F = 34$, $p < 0.0001$) between the AE Cd and the sum of the individual fractions was found, suggesting this to be the case. In addition, if the sediment was partially anoxic, exposure to air during the extraction process may have affected the recoveries of Cd among sediment fractions due to the introduction of metal sulfides.

Rapin et al. [41] report an approx. 10-fold increase in readily exchangeable Cd (included in our ER fraction) in anoxic sediments exposed to adventitious oxygen relative to samples treated in a N_2 -atmosphere during a sequential extraction procedure. The simultaneous extraction procedure is designed for oxidized sediments and therefore could produce unpredictable results if used on anoxic sediments. Because of the operational nature of the sediment extractions, references to specific geochemical phases are tentative.

Small but statistically significant treatment level–related differences in the partitioning of Cd in all of the sediment fractions were observed in postspike sediments and after 2 and 8 weeks. Repeated-measures ANOVA on Cd concentration data obtained at 2, 8, and 10 weeks found significant treatment level effects for Cd in the ER, R-ER, and ORG fractions and for the proportion of Cd associated with ER and R-ER fractions relative to the AE fraction (Table 4). A significant interaction was found for treatment level \times week for Cd in the AE and ER fractions (Table 4). Specific differences among treatment levels in each of the geochemical fractions are described below.

One day after spiking the sediments with Cd and the metal mixture, the highest proportion of Cd was recovered from the ER fraction (~ 55 – 75%) (Fig. 3a). Treatment levels with the highest concentrations of the metal mixture also had the highest concentration of Cd associated with the ER fraction, [Cd(ER)], such that treatment levels 3 and 4 [Cd(ER)] were significantly higher than those in treatment levels 1 and 2 ($F = 9.6$, $p < 0.01$). The relationship was reversed after 2 and 8 weeks, when the proportion of Cd in the ER fraction in treatment levels 3 and 4 decreased to levels significantly lower than those in treatment levels 1 and 2 ($p < 0.05$) (Fig. 4b and

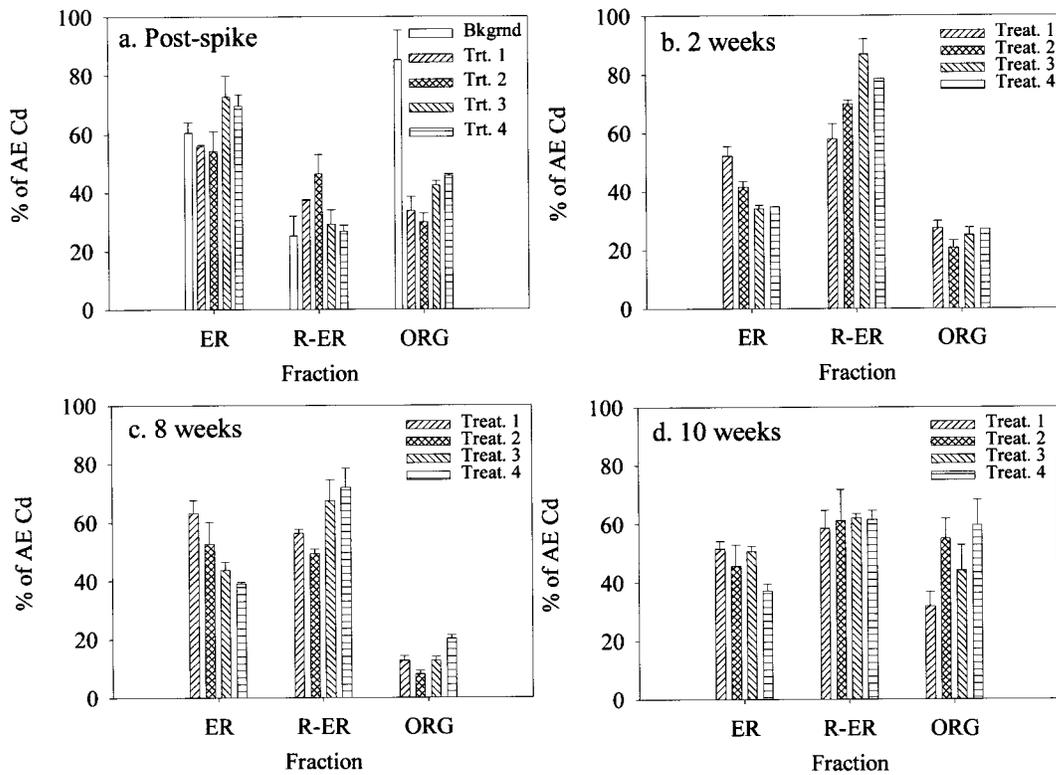


Fig. 4. Percentage of total acid-extractable Cd associated with the easily reducible (ER), reducible (R-ER), and alkaline-extracted (ORG) fractions. Shown are background and postspike samples (a) and samples at 2 weeks, (b), 8 weeks (c), and 10 weeks (d). Values are mean \pm SE ($n = 3$).

Table 4. Results of repeated-measures analysis of variance for differences in the partitioning of Cd into geochemical fractions among treatment levels over 2, 8, and 10 weeks

Geochemical fraction ^a	Factor	df	F	p
AE	Treatment	3	4.85	0.03
	Tray (treatment)	8	4.34	0.006
	Week	2	51.4	<0.001
	Treatment \times week	6	3.43	0.02
ER	Treatment	3	5.19	0.03
	Tray (treatment)	8	2.52	0.05
	Week	2	19.6	<0.001
	Treatment \times week	6	3.76	0.02
R-ER	Treatment	3	6.06	0.02
	Tray (treatment)	8	2.27	0.08
	Week	2	30.5	<0.001
	Treatment \times week	6	2.50	0.07
ORG	Treatment	3	8.67	0.007
	Tray (treatment)	8	0.85	0.57
	Week	2	51.2	<0.001
	Treatment \times week	6	2.56	0.06
ER % of AE	Treatment	3	7.71	0.01
	Tray (treatment)	8	3.11	0.03
	Week	2	10.32	0.001
	Treatment \times week	6	3.21	0.03
R-ER % of AE	Treatment	3	2.68	0.012
	Tray (treatment)	8	2.69	0.04
	Week	2	13.3	<0.001
	Treatment \times week	6	1.97	0.13
ORG % of AE	Treatment	3	3.33	0.08
	Tray (treatment)	8	1.28	0.32
	Week	2	66.1	<0.001
	Treatment \times week	6	2.96	0.04

^a AE = acid extractable; ER = easily reducible; ORG = alkaline extracted; R-ER = reducible.

c). After 10 weeks, the proportion of Cd associated with the ER fraction was lower than in postspike sediments, accounting for approx. 35 to 55% of the total Cd (Fig. 4d). No significant differences were observed among treatment levels in the ER fraction after 10 weeks (Fig. 3d).

In postspike sediments, there were no significant differences in the partitioning of Cd in the R-ER fraction among treatment levels, although [Cd(R-ER)] appeared somewhat lower in treatment levels 3 and 4 relative to treatment levels 1 and 2 (Fig. 3a). After 2 weeks, [Cd(R-ER)] significantly increased in treatment levels 3 and 4 from postspike levels corresponding to the lower Cd recoveries from the ER fraction in treatment levels 3 and 4. Small increases in treatment levels 1 and 2 [Cd(R-ER)] over postspike concentrations were also observed at 2 weeks, although values were significantly lower than those in treatment levels 3 and 4 ($p < 0.05$). Similar differences in concentrations among treatment levels were found at 8 weeks and for the proportion of Cd associated with the R-ER fraction (Fig. 4b to d). A significant inverse correlation between treatment level [Cd(R-ER)] and treatment level [Cd(ER)] was found in 2-, 8-, and 10-week samples ($r = -0.439$, $p = 0.007$).

The ORG fraction was not a major site for Cd during the 10-week exposure, except in postspike sediments and after 10 weeks in some treatment levels (Figs. 3 and 4). Approximately 30 to 45% of the Cd in postspike sediments was associated with the ORG fraction, which was slightly higher than that associated with the R-ER fraction (Fig. 4a). The [Cd(ORG)] in postspike sediments was significantly higher in treatment level 4 than in treatment levels 1 and 2 and in treatment level 3 than in treatment level 2 ($F = 6.2$, $p = 0.02$). This same trend was found after 8 weeks of exposure ($F = 30$, $p = 0.0001$) (Fig. 3c). Some variability in the overall Cd concen-

Table 5. Changes in % dry matter in *Eriocaulon septangulare* over the 10-week exposure period compared to plants freshly collected from Lake 104^a

Part	Week	Lake 104	% Dry matter			
			Treatment 1	Treatment 2	Treatment 3	Treatment 4
Root	0	5.47				
	2	NA	9.06 ± 3.87	5.78 ± 1.27	8.73 ± 1.06	7.24 ± 0.46
	10	4.47	5.22 ± 0.75	6.91 ± 1.07	6.66 ± 1.06	7.99 ± 0.98*
Shoot	0	7.33				
	2	NA	11.79 ± 0.78	13.0 ± 3.0	17.0 ± 0.9	11.3 ± 0.5
	10	14.9	14.7 ± 2.2	18.4 ± 3.2	16.3 ± 2.2	24.9 ± 4.3**

^a Values are mean ± SE ($n = 3$), except in Lake 104, for which $n = 1$ (pooled sample).

* Treatment level 4 is significantly different from treatment level 1 and Lake 104 plants ($p < 0.05$).

** Treatment level 4 is significantly different from all other treatment levels and Lake 104 plants ($p < 0.01$).

tration in the ORG fraction was observed among 2-, 8-, and 10-week samples that could not be explained.

Changes in percentage of dry matter in plants

Overall, the percentage of dry matter in shoots increased from the beginning of the exposure to 10 weeks in treatment levels and in Lake 104 specimens. The proportion of dry matter in the roots and shoots of transplanted *E. septangulare* in treatment level 4 after 10 weeks in Roddy Lake was significantly higher than that measured in specimens collected from Lake 104 (Table 5). The percentage of dry matter in treatment level 4 plants was also significantly higher than in the roots of treatment level 1 plants and in the shoots of plants from all of the other treatment levels.

Adsorbed Cd in H₂O-washed plants— External concentrations

The TiCl₃ wash removed significant amounts of metal adsorbed on the plants. Adsorbed Cd accounted for 57 and 35% of the total Cd extracted from the roots and shoots, respectively (Fig. 5). The difference in Cd concentration in TiCl₃-washed and H₂O-washed plants was highly significant in the roots on all sample days (2–10 weeks) and in the shoots from 4 to 8 weeks ($p < 0.05$). No treatment level-related differences in the amount of Cd adsorbed on either the roots or shoots were observed, although the proportion of adsorbed Cd on the roots

tended to decrease as the concentration of the metal mixture increased.

Cadmium accumulation in TiCl₃-washed plants— Internal concentrations

Cadmium accumulated in the roots and shoots of *E. septangulare* over the 10-week exposure period, as shown by the analysis of TiCl₃-washed plants (Fig. 6a and b). Cadmium concentrations were approx. 10 times higher in the roots than in the shoots. Cadmium uptake in the roots was initially slow during the first 4 weeks of exposure but increased steadily after this time. This increase may have continued after 10 weeks, except in treatment level 4, which showed a steady increase from the beginning of the exposure. Root Cd concentrations were significantly different from background concentrations in Lake 104 plants (2.43 µg Cd/g dry weight) after 6 weeks of exposure ($F = 10.74$, $p = 0.003$). In the shoots, there was an initial increase in Cd accumulation, followed by a slight decrease from 4 to 6 weeks and then steady increase thereafter until week 10. Cadmium concentrations in shoot in all treatment levels were significantly different from background levels (0.16 µg Cd/g dry weight) after 2 weeks of exposure ($F = 10.5$, $p = 0.003$). As with the roots, shoot Cd concentrations did not show signs of reaching steady state after 10 weeks in situ.

Both root and shoot Cd concentrations increased significantly over time (Table 6). No statistically significant effects of treatment level on root Cd concentration were observed during the entire exposure period. On the other hand, the repeated-measures test over the entire exposure period indicated significantly higher shoot Cd concentrations in treatment levels 1 and 4 compared to treatment levels 2 and 3.

DISCUSSION

Effect of the metal mixture on the distribution of Cd in spiked sediments

The metal mixture added with Cd to uncontaminated lake sediments appeared to influence the distribution of Cd among operationally defined geochemical fractions. At the highest metal mixture concentrations, Cd initially sorbed onto the ER fraction (postspike) but was later recovered in higher proportions from the R-ER fraction (2 and 8 weeks). The literature on Cd binding to sediment reports that the affinity of Cd for natural substrates follows the order Mn > Fe (amorphous) > chlorite > Fe (crystalline) = illite = humics > kaolinite >

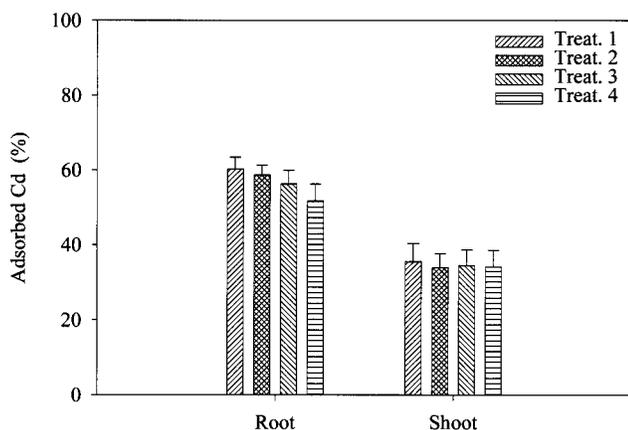


Fig. 5. Percentage of total Cd adsorbed on roots and shoots of *Eriocaulon septangulare*. Values are means ($n = 3$) of four plants collected over the entire 10-week exposure period. % Adsorbed = $1 - [\text{Cd}(\text{TiCl}_3 \text{ washed})]/[\text{Cd}(\text{H}_2\text{O washed})] \times 100$.

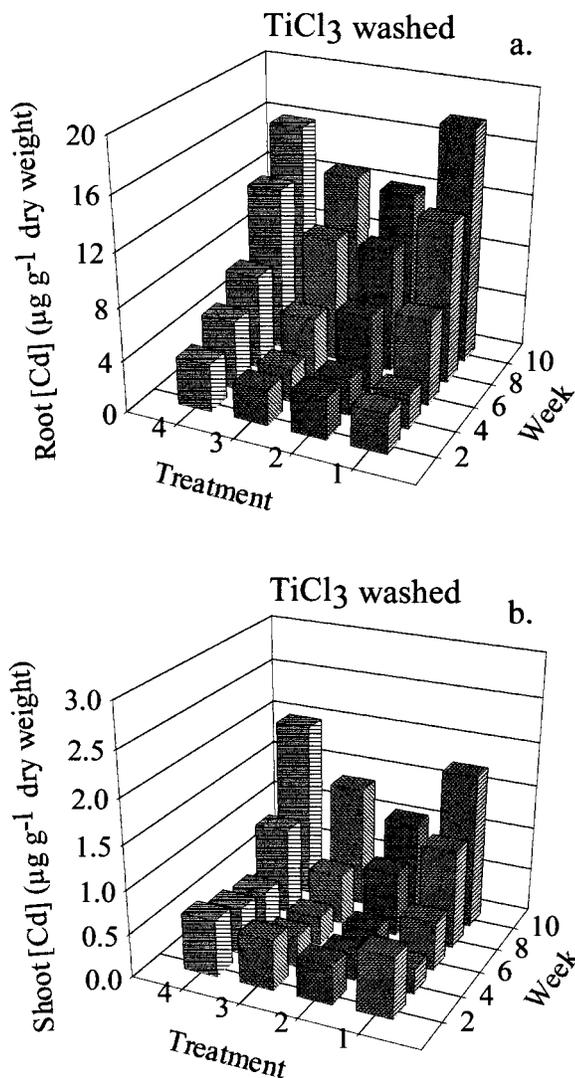


Fig. 6. Accumulation of Cd in $TiCl_3$ -washed *Eriocaulon septangulare* over the 10-week exposure period. (a) Roots. (b) Shoots. Values are means ($n = 3$).

silica [42], and there are examples of spiked Cd rapidly partitioning onto manganese oxides. For example, Cd applied to estuarine sediments quickly partitioned onto an ER (manganese oxide) fraction and repartitioned onto an organic and sulfide fraction with time [7,43]. The repartitioning of Cd onto the iron oxides over time might be expected through diagenetic

processes. For example, Cd added experimentally to the epilimnion of Lake 382 during the ice-free season associated with manganese oxides and the ORG fraction in depositional zones and with iron oxides in shallow sandy sediment (same extraction scheme used here) [44]. The analysis of background sediments from Roddy Bay indicated that a large portion of the Cd was associated with the ORG fraction. Tessier et al. [4] concluded that the majority of the Cd bound to natural littoral sediments in 38 Ontario and Quebec lakes receiving trace metals from atmospheric deposition was associated with organic matter. The repartitioning of Cd in treatment levels with the metal mixture among the different sediment fractions may have been caused by changing redox conditions and competition for binding sites on the preferred manganese oxide fraction. If the spiked sediment developed an anoxic gradient after the first 2 weeks in situ (stimulated by oxidative metabolism of organic matter supplied by the mixing of fresh sediments at the start of the experiment) then the manganese and iron oxides at the bottom of the sediment trays would be reduced. Dissolved Mn^{2+} and Fe^{2+} would then migrate to the oxic upper sediments and precipitate as oxyhydroxides, which readily adsorb Cd. The fact that the iron oxides tend to precipitate faster than manganese oxides might explain the higher recoveries of Cd from the R-ER fraction after 2 weeks. The inequitable partitioning of Cd among treatment levels might be explained by competition between Cd and the other metals for binding sites on manganese or iron oxides. The relative affinities (calculated as $K_d = \text{concentration of sorbed metal} / \text{concentration of dissolved metal}$) of the different metals for a synthetic manganese oxide substrate (Buserite) followed the order of $Pb > Zn > Cd \approx Ni$ [45]. Based on the overall lower affinity of Cd for manganese oxides compared to the other metals, it is possible that the other metals bound to the potentially limited manganese oxide sites and forced Cd onto the other available substrates, including iron oxides and organic material. An increasing proportion of Zn was observed in the ER fraction with increasing metal mixture treatment level at 2, 8, and 10 weeks (data not shown), which suggests that Zn may have been involved in the repartitioning of Cd onto the R-ER fraction. At the highest treatment level, the molar ratio of Zn to Cd was approx. 40:1. Cadmium has an overall lower affinity for freshly precipitated iron oxides than other metals ($Pb > Cu > Zn > Ni > Cd$) that should have led to the exclusion of Cd from the iron oxides [46–48]. The fact that this did not occur suggests that the number of binding sites on the iron oxides was not limiting, leading to higher adsorption of Cd onto the iron oxide as concentrations of the metal mixture increased.

Table 6. Results of repeated-measures analysis of variance for differences in root and shoot Cd concentrations among treatment levels over the entire exposure period (2–10 wk)

Factor		Root			Shoot		
		df	F	p	df	F	p
TiCl ₃ -washed plants	Treatment	3	0.99	0.44	3	6.79	0.01*
	Tray (treatment)	8	1.36	0.26	8	1.30	0.28
	Week	4	52.1	<0.001*	4	48.4	<0.001*
	Treatment × week	12	0.88	0.57	12	0.79	0.66
H ₂ O-washed plants	Treatment	3	1.70	0.24	3	3.58	0.05
	Tray (treatment)	8	2.09	0.07	8	1.27	0.31
	Week	4	66.4	<0.001*	4	34.8	<0.001*
	Treatment × week	12	0.90	0.56	12	0.58	0.84

* Significant.

After 10 weeks in situ, the distribution of Cd became more equitable between the ER and R-ER fractions, and overall metal concentrations had decreased in all fractions, except the organic fraction. Losses were predominantly in the treatment levels with the metal mixture and were most obvious at the highest treatment level concentrations. The role of *E. septangulàre* in determining the distribution of Cd in sediments after 10 weeks is discussed below.

Effect of transplantation and metal exposure on plant percentage dry matter

Transplanting *E. septangulàre* from one lake to another and exposure to metals did not appear to have a negative effect on plant condition, because the percentage of dry matter in the roots and shoots of transplanted *E. septangulàre* was the same as or higher than that in Lake 104 plants after 10 weeks. The significant increase in the percentage of dry matter in treatment level 4 roots and shoots relative to the other treatment levels and Lake 104 plants indicates that transplanting and/or exposure to the metals may have influenced condition in *E. septangulàre*. The fact that increases were observed only in treatment levels 2 to 4 suggests that exposure to the metal mixture may have led to the increase in the percentage of dry matter, possibly through increased availability of essential metals (Cu, Zn, and Ni) or by a fertilization effect due to the addition of $\text{Pb}(\text{NO}_3)_2$.

Despite the positive changes in the percentage of dry matter, measured at the end of the 10-week exposure, the time course of metal accumulation in the roots and shoots is interpreted as indicating that the plants experienced a 4- to 6-week period of transplant stress. Metal uptake in the roots and shoots showed an initial lag period of approx. 4 weeks that was followed by a steady increase in metal accumulation. This period of steady increase may indicate that the plant had become established and was physiologically stable, although it is impossible to be sure because there was no transplant control (i.e., tray of plants in background sediment). Adverse effects from exposure to Cd and the metal mixture may have been possible, but no toxicological data on this species exists. The toxicity threshold (median effective concentration) has been measured at 130 to 1,200 $\mu\text{g Cd/g}$ dry weight for *Lemna trisulca* [49], which is considerably higher than the highest root Cd concentration ($<50 \mu\text{g/g}$ dry weight) observed after 10 weeks of in situ exposure in the present experiment.

Effect of the metal mixture on the accumulation of Cd by plants

The repartitioning of Cd from the manganese oxide or ER to the iron oxide or R fraction should have indirectly reduced the bioavailability of the adsorbed Cd. Iron oxides are reductively dissolved at lower redox states than manganese oxides, which favors the stability of the iron oxide substrates and prevents the release of Cd into the sediment pore water, where it can be accumulated by the plants. Nevertheless, Cd accumulation in the roots and shoots of *E. septangulàre* among treatment levels was not consistent with this hypothesis. Despite the apparent increase in the amount of Cd associated with the less ER fractions, Cd concentrations in treatment level 4 roots and shoots were similar to those measured in treatment level 1 and higher than those in treatment levels 2 and 3. The higher Cd concentrations in shoots from treatment level 1 corresponds to the higher Cd concentration in treatment level 1 associated with the most easily reducible fraction.

We anticipated that any differences in the partitioning of Cd in the sediment added with or without a mixture of metals might affect the availability of Cd for uptake by macrophytes, as judged by differences in plant tissue Cd concentrations. Because of the small differences among treatment levels in sediment Cd concentrations and the inconsistent trend among treatment levels in plant Cd concentrations, it is difficult to draw a relationship between the partitioning of Cd in the sediment and Cd availability to the plants. Alternatively, the apparent differences in plant tissue Cd concentrations might be simply explained by competition among metals for uptake sites on the plant surface. The higher shoot Cd concentrations in plants from treatment level 1 relative to treatment levels 2 and 3 might be expected due to the lack of competition from the other metals. The high shoot Cd concentration in treatment level 4 does not conform to this theory but may have an alternative explanation. At the high exposure concentrations in treatment level 4, it is possible that the cell membranes became leaky as a result of cellular damage, thus allowing more metal to be absorbed relative to treatment levels 2 and 3. Damage to the plasma membrane caused by metals such as Cu has been associated with leakage of soluble constituents of the cell [50].

Alternatively, Huebert and Shay [8] found that in *Lemna trisulca* with low external Zn concentrations (axenic culture, aqueous exposure; 0.08–6.12 μM), Cd uptake was either not affected or decreased; however, with high levels of Zn (12.2 μM), Cd uptake was enhanced. The investigators suggested that above a certain concentration threshold, interactions with other metals may biochemically enhance Cd uptake. Accumulation of elements in plants is thought to be mediated by element-specific transport proteins in the cell membrane [37]. Absorption of some elements may take place inadvertently via ion pumps for other essential cations (e.g., Cd in a Ca pump). A mechanism by which one metal could enhance the uptake of another using these transport processes has not been described.

Effect of the metal mixture on adsorbed Cd

Macrophytes that possess the isoetid growth form are particularly effective at oxygenating their substratum by releasing oxygen along the length of their root. This is thought to be an adaptive response to living in areas with low dissolved inorganic carbon, because the oxygen stimulates CO_2 -generating decomposition processes in the sediment [51]. Oxygen released into the sediment has been shown to cause the precipitation of Fe and Mn as oxides, which also results in the removal of other trace metals from the sediment pore water. The TiCl_3 wash removed a larger proportion of adsorbed metal from the roots compared to the shoots, indicating that more metals were precipitated on the roots, consistent with leakage of oxygen from the roots [52]. Although no significant differences among treatment levels were found, the decreasing trend of adsorbed Cd with increasing concentration of the metal mixture might be expected from increased competition from other metals for limited binding sites on the plant surface.

The oxygenating effect of *E. septangulàre* may have played a role in the equitable distribution of Cd between ER and R-ER fractions after 10 weeks. At this time, one-fourth of the plants had been removed from the trays; as a consequence, the sediments would have been less oxygenated. The oxidizing effect of isoetid roots on the sediment is a local phenomenon along the length of the root [53] and in very sandy sediments, similar to those in this study, extends outward less than 0.1

m², which makes the above suggestion possible [50]. Furthermore, photosynthesis in *E. septanguläre* at this time of year would have been relatively lower compared to other sample periods, thus reducing the release of oxygen to acquire photosynthetic inorganic CO₂.

CONCLUSIONS

A metal mixture of Cu, Zn, Pb, and Ni influenced the distribution of Cd among geochemical fractions in spiked littoral sediments over a 10-week exposure period. At the highest concentrations of the metal mixture, small amounts of Cd re-partitioned from the manganese oxide fraction to the iron oxide and ORG fractions, where metals are considered to be potentially less available for uptake by plants. The highest Cd concentrations in the roots and shoots of *E. septanguläre* were found in treatment levels with Cd alone or with the highest concentration of the metal mixture. Partitioning of Cd among geochemical fractions in sediment alone did not explain treatment level-related differences in tissue Cd concentrations.

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REFERENCES

- Campbell PGC, Stokes PM. 1985. Acidification and toxicity of metals to aquatic biota. *Can J Fish Aquat Sci* 42:2034–2049.
- Stephenson M, Mackie GL. 1989. A laboratory study of the effects of waterborne cadmium, calcium and carbonate concentrations on cadmium concentrations in *Hyalella axteca* (Crustacea: Amphipoda). *Aquat Toxicol* 15:53–62.
- Wagemann R, Capel MJ, Hesslein R, Stephenson M. 1994. Sediment-water distribution coefficients and speciation of cadmium in a Canadian Shield lake. *Can J Aquat Sci* 51:1951–1958.
- Tessier A, Couillard Y, Campbell PGC, Auclair JC. 1993. Modeling Cd partitioning in oxic lake sediments and Cd concentrations in the freshwater bivalve *Anodonta grandis*. *Limnol Oceanogr* 38:1–17.
- Jaagumagi R. 1993. Development of Ontario provincial sediment quality guidelines for arsenic, cadmium, chromium, copper, lead, manganese, mercury, nickel and zinc. Technical Report 92-2309-069. Ontario Ministry of the Environment, Toronto, ON, Canada.
- Canadian Council of Resource and Environment Ministers. 1987. Canadian water quality guidelines. Task Force on Water Quality Guidelines, Environment Canada, Ottawa, ON.
- Rule JH, Alden RW III. 1996. Interactions of Cd and Cu in anaerobic estuarine sediments. I: Partitioning in geochemical fractions of sediments. *Environ Toxicol Chem* 15:460–465.
- Huebert DB, Shay J. 1992. Zinc toxicity and its interaction with cadmium in the submerged aquatic macrophyte *Lemna trisucla* L. *Environ Toxicol Chem* 11:715–720.
- Santschi PH, Nyffeler UP, Anderson RF, Schiff SL, O'Hara P. 1986. Response of radioactive trace metals to acid-base titrations in controlled experimental ecosystems: Evaluation of transport parameters for application to whole-lake radiotracer experiments. *Can J Fish Aquat Sci* 43:60–77.
- Nriagu JO. 1990. Global metal pollution. Poisoning the biosphere? *Environment* 32:7–33.
- Aquamin Sterring Group. 1996. Assessment of the aquatic effects of mining in Canada. Environment Canada, Ottawa, ON.
- Kelly M. 1988. *Mining and the Freshwater Environment*. Elsevier, London, UK.
- Naddy RB, La Point TW, Klaine SJ. 1995. Toxicity of arsenic, molybdenum and selenium combinations to *Ceriodaphnia dubia*. *Environ Toxicol Chem* 14:329–336.
- Kraak MHS, Lavy D, Schoon H, Toussaint M, Peeters WHM, van Straalen NM. 1994. Ecotoxicity of mixtures of metals to the zebra mussel *Dreissena polymorpha*. *Environ Toxicol Chem* 13:109–114.
- Enserink EL, Maas-Diepeveen JL, van Leeuwen CJ. 1991. Combined effects of metals: An ecotoxicological evaluation. *Water Res* 25:679–687.
- Keller AE, Zam SG. 1991. The acute toxicity of selected metals to the freshwater mussel, *Anodonta imbecilis*. *Environ Toxicol Chem* 10:539–546.
- Luoma SN, Fisher N. 1996. Uncertainties in assessing contaminant exposure from sediments. In Ingersoll CG, Dillon T, Bidinger GR, eds, *Critical Issues in Methodological Uncertainty*. SETAC Special Publication Series. Lewis Publishers, Boca Raton, FL, USA, pp 211–237.
- Jenne EA, Luoma SN. 1977. Forms of trace elements in soils, sediments, and associated waters: An overview of their determination and biological availability. In Drucker H, Wildung IR, eds, *Biological Implications of Metals in the Environment*. National Technical Information Center, Springfield, VA, USA, pp 110–143.
- Luoma SN, Bryan GW. 1981. Statistical assessment of the form of trace metals in oxidized estuarine sediments employing chemical extractants. *Sci Total Environ* 17:165–196.
- St-Cyr L, Campbell PGC. 1994. Trace metals in submerged plants of the St. Lawrence River. *Can J Bot* 72:429–439.
- St-Cyr L, Campbell PGC, Guertin K. 1994. Evaluation of the role of submerged plant beds in the metal budget of a fluvial lake. *Hydrobiologia* 291:141–156.
- Hutchinson GE. 1975. *A Treatise on Limnology III: Limnological Botany*. Wiley-Interscience, New York, NY, USA.
- Yan ND, Miller GE, Wile I, Hitchin GG. 1985. Richness of aquatic macrophyte floras of soft water lakes of differing pH and trace metal content in Ontario, Canada. *Aquat Bot* 23:27–40.
- France RL, Stokes PM. 1988. Isoetid-zoobenthos associations in acid sensitive Ontario, Canada, lakes. *Aquat Bot* 32:99–114.
- Hitchin GG, Wile I, Miller GE, Yan ND. 1984. Macrophyte data from 46 southern Ontario soft-water lakes of varying pH. Data Report 84/1. Ontario Ministry of Environment, Dorset, ON, Canada.
- Boston HL, Adams MS, Pienkowski TP. 1987. Utilization of sediment CO₂ by selected North American isoetids. *Ann Bot* 60:485–494.
- Jackson LJ, Kalff J, Rasmussen JB. 1993. Sediment pH and redox potential affect the bioavailability of Al, Cu, Fe, Mn, and Zn to rooted aquatic macrophytes. *Can J Fish Aquat Sci* 50:143–148.
- Jackson LJ, Kalff J. 1993. Patterns of metal content of submerged aquatic macrophytes: The role of plant growth form. *Freshwater Biol* 29:351–359.
- Brunskill GJ, Schindler DW. 1971. Geography and bathymetry of selected lake basins, Experimental Lakes Area, northwestern Ontario. *J Fish Res Board Can* 28:139–155.
- Campbell PGC, Tessier A, Bisson M, Bougie R. 1985. Accumulation of copper and zinc in the yellow water lily, *Nuphar variegatum*: Relationships to metal partitioning in the adjacent lake sediments. *Can J Fish Aquat Sci* 42:23–32.
- Howell ET. 1990. Effects of the isoetid macrophytes *Eriocaulon septangulare* and *Lobelia dortmanna* on the periphyton of littoral sediments. *Can J Bot* 68:1903–1910.
- Hesslein RH. 1976. An in situ sampler for close interval pore water studies. *Limnol Oceanogr* 21:912–914.
- Tessenow U, Baynes Y. 1978. Experimental effects of *Isoetes lacustris* L. on the distribution of Eh, pH, Fe and Mn in lake sediment. *Int Assoc Theor Appl Limnol Proc* 20:2358–2362.
- Hudson RJM, Morel FMM. 1989. Distinguishing between extra- and intracellular iron in marine phytoplankton. *Limnol Oceanogr* 34:1113–1120.
- Morel FMM. 1983. *Principals of Aquatic Chemistry*. Wiley-Interscience, New York, NY, USA.
- Salisbury FB, Ross CW. 1992. *Plant Physiology*, 4th ed. Wadsworth, Belmont, CA, USA.
- Bendell-Young L, Dutton M, Pick FR. 1992. Contrasting two methods for determining trace metal partitioning in oxidized lake sediments. *Biogeochemistry* 17:205–219.
- Stewart AR. 1998. Effect of a metal mixture (Cu, Zn, Pb and Ni) on the bioavailability and bioaccumulation of cadmium in natural systems. PhD thesis. University of Manitoba, Winnipeg, MB, Canada.

39. SAS Institute. 1989. *SAS/STAT® User's Guide*, Version 6, 4th ed, vol 2. Cary, NC, USA.
40. Stephenson M, Mackie GL. 1988. Total cadmium concentrations in the water and littoral sediments of central Ontario lakes. *Water Air Soil Pollut* 38:121–136.
41. Rapin F, Tessier A, Campbell PGC, Carignan R. 1986. Potential artifacts in the determination of metal partitioning in sediments by a sequential extraction procedure. *Environ Sci Technol* 20: 836–840.
42. Laxen DPH. 1983. Cadmium adsorption in freshwaters: A quantitative appraisal of the literature. *Sci Total Environ* 30:129–146.
43. Rule JH, Alden RW III. 1992. Partitioning of Cd in geochemical fractions of anaerobic estuarine sediments. *Estuarine Coastal Shelf Sci* 34:487–499.
44. Stephenson M, et al. 1996. Sedimentation of experimentally added cadmium and ¹⁰⁹Cd in Lake 382, Experimental Lakes Area, Canada. *Can J Fish Aquat Sci* 53:1888–1902.
45. Balistrieri LS, Murray JW. 1986. The surface chemistry of sediments from the Panama Basin: The influence of Mn oxides on metal adsorption. *Geochim Cosmochim Acta* 50:2235–2243.
46. Kinniburgh DG, Jackson ML, Syers JK. 1976. Adsorption of alkaline earth, transition, and heavy metal cations by hydrous oxide gels of iron and aluminum. *Soil Sci Soc Am Proc* 40:796.
47. Laxen DPH. 1985. Trace metal adsorption/coprecipitation on hydrous ferric oxide under realistic conditions. *Water Res* 19:1229–1236.
48. Laxen DPH. 1984. Adsorption of Cd, Pb and Cu during the precipitation of hydrous ferric oxide in a natural water. *Chem Geol* 47:321–332.
49. Woolhouse HW. 1983. Toxicity and tolerance in the responses of plants to metals. In Lange OL, Nobel PS, Osmond CB, Ziegler H, eds, *Encyclopedia of Plant Physiology*, Vol 12 C. Springer-Verlag, Berlin, Germany, pp 245–300.
50. Huebert DB. 1992. The effect of biological and chemical factors on cadmium uptake and toxicity in *Lemna trisulca* L. PhD thesis. University of Manitoba, Winnipeg, MB, Canada.
51. Wium-Andersen S, Andersen JM. 1972. Carbon dioxide content in the interstitial water in the sediment of Grane Langsø, a Danish *Lobelia* lake. *Limnol Oceanogr* 17:943–947.
52. Wium-Andersen S. 1971. Photosynthetic uptake of free CO₂ by the roots of *Lobelia dortmanna*. *Physiol Plant* 25:245–248.
53. Smits AJM, Laan P, Their RH, van der Velde G. 1990. Root aerenchyma, oxygen leakage patterns and alcoholic fermentation ability of the roots of some nymphaeid and isoetid macrophytes in relation to the sediment type of their habitat. *Aquat Bot* 38:3–17.