Accumulation of Cd by a freshwater mussel (*Pyganodon grandis*) is reduced in the presence of Cu, Zn, Pb, and Ni

A.R. Stewart

Abstract: The effects of a metal mixture on Cd bioavailability and uptake in the freshwater mussel *Pyganodon grandis* (formerly *Anodonta grandis grandis*) were investigated in a limnocorral experiment in a Precambrian Shield lake during the summer of 1992. Differences in the partitioning of Cd in water, sediment, and mussels were identified between limnocorrals treated with Cd alone or with Cd and a mixture of metals (Cu, Zn, Pb, and Ni) at three concentration levels. Loss of Cd from the water column was slower in treatments with the metal mixture (22- to 34-day half-life) than in the treatment with Cd alone (11-day half-life). Despite the higher concentrations of Cd in the water column in treatments with the mixture of metals, the mussels accumulated proportionally less Cd as the metal concentrations increased. These relationships were observed in mussels exposed for 40 days ([Cd] <4.4 $\mu g \cdot L^{-1}$) and 80 days ([Cd] = 4–14 $\mu g \cdot L^{-1}$). The uncoupling of the effects of the metal mixture on Cd bioavailability and uptake suggests that laboratory studies may be appropriate for characterizing metal uptake in mussels exposed to mixtures of metals under nonequilibrium conditions. The significant deviation in the behavior of Cd in the presence of the metal mixture emphasizes the need to further investigate regulatory approaches that focus on individual contaminants.

Résumé : On a étudié les effets d'un mélange de métaux sur la biodisponibilité du Cd et l'absorption de ce métal par la moule d'eau douce *Pyganodon grandis* (anciennement *Anodonta grandis grandis*) dans le cadre d'une expérience en enceintes limnologiques menée dans un lac du Bouclier précambrien durant l'été de 1992. On a observé des différences dans la répartition du Cd dans l'eau, les sédiments et les moules entre l'enceinte dans laquelle on a introduit seulement du Cd et les enceintes dans lesquelles on a introduit du Cd et un mélange de métaux (Cu, Zn, Pb et Ni) à trois concentrations. La diminution de la concentration de Cd dans la colonne d'eau était plus lente dans les traitements utilisant le mélange de métaux (demi-vie de 22–34 jours) que dans le traitement utilisant le Cd seul (demi-vie de 11 jours). Malgré les plus fortes concentrations de Cd dans la colonne d'eau dans les traitements utilisant le mélange de métaux, les moules accumulaient proportionnellement moins de Cd quand on passait à une concentration de métaux supérieure. Ces relations ont été observées chez les moules exposées durant 40 jours ([Cd] <4,4 $\mu g \cdot L^{-1}$) et 80 jours ([Cd] = 4–14 $\mu g \cdot L^{-1}$). L'effet restrictif du mélange de métaux sur la biodisponibilité et l'absorption du Cd laisse penser que des études en laboratoire pourraient être utiles pour caractériser l'absorption des métaux par les moules exposées à des mélanges de métaux dans des conditions non équilibrées. La modification significative du comportement du Cd en présence du mélange de métaux met en lumière la nécessité de réviser les méthodes de réglementation qui considèrent les contaminants individuellement.

[Traduit par la Rédaction]

Introduction

In the environment, contaminants are generally present as mixtures. For example, toxic heavy metals from nonferrous metal mining, smelting, and refining processes are released together into aquatic ecosystems through atmospheric deposition, surface runoff, and milling effluents (AQUAMIN 1996; Nriagu 1990). The resulting mixture of heavy metals in the receiving waters may interact and mutually influence metal toxicity in aquatic organisms. Metal mixture toxicity has been shown to deviate from that expected for individual metals for numerous species tested in the laboratory (Voyer and Heltshe 1984; Spehar and Fiandt 1986; European Inland Fisheries Advisory Commission 1987; Keller and Zam 1991; Kraak et al. 1994). Virtually nothing is known about metal mixture toxicity in the field.

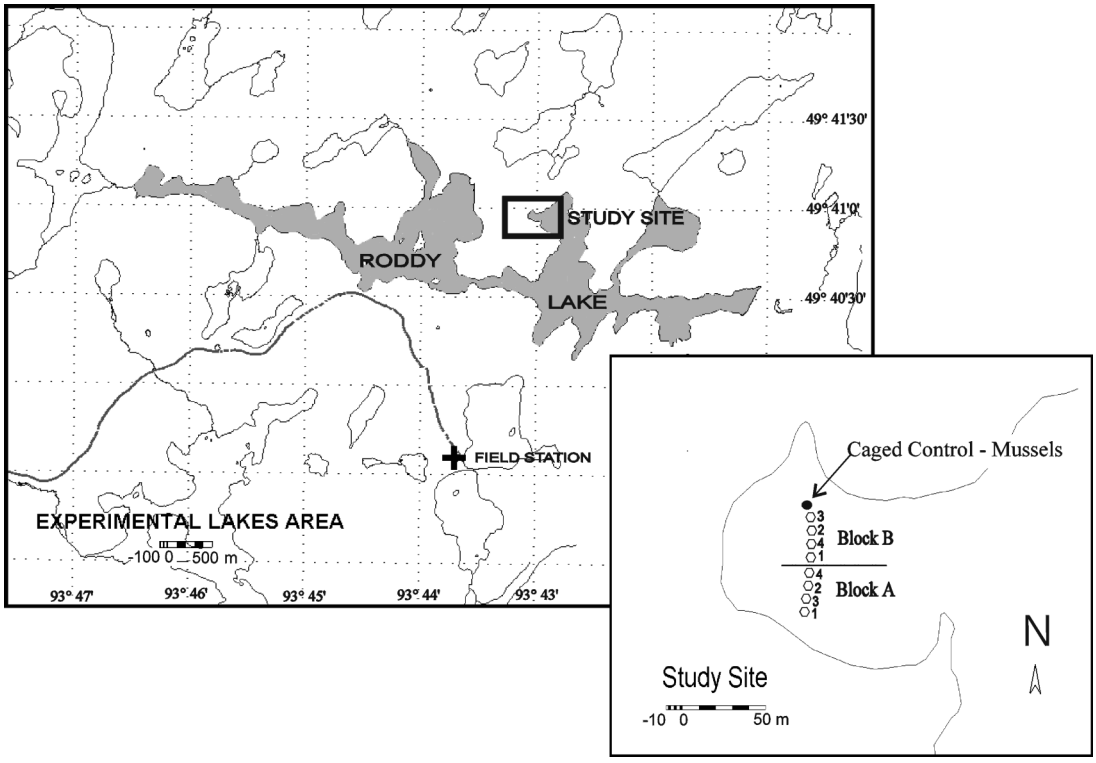
Metal toxicity in nature is determined to a great extent by the availability of individual metals for uptake by organisms. In the aquatic environment, metal transport, partitioning, and bioavailability are determined by a host of physicochemical factors including organic matter, redox potential, pH, and substrate type. Metal mixtures may also influence metal bioavailability, depending on metal concentrations, relative binding strengths of the metals for various substrates (K_d or partitioning coefficient), and the physiological roles of the metals. Metals introduced into surface waters are eventually lost to and accumulate in the sediment. Transport to the sedi-

Received May 5, 1998. Accepted October 23, 1998. J14571

A.R. Stewart. Department of Botany, University of Manitoba, Winnipeg, MB R3T 2N2, Canada.

[1]Corresponding address: Eric Marshall Aquatic Research Library, Department of Fisheries and Oceans, Freshwater Institute, 501 University Crescent, Winnipeg, MB R3T 2N6, Canada. e-mail: library-fwi@dfo-mpo.gc.ca

Fig. 1. Location of study site in Roddy Lake at the Experimental Lakes Area. Locations of limnocorrals and treatment assignment between blocks are also shown.



ment can occur by metals adsorbing onto settling particles in the water column or by metals directly adsorbing onto particles at the sediment–water interface (Luoma 1983; Santschi et al. 1986; Campbell and Tessier 1996). It is hypothesized that in the presence of large quantities of metals such as in mining or industrial effluents, binding sites on particles may become limiting, thus influencing the removal of metals from the water column. Metals remaining in the water column can be accumulated by phytoplankton, zooplankton, filter-feeding macroinvertebrates, and fish (Morel 1983; Campbell and Tessier 1996). If metal mixtures influence the bioavailability of metals in the water column, then corresponding changes in tissue metal concentrations would be expected. Field studies representing the complexity of natural processes are needed to elucidate the behavior of metal mixtures, their effect on the bioavailability of metals in natural systems, and consequently their toxicity to aquatic organisms.

To investigate the effects of a metal mixture on Cd bioavailability, differences in the partitioning of Cd in water, sediment, and the freshwater floater mussel *Pyganodon grandis* (Say) Hoeh (formerly *Anodonta grandis grandis*) were identified between limnocorrals treated with Cd alone or with Cd and a mixture of metals (Cu, Zn, Pb, and Ni) at three concentration levels. The experiment was conducted in a lake at the Experimental Lakes Area (ELA), northwestern Ontario. Cadmium, a priority substance under the Canadian Environmental Protection Act, is highly toxic to aquatic organisms and is commonly released with the metals Cu, Zn, Pb, and Ni during mining, smelting, and other industrial processes (Nriagu 1990). *Pyganodon grandis* is useful in assessing the bioavailability of metals in contaminated environments (Couillard et al. 1993, 1995; Tessier et al. 1993; Malley et al. 1995; Stewart 1997). Limnocorrals were dosed so that the resulting sediment metal concentrations were in the range of sediment concentrations found in lakes near base-metal smelters (Harrison and Klaverkamp 1990). The aim of this study was to determine if metal mixtures affect the bioavailability of Cd in the aquatic environment and whether or not the resulting environmental metal concentrations can be used to explain tissue metal concentrations. Conclusions of this field study are used to evaluate the relevance to natural systems of metal mixture studies conducted in the laboratory.

Methods

Experimental site

The ELA is located 52 km southeast of Kenora, Ontario, on the southwestern part of the Precambrian Shield (Brunskill and Schindler 1971). The ELA comprises some of the most oligotrophic lakes in the world. They are representative of small lakes especially prone to acidification and contamination from metals and organic pollutants (Schindler 1988). Apart from small-scale logging in previous years, limited fishing and hunting, past occurrence of forest fires, and regional LRTAP deposition, the ELA remains pristine.

The site of the limnocorral experiment was a shallow, protected bay in Roddy Lake (93°43′W, 49°41′N) (Fig. 1). Water chemistry for Roddy Lake is shown in Table 1. The experimental site consisted of littoral sediment composed of sand and gravel (1–2% loss on ignition) down to a depth of 6–8 cm, below which there was clay. There was a slight gradient of increasing sedimentary organic matter (~10% difference) from north to south along the transect where the limnocorrals were situated. The bay contained isoetid

Table 1. Surface water chemistry of Roddy Lake and Lake 104.

Parameter	Roddy Lake	Lake 104
NH_4-N ($\mu g \cdot L^{-1}$)	9.7±4.0	9
Dissolved inorganic carbon ($\mu mol \cdot L^{-1}$)	143±8	120
Dissolved organic carbon ($\mu mol \cdot L^{-1}$)	413±23	940
Na ($mg \cdot L^{-1}$)	1.00±0.05	0.98
K ($mg \cdot L^{-1}$)	0.43±0.07	0.44
Ca ($mg \cdot L^{-1}$)	2.41±0.12	2.53
Mg ($mg \cdot L^{-1}$)	0.68±0.02	0.68
Fe ($mg \cdot L^{-1}$)	0.02±0.01	0.14
Mn ($mg \cdot L^{-1}$)	0.01±0.0	0.01
Cl ($mg \cdot L^{-1}$)	0.37±0.02	0.24
SO_4 ($mg \cdot L^{-1}$)	3.23±0.09	1.64
Alkalinity ($\mu equiv. \cdot L^{-1}$)	134±4.7	123
pH	6.74–7.27[a]	6.59
O_2 ($mg \cdot L^{-1}$)	9.15±0.41	8.7

Note: Values for Roddy Lake are means ± SD of six samples from 8 June to 15 October 1992. Values for Lake 104 are based on a single sample taken on 15 September 1992.
[a]Range.

macrophytes including *Eriocaulon septangulare* and *Juncus* sp. and a few resident *P. grandis*.

Experimental design

The experiment consisted of four duplicate treatments (n = 2) for a total of eight limnocorrals (Fig. 1). Treatments 1–4 received Cd additions targeted to raise sediment Cd concentrations by seven times above background (Table 2). Treatments 2–4 also received a mixture of Cu, Zn, Pb, and Ni to raise sediment Cu, Zn, Pb, and Ni concentrations by three, four, or seven times above background, respectively (Table 2). To compensate for the small gradient in organic matter that could affect metal bioavailability and confound the results, a randomized block ANOVA design was used. Each treatment duplicate was randomly assigned within a southern block (A, lower organic matter) and a northern block (B, higher organic matter) (Fig. 1). This experimental design reduced the chance that statistical differences among treatments were the result of the gradient in organic matter rather than a treatment effect (Hurlbert 1984). No statistically significant differences between the two blocks were found ($P > 0.05$) in metal concentrations in water, sediments, or mussels.

Mussels were introduced into the eight limnocorrals 3 weeks after the metal additions to the limnocorrals were completed and when metal levels in the water column were still high. A second group of mussels was introduced after 40 days when metal levels were low to compare responses to two exposure levels. Additional mussels were introduced into a cage outside the limnocorrals on days 0 and 40 to serve as transplant controls (Fig. 1). The cage was open to the sediment and was composed of plastic mesh (1 cm in diameter, 250-cm-high borders), allowing unrestricted exposure of the mussels to lake water. The aquatic macrophyte *E. septangulare* was also introduced into the limnocorrals on day 0. The accumulation of metals in *E. septangulare* will be described elsewhere.

The limnocorrals were 2-m-diameter cylindrical tubes about 1.5 m deep, composed of impermeable, reinforced woven translucent plastic (Curry Industries, Winnipeg, Man.). At the lake surface, the limnocorrals were supported by styrofoam floats, each in a reinforced vinyl pocket closed with plastic ties and supported by an external aluminum frame. The bottom of the limnocorrals ended in skirts that extended outward onto the sediment surface and were secured on the sediment with sandbags placed by snorkel divers.

Metals as salts ($CdCl_2 \cdot 2.5H_2O$, $CuCl_2 \cdot 2H_2O$, $ZnCl_2$, $Pb(NO_3)_2$, and $NiCl_2 \cdot 6H_2O$; Fisher Scientific Co., Winnipeg, Man., ACS grade) dissolved in 1 L of deionized distilled water were added to limnocorrals in five separate additions on 5, 16, 17, 19, and 22 June 1992. The exposure experiment began on 13 July 1992 (day 0), 3 weeks after the final metal addition. Chloride samples were taken weekly from the limnocorrals and Roddy Lake beginning 15 July 1992 until the end of the experiment to monitor the limnocorrals for leakage. Because most of the metals were added as chloride salts and Cl^- does not adsorb onto particulates or sediment, Cl^- levels should have remained elevated at a constant concentration above the ambient lake water level. From the time of final metal addition until day 80, Cl^- levels in the treatments decreased by about 2–12%, after adjusting for changes in limnocorral water volume due to precipitation and evaporation. This amount of leakage is less than that observed in other ELA limnocorral experiments (Santschi et al. 1986).

Table 2. Experimental design.

	Treatment			
Metal	1	2	3	4
Cd	7×	7×	7×	7×
Cu	1×	3×	4×	7×
Zn	1×	3×	4×	7×
Pb	1×	3×	4×	7×
Ni	1×	3×	4×	7×

Note: Values are the factors by which sediment metal concentrations were targeted to be increased above background. Each treatment was represented by two limnocorrals.

Collection and deployment of mussels

The source of *P. grandis* introduced into the limnocorrals was Lake 104 (93°50′W, 49°41′N). Lake 104 is a brown-water lake with higher dissolved organic carcon and slightly lower pH than Roddy Lake (Table 1). Transplantation of the mussels to Roddy Lake did not expose them to an appreciable change in water quality. On day 0 (13 July) and day 40 (27 August), 180 and 25 mussels, respectively, of about 9 years of age and 10 ± 0.8 cm in length (mean ± SD) were collected by snorkel divers and transported in coolers to Roddy Lake. These mussels were introduced into the limnocorrals and control cage. Mussels were free to move within the limnocorrals and the cage. Mussels were also collected from Lake 104 on days 0, 40, and 80 (15 October) for background metal determinations.

Water and sediment sampling

To monitor the loss of metals from the water column, water samples were collected from Roddy Lake and limnocorrals in acid-washed polyethylene Nalgene bottles every 2 days from 5 June 1992 to 28 July 1992 and once a week thereafter until the experiment was terminated on 15 October (day 80). Total water samples (unfiltered) were acidified (0.5% HNO_3 Baker analyzed for trace metal analysis) immediately upon returning to the laboratory. The partitioning of metals in the water column of Roddy Lake and limnocorrals on days 0, 40, and 80 was determined in water samples drawn from 1 m depth using a peristaltic pump. The 1-m water samples were filtered through acid-washed 10-μm nylon mesh netting into a acid-washed Nalgene carboy to avoid clogging of the 1-μm filter; >10-μm particulates were discarded. At the field laboratory, the remaining water (~2.5 L) was filtered through a 1-μm-mesh, 142-mm-diameter polycarbonate membrane filter (Poretics Corp., Mississauga, Ont.) using acid-washed tubing and a vacuum pump. Samples of the <1-μm filtrate and a laboratory blank of 1-μm-filtered deionized distilled water were taken. The 1-μm laboratory blanks (n = 3) were all below the analytical detection limit

for each metal (Cd, <0.02 $\mu g \cdot L^{-1}$; Cu, <0.5 $\mu g \cdot L^{-1}$; Zn, <0.5 $\mu g \cdot L^{-1}$; Pb, <0.3 $\mu g \cdot L^{-1}$; Ni, <0.5 $\mu g \cdot L^{-1}$). A 1-μm mesh was chosen rather than the commonly used 0.45 μm because the 1-μm cutoff is closer to the particle size retained by filter-feeding unionid bivalves. Analysis of stomach contents suggests that the smallest particle size retained by *Pyganodon* species is 8 μm in diameter (Tankersley and Dimmock 1993). Furthermore, the ELA Chemical Laboratory uses a mesh size of 1 μm to operationally define suspended and dissolved fractions.

Additional water samples were taken from Roddy Lake and limnocorrals 1A, 2B, 3A, and 4B once a month in June, July, August, and September for analysis of major ions (Na, K, Ca, Mg, Fe, and Mn), pH, alkalinity, dissolved organic carbon, dissolved inorganic carbon, sulfate, and oxygen. Chemical analyses were performed using methods from Stainton et al. (1977) with the following modifications. Dissolved organic carbon samples were analyzed by a heated persulfate digestion followed by total carbon analysis (total carbon analyzer model 700, OI Corp. College Station, Tex.) and dissolved inorganic carbon was measured using infrared detection of carbon dioxide.

To determine if target levels were achieved in limnocorral sediments, metal concentrations were measured in sediments on days 0, 40, and 80. Samples were collected from each limnocorral in triplicate with a 5-cm internal diameter plexiglass coring tube. The top 2 cm of the core was placed in an acid-washed centrifuge tube and centrifuged at 4000 rpm for 30 min to remove porewater. Sediments were then frozen at –30°C until analyzed for metals.

Sampling and processing of mussels

On day 80, mussels were collected from the limnocorrals by SCUBA divers, placed in polypropylene bags, and transported to the field laboratory for processing. Mussels introduced into the limnocorrals or control cage on day 0 and removed on day 80 were designated 0- to 80-day mussels, and mussels introduced on day 40 and removed on day 80 were designated 40- to 80-day mussels. Of the 205 mussels introduced into the limnocorrals and cage, six died (three from limnocorral 4B) and 30 mussels were not recovered until the following spring. The spring mussels were not included in the following analyses. Mussels were maintained without water at 7.5 ± 0.5°C (lake temperature on day 80) until they were processed within 12 h. No specific attempt was made to clear gut contents, since previous metal uptake experiments carried out at the ELA showed that gut clearing had little effect on whole-body or tissue contents of Cd (Malley et al. 1989).

Mussels were blotted dry and their shell length, height, thickness, and live weight were measured. There were no significant differences ($P > 0.05$) in mussel length, height, depth, and live weight among groups in the limnocorrals or cage. Mussels were removed from the shells and whole bodies were frozen in Whirl-pak bags or they were dissected into mantle, gill, foot, kidney, and remainder of body, i.e., viscera. Mussel shells and bodies were freeze-dried (Lab Con Co. Freeze Dry 5, Fisher Scientific Co., Winnipeg, Man.) and condition of the mussels was determined by the ratio of freeze-dried (fd) body weight to freeze-dried shell weight after Davenport and Chen (1987):

$$\text{Condition} = \frac{\text{fd body wt (g)}}{\text{fd shell wt (g)}} .$$

With few exceptions, mussels bearing glochidia were not included in the statistical analyses; the mass of glochidia in the gill can potentially confound comparisons of metal concentrations, which are calculated on a per weight basis. Whole-body metal concentrations were determined for 0- to 80-day and 40- to 80-day mussels from each limnocorral and for the cage (five animals per limnocorral or cage). Tissue metal concentrations were also determined for 0- to 80-day mussels from limnocorrals 1A, 2B, 3A, and 4B (four or five animals) and the cage (two animals). Background metal concentrations were determined for the whole bodies of Lake 104 mussels on days 0, 40, and 80 (minimum of six animals) and in individual tissues on day 80 (three animals).

Analysis of water, sediment, and tissues

Total water samples and <1-μm filtrate were analyzed for metals by graphite furnace atomic absorption spectrometry (GFAAS) and flame atomic absorption spectrometry (FAAS) on a Varian GTA-95 (Varian Instruments, Georgetown, Ont.). Precautions were taken to prevent contamination by soaking all glassware in concentrated HNO_3 (reagent grade) and rinsing it three times in deionized distilled water.

Sediments were freeze-dried, digested by Aqua Regia (4:1 HCl–HNO_3, reagent grade), and extracts analyzed for metals using FAAS and GFAAS. Duplicate samples (~100 mg) of National Research Council of Canada reference sediments MESS-1 and PACS-1 were analyzed with almost every sample run. Mean (micrograms per gram dry weight ± SD, n = 4–6) metal concentrations obtained here for the reference materials were within the range specified for certified values (given in parentheses): Cd, 2.47 ± 0.2 (PACS-1, 2.38 ± 0.2); Cu, 22.2 ± 1.8 (MESS-1, 25.1 ± 3.8); Zn, 161 ± 6.4 (MESS-1, 191 ± 17); Pb, 29.7 ± 4.4 (MESS-1, 34.0 ± 6.1); Ni, 42.0 ± 3.6 (PACS-1, 44.1 ± 2.0). Variability (coefficient of variation (CV)) among triplicate cores from individual limnocorrals for the different metals was follows: Cd, ~15%; Cu, ~20%; Zn, ~15%; Pb, ~21%; Ni, ~21%. All sediment concentrations were expressed on a dry weight basis unless otherwise specified.

Freeze-dried mussel bodies and tissues were ground in a coffee grinder for at least 15 s. Tissues were then digested using concentrated HNO_3 and an H_2O_2 oxidation step according to Malley et al. (1989). Metal concentrations were measured with FAAS and GFAAS using a Varian GTA-95 or polarized Zeeman Z-8200 with Zeeman background correction (Hitachi Scientific Instruments, Rexdale, Ont.). Duplicate samples of National Research Council of Canada reference materials Dolt-2 and Dorm-1 (~100 mg) were analyzed with every set of mussel samples. Measured Cd, Cu, and Zn concentrations in reference materials varied little over time (CV = 3–11%, n = 7–16) and were within the specified ranges for each metal. Lead and Ni concentrations in reference materials were more variable (CV = 18–45%, n = 10), but their means were within the certified ranges. Average CV's for whole-body metal concentrations calculated using replicate mussels within each limnocorral were as follows: Cd, 18%; Cu, 21%; Zn, 16%; Pb, 28%; Ni, 27%. The variations in metal concentrations of individual tissues were generally 1.5–2 times higher than the above CV's. The above CV's for whole bodies were similar to those reported in other field studies with *P. grandis* (Couillard et al. 1993). All whole-body and tissue metal levels were expressed on a dry weight basis unless otherwise specified.

Calculations and statistics

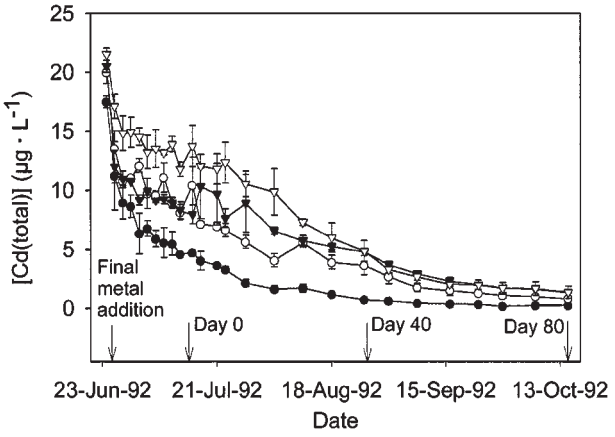
The loss rate for Cd from the water column in the different treatments was calculated as a half-life by fitting an exponential decay curve:

$$b = \frac{\ln C_0 - \ln C_t}{t}$$

$$t_{1/2} = \frac{\ln 2}{b}$$

where b is the loss rate coefficient, C_0 is the initial total water metal concentration, C_t is the final total water metal concentration, and t is the number of days between C_0 and C_t. The loss rate coef-

Fig. 2. Loss of Cd from the water column after the final metal addition. Values are means ± SE concentrations for each treatment (n = 2). Treatment 1 (solid circles), 11-day half-life; treatment 2 (open circles), 25-day half-life; treament 3 (solid triangles), 34-day half-life; treatment 4 (open triangles), 33-day half-life. Half-lives are calculated by fitting an exponential decay curve ($b = (\ln C_0 - C_t)/t$, where b is the loss rate coefficient, C_0 is the initial total water metal concentration, C_t is the final total water metal concentration, and t is the number of days between C_0 and C_t).



ficient for the exponential decay curve was optimized through a series of iterations using SigmaPlot version 4.0 (SPSS Inc. 1997).

Apparent partitioning coefficients, K_d^a, for Cd on particulate material and sediment were calculated for days 0, 40, and 80 according to Wood et al. (1995):

$$\text{Particulate } K_d^a = \frac{c_p}{c_f c_{ss}}$$

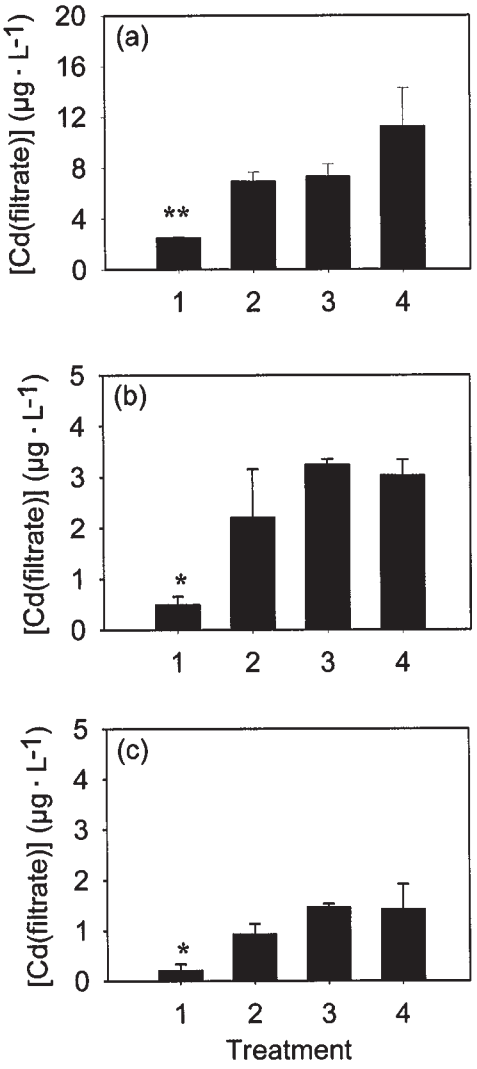
$$\text{Sediment } K_d^a = \frac{c_s}{c_f c_{ss}}$$

where c_p is the concentration of Cd adsorbed onto >1-µm particles (moles per kilogram), which is equal to the Cd concentration in the total water sample ([Cd(total)]) minus the Cd concentration in the 1-µm filtrate ([Cd(filtrate)]), c_s is the concentration of Cd in sediments (moles per kilogram of dry sediment), c_f is [Cd(filtrate)] (moles per kilogram), and c_{ss} is the concentration of suspended solids (milligrams per litre). Partitioning coefficients were termed "apparent", since the system was not in equilibrium.

The Cd body burden or "content" for mussel whole bodies and tissues was calculated as Cd body burden (micrograms) = Cd concentration (micrograms per gram dry weight) × body or tissue weight (grams dry weight).

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 mussel size and metal concentrations in water, particulate material, and mussels among treatments were determined using SAS version 6.08 ANOVA, randomized blocks design (SAS Institute Inc. 1989). Differences in mussel condition and background tissue metal concentrations among Lake 104, the cage, and the treatments were also tested using ANOVA.

Fig. 3. Cd concentration in filtered water samples (<1 µm) at (*a*) day 0, (*b*) day 40, and (*c*) day 80. Values are means ± SE (n = 2). Treatment 1 is significantly different from the other treatments at $^*P < 0.05$ and $^{**}P < 0.01$.



Results

Loss of Cd and other metals from the water column

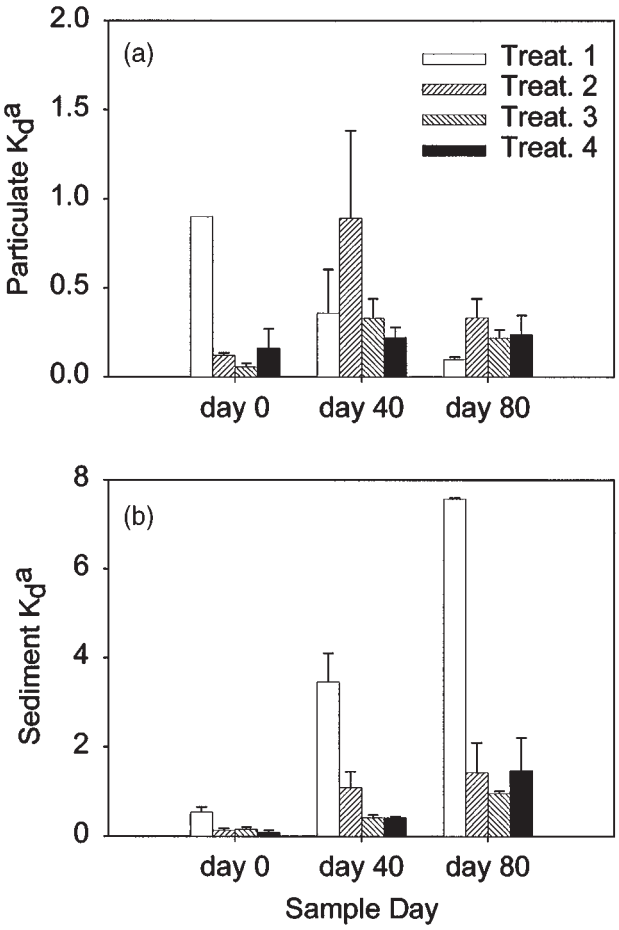
Cadmium remained in the water column longer in treatments with the metal mixture than with Cd alone (Fig. 2; Table 3). The half-life for Cd in treatment 1 was 11 ± 1 days (mean ± SE) compared with 25 ± 2, 34 ± 5, and 33 ± 3 days for treatments 2, 3, and 4, respectively. Water column [Cd(total)] in treatment 1 was significantly less than in treatments with the metal mixture on days 0 ($F_{3,3}$ = 19.97, P = 0.02) and 40 ($F_{3,3}$ = 40.54, P = 0.006) but not on day 80 (Table 3). As expected, Cu, Zn, Pb, and Ni concentrations in the water column of treatments 2–4 increased with increasing concentration of the metal mixture and declined with time (Table 3). Non-Cd metal concentrations in treatment 1 generally were indistinguishable from background concentrations as represented by Roddy Lake values (Table 3).

Table 3. Metal concentrations ($\mu g \cdot L^{-1}$) in unfiltered water on days 0, 40, and 80.

	Day	pH	Cd	Cu	Zn	Pb	Ni
Roddy Lake	0	7.13	<0.02	<0.5	0.63	<0.3	<0.5
Treatment 1	0	6.89	4.61±0.02	<0.5	3.82±2.06	<0.3	<0.5
Treatment 2	0	7.04	9.30±0.87	11.9±1.6	128±8	29.9±5.4	18.5±0.7
Treatment 3	0	6.82	7.95±0.81	17.4±1.0	209±14	46.3±1.8	24.6±0.7
Treatment 4	0	6.78	13.7±1.7	30.8±0.6	414±4	87.0±2.0	56.7±5.4
Roddy Lake	40	7.27	<0.02	<0.5	1.55	0.52	<0.5
Treatment 1	40	7.33	0.66±0.14	<0.5	1.19±0.08	<0.3	<0.5
Treatment 2	40	7.02	3.20±0.63	4.55±0.47	38.0±5.4	4.38±0.62	6.15±1.06
Treatment 3	40	7.49	4.34±0.17	6.7±0.34	78.9±2.7	7.41±0.57	12.5±0.8
Treatment 4	40	7.56	4.00±0.17	10.7±0.4	155±21	12.1±1.5	21.9±0.7
Roddy Lake	80	6.74	<0.02	0.72	1.4	0.93	<0.5
Treatment 1	80	7.49	0.71±0.02	<0.5	2.50±1.69	<0.3	<0.5
Treatment 2	80	6.77	1.02±0.09	2.97±0.06	23.4±1.1	1.30±0.003	4.03±0.31
Treatment 3	80	7.59	1.66±0.03	4.12±0.17	47.6±1.0	1.68±0.26	7.59±0.23
Treatment 4	80	7.43	1.60±0.50	5.19±0.74	82.6±19.3	2.49±0.95	11.2±1.6

Note: Values are means ± SE (n = 2), except pH (n = 1).

Fig. 4. (*a*) Particulate (>1 μm) and (*b*) sediment apparent partitioning coefficients for Cd in the limnocorrals on each sample day. Partitioning coefficients are calculated as ratios of the Cd concentration adsorbed onto particles or sediment to [Cd(filtered)]. Particulate partitioning coefficients are corrected for the concentration of suspended solids in the water.



The trends in the Cd concentration in the <1-μm filtrate ([Cd(filtrate)]) among treatments (Fig. 3) were similar to those for [Cd(total)] (Table 3) on each sample day. Values for [Cd(filtrate)] were significantly higher in treatments with the metal mixture compared with those in treatment 1 on days 0 ($F_{3,3}$ = 32, P < 0.01), 40 ($F_{3,3}$ = 13, P < 0.05), and 80 ($F_{3,3}$ = 13, P < 0.05) (Fig. 3).

The trends in apparent partitioning coefficients for Cd on particles (K_d^a) (Fig. 4*a*) were less clear than those for the sediment K_d^a (Fig. 4*b*). The particulate K_d^a for treatment 1 progressively decreased from day 0 (c_p = 0.087, $c_f c_{ss}$ = 0.097) to day 80 (c_p = 0.001, $c_f c_{ss}$ = 0.014), corresponding to the loss of Cd from the water column to the sediments over the same time period (Fig. 4*a*). On day 0, particulate K_d^a in treatment 1 was considerably larger than those in treatments 2–4, but by day 40 was similar to those in treatments 3 and 4 that had increased from day 0. At the same time, treatment 2 particulate K_d^a was somewhat elevated above those in all other treatments, although its standard error was quite high. By day 80, values of particulate K_d^a in treatments 2–4 were larger than that in treatment 1. The K_d^a for the sediment in treatment 1 was consistently larger than those in treatments 2–4 and increased over time (Fig. 4*b*). This is consistent with the greater loss of Cd from the water column, represented by a smaller half-life (Fig. 2), and its sorption onto the surface sediment in treatment 1 relative to treatments 2–4.

Metal concentrations in sediments

Cadmium concentrations in limnocorral sediments reached target concentrations by day 0 in treatment 1 and by day 40 in treatments 2 and 3 but not until day 80 in treatment 4 (Table 4). Target concentrations of the other metals were reached in the sediments by 40 or 80 days (Table 4). Nickel concentrations in all treatments were slightly below target levels, and Pb concentrations in treatment 3 and 4 limnocorrals exceeded target levels. Unexpectedly, all of the metals were elevated in limnocorral 2A on day 40 relative to day 80.

Table 4. Metal concentrations in limnocorral sediments on days 0, 40, and 80 compared with background sediment metal concentrations in Roddy Lake (background) and target metal concentrations.

			Metal concentration (μg·g dry weight^{-1})			
Metal	Background	Treatment	Target	Day 0	Day 40	Day 80
Cd	0.2±0.03	1	1.5	1.4±0.1	1.7±0.1	1.8±0.03
		2	1.5	1.0±0.1	2.1±0.2	1.4±0.2
		3	1.5	1.1±0.1	1.4±0.1	1.4±0.02
		4	1.5	1.0±0.1	1.3±0.1	1.7±0.2
Cu	0.6±0.07	1	Background	0.7±0.2	0.7±0.05	0.9±0.1
		2	1.6	1.0±0.2	1.7±0.3	1.4±0.4
		3	2.2	1.1±0.1	1.8±0.1	2.1±0.2
		4	3.8	1.9±0.2	3.2±0.3	3.6±0.6
Zn	5.3±0.8	1	Background	7.5±0.2	6.6±0.2	6.3±0.9
		2	16	11.1±1.2	18.0±2.1	12.3±2.9
		3	21	13.7±1.4	18.5±0.8	20.3±1.1
		4	37	17.3±1.0	27.2±2.0	34.3±4.5
Pb	1.8±0.04	1	Background	1.5±0.1	2.0±0.2	1.9±0.1
		2	5.4	3.6±0.3	9.5±1.4	5.3±1.6
		3	7.2	5.3±0.8	9.0±0.6	10.2±1.8
		4	13	9.3±1.6	17.3±1.1	23.1±5.6
Ni	1.0±0.4	1	Background	0.9±0.04	1.0±0.1	1.3±0.2
		2	3.1	1.9±0.3	2.9±0.7	2.5±0.7
		3	4.1	1.8±0.3	3.3±0.2	3.8±0.2
		4	7.2	2.5±0.3	4.6±0.3	6.5±0.9

Note: Values are means ± SE.

Table 5. Condition indices of mussels collected in October 1992 from the treatments and cage in Roddy Lake and source Lake 104.

	Condition index		
	Fresh, day 80	40–80 days	0–80 days
Lake 104	0.183±0.03	—	—
Cage	—	0.182±0.03	0.168±0.01
Treatment 1	—	0.179±0.01	0.129±0.01
Treatment 2	—	0.155±0.01	0.146±0.01
Treatment 3	—	0.189±0.02	0.146±0.004
Treatment 4	—	0.193±0.01	0.127±0.01

Note: Values for Lake 104 and the cage are means ± SE of five or six animals each. Values for treatments are means of replicate treatments consisting of means of five animals in each.

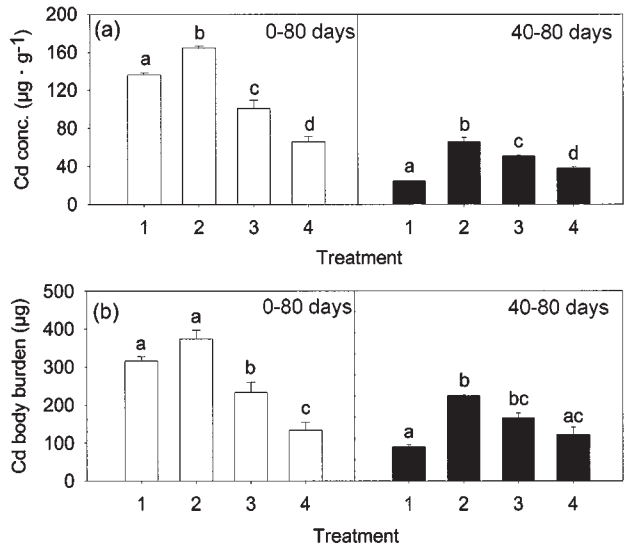
Condition of the mussels

Transplanting mussels from Lake 104 to the cage in Roddy Lake for 80 days did not significantly affect condition. Mussels collected from the cage in Roddy Lake in October had a condition index of 0.168, which was not significantly different from the condition index of 0.183 for mussels freshly collected from Lake 104 in October (Table 5). Enclosure in the limnocorrals for 80 days relative to caged mussels in Roddy Lake, nevertheless, was associated with a decline in condition in treatments 1 and 4 but not in treatments 2 and 3 ($F_{5,1}$ = 11, P = 0.04) (Table 5). This difference between mussels in the limnocorrals and the cage was not seen in the 40- to 80-day mussels. The longer the mussels were held in the limnocorrals, the lower their condition. For example, 0- to 80-day mussels in treatment 4 were lower in condition than 40- to 80-day mussels in treatment 4 ($F_{5,1}$ = 9, P = 0.03). The intensity of the treatment did not affect condition. Condition did not vary significantly among the four treatments for either 0- to 80- or 40- to 80-day mussels.

Accumulation of Cd by mussels

Caging mussels in Roddy Lake for 80 days did not result in a change in whole-body Cd concentrations ([Cd(body)]) compared with Lake 104 mussels freshly collected on the same day (1.62 versus 1.29 μg·g dry weight^{-1}) (P > 0.05). Treatment had a significant effect on Cd concentrations in 0- to 80-day mussels. Not surprisingly, treatment 1 mussels had lower [Cd(body)] than mussels in treatment 2 in the 0- to 80-day group and in treatments 2–4 in the 40- to 80-day group. This is related to lower water column Cd concentrations in treatment 1 compared with the other treatments, not only on days 0 and 40 when the mussels were introduced into the limnocorrals (P < 0.005) but over the entire exposure period (Fig. 2). Among treatments with the metal mixture, [Cd(body)] declined with increasing addition of the metal mixture ($F_{3,3}$ = 65, P < 0.005) (Fig. 5*a*). As expected, the 40- to 80-day mussels, introduced into the limnocorrals on day 40, accumulated significantly less Cd than did the 0- to 80-day mussels (P < 0.0001). Despite the lower exposure levels, the 40- to 80-day mussels also showed the decline in [Cd(body)] with increasing addition of the metal mixture ($F_{3,3}$ = 57, P = 0.005).

Fig. 5. Cd (*a*) concentration and (*b*) content in whole bodies (dry weight) of 0–80 and 40–80 day mussels. Bars are means ± SE for each treatment (n = 2). Bars with the same letter are not different at the P = 0.05 level (0–80 and 40–80 day mussels are statistically separate).



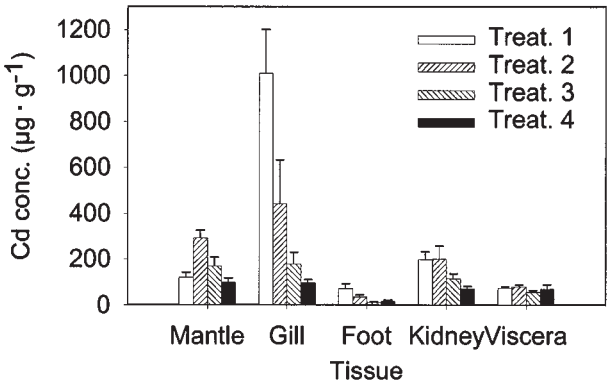
Expressing metal levels as body burdens, which adjusts for variable contribution by soft tissues to total body weight, resulted in fewer statistical differences among treatments. Nevertheless, whole-body Cd burdens among treatments showed the same trends as [Cd(body)] (Fig. 5*b*).

In general, the highest tissue Cd concentration was found in the gill followed by kidney and mantle. Like whole-body concentrations and body burdens, tissue Cd concentration declined with increasing metal mixture concentration (Fig. 6). In the gill, Cd concentrations were highest in mussels from treatment 1 and decreased with increasing concentration of the metal mixture. This trend was also observed for Cd concentrations in the foot. In the kidney, Cd levels were similar in treatments 1 and 2 and lower in treatments 3 and 4. In the mantle, Cd concentration decreased from treatment 2 to 4 with the increasing concentration of the metal mixture. There were no obvious differences among treatments in viscera Cd concentration.

Accumulation of Cu, Zn, Pb, and Ni by mussels

The 0- to 80- and 40- to 80-day mussels in the cage and in treatment 1 had background concentrations of Cu, Zn, Pb, and Ni (Fig. 7). In treatments with the metal mixture, mussels accumulated substantial amounts of Cu, Zn, Pb, and Ni (Fig. 7). Two different accumulation patterns among metals were found. Whole-body Cu, Zn, and Ni concentrations in 0- to 80- and 40- to 80-day mussels generally did not reflect the proportional increase in metals added to the limnocorrals in treatments 2–4. Lead was the only metal to reflect the increasing metal concentrations among treatments; this was observed in both the 0- to 80- and 40- to 80-day mussels (Fig. 7*c*). Whole-body Pb concentrations in treatment 4 mussels were significantly higher than in treatment 2 mussels (40- to 80-days, $F_{3,3}$ = 348, P < 0.0005; 40- to 80-days, $F_{3,3}$ = 69, P < 0.005).

Fig. 6. Cd concentration in five tissues (dry weight) of 0–80 day mussels. Bars are means ± SE for each treatment (n = 1).

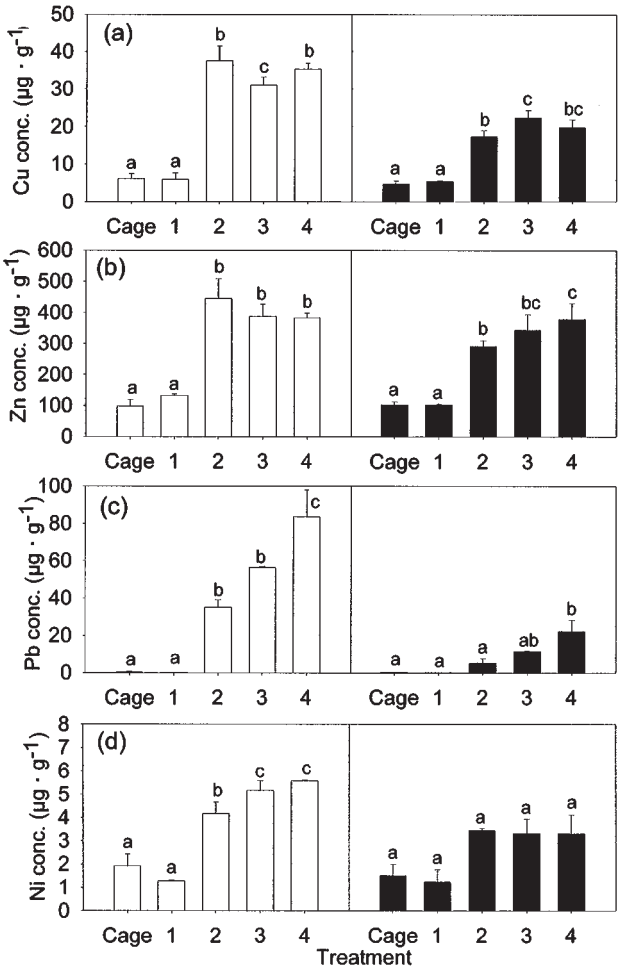


Discussion

Partitioning of Cd in the water column

The presence of the metal mixture had a significant effect on the partitioning of Cd in the water, resulting in increased residence times for Cd in the water column and higher concentrations in the water at each sampling compared with the treatment without the mixture. Radioisotope studies performed at the ELA using shallow limnocorrals showed that metals are removed from the water column by two primary mechanisms: (*i*) adsorption onto particulate material and subsequent settling to the bottom of the limnocorrals and (*ii*) adsorption directly onto sediments after diffusion through a stagnant film (diffusive sublayer) overlaying the sediments (Santschi et al. 1986). In the present study, both removal processes may have been influenced by some form of competition among metals for binding sites resulting in longer residence time for Cd in the water column. For example, the lower particulate K_d^a in treatments 2–4 on day 0 suggests that there was less Cd adsorbed onto particles in the presence of other metals, thus reducing the removal of Cd from the water column by settling particles. Furthermore, the distinctly lower sediment K_d^a in treatments with the metal mixture strongly suggests that binding sites on the surface sediment (approximately the top 1 mm) may have initially been saturated by the metals, leading to increased competition and decreased adsorption. Santschi et al. (1986) found that the apparent K_d for ^{59}Fe adsorption onto suspended particles was smaller in a limnocorral that received an addition of stable Fe, resulting in a slower removal of stable Fe from the water column. The authors suggested that the increased Fe loading saturated the adsorption capacity of the particles. Despite the possibility of initial saturation of surface sediments in this experiment, it is unlikely that the sediments were completely saturated and unable to absorb more metal because by day 80, Cd concentrations in sediment were similar among treatments. Over time, metal-laden particles move deeper into sediments by physical processes and molecular diffusion opening up new binding sites at the sediment–water interface (Santschi et al. 1986). Adsorption onto limnocorral walls and colonizing organisms was not found to play a significant role in the removal of metals from the water column in the Santschi et al. (1986) experiments (<5%) and therefore was not considered in the present study. This ex-

Fig. 7. Concentrations of (*a*) Cu, (*b*) Zn, (*c*) Pb, and (*d*) Ni in whole bodies (dry weight) of 0–80 day mussels (open bars) and 40–80 day mussels (solid bars). Bars are means ± SE for each treatment (n = 2). Bars with the same letter are not different at the P = 0.05 level (0–80 and 40–80 day mussels are statistically separate).



trapolation was valid, since the two experiments were similar in conception (materials, wall to sediment surface area ratio, and chemistry), and mass balance estimates in the present study for water and sediment account for all of the added Cd on day 0, prior to the addition of the mussels.

Various metals are thought to sorb predominantly to different molecular binding sites, but some sites may be mutually shared (Sigg et al. 1987). The result would be competition among metals for mutually shared sites. For example, Benjamin and Leckie (1980) found that Zn had a greater effect than Cu or Pb on Cd adsorption onto oxides, suggesting that Cd and Zn may have some shared binding sites. On a molar basis, there was five to seven times more Cu, Pb, and Ni and 43 times more Zn than Cd added to the limnocorrals in the present study. The high ratio of Zn to Cd makes it likely that Zn had the greatest impact on the sorption of Cd to particles.

Competition from Ca and Mg has also been shown to decrease Cd adsorption onto particulate matter (Laxen 1985; Cowan et al. 1991). It has been shown that Ca (2.5 mM) reduced the sorption of Cd (1 µM) to amorphous iron oxyhydroxide, due to competition for mutually accessible surface sites (Cowan et al. 1991). Furthermore, Ca concentrations as low as 0.25 mM were also found to cause the desorption of Cd from amorphous iron oxyhydroxide. Chemical analysis of the water showed relatively low Ca concentrations that increased with metal addition on days 0, 40, and 80 (0.06 mM in treatment 1 versus 0.1 mM in treatment 4 on day 80).

Differences in pH among the treatments (6.78–7.04) on day 0 were sufficient to influence the partitioning of Cd in the water column, but the result was contrary to that predicted. The higher pH in treatments 3 and 4 relative to treatments 1 and 2 on day 40 should have resulted in a higher particulate K_d^a, which was not the case (see Fig. 3). Wagemann et al. (1994) reported a significant shift of Cd from ionic Cd^{2+} to particulate-bound Cd at pH > 6.5, where the percentage of particulate Cd exceeded Cd^{2+} at around pH 7.4 (for 1.0 mg suspended sediment·L^{-1}).

The addition of the metal mixture along with Cd resulted in a decrease in the loss rate of Cd from the water column and a greater opportunity for uptake by the mussels. It is important to note that the sediments used in this study had very low background metal concentrations and that more contaminated sediments nearing saturation may have resulted in an even greater reduction in Cd removal from the water column by the metal mixture.

Factors affecting mussel condition

The condition of mussels transplanted and caged outside the limnocorrals for 80 days did not significantly differ from the condition of mussels from the source lake, suggesting that the animals did not experience significant transplant stress. This is consistent with the high degree of similarity between Roddy Lake and Lake 104 water chemistry. However, mussels held in the limnocorrals showed a loss of condition that worsened with time, suggesting stress due to the enclosure or exposure to metals. Results from acute laboratory exposures of juvenile *Anodonta imbecilis* (Keller and Zam 1991) indicate that Cu, Zn, and Cd may have been at lethal concentrations in treatment 4 at the beginning of the experiment and may have caused the observed loss in mussel condition and mortalities in this treatment. However, this observation is not supported by the fact that the loss of condition in treatment 4 mussels was only slightly greater than in mussels in treatment 1 not exposed to toxic metal mixture concentrations. The lack of a significant difference in condition among treatments in either exposure group (0–80 or 40–80 days) suggests that the length of enclosure was more important than was the treatment concentration in causing the loss of condition in the mussels. Loss of condition may be attributed to a variety of factors including metal toxicity and the abundance and nutritional quality of suspended particles filtered by the mussels. Given that the control cage (exchange with lake water) was not a true control for the enclosure effect of the limnocorral (no exchange with lake water), it is not possible to distinguish unequivocally between the effects of the enclosure and metal exposure on mussel condition.

A consequence of lower condition in mussels is artificially inflated tissue metal concentrations that can affect the conclusions drawn from a study (Salazar et al. 1996). Expressing metal accumulation on a content basis controls for losses in soft tissue weights and allows treatment groups to

be more accurately compared. In the present study, the conclusions were essentially the same whether the metals were expressed as concentrations or as contents (Fig. 5).

Effect of the metal mixture on Cd accumulation

Despite the higher water column Cd concentrations in the presence of the metal mixture, Cd accumulation in the 0- to 80-day mussels was progressively lower with increasing concentrations of the metal mixture. This same trend was observed in 40- to 80-day mussels exposed to overall lower metal concentrations (70–90% lower, see Table 4). These results are consistent with those from other studies that examined the interactions between Cd and one or two of the metals in the mixture. Hemelraad et al. (1987) exposed the freshwater mussel *Anodonta cygnea* to Cd (25 $\mu g \cdot L^{-1}$) and to a mixture of Cd (25 $\mu g \cdot L^{-1}$) and Zn (2.5 $mg \cdot L^{-1}$) in the laboratory for 16 weeks, without food. In the presence of Zn, Cd accumulation was reduced by 50% in the whole animal and by over 50% in the gill and mantle, although it was not affected in the kidney. A similar reduction in Cd accumulation in the presence of Zn was observed in *Mytilus edulis* exposed in a laboratory factorial experiment to 20 $\mu g\ Cu \cdot L^{-1}$ and 200 $\mu g\ Zn \cdot L^{-1}$ (Elliott et al. 1986). In the present limnocorral experiment, Cd accumulation at the highest mixture concentrations in treatments 3 and 4 was lower than in treatment 1 by 26 and 50% in the whole body and by 42 and 64% in the kidney, respectively. Reduced uptake in the gill was observed at all concentrations of the metal mixture compared with treatment 1 and ranged from 56 to 91%.

A possible explanation for the reduced Cd uptake at higher concentrations of the metal mixture is that mussels spent more time with their valves closed. A reduction in the periods of valve openness and time actively filtering might be expected to reduce metal uptake. Salánki and V.-Balogh (1989) found a decrease in the duration of active filtration periods in the unionid mussel *A. cygnea* from 20 h down to 8 h and from 30–60 h down to 7 h during 240-h exposures to 10 $\mu g\ Cu \cdot L^{-1}$ and 50 $\mu g\ Pb \cdot L^{-1}$, respectively. The effect of reduced filtration activity on metal uptake in mussels is not well understood at this time. Pynnönen (1995) found that Cd concentrations in the gill of *A. cygnea* exposed in the laboratory for 2 weeks were less after exposure to 200 $\mu g\ Cd \cdot L^{-1}$ than after exposure to 50 $\mu g\ Cd \cdot L^{-1}$. Further studies identified a positive correlation between filtration activity and Cd accumulation in *A. cygnea* exposed to 50 $\mu g\ Cd \cdot L^{-1}$ (K. Pynnönen, Department of Zoology, University of Helsinki, Helsinki, Finland, personal communication). In contrast, an experiment that studied the effects of Cd, Cu, and Zn interactions on the filtration rate of the zebra mussel *Dreissena polymorpha* found that Cd was accumulated at all concentrations (10–1000 $\mu g\ Cd \cdot L^{-1}$), despite up to an 80% decrease in filtration rate (Kraak et al. 1994). Two lines of evidence in the present study suggest that reduced Cd accumulation with increasing metal mixture concentration cannot be simply explained by valve closure. It would be expected that increasing concentrations of a metal mixture would have led to progressive reduction in active filtration periods and to a reduction in food intake and consequently a loss in condition. Nevertheless, a loss of condition was not correlated with metal mixture concentration. Furthermore, reduced uptake of all the metals at higher concentrations of the mixture would have been expected and this was not observed (Fig. 6). For example, a treatment-dependent increase in Pb was observed in 0- to 80-day mussels. Relatively similar concentrations of Cu, Zn, and Ni were observed among the treatments, but concentrations of the essential metals Cu and Zn may have been determined by physiological regulation. The relationships among environmental concentrations of metals, filtering activity, and metal uptake are very important in assessing the value of mussels in bioaccumulation monitoring programs; thus, a better understanding of these relationships is needed. Specifically, studies are needed that use environmentally relevant metal concentrations and chronic exposure periods (weeks) that link thresholds for changes in filtration activity to impacts on metal uptake.

The soft tissue metal concentrations observed in this study were similar to values obtained for *P. grandis* in mining areas. Cadmium concentrations in the treated 0- to 80- and 40- to 80-day mussels ([Cd(body)] = 24–153 $\mu g \cdot g^{-1}$) were in the range of resident *P. grandis* ([Cd(body)] = 19–129 $\mu g \cdot g^{-1}$) collected along a metal contamination gradient in the Rouyn-Noranda mining region, northwestern Quebec (Couillard et al. 1993). However, Cd levels in the gill of 0- to 80-day mussels from treatments 1 and 2 (400–1000 $\mu g\ Cd \cdot g^{-1}$) were nearly four times higher than those measured in *P. grandis* collected from Rouyn-Noranda (38–270 $\mu g\ Cd \cdot g^{-1}$). The higher Cd concentrations in the gill may reflect higher water column concentrations in the present experiment compared with the exposure concentrations of the Rouyn-Noranda mussels. Furthermore, 80 days may be insufficient for metals accumulated in the gills to be translocated to other organs for storage.

Competition among metals in the environment and on biological surfaces

The aim of this experiment was to determine if metal mixtures affect the bioavailability of Cd in the aquatic environment and if the resulting available Cd concentrations influence Cd uptake in freshwater mussels. The partitioning of Cd in the water on particles and in the sediment over the course of the experiment showed that the metal mixture slowed the removal of Cd from the water column, possibly through competition for sites on settling particles and on the surface sediment. This resulted in more Cd in the water column available for uptake by the mussels in the presence of the mixture. Nevertheless, the higher water column Cd concentrations did not result in increased accumulation of Cd by freshwater mussels. It appeared that some mechanism related to the mussel and not to the distribution of Cd in the environment influenced Cd uptake.

Assuming that valve closure was not a determining factor, there are two possible explanations for these results, depending on the primary pathway of Cd uptake in freshwater mussels. If the mussels accumulate most of their metal burden from the water, then direct competition among metals for sites on the mussels (i.e., gill epithelia) might have reduced Cd uptake. Recent studies by Wang et al. (1996) using the marine mussel *M. edulis* suggest that although Cd is accumulated from both dissolved and particulate pathways, the largest portion of Cd is obtained from dissolved sources (>50–80%). The principal mechanism of Cd uptake by freshwater organisms is through binding to membrane trans-

port ligands or by incorporation of Cd into an active pump for a major ion that deposits the metals into the interior of the cell (Rainbow and Dallinger 1993). Competition among metals may have occurred at these transport sites. A less likely alternative is that the portion of Cd accumulated from particles or "food" could have been reduced at the highest concentration of the metal mixture due to reduced sorption on particles. The lower particulate K_d^a in the treatments with the mixture on day 0 seems to suggest this, although sample variability on day 40 makes it difficult to confirm the relationship.

Current knowledge of the behavior of trace metals under varying degrees of chemical equilibrium is insufficient to allow for the broad generalization of these results. For this reason, further testing of metal mixtures at chemical equilibrium is needed. Under nonequilibrium conditions, the uncoupling of the effects of the metal mixture on Cd bioavailability and uptake suggests that laboratory studies may be appropriate for characterizing metal uptake in mussels exposed to mixtures of metals. The effect of the metal mixture to prolong the high water column Cd concentrations and reduce uptake in the mussels is a significant deviation from the behavior of Cd added alone. These results emphasize the need to further examine the appropriateness of water and sediment quality criteria developed for individual contaminants (Canadian Council of Resource and Environment Ministers 1987; Jaagumagi 1992).

Acknowledgements

This research was supported by the Green Plan Toxic Chemicals Program and a University of Manitoba Fellowship to R. Stewart. D. Tenkula, R. Herzog, and M. Holoka greatly assisted in the field, R. Hunt, C. Ranson, and K. Ramlal advised on metal analyses, B. de March and S. Kasian advised on experimental design and statistics, and Drs. D.F. Malley, J. Klaverkamp, and K. Pynnönen and three anonymous reviewers provided constructive comments on the manuscript.

References

AQUAMIN. 1996. Assessment of the aquatic effects of mining in Canada. Prepared for AQUAMIN Steering Group. (Available from Environment Canada, Evaluation and Interpretation Branch, AQUAMIN Secretariat, Ottawa, ON K1A 0H3, Canada.)

Benjamin, M.M., and Leckie, J.O. 1980. *In* Contaminants and sediments. Vol. 2. *Edited by* R.A. Baker. Ann Arbor Science Publishers, Ann Arbor, Mich.

Brunskill, G.J., and Schindler, D.W. 1971. Geography and bathymetry of selected lake basins, Experimental Lakes Area, northwestern Ontario. J. Fish. Res. Board Can. **28**: 139–155.

Campbell, P.G.C., and Tessier, A. 1996. Ecotoxicology of metals in the aquatic environment: geochemical aspects. *In* Ecotoxicology. A hierarchical treatment. *Edited by* M.C. Newman and C.H. Jagoe. Lewis Publishers, Chelsea, Mich. pp. 11–58.

Canadian Council of Resource and Environment Ministers. 1987. Canadian water quality guidelines. Task force on water quality guidelines. Environment Canada, Water Quality Objectives Division, Ottawa, Ont.

Couillard, Y., Campbell, P.G.C., and Tessier, A. 1993. Response of metallothionein concentrations in a freshwater bivalve *Anodonta grandis* along an environmental cadmium gradient. Limnol. Oceanogr. **38**: 299–313.

Couillard, Y., Campbell, P.G.C., Tessier, A., Pellerin-Massicotte, J., and Auclair, J.C. 1995. Field transplantation of a freshwater bivalve, *Pyganodon grandis*, across a metal contamination gradient. I. Temporal changes in metallothionein and metal (Cd, Cu, and Zn) concentrations in soft tissues. Can. J. Fish. Aquat. Sci. **52**: 690–702.

Cowan, C.E., Zachara, J.M., and Resch, C.T. 1991. Cadmium adsorption on iron oxides in the presence of alkaline-earth elements. Environ. Sci. Technol. **25**: 437–446.

Davenport, J., and Chen, X. 1987. A comparison of methods for the assessment of condition in the mussel (*Mytilus edulis* L.). J. Molluscan Stud. **53**: 293–297.

Elliott, N.G., Swain, R., and Ritz, D.A. 1986. Metal interactions during accumulation by the mussel *Mytilus edulis planulatus*. Mar. Biol. **93**: 395–399.

European Inland Fisheries Advisory Commission. 1987. Water quality criteria for European freshwater fish. EIFAC (Eur. Inland Fish. Advis. Comm.) Tech. Pap. 37. Rev. 1.

Harrison, S.E., and Klaverkamp, J.F. 1990. Metal contamination in liver and muscle of northern pike (*Esox lucius*) and white sucker (*Catostomus commersoni*) and in sediments at Flin Flon, Manitoba. Environ. Toxicol. Chem. **9**: 941–956.

Hemelraad, J., Kleinveld, H.A., De Roos, A.M., Holwerda, D.A., and Zandee, D.I. 1987. Cadmium kinetics in freshwater clams. III. Effects of zinc on uptake and distribution of cadmium in *Anodonta cygnea*. Arch. Environ. Contam. Toxicol. **16**: 95–101.

Hurlbert, S.H. 1984. Pseudoreplication and the design of ecological field experiments. Ecol. Monogr. **54**: 187–211.

Jaagumagi, R. 1992. Development of Ontario provincial sediment quality guidelines for arsenic, cadmium, chromium, copper, lead, manganese, mercury, nickel and zinc. Ontario Ministry of the Environment, Toronto, Ont.

Keller, A.E., and Zam, S.G. 1991. The acute toxicity of selected metals to the freshwater mussel, *Anodonta imbecilis*. Environ. Toxicol. Chem. **10**: 539–546.

Kraak, M.H.S., Lavy, D., Schoon, H., Toussaint, M., Peeters, W.H.M., and Van Straalen, N.M. 1994. Ecotoxicity of mixtures of metals to the zebra mussel *Dreissena polymorpha*. Environ. Toxicol. Chem. **13**: 109–114.

Laxen, D.P.H. 1985. Trace metal adsorption/coprecipitation on hydrous ferric oxide under realistic conditions. Water Res. **19**: 1229–1236.

Luoma, S.N. 1983. Bioavailability of trace metals to aquatic organisms — a review. Sci. Total Environ. **28**:1–22.

Malley, D.F., Chang, P.S.S., and Hesslein, R.H. 1989. Whole lake addition of cadmium-109: radiotracer accumulation in the mussel population in the first season. Sci. Total Environ. **87/88**: 397–417.

Malley, D.F., Stewart, A.R., and Hall, B.D. 1995. Uptake of methyl mercury by the floater mussel, *Anodonta grandis grandis* (Bivalvia, Unionidae) caged in a flooded wetland. Environ. Toxicol. Chem. **15**: 928–936.

Morel, F.M.M. 1983. Principles of aquatic chemistry. John Wiley & Sons, Inc., Toronto, Ont.

Nriagu, J.O. 1990. Global metal pollution. Poisoning the biosphere? Environment (Washington, D.C.), **32**: 7–33.

Pynnönen, K. 1995. Effect of pH, hardness and maternal preexposure on the toxicity of Cd, Cu and Zn to the glochidial larvae of a freshwater clam *Anodonta cygnea*. Water Res. **29**: 247–254.

Rainbow, P.S., and Dallinger, R. 1993. Metal uptake, regulation and excretion in freshwater invertebrates. *In* Ecotoxicology of metals in invertebrates. *Edited by* R. Dallinger and P.S. Rainbow. SETAC Spec. Publ. Ser. Lewis Publishers, Boca Raton, Fla. pp. 119–132.

Salánki, J., and V.-Balogh, K. 1989. Physiological background for using freshwater mussels in monitoring copper and lead pollution. Hydrobiologia, **188/189**: 445–454.

Salazar, S.M., Beckvar, N., Salazar, M.H., and Finkelstein, K. 1996. An in situ assessment of mercury contamination in the Sudbury River, Massachusetts, using bioaccumulation and growth in transplanted freshwater mussels. NOAA Tech. Memo. NOS ORCA 89. National Oceanic and Atmospheric Administration, Seattle, Wash.

Santschi, P.H., Nyffeler, U.P., Anderson, R.F., Schiff, S.L., and 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.

SAS Institute Inc. 1989. SAS/STAT user's guide, version 6, edition 4. Vol. 2. SAS Institute Inc., Cary, N.C.

Schindler, D.W. 1988. Experimental studies of chemical stressors on whole lake ecosystems. Verh. Int. Ver. Theor. Angew. Limnol. **23**: 11–41.

Sigg, L., Sturm, M., and Kistler, D. 1987. Vertical transport of heavy metals by settling particles in Lake Zurich. Limnol. Oceanogr. **32**: 112–130.

Spehar, R.L., and Fiandt, J.T. 1986. Acute and chronic effects of water quality criteria-based metal mixtures on three aquatic species. Environ. Toxicol. Chem. **5**: 917–931.

SPSS Inc. 1997. SigmaPlot 4.0 for Windows. User's manual. SPSS Inc., Chicago, Ill.

Stainton, M.P., Capel, M.J., and Armstrong, F.A.J. 1977. The chemical analysis of freshwater. 2nd ed. Can. Fish. Mar. Serv. Misc. Spec. Publ. No. 25.

Stewart, A.R. 1997. Technical evaluation of molluscs as a biomonitoring tool for the Canadian mining industry. Aquatic Effects Technical Evaluation Program Project 2.3.1. Natural Resources Canada, Ottawa, Ont.

Tankersley, R.A., and Dimock, R.V., Jr. 1993. The effect of larval brooding on the filtration rate and particle retention efficiency of *Pyganodon cataracta* (Bivalvia: Unionidae). Can. J. Zool. **71**: 1934–1944.

Tessier, A., Couillard, Y., Campbell, P.G.C., and Auclair, J.C. 1993. Modeling Cd partitioning in oxic lake sediments and Cd concentrations in the freshwater bivalve *Anodonta grandis grandis*. Limnol. Oceanogr. **38**:1–17.

Voyer, R.A., and Heltshe, J.F. 1984. Factor interactions and aquatic toxicity testing. Water Res. **18**: 441–447.

Wagemann, R., Capel, M.J., Hesslein, R., and Stephenson, M. 1994. Sediment–water distribution coefficients and speciation of cadmium in a Canadian Shield lake. Can. J. Fish. Aquat. Sci. **51**: 1951–1958.

Wang, W.X., Fisher, N.S., and Luoma, S.N. 1996. Kinetic determinations of trace element bioaccumulation in the mussel *Mytilus edulis*. Mar. Ecol. Prog. Ser. **140**: 91–113.

Wood, T.M., Baptista, A.M., Kuwabara, J.S., and Flegal, A.R. 1995. Diagnostic modeling of trace metal partitioning in south San Francisco Bay. Limnol. Oceanogr. **40**: 345–358.