A FIELD STUDY OF CADMIUM DYNAMICS IN PERIPHYTON AND IN *Hyalella azteca* (CRUSTACEA: AMPHIPODA)

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Abstract. We studied the dynamics of Cd uptake and depuration in epilithic periphyton and in the grazing amphipod *Hyalella azteca*. Both stable Cd, sufficient to achieve an aqueous concentration of 90 ng L^{-1}, and its radiotracer ^{109}Cd, were added during 1987 to the epilimnion of oligotrophic Lake 382 of the Experimental Lakes Area in northwestern Ontario. Cadmium dynamics within both periphyton and *Hyalella* were rapid, with equilibrium being approached within two weeks. For periphyton, the Cd uptake rate constant (K_{uw}) was 3.8×10^4 d^{-1} with a depuration rate of 0.29 d^{-1}. For *Hyalella* the depuration rate was 0.36 d^{-1}, 10% due to growth dilution and 90% to excretion or desorption. The total Cd uptake rate (k_{ut}) by *Hyalella* was 6.1×10^4 d^{-1}, with more of the uptake (58%) derived from food (periphyton) than from water. *Hyalella* assimilated 80% of ingested Cd. Steady-state bioconcentration factors (BCF) were at least 10-fold higher than previously published values for Amphipoda. In periphyton and *Hyalella* the BCF were 1.2×10^5 and 3.2×10^5, respectively.

1. Introduction

Cadmium is a toxic, nonessential trace metal which is being released into the global environment by human activities at a rate that far exceeds the natural rate of release (Nriagu, 1980). Atmospheric transport and subsequent deposition (Chan *et al.*, 1986) is a major source of Cd loading to many remote lakes. As a result, aqueous Cd concentrations are elevated in many soft water lakes in central Canada (Stephenson and Mackie, 1988a) and elsewhere (Borg, 1983; Laxen, 1984). Recent lake sediments in many remote areas of North America contain Cd concentrations that are elevated with respect to older sediments (Galloway and Likens, 1979; Dillon and Smith, 1984).

Even low concentrations of Cd in water or sediments have been shown to be harmful to freshwater crustaceans (Guidici *et al.*, 1986; Nebeker *et al.*, 1986; Marshall *et al.*, 1981). The freshwater amphipod *Hyalella azteca* is particularly sensitive to Cd in both water and sediments (Borgmann *et al.*, 1989; Nebeker *et al.*, 1986). Increased mortality during chronic exposure was noted for *Hyalella* at 1 μg L^{-1}, but reproduction was not impaired at this concentration (Borgmann *et al.*, 1989).

Despite the well-documented sensitivity of many aquatic organisms to Cd, little is known about the sublethal effects of low (<1 μg L^{-1}) Cd concentrations on aquatic biota or communities, or about the pathways or mechanisms by which

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Cd is accumulated. To address these deficiencies a whole-lake Cd addition experiment, with ^{109}Cd added simultaneously as a tracer of the stable Cd, was initiated in 1987 by the Department of Fisheries and Oceans, Canada, at the Experimental Lakes Area (ELA) in northwestern Ontario (Malley *et al.*, 1989). This paper reports the results of a study of the uptake and depuration of Cd by epilithic periphyton and by a grazing freshwater amphipod (*Hyalella azteca* Sassure). *Hyalella* is known to readily accumulate Cd to high concentrations in response to aqueous Cd (Stephenson and Mackie, 1988b, 1989). However, a field study was unable to demonstrate any correlation between Cd concentrations in *Hyalella* and the Cd concentration of littoral sediments at the collection site (Stephenson and Mackie, 1988b). Since *Hyalella* may consume only selected materials from the bulk sediment, and may absorb only a portion of the ingested Cd, the importance of dietary Cd may be underestimated. In the present study we model the ^{109}Cd dynamics within periphyton and *Hyalella*, and assess the importance of periphyton as a dietary source of Cd for *Hyalella*.

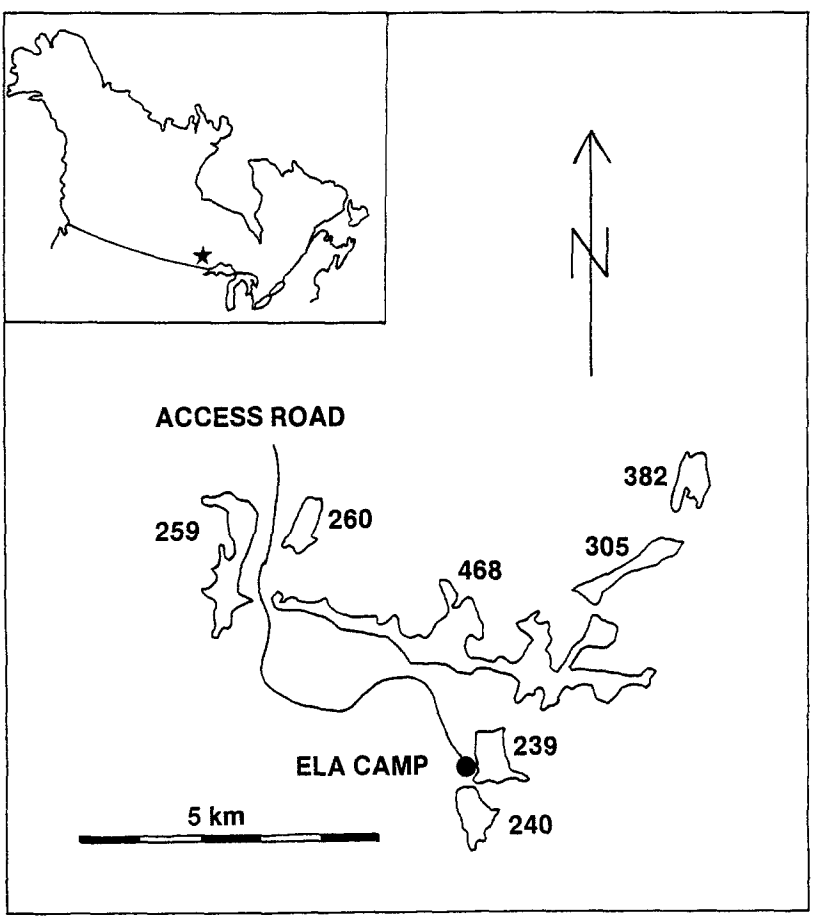

Fig. 1. (a) A map of the Experimental Lakes Area (ELA) showing the locations of Lakes 239 and 382. The inset map of Canada shows the location of ELA.

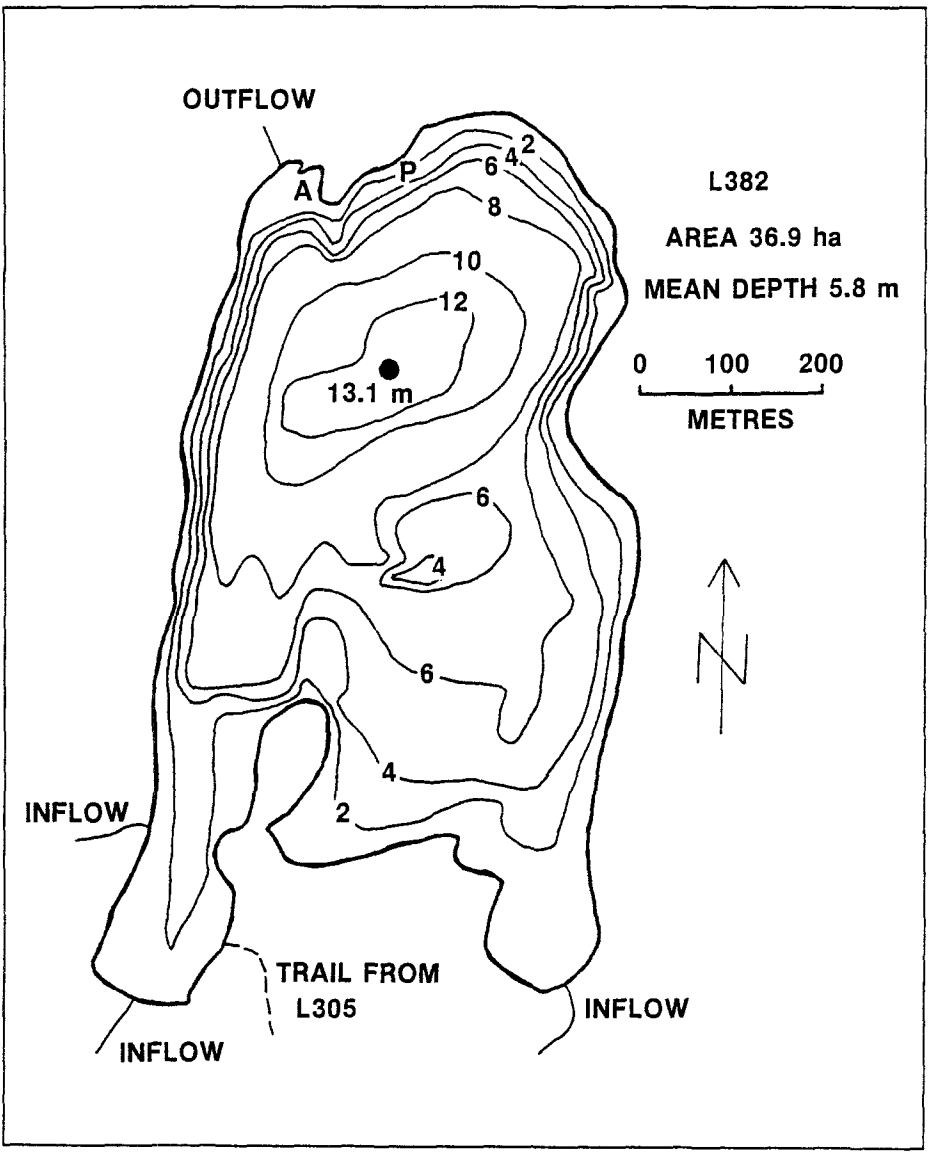

Fig. 1. (b) A bathymetric map of L382, ELA, showing the locations where amphipods (A) and periphyton (P) were held in 1987.

2. Study Area and Methods

2.1. Study Area

Lakes 239 (L239) and 382 (L382) are oligotrophic soft water lakes at the Experimental Lakes Area (ELA) in northwestern Ontario (Figure 1a). Lake 382, which was treated with Cd in 1987, has an area of 37 ha and a mean depth of 5.8 m (Figure 1b).

TABLE I

Selected chemical characteristics of Lakes 239 and 382 at the ELA in northwestern Ontario

Parameter	Lake 239			Lake 382		
	Mean	SD	n	Mean	SD	n
Alkalinity[a]	156	4.6	18	98	3.9	17
Na[b]	1.47	0.16	18	1.09	0.18	17
K[b]	0.80	0.07	18	0.46	0.04	17
Ca[b]	3.38	0.35	18	2.22	0.13	17
Mg[b]	1.03	0.08	18	0.68	0.05	17
SO_4[b]	5.39	0.24	18	3.39	0.17	17
Cl[b]	0.50	0.04	18	0.31	0.10	17
DOC[c]	454	118	18	601	60	17

Data are ice-free season epilimnetic means for 1987, provided by G. A. Linsey, Freshwater Institute, Science Laboratories, Winnipeg, Manitoba.

[a] $\mu eq\ L^{-1}$

[b] $mg\ L^{-1}$

[c] $\mu m\ L^{-1}$

Lake 239 is somewhat larger (56 ha) and deeper (mean depth 10.5 m). Water chemistry characteristics of the two lakes are given in Table I. Background Cd concentrations in ELA lakes are very low. Prior to the experimental addition, L382 had a measured Cd concentration of 1.6 ng L^{-1} (Malley *et al.*, 1989). Lake 239 was presumed to be similarly pristine.

During the experimental Cd addition in 1987, a Cd concentration of about 90 ng L^{-1} was maintained in the epilimnion of L382 throughout late July and August (Figure 2). This concentration was achieved through 29 additions of Cd as $CdCl_2 \cdot 2H_2O$ to the epilimnion of L382, starting on 23 June and ending on 29 October, 1987. In addition to the stable Cd, a small quantity of ^{109}Cd was simultaneously added to the lake as a radioactive tracer, such that the detection of 1 Bq ^{109}Cd indicated the presence of 164 ng Cd (Malley *et al.*, 1989). This tracer greatly simplified analytical procedures for the added Cd. The Cd uptake and depuration experiments described here were conducted between 4 and 15 August, 1987.

2.2. Methods

2.2.1. *Periphyton*

Periphyton developed in L239 and L382, prior to the experimental Cd addition, on 48 mm × 48 mm × 6 mm unglazed vitrified brown clay tiles. A sufficient number of tiles for all uptake, depuration, and feeding studies was placed in the lakes at a depth of 0.75 m, on flat bedrock six weeks prior to the transplant study. The tiles were rapidly colonized on the upper surface by an assemblage of algae, detritus, and other living and non-living material, collectively termed periphyton. By the time of the transplant study, the tiles had developed periphytic assemblages

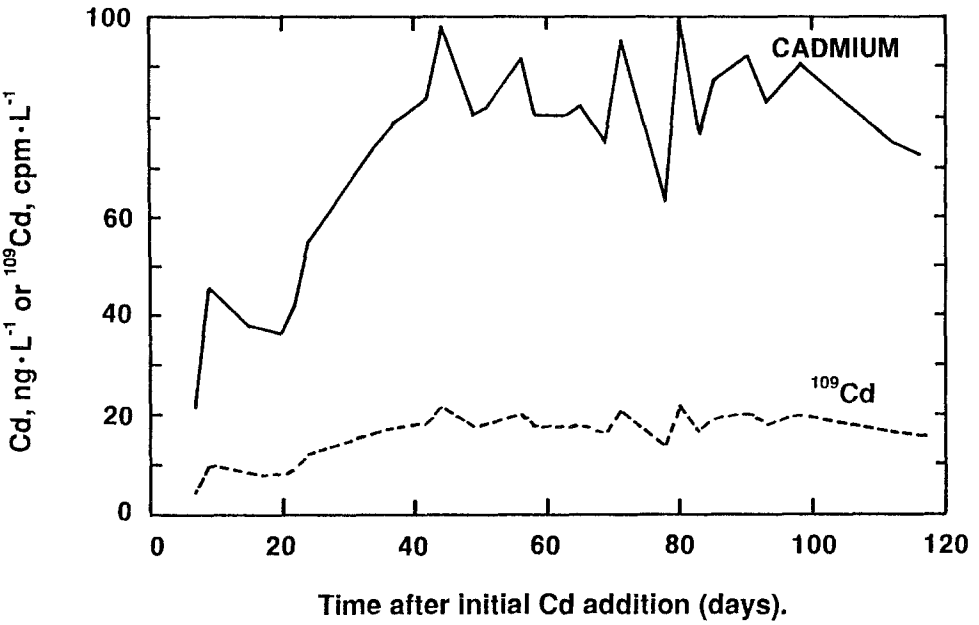

Fig. 2. Stable cadmium and ^{109}Cd concentrations in the epilimnion of L382 in 1987. Redrawn from Malley *et al.* (1989).

apparently similar to those on the adjacent rock surfaces. Taxonomic analysis of periphyton grown on tiles in L239 found that the algal assemblages were dominated by Cyanophyceae, Diatomae, and Chlorophyceae, with occasional Cryptophyceae and Peridineae. The most abundant taxa included *Anabaena*, *Lyngbya*, *Navicula* and *Bulbochaete*. At least 24 algal taxa were present. Tuchman and Stevenson (1980) demonstrated that, for algae, substrata of this type placed in lakes develop populations which are similar to natural assemblages found on rock.

On 4 August 1987, a set of colonized tiles was transported from contaminated L382 to pristine L239 to study the loss of ^{109}Cd from periphyton. Simultaneously, a comparable set of colonized tiles was transported from L239 to L382 to study ^{109}Cd uptake. In both cases tiles were again placed at a depth of 0.75 m on flat bedrock for the duration of the study. In each case periphyton on 3 replicate tiles was sampled prior to transplantation, and then at times 0 and 4 hr, and 1, 2, 4, 7 and 11 d. Tiles were not replaced.

Tiles were collected in the field by carefully placing them underwater into plastic bags (Whirl-Pak) with a minimal quantity of lake water to avoid the loss of loosely attached material. The bags were sealed and returned to the laboratory for processing. In the laboratory, the tiles were first scraped with a plastic spatula to remove the periphyton and then rinsed with lake water. The scrapings and rinsings were poured into a measuring cylinder, which was made up to a standard volume (92 mL) with lake water. After mixing, a subsample of 20 mL was removed and filtered through a pre-weighed, pre-ashed Whatman GFC filter for carbon analysis, while the

remainder of the sample was allowed to settle for ≥ 30 min. The settled periphyton, in approximately 10 mL of water, was preserved with Lugol's solution and transferred to a glass vial for gamma counting of the ^{109}Cd. Carbon analysis was by combustion of the samples, using a Perkin-Elmer model 240 elemental analyzer, following procedures described by Stainton *et al.* (1977).

2.2.2. *Amphipods*

Throughout this study, *Hyalella* were collected using a sweep net in littoral vegetation at a depth of approximately 0.5 m, and were separated from the associated debris by hand, using disposable polyethylene eyedroppers.

Prior to the Cd-addition experiment, stable Cd analyses were performed on individual amphipods collected from several ELA lakes (including L382 and L239). These amphipods were collected between 3 and 4 November 1986, oven dried at 60 °C, and weighed using a Perkin-Elmer AD-6 microbalance. Animals were digested using a HNO_3/H_2O_2 microdigestion technique described by Stephenson and Mackie (1988b). Samples of NBS standard reference material (1566 Oyster Tissue) were digested and analysed simultaneously. Since the measured Cd concentration in this tissue was 2.98 $\mu g\ g^{-1}$, lower than the certified value of 3.5 $\mu g\ g^{-1}$, a correction factor of 1.175 was applied to the measured data. Cadmium analysis of the digestate solutions was by graphite furnace atomic absorption spectrophotometry (Varian GTA-95).

To monitor the response of Cd concentrations in *Hyalella* to Cd addition to L382, batches of approximately 100 amphipods were collected for ^{109}Cd assay. The first collection was made immediately prior to the Cd addition on 23 June 1987. Collections were repeated at 15, 22, 44, 50, 64, 70, 88, and 116 d after the first Cd addition. These animals were oven dried at 60 °C, weighed on a Perkin-Elmer AD-6 microbalance, and assayed for ^{109}Cd as described below.

To study the Cd uptake and depuration rates of *Hyalella* from aqueous and dietary sources, animals were caged in Lakes 382 and 239 under carefully controlled conditions. In each lake, groups of 20 animals were held in cages made from acrylic tubing and nitex screen. The cages were made from two identical halves held together by a rubber band. Each half consisted of a 3.8 cm length of 7 cm inside diameter, 7.6 cm outside diameter clear acrylic tubing, with nitex mesh (200-μm opening) glued across one end. Each half also had three lugs glued equilaterally to the outside, with projections extending beyond the open (unscreened) end of the tube. These lugs aligned the two cages halves so that the unscreened ends met without gaps, and provided attachment points for the rubber band, which held the halves together. The cages were held with their screened faces vertical in an acrylic rack, which was weighted down with rocks. The racks were installed at a depth of 0.5 m in both L382 and L239. Depending on the treatment (see next section) each cage was furnished with a previously colonized periphyton tile from either L382 or L239, which was installed with its colonized face uppermost.

2.2.3. *Experimental Treatments*

Three treatments were established. The first treatment (Cd loss) studied the loss of Cd by *Hyalella*. Animals collected from L382 (and exposed to ^{109}Cd since the beginning of the Cd addition on 27 June, 1987) were held in L239 and were provided with a tile colonized with unlabelled L239 periphyton for food. The second treatment (total uptake) studied the uptake of Cd by *Hyalella*. Animals from L239 were held in L382, with labelled periphyton from L382. The third treatment (food uptake) studied the uptake of Cd by *Hyalella* from food, using caged animals from L239 held in L239 and fed labelled periphyton on tiles from L382. Fresh labelled periphyton tiles were placed in these cages daily. These tiles had been incubated in L382 for six weeks prior to the feeding experiment. We will show that this exposure was sufficient for the achievement of equilibrium [Cd] in periphyton. Animals were sampled from each treatment by sacrificing one cage daily for eleven days. Upon collection each group of amphipods was oven-dried at 60 °C and transferred to a polypropylene centrifuge tube for ^{109}Cd analysis.

2.2.4. *Cadmium-109 Analysis*

Analysis for ^{109}Cd was by gamma-counting using an LKB-Wallac Compugamma counter. Counting efficiency was determined by counting solutions of known activity in vials with geometry identical to those used for samples. Counts were back-calculated for radioactive decay to 23 June 1987, when the isotope-labelled Cd solution was prepared. The stable Cd addition (diluted in the epilimnion to 90 ng L^{-1}) overwhelmed the background Cd concentration in L382 water (<2 ng L^{-1}), and we isolated amphipods and periphyton from the accumulated background pool of Cd in sediments. Thus, we disregarded effects due to dilution of the specific activity of ^{109}Cd by background stable Cd in L382 and L239. In this way, using the specific activity on 23 June 1987, ^{109}Cd was used to calculate stable Cd concentrations in biota. Cadmium-109 concentrations were expressed on a per gram dry mass basis for the amphipods and on a per gram carbon basis for the periphyton.

2.2.5. *Determination of Grazing Rates*

To determine grazing rates of *Hyalella* we conducted an experiment to measure periphyton loss from tiles grazed by amphipods. We removed tiles from L239 and gently rinsed them with lake water to remove loosely attached periphyton. These tiles were then incubated in groups of three, each with 30 amphipods, for 48 hr. After this time, the tiles were removed from the test containers and again gently rinsed to remove amphipods and fecal pellets. Amphipods form discrete, membrane-bound fecal pellets that do not adhere to surfaces, and which were observed to be completely removed from the tiles by rinsing. Next the tiles were scraped clean and rinsed. The scrapings were recovered on pre-weighed filters (Whatman GFC), oven dried at 60 °C, and re-weighed to determine the mass of periphyton. Duplicate groups of tiles were incubated simultaneously without amphipods to determine the

mass of periphyton without grazing. The amphipods were also dried at 60 °C and weighed to determine their mean mass.

2.3. Modeling

Cadmium dynamics in periphyton and amphipods were described mathematically as first-order processes. Depuration was described by

$$C_t = C_0 \times e^{(-K_d \times t)}, \tag{1}$$

where

C_t = Cd concentration in biota at time t (mg kg^{-1})
C_0 = Cd concentration in biota at time 0 (mg kg^{-1})
e = base of natural logarithms (2.718)
K_d = depuration rate constant (d^{-1})
t = time in days (d).

Once depuration rates had been established, Cd uptake rates were calculated from

$$C_t = (U/K_d) \times [1 - e^{(-k_d \times t)}] \tag{2}$$

where U = an empirical uptake constant (mg kg^{-1} d^{-1}).

For periphyton, where all uptake is assumed to be directly from water

$$U_p = K_{uw} \times C_w, \tag{3}$$

where

K_{uw} = uptake rate constant from water (d^{-1})
C_w = Cd concentration in water (mg kg^{-1}).

For amphipods, where Cd uptake may be by aqueous or dietary pathways, a total uptake rate, U_a can be defined as if all uptake were directly from water, so that:

$$U_a = K_{ut} \times C_w, \tag{4}$$

where

K_{ut} = total uptake rate constant (d^{-1})
C_w = Cd concentration in water (mg kg^{-1}).

However, U_a is more properly defined as

$$U_a = (K_{uf} \times C_f) + (K_{uw} \times C_w) \tag{5}$$

where

K_{uf} = uptake rate constant from food (d^{-1})
C_f = Cd concentration in food (mg kg^{-1}).

For the food-only treatment, we can define U as the uptake rate from food,

such that

$$U_f = K_{uf} \times C_f. \tag{6}$$

A second way to view Cd accumulation from food is to subdivide K_{uf}, so that

$$K_{uf} = a \times R \tag{7}$$

and

$$U_f = a \times R \times C_f, \tag{8}$$

where

a = assimilation efficiency of Cd from food (mg Cd absorbed per mg Cd ingested)
R = feeding rate (kg food ingested per kg body weight per day).

The use of U allows us to compare uptake rates between periphyton and amphipods, and between amphipod total uptake and food uptake treatments, without making assumptions about the mechanism of uptake. Subdividing U into K_{uf} and K_{uw} allows us to evaluate the importance of dietary and aqueous sources of Cd uptake to *Hyalella*. Subdividing K_{uf} into feeding rate and assimilation efficiency allows us to estimate the fraction of the total ingested Cd that is assimilated by *Hyalella*.

Finally, a dimensionless bioconcentration factor (BCF) can be estimated from the instantaneous total uptake and depuration rates, using

$$\text{BCF} = K_{ut}/K_d, \tag{9}$$

or

$$\text{BCF} = K_{uw}/K_d. \tag{10}$$

The use of a BCF calculated from K_u and K_d data facilitates comparisons between the treatments which would not be possible using a BCF calculated from measured Cd concentrations in biota, water and food.

All calculations were performed on a microcomputer using a statistical software package (SYSTAT, Version 4.0). Depuration rate constants from L239 were estimated by linear regression. Uptake rate constants were estimated by nonlinear regression (quasi-Newton algorithm) using two models. Model 1 calculated uptake rates given the depuration rates measured in L239. Model 2 allowed the nonlinear regression program to estimate both uptake and depuration rate parameters simultaneously.

3. Results

3.1. Background cadmium concentrations

Background Cd concentrations in *Hyalella* collected from five ELA lakes in November 1986 were between 1.09 and 2.34 mg kg^{-1} dry mass (Table II). In L382, the mean concentration was 1.38 mg kg^{-1}.

TABLE II

Background Cd concentrations of amphipods collected from ELA lakes in November 1986

Lake	L239	L259	L260	L305	L382
Cd concentration	1.96	2.34	1.09	1.31	1.38
Standard deviation	0.64	0.63	0.49	0.31	0.66

Values given are the mean and standard deviation for 10 replicate determinations of individual animals, expressed as mg Cd per kg animal dry mass.

3.2. Cadmium addition to L382

The Cd additions to L382 resulted in a rapid rise in the epilimetic aqueous [Cd] between 23 June and 2 August 1987. Between 2 August and 1 October 1987, [Cd] in the epilimnion of L382 remained fairly constant at about 90 ng L^{-1} (Figure 2). Cadmium concentrations in *Hyalella* living freely in the lake immediately responded to the Cd addition and stabilized at approximately 23 mg Cd kg^{-1} dry mass by 6 August 1987 (Figure 3).

3.3. Transfer experiments

Cadmium depuration from periphyton and amphipods collected from L382 and relocated to L239 (Figures 4, 5) was consistent with the assumption of first-order dynamics. Loss rates were 0.089 d^{-1} and 0.092 d^{-1} respectively (Table III).

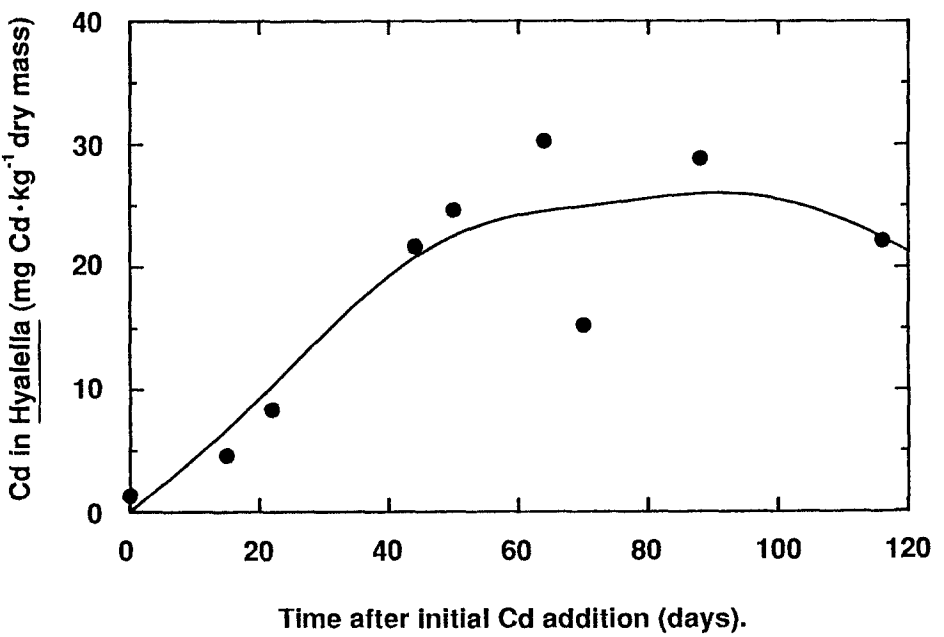

Fig. 3. Cadmium concentrations in *Hyalella azteca* collected from L382 before and during ^{109}Cd addition in 1987. Line drawn by hand.

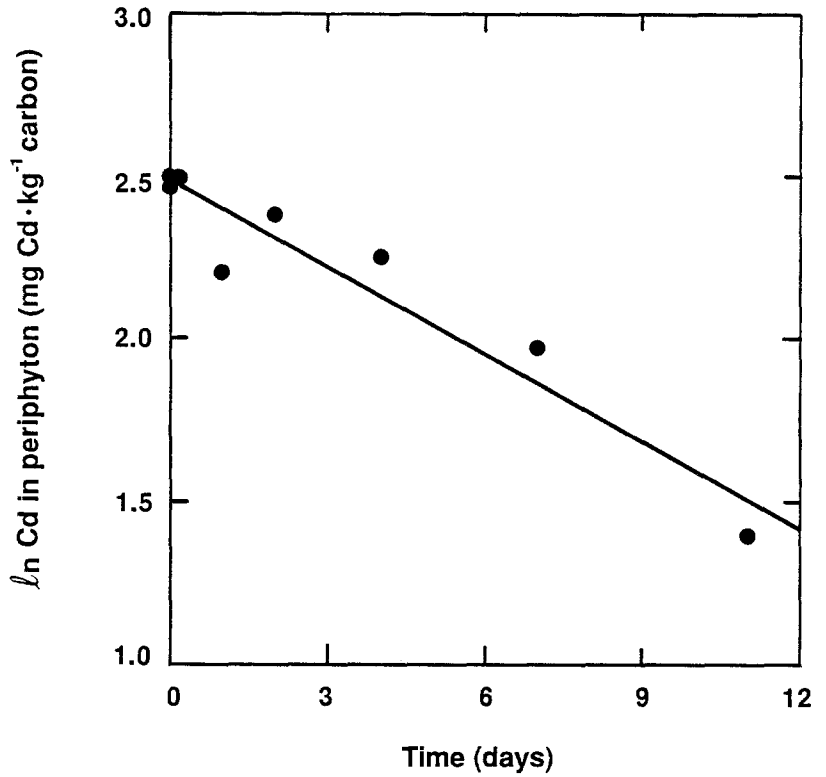

Fig. 4. Cadmium depuration from periphyton cultured in L382 and subsequently held in L239.

TABLE III

Mean uptake ($U = K_u \times C_w$) and depuration (K_d) rates measured for periphyton and amphipods held in Lakes 239 and 382

Depuration L239	K_d	lcl	ucl	U	lcl	ucl
Periphyton	0.089	0.062	0.116	–	–	–
Amphipods	0.092	0.067	0.115	–	–	–
Total uptake L382						
Periphyton Model 1	0.089	–	–	1.85	1.44	2.26
Periphyton Model 2	0.292	0.091	0.493	3.39	1.77	5.01
Amphipods Model 1	0.092	–	–	2.63	2.28	2.99
Amphipods Model 2	0.355	0.190	0.521	5.49	3.54	7.44
Food Uptake L239						
Amphipods Model 1	0.092	–	–	1.69	1.09	2.30
Amphipods Model 2	0.327	-0.362	1.016	3.20	-1.63	8.02

Upper and lower 95% confidence limits about the mean are indicated by ucl and lcl respectively. K_d has units of d^{-1}. U has units of mg kg^{-1} d^{-1}.

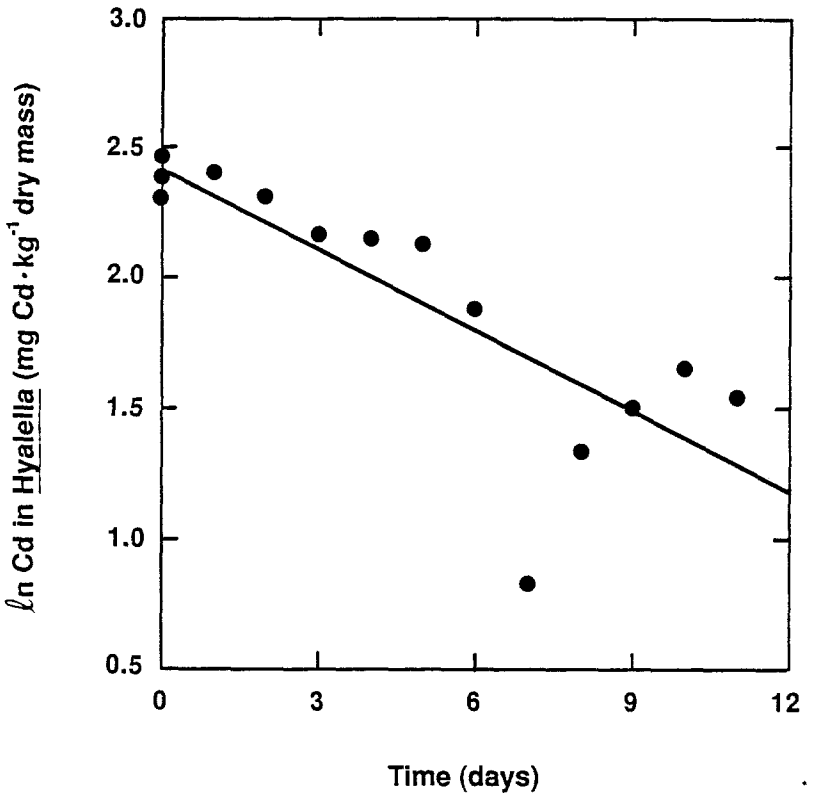

Fig. 5. Cadmium depuration from amphipods collected in L382 and subsequently held in L239.

Cadmium uptake was rapid. Both periphyton (Figure 6) and amphipods (Figure 7) approached equilibrium [Cd] by 11 d after transplantation from L239 to L382. Uptake rates (U) are summarized for the various treatments and models in Table III. The instantaneous Cd-uptake rate (K_{uw}) of periphyton, estimated using the measured depuration rate and taking C_w to be 90×10^{-6} mg kg^{-1} (Model 1), was 2.06×10^4 d^{-1}, based upon equation (3). However, these uptake and depuration rates produced a Cd-uptake curve that did not fit the observed data particularly well. The Cd concentration in periphyton was consistently underestimated early in the uptake curve, while the predicted equilibrium [Cd] was higher than that observed. When the nonlinear regression model was allowed to fit the Cd-uptake data by calculating both uptake and depuration rates (Model 2), a better fit was achieved, with somewhat higher instantaneous uptake ($K_{uw} = 3.77 \times 10^4$ d^{-1}) and depuration ($K_d = 0.29$ d^{-1}) rates.

The instantaneous rate of Cd uptake (K_{ut}) by amphipods calculated using Model 1 and assuming $C_w = 90 \times 10^{-6}$ mg kg^{-1}, was 2.92×10^4 d^{-1} for the total uptake treatment, and 1.88×10^4 d^{-1} food-uptake treatment. When the parameters were fitted using Model 2 the rates were again higher. Total instantaneous uptake and

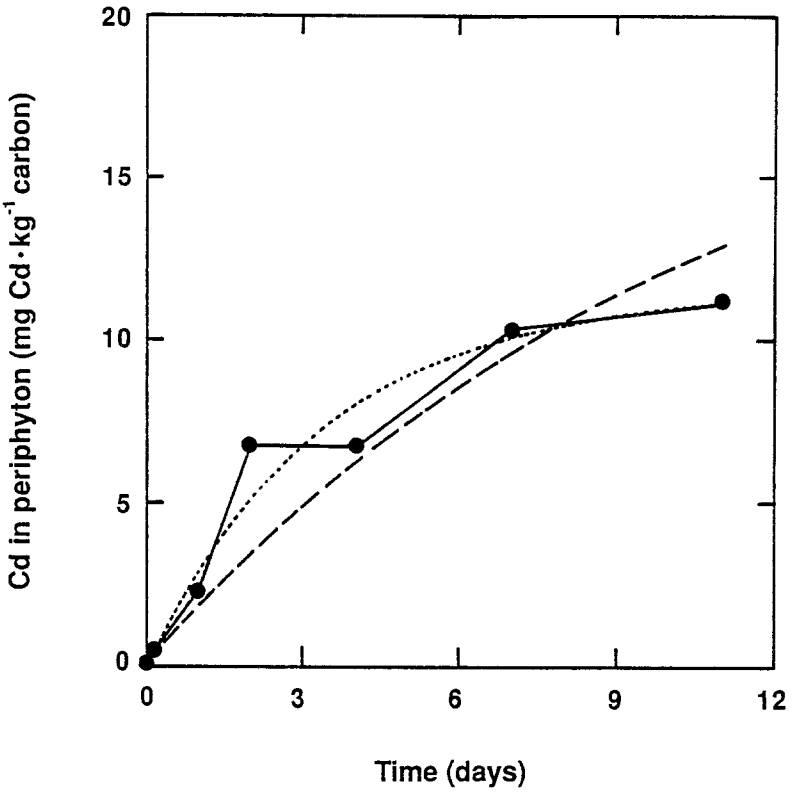

Fig. 6. Cadmium uptake by periphyton cultured in L239 and transported to L382. Curves fitted to the data are based on Model 1 (– – – – –) or Model 2 (. . . . .) parameters.

TABLE IV

Determination of periphyton grazing rates by *Hyalella azteca*

Treatment	Periphyton mass (mg)	Loss due to grazing (mg)	Amphipod mass (mg)	Grazing rate mg mg^{-1} d^{-1}
Ungrazed				
Rep. 1	43.85			
Rep. 2	33.75			
Mean	38.8			
Grazed				
Rep. 1	24.65	14.15	6.7	1.06
Rep. 2	16.50	22.30	12.4	0.90

Tiles with periphyton were grazed for 48 hr. The mean estimated grazing rate of *Hyalella* is 0.98 mg periphyton consumed per mg amphipod per day. For a median animal with a mass of 210 μg, this represents a periphyton consumption of 206 μg d^{-1}.

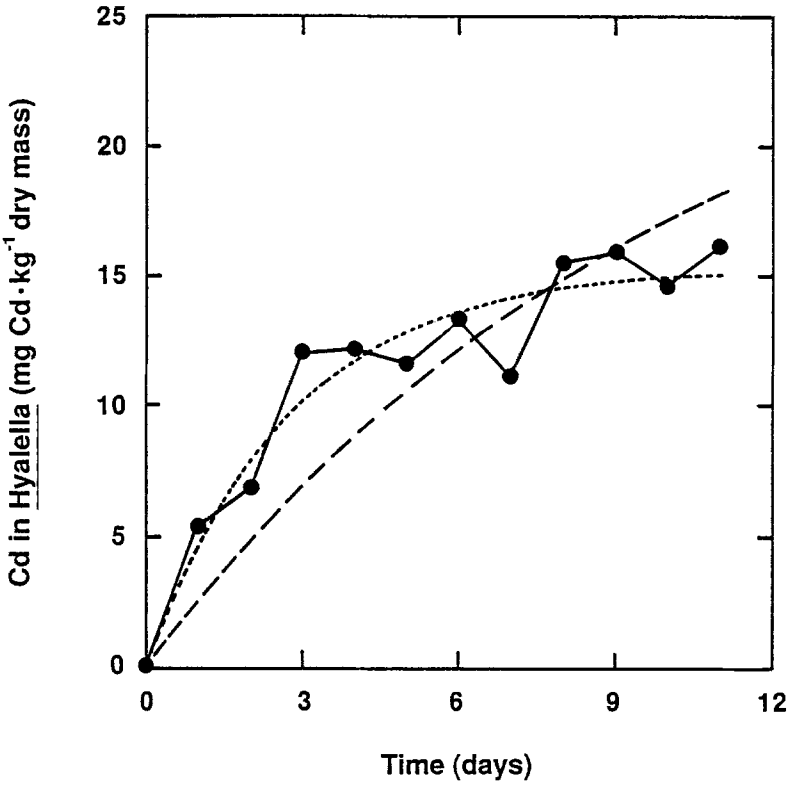

Fig. 7. Cadmium uptake by amphipods collected in L239 and transplanted into L382. Curves fitted to the data are based on Model 1 (-----) or Model 2 (.....) parameters.

depuration rates were 6.10×10^4 d^{-1} and 0.36 d^{-1}, respectively, whereas uptake and depuration rates for animals in the food-only treatment (Figure 8) were 3.56×10^4 d^{-1} and 0.33 d^{-1}.

For the food-uptake treatment, an assimilation efficiency (K_{uf}) can be calculated by setting the Cd concentration in food to 4.1 mg kg^{-1}, the concentration measured in L382 periphyton on 1 September, 1987. With $U = 3.2$ (Model 2) K_{uf} is 0.780 d^{-1}. The grazing experiment (Table IV) indicated that *Hyalella* consumed periphyton at a daily rate of 206 μg per average adult with a mass of 210 μg; or at a standard rate of (0.98 kg periphyton)/(kg amphipod × d). At this grazing rate, the food-uptake rate (k_{uf}) estimated by Model 2 can be transformed into a Cd assimilation efficiency of 0.80-μg Cd assimilated per μg Cd in food consumed, or 80%.

4. Discussion

4.1. Cadmium in Lake 382 amphipods

The studied biota of L382 accumulated Cd quickly. Cadmium concentrations in

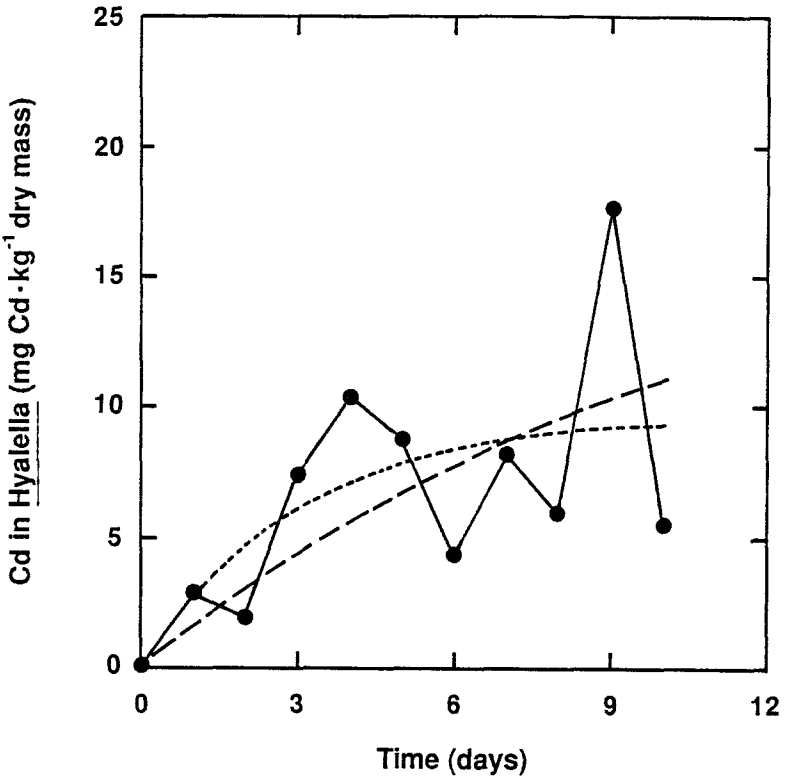

Fig. 8. Cadmium uptake by amphipods collected and held in L239 and fed periphyton cultured in L382. Curves fitted to the data are based on Model 1 (-----) or Model 2 (.....) parameters.

L382 amphipods (Figure 3) closely followed the epilimnetic aqueous [Cd] (Figure 2) over the four-month study period. By the start of the transplant experiments, *Hyalella* in L382 has reached equilibrium Cd concentrations with their environment. The equilibrium concentration achieved (approximately 23 mg kg^{-1}, Figure 3) is consistent with the predictions of the empirical model of Stephenson and Mackie (1988b):

$$\log[Cd_{Hy}] = 1.182 - (1.154 \times \log[Ca_{Aq}]) + (0.643 \times \log[Cd_{Aq}]) - (0.805 \times \log[DOC_{Aq}]), \quad (10)$$

where

Cd_{Hy} = the Cd concentration of *Hyalella* (mg kg^{-1})
Ca_{Aq} = the aqueous Ca concentration (mg L^{-1})
Cd_{Aq} = the aqueous Cd concentration (ng L^{-1})
DOC_{Aq} = the aqueous dissolved organic carbon concentration (mg L^{-1}).

Substituting data from Table I into this equation, and assuming Cd_{Aq} = 90 ng L^{-1}, yields a predicted Cd concentration of 22.3 mg kg^{-1} dry mass in *Hyalella*.

4.2. Transfer studies

In the transfer experiments, both periphyton and amphipods approached equilibrium Cd concentrations within 11 d. The dynamics of Cd accumulation, however, were more complex than we expected. The depuration rates measured in periphyton and in amphipods transferred to L239 cannot be reconciled with the faster rates required to fit the observed uptake curves in L382. This problem has two major implications. Either the model is unsuitable and neglects significant uptake or depuration processes, or the rates are different in the two systems and we must account for that difference.

Although it can be argued that our model is simplistic, treating uptake and depuration as single, rather than as multiple processes, we feel that it fits the observed data well when suitable parameters are selected. These parameters are uptake and depuration rates which we can mechanistically subdivide into process-specific rates. Given that the model appears to adequately describe Cd dynamics in the studied biota, we reject the first alternative. Consequently, we believe that the depuration rate of periphyton and amphipods observed in L239 was significantly slower than in L382.

Since the two lakes are chemically similar (Table I), we believe that the different rates are unlikely to be due to differences in the major ion water chemistry. The major chemical differences between L239 and L382 are that L239 has somewhat higher Ca and lower DOC concentrations. Although Stephenson and Mackie (1988b) showed that [Cd] in *Hyalella* from Precambrian Shield lakes in central Ontario is negatively correlated with aqueous [Ca] and [DOC], any effects due to the chemical differences between L239 and L382 would be slight. Furthermore, their net effect would be to lower [Cd] in *Hyalella* from L239, and presumably enhance depuration, the opposite of what was observed.

One explanation for the different Cd depuration rates is that depuration may be a function of the uptake rate or exposure concentration. Cadmium exposure can stimulate the synthesis of metallothionein or metallothionein-like proteins by many organisms (Klaverkamp *et al.*, 1984). Metallothioneins act as sinks and regulators for toxic heavy metals by chelating them and removing them from intracellular fluids (cytosol). If metallothioneins are inducible, then so may be other strategies for coping with an influx of toxic metals such as Cd, with the net effect of enhancing depuration rates or reducing uptake rates with increasing exposure to Cd.

Other researchers also have concluded that depuration rate is a function of exposure concentration. Giesy *et al.* (1980), studying Cd and Zn accumulation and elimination by crayfish, concluded that steady-state Cd concentrations in aquatic macroinvertebrates are concentration dependent, and that uptake coefficients may decrease with increasing Cd exposure concentrations. Consequently, coefficients determined at one exposure concentration may be of limited value when predicting accumulation from exposure to different concentrations. Similarly, van Hattum *et al.* (1989), in

a study of Cd bioaccumulation by the aquatic isopod *Asellus aquaticus*, found that depuration constants tended to increase (from 0.014 to 0.049 d^{-1}) with increasing exposure to Cd. However, in their study, the differences were not significant owing to the relatively large confidence limits on individual estimates.

4.3. Periphyton

Newman and McIntosh (1989) discuss at least nine mechanisms by which algal *Aufwuchs* (periphyton) can lose trace metals. These include biological (dilution with growth, release at death, grazing, elimination), physical (sloughing, dilution by non-binding material, desorption), and chemical (dissolution, desorption) mechanisms. Our measured depuration rate for periphyton implicitly includes all of these possible mechanisms. Although we could provide rate estimates for some of these mechanisms (e.g., growth as net production), the large number of undefined rates limits the value of the exercise. Clearly, describing mechanistic contaminant loss rates from a biological compartment as poorly defined as periphyton requires considerable study.

Newman and McIntosh (1989) further point out that because of the heterogeneous composition of procedurally defined *Aufwuchs* (periphyton) it is not clear that algal, or even biological processes, control the concentrations of trace metals measured in the assemblage. Among other inorganic materials frequently found in periphyton are Fe and Mn hydrous oxides, which are particularly effective scavengers of trace metals.

We are not aware of Cd dynamic data for periphyton elsewhere in the literature. However, the depuration rates we measured for ^{109}Cd in periphyton were similar to rates reported by Nucho and Baudin (1989) for ^{60}Co loss from *Scenedesmus obliquus*. They reported a two-phase depuration curve, with an initial rapid rate and a secondary slow rate. Their slower depuration rate had a K_d value of 0.143 d^{-1} for algae exposed for 12 d to ^{60}Co in solution. They attributed the rapid phase of depuration to the desorption of ^{60}Co bound to the cell surfaces and postulated that the slower rate was due to the loss of ^{60}Co, which had penetrated the cell wall to bind with intracellular proteins. We found no evidence for two-phase depuration in the present study.

Cadmium uptake rates for periphyton and other phytobenthos are also typically rapid. Kinkade and Erdman (1975) found that the alga *Nitella flexilis* equilibrated with ^{115m}Cd in approximately 14 d, and the macrophyte *Elodea* responded in a similar manner. Conversely, van Hattum *et al.* (1989) found that *Elodea* continued to accumulate Cd at an almost linear rate until 16 d of exposure, and that there was no evidence of steady state being achieved.

4.4. Amphipods

Depuration rates of amphipods appeared to be positively correlated with their exposure to Cd, as noted earlier. Consequently, depuration rates between 0.092 and 0.355 d^{-1} are indicated by this study. Hare *et al.* (1991) reported a Cd depuration

rate of 0.088 d^{-1} for the burrowing mayfly *Hexagenia rigida* exposed to ^{109}Cd in lake sediment. Janssen *et al.* (1991) measured Cd depuration rates of 0 to 0.375 d^{-1} for four species of soil arthropods, and summarized data from other studies showing that for soil invertebrates, Cd excretion rates tend to be <0.10 d^{-1}.

Since *Hyalella* are clearly a better-defined compartment than periphyton, it is feasible to attempt to resolve some of their net depuration rate into process-specific fractions. The most practical approach is to view the net depuration rate as including growth dilution and net excretion of Cd. Growth dilution is a potentially significant mechanism by which organisms can reduce their contaminant concentrations. Because the amphipods used in this study were actively growing, we have included the diluting effect of their growth on [Cd] in our estimates of depuration. We can correct for growth by subtracting the production or growth rate of amphipods from the measured depuration rate. A reasonable estimate of their summer growth rate is 0.035 d^{-1} (Stephenson, unpublished). Thus, for amphipods growth dilution accounts for approximately 10 to 30% of the measured depuration rates, depending on whether Model 2 or Model 1 parameters are chosen.

Using values of U calculated for the food-only and the total uptake treatments, the relative importance of food and water as sources of Cd for *Hyalella* can be assessed. Using Model 2 data, a comparison of U suggests that amphipods exposed to ^{109}Cd via food alone have an uptake rate (3.20 mg kg^{-1} d^{-1}) equivalent to 58% of the total uptake rate (5.49 mg kg^{-1} d^{-1}). This is a large percentage of the total uptake rate to be derived from food. Previously, van Hattum *et al.* (1989) reported that *Asellus* obtained from 2% to 50% of its Cd from dietary sources. However, their highest values were obtained under artificial conditions with highly contaminated food (239 μg Cd g^{-1} in *Elodea*) and relatively little (1.1 μg L^{-1}) in water. Under more realistic conditions in their study, between 2% and 11% of total Cd uptake was from dietary sources. Similarly, Giesy *et al.* (1980) concluded that food was a significant source of Cd for the crayfish *Procambarus acutus*, and Dudderidge and Wainwright (1980) showed that the amphipod *Gammarus pulex* accumulates Cd from eating fungal (*Pythium* sp.) mycelia. However, since in these studies the Cd concentration in food was not related to exposure concentrations in water, it is not possible to derive a relationship between dietary and aqueous sources of Cd.

Our estimate of the assimilation efficiency with which *Hyalella* absorbs Cd from natural periphyton (80%) is also high in comparison with the literature. Van Hattum *et al.* (1989) reported an efficiency of approximately 1% for the isopod *Asellus*, and carnivorous marine crustaceans appear to assimilate only about 10% of ingested Cd (Jennings and Rainbow, 1979; Benayoun *et al.*, 1974). Janssen *et al.* (1991) summarized Cd assimilation efficiencies for a number of soil invertebrates and reported values ranging between 7 and 90%. They noted that high efficiencies occurred in predatory organisms, although high efficiencies were also found in snails and isopod crustaceans. Possibly the high metabolic Ca requirement of Crustacea and Mollusca for shell formation and calcification leads to high Cd assimilation

efficiencies. Both Ca and Cd bear the same charge in solution (+ 2), and both have similar ionic radii (0.99 and 0.97 angstroms respectively, Weast, 1975). *Hyalella* in Canadian Shield lakes inhabit a Ca-poor environment, and increasing Ca concentration in solution has been shown to reduce Cd uptake by *Hyalella azteca* (Stephenson and Mackie, 1989). The high Cd assimilation efficiency may result from coincidental Cd uptake while the animals attempt to obtain sufficient Ca from a Ca-depleted environment.

Given the high Cd assimilation efficiency estimated in the present study, it is worthwhile considering sources of error in our estimate. In order to inflate our estimate of assimilation efficiency, our grazing rates would have to underestimate the true rate. This is unlikely since our grazing rates were measured under conditions very similar to the food-only exposure conditions, and we chose the highest grazing rate indicated by three experiments (range 0.78 to 0.98 μg periphyton consumed per μg amphipod per day). Furthermore, our handling procedures may have caused mechanical losses of periphyton from the tiles, causing us to overestimate the grazing rate. A second check is to consider the growth rate of *Hyalella* (0.035 d^{-1}). Assuming a typical animal with a mass of 210 μg is growing at this rate, it elaborates approximately 7.4 μg of tissue daily. If it achieves this by consuming 206 μg or periphyton, it has a conversion efficiency of about 3.6%. This is an intuitively reasonable figure for a grazing invertebrate. Therefore, we are confident that there is no major error in the estimated grazing rate.

A second possible error in our estimate of assimilation efficiency is that the instantaneous Cd uptake rate (K_{uf}) is inflated. This is possible owing to the very large confidence interval about the estimated value (Table III). However, it is clear from Figure 8 that a significant amount of Cd was accumulated by these amphipods, even if the scatter is large. Since we have rejected Model 1 because of its inconsistency with measured depuration rates, we prefer the estimate based on Model 2 parameters, and conclude that *Hyalella* assimilates approximately 80% of ingested Cd.

4.5. Bioconcentration factors

When treated as bioconcentration factors (BCF), the data reported here are high in comparison with the literature. Taylor (1983) reviewed Cd accumulation in aquatic biota and reported BCFs of 8.0×10^2 to 1.0×10^4 for freshwater algae, and 10 to 1.5×10^3 for freshwater Crustacea. Vymazal (1987) likewise reviewed literature values for Cd bioconcentration in freshwater algae and reported values between 1.2×10^4 and 1.5×10^5. Our values (1.3×10^5 and 1.7×10^5 for periphyton and amphipods respectively) are in the high range of reported values. There are several probable reasons for this. Firstly, water in L382 is very dilute, with a low [Ca] in solution. Most previous studies have been conducted at much higher aqueous [Ca]. Increasing the [Ca] of test solutions from 2.5 to 6.5 mg L^{-1} interferes with Cd uptake and reduces [Cd] in *Hyalella azteca* (Stephenson and Mackie, 1989). Similar effects have been reported in a study of Cd accumulation by the alga *Chlorella pyrenoidosa* (Gipps and Coller, 1982). Secondly, if K_{ut} and K_d are a function of

the exposure concentration, with K_{ut} tending to decrease and K_d to increase with increasing exposure, then BCF values will decrease with increasing [Cd] in the environment, as was noted by Taylor (1983). Since most BCF data are generated in laboratory studies at rather high aqueous [Cd] (1 to 1000 $\mu g\ L^{-1}$) in comparison with the environmentally relevant concentration used in this study (0.09 $\mu g\ L^{-1}$), again it is not surprising that our BCF values are higher than most of those previously published. Finally, some laboratory studies are designed to calculate a BCF value in the classical way, such that only aqueous exposure routes are available. Clearly, in the present situation neglecting dietary uptake would have resulted in a lower BCF value for amphipods.

Our results, and those of other workers (Van Hattum *et al.*, 1989; Giesy *et al.*, 1980), have strongly suggested that uptake and depuration rates may be dependent upon exposure concentration. Consequently, the BCF approach itself may be seriously flawed. If it is hoped that a BCF value will permit the extrapolation of results from one system to another, then care must be exercised to ensure that the systems (exposure conditions) are indeed comparable. Alternatively, as Giesy *et al.* (1980) noted, we should be fitting kinetic data to isotherms so that changes in the rate constants as a function of the exposure concentration can be accounted for. Otherwise, models using BCF or analogous transfer coefficients may seriously underestimate the environmental consequences of low contaminant concentrations in water.

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