Environmental Pollution xxx (2009) 1–6

Contents lists available at ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol

Trophic transfer of Cd from natural periphyton to the grazing mayfly *Centroptilum triangulifer* in a life cycle test

Lingtian Xie [a], David H. Funk [b], David B. Buchwalter [a,*]

[a] *Department of Environmental and Molecular Toxicology, North Carolina State University, Raleigh, NC 27695, USA*
[b] *Stroud Water Research Center, Avondale, PA 19311, USA*

Periphyton is a major source of Cd bioaccumulation in a grazing mayfly.

ARTICLE INFO

Article history:
Received 13 May 2009
Received in revised form
2 July 2009
Accepted 12 July 2009

Keywords:
Trophic transfer
Cadmium
Periphyton
Mayfly
Centroptilum triangulifer

ABSTRACT

In streams, periphyton biofilms are important sinks for trace metals such as cadmium and are primary food sources of many invertebrate consumers. To study Cd trophic transfer, we produced differentially contaminated diets by exposing natural periphyton to environmentally relevant dissolved Cd ranging from 0 to 10 $\mu g\ L^{-1}$ for 6–7 days using a radiotracer approach. On average, periphyton grown during three different seasons bioconcentrated Cd similarly – approximately 1315 (±442) -fold above dissolved concentrations. However, mayfly larvae (*Centroptilum triangulifer*) raised on these differentially contaminated diets (first instar through adulthood) had significantly higher trophic transfer factors from periphyton grown in Aug and Nov 2008 (4.30 ± 1.55) than from periphyton grown in Jan 2009 (0.85 ± 0.21). This Cd bioaccumulation difference is only partially explained by apparent food quality and subsequent growth differences. Taken together, these results suggest that primary producers at the base of food webs drive metal bioaccumulation by invertebrate grazers.

1. Introduction

Insects are the dominant invertebrate faunal group in most lotic systems and play critical ecological roles. It is important that their bioaccumulation of contaminants such as metals is well understood, both in terms of toxicity issues to the insects themselves, and also their contribution to the diets (and thus trophic transfer) to fish and birds. A growing number of studies highlight the importance of diet in the bioaccumulation of metals in aquatic insects (Croisetiere et al., 2006; Croteau and Luoma, 2008; Irving et al., 2003; Martin et al., 2007; Munger and Hare, 1997). That diet is an important route of exposure may help explain the vast discrepancies (e.g. Buchwalter et al., 2007) that exist between observations of insect sensitivities to metals in nature (Cain et al., 2004; Clements, 1991; Clements et al., 2000) and the apparent insensitivities of insects in standard laboratory toxicity tests (Brix et al., 2005) that only use dissolved exposures. Since primary producers often concentrate metals to several orders of magnitude above dissolved concentrations, this exposure pathway is likely highly important in determining responses of insects to metals in nature.

Laboratory attempts to understand trophic transfer of metals often use simplified diets such as a single algal species or a single prey type. While the use of standardized diets may reduce experimental variability, they do not reflect the complex diets that many organisms experience in nature. In streams, many species of insects graze on periphyton – complex biofilms consisting of diatoms, algae, fungi, bacteria, detritus and extracellular matrix – which can be a sink for metals such as Cd (Brooks et al., 2004; Hill et al., 2000; Meylan et al., 2003; Morin et al., 2008a,b; Selby et al., 1985). Therefore, characterizing the bioaccumulation of metals in periphyton is critical to understanding their subsequent transfer to invertebrate grazers.

Here, we used a radiotracer approach to quantify Cd bioaccumulation into natural periphyton communities, which are the natural diets of many mayfly species including *Centroptilum triangulifer* (Sweeney and Vannote, 1984). We exposed periphyton to a range of dissolved Cd concentrations for 6–7 days prior to transferring them to clean water. Larvae of the parthenogenetic mayfly *C. triangulifer* (Ephemeroptera: Baetidae) were raised on these differentially contaminated diets through adulthood. We combined these dietary bioaccumulation studies with dissolved bioaccumulation studies to approximate the relative contribution of Cd in mayfly tissues derived from dissolved and dietary sources. The importance of diet is discussed in the context of setting water quality criteria.

---

* Corresponding author. Tel.: +1 919 513 1129; fax: +1 919 515 7169.
E-mail address: david_buchwalter@ncsu.edu (D.B. Buchwalter).

0269-7491/$ – see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.envpol.2009.07.010

2. Materials and methods

2.1. Test animals

The mayfly *Centroptilum triangulifer* (Ephemeroptera: Baetidae) was obtained from culture at the Stroud Water Research Center (Avondale, PA). The same clone (WCC-2) was used for all studies. Originally described as *Cloeon triangulifer* (McDunnough, 1931), this species has been used in studies of temperature and development (Sweeney and Vannote, 1984), chlordane (Sweeney et al., 1993), and aluminum exposure (Tabak and Gibbs, 1991). Given its suitability to laboratory culture, its development as a standard test organism is warranted and ongoing.

2.2. Experimental design

2.2.1. Labeling periphyton with cadmium

Acrylic plates (6.5 × 23 × 0.15 cm) were colonized by periphyton by streaming fresh stream water from White Clay Creek, PA (39°51′47″N, 75°47′07″W) continuously for 2–4 weeks in a greenhouse. These periphyton communities were harvested in Aug 2008, Nov 2008 and Jan 2009 for three distinct sets of experiments. Plates were harvested when periphyton reached a thickness of approximately 1–2 mm and express shipped to North Carolina State University. At this stage, periphyton consisted primarily of diatoms with some bluegreen and green algae, along with some naturally-colonizing consumers (predominantly micro- and meiofauna) (Xie et al., 2009). Microbial and fungal constituents were not characterized.

In each study, the colonized plates were placed in 2.0 L glass bottles holding 1.8 L American Society for Testing and Materials (ASTM) artificial soft water (48 mg L^{-1} $NaHCO_3$, 30 mg L^{-1} $CaSO_4 \cdot 2H_2O$, 30 mg L^{-1} $MgSO_4$, and 2 mg L^{-1} KCl, pH 7.4). The Aug 2008 plates were exposed to dissolved Cd at nominal concentrations of 0, 0.1, 0.5, 2.5 and 10 μg L^{-1} with two replicates per treatment. Each Cd treatment received ^{109}Cd as $CdCl_2$ (specific activity: 570.2 Bq ng^{-1}), with stable $CdCl_2$ providing the remainder of the ambient Cd in solution. During labeling the cadmium concentration in the water for each replicate was monitored daily and was calculated based on the mean radioactivity of duplicate 1-mL subsamples. As loss of Cd from solution was rapid, appropriate amounts of ^{109}Cd and $CdCl_2$ were added to each replicate after 3 days of exposure to maintain designated concentrations. After six days of Cd exposure, the plates were removed from their respective solutions and placed in bottles containing uncontaminated ASTM soft water. After 1 day of equilibration in clean water, duplicate algal samples (approximately 1 cm^2) were collected from the center of the plate from each bottle, dried, weighed and assayed for radioactivity. Duplicate 2 mL water samples were collected from each bottle as described above. These dissolved exposures generated differentially contaminated diets for the subsequent mayfly tests (see below).

As mayfly larvae did not survive the highest 2 dietary treatments from the Aug 2008 experiments, the Nov 2008 plates were exposed to a lower range of ambient Cd at 0, 0.02, 0.1, 0.5, and 2.5 μg L^{-1}. As was the case in the previous experiment, identical amounts of Cd as $^{109}CdCl_2$ (specific activity: 492.1 Bq ng^{-1}) were added to each Cd exposure, with stable $CdCl_2$ providing the remainder of the ambient Cd in solution. Cadmium was replenished after 3 days of Cd exposure as described above, and the duration of exposure for this set of experiments was 7 days. A parallel experiment was conducted exclusively with stable Cd to rule out the potential influence of radioactivity on mayfly performance. Generally poor survivorship was observed in both radioisotope (44%) and stable experiments (32%) prompting a third study. Finally, the Jan 2009 plates were exposed to dissolved Cd: 0, 0.15, 0.625, 2.5 and 10 μg L^{-1} for 6 days, with additions of Cd at day 3 as described above. Each concentration had two replicates. The 0.15 μg L^{-1} treatment used exclusively $^{109}CdCl_2$ (specific activity: 454.7 Bq ng^{-1}), while the other three treatments were prepared by adding the same amount of ^{109}Cd and appropriate amounts of $CdCl_2$ to achieve a final concentration of 0.625, 2.5 and 10 μg L^{-1} respectively.

2.2.2. Mayfly life cycle exposure to dietary Cd

After the periphyton plates were loaded with Cd, equilibrated in clean water for 24 h and sampled, 4–6 day old larvae were added to each replicate bottle (2 replicates per concentration). Thirty larvae were added to each bottle for the Aug 08 experiments, and 20 larvae were added to each bottle in subsequent experiments. Each bottle was gently aerated, and the light:dark cycle was natural for the given season with ambient light provided by large laboratory windows. Laboratory temperatures ranged from 19 to 22 °C in all experiments. Cadmium water concentrations were monitored twice per week. Cadmium in the periphyton was determined approximately every 10 days as described above. All endpoints are reported for adult mayflies and include Cd bioaccumulation, growth and survival. *Subimago exuvia* and egg masses collected from adults were also assayed for radioactivity.

Subimagos were collected upon emergence (typically ∼30 days) and transferred to humid chambers overnight for the final molt to adulthood. Radioactivity in the adults was determined before and after egg delivery. Adults were stimulated to release eggs by wetting the abdomen with ASTM soft water. Individual adults were then placed in a microcentrifuge tube and frozen at −20 °C. Adults were subsequently oven dried at 60 °C for approximately 48 h and the dry weight of each adult was obtained on a Sartorius CP225D microbalance to the nearest 0.01 mg.

2.3. Dissolved uptake and efflux

As monitoring of dissolved Cd over the life cycle test revealed some movement of Cd from the periphyton to the surrounding water, we performed dissolved uptake and elimination experiments to allow us to estimate the relative contributions of dissolved Cd to the overall Cd bioaccumulation in the mayflies. Only the highest (217.8 ng L^{-1}; Specific activity: 404 Bq ng^{-1}) dissolved concentration measured in the Jan 09 experiments was used to determine the uptake and efflux rate constants to ensure adequate radioactivity in the animals after 24 h of exposure. Five mixed-sized larvae (approximately 15–20 days post hatch) used for each of the three replicates were transferred to a Teflon beaker with 80 mL soft water without aeration at the same light regime as the dietary exposure at room temperature. Radioactivity in the larvae was measured at 3, 6, 12, and 24 h after being thoroughly rinsed in clean soft water. Larvae were not fed during exposures. After 24 h of exposure, the animals from each replicate were transferred to plastic bottles with 1 L clean soft water to monitor Cd efflux. Radioactivity in the larvae was determined daily for seven days with water renewed every day.

2.4. Acute toxicity test

To assess the acute toxicity of the presumed most sensitive life stage to dissolved Cd, a 48-h acute toxicity test was conducted on first instar larvae (1–2 days post hatch). Seven larvae were exposed to replicate concentrations of 0, 10, 100, 1000, and 10,000 μg L^{-1} Cd in a 8.4 cm petri dish at room temperature. Survivorship was evaluated by microscopic examination after 48 h exposure.

2.5. Radioactivity measurement

All measurements of radioactivity in water, periphyton, exuvia, *C. triangulifer* adults and eggs were measured using a Perkin–Elmer Wallac Wizard 1480 automatic gamma counter (Shelton, Connecticut, USA) at 16–32 keV. Samples were counted for 3 min. All counting errors were generally <5%. Appropriate corrections were made for radioactive decay and counting efficiency.

2.6. Statistical analysis

All data are expressed as mean ± standard deviation. Unless otherwise stated, all data analysis was performed using SAS (version 8.02). Bioconcentration of Cd from water to periphyton was determined by dividing Cd concentrations in periphyton (μg g^{-1}) by Cd in the water (μg L^{-1}), assuming 1 L of soft water weighs 1000 g. Note that these are not steady state bioconcentration factors (BCFs) as they represent periphyton concentrations in a non-equilibrium scenario. Trophic transfer factors (TTFs) were determined by dividing Cd body burden in *C. triangulifer* by mean Cd concentrations in the periphyton over the duration of the experiment after considering dissolved Cd contributions. Cadmium uptake and efflux rate constants were calculated based on models published elsewhere (Schlekat et al., 2002). Cadmium body burden (μg g^{-1} wet weight) in the organisms at steady state (C_{ss}) from the dissolved route was estimated using the equation: $C_{ss} = (k_u/k_e) * C_w$, where k_u is the uptake rate constant (L g^{-1} day^{-1}), k_e is the efflux rate constant (day^{-1}), and C_w is Cd concentration in the exposure media (μg L^{-1}). Regression analysis and One-way analysis of variance (ANOVA) were applied when appropriate with significance at $P < 0.05$. LC50 was determined using GraphPad Prism 5 software.

3. Results

3.1. Cadmium loading of periphyton

The bioconcentration of Cd by periphyton communities was relatively consistent across three separate experiments performed on periphyton grown in three separate seasons (Fig. 1). Average periphyton bioconcentration of Cd from solution was 1315-fold (range 629–2041) higher than ambient dissolved Cd concentrations (Table 1). Periphyton Cd bioconcentration did not show saturation with increasing ambient Cd. We assume that steady state was either reached or approached over the 6–7 days of exposure. The effect of seasonality on the uptake of Cd by periphyton was not statistically significant ($P = 0.051$) with analysis confounded by differences in exposure duration (6 vs. 7 days).

As each biofilm provided food for a cohort of mayflies for the entire developmental period, we monitored changes in periphyton Cd approximately every 10 days during the mayfly exposures. This schedule allowed us to detect any gross changes in dietary Cd while minimizing the possibility of inadvertently removing larvae during periphyton sampling. Generally, the periphyton maintained

Please cite this article in press as: Xie, L., et al., Trophic transfer of Cd from natural periphyton to the grazing mayfly *Centroptilum triangulifer*..., Environ. Pollut. (2009), doi:10.1016/j.envpol.2009.07.010

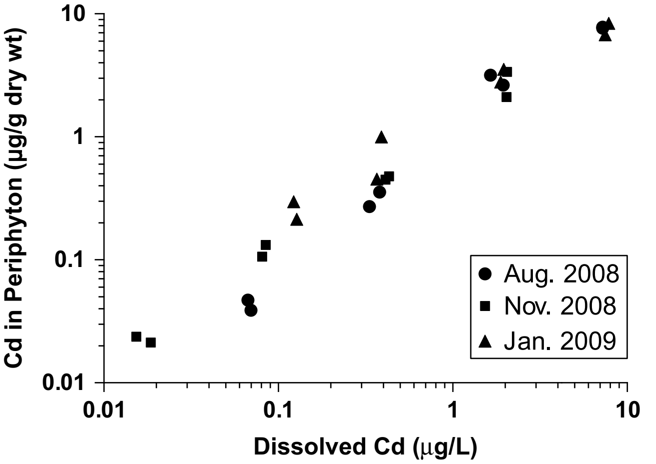

Fig. 1. Correlations between dissolved Cd exposure concentration and Cd associated with periphyton biofilms (Pearson $r > 0.97$ for each of the three experiments). Exposure durations were 6 days (Aug 2008 and Jan 2009 experiments) or 7 days (Nov 2008 experiments), with replenishment of ambient Cd at day 3 in each experiment.

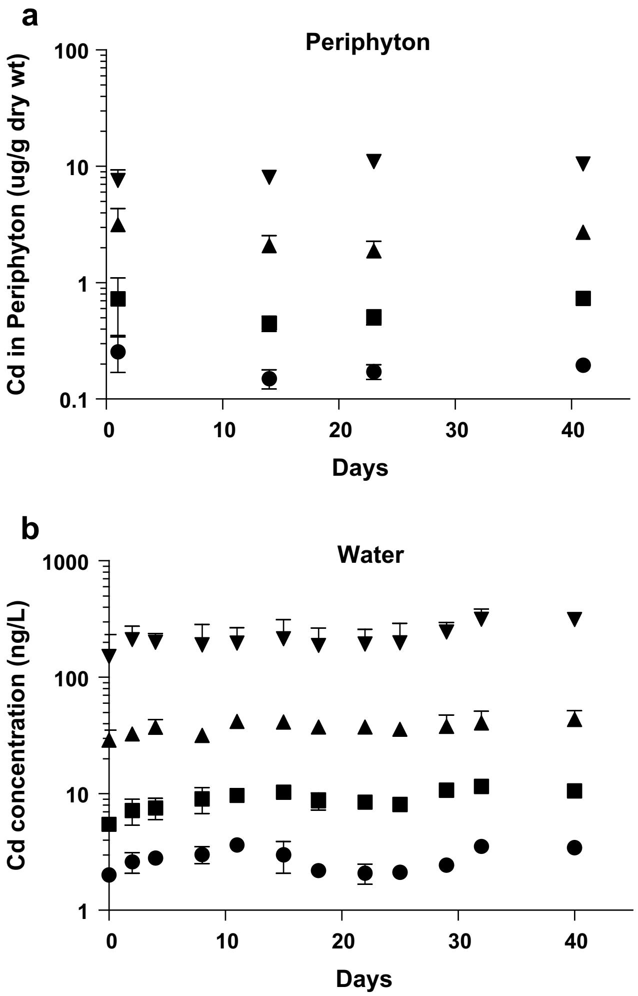

Fig. 2. Example of measured Cd concentrations in water (Panel b) and periphyton (Panel a) over the course of *C. triangulifer* life cycle exposure (Jan 2009 experiments). Symbols represent initial nominal dissolved Cd treatments of periphyton (µg L^{-1}) – ● – 0.15, ■– 0.625, ▲ – 0.5, ▼ – 10.0. Error bars represent standard deviations.

consistent Cd concentrations throughout the mayfly developmental tests (Fig. 2a). Monitoring of Cd in water was more routine (every 2–3 days), and an equilibrium between water and periphyton appeared to be established for each Cd treatment (Fig. 2b).

3.2. *Life cycle accumulation of cadmium in* C. triangulifer

Within each experiment, Cd concentrations in *C. triangulifer* adults were strongly correlated with Cd concentrations in the periphyton (Pearson $r > 0.96$ for all three experiments) (Fig. 3). Despite similar total Cd loading in periphyton across experiments, the accumulation by mayflies was different from experiment to experiment. For example, mayflies grown on periphyton with a Cd concentration of 0.53 µg g^{-1} (Nov 2008 experiment) accumulated approximately 3-fold more Cd than mayflies grown on a slightly higher Cd concentration of 0.61 µg g^{-1} in the Jan 2009 experiments. Trophic transfer factors from the Aug 2008 (3.74 ± 2.60) did not differ from the Nov 2008 (4.50 ± 2.16) experiments but both were larger than those from the Jan 2009 experiments (0.85 ± 0.21) ($P < 0.01$). Residual periphyton in the gut is not an issue in adult

Table 1

Summary of results from three trophic transfer experiments. Cd in periphyton is reported as the periphyton concentration when mayfly larvae were introduced to differentially contaminated diets. Dissolved Cd concentrations represent the mean of all measured Cd values over the mayfly life cycle exposures. Trophic transfer factors are estimated after accounting for possible dissolved accumulation based on k_u and k_e estimates. Percent survival is reported as the mean survival of the 2 replicate exposures. Control survivorship was 98% (Aug. 2008), 50% (Nov. 2008), and 90% (replicate 1), 30% (replicate 2) (Jan. 2009). N/A: not available. All values are reported ± standard deviation.

Periphyton treatments (nominal)	Cd in periphyton (µg g^{-1} dry wt)	Periphyton bioconcentration (fold increase)	Dissolved Cd (ng L^{-1})	Cd in mayfly adults (µg g^{-1} dry wt)	Trophic transfer factor	% Cd from solution	% Cd from diet	% Survival
Aug 2008								
0.1 µg L^{-1}	0.043 ± 0.016	629 ± 84	6.8 ± 4.0	0.16 ± 0.17	3.95	29.5	70.5	57
0.5 µg L^{-1}	0.313 ± 0.064	877 ± 166	16.0 ± 7.0	1.00 ± 0.74	3.54	12.5	87.5	40
2.5 µg L^{-1}	2.907 ± 0.750	1617 ± 212	133.5 ± 58.6	N/A	N/A	N/A	N/A	0
10 µg L^{-1}	7.722 ± 2.009	1067 ± 15	568.6 ± 275.7	N/A	N/A	N/A	N/A	0
Nov 2008								
0.02 µg L^{-1}	0.022 ± 0.003	1326 ± 103	0.8 ± 0.5	0.08 ± 0.03	3.38	7.0	93.0	25
0.1 µg L^{-1}	0.119 ± 0.023	1436 ± 222	3.2 ± 1.1	0.84 ± 0.41	7.29	3.0	97.0	40
0.5 µg L^{-1}	0.463 ± 0.206	1099 ± 46	11.4 ± 3.5	2.61 ± 1.31	4.51	3.6	96.4	50
2.5 µg L^{-1}	2.737 ± 0.884	1344 ± 438	77.2 ± 28.2	9.68 ± 3.43	3.08	7.0	93.0	60
Jan 2009								
0.15 µg L^{-1}	0.255 ± 0.085	2041 ± 466	2.7 ± 0.6	0.19 ± 0.06	0.87	10.9	89.1	85
0.625 µg L^{-1}	0.726 ± 0.376	1918 ± 120	9.0 ± 1.9	0.66 ± 0.27	0.93	10.7	89.3	78
2.5 µg L^{-1}	3.160 ± 1.170	1643 ± 276	37.2 ± 6.0	1.85 ± 0.78	0.64	14.9	85.1	65
10 µg L^{-1}	7.568 ± 1.714	787 ± 150	217.8 ± 72.0	11.00 ± 4.30	0.95	16.8	83.2	75

Please cite this article in press as: Xie, L., et al., Trophic transfer of Cd from natural periphyton to the grazing mayfly *Centroptilum triangulifer*..., Environ. Pollut. (2009), doi:10.1016/j.envpol.2009.07.010

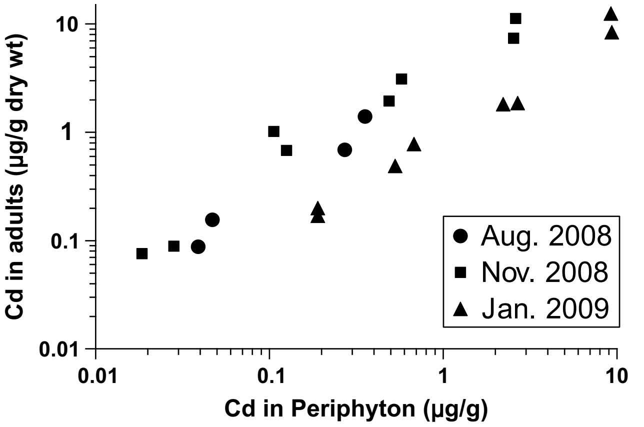

Fig. 3. Relationship between periphyton Cd concentration and Cd body burdens in *C. triangulifer* adults (Pearson $r > 0.96$ for each of the three experiments).

mayflies, as larvae cease feeding prior to emergence to allow air to fill the gut in the winged life stages (Funk et al., 2008). In all experiments, there was very limited cadmium present in the subimago exuvia and maternal transfer of Cd to eggs was rare (data not shown). A few (<3%) aberrant individuals transferred measurable radioactivity to eggs, but there was no discernable pattern associated with maternal or egg Cd burdens. There was no significant difference in egg production between the control (1505 ± 383, $n = 6$) and combined treatment group adults (1373 ± 288, $n = 6$) ($P > 0.05$).

3.3. Dissolved vs. dietary contributions

To estimate the potential contribution of dissolved Cd in mayfly tissues, we estimated the uptake rate ($k_u = 1.008 \pm 0.26$ (L g^{-1} day^{-1})) and efflux rate ($k_e = 0.041 \pm 0.006$ day^{-1}) constants. We applied these constants to the measured dissolved Cd concentrations in each exposure, estimating an assumed steady state Cd body burden derived from solution only. Despite the fact that the k_u for *C. triangulifer* is the highest reported for an aquatic insect to date (see Buchwalter et al., 2008), dietary Cd was the predominant exposure route. Averaged across all experiments, dietary Cd accounted for 88% of the Cd body burden measured in adult mayfly tissues (Table 1).

3.4. Mayfly performance

The survivorship of mayflies (Table 1) was highly variable throughout these studies, perhaps as a result of food quality differences. An apparent concentration–response relationship was observed in the Aug 2008 experiment with excellent control survivorship. Mortality occurred in the highest 2 treatment groups before larvae reached sizes visible to the naked eye. Residual food was present in all treatment groups (excluding controls) in this experiment, suggesting that food availability was not a primary factor in the performance of the Cd treatment groups. A repeat of that experiment (Jan 2009) with similar Cd dietary treatments did not produce any apparent effect on the mayflies. However, as noted above, Cd bioaccumulation was much lower in this experiment. Mayfly survivorship in the Nov 2008 experiments was generally poor in both radioisotope and stable Cd parallel experiments. The average survivorship across all experiments was approximately 60%.

3.5. Acute toxicity test

A dissolved acute toxicity assay with newly hatched larvae failed to show toxicity at environmentally relevant concentrations. The no observed effect concentration (NOEC) of cadmium for the newly hatched *C. triangulifer* larvae was estimated to be 10 µg L^{-1}. The 48-h LC50 was determined to be 352 µg L^{-1} – a concentration 2–3 orders of magnitude higher than what might be encountered in nature.

4. Discussion

Periphyton is a sink for Cd in aquatic environments. In nature, reported Cd concentrations in periphyton vary greatly from different aquatic systems depending on water chemistry and contamination history. For example, in Southeast Missouri streams, Cd in periphyton ranged from 0.22 to 0.43 µg g^{-1} in streams unaffected by mining activity, and up to 5.2 µg g^{-1} (dry wt) in streams affected by lead mining activity (Besser et al., 2007). Periphyton from East Fork Poplar Creek in East Tennessee, a mercury contaminated stream had a Cd level of ~11 µg g^{-1} (Hill and Larsen, 2005). Cadmium in periphyton from the Boulder River watershed (Jefferson County, Montana) ranged from 0.54 to 70.3 µg g^{-1} dry wt (Farag et al., 2007; Rhea et al., 2006). Extreme values (143–1809 µg g^{-1}) for Cd in periphyton were reported from the Riou Mort River in Southeast France (Morin et al., 2008a,b). Thus, Cd in periphyton from our experiments (0.043–7.7 µg g^{-1}) is representative of what might be encountered in mildly to moderately contaminated streams.

The high assimilative capacity of periphyton for Cd is also evident from laboratory studies, where exposure to extreme Cd concentrations results in tremendous Cd bioaccumulation in periphyton. For example, exposure to 64 µg L^{-1} cadmium for three weeks (Ivorra et al., 2002) and 25 µg L^{-1} of cadmium for 10 days (Brooks et al., 2004), resulted in Cd concentrations of approximately 1000 and 600 µg g^{-1} dry wt, respectively. This suggests that periphyton is difficult to saturate with Cd under natural conditions and acts as a major Cd sink. In our experiments, periphyton bioconcentrated Cd 1315 ± 442 fold from water. This value is within the range of the reported Cd BCFs of 8.0×10^2–1.0×10^4 for freshwater algae (Taylor, 1983), but less than that reported in a marine diatom, which is in the range of 10^4–10^5 (Wang and Ke, 2002) and a freshwater diatom *Asterionella formosa* which had a BCF of 40,000 (Conway, 1978).

While periphyton Cd bioaccumulation was comparable among three different seasonal periphyton communities, the subsequent transfer of Cd to grazing mayflies was not. In two of the three experiments, TTFs were quite large (3–4), whereas they were modest (<1) in another. Possible reasons for these bioaccumulation differences among experiments include food quality/growth, and Cd bioavailability differences associated with periphyton flora. Animals grown on the Jan 2009 periphyton were 35% larger on average than animals grown in the other studies ($P < 0.001$). This growth difference was also reflected in the controls of these experiments, suggesting that food quality was superior in the Jan 2009 periphyton.

It is also possible that subcellular Cd compartmentalization varied among the more dominant species in the different periphyton communities. Cadmium is generally adsorbed to the cell wall (insoluble fraction) and absorbed into cytosol (soluble fraction) (Ettajani et al., 2001; Wang and Wang, 2008) in algae. The insoluble fraction is considered to be generally less bioavailable, whereas the cytosolic Cd is considered to be highly bioavailable (Cheung et al., 2006; Ettajani et al., 2001; Rainbow et al., 2007; Wallace and Lopez, 1997; Wang and Wang, 2008). Distributions of Cd can vary widely from species to species (Ettajani et al., 2001; Wang and Wang, 2008). Thus, seasonal differences in periphyton flora may strongly affect Cd bioavailability to grazers. While these compartmentalization differences are tractable in laboratory studies with single

Please cite this article in press as: Xie, L., et al., Trophic transfer of Cd from natural periphyton to the grazing mayfly *Centroptilum triangulifer*..., Environ. Pollut. (2009), doi:10.1016/j.envpol.2009.07.010

algal species as diets, natural periphyton communities are exceptionally complex. Historical studies of White Clay Creek (Stroud Water Research Center, 1974) report that algal communities are typically comprised of 128–223 species (mean = 168), with the dominant taxa rarely representing more than 15% of the individuals in the algal community.

Our results show that diet is a predominant Cd exposure route and that Cd can be strongly biomagnified from periphyton to *C. triangulifer* in a life cycle assay. Trophic transfer studies for Cd from freshwater primary producers are surprisingly rare, but include TTF estimates of >1 (up to 6) in *Daphnia pulex* (Mathews and Fisher, 2008), and >1 (up to 8) in *Daphnia magna* (Guan and Wang, 2004). In a freshwater food web, Croteau et al. (2005) provide evidence of Cd biomagnification in nature – particularly among invertebrates.

Given that periphyton is a sink for metals, and that metals associated with periphyton can be highly bioavailable, it follows that periphyton diets are a potentially important exposure route for grazing insects in nature. Aside from growth issues associated with dietary exposures (Irving et al., 2003), and potential toxicity observed in the Aug 2008 experiments, the link between bioaccumulation and toxicity remains somewhat elusive. However, the importance of dietary metal bioaccumulation in invertebrates (Hook and Fisher, 2001) calls to question the relevance of toxicity testing that ignores this exposure route. Test systems that incorporate environmentally realistic dietary exposures should be considered in the context of setting water quality criteria.

Acknowledgements

The authors acknowledged the editorial contributions of G.A. LeBlanc, D.J. Cain, E. Cable, and J.M. Conley, and four anonymous reviewers which greatly improved the presentation of this work. This research was supported by the College of Agriculture and Life Science, North Carolina State University.

References

Besser, J.M., Brumbaugh, W.G., May, T.W., Schmitt, C.J., 2007. Biomonitoring of lead, zinc, and cadmium in streams draining lead-mining and non-mining areas, Southeast Missouri, USA. Environmental Monitoring and Assessment 129, 227–241.

Brix, K.V., DeForest, D.K., Burger, M., Adams, W.J., 2005. Assessing the relative sensitivity of aquatic organisms to divalent metals and their representation in toxicity datasets compared to natural aquatic communities. Human and Ecological Risk Assessment: An International Journal 11, 1139–1156.

Brooks, B.W., Stanley, J.K., White, J.C., Turner, P.K., Wu, K.B., La Point, T.W., 2004. Laboratory and field responses to cadmium: an experimental study in effluent-dominated stream mesocosms. Environmental Toxicology and Chemistry 23, 1057–1064.

Buchwalter, D.B., Cain, D.J., Clements, W.H., Luoma, S.N., 2007. Using biodynamic models to reconcile differences between laboratory toxicity tests and field biomonitoring with aquatic insects. Environmental Science and Technology 41, 4821–4828.

Buchwalter, D.B., Cain, D.J., Martin, C.A., Xie, L., Luoma, S.N., Garland, T., 2008. Aquatic insect ecophysiological traits reveal phylogenetically based differences in dissolved cadmium susceptibility. Proceedings of the National Academy of Sciences of the United States of America 105, 8321–8326.

Cain, D.J., Luoma, S.N., Wallace, W.G., 2004. Linking metal bioaccumulation of aquatic insects to their distribution patterns in a mining-impacted river. Environmental Toxicology and Chemistry 23, 1463–1473.

Cheung, M.S., Fok, E.M.W., Ng, T.Y.T., Yen, Y.F., Wang, W.X., 2006. Subcellular cadmium distribution, accumulation, and toxicity in a predatory gastropod, *Thais clavigera*, fed different prey. Environmental Toxicology and Chemistry 25, 174–181.

Clements, W.H., 1991. Community responses of stream organisms to heavy metals: a review of observational and experimental approaches. In: Newman, M.C., McIntosh, A.W. (Eds.), Metal Ecotoxicology Concepts and Applications. Lewis Publishers, Chelsea, MI, pp. 363–386.

Clements, W.H., Carlisle, D.M., Lazorchak, J.M., Johnson, P.C., 2000. Heavy metals structure benthic communities in Colorado mountain streams. Ecological Application 10, 626–638.

Conway, H.L., 1978. Sorption of arsenic and cadmium and their effects on growth, micronutrient utilization, and photosynthetic pigment composition of *Asterionella formosa*. Journal of Fisheries Research Board of Canada 35, 286–294.

Croisetiere, L., Hare, L., Tessier, A., 2006. A field experiment to determine the relative importance of prey and water as sources of As, Cd, Co, Cu, Pb, and Zn for the aquatic invertebrate *Sialis velata*. Environmental Science and Technology 40, 873–879.

Croteau, M.-N., Luoma, S.N., Stewart, A.R., 2005. Trophic transfer of metals along freshwater food webs: evidence of cadmium biomagnification in nature. Limnology and Oceanography 50, 1511–1519.

Croteau, M.N., Luoma, S.N., 2008. A biodynamic understanding of dietborne metal uptake by a freshwater invertebrate. Environmental Science and Technology 42, 1801–1806.

Ettajani, H., Berthet, B., Amiard, J.C., Chevolot, L., 2001. Determination of cadmium partitioning in microalgae and oysters: contribution to the assessment of trophic transfer. Archives of Environmental Contamination and Toxicology 40, 209–221.

Farag, A.M., Nimick, D.A., Kimball, B.A., Church, S.E., Harper, D.D., Brumbaugh, W.G., 2007. Concentrations of metals in water, sediment, biofilm, benthic macroinvertebrates, and fish in the Boulder River watershed, Montana, and the role of colloids in metal uptake. Archives of Environmental Contamination and Toxicology 52, 397–409.

Funk, D.H., Jackson, J.K., Sweeney, B.W., 2008. A new parthenogenetic mayfly (Ephemeroptera:Ephemerellidae:Eurylophella Tiensuu) oviposits by abdominal bursting in the subimago. Journal of the North American Benthological Society 27, 269–279.

Guan, R., Wang, W.-X., 2004. Dietary assimilation and elimination of Cd, Se, and Zn by *Dapnhia magna* at different metal concentrations. Environmental Toxicology and Chemistry 23, 2689–2698.

Hill, W.R., Bednarek, A.T., Larsen, I.L., 2000. Cadmium sorption and toxicity in autotrophic biofilms. Canadian Journal of Fisheries and Aquatic Sciences 57, 530–537.

Hill, W.R., Larsen, I.L., 2005. Growth dilution of metals in microalgal biofilms. Environmental Science and Technology 39, 1513–1518.

Hook, S.E., Fisher, N.S., 2001. Sublethal effects of silver in zooplankton: importance of exposure pathways and implications for toxicity testing. Environmental Toxicology and Chemistry 20, 568–574.

Irving, E.C., Baird, D.J., Culp, J.M., 2003. Ecotoxicological responses of the mayfly *Baetis tricaudatus* to dietary and waterborne cadmium: implications for toxicity testing. Environmental Toxicology and Chemistry 22, 1058–1064.

Ivorra, N., Hettelaar, J., Kraak, M.H.S., Sabater, S., Admiraal, W., 2002. Responses of biofilms to combined nutrient and metal exposure. Environmental Toxicology and Chemistry 21, 626–632.

Martin, C.A., Luoma, S.N., Cain, D.J., Buchwalter, D.B., 2007. Cadmium ecophysiology in seven stonefly (Plecoptera) species: delineating sources and estimating susceptibility. Environmental Science and Technology 41, 7171–7177.

Mathews, T., Fisher, N.S., 2008. Evaluating the trophic transfer of cadmium, polonium, and methylmercury in an estuarine food chain. Environmental Toxicology and Chemistry 27, 1093–1101.

McDunnough, J., 1931. New species of North American Ephemeroptera. Canadian Entomologist 63, 82–93.

Meylan, S., Behra, R., Sigg, L., 2003. Accumulation of copper and zinc in periphyton in response to dynamic variations of metal speciation in freshwater. Environmental Science and Technology 37, 5204–5212.

Morin, S., Duong, T.T., Dabrin, A., Coynel, A., Herlory, O., Baudrimont, M., Delmas, F., Durrieu, G., Schafer, J., Winterton, P., Blanc, G., Coste, M., 2008a. Long-term survey of heavy-metal pollution, biofilm contamination and diatom community structure in the Riou Mort watershed, South-West France. Environmental Pollution 151, 532–542.

Morin, S., Duong, T.T., Herlory, O., Feurtet-Mazel, A., Coste, M., 2008b. Cadmium toxicity and bioaccumulation in freshwater biofilms. Archives of Environmental Contamination and Toxicology 54, 173–186.

Munger, C., Hare, L., 1997. Relative importance of water and food as cadmium sources to an aquatic insect (*Chaoborus punctipennis*): implications for predicting Cd bioaccumulation in nature. Environmental Science and Technology 31, 891–895.

Rainbow, P.S., Amiard, J.C., Amiard-Triquet, C., Cheung, M.S., Zhang, L., Zhong, H., Wang, W.X., 2007. Trophic transfer of trace metals: subcellular compartmentalization in bivalve prey, assimilation by a gastropod predator and in vitro digestion simulations. Marine Ecology Progress Series 348, 125–138.

Rhea, D.T., Harper, D.D., Farag, A.M., Brumbaugh, W.G., 2006. Biomonitoring in the Boulder River watershed, Montana, USA: metal concentrations in biofilm and macroinvertebrates, and relations with macroinvertebrate assemblage. Environmental Monitoring and Assessment 115, 381–393.

Schlekat, C.E., Lee, B.G., Luoma, S.N., 2002. Dietary metals exposure and toxicity to aquatic organisms: implications for ecological risk assessment. In: Newman, M.C., Roberts Jr., M.H., Hale, R.C. (Eds.), Coastal and Estuarine Risk Assessment. Lewis Publisher, Boca Raton, pp. 151–188.

Selby, D.A., Ihnat, J.M., Messer, J.J., 1985. Effects of subacute cadmium exposure on a hardwater mountain stream microcosm. Water Research 19, 645–655.

Stroud Water Research Center, 1974. A Study of the White Clay Creek Watershed 1969–1974. Final report to the Rockefeller Foundation and the National Science Foundation. Stroud Water Research Center, Limnology Department, Academy of Natural Sciences of Philadelphia, Philadelphia, Pennsylvania.

Sweeney, B.W., Funk, D.H., Standley, L.J., 1993. Use of the stream mayfly *Cloeon triangulifer* as a bioassay organism – life-history response and body burden following exposure to technical Chlordane. Environmental Toxicology and Chemistry 12, 115–125.

Sweeney, B.W., Vannote, R.L., 1984. Influence of food quality and temperature on life-history characteristics of the parthenogenetic mayfly, *Cloeon triangulifer*. Freshwater Biology 14, 621–630.

Tabak, L.M., Gibbs, E., 1991. Effects of aluminum, calcium and low pH on egg hatching and nymphal survival of *Cloeon triangulifer* McDunnough (Ephemeroptera, Baetidae). Hydrobiologia 218, 157–166.

Taylor, D., 1983. The significance of the accumulation of cadmium by aquatic organisms. Ecotoxicology and Environmental Safety 7, 33–42.

Wallace, W.G., Lopez, G.R., 1997. Bioavailability of biologically sequestered cadmium and the implications of metal detoxification. Marine Ecology Progress Series 147, 149–157.

Wang, M.J., Wang, W.X., 2008. Cadmium toxicity in a marine diatom as predicted by the cellular metal sensitive fraction. Environmental Science and Technology 42, 940–946.

Wang, W.X., Ke, C.H., 2002. Dominance of dietary intake of cadmium and zinc by two marine predatory gastropods. Aquatic Toxicology 56, 153–165.

Xie, L., Flippin, J.L., Deighton, N., Funk, D.H., Dickey, D.A., Buchwalter, D.B., 2009. Mercury(II) bioaccumulation and antioxidant physiology in four aquatic insects. Environmental Science and Technology 43, 934–940.

Please cite this article in press as: Xie, L., et al., Trophic transfer of Cd from natural periphyton to the grazing mayfly *Centroptilum triangulifer*..., Environ. Pollut. (2009), doi:10.1016/j.envpol.2009.07.010