Bioavailability of sediment-associated Cu and Zn to Daphnia magna

P.L. Gillis a,∗, C.M. Wood a, J.F. Ranville b, P. Chow-Fraser a

a McMaster University, Hamilton, Ont., Canada L8S 4K1
b Colorado School of Mines, Golden, CO 80401, USA

Received 27 October 2005; received in revised form 11 January 2006; accepted 13 January 2006

Abstract

Exposures to mining-impacted, field-collected sediment (Clear Creek, CO, USA) contaminated with Cu (2.4 mg/g) and Zn (5.2 mg/g) were acutely toxic to juvenile Daphnia magna. Dissolved Cu and Zn in the overlying water (sediment + reference water) were at levels that could cause acute toxicity. To reduce dissolved metals below toxic levels, the sediment was repeatedly rinsed to remove any easily mobilized metals. Washing the sediment reduced dissolved Cu by 60% and Zn by 80%. D. magna exposed to washed sediment experienced higher survival (95%) compared to those exposed to the original sediment (<50%). Cu and Zn that remained associated with suspended sediment after washing were not bioavailable, since survival and tissue metal concentrations in D. magna exposed to both filtered (>0.45 μm) and unfiltered overlying water were statistically similar. Multiple regression analysis indicated that only dissolved Cu significantly contributed to mortality of D. magna whereas particulate Cu, particulate Zn, and dissolved Zn did not. Regression analysis on a combined dataset from all Clear Creek exposures (washed and unwashed), revealed a significant (p<0.0001, r²=0.76) relationship between the concentration of dissolved copper in the overlying water and the mortality of exposed Daphnia, yielding an estimated LC50 of 26 μg/L dissolved copper (hardness approximately 140 mg/L). The results of this study indicate that if the sediment of Clear Creek was subjected to a resuspension event that there would be a significant efflux of metals from the sediment into the water column, resulting in potentially toxic levels in the water column.

© 2006 Elsevier B.V. All rights reserved.

Keywords: Metal bioavailability; D. magna; Sediment associated metals; Labile metals; Copper; Zinc

1. Introduction

Aquatic organisms may be exposed to metals through the dissolved phase and/or through contact with metal-contaminated particles. Previously the dissolved phase was thought to be the main source of metal exposure for planktonic organisms, but recently a number of studies have demonstrated that dietary exposure is also an important route for metal accumulation (Taylor et al., 1998; Wang and Fisher, 1998; Hooke and Fisher, 2001; Adam et al., 2002; Barata et al., 2002; Fisher and Hooke, 2002; Yu and Wang, 2002a,b). These dietary studies have focused on the uptake of metals from organic particles, in particular from metal-contaminated algae, but the uptake of metals from suspended inorganic particles and sediments remains relatively unexplored. A better understanding of the fate of sediment-associated metals is needed because if dissolved metal concentrations alone are used to predict toxicity, then environmental risk could be underestimated if there is bioavailable metal associated with inorganic particles.

Filter-feeding planktonic organisms such as Daphnia may be exposed to sediment-associated metals in two ways. Metals can be released from the sediment into the pore water by changes in geochemical speciation, and then by simple diffusion and convection can move into the overlying water. Metals released in this way will be incorporated into the dissolved phase and depending on the ionic composition of the water are typically considered bioavailable. In addition, metal-contaminated sediment particles may become resuspended into the water column by convection associated with bioturbation and currents. Metals associated with suspended sediment particles have the potential to be ingested by filter-feeding organisms but whether or not they are bioavailable will depend on the nature of the association. Filter-feeding organisms are often exposed to both dissolved and particle-associated metals simultaneously.

As filter-feeders, the preferred food of Daphnia magna is algae but any suspended particles over 0.45 μm will be retained
on the filtering appendages and may be ingested (Brendelberger, 1985). D. magna will periodically browse at the sediment–water interface if the availability of suspended food (i.e. algae) falls below a threshold (Horton et al., 1979). In order to overcome periods of food shortage, D. magna will actively stir up sediments and detritus by scraping the bottom of shallow waters with their thoracic appendages and then will produce water currents to remove particles from the water (Lampert, 1987). By grazing at the sediment–water interface Daphnia combines epiphitic and suspension feeding (Martinez-Madrid et al., 1999). Recently we demonstrated that D. magna does ingest sediment, by showing the presence of sediment particles in the gut after exposure in whole sediment bioassays (Gillis et al., 2005).

Based on the limited data available to date, the bioavailability of sediment-associated metals appears to depend on the metal, the composition of the particle and possibly whether the particles were naturally or artificially contaminated. Weltens et al. (2000) reported enhanced accumulation of Cd and higher mortality in D. magna exposed to Cd- and Zn-spiked particles than in D. magna exposed to only the equivalent dissolved concentration. Weltens et al. (2000) suggested that the physicochemical condition of the gut would favor desorption of metals during the digestion process; therefore, sediment bound particles could be a source of bioavailable metals. In contrast, Erickson et al. (1995) reported that Cu bound to (spiked) particles did not significantly contribute to toxicity in Ceriodaphnia dubia any more than exposures to the dissolved phase alone. Ma et al. (2002) demonstrated that toxicity in C. dubia decreased with the binding of free Cu (Cu(II)) since there was a linear relationship between survival and Cu(II). Weltens et al. (2001) found that suspended solids collected from rivers polluted with a range of metals and organics, released contaminants into the surrounding water (laboratory exposures) creating an acutely toxic environment but there was no further toxicity associated with contaminants that remained bound to the particles.

The overall goal of this study was to determine if the metals associated with field-collected sediment are bioavailable to D. magna. This was accomplished by determining the relative contribution of dissolved and particle-associated metals to levels of accumulation in D. magna and any observed acute toxicity. Sediment collected from a mining-impacted stream was employed, where preliminary investigations determined that copper and zinc, and not others, were of primary concern for toxicity. Our initial screening of the overlying water revealed that substantial Cu and Zn desorbed from the sediment into the overlying water, resulting in acutely toxic concentrations of Cu and Zn in the overlying water. Therefore, it was necessary to significantly reduce the amount of easily mobilized metals associated with the sediment through a series of rinsings. After exposure to the ‘washed’ sediment demonstrated that dissolved metals had been reduced below acutely toxic levels, the final aim was to separate the dissolved fraction from the particulate fraction to determine which phase was the main source of bioavailable metal. Survival and tissue Cu and Zn concentrations were determined for the exposed organisms and compared with the amounts of dissolved and particle-associated metal in the overlying water.

2. Materials and methods

2.1. Algae cultures

A pure culture of Pseudokircheriella subcapitata purchased from University of Toronto Culture Collection (Toronto, Ont., Canada) in November 2002, was cultured with Bristol’s medium according to USEPA protocol 16.6049002F (1993). Algae were cultured in 5 L volumetric flasks and held under 24 h light in climate-controlled chambers at 25 ± 2 C.

2.2. D. magna cultures

A D. magna clone (lot #090600 DM) purchased from Aquatic Research Organisms (Hampton, NH, USA) was held (20–22 C) in continuous culture according to USEPA protocol 16.6049002F (1993). Daphnia were fed a combined diet of yeast, cerophyll and trout chow (YCT) and unicellular algae (P. subcapitata) daily. Culture media was changed three times per week. Neonates were used to initiate new cultures once a week. Dechlorinated Hamilton city tap water (Lake Ontario) was used as culture water, the overlying water in exposures and the water for gut clearing. This water (herein referred to as reference water) was dechlorinated on site and routinely monitored for chlorine, metals and major ions. Ionic composition in mM: [Na+] = 0.86, [Cl−] = 1.0, [Ca2+] = 1.0, [K+] = 0.05, [Mg2+] = 0.2. Hardness was approximately 140 mg/L (as CaCO3), pH was 7.8–8 and dissolved organic carbon was approximately 3.0 mg C/L. Background Cu was 2–4.0 µg/L and Zn was <50 µg/L.

Rather than D. magna neonates, we used 5-day-old juveniles because their larger size provided more tissue for metal analysis. We did not use adults to avoid confounding effects of changes in size and ionic status that accompany brood production and release.

2.3. Sediments

The metal-contaminated sediment used in this study was collected from Clear Creek, CO, USA (39°44'54"N, 105°23'55"W). The reference sediment used to dilute the Clear Creek sediment was collected from Long Point, Lake Erie, Ont., Canada (42°33'54"N, 80°02'28"W). Bulk sediments were digested using a 1:1 HNO3:HCl mixture using trace element grade acid (Fisher Scientific) and then analyzed by inductively coupled mass spectrometry. Sediment metal concentrations are given in Table 1.

Clear Creek is a high-gradient stream which receives metal-rich effluent from a number of mining sites, and has elevated metal levels in both the water and sediments (Table 1). Clear Creek has been designated as a USEPA ‘Superfund site’, indicating that the creek is significantly contaminated and requires study and remediation. Clear Creek streambed sediments are dominated by amorphous iron oxyhydroxide precipitates (i.e. schwertmannite, ferrihydrite, and goethite). These phases coat other detrital silicate minerals and form within the water column as a result of ferrrous iron oxidation and precipitation due to upstream inputs of acid mine drainage. The composition of...
the streambed sediments vary seasonally with silicates dominating after high flow periods and iron precipitates during low flow periods (Harvey et al., 2003). The sediment used in this study contains an intermediate amount of the iron-rich precipitate. Although the concentrations of Cu, Zn, Pb and Cd in Clear Creek sediment were all elevated above what Persaud et al. (1992) designated to be the severe effects level, our initial investigations indicated that only Cu and Zn were released from the sediment in such quantities that the concentrations in the overlying water were within the acutely toxic range for *D. magna* (De Schamphelaere et al., 2002; De Schamphelaere and Janssen, 2002; Muyssem and Janssen, 2001). The concentration of cadmium in the overlying water of the initial exposures (unwashed sediment) was less than 10 μg/L, whereas Stuhlbacher et al. (1993) reported that in water with similar hardness (170 mg/L) to the water used in this study (140 mg/L), the Cd LC50s for juvenile (3-6-day old) *D. magna* ranged from 49 to 250 μg/L, depending on the clone used, and similarly, Barata et al. (1998) reported that Cd LC50s for neonates (hardness of 179 mg/L) ranged from 23 to 233 μg/L, depending on the clone. The concentration of Pb in the overlying water of the unwashed sediment exposures was deemed non-toxic since all concentrations were below 5 μg/L and the LC50s for neonate *Daphnia* are reported to be in the range of hundreds of micrograms to milligrams per liter (Biesinger and Christensen, 1972; Chapman, 1980; Fargašová, 1994; Arambašić et al., 1995; Carvalho et al., 1998). Therefore, based on our understanding of the stream, the concentrations of metals in the overlying water of our sediment exposures along with published *Daphnia* metal toxicity data, we chose to focus on the bioavailability and toxicity of Cu and Zn from the Clear Creek sediments.

### 2.4. *D. magna* exposures: initial exposure

Fig. 1 provides an overview of the study design and illustrates the sequence of experiments conducted in this study. *Daphnia* exposures were conducted in 250 mL glass beakers at room temperature (18-21 °C) for 48 h without aeration, addition of supplemental food or renewal of overlying water. Reference water was used as overlying water except in treatments where site water was required (initial exposure only). In all cases the water-to-sediment ratio was 4:1. Dissolved oxygen, pH, and dissolved (filtered through an Acrodisc 0.45 μm in-line syringe-tip filter) and total (unfiltered) Cu and Zn in the overlying water were measured at initiation and upon completion of the exposure. To determine if the metals associated with the field-collected sediment were bioavailable, survival and tissue Cu and Zn concentrations were determined for *D. magna* exposed to: (1) reference water alone; (2) site water alone; (3) reference water + sediment; (4) site water + sediment. Ten juvenile *D. magna* were added to each of four replicate beakers. Mortality was recorded at the end of the exposure (48 h). Surviving *D. magna* were transferred to reference water for one hour to purge their gut before being analyzed for tissue metals.

### 2.5. Sediment ‘washing’

The dissolved concentrations of Cu and Zn in the overlying water in the Initial Exposure were within the range of reported EC50s (Muyssem and Janssen, 2001; De Schamphelaere et al., 2002; De Schamphelaere and Janssen, 2002). Therefore, in order to differentiate toxicity associated with the dissolved fraction from toxicity associated with the particle-bound metals, sediments were subjected to a series of ‘washings’ (using reference water) to remove any easily mobilized metals from the sediment particles. Two methods of washing were compared. In the first (Method A), the sediments that were used in the initial exposure underwent a series of resuspensions by being stirred for 10 min and then allowed to settle for 1 week, after which the over-

![Fig. 1. Flowchart of study design and experiments conducted. Items 1–3, and 5 are part of the current study, item 4 was the subject of a separate investigation with results presented in Gillis et al. (2005).](image-url)
lying water was replaced. This was repeated three times over a month. In the second (Method B), fresh sediments were subjected to a series of mixing by a Vortex mixer for 5 min followed by centrifugation (3000 rpm for 10 min) and finally replacement of overlying water. This process was repeated six times in the course of a day. In both methods, the overlying water was sampled for dissolved metals after each ‘wash’ before it was gently decanted and replaced with new reference water for the next ‘wash’. The water-to-sediment ratio in the washing vessels was approximately 3:1.

### 2.6. Washed sediment exposure

Following the removal of the easily mobilized metals from the sediment, *D. magna* were exposed to the washed sediments with the same experimental conditions described for the initial exposure. Survival (48 h) and tissue metal concentrations (1 h with the same experimental conditions described for the initial *D. magna* exposure) of sediments were measured with 50 mL of washed sediment were added and left to settle for 1 week (18–21 °C) before it was transferred to a new beaker (referred to as ‘filtered’). In a third treatment, the beakers (containing sediment and reference water) were used without any manipulation; therefore, the overlying water in this final treatment was also unfiltered. Five replicate beakers were prepared for each treatment.

Ten *D. magna* were added to each exposure vessel and held for 48 h under the same experimental conditions (temperature, without aeration, etc.) described above for the other exposures. Following exposure, *D. magna* were transferred to 200 mL of reference water containing $5 \times 10^4$ cells of *P. subcapita* and allowed to purge their guts for 8 h prior to metal analysis. This extended period of purging in the presence of algae was necessitated by closer examination of the *D. magna* from the initial exposure and the washed sediment exposures which revealed that there were still sediment particles in their gut after 1 h of clearance in water. After further investigation into the gut clearance patterns of *Daphnia*, we concluded that only when *D. magna* were held in the presence of algae for 8 h were they able to clear their gut of metal-contaminated sediment particles (Gillis et al., 2005).

### 2.8. Metal analysis

After gut clearing, *D. magna* from one replicate beaker were combined into a single sample for tissue metal analysis due to minimum requirements for analysis (approximately 0.3 mg). All tissue metal concentrations presented here are whole-body concentrations. Tissues were dried at 60 °C for 24 h, weighed, and then digested with 50 μL of concentrated metals grade nitric acid (in 2 mL micro-centrifuge tubes) for 24 h at 60 °C. Samples were brought up to a final volume of 1.5 mL with 1% nitric acid prior to metal analysis. Tissue and water concentrations of Cu were measured with graphite furnace atomic absorption spectroscopy (220 FS, Varian). Method blanks (5) and Fisher Scientific calibration standards (every 20 samples) were included in every run. A maximum of 5% difference between duplicates was accepted. The detection limit for Zn (flame) was 50 μg/L and Cu (furnace) was 2 μg/L.

### 2.9. Statistical analysis

Means are given ±S.E. Statistical analyses were conducted with the software SPSS version 10.0 and Sigma Stat version 3.0. Comparisons between treatments were made using analysis of variance followed by Tukey’s multiple comparison test to determine differences between treatments ($p < 0.05$). Simple linear regressions and stepwise linear regression were used to determine the relationships between variables (dissolved, particulate, and tissue metal) in the phase separation experiment. Nonlinear regression analyses were used to determine which variables (particulate and dissolved Cu and Zn) contributed significantly to the prediction of *D. magna* survival in a composite dataset from all exposures (initial, washed, and phase separation).

### 3. Results

#### 3.1. Initial exposure

Dissolved oxygen remained relatively constant throughout the exposure (range 7.0–9.5 mg/L). The pH in the treatments shifted slightly downwards during the exposure from a median
406

P. L. Gillis et al. / Aquatic Toxicology 77 (2006) 402–411

of 7.42 at the beginning of the exposure, to a median of 7.03 at the end of the exposure. The concentrations of Cu (2–3 μg/L) and Zn (<50 μg/L) in the reference water were significantly lower than in any of the other exposures (e.g. sediment + reference water) (Fig. 2A and B). The concentrations of Cu and Zn in the overlying water (dissolved and total) were significantly (p < 0.05) higher in the ‘site water + sediment’ treatment than in either the ‘site water only’ or the ‘reference water + sediment’ treatments, and this suggested that both the sediment and the overlying site water could serve as a source of metals (Fig. 2A and B). In all treatments, dissolved Cu (range 28–50 μg/L) accounted for over 60% of the total Cu while dissolved Zn (range 423–1143 μg/L) accounted for more than 85% of the total Zn in the overlying water at the beginning of the exposure (Fig. 2A and B; Table 2). Survival was significantly lower (<50%) in treatments containing sediment compared to the site-water-only treatment (>80%) (Fig. 2C). Tissue concentrations of Cu and Zn were about 2–10-fold higher in treatments containing sediment compared to reference water controls (Fig. 2D), suggesting that there was substantial bioavailable metal associated with these sediments. For Zn, tissue levels were similar for the three experimental exposures, but for Cu, tissue levels were significantly higher in the ‘site-water + sediment’ treatment than in the ‘site-water only’ treatment.

3.2. Sediment washing

Cu and Zn levels in the overlying water decreased with repeated washing of the sediment (Fig. 3). After washing, Cu concentrations were reduced to approximately 40% (Method A: stir-and-settle: 38%, Method B: vortex-and-centrifuge 42%) and Zn concentrations reduced to approximately 20% (Method A 20%, Method B 23%) of initial concentrations in the overlying water of unwashed sediments. The final dissolved Cu concentrations were 16 (±2.5) μg/L and 12 (±0.3) μg/L after washing with Methods A and B, respectively. The final dissolved concentration of Zn in the overlying water was 89 (±21.0) μg/L after washing with Method A (stir-and-settle) and 194 (±5.6) μg/L using Method B (vortex-and-centrifuge).

3.3. Washed sediment exposures

Dissolved oxygen remained constant throughout the exposure (range 8.0–10.0 mg/L). The median pH shifted downward during the exposure, from 7.18 at the beginning to 6.44 at the end of the exposure. Dissolved Cu (10–14 μg/L) and Zn (63–80 μg/L) in the overlying water of washed sediment were significantly lower than in exposures with unwashed sediment (Fig. 4A and B, Table 3). The concentration of total (unfiltered) Zn and Cu in the overlying water of sediment washed by Method A (stir-and-settle) was significantly reduced compared to total metal levels in the overlying water of unwashed sediments. In contrast, the concentration of total Zn in the overlying water of Method B washed sediment (vortex-and-centrifuge) was statistically similar to the amount of total Zn for unwashed sediment, and the concentration of total Cu was significantly higher in the overlying water of Method B sediment compared to unwashed sediment.
3.4. Separation of phases

The separation of phases of the unfiltered treatments ranged from 150 to 480 d. Particle-associated Zn in the overlying water and associated Zn in the reference water control were all near the detection limit. However, Cu was below the detection limit in the reference water and the filtered treatments but was 30 g/L in the overlying water of the unwashed sediment (74% ± 8.7; unfiltered + sediment 44% ± 6.8) was not significantly different (p > 0.05) than survival in the treatments with suspended particles (unfiltered, 54% ± 8.7; unfiltered + sediment 44% ± 6.8) (Fig. 5A). Similarly, tissue concentrations of Cu and Zn were statistically similar across all treatments (Fig. 5B).

Linear regression analysis revealed a significant relationship (p = 0.0003, r^2 = 0.61) between the concentration of dissolved Cu in the overlying water and the concentration of Cu in the tissue of D. magna. The relationship between particle-associated Cu and tissue Cu was also significant (p = 0.04, r^2 = 0.27) but explained far less of the variation in the data. No significant relationships were found between the concentration of Zn (dissolved or particulate) in the overlying water and the concentration of Zn in D. magna tissues. Similarly, stepwise linear regression using the concentrations of dissolved Cu and Zn and the concentrations of particulate Cu and Zn, revealed that, only the concentration of dissolved Cu significantly contributed to D. magna mortality.

### Table 2

Mean (±S.E., n = 4) total and dissolved concentrations (μg/L) of Zn and Cu in the overlying water at the beginning and the end of the 48 h exposure with unwashed sediment

<table>
<thead>
<tr>
<th>Metal</th>
<th>Treatment</th>
<th>T=0h Total metal</th>
<th>T=48h Total metal</th>
<th>T=0h Dissolved</th>
<th>T=48h Dissolved</th>
<th>T=0h % dissolved</th>
<th>T=48h % dissolved</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zn</td>
<td>Reference water (control)</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>Sediment + reference water</td>
<td>482 (53.5)</td>
<td>423 (50.0)</td>
<td>87.8</td>
<td>454 (45)</td>
<td>443 (47)</td>
<td>97.6</td>
</tr>
<tr>
<td>Cu</td>
<td>Reference water (control)</td>
<td>13.9 (5.2)</td>
<td>6.0 (2.6)</td>
<td>43.2</td>
<td>15.4 (6.0)</td>
<td>12.8 (5.7)</td>
<td>95.5</td>
</tr>
</tbody>
</table>

Note: ND = not detected (<50 μg/L).

### Table 3

Mean (±S.E.) total and dissolved concentrations (μg/L) of Zn and Cu in the overlying water at the beginning and the end of the 48 h exposure with washed sediment

<table>
<thead>
<tr>
<th>Metal</th>
<th>Treatment</th>
<th>T=0h Total metal</th>
<th>T=48h Total metal</th>
<th>T=0h Dissolved</th>
<th>T=48h Dissolved</th>
<th>T=0h % dissolved</th>
<th>T=48h % dissolved</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zn</td>
<td>Reference water</td>
<td>45.7 (4.0)</td>
<td>28.4 (1.3)</td>
<td>62.1</td>
<td>45.4 (3.6)</td>
<td>37.3 (2.8)</td>
<td>82.2</td>
</tr>
<tr>
<td></td>
<td>Sediment + reference water</td>
<td>78.0 (5.9)</td>
<td>49.5 (0.8)</td>
<td>63.5</td>
<td>67.9 (1.8)</td>
<td>53.0 (1.9)</td>
<td>78.1</td>
</tr>
</tbody>
</table>

Note: ND = not detected (<50 μg/L).
tissue metal concentrations from those exposures could not be combined with the tissue metal data from the phase separation experiment for similar analysis.

4. Discussion

4.1. Sediment toxicity

The low survival (<50%) of _D. magna_ exposed to unwashed sediment demonstrated that there was substantial bioavailable metal associated with the Clear Creek sediment. The concentrations of Cu and Zn in the overlying water of unwashed sediment fall within the range of published EC50s for neonate (<24 h old) _D. magna_ (De Schamphelaere et al., 2002; De Schamphelaere and Janssen, 2002; Muyssen and Janssen, 2001). Although 5-day-old _D. magna_ used in this study may be less sensitive to metal exposure than neonates (Stühlbacher et al., 1993) some toxicity would be expected at these levels of dissolved Cu and Zn. Therefore, in order to reduce dissolved Cu and Zn below the toxic range, it was necessary to remove as much as possible of the easily mobilized metals from the sediment. Our preliminary analyses (see Section 2) demonstrated that Cd and Pb were not of concern in these exposures. The significant decrease in water-borne Cu and Zn, along with the corresponding increase in survival in washed sediment exposures (compared to unwashed) confirmed that Zn and/or Cu in the overlying water...
of unwashed sediments were likely responsible for *D. magna* mortality.

### 4.2. Sediment washing

Repeated rinsing of the sediment was successful at removing most of the labile metals from the sediment. Both methods of washing were adequate for this task but the quicker, vortex- and-centrifuge, Method B, was favored in the interest of time. It should be noted that the level of suspended particle-associated metal in the overlying water was enhanced when sediments were washed this way (Fig. 4A and B). We suspect that ‘vortex’ mixing may increase the bioavailability of particle-bound metals to *Daphnia*. Tissue Cu concentrations in *D. magna* exposed to sediments washed with Method B were significantly higher than those in animals exposed to sediment washed by the stir- and-settle Method A or to unwashed sediment (Fig. 4D). After undertaking a more detailed investigation into the gut clearing patterns of *D. magna* (Gillis et al., 2005), we strongly suspect that at least some of this ‘tissue Cu’ reported for the unwashed (initial) and washed sediment exposures after only 1 h of purging in reference water alone was due to Cu-bearing particles that remained in the gut because of incomplete gut clearance. Based on the calculations of Gillis et al. (2005) we estimate that over 60% of the whole-body Cu concentration (400 μg/g) for the unwashed and washed sediment exposures could be attributed to Cu-laden particles in the gut. Therefore, we suggest that the significant increase in tissue Cu in *D. magna* exposed to sediment washed using Method B (Fig. 4D), was due to an increase in sediment-associated Cu in the gut. The vigorous action of ‘vortex’ mixing likely broke up the larger aggregates into smaller particles. Smaller particles would not only have stayed in suspension longer (as was reflected in the significant increase in total Cu in the overlying water of Method B washed sediment) but they may also now be within the size range that *D. magna* can retain on their filtering appendages, thereby contributing to the increase in tissue Cu. For Zn, the concentration of total Zn in the overlying water of sediment washed by Method B was only slightly (not significantly) reduced compared to levels in unwashed sediment, with tissue Zn concentration in washed sediment significantly lower than in unwashed sediment exposures. The reason for these differences are unknown but may be related to differences in the association between the metals and the sediment particles. Nevertheless the lack of a corresponding negative effect on the survival of *D. magna* exposed to ‘vortex-washed’ sediment further supports the conclusions that this ‘tissue Cu’ had not actually bioaccumulated.
4.3. Separation of phases

Once the amount of metal dissociating from the Clear Creek sediment had been reduced through sediment washing and dilution with clean sediment, we could begin to investigate whether the particle-associated metals were bioavailable to *D. magna*. By removing the suspended particles (>0.45 μm) through filtration, the amount of metal accumulated from the dissolved and the particulate phase could be quantified. Although there was no significant difference in survival between the treatments, there was a trend of decreasing survival from the filtered, to unfiltered, to the unfiltered + sediment exposure (Fig. 5A). Because the concentration of dissolved copper was similar across all treatments (24–27 μg/L), we suggest that this trend is not the result of differential copper exposure across the treatments. Since *D. magna* exposed to filtered and unfiltered overlying water, even in the presence of sediment, accumulated similar amounts of Cu and Zn (Fig. 5B), we concluded that only the remaining labile metal was bioavailable and that any Cu or Zn that remained associated with the sediment after washing (i.e. the particle-associated metal) was not available to *Daphnia*. The significant relationship between dissolved Cu and survival (Fig. 6A) and the lack of a significant relationship between the particle-associated Cu and survival in the combined dataset (Fig. 6B) supports an earlier study by Erickson et al. (1995) who reported that Cu associated with sediment particles was not bioavailable to *C. dubia*. However, our findings contrast with Welten et al. (2000), who found that particle-associated Cd was bioavailable and thus toxic to *C. dubia*. The difference in toxicity between the Welten et al. (2000) study and this study could be attributed to differences in sorption behavior of sediment-associated Cd versus Cu.

4.4. Combined dataset

Using our combined dataset (all exposures in this study) we observed 50% mortality (LC50) when the dissolved Cu concentration reached 26 μg/L (Fig. 6A). The Biotic Ligand Model (BLM) (Hydroquel, 2005) was used to predict copper toxicity across the pH range observed in our exposures (6.44–7.42). According to the BLM, the predicted LC50 for Cu for *D. magna* would be between 9 and 35 μg/L. This appears to be a reasonable prediction, considering that the sediment used in these exposures was mining-impacted, field-collected sediment which is contaminated by numerous metals, and that BLM predictions are based upon single metal (Cu) exposures.

4.5. Environmental relevance

There has been considerable interest in the fate of metal-contaminated sediment during natural resuspension events and dredging. Bonnet et al. (2000) simulated a resuspension event by stirring a number of moderately contaminated sediments (Cu 40–70 μg/g, Zn 140–200 μg/g) and found that 24 h after mixing that only a small fraction of the sediment-associated metals were released into the overlying water and that no toxicity to *Hydra attenuata* and *D. magna* was observed. By contrast in the present study, there was a higher potential for desorption of metals into the overlying water due to the much higher metal load in the Clear Creek sediments (unwashed: Cu 2424 μg/g, Zn 5150 μg/g; Table 1). Carvalho et al. (1998) also investigated the effect of sediment resuspension and thus oxidation of metal-contaminated anoxic sediment, and reported that Cu and Zn were mobilized from the sediments resulting in concentrations well in excess of acutely toxic levels, which was reflected in the toxicity to *D. similis*. The watershed of Clear Creek has been identified as an area of high priority because of metal pollution from historic mining activities in the area. Recently, USEPA (2005) concluded that dissolved metals in the surface waters of Clear Creek were not likely to have a significant acute or chronic impact to aquatic biota but that sediment leachate tests and sediment toxicity tests both indicated that portions of Clear Creek are toxic for survival and growth. Based on the results of our laboratory study, we would expect that if the sediment of Clear Creek was subjected to a resuspension event, there could be a large efflux of metals from the sediment into the water column resulting in an acutely toxic environment for sensitive organisms such as *D. magna*.

5. Conclusions

Exposure to field-collected mining-impacted sediment was acutely toxic to *D. magna*, at least in part due to high levels of Cu in the dissolved phase of the overlying water. Once the labile metals had been exhausted, the Cu and Zn that remained associated with the particles were not bioavailable *D. magna*.

Acknowledgements

This study was funded by the Center for the study of Metal in the Environment (USEPA). CMW is supported by the Canada Research Chair Program. We would like to thank Sonia Sharma and Andrée McCracken for assistance in the laboratory and Dr. Chris Glover for input into this study.

References

Adams, C., Garnier-Laplace, J., Baudin, J.P., 2002. Bioaccumulation of 110mAg, 137Cs, 14Cd and 54Mn by the freshwater cyanobacterium *Daphnia magna* from dietary sources (*Scenedesmus obliquus* and *Cyclotella meneghiana*). Water Air Soil Pollut. 136, 125–146.


Fisher, N.S., Hooke, S.E., 2002. Toxicology tests with aquatic animals need to consider the trophic transfer of metals. Toxicology 181/182, 531–536.


