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# Does biofilm contribute to diel cycling of Zn in High Ore Creek, Montana?

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**Abstract.** Concentrations of metals cycle daily in the water column of some mining-impacted streams in the Rocky Mountains of the western USA. We hypothesized that biofilm in High Ore Creek, Montana, USA, sorbs and releases Zn on a diel cycle, and this uptake-and-release cycle controls the total and dissolved (0.45-$\mu$m filtered) Zn concentrations. We collected water samples from three sites (upstream, middle and downstream at 0, 350 and 650 m, respectively) along a 650-m reach of High Ore Creek during a 47-h period in August 2002 and from the upstream and downstream sites during a 24-h period in August 2003; we also collected biofilm samples at these sites. In 2002 and 2003, total and dissolved Zn concentrations did not exhibit a diel cycle at the upstream sampling site, which was ~30 m downstream from a settling pond through which the creek flows. However, total and dissolved Zn concentrations exhibited a diel cycle at the middle and downstream sampling sites, with the highest Zn concentrations occurring at dawn and the lowest Zn concentrations occurring during late afternoon (>2-fold range of concentrations at the downstream site). Based on (1) concentrations of Zn in biofilm at the three sites and (2) results of streamside experiments that demonstrated Zn uptake and release by naïve biofilm during the light and dark hours of a photocycle, respectively, we conclude that Zn uptake in photosynthetic biofilms could contribute a large percentage to the cycling of Zn concentrations in the water column in High Ore Creek.

## Introduction

Concentrations of metals cycle daily in the water column of some mining-impacted streams in the Rocky Mountains of the western USA (Brick and Moore 1996; Nimick et al. 2003). For example, concentrations of dissolved cadmium (Cd), manganese (Mn), and zinc (Zn) fluctuate daily by 2- to 6-fold in several streams including High Ore Creek (Nimick et al. 2003), a montane stream in Montana impacted by mine drainage. Usually, dissolved concentrations of Cd, Mn, and Zn are highest at dawn and lowest in late afternoon. Although the cause of this phenomenon is uncertain, these metal concentrations are negatively correlated with pH, water temperature, and solar radiation. The purpose of our study was to determine the extent to which biofilm contributes to diel cycling of Zn concentrations in the water column of High

Ore Creek. Herein we define biofilm broadly as the attached community and its associated exopolymer gel and embedded abiotic particles.

Several laboratory studies have demonstrated that photoautotrophs can accumulate and release cationic metals during photosynthetic cycles. Gray and Hill (1995) found that photosynthetic biofilm sorbed 76% more nickel (Ni) under lighted conditions (PAR (photosynthetically active radiation) $>0.050$ mmol/m$^2$/s) than in darkness. They suggested that under lighted conditions, photosynthetically driven pH increases in the biofilm micro-environment might be responsible for increased Ni sorption through cation exchange or precipitation of Ni-carbonates and (or) Ni-hydroxides. Similarly, Ting et al. (1989) reported the Zn concentration in green algae (*Chlorella vulgaris*) exposed to 0.075–0.080 mmol PAR/m$^2$/s increased 10.8-fold and the total Zn in their experimental chambers concurrently decreased 8.2-fold during a 35-h period. They suggested surface adsorption onto the algae and membrane transport as possible mechanisms for Zn uptake.

However, contributions of photosynthetic processes to the cycling of cationic metals in natural aquatic ecosystems have not been reported. Based on the laboratory studies cited above and some of our preliminary results with lab-cultured biofilm (unpublished data), we hypothesized that photosynthetic biofilm in High Ore Creek sorb metal during the light period and release metal during the dark period of the photocycle, thus contributing to the observed diel cycle of Zn concentrations in the water column.

## Methods

### *Overview*

We conducted two streamside experiments at High Ore Creek to determine if Zn uptake in laboratory-cultured biofilm is affected by photocycle when the biofilm is exposed to filtered creek water. We also collected water and biofilm samples from High Ore Creek during two consecutive summers to evaluate trends in diel Zn cycling in our study reach and to perform mass balance calculations.

### *Study site*

High Ore Creek is a low-order stream in the Boulder River watershed, in Jefferson County, Montana, USA. It descends from its origin at ~2260 m a.s.l. to its confluence with the Boulder River at ~1550 m a.s.l. The Comet Mine has existed near the headwaters of High Ore Creek for more than 120 years. In 1997, the state of Montana and the Bureau of Land Management initiated remediation efforts to decrease instream trace-metal concentrations in High Ore Creek by (1) removing tailing and waste rock piles at the

mine site and (2) removing sediment from the streambed and floodplain directly below the mine (Gelinas and Tupling 2004). However, elevated concentrations of trace metals, including Zn, still exist in High Ore Creek. We established sampling sites along a 650-m reach of this second-order segment of the creek. The upstream, middle, and downstream sampling sites were at 0, 350, and 650 m along this reach, which began ~30 m below the second of two settling ponds through which the creek flows. The ponds are located directly below the old mine and mill complex (Figure 1). Average stream width in this reach was 0.94 m, and average discharge was 0.008 and 0.006 $m^3/s$ when we sampled water and biofilm in August 2002 and 2003, respectively. Nearly the entire streambed of this 650-m reach was carpeted with a ~5 to 20 mm-thick layer of biofilm in August 2002 and 2003.

*Streamside experiments*

*Experiment 1 – raceway biofilm*

In April 2001, we placed glass aquaria containing 96.5-$cm^2$ glass slides in an outdoor cement spillway that delivers artesian well water to several raceways at the University of Wyoming's Red Buttes Environmental Biology Laboratory in Laramie, Wyoming, USA. A mixed community of biofilm species colonized the glass slides during Spring and Summer 2001.

We transported these glass slides in a plastic cooler to High Ore Creek on 9 August 2001, placed fifteen slides in each of two large fiberglass tanks situated on the creek bank, and pumped filtered water from the creek through each tank. The filtration step consisted of a series of 20-, 5-, and 0.45-$\mu$m filters, and the filtered water was divided equally between the two tanks (75 l volume in each tank, through which the average flow was 3.9 l/min; i.e., ~22 theoretical volume replacements/d, calculated from Figure 2 in Weber 1993). This 25.5-h exposure began at 20:30 h on 9 August and ended at 22:00 h on 10 August. Both exposure tanks were uncovered until 05:00 h on 10 August, at which time we covered one of the two aquaria with black plastic.

We collected water samples from each tank at 0, 9, 13.5, 17.5, 21.5 and 25.5 h. Water quality parameters measured at each sampling period included pH, temperature, dissolved oxygen (D.O.), and dissolved Zn. Because water entering the tanks had been filtered to 0.45 $\mu$m, water samples were not filtered after collection. Samples for dissolved Zn analyses were acidified to pH $\leq$ 2 with concentrated trace-metal grade $HNO_3$ and later analyzed for Zn by flame atomic absorption spectrophotometry (FAAS; Perkin–Elmer Model 372; use of trade names does not imply endorsement by the U.S. Government). At each sampling time, we scraped biofilm off three slides from each aquarium into an acid-washed 7-ml plastic scintillation vial using the edge of a clean glass slide, placed the vials in ice for transport to Laramie, stored them at −80 °C, and later analyzed the biofilm for Zn.

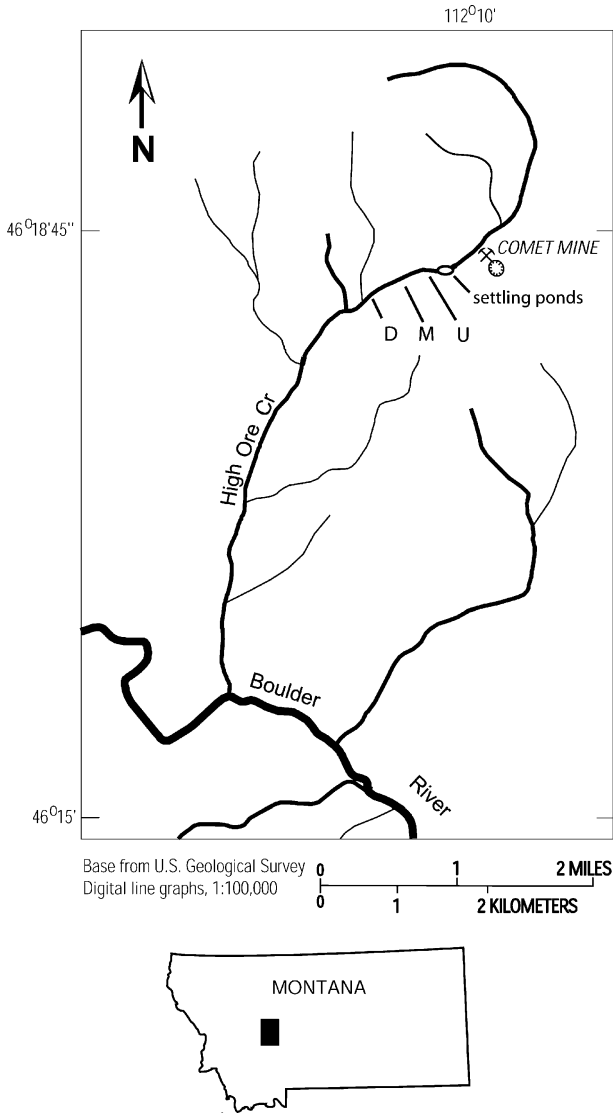


*Figure 1.* Map of High Ore Creek, Montana, USA. Letters U, M, and D represent the upstream, middle, and downstream sampling sites on High Ore Creek, respectively.

*Experiment 2 – High Ore Creek biofilm cultured in the laboratory*

Biofilm scraped from rocks in High Ore Creek was stored in plastic containers and shipped in ice to Laramie in October 2001. We placed that biofilm in 5-l glass aquaria containing clean glass slides and a nutrient medium (same medium used in Stein 1973, pp. 16–18 except we did not add $Na_2SiO_3 \cdot 9H_2O$) under a combination of 40-W GE® softlight and grow-lux (aquarium lighting)

fluorescent bulbs (~0.080 mmol PAR/$m^2$/s at the water surface). After 8 weeks, the glass slides in each aquarium were visibly covered with biofilm, which was used to seed new cultures or conduct laboratory and field experiments. We determined that this culture contained a mixed community of cyanobacteria, green algae, and diatoms by observation under a light microscope (40 × magnification).

In August 2002, we transported the 96.5-$cm^2$ glass slides on which this lab-cultured biofilm was grown to High Ore Creek. We pumped stream water through a series of filters (20, 10, 1 and 0.45 $\mu$m) and divided that flow among three 8-l glass aquaria (flow rate of ~200 ml/min/aquarium; i.e., ~18 theoretical volume replacements/d, calculated from Figure 2 in Weber 1993), each of which contained 16 biofilm-covered glass slides. All of the aquaria were exposed to natural light. This 54.5-h exposure began at 13:00 h on 11 August and ended at 19:30 h on 13 August. Water quality parameters measured during each sampling period included alkalinity, conductivity, D.O., dissolved Zn, pH, and temperature. Photosynthetically active radiation was measured automatically every 15 min with a Li-Cor LI-1400 data logger equipped with a calibrated underwater light sensor.

We collected water samples from each aquarium at 4.5, 7.5, 17.5, 25, 28.5, 31.5, 41.5, 49, 52.5, and 54.5 h, acidified them to pH ≤ 2 with concentrated trace-metal grade $HNO_3$, and stored them in ice. Because water entering the aquaria had been filtered to 0.45 $\mu$m, water samples were not filtered after collection. These samples were transported to Laramie and later analyzed for Ca, K, Mg, Na, and Zn. We collected water for dissolved organic carbon (DOC) analysis in baked (500 °C for 2 h) amber glass bottles, acidified these to pH 2–3 with 2 N reagent-grade HCl, and stored them in ice in the field and during transport to Laramie, where they were refrigerated at 4 °C. Additional water samples were collected for major anion ($Cl^-$, $F^-$, $NO_3^-$, $SO_4^{2-}$) analyses at each sampling period in acid-washed 7-ml plastic scintillation vials and stored in ice in the field and during transport to Laramie, where they were refrigerated at 4 °C. At each sampling time, we scraped biofilm from one slide from each of the three aquaria into an acid-washed 7-ml plastic scintillation vial, placed these vials in ice for transport to Laramie, stored them at −80 °C, and later analyzed the biofilm for Zn. We also analyzed subsamples of biofilm for ash, ash free dry weight (AFDW), organic carbon (OC), and chlorophyll *a* (chl *a*).

### *Instream monitoring*

#### *August 2002*

*Solar radiation*. The Li-Cor datalogger recorded streamside measurements of PAR at the middle site every 15 min from 16:30 h on 22 August to 15:00 h on 24 August.

*Discharge and tracer study*. We measured stream velocity in one reach between the upstream and middle sites on 25 August using a Swoffer model 2100 current meter and then calculated discharge from the measured velocity and stream morphology. Additionally, we measured discharge directly below the downstream site from 22 to 25 August using a free-flowing, 15-cm Parshall flume maintained by the U.S. Geological Survey. Flow was not measured at the upstream site because the stream was not deep enough for an accurate velocity measurement. Additionally, we added NaCl to High Ore Creek at the upstream site on 25 August and monitored the conductivity at the downstream site to estimate the transit time of water through the 650-m study reach.

*Water*. We intensively sampled water from High Ore Creek from 16:00 h on 22 August to 15:00 h on 24 August. Water samples were collected from the upstream, middle, and downstream sites simultaneously every hour for 47 h using automatic pumping samplers. Water samples remained on ice in the samplers prior to collection for no more than 5 h (at night) in 2002 and 4 h (at night) in 2003. Upon collection, two subsamples were acidified (5 ml of water filtered through 0.45-$\mu$m Whatman® cellulose nitrate membrane filters, and 20 ml of unfiltered water) to pH $\leq$ 2 with concentrated trace-metal grade $HNO_3$. We transported these water samples to Laramie and analyzed the filtered water for Zn and the unfiltered water for Ca, K, Mg, Na, and Zn. We also measured water temperature, pH, and D.O. at each of the three sites at eight times during the 47-h sampling period. We calculated particulate Zn as the difference between total and dissolved Zn.

*Biofilm*. We scraped biofilm from three rocks at each of the three water-sampling sites into acid-washed 7-ml plastic scintillation vials using a small plastic spatula, at 16:00 h on 24 August and at 06:30 h on 25 August, stored these vials in dry ice and transported them to Laramie, transferred them to a $-80$ °C-freezer and later analyzed the biofilm for Zn.

We collected additional biofilm samples from each site at 16:00 h on 25 August for analysis of total dry weight per area, loss on ignition (ash and AFDW), OC, and chl *a*. We removed three rocks from the streambed at each of the three sampling sites, and lightly rinsed the surface of each rock with reverse osmosis/de-ionized (RO/DI) water and scraped it with a stiff plastic brush to remove the loosely attached biofilm matrix. This material was collected in a plastic tray and rinsed into an acid-washed Nalgene® bottle using RO/DI water. Then we wrapped aluminum foil over the scraped area, cut the foil to match the pattern of this area, and weighed each piece of foil to calculate the scraped area. We transported these biofilm samples in ice to Laramie, and stored them at 4 °C. Later, we centrifuged the contents of each bottle at 4000 RPM for 5 min, and dried the pellet to constant weight at 65 °C. Then we weighed the dried pellet to determine total mass, ground the pellet using a mortar and pestle, and finally analyzed for AFDW, ash, and OC content.

*August 2003*

We used a similar water-monitoring scheme from 16:00 h on 11 August to 16:00 h on 12 August 2003 as in August 2002; however, we only collected water samples at the upstream and downstream sites, and these were collected every 2 h instead of every hour. In addition, we measured pH, temperature, and D.O. at each site every 2 h and subsampled the bi-hourly water samples for alkalinity, DOC, conductivity, dissolved calcium, and major anion analyses. We filtered (0.45 $\mu$m) water samples for major anion analyses, collected the filtrates in acid-washed 7-ml plastic scintillation vials, shipped them in ice to Laramie, stored them at $-4$ °C, and analyzed them by ion chromatography (Dionex model DX-100) within 1 week of sampling. We also collected biofilm samples after the 24-h water collection period (at 06:45 and 14:00 h on 15 August) and analyzed them as described for August 2002. Discharge was measured in the Parshall flume installed at the downstream site (as in 2002) and also in a second Parshall flume that we installed at the upstream site in June 2003.

*Chemical analyses*

We measured pH with an Orion® Model 290A pH meter equipped with an Orion® Model 8163BN Ross combination pH probe and an Orion® Model 917005 ATC-Probe thermometer. This instrument was calibrated before each use with pH 4, 7, and 10 buffers. We measured conductivity using a VWR® digital conductivity meter calibrated with VWR® traceable conductivity standard, and water temperature and D.O. using a YSI® Model 58 D.O. meter equipped with a YSI® Model 5739 probe. We air calibrated this latter instrument according to the manufacturer's instructions, and adjusted all saturation calculations for atmospheric pressure using a stream elevation of 1900 m. Samples for alkalinity analysis were collected in 50-ml Nalgene® bottles and placed in ice while they were transported to Laramie, and stored at 4 °C until we analyzed them using standard methods (APHA et al. 1995).

We analyzed water samples for DOC on a Shimadzu TOC-5000A total organic carbon analyzer. We also analyzed water samples for major anions on a Dionex DX-100 ion chromatograph and for total and dissolved Zn, Ca, K, Mg, and Na by FAAS.

We digested weighed subsamples of dried, ground biofilm in concentrated trace-metal grade $HNO_3$ at 55 °C for 24 h, and then analyzed the digest for Zn content by FAAS. We combusted other weighed subsamples of dried, ground biofilm at 500 °C for 5 h in aluminum dishes and then weighed them to determine ash and AFDW content, according to Standard Methods 2540 B and 2540 E (APHA et al. 1995). Organic carbon was analyzed in a modification of Standard Method 5310 B (this procedure details organic carbon analysis in water; APHA et al. 1975). To measure the OC content of biofilm, inorganic carbon was removed by submersing dried, ground subsamples of biofilm ($\sim$100 mg each) in $\sim$2 ml of 7.6 mM $H_3PO_4$ (pH$\sim$1.8) in a glass tube. After

mixing, each tube was placed on a heating block at ~40 °C and gently aerated with $N_2$ until dry to volatize inorganic carbon from the samples. We then analyzed the carbon content on a Carlo Erba Model 1106 Elemental Analyzer.

Chlorophyll *a* content was analyzed according to Wetzel and Likens (1991). After grinding frozen biofilm samples that had been scraped from known areas of rock with a porcelain mortar and pestle using 90% acetone, we measured the absorbance of the supernatant from each sample at 750 and 665 nm on a Milton Roy Model 301 Spectronic spectrophotometer and calculated chl *a* content based on a standard curve that we generated prior to sample analysis using purified chl a (Sigma Chemical Company, product #C6144) dissolved in 90% acetone.

To insure analytical accuracy, we ran sample blanks, filter blanks, analytical and digestion spikes, sample replicates, and external reference standards on water and biofilm analyses when possible. The concentrations of Zn in our sample and filter blanks were below detection limit. The instrument detection limits for our analytes were as follows (mg/l for everything except particulate C (mg)): aqueous C, 0.087; particulate C, 0.0014; $Ca^{2+}$, 0.011; chl *a*, 0.18; $Cl^{-}$, 0.19; $F^{-}$, 0.02; $K^{+}$, 0.017, $Mg^{2+}$, 0.0021; $NO_3^{-}$, 0.13; $Na^{+}$, 0.012; $SO_4^{2-}$, 0.15; $Zn^{2+}$, 0.0099.

### *Geochemical speciation calculations*

We used MINEQL+ Version 4.0 for Windows (Schecher and McAvoy 1998) to calculate saturation indices for all of the Ca-, Mg-, Na-, K-, and Zn-containing minerals included in the MINEQL+ thermodynamic database (including 21 Zn-carbonates, Zn-hydroxides, and Zn-oxides). Additionally, we entered hydrozincite (log $K = -9.65$ for $5Zn^{2+} + 2CO_3^{2-} + 6H_2O \leftrightarrow Zn_5(OH)_6(CO_3)_2(s) + 6H^{+}$; Zachara et al. 1989) into the thermodynamic database because it has been reported to precipitate in some other Zn-contaminated streams (e.g., Podda et al. 2000). For these MINEQL+ calculations, we input pH, temperature, alkalinity, concentrations of dissolved Ca, Cl, F, $NO_3$, $SO_4$, and Zn, and concentrations of total K, Na, and Mg at each sampling period at the upstream and downstream sites in 2003. Because Ca and Zn (the two elements most likely to form mineral precipitates at the pH and alkalinity values in High Ore Creek) were entered as dissolved concentrations, we did not allow any Ca- or Zn-containing minerals to precipitate in the speciation calculations.

### *Statistical analyses*

For the 2001 streamside experiment and all instream-monitoring parameters in 2002 and 2003 for which we did not compare daytime values to night-time values (i.e., all parameters listed in Tables 3 and 4), we conducted statistical

comparisons with ANOVA ($\alpha = 0.05$) followed by Tukey HSD post hoc pairwise comparisons where applicable, using Minitab$^{TM}$ Version 13.31 (Minitab Statistical Software, Minitab Inc.). Because only three subsamples were collected from each of the two treatment tanks during the 2001 streamside experiment at each sampling period for analysis of Zn in biofilm (Figure 2) and only three subsamples were collected from each sampling site in 2002 and 2003 along our study reach for analyses of AFDW, ash, OC, and chl *a* (Tables 2 and 3), the statistical inferences we make in the following sections are based on the variances obtained from these subsamples and not from true replication.

For instream-monitoring parameters in 2002 and 2003 for which we compared daytime values to night-time values (i.e., all parameters shown in Figures 5 and 7), we attempted to perform repeated-measures analyses using The SAS System for Windows Version 8.02. However, due to the insignificance ($p > 0.05$) of the orthogonal components with time of day in our model for each parameter, we concluded that the three subsamples collected during the day or night at each site could not be treated as true replicates. Therefore, we averaged over the day and night subsamples when comparing these values among the different sites along our study reach during a particular year, which gave us two replicates per site (the average of the day and the average of the night samples).

Coefficients of determination ($R^2$) measured between parameters such as $\Delta$Zn and PAR were based on Pearson or Spearman correlation coefficients, depending on whether the trend of the data was linear or non-linear, respectively.

## Results

### *Streamside experiments*

#### *Experiment 1 – raceway biofilm*

Because of instream diel fluctuations of temperature, pH, D.O., and dissolved Zn, these parameters also fluctuated on a diel cycle in the streamside tanks through which filtered creek water was pumped. For example, temperature ranged from 10.4 °C at night to 22.8 °C during the daylight, pH ranged from 7.7 at night to 8.6 during the daylight, and D.O. ranged from 78% of saturation at night to 101% of saturation during daylight (Table 1). In contrast, dissolved Zn concentrations decreased during daylight (minimum of 0.31 mg Zn/l at 18:00 h) and increased at night (maximum of 0.98 mg Zn/l at 05:30 h; Figure 2a). However, water quality was always nearly identical in the light and dark tanks. Despite the decreasing dissolved Zn concentrations in the water column during daylight, the mean Zn concentration ($\pm$S.D., $n = 3$) in biofilm in the light tank increased to $1.65 \pm 0.14$ mg Zn/g dw biofilm during daylight and was 1.85× higher than the mean biofilm Zn concentration of $0.89 \pm 0.19$ mg Zn/g dw biofilm in the dark tank during daylight (Figure 2b; $p \leq 0.05$).

*Table 1.* Water quality in streamside exposure tanks along High Ore Creek, Montana, USA, from 9 to 10 August 2001.

| Time | Temperature (°C) | | pH | | Dissolved $O_2$ (% saturation) | |
|---|---|---|---|---|---|---|
| | L | D | L | D | L | D |
| 21:30 h | 14.9 | 14.9 | 7.97 | 7.97 | 78 | 78 |
| 05:30 h | 10.4 | 10.6 | 7.72 | 7.84 | 90 | 89 |
| 10:00 h | 12.6 | 13.3 | 8.02 | 8.09 | 97 | 96 |
| 14:00 h | 22.1 | 22.1 | 8.58 | 8.58 | 100 | 101 |
| 18:00 h | 22.7 | 22.8 | 8.56 | 8.55 | 99 | 97 |
| 22:00 h | 15.9 | 16.1 | 7.88 | 7.93 | 88 | 88 |

Creek water was filtered through a 0.45-$\mu$m membrane before flowing through each exposure chamber. L = tank exposed to natural light; D = tank covered with black plastic.

*Experiment 2 – High Ore Creek biofilm cultured in the laboratory*

As in Experiment 1, temperature, pH, D.O., and dissolved Zn fluctuated on diel cycles in the streamside aquaria through which filtered creek water was pumped. Temperature ranged from 8.7 to 22.8 °C, pH ranged from 7.4 to 8.3, and D.O. ranged from 90 to 107% of saturation (Table 2). Mean dissolved Zn concentrations ($\pm$S.D., $n = 3$) in the three aquaria decreased during daylight (minimum of $0.69 \pm 0.01$ mg Zn/l at 17:30h during the second light period) and increased at night (maximum of $2.31 \pm 0.04$ mg Zn/l at 06:30 h during the second dark period; Figure 3). Concentrations of the other cations and the anions did not vary considerably, as follows (mean $\pm$ S.D., $n = 3$; as mg/l): $Ca^{2+}$, $53.8 \pm 0.5$; $K^{+}$, $2.0 \pm 0.1$; $Mg^{2+}$, $13.1 \pm 0.2$; $Na^{2+}$, $5.0 \pm 0.03$; $F^{-}$, $0.11 \pm 0.01$; $Cl^{-}$, $1.1 \pm 0.3$; $NO_3^{-}$, $0.25 \pm 0.25$; and $SO_4^{2-}$, $132.5 \pm 4.1$. Alkalinity varied by only 17% during the experiment.

In contrast to the dissolved Zn concentrations, the mean Zn concentration in biofilm in these aquaria increased during daylight (maximum of $23.4 \pm 1.2$ mg Zn/g dw biofilm at 19:30 h during the second light period) and decreased at night (minimum of $2.6 \pm 0.4$ mg Zn/g dw biofilm at 06:30 during the first dark period; Figure 3). The Zn concentration in the biofilm prior to exposure to filtered water from the creek was $0.027 \pm 0.016$ mg Zn/g dw biofilm. The mean concentrations ($\pm$S.D.; as mg/g dw biofilm) of ash, AFDW, and OC in biofilm sampled during daylight were $154 \pm 57$ ($n = 3$), $846 \pm 57$ ($n = 3$) and $451 \pm 5.8$ ($n = 2$), respectively. Photosynthetically active radiation ranged from 0 to 2.6 mmol/$m^2$/s (Figure 3).

*Instream monitoring*

*August 2002*

*Solar radiation*. Photosynthetically active radiation ranged from 0 to 2.2 mmol/$m^2$/s at the middle site on High Ore Creek during the 47-h water-sampling period from 22 August to 24 August (Figure 4a).

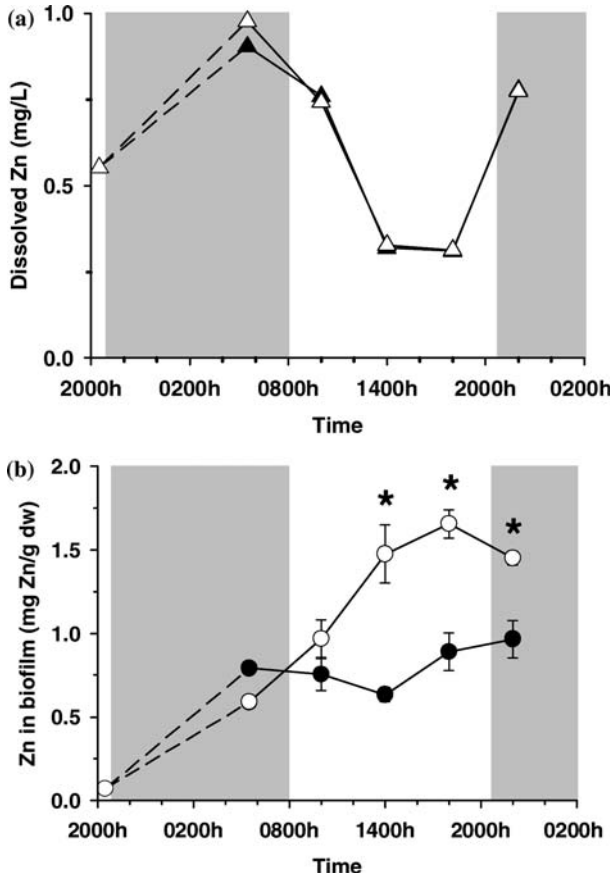


*Figure 2*. Zn concentrations in stream water (filtered to 0.45$\mu$m) and in naïve biofilm exposed to that water during a 25.5-h streamside exposure along High Ore Creek, Montana, USA, from 9 to 10 August 2001. The biofilm was grown on glass plates in a cement spillway at Red Buttes Environmental Biology Laboratory in Laramie, Wyoming, USA. The dashed line between the first and second data points in each panel indicates that the trend is interpolated because no samples were taken during that 9-h period. ● = Zn in biofilm exposed to complete darkness, ○ = Zn in biofilm exposed to natural light; ▲ = dissolved Zn in the tank exposed to complete darkness, and △ = dissolved Zn in the tank exposed to natural light. Error bars are ±1 standard error of the mean ($n$ = 3). Shaded areas indicate night. *Significantly greater Zn concentration in biofilm in the light tank ($p < 0.05$).

*Stream discharge and tracer study*. The weighted mean discharge measured in the Parshall flume at the downstream site during the 47-h water-sampling period was 0.0083 $m^3/s$ (Figure 4b). Discharge measured at a site between the upstream and middle sites using the current meter on 25 August was 0.0073 $m^3/s$, whereas discharge at the same time at the lower site was 0.0079 $m^3/s$. Therefore, these measurements suggest an 8% net dilution of the stream flow by inflowing groundwater.

*Table 2*. Water quality in streamside exposure aquaria along High Ore Creek, Montana, USA, from 11 to 13 August 2002.

| Date in August 2002 | Time | Temperature (°C) | pH | Dissolved $O_2$ (% saturation) | Alkalinity (mg/l as $CaCO_3$) |
|---|---|---|---|---|---|
| 11 | 17:30 h | 15.2 ± 0.9 | 7.78 ± 0.17 | 101 ± 4 | 80 |
| 11 | 20:30 h | 12.5 ± 0.2 | 7.76 ± 0.12 | 91 ± 1 | 95 |
| 12 | 06:30 h | 8.7 ± 0.6 | 7.37 ± 0.07 | 90 ± 3 | 93 |
| 12 | 14:00 h | 18.0 ± 0.4 | 7.76 ± 0.16 | 107 ± 8 | 83 |
| 12 | 17:30 h | 18.8 ± 0.4 | 8.06 ± 0.05 | 101 ± 4 | 88 |
| 12 | 20:30 h | 14.9 ± 0.4 | 7.96 ± 0.04 | 96 ± 1 | 82 |
| 13 | 06:30 h | 9.4 ± 0.4 | 7.81 ± 0.11 | 94 ± 2 | 89 |
| 13 | 14:00 h | 20.7 ± 0.7 | 8.17 ± 0.06 | 102 ± 2 | 79 |
| 13 | 17:30 h | 22.8 ± 1.2 | 8.31 ± 0.08 | 99 ± 4 | 81 |
| 13 | 19:30 h | 18.7 ± 1 | 8.17 ± 0.1 | 95 ± 3 | 85 |

Creek water was filtered through a 0.45-$\mu$m membrane before flowing through each exposure chamber. All results are mean ± S.D. ($n$ = 3), except for alkalinity ($n$ = 1).

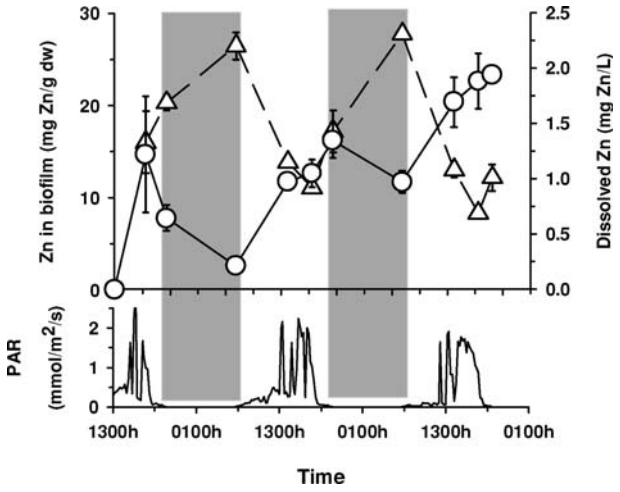


*Figure 3*. Photosynthetically active radiation (PAR), and Zn concentrations measured in stream water (filtered to 0.45 $\mu$m) and naïve biofilm during a 54.5-h streamside exposure along High Ore Creek, Montana, USA, from 13:00 h 11 August to 19:30 h 13 August 2002. The biofilm was seeded from High Ore Creek biofilm that was collected in October 2001 and subsequently cultured on glass plates in particle-free laboratory water prior to being exposed to the filtered stream water in streamside aquaria. △ = dissolved Zn, and ○ = Zn in biofilm. Error bars are ± 1 standard error of the mean ($n$ = 3). Shaded areas indicate night.

Nominal transport time measured during the NaCl tracer study was 1.25 h.

*Water*. Several water-quality parameters followed a diel cycle that correlated to varying degrees with PAR. Temperature, pH, D.O. (% saturation), and particulate Zn increased from morning to late afternoon while total and

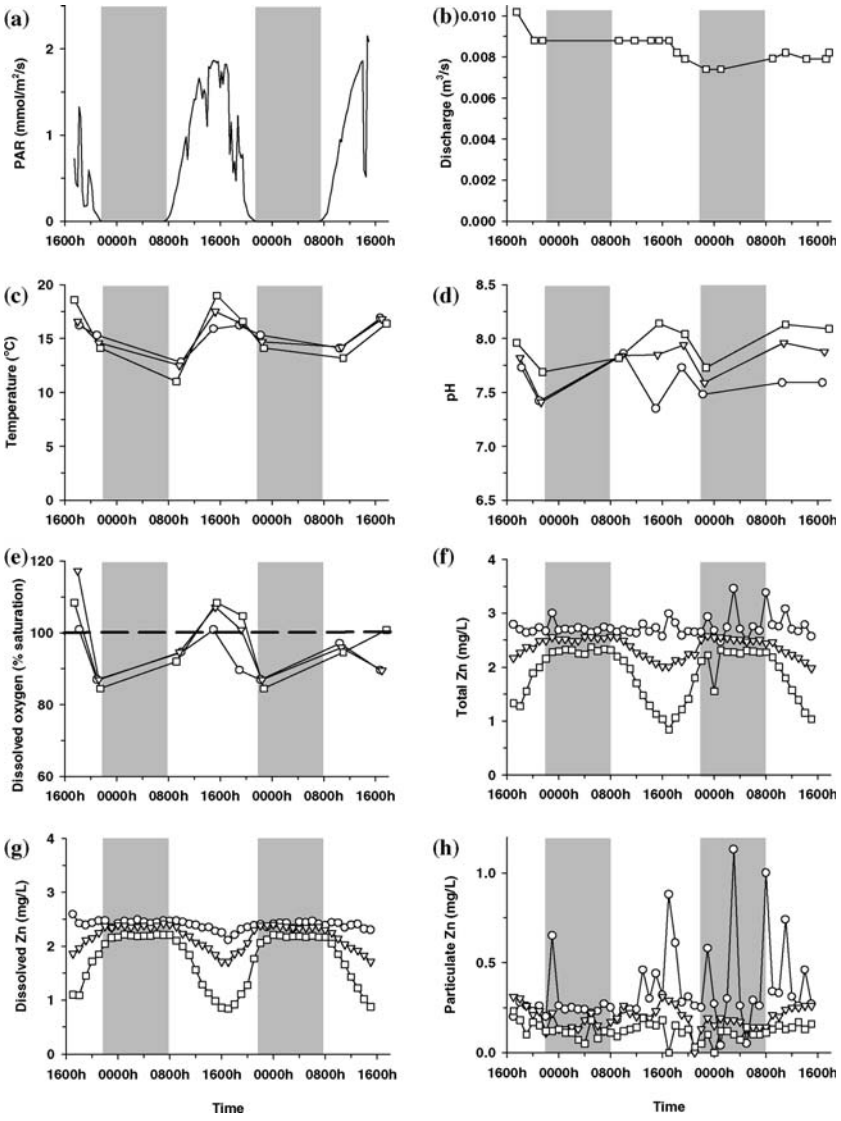


*Figure 4*. Photosynthetically active radiation (PAR; measured at the middle site) and various water-quality parameters measured during 47 h along a 650-m reach of High Ore Creek, Montana, USA, from 22 to 24 August 2002. ○ = upstream (0 m), ▽ = middle (350 m) and □ = downstream (650 m) sites. Shaded areas indicate night.

dissolved Zn decreased (Figure 4). Cation concentrations at the upstream, middle, and downstream sites did not exhibit diel cycles and were, respectively (mean ± S.D., $n = 48$; as mg/l): $Ca^{2+}$, 52.3 ± 1.0, 54.0 ± 1.1 and 55.2 ± 3.0; $K^{+}$, 1.7 ± 0.1, 1.8 ± 0.1 and 1.7 ± 0.1; $Mg^{2+}$, 13.6 ± 0.2, 13.8 ± 0.2 and 14.1 ± 0.8; and $Na^{+}$, 5.0 ± 0.4, 5.1 ± 0.1 and 5.1 ± 0.3.

*Biofilm*. We compared the concentrations of Zn in biofilm among the upstream, middle, and downstream sites based on six separate normalization parameters: total mass of biofilm (organic and inorganic matter), area of substrate sampled, mg of chl *a*, g of AFDW, g of ash, and g of OC. Concentrations of Zn in biofilm collected during the light and dark periods of the photocycle did not differ significantly within each site ($p > 0.05$), for any of the six methods of normalizing the results (Figure 5). Biofilm at the upstream site contained significantly more Zn per g dw of biofilm (1.8×) and per g of ash (2.6×) than at the downstream site (Figures 5a and 5b; $p \leq 0.05$), whereas biofilm from the downstream site contained significantly more Zn per $m^2$ (3.5×), per mg of chl *a* (3.5×), per g of OC (2.5×), and per g of AFDW (1.6×) than biofilm from the upstream site (Figures 5c–f; $p \leq 0.05$).

The amount of AFDW/g dw biofilm decreased from the upstream site to the downstream site; conversely, the amount of ash/$m^2$ and the amount of ash/g dw biofilm increased from the upstream site to the downstream site. Also, we found more OC and chl *a*/g dw biofilm at the upstream site than at the middle and downstream sites. Total amount of biofilm/$m^2$, AFDW/$m^2$, OC/$m^2$, OC/g AFDW, chl *a*/$m^2$, chl *a*/g AFDW, and chl *a*/g OC did not appear to differ among the three sites (Table 3).

*August 2003*

*Solar radiation*. Photosynthetically active radiation was not measured during this 24-h monitoring period due to equipment malfunction.

*Stream discharge and tracer study*. The mean discharges in the Parshall flumes at the upstream and downstream sites during the 24-h water-sampling period (11–12 August) were 0.0059 and 0.0061 $m^3$/s, respectively (Figure 6b). Therefore, these measurements suggest a 3% net dilution of the stream flow by inflowing groundwater.

Nominal transport time measured during the NaCl tracer study was 1.75 h.

*Water*. Water-quality parameters followed the same diel pattern observed in August 2002 (Figure 6). There were no diel variations in the cation or anion concentrations, alkalinity, or conductivity at the upstream and downstream sites. Cation and anion concentrations at the upstream and downstream sites were, respectively (mean ± S.D., $n = 13$; as mg/l): $Ca^{2+}$, 64.2 ± 0.6 and 63.4 ± 0.7; $K^+$, 2.1 ± 0.04 and 1.8 ± 0.1; $Mg^{2+}$, 16.7 ± 0.1 and 16.6 ± 0.1; $Na^+$, 5.8 ± 0.1 and 5.7 ± 0.1; $F^-$, 0.13 ± 0.01 and 0.13 ± 0.01; $Cl^-$; 0.85 ± 0.08 and 0.87 ± 0.05; $NO_3^-$, ≤ 0.13 (below detection) and 0.20 ± 0.08; $SO_4^{2-}$, 154.6 ± 4.0 and 152.9 ± 2.6. Alkalinity (mg/l as $CaCO_3$) and conductivity ($\mu$S/cm) values at the upstream and downstream sites were, respectively (mean ± S.D., $n = 13$): alkalinity, 68 ± 7 and 73 ± 3; and conductivity, 441 ± 5 and 449 ± 4.

Because the membrane filters used in the field contaminated the DOC samples, we could not determine reliable DOC concentrations; however, there

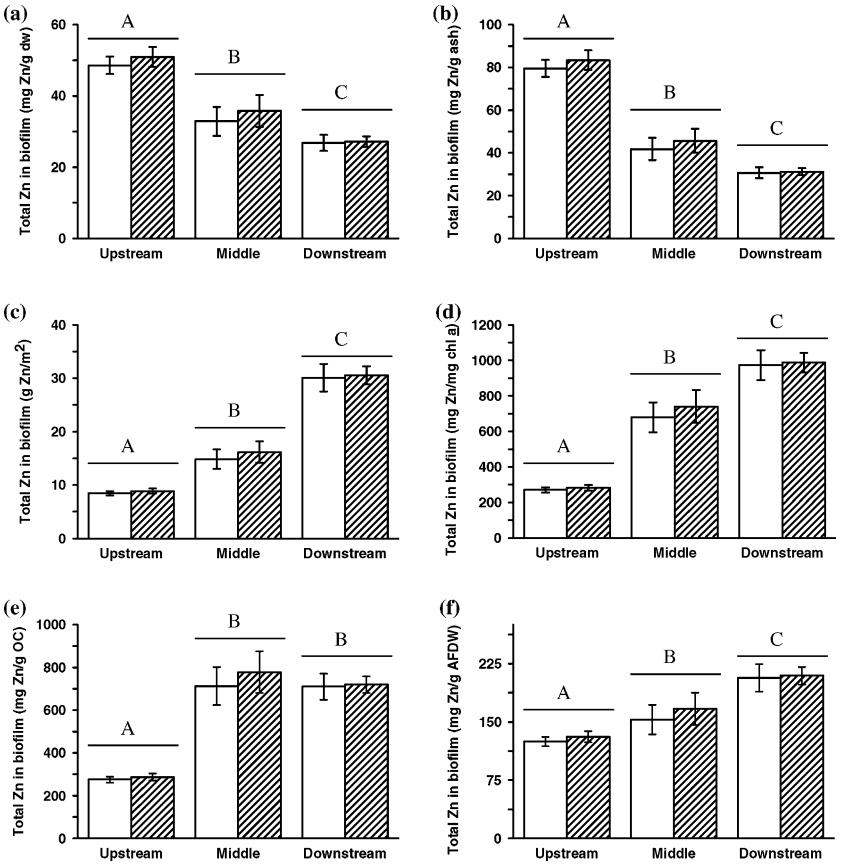


*Figure 5*. Mean Zn concentrations in biofilm normalized to various measurements of biofilm and substrate area in samples collected from the upstream, middle, and downstream sampling sites located at 0, 350, and 650 m along a 650-m reach of High Ore Creek, Montana, USA. Daylight samples (open bars) were collected at 18:15 h on 24 August, and night samples (shaded bars) were collected at 06:30 h on 25 August 2002. Error bars represent $\pm 1$ standard error of the mean ($n = 3$). Different capital letters above any pair of bars (day and night) within a panel indicate significant differences ($p < 0.05$) between those two sites.

were no diel variations in the total organic carbon (TOC) concentrations analyzed in the unfiltered samples. The TOC concentrations at the upstream and downstream sites were, respectively (mean $\pm$ S.D., $n = 13$; as mg/l) $2.30 \pm 0.23$ and $3.16 \pm 1.77$.

*Biofilm*. We compared the concentrations of Zn in biofilm among the upstream and downstream sites based on the same six normalization parameters used in 2002. Concentrations of Zn in biofilm collected during the light and dark periods of the photocycle did not differ significantly ($p > 0.05$) within

*Table 3*. Concentrations of ash free dry weight (AFDW), ash, organic carbon (OC), and chlorophyll *a* (chl *a*) in biofilm collected from High Ore Creek, Montana, USA, at 16:00 h on 25 August 2002.

| Parameter | Upstream | Middle | Downstream |
|---|---|---|---|
| Biofilm (g dw/m$^2$) | $174.2^{a} \pm 16.8$ | $451.6^{a} \pm 180.0$ | $1123.8^{a} \pm 877.9$ |
| AFDW (g/m$^2$) | $67.9^{a} \pm 21.3$ | $97.0^{a} \pm 30.2$ | $145.8^{a} \pm 112.1$ |
| AFDW (mg/g dw biofilm) | $389.7^{a} \pm 122.3$ | $214.8^{a,b} \pm 66.8$ | $129.7^{b} \pm 99.8$ |
| Ash (g/m$^2$) | $106.3^{a} \pm 21.3$ | $354.6^{b} \pm 30.2$ | $978.1^{c} \pm 112.1$ |
| Ash (mg/g dw biofilm) | $610.3^{a} \pm 122.3$ | $785.2^{a,b} \pm 66.8$ | $870.3^{b} \pm 99.8$ |
| OC (g/m$^2$) | $30.8^{a} \pm 9.1$ | $20.8^{a} \pm 2.5$ | $42.4^{a} \pm 31.6$ |
| OC (mg/g dw biofilm) | $176.8^{a} \pm 52.4$ | $46.0^{b} \pm 5.4$ | $37.7^{b} \pm 28.1$ |
| OC (mg/g AFDW) | $453.7^{a} \pm 134.5$ | $214.3^{a} \pm 25.4$ | $290.9^{a} \pm 216.6$ |
| Chl *a* (mg/m$^2$) | $31.3^{a} \pm 6.5$ | $21.6^{a} \pm 14.8$ | $30.9^{a} \pm 19.9$ |
| Chl *a* (mg/g dw biofilm) | $0.18^{a} \pm 0.04$ | $0.05^{a,b} \pm 0.03$ | $0.028^{b} \pm 0.02$ |
| Chl *a* (mg/g AFDW) | $0.5^{a} \pm 0.1$ | $0.2^{a} \pm 0.2$ | $0.2^{a} \pm 0.1$ |
| Chl *a* (mg/g OC) | $1.0^{a} \pm 0.2$ | $1.1^{a} \pm 0.7$ | $0.7^{a} \pm 0.5$ |

All results are mean ± S.D. ($n = 3$), except for Downstream OC analyses ($n = 2$). Means with different superscript letters within a row indicate significant differences ($p < 0.05$) between sampling sites for that parameter. Statistical analysis was conducted using three subsamples from each site as replicates to get a sense of the true variability between sites.

each site, for any of the six methods of normalizing the results (Figure 7). However, the amounts of Zn per mg chl *a*, per g OC, and per g AFDW decreased significantly ($p < 0.05$) from the upstream site to downstream site (Figures 7d–f), whereas the amounts of Zn per g dw, per g ash, and per m$^2$ did not differ significantly ($p > 0.05$) between the two sites (Figures 7a–c).

The amounts of AFDW/m$^2$, AFDW/g dw biofilm, OC/m$^2$, OC/g dw biofilm, chl *a*/m$^2$, and chl *a*/g dw biofilm increased from the upstream site to the downstream site. Conversely, the amounts of ash/g dw biofilm decreased from the upstream site to the downstream site. Total amount of biofilm/m$^2$, ash/m$^2$, OC/g AFDW, chl *a*/g AFDW, and chl *a*/g OC did not appear to differ between the two sites (Table 4).

### *Zn mass balance calculations*

Based on estimated stream dilution and biomass of biofilm in the 650-m stream reach, we calculated the net uptake of Zn along the reach using a simple mass balance. Because (1) the difference between the discharge measured above the middle site using the current meter and at the downstream site using the Parshall flume in August 2002 was small (8% net dilution complicated by the possibility for error using a current meter in such a shallow stream) and (2) the difference in average discharge between the upstream and downstream sites using the Parshall flumes in August 2003 was even smaller (3% net dilution), we assumed no net dilution from the upstream site to the downstream site in August 2002 and 2003 in our calculations of Zn uptake into biofilm.

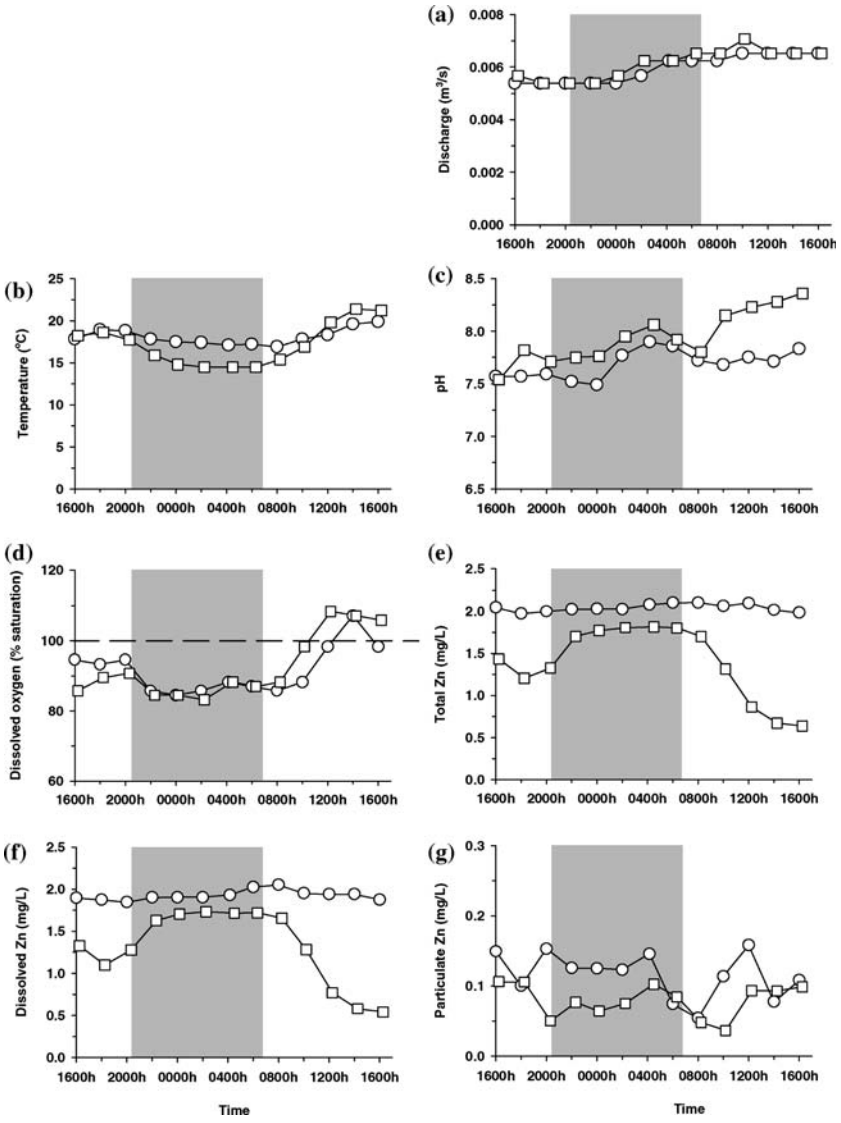


*Figure 6*. Various water-quality parameters measured during 24 h along a 650-m reach of High Ore Creek, Montana, USA, from 11 to 12 August 2003. ○ = upstream (0 m) and □ = downstream (650 m) sites. Shaded areas indicate night.

We estimated a total of 318 and 89 kg dw of biofilm in the entire 650-m study reach in August 2002 and 2003, respectively (using our measurements of biofilm per $m^2$ at each sampling site multiplied by the estimated water surface area along that reach). Because this estimate of biomass assumes the streambed is flat, we probably underestimated the biomass in the study reach.

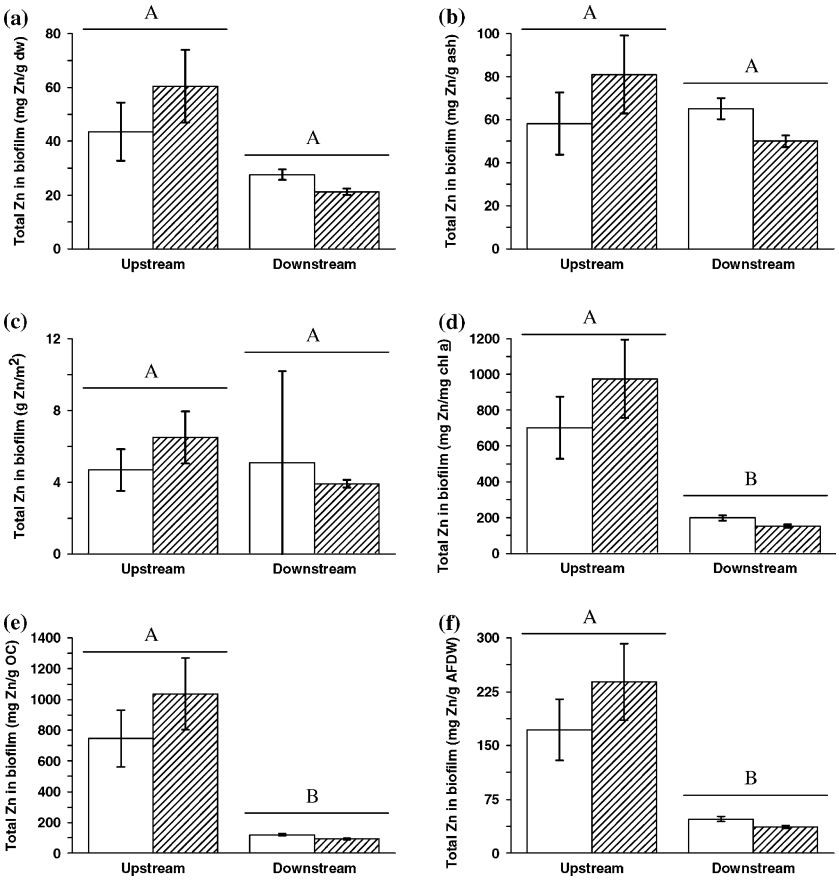


*Figure 7.* Mean Zn concentrations in biofilm normalized to various measurements of biofilm and substrate area in samples collected from the upstream and downstream sampling sites located at 0 and 650 m along a 650-m reach of High Ore Creek, Montana, USA. Daylight samples (open bars) were collected at 16:00 h on 15 August and night samples (shaded bars) were collected at 06:45 h on 15 August 2003. Error bars represent $\pm 1$ standard error of the mean ($n = 3$). Different capital letters above each pair of bars (day and night) within a panel indicate significant differences ($p < 0.05$) between those two sites.

In August 2002 and 2003, we calculated an average net loss of 0.86 and 0.76 mg Zn/l, respectively, from the water column in this 650-m reach of High Ore Creek for the 24-h period from 20:00 h on 22 August to 20:00 h on 23 August 2002 and for the 24-h period from 16:00 h on 11 August to 16:00 h on 12 August 2003. Based on average discharges at the downstream site of 0.0083 and 0.0061 $m^3/s$ in each year, we estimate net uptakes of 635 and 401 g Zn/d over this reach of High Ore Creek in August 2002 and August 2003, respectively. Assuming all of the Zn lost from the water column was accumulated by biofilm on the stream substrate, this translates into average net Zn uptake rates

*Table 4*. Concentrations of ash free dry weight (AFDW), ash, organic carbon (OC) and chlorophyll *a* (chl *a*) in biofilm collected from High Ore Creek, Montana, USA, at 12:00 h on 14 August 2003.

| Parameter | Upstream | Downstream |
|---|---|---|
| Biofilm (g dw/m$^2$) | $107.3^a \pm 59.6$ | $184.5^a \pm 29.6$ |
| AFDW (g/m$^2$) | $27.2^a \pm 10.8$ | $106.4^b \pm 22.9$ |
| AFDW (mg/g dw biofilm) | $253.2^a \pm 0.1$ | $576.8^b \pm 0.1$ |
| Ash (g/m$^2$) | $80.1^a \pm 10.8$ | $78.1^a \pm 22.9$ |
| Ash (mg/g dw biofilm) | $746.8^a \pm 0.1$ | $423.2^b \pm 0.1$ |
| OC (g/m$^2$) | $6.3^a \pm 1.3$ | $42.2^b \pm 11.1$ |
| OC (mg/g dw biofilm) | $58.3^a \pm 12.4$ | $228.9^b \pm 60.4$ |
| OC (mg/g AFDW) | $230.2^a \pm 48.8$ | $396.9^a \pm 104.7$ |
| Chl *a* (mg/m$^2$) | $34.8^a \pm 31.4$ | $152.9^b \pm 17.0$ |
| Chl *a* (mg/g dw biofilm) | $0.3^a \pm 0.3$ | $0.8^b \pm 0.1$ |
| Chl *a* (mg/g AFDW) | $1.3^a \pm 1.2$ | $1.4^a \pm 0.2$ |
| Chl *a* (mg/g OC) | $5.6^a \pm 5.0$ | $3.6^a \pm 0.4$ |

All results are mean ± S.D. ($n = 3$). Means with different superscript letters within a row indicate significant differences ($p < 0.05$) between sampling sites for that parameter. Statistical analysis was conducted using three subsamples from each site as replicates to get a sense of the true variability between sites.

of 2.0 and 4.5 mg Zn/g dw biofilm/d in 2002 and 2003, respectively (or 10.7 and 10.9 mg Zn/g AFDW/d).

The estimated net Zn uptakes during the light period were 1.5 and 3.7 mg Zn/g dw biofilm in 2002 and 2003, respectively. These accumulations are similar to the measured uptakes in the naïve biofilm in our streamside experiments that were 1.1 and 13.6 mg Zn/g dw biofilm during the first daylight period in 2002 and 2003, respectively (Figures 2b and 3).

## Discussion

Diel cycles in the concentrations of heavy metals and metalloids have been demonstrated in the water column of several mining-impacted streams in the Rocky Mountain region of the western USA (Fuller and Davis 1989; Brick and Moore 1996; Scott et al. 2002; Nimick et al. 2003; Jones et al. 2004). Possible mechanisms for diel metal cycling are not well understood and might vary depending on the dominant mechanisms at a particular site, including (1) photoreduction of oxidized minerals on the stream substrate, (2) reductive dissolution of oxidized minerals by microbial action at night, (3) diel variation in mineral solubility caused by diel changes in water-column pH and temperature, (4) enhanced oxidation of minerals in the presence of photosynthesizing organisms due to high D.O. concentrations during the day, and (5) sorption of metals to mineral oxides and (or) co-precipitation of metals with mineral oxides. Specifically, such cycles have been attributed to (1) water-column pH changes that control adsorption and desorption of anions from

mineral surfaces (e.g., arsenate sorption at lower pH during the night and desorption at higher pH during the day: Fuller and Davis 1989; Nimick et al. 1998), (2) adsorption and desorption of cations from mineral and biological surfaces (Nimick et al. 2003), (3) removal of Mn and Zn through enhanced oxidation and precipitation of Mn oxides in the presence of photosynthetic organisms during the day (Scott et al. 2002), and (4) photoreduction of Mn oxides and consequent release of trace metals during the day (Scott et al. 2002). In this paper, we have presented evidence for a diel Zn cycle that is negatively correlated with solar radiation in High Ore Creek (i.e., lowest Zn concentrations during the day and highest Zn concentrations at night), similar to the diel cycles reported for Cd, Mn, and Zn in High Ore Creek and other Rocky Mountain streams by Nimick et al. (2003). We believe much of this cycle in High Ore Creek can be explained by adsorption and precipitation in the high-pH microenvironment in the biofilm matrix, independent of abiotic photochemical reactions and only indirectly related to changes in water-column pH.

### *Zn uptake in naïve, laboratory-cultured biofilm*

Both of the streamside experiments demonstrated that lab-cultured biofilm can take up Zn in daylight and at least partially release it at night, in the absence of particles suspended in the water column (because the stream water delivered to the streamside chambers was pre-filtered) and particles embedded in the biofilm (because the biofilm was cultured on glass plates in particle-free water prior to the beginning of each experiment). In contrast, in continuous darkness (the 2001 streamside experiment), lab-cultured biofilm did not exhibit a daily uptake and release cycle. Although both the natural light and complete darkness treatments exhibited an initial uptake during the first dark period of the 2001 streamside experiment, this uptake most likely reflects Zn sorption to binding sites on the naïve algal cells. However, based on the release of Zn from biofilm at night during the 2002 streamside experiment, we do not believe this initial uptake rate in darkness in the 2001 streamside experiment is representative of the uptake rate in acclimated biofilm. Furthermore, Zn concentrations measured in the biofilm used in our streamside exposures were similar to the concentrations measured by Ting et al. (1989), who showed that *Chlorella vulgaris* exposed to 1.3 mg Zn/l for 35 h under 0.075 to 0.080 mmol PAR/$m^2$/s accumulated 1.4 mg Zn/g dw algae. For comparison, the biofilm in our streamside chambers was exposed to ~0.5–2.5 mg Zn/L under natural light (~2–2.5 mmol PAR/$m^2$/s) and accumulated 1.1–13.6 mg Zn/g dw during the first light period in 2002 and 2003, respectively (Figures 2b and 3). The uptake rates differed between 2002 and 2003 probably because the biofilm used during each year was cultured from completely different sources. The biofilm used in the 2002 streamside experiment appeared to be dominated by diatoms, whereas the biofilm used in the 2003 streamside experiment was a mixed community of diatoms, green algae, and cyanobacteria.

The most interesting feature of the diel uptake-and-release cycle in the streamside experiments is that the highest Zn concentrations in the biofilm occurred during daylight, when dissolved Zn concentrations in the water column were lowest; whereas the lowest Zn concentrations in the biofilm occurred at night when dissolved Zn concentrations in the water column were highest. Although it could be argued that the pH of the water column controlled sorption (at the higher pH values during daylight) and desorption (at the lower pH values at night) that drove the diel uptake and release of Zn from the biofilm-covered glass plates in the streamside experiments, we have demonstrated similar diel uptake and release of Zn from biofilm in the laboratory at constant water-column temperature and pH (unpublished data). Therefore, biogeochemical processes and water quality within biofilm appear to contribute to the diel cycles of uptake and release of Zn, in the absence of iron/manganese oxides and cycles of water-column chemistry. We believe Zn adsorption/precipitation and desorption/dissolution is driven by pH changes in the biofilm matrix caused by photosynthesis and respiration (Jørgensen et al. 1983).

*Instream monitoring*

Instream monitoring demonstrated that almost all of the diel Zn cycle is caused by uptake of the metal during the day, and that none of the diel cycle is caused by release of the metal at night (Figures 4f and 6e). Because the particulate Zn concentrations were approximately an order of magnitude smaller than the amplitudes of the dissolved Zn cycles (compare Figures 4g and h, and 6f and g), the change in dissolved Zn concentrations cannot be attributed to sorption to and desorption from suspended particles. This conclusion is further supported by a diel cycle of total Zn (Figures 4f and 6e) whose amplitude is approximately equal to the amplitude of the dissolved Zn cycle (Figures 4g and 6f). Additionally, our unpublished microscopic analysis of particles filtered from High Ore Creek water in August 2003 indicates that the composition of particles in the water column does not change between day and night, and the concentration of particles does not increase during the day. Analysis of these particles using energy dispersive spectroscopy indicates that Zn is not associated with diatoms but is associated with other algal cells and unidentified particles.

*Zn sorption/precipitation in water column vs. biofilm matrix*

In concept, the loss of Zn from the water column of High Ore Creek during daylight could have been caused by precipitation of Zn-containing minerals out of the water column, sorption to inorganic substrates on the streambed, or uptake by biofilm. However, our geochemical speciation calculations suggest the pH in the water column did not reach a high enough value during the day

to cause precipitation of Zn-oxides, Zn-hydroxides or Zn-carbonates. For example, values of the saturation index ($=\log (IAP/K_{sp})$, where IAP is the ion activity product and $K_{sp}$ is the solubility product) for $ZnCO_3$, ZnO, and hydrozincite ranged from $-3.01$ to $-0.91$, $-6.41$ to $-4.15$, and $-21.50$ to $-10.98$, respectively. This undersaturation of Zn-containing minerals was due to the sequestration of $>97\%$ of the total dissolved Zn in two dissolved complexes: $ZnCO_3^0$ and $Zn(CO_3)_2^{2-}$.

Although the geochemical speciation calculations indicated that four Ca-containing minerals (aragonite, calcite, dolomite, and huntite) were supersaturated day and night at the upstream and downstream sites in High Ore Creek in 2003, we do not believe Zn co-precipitated with these minerals in either year because the molar $\Delta$Ca:$\Delta$Zn ratio was 3.6 for the 47-h sampling period in 2002 and 3.3 for the 24-h sampling period in 2003. These molar $\Delta$Ca:$\Delta$Zn ratios are a measure of concurrent losses of Ca and Zn from the water column. If Zn were co-precipitating as a minor impurity in calcite, this ratio should be closer to 50 (personal communication, Dr James Drever, Department of Geology and Geophysics, University of Wyoming, Laramie, Wyoming, USA). Supporting the argument against considerable precipitation of Ca- or Zn-containing minerals from the water column, we never observed a white precipitate on the streambed of High Ore Creek (anecdotal visual observations) or on the surface of biofilm in samples we brought to the laboratory and inspected at 400× under a scanning electron microscope (unpublished data).

The argument against sorption of Zn to inorganic/abiotic streambed substrates is apparent from the pH and Zn data for August 2003. During this intensive 24-h sampling, the difference in Zn concentration (total or dissolved) between the upstream and the downstream sites did not always increase as pH increased (compare Figures 6c, e, and f; $R^2 = 0.54$ for the correlation of $\Delta$Zn and downstream pH). This is not what would be expected for adsorption/desorption reactions between a cationic metal and inorganic substrate at a pH edge (e.g., see Drever 1997, p. 192). In other words, the diel Zn cycle in High Ore Creek was unaffected as water-column pH gradually increased during this 24-h period. Furthermore, although Zn sorption can be positively correlated with water temperature (Nimick et al. 2003), the water-column Zn concentration remained constant instead of increasing while the water temperature gradually decreased at night (compare Figures 4c, f, and g, and 6b, e, and f). Therefore, we believe the majority of the Zn removal from the water column occurred within the biofilm layer and not by mineral precipitation in the water column or sorption to inorganic substrates.

Although pH usually is lower at night than during the day because of photosynthesis during the daylight hours and respiration at night, the relatively low pH values during daylight on 22 August 2003 were probably caused in part by an overcast sky and rain showers in the afternoon that decreased the intensity of photosynthesis in the creek and possibly decreased the stream pH. Additionally, we measured large decreases in pH (e.g., from 7.95 to 5.60,

presumably due to acid drainage) in the water column of High Ore Creek above the settling ponds in June 2003 following storms. Therefore, rain showers might have also lowered the pH in August 2003.

*Diel variation in Zn concentrations in biofilm*

As a corollary to our hypothesis that uptake and release of Zn is controlled by biofilm, we expected to see major differences between day and night in the amount of Zn accumulated in the biofilm collected from High Ore Creek. However, Zn concentrations in the biofilm did not differ significantly between day and night at any of the three sampling sites. In hindsight, this lack of significant differences can be explained by the large amount of Zn already accumulated in the biofilm and the high variability of Zn concentrations in biofilm at each site. Specifically, without larger sample sizes than we collected, it would be difficult to infer statistical significance for the relatively small increases of 1.5–3.7 mg Zn/g dw biofilm that we predicted in the biofilm over the course of a light period because we found high variability in Zn concentrations in biofilm at the downstream site in 2002 and 2003 (average standard deviations were 3.7 and 12.7 mg Zn/g dw biofilm in 2002 and 2003, respectively). The high variability in Zn concentrations in biofilm is probably due to the high Zn content (27–51 mg Zn/g dw biofilm in 2002 and 2003) and the high and variable ash content (42–87% in 2002 and 2003) of this biofilm.

*Zn uptake in High Ore Creek biofilm vs. naïve, laboratory-cultured biofilm*

Based on our mass balance calculations, we estimated average Zn uptake rates of 0.12 and 0.26 mg Zn/g dw biofilm/h (0.62 and 0.64 mg Zn/g AFDW/h) in High Ore Creek biofilm during daylight in 2002 and 2003, respectively. These values are lower than the measured Zn uptake by naïve, lab-cultured biofilm from High Ore Creek during the light interval in the streamside experiment in 2002 (0.95 mg Zn/g dw biofilm/h (based on average Zn uptake between two sets of samples taken at 06:30 and 17:30 h on 11 and 12 August; Figure 3), or 1.1 mg Zn/g AFDW/h). Because the lab-cultured biofilm contained more organic matter and less ash than *in situ* High Ore Creek biofilm, the higher uptake rate of Zn/g AFDW/h calculated for lab-cultured biofilm in the streamside experiment suggests that organic matter is involved in Zn uptake in biofilm. For instance, the AFDW content in High Ore Creek biofilm ranged from 13 to 39% in 2002 and from 25 to 58% in 2003, whereas the lab-cultured biofilm contained 85% AFDW. Furthermore, because the calculated uptake rates of Zn in biofilm in High Ore Creek were less than the measured uptake rates in biofilm in the streamside experiment, we conclude that the calculated uptake rates in the field are well within the capability of the biofilm growing in this reach.

*Evidence for two-compartment Zn accumulation in the stream substrate*

Although our mass balance calculations can explain the net Zn loss in this reach of High Ore Creek over the course of a few days, the measured concentrations of Zn in biofilm (~25 and 50 mg Zn/g dw biofilm at the downstream and upstream sites, respectively, in 2002 and 2003; Figures 5a and 7a) do not support the hypothesis that the daily net loss of Zn is accumulating exclusively in biotic tissues or on photosynthetic cells over a period of weeks to months. For example, the measured Zn concentrations in biofilm were 28 mg Zn/g AFDW in the lab-cultured biofilm and 42 to 208 mg Zn/g AFDW in High Ore Creek biofilm. This suggests a disproportionate amount of Zn accumulates in the biofilm in High Ore Creek in relation to its organic content when compared to the naïve biofilm in the streamside experiments. If we attribute the net loss of Zn in High Ore Creek entirely to uptake by biofilm, it would only take 4 to 20 d to accumulate the measured concentrations of Zn in biofilm at the downstream site, assuming that little of the biofilm matrix is lost to death and (or) sloughing and that invertebrate grazing has little impact on biomass.

We do not believe death and (or) sloughing of the biofilm matrix has a large impact on the overall biomass in this reach at this time of year based on the robust appearance of the biofilm mats. Furthermore, although invertebrate grazing could also decrease the biofilm biomass, our field observations and preliminary invertebrate sampling indicated few (if any) invertebrate grazers inhabit this reach of stream, most likely due to toxic concentrations of heavy metals. This observation is supported by the presence of grazers such as mayflies and an obvious decrease in biofilm biomass directly below the downstream site of our study reach, where an unpolluted tributary enters High Ore Creek and dilutes the stream water by about 20%.

Therefore, we suggest the photosynthetic biofilm covering the streambed acts as a pH-activated, light-dependent gate between the water column and a mineral sink in a two-compartment system, wherein the dominant Mn-oxide crust described by Shope (2003) underlying the biofilm on much of the substrate in High Ore Creek might be the mineral sink. We observed similar Mn-oxide crusts on the substrate beneath the biofilm that covered most of the streambed in our study reach, and we believe the biofilm acts as the initial Zn uptake compartment. This Zn subsequently transfers from the biofilm into the Mn-oxide crust, which becomes the second and final compartment. Xie (2002) reported Mn-oxide crust from the mouth of High Ore Creek contained approximately 12% Zn and 23% Mn by weight, and Shope (2003) reported Mn-oxide crust from the upper reaches of High Ore Creek (near the settling ponds) contained 25% Zn and 25% Mn by weight. Our initial screening of crust samples indicated that the Mn-oxide crust from the upstream and downstream sampling sites of our study reach contained approximately 3 to 15% Zn and 6 to 26% Mn by weight (unpublished data). Therefore, we can conclude that this Mn-oxide crust is highly enriched in both Zn and Mn.

*Importance of water column and substrate interactions in diel Zn cycling*

The settling pond 30 m above the upstream site on High Ore Creek supplied a nearly constant concentration of total and dissolved Zn to the sampling reach during both sampling periods in 2002 and 2003 (Figures 4f and g, and 6e and f). Bearing this in mind, it is interesting that the diel cycle of total and dissolved Zn became more pronounced the farther downstream we sampled (e.g., decreases of 15, 29, and 62% dissolved Zn during daylight in 2002 at the upstream, middle, and downstream sites, respectively, Figure 4g; and decreases of 10 and 69% dissolved Zn during daylight in 2003 at the upstream and downstream sites, respectively, Figure 6f). The increase in the difference between the morning and afternoon Zn concentrations as distance downstream from the settling pond increased suggests that the factors responsible for this diel cycling might be related to the much higher benthic surface area:water volume ratio in the stream than in the pond, especially because nearly the entire streambed of this reach was carpeted with a thick mat of photosynthetic biofilm.

## Conclusions

We rejected the hypothesis that diel Zn cycling in the water column in High Ore Creek is caused by precipitation of Zn from the water column because (1) geochemical speciation calculations indicate that the pH of the water column does not become high enough to reach saturation of Zn-containing minerals, and (2) Zn removal was not well-correlated with water-column pH in 2003. Similarly, we rejected the hypothesis that a diel cycle of pH- or temperature-controlled sorption to and desorption from abiotic surfaces on the streambed causes diel cycling of Zn concentrations in the water column because (1) Zn removal was not well-correlated with water-column pH in 2003 and (2) Zn removal was not correlated with temperature at night. Instead, the diel Zn cycling appears to be driven by light-mediated biological processes in the biofilm (i.e., photosynthesis). The gradual increases and decreases in the differences in Zn concentrations during daylight at the middle and downstream sites, which have a direct, negative correlation with solar radiation (e.g., $R^2 = 0.91, p = 0.001$ for the correlation of $\Delta$Zn and downstream pH in 2003), suggest that decreases in water-column Zn concentrations are caused by sorption and (or) precipitation in the biofilm matrix as the pH of the water in this matrix increases during daylight.

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## References

APHA, AWWA, WEF (American Public Health Association, American Water Works Association, Water Environment Federation) 1975. Standard Methods for the Examination of Water and Wastewater. 14th ed. American Public Health Association, Washington, District of Columbia, USA.

APHA, AWWA, WEF (American Public Health Association, American Water Works Association, Water Environment Federation) 1995. Standard Methods for the Examination of Water and Wastewater. 19th ed. American Public Health Association, Washington, District of Columbia, USA.

Brick C.M. and Moore J.N. 1996. Diel variation of trace metals in the upper Clark Fork River, Montana. Environ. Sci. Technol. 30: 1953–1960.

Drever J.I. 1997. The Geochemistry of Natural Waters: Surface and Groundwater Environments. 3rd ed. Prentice-Hall, Inc., Upper Saddle River, New Jersey, USA.

Fuller C.C. and Davis J.A. 1989. Influence of coupling of sorption and photosynthetic processes on trace element cycles in natural waters. Nature 340: 52–54.

Gelinas S.L. and Tupling R. 2004. Monitoring remediation? Have mine-waste and mill-tailings removal and flood-plain restoration been successful in the High Ore Creek valley?. In: Nimick D.A., Church S.E. and Finger S.E. (eds), Integrated Investigation of Environmental Effects of Historical Mining in the Basin and Boulder Mining Districts, Boulder River Watershed, Jefferson County, Montana, U.S. Geological Survey Professional Paper 1652. Chapter E2. pp. 457–474.

Gray B.R. and Hill W.R. 1995. Nickel sorption by periphyton exposed to different light intensities. J. N. Amer. Benthol. Soc. 14: 299–305.

Jones C.A., Nimick D.A. and McCleskey R.B. 2004. Relative effect of temperature and pH on diel cycling of dissolved trace-elements in Prickly Pear Creek, Montana. Water, Air, Soil Poll. 153: 95–113.

Jørgensen B.B., Revsbech N.P. and Cohen Y. 1983. Photosynthesis and structure of benthic microbial mats: microelectrode and SEM studies of four cyanobacterial communities. Limnol. Oceanogr. 28: 1075–1093.

Nimick D.A., Gammons C.H., Cleasby T.E., Madison J.P., Skaar D. and Brick C.M. 2003. Diel cycles in dissolved metal concentrations in streams–Occurrence and possible causes. Water Resources Res. 39(9): citation no. 1247, doi:10.1029/WR001571.

Nimick D.A., Moore J.N., Dalby C.E. and Savka M.W. 1998. The fate of geothermal arsenic in the Madison and Missouri Rivers, Montana and Wyoming. Water Resources Res. 34: 3051–3067.

Podda F., Zuddas P., Minacci A., Pepi M. and Baldi F. 2000. Heavy metal coprecipitation with hydrozincite [$Zn_5(CO_3)_2(OH)_6$] from mine waters caused by photosynthetic microorganisms. Appl. Environ. Microbiol. 66: 5092–5098.

Schecher W.D. and McAvoy D.C. 1998. MINEQL + Chemical Equilibrium Modeling System, Version 4.0 for Windows. Environmental Research Software, Hallowell, Maine, USA.

Scott D.T., McKnight D.M., Voelker B.M. and Hrncir D.C. 2002. Redox processes controlling manganese fate and transport in a mountain stream. Environ. Sci. Technol. 36: 453–459.

Shope C.L. 2003. Field and Laboratory Investigation of the Hydrogeochemistry of High Ore Creek, Basin, Montana. MS Thesis, Montana Tech of the University of Montana, Butte, Montana, USA.

Stein J.R. 1973. Handbook of Phycological Methods: Culture Methods and Growth Measurements. Cambridge University Press, London, England.

Ting Y.P., Lawson F. and Prince I.G. 1989. Uptake of cadmium and zinc by the alga *Chlorella vulgaris*: Part 1. Individual ion species. Biotechnol. Bioeng. 34: 990–999.

Weber C.I. (ed.) 1993. Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms. 4th ed. EPA/600/4–90/027F, U.S. Environmental Protection Agency, Cincinnati, Ohio, USA.

Wetzel R.G. and Likens G.E. 1991. Limnological Analyses. 2nd ed. Springer-Verlag, New York, NY, USA.

Xie Y. 2002. Experimental Investigation of the Causes of Diurnal Cycling of Zinc in a Stream Impacted by Mine Drainage. MS Thesis, Montana Tech of the University of Montana, Butte, Montana, USA.

Zachara J.M., Kittrick J.A., Dake L.S. and Harsh J.B. 1989. Solubility and surface spectroscopy of zinc precipitates on calcite. Geochim. Cosmochim. Acta 53: 9–19.