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Photosynthetically Mediated Zn Removal from the Water Column in High Ore Creek, Montana

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Abstract We collected cobbles covered in biofilm from High Ore Creek, Montana, placed them in 12 transparent PVC plastic chambers, and exposed the chambers to four treatments: Sunlight, Sunlight-occluded, DCMU (photosynthesis inhibited), and Formalin. Total aqueous zinc (Zn) concentrations in the Sunlight treatment decreased during the 4-h experiment and were significantly lower ($P \leq 0.05$) than in the other three treatments, in which the total aqueous Zn concentrations did not decrease significantly. Therefore, we believe photosynthesis in the biofilm played a role in causing total aqueous Zn concentrations in the Sunlight treatment to decrease, and we believe a similar process contributes to diel Zn cycling in High Ore Creek and some other metals-contaminated streams.

Keywords biofilm · diel cycling · DCMU · diuron · formalin · photosynthesis

1 Introduction

Cadmium, manganese and zinc (Zn) concentrations cycle daily in some mining-impacted streams in the Rocky Mountains of the USA (Nimick et al., 2003). Our research on High Ore Creek (HOC), Montana, has demonstrated that the diel Zn cycle is strongly associated with photoperiod because Zn concentrations decrease during the day and return to a constant, higher concentration at night (Morris, Nimick, Farag, & Meyer 2005). Additionally, laboratory and streamside experiments using lab-cultured biofilm, free of abiotic particles, indicate that photosynthetic biofilm accumulates Zn during daytime and releases Zn at night (Morris, 2005; Morris, Farag, Nimick, & Meyer 2006; Morris et al., 2005). The objective of this streamside experiment was to determine if Zn uptake in biofilm collected from HOC was caused by processes occurring in the biofilm matrix, which includes living algae and bacteria, dead organic matter, and abiotic particles. These processes might include (1) photosynthesis increasing the pH in the biofilm, which could cause precipitation of Zn-containing minerals and (or) adsorption of Zn to particles in the biofilm matrix, (2) absorption of Zn into algal and bacterial cells in the biofilm matrix, and (3) photoreactions with abiotic particles embedded in the biofilm.

2 Methods

2.1 Water

We pumped water from HOC through a series of filters (5, 1 and 0.45 μm) into an acid-washed plastic carboy prior to sunrise at 0500 h on 14 August 2003. The carboy

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was covered in black plastic to occlude sunlight and was placed in the creek to maintain the water temperature in the carboy at about the same as the creek temperature. About 2 h before the exposure began, we divided this filtered creek water into three batches. We added the photosynthesis inhibitor diuron (DCMU, $C_9H_{10}Cl_2N_2O$; Sigma # 45463) to the first batch of water to make the final concentration 0.5 mM DCMU. Formaldehyde was added to the second batch of water to make the final concentration 4% formaldehyde (10% formalin). We added nothing to the third batch of water, which was used in the Sunlight and Sunlight-occluded treatments.

2.2 Biofilm

At 0500 h on 14 August 2003, we removed biofilm-covered cobbles (~10 cm diameter) from the streambed of HOC and placed them in an acid-washed, opaque green plastic container that had several 0.5-cm holes punched in the sides of the tub (to allow creek water to flow through). After attaching the lid, this tub was also placed in the creek. The biofilm layer on the cobbles was ~1 cm thick.

2.3 Exposure chambers

We constructed twelve 2-L cylindrical exposure chambers with transparent PVC plastic pipe (26 cm long, 10-cm inner diameter). For each chamber, we removed one cobble from the holding tub and placed it in the chamber at about 1430 h on 14 August 2003. Then we sealed the open end of the chamber with a rubber sheet covered in plastic wrap, which was secured with a plastic flange and stainless steel nuts and bolts; and we poured the appropriate test water into the chamber (three replicate chambers for each of the four treatments) through a filling spout on the top so no air remained in the chamber. All chambers were placed in a water bath situated on the creek bank, and creek water flowed through the water bath to maintain each chamber at about the same temperature as the stream throughout the experiment. Each chamber was fitted with a 60-ml syringe that we used to mix the water in the chambers and to periodically draw 10 ml of water for pH and Zn analyses.

2.4 Chemical analyses

After all 12 chambers were filled and sealed, we collected a 20-ml water sample from each of the three stock solutions, acidified it to pH <2 using trace-metal grade HNO_3, and later analyzed it for total Zn, Ca, K, Mg and Na by flame atomic absorption spectrophotometry (FAAS; Perkin-Elmer Model 372). In addition, 10 ml of each stock solution was filtered (0.45 μm), acidified, and later analyzed for dissolved Ca and Zn by FAAS. A second 10-ml water sample was collected from each stock solution and placed on ice for later analysis of F^-, Cl^-, NO_3^-, and SO_4^{2-} on a Dionex DX-100 ion chromatograph. Additional water samples were collected from each stock solution and immediately analyzed for pH (using an Orion® Model 290A pH meter equipped with an Orion® Model 8163BN Ross combination pH electrode) and dissolved oxygen (D.O.; using a YSI® Model 58 D.O. meter equipped with a YSI® Model 5739 probe).

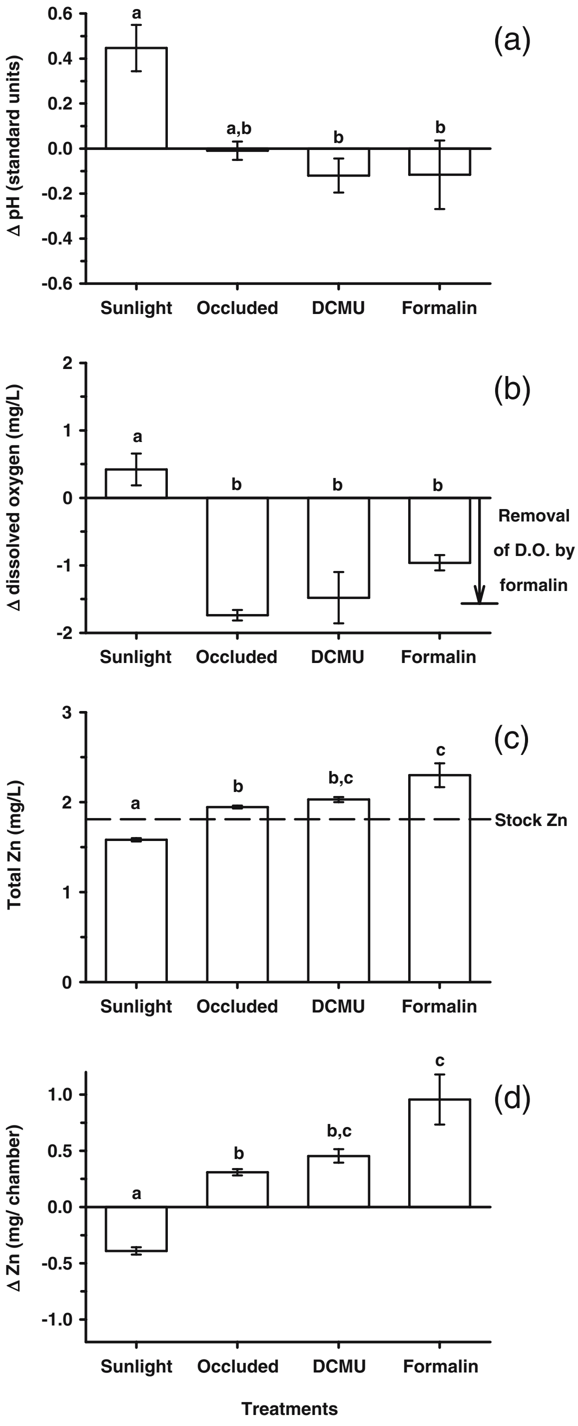
2.5 Experiment

This 4.5-h experiment began at 1430 h and ended at 1900 h on 14 August 2003. The experiment was terminated when the pH of the water in one of the three Sunlight-treatment chambers reached 8.26 (the highest pH measured in HOC on 13 August 2003). We withdrew 10-ml water samples from each chamber at 51, 104, 158, and 215 min. One ml from each of four 10-ml samples collected from each chamber throughout the experiment was composited for a total of 4 ml, which we acidified and later analyzed for total Zn for mass-balance calculations. Then we immediately analyzed the pH of the remaining 8 ml of each 10-ml sample.

At the end of the experiment, we withdrew 120 ml of water from each chamber. A subsample of this 120 ml was immediately analyzed for D.O. and pH; and the remainder was analyzed for total and dissolved Zn, and major inorganic cations and anions as described above. After all water samples were collected, we removed the cobbles from each chamber and measured the remaining volume of water for mass-balance calculations. Photosynthetically active radiation (PAR) was measured automatically at the streamside every 15 min with a Li-Cor LI-1400 data logger equipped with an underwater light sensor (serial # UWQ 6414).

2.6 Geochemical speciation calculations and statistical analysis

We used MINEQL+ Version 4.0 for Windows (Schecher & McAvoy, 1998) to calculate saturation indices (S.I.



◀ **Figure 1** Water-quality parameters in transparent PVC plastic chambers containing 0.45-μm-filtered water and biofilm-covered cobbles from High Ore Creek, Montana, during a streamside experiment from 1430 h to 1900 h on 14 August 2003. The Sunlight treatment was exposed to full sunlight, the Occluded treatment was maintained in complete darkness, the DCMU treatment contained a photosynthesis inhibitor (0.5 mM diuron) and was exposed to full sunlight, and the Formalin treatment contained 4% formaldehyde (10% formalin) and was exposed to full sunlight. All Δ values are changes relative to the initial value in the exposure chamber, and the Δmg of Zn in panel **d** is the loss or gain of total Zn in each chamber (accounting for the Zn in the water that was periodically withdrawn from the chamber for analytical samples). *Different letters above a pair of bars* indicate significant ($P<0.05$) differences; error bars are ±1 standard deviation from the mean (n=3). The *arrow* in panel **b** indicates the range of dissolved oxygen (D.O.) removal from water by 10% formalin in separate laboratory experiments. The *dashed line* in panel **c** indicates the Zn concentration (1.85 mg Zn/L) of the stock solution for the Sunlight and Occluded treatments at the beginning of the experiment; the Zn concentrations in the stock solutions for the DCMU and Formalin treatments were within 4% of this concentration.

values; where S.I.=IAP/K_{sp}, IAP=the ion activity product of the ions involved in formation of the mineral, and K_{sp}=the solubility product of the mineral) for all the Ca-, Mg-, Na-, K-, and Zn-containing minerals included in the MINEQL+ thermodynamic database (including 21 Zn-carbonates, Zn-hydroxides, and Zn-oxides), based on the pH, alkalinity, and concentrations of major inorganic ions and Zn measured at the end of the experiment. Additionally, we entered hydrozincite (log K=−9.65 for $5Zn^{2+}+2CO_3^{2-}+6H_2O \leftrightarrow Zn_5(OH)_6(CO_3)_2(s)+H^+$; Zachara, Kittrick, Dake, & Harsh 1989) into the thermodynamic database because it has been reported to precipitate in some other Zn-contaminated streams (e.g., Podda, Zuddas, Minacci, Pepi, & Baldi 2000). Equilibrium equations used for all these Zn-containing minerals are listed in Morris et al. (2005). We conducted statistical comparisons with ANOVA ($\alpha=0.05$) followed by Tukey HSD post hoc pairwise comparisons using Minitab™ Version 13.31 (Minitab Statistical Software, Minitab Inc.).

3 Results

3.1 Water quality and changes in Zn concentrations

The pH in the Sunlight treatment increased while the pH in the other three treatments either did not change or decreased (Figure 1a). Similarly, the D.O. concentration in the Sunlight treatment increased, but it decreased in the other three treatments (Figure 1b). The total Zn concentration of the Sunlight and Sunlight-occluded stock solution was 1.85 mg Zn/L, and the total Zn concentrations of the stock solutions for the DCMU and Formalin treatments were within 4% of this concentration. At the end of the experiment, the total Zn concentration in the Sunlight treatment (1.6 mg Zn/L) was significantly (P=0.001) lower than the total Zn concentrations in the Sunlight-occluded, DCMU, and Formalin treatments (1.9, 2.0, and 2.3 mg Zn/L, respectively; Figure 1c). Consequently, the change in the total amount of Zn per chamber in the Sunlight treatment was negative and differed significantly (P<0.001) from the other three treatments, in which the total amount of Zn increased (Figure 1d). The increases in total Zn concentration and ΔZn/chamber in the Sunlight-occluded, DCMU, and Formalin treatments indicate that Zn was released from the biofilm-covered cobbles in these treatments.

3.2 Effects of chemical treatments

Because pH and D.O. increased in the Sunlight treatment, the biofilm in that treatment was actively photosynthesizing. Conversely, because pH and D.O. decreased in the Sunlight-occluded and DCMU treatments, photosynthesis either decreased or was not occurring in these treatments, but respiration probably continued. Although the purpose of the Formalin treatment was to kill all living organisms, it is difficult to determine if respiration occurred due to the possibility of the formalin reacting with and removing D.O. from the water. In separate experiments without biofilm and cobbles, 10% formalin removed as much as 1.6 mg D.O./L. We also determined that the PVC plastic attenuated ~90% of UV-B radiation and ~30% of PAR. However, based on the pH and D.O. increases in the Sunlight chambers, this decrease in PAR was not enough to inhibit photosynthesis. Throughout the experiment, PAR at the streamside ranged from ~200 to 1,550 μmol/m^2/s.

3.3 Geochemical speciation calculations

Hydrozincite was the only Zn-containing mineral that was supersaturated in the water column of our Sunlight treatment. Its average $\log_{10}$(S.I.) was 2.6±0.8 (n=3), where $\log_{10}$(S.I.)>0.0 indicates supersaturation (Drever, 1997). However, we do not believe this mineral was precipitating in the water column because (1) we saw no precipitates in the water or on the 0.45-µm filters and (2) the difference between the average total and dissolved Zn concentrations was only ~20%, which is similar to the 15% difference in the average total and dissolved Zn concentrations in our Sunlight-occluded treatment. Furthermore, hydrozincite was also supersaturated (average $\log_{10}$(S.I.)=0.4±0.5; n=3) in our Sunlight-occluded treatment but total Zn concentration did not decrease (Figure 1c). Because Zn concentration increased in the DCMU treatment, non-photosynthetic photoreactions (e.g., photoreductions or photo-oxidations) caused by sunlight do not appear to drive Zn removal from the water column.

4 Discussion

We believe Zn removal from the water column occurs at the surface of the biofilm matrix and within the matrix, where photosynthesis causes the pH to exceed 10 (as measured in previous laboratory experiments; Morris, 2005). Elevated pH in the biofilm matrix could cause adsorption of Zn to the surfaces of biotic and abiotic particles and (or) precipitation of Zn-containing minerals in the biofilm matrix. However, we believe only a small percentage of Zn is absorbed into algal and bacterial cells in the biofilm in HOC because (1) previous laboratory experiments that indicate ~70% of Zn accumulated in lab-cultured biofilm (free of abiotic particles) is located on the outside of the microbial cells and only ~30% is internalized (Morris & Meyer, 2006), and (2) the ash concentration in biofilm in HOC (42–87%) is much higher than in lab-cultured biofilm (15%; Morris et al., 2005), indicating that biofilm from HOC contains more abiotic material and a much smaller percentage of biotic particles capable of absorbing Zn than lab-cultured biofilm contains. Therefore, the relatively small amount of Zn absorbed into algal and bacterial cells during daylight in HOC would not contribute considerably to diel Zn cycling in the water column.

Regardless of the underlying mechanism(s), removal of dissolved Zn from the interstitial water in the biofilm matrix should produce a steep Zn diffusion gradient from the water column into the biofilm during daylight. That diffusion gradient could at least partially drive diel Zn cycling in the water column of HOC and some other metals-contaminated streams, at least temporarily transferring Zn into the biofilm. Therefore, researchers, modelers, regulators, and reclamation teams working in mining-impacted areas should be aware of photosynthetic biofilm's potential to influence and (or) control diel metal cycling.

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