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Influence of Total Organic Carbon and UV-B Radiation on Zinc Toxicity and Bioaccumulation in Aquatic Communities

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The effects of total organic carbon (TOC) and UV-B radiation on Zn toxicity and bioaccumulation in a Rocky Mountain stream community were assessed in a 10-d microcosm experiment. We predicted that TOC would mitigate Zn toxicity and that the combined effects of Zn and UV-B would be greater than Zn alone. However, TOC did not mitigate Zn toxicity in this study. In fact, treatments with TOC plus Zn had significantly lower community respiration as compared with the controls and Zn concentrations associated with the periphyton increased in the presence of TOC. UV-B had no additive effect on periphyton Zn accumulation or community respiration. Heptageniid mayflies (Ephemeroptera) were particularly sensitive to Zn, and reduced abundances were observed in all Zn treatments. UV-B did not additionally impact Heptageniid abundances; however UV-B did have a greater effect on macroinvertebrate drift than Zn alone. Ephemeroptera, Plecoptera, and Trichoptera (groups typically classified as sensitive to disturbance) were found in highest numbers in the drift of UV-B + Zn treatments. Measures of Zn accumulation in the caddisfly *Arctopsyche grandis*, periphyton biomass, and total macroinvertebrate abundance were not sufficiently sensitive to differentiate effects of TOC, UV-B, and Zn. These results indicate that UV-B and TOC affect Zn bioavailability and toxicity by impacting species abundance, behavior, and ecosystem processes.

Introduction

Most ecosystems are subjected to multiple perturbations whether natural or human induced. Paine et al. (*1*) provides a compelling argument that more serious ecological consequences result from multiple disturbances as opposed to single perturbations. Metal pollution from historic mining operations in Colorado has been a major concern in Rocky Mountain streams for the last century (*2*, *3*). In addition, these high elevation streams may be particularly susceptible to UV-B (280−320 nm) because solar intensity increases with elevation and because these streams generally have naturally low levels of organic matter, which reduces UV-B exposure (*4*, *5*). Although UV-B is a natural stressor in these ecosystems, anthropogenic release of chlorofluorocarbons and ozone depletion have resulted in a 10−20% increase in UV-B per decade at the earth surface in the Northern Hemisphere (*6*).

UV-B exposure can cause cellular damage to organisms (*7*), resulting in adverse impacts on community structure and ecosystem function (*8*, *9*). Recent studies suggest that UV-B may influence the distribution and abundance of freshwater organisms (*8*−*10*), potentially disrupting basic ecosystem functions such as respiration, primary productivity, nutrient cycling, and food web dynamics (*11*). Several studies also document aquatic macroinvertebrate behavioral changes associated with UV-B exposure. Donahue and Schindler (*12*) found that UV-B influences the diel drift of simuliidae larvae. Similarly, Kiffney et al. (*13*) documented an increase in drifting stream macroinvertebrates associated with elevated UV-B exposure. In contrast, other investigators have found that ambient UV-B exposure has no inhibitory effect on stream benthic algae or invertebrates (*14*, *15*).

The depth to which UV-B penetrates aquatic systems is influenced by the quantity of natural organic matter (NOM) present in the system (*16*). The composition of NOM is variable but typically contains a large proportion of humic substances together with a host of other polyelectrolyte macromolecules (*17*, *18*). NOM plays an important role in aquatic photochemistry (*14*) and provides protection to aquatic communities from UV-B. However, UV-B degrades NOM into low molecular weight compounds through a process known as photobleaching (*19*, *20*). Because of photodegradation of NOM (*21*), UV-B penetration and exposure may increase in shallow, high elevation streams.

Previous research has demonstrated that metal impacts can structure benthic macroinvertebrate communities. Clements et al. (*2*) found that metal concentration was the most important predictor of benthic community structure in a study investigating 95 sites in the Rocky Mountains of Colorado. Adverse physiological and individual responses to metal exposure have been observed in algae and macroinvertebrates, including reduced growth rates (*22*−*24*) and increased mortality (*24*). Changes in behavior associated with metal exposure have also been documented. Balch et al. (*25*) found a correlation between high Zn body burdens and the production of abnormal food capture nets in the filter-feeding caddisfly larvae (*Hydropsyche betteni*). Clements (*26*) reported an increase in drifting stream macroinvertebrates associated with metal exposure.

In addition to concerns over metal pollution and increases in UV-B radiation, recent evidence has indicated that these streams may experience changes in natural dissolved organic material due to changes in hydrologic regime associated with climate change (*27*). Organic matter plays an important role in both metal bioavailability and UV-B exposure. Interactions among metals, organic matter, and UV-B are complex, and the ability of organic matter to bind metals and attenuate light varies with source (e.g., allochthonous versus autochthonous) and chemical composition. Organic matter forms complexes with heavy metals in aquatic ecosystems, thereby reducing metal bioavailability and toxicity (*21*, *28*). Prusha and Clements (*29*) found an inverse relationship between dissolved organic carbon (DOC), which typically comprises 50% of the mass of NOM (*30*), and Zn body burdens in the filter-feeding caddisfly *Arctopsyche grandis* in metal-contaminated streams. On the other hand, NOM may also increase metal bioavailability. Parent et al. (*31*) found that the total amount of aluminum associated with the algae was

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TABLE 1. Comparison of Physicochemical Characteristics and UV-B Levels (Measured above the Water Surface) among Treatments in a 10-d Microcosm Study Examining the Effects of TOC, Zn, and UV-B on Macroinvertebrate Assemblages and Bioaccumulaton[a]

variable	control ($n = 2$)	TOC + Zn ($n = 4$)	Zn ($n = 4$)	TOC + UV + Zn ($n = 4$)	UV + Zn ($n = 4$)	*p* value (ANOVA)
temp (°C)	16.1 ± 0.9	16.6 ± 0.9	16.3 ± 0.9	16.6 ± 0.9	16.4 ± 0.9	0.97
pH	8.0 ± 0.1	7.9 ± 0.8	7.9 ± 0.1	7.9 ± 0.8	7.9 ± 0.1	0.77
DO (mg L^{-1})	6.5 ± 0.1	6.5 ± 0.1	6.6 ± 0.1	6.4± 0.1	6.5 ± 0.1	0.91
conductivity ($\mu\Omega^{-1}$ s^{-1})	71.1 ± 1.2	74.0 ± 1.4	71.8 ± 1.1	75.2 ± 1.5	73.0 ± 1.1	0.17
hardness (mg of CO_3^{-1})	37.5 ± 10.0	42.6 ± 6.6	42.0 ± 6.0	37.6 ± 12.6	44.8 ± 2.3	0.96
alkalinity (mg L^{-1})	43.8 ± 7.8	41.3 ± 5.3	45.9 ± 7.4	44.8 ± 8.8	42.3 ± 5.8	0.99
TOC (mg L^{-1})[b]	2.5 ± 0.1	4.7 ± 0.2	2.2 ± 0.1	4.7 ± 0.9	2.3 ± 0.1	<0.001
Zn (μg L^{-1})[b]	14.5 ± 0.05	501.8 ± 18.7	469.5 ± 31.5	522.3 ± 32.7	510.5 ± 40.6	<0.001
NH_4 (mg L^{-1})[c]	0.05	0.01	0.03	0.01	0.03	
NO_3-N (mg L^{-1})[c]	0.04	0.01	0.02	0.01	0.02	
P (mg L^{-1})[c]	<0.001	<0.001	0.001	0.001	0.001	
UV-B (mW cm^{-2})[b]	<0.005	<0.005	<0.005	0.20 ± 0.0	0.20 ± 0.0	<0.001
chlorophyll *a* (μg cm^{-2})	0.168 ± 0.1	0.199 ± 0.0	0.184 ± 0.0	0.297 ± 0.2	0.146 ± 0.1	0.75
AFDW (mg cm^{-2})	0.029 ± 0.0	0.019 ± 0.0	0.026 ± 0.0	0.026 ± 0.0	0.023 ± 0.0	0.94

[a] Values are means ± standard errors. [b] Variables manipulated among treatments. [c] These values represent a one-time measurement and therefore were not statistically compared among treatments.

higher in the presence of fulvic acid, a component of NOM. Thus, qualitative and quantitative changes in NOM resulting from photodegradation may increase metal bioavailability and UV-B exposure to stream organisms (*19*, *20*). Organic matter complexes metals and often results in decreased metals availability to aquatic organisms (*21*). Thus, losses in NOM resulting from UV-B exposure may release metals previously bound to the NOM increasing their availability to stream organisms.

Community and ecosystem responses to multiple perturbations are poorly understood because of the inherent difficulties conducting field experiments that quantify individual effects. The goal of this research was to investigate benthic community responses to combined metal and UV-B exposure and to test the hypothesis that the combined effects of Zn and UV-B would be greater than Zn alone.

Methods

To determine how Zn toxicity and the corresponding response of aquatic communities were influenced by organic material and UV-B radiation, natural benthic communities were transferred into the laboratory and exposed to UV-B, Zn, and humic acid (HA) in stream microcosms. In this study we used HA (Sigma Chemical; St. Louis, MO), a commercially available organic material, to test the hypothesis that organic matter influences Zn and UV-B effects. Periphyton and macroinvertebrate communities were collected during October 2002 from a reference site on the Arkansas River (elevation 2835 m) near Leadville, CO. Macroinvertebrates were collected using a previously described procedure (*27*) in which colonization trays (10 × 10 × 6 cm) filled with pebbles and small cobbles were placed in the stream. Sixty trays were secured on racks anchored in riffle areas of the streambed. Glazed ceramic tiles (5 × 5 cm) were used as colonization substrates for periphyton. Fifty-four tiles were attached to plastic trays and anchored to the streambed near the colonization trays. After a 40-d colonization period, trays and tiles were removed from the stream, placed in aerated coolers, and transported to the Stream Research Laboratory (SRL) located at Colorado State University (CSU; Fort Collins, CO).

The SRL consists of 18 experimental stream microcosms (76 × 46 × 14 cm) that receive water similar in physicochemical characteristics to unpolluted Rocky Mountain streams (*26*). Turnover rate in each flow-through microcosm was 24 h, and current was generated by paddlewheels. The experimental streams are located within a greenhouse that filters out 97% of incoming solar UV radiation (280−400 nm), while allowing transmission of 50−80% of photosynthetically active radiation (PAR; 400−500 nm; *13*).

Three trays and three tiles were placed in each microcosm and randomly assigned to five treatments manipulating UV-B radiation, Zn, and HA content. Organic matter was measured as total organic carbon (TOC) in order to demonstrate the full spectrum of binding, both particulate and dissolved. Treatments consisted of controls, Zn only, Zn + TOC, Zn + UV-B, and Zn + TOC + UV-B. All treatments except controls were assigned to four streams; controls were assigned to two streams. All Zn treated streams were exposed to a target concentration of 1000 μg of Zn/L as $ZnSO_4$. HA-treated streams received 12 mg/L TOC, a concentration sufficient to increase TOC approximately 2× above background levels. Previous research has shown that this level of HA has no toxic effects on stream communities (Kashian, unpublished data). Background TOC concentrations were a natural component of the source water. Stock solutions of $ZnSO_4$ and HA were delivered to each treated stream from separate 20-L carboys using peristaltic pumps. In Zn + TOC treatments, HA and $ZnSO_4$ were simultaneously added to the same carboys 1 h prior to dosing to allow Zn to bind to TOC. Streams were then dosed at the slow rate of 10 mL/min, which allowed additional binding time to occur in the carboy through the course of the 24-h dosing cycle. UV-B radiation was generated by two SF 20 lamps per stream (UVB-313; National Biological, Twinsbury, OH) suspended 15 cm above the water surface and directly above the colonization trays and tiles. UV-B exposure occurred from 0600 to 1600 h each day to bracket solar noon. Experiments were conducted for 10 d.

Stream temperature, pH, conductivity, and dissolved oxygen were measured daily in experimental streams and in the field when trays were collected**.** Water samples (500 mL) were collected for determination of hardness and alkalinity (*32*, *33*) on days 5 and 10 of the experiment. Levels of UV-B radiation were measured at the beginning and end of the experiment using an Optronics model 754 spectroradiometer (Optronics Laboratories Inc., Orlando, FL). Nutrient samples were collected on day 10 and filtered through a 0.45-μm Millipore filter and sent to Colorado State University's Soil Testing laboratory for analysis of NO_3-N, NH_4-N, and soluble reactive phosphate. All samples were tested at a detection limit of 0.001 mg/L. To verify target metal levels in the microcosms, water samples (14 mL) were collected on days 5 and 10 and acidified with nitric acid (pH ≤ 2.0). Zn concentrations were determined using flame atomic absorp-

TABLE 2. Total Macroinvertebrate Abundance, Number of Taxa, and Abundance of Major Macroinvertebrate Groups (Mean ± 1 SE) at the End of a 10-d Microcosm Experiment Examining the Effects of TOC, UV-B Radiation, and Zn on Macroinvertebrate Assemblages

variable	control	TOC + Zn	Zn	TOC + UV + Zn	UV + Zn
total abundance	2190.0 ± 915.0	1237.5 ± 503.5	1627.3 ± 337.6	2028.3 ± 736.2	1306.0 ± 530.6
no. of taxa	35.5 ± 2.5	26.8 ± 2.2	30.5 ± 1.3	33.0 ± 2.5	26.8 ± 2.3
Ephemeroptera	521.5 ± 78.5	209.3 ± 43.7	363.8 ± 30.9	327.5 ± 86.1	271.3 ± 55.6
Plecoptera	45.0 ± 5.0	39.0 ± 3.6	40.0 ± 6.5	47.0 ± 10.3	42.8 ± 4.4
Trichoptera	57.0 ± 14.0	20.8 ± 3.5	34.0 ± 2.5	42.8 ± 19.6	37.3 ± 7.4
Chironomids	1538.0 ± 813.0	946.3 ± 458.3	1165.3 ± 319.5	1581.3 ± 630.4	932.8 ± 466.5

tion spectrophotometry (detection limit = 45 μg/L). TOC concentrations were analyzed on days 5 and 10 to verify target concentrations. Water samples for analysis of TOC were acidified to a pH of 2 with hydrochloric acid and measured with a Shimadzu TOC-5050A (detection limit = 0.5 mg/L).

The biological response variables measured included a suite of ecologically relevant structural (total abundance; number of taxa; abundance of Ephemeroptera, Plecoptera, and Trichoptera (EPT); macroinvertebrate drift; periphyton biomass) and functional (community respiration) characteristics known to be indicators of ecosystem health (*8*, *11*, *34*−*37*). Drift was measured in each experimental stream by placing a small net immediately downstream from the trays. Drift samples were collected on day 5 during the 10-h UV-B exposure (0600 to 1600 h) and at night (1900 to 0500 h). Organisms caught in the drift net were removed from the stream and preserved in 80% ethanol (EtOH). Community respiration was measured in the microcosms at the conclusion of the experiment using a modified technique developed for EPA's Environmental Monitoring and Assessment Program (*38*). A single tray was removed from each stream on day 10 and placed into a separate respiration chamber (20 cm × 20 cm × 10 cm). Dissolved oxygen was measured initially and after a 1-h incubation period using a calibrated (Winkler titration method) YSI dissolved oxygen meter. A change in dissolved oxygen concentration in the overlying water of these chambers provided an estimate of community respiration. After measuring respiration, the three trays from each stream were combined and the contents rinsed through a 350-μm-mesh sieve and preserved with 90% EtOH. Organisms were sorted and all individuals (except Chironomidae) were identified to genus or species. Chironomids were identified to subfamily or tribe.

To assess potential indirect effects of Zn, TOC, and UV-B on macroinvertebrate food resources, ash-free dry weight (AFDW) and chlorophyll *a*, indicators of periphyton biomass and quality, were measured at the end of the exposure period (*39*). Periphyton samples were collected from the ceramic tiles for these analyses. One tile from each stream was used for chlorophyll *a* (μg/cm^2) analysis, and one tile was used for AFDW. Periphyton for both chlorophyll *a* and AFDW was removed from individual tile substrates using a battery-operated, high-speed (7500 rpm) rotary tool (Dremel; Robert Bosch Tool Corporation, Mount Prospect, IL) with a nylon bristle attachment. Periphyton and rinse water were filtered through a 0.45-μm glass-fiber filter. Laboratory analysis of chlorophyll *a* and AFDW followed procedures outlined by the American Public Health Association (*33*). The extraction for chlorophyll *a* followed the protocol developed by Biggs and Kilroy (*39*).

To estimate Zn bioavailability to macroinvertebrates, the caddisfly *A. grandis* (Banks) was exposed to each treatment in the microcosm experiment. Organisms (n = 90) were collected from the Arkansas River and transported to the SRL. Five individuals were placed in fine mesh (750-μm) cages in each stream microcosm. After the 10-d exposure, organisms were removed for metals analysis. In the laboratory, individuals from each treatment were rinsed with DI water, dried at 55 °C, weighed, and digested in a mixture of nitric acid and hydrogen peroxide. Rinsing was done to remove any surface film that may contain Zn or TOC. Samples were diluted with Milli-Q water and placed in a hot water bath for 8 h. All samples were analyzed with an Instrumentation Laboratory Video 22 air acetylene flame atomic absorption spectrophotometer with Smith−Hieftje background correction (Franklin, MA). Quality assurance and quality control (QA/QC) procedures included the use of a bovine standard (± 15%), acid blanks (± 36 μg/L), and external QA/QC (± 10%). To estimate Zn concentrations associated with the periphyton community, biofilm was removed from one tile in each stream using a Dremel tool and rinsed into a preweighed labeled centrifuge tube. Samples were analyzed for Zn as described above.

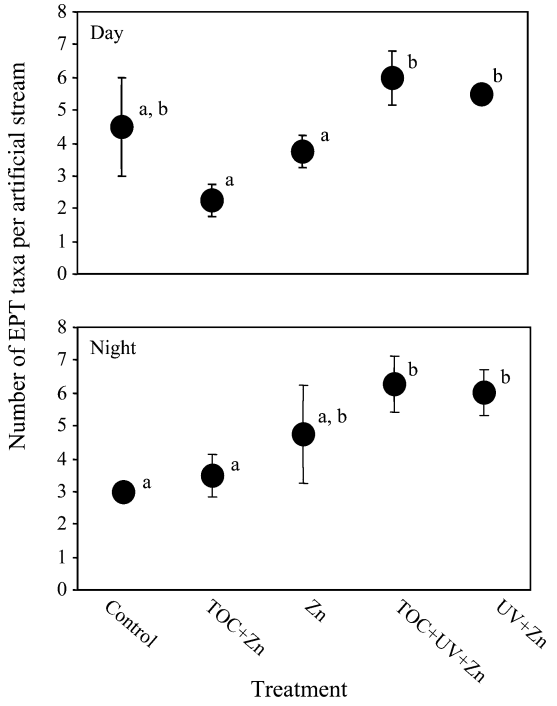

FIGURE 1. Mean (± 1 SE) number of Ephemeroptera, Plecoptera, and Trichoptera (EPT) taxa in samples from stream microcosms. Drift response of EPT taxa was measured during the 10-h UV-B enhanced exposure and 10 h during the night. Bars with the same letters were not significantly different (p < 0.05) as determined by ANOVA with a pair-wise LSD.

We predicted that Zn toxicity and bioaccumulation would be mitigated in streams treated with TOC and that the combined effects of Zn and UV-B would be greater than Zn alone. Thus, we hypothesized the following sequence of stressors: controls < [TOC + Zn] < Zn < [UV + TOC + Zn] ≈ [UV + Zn]. One-way ANOVA (*40*) was used to test for differences in benthic community indicators (e.g., abundance, number of Heptageniid mayflies, species richness)

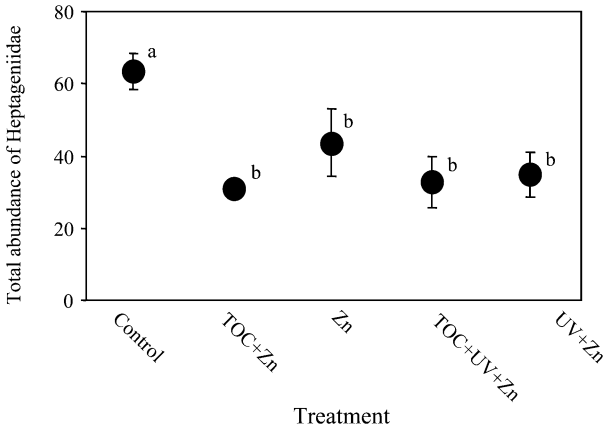

FIGURE 2. Effects of UV-B, TOC, and Zn on the abundance of Heptageniidae mayflies in a 10-d microcosm experiment. Values given as means ± 1 SE. Bars with the same letters were not significantly different ($p < 0.05$) as determined by ANOVA with a pair-wise LSD.

and differences in Zn bioaccumulation (i.e., periphyton and *A. grandis*) among treatments. If ANOVA indicated significant main effects ($p < 0.05$), a least significant difference (LSD) test was used to test for differences among treatments. Assumptions of ANOVA were tested using F_{max} tests and by inspection of residual plots. Where necessary, data were log-transformed to satisfy assumptions of homogeneity of variance.

Results

Except for variables intentionally manipulated (e.g., TOC and Zn), physicochemical characteristics were similar among treatments (Table 1). Nutrient concentrations were low in all streams and did not differ among treatments. In treated streams, mean concentrations of Zn ranged from 470 to 522 μg/L, and addition of HA increased mean background TOC levels by approximately 2.3 mg/L above untreated streams (Table 1). UV-B levels were similar to ambient solar UV-B (0.22 mW/cm^2) measured at the colonization site on a cloudless July day around 1:00 pm CMT (Kashian, unpublished data).

The mayfly *Baetis bicaudatus* dominated drift samples in all treatments and accounted for 53−72% of all organisms in day and night collections. Drift of metal-sensitive taxa (i.e., EPT) increased at night along the predicted stress gradient and was approximately 2× greater in UV-B-treated streams as compared to controls (Figure 1). Similar patterns were observed during the day in treated streams; however, treatments were not significantly different from the control.

Total macroinvertebrate abundance ($p = 0.76$), number of taxa (0.08), abundance of the major macroinvertebrate groups ($p = 0.48$), and periphyton biomass ($p = 0.94$), and quality as represented by chlorophyll *a* content ($p = 0.75$) in the microcosms did not significantly differ among treatments at the end of the 10-d experiment (Tables 1 and 2). Chironomidae dominated the benthic communities and typically compromised 70% of all samples. The most sensitive indicator of stress associated with community structure in this study was the abundance of heptageniid mayflies (Figure 2). Abundance of the two dominant heptageniids (*Epeorus* spp. and *Rhithrogena* spp.) was significantly lower in treated streams compared to controls ($p < 0.05$; Figure 2). There was relatively little difference in abundance of these organisms among treated streams, suggesting that effects were primarily a result of Zn exposure. Furthermore, the reduced abundance of heptageniids was not an artifact of loss of individuals associated with drift because very few of these individuals were present in the drift. Therefore, reduced abundances were likely the result of increased mortality associated with Zn exposure.

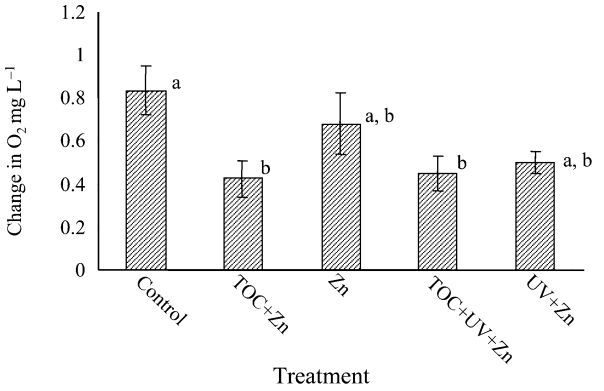

FIGURE 3. Effects of Zn, TOC, and UV-B radiation on community respiration. Respiration was calculated as the rate of change in dissolved O_2 concentration in a respirometer chamber (20 cm × 20 cm × 10 cm) over 1 h. Values given as means ± 1 SE. Bars with the same letters were not significantly different ($p < 0.05$) as determined by ANOVA with a pair-wise LSD.

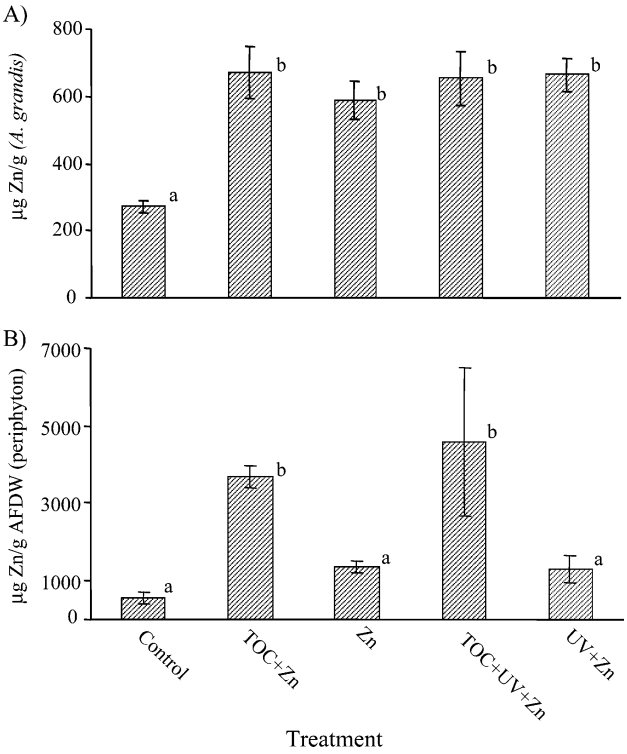

FIGURE 4. Average Zn concentrations in (A) *Arctopsyche grandis* and (B) periphyton (adjusted to AFDW) after a 10-d microcosm experiment. Values given as means ± 1 SE. Bars with the same letters were not significantly different ($p < 0.05$) as determined by ANOVA with a pair-wise LSD.

Community respiration varied among treatments, and was significantly reduced in treatments containing both Zn and TOC (Figure 3). Respiration in the TOC + Zn and the TOC + UV-B + Zn treatments was reduced by approximately 50%. Although respiration was lower in the Zn and Zn + UV treatments as compared to controls, these differences were not statistically significant.

Zn concentration in the caddisfly *A. grandis* was significantly greater in treated streams as compared to controls (Figure 4A). However, there were no differences in Zn levels among the treated streams. In contrast to our predictions, metal levels in caddisflies from streams treated with TOC

were similar to those from Zn alone and Zn + UV streams. Metal levels associated with the periphyton also increased in treated streams; however, periphyton differentially accumulated Zn among treatments (Figure 4B). Zn concentration associated with periphyton from streams with TOC was approximately 8× greater than with periphyton from controls. The presence of TOC also significantly increased Zn concentration in the TOC + Zn and TOC + Zn + UV treatments as compared to the Zn-only and Zn + UV treatments.

Discussion

Results of this study generally show that complex interactions of heavy metals, UV-B, and TOC structure benthic macroinvertebrate communities. The combination of UV-B and Zn had a greater effect on macroinvertebrate drift than the Zn and Zn + TOC treatments, suggesting that UV-B may increase the susceptibility of benthic organisms to Zn. The mechanism of this effect is unknown. Although, we did not test the independent effects of UV-B on drift, we suspect some degree of this effect resulted from the combination of Zn and UV-B and not just UV-B alone because a pattern of increased drift in the UV-B treatments occurred even when the UV-B lamps were turned off at night (Figure 1). Additive or synergistic effects related to oxidative stress, damaged DNA, or inhibitions of DNA repair mechanisms are potential mechanisms for enhanced toxicity. It is unlikely that increased drift in Zn plus UV-B exposures was due to photoenhanced toxicity as exhibited by interactions between UV-B and organic contaminants such as polycyclic aromatic hydrocarbons (PAH's). Drift has been previously documented as a behavioral response to unfavorable environmental conditions, including metals and UV-B radiation (*13*, *37*, *41*). Kiffney et al. (*13*) documented increases in drift of mayflies and caddisflies in UV-B-treated streams and suggested that mayflies and noncased caddisflies are particularly sensitive because they inhabit rock surfaces with naturally high UV-B exposure. Kiffney et al. (*13*) did not observe stoneflies drifting in significant numbers when exposed to UV-B. Kiffney and Clements (*42*) reported that stoneflies drifted in response to metals. Our data agreed with these studies, as taxa with known sensitivities to UV-B and metals (i.e., EPT taxa) generally increased in the drift along a predicted stress gradient.

We identified several macroinvertebrate taxa sensitive to Zn and TOC + Zn, but these measures did not provide evidence of a Zn by TOC interaction. We identified a few species sensitive to Zn by examining species abundance. The most sensitive indicator of stress associated with community structure was abundance of heptageniid mayflies, a finding consistent with other studies (*41*, *43*). In our study, reduced abundances of heptageniid mayflies were limited to treatments with elevated Zn concentration. TOC did not mitigate Zn toxicity to heptageniids, and UV-B had little additional effects on abundance. The driving force of this response appears to be solely associated with Zn toxicity. TOC concentrations in this study may have not been high enough or the quality of HA may not have been sufficient to bind all the available Zn. Although many bioavailability studies conducted to date have used commercial humic acids similar to those used in this study, molecular weights of commercial humic acids are significantly larger than those of aquatic fulvic acids (*44*). Nonetheless, Haizer et al. (*45*) reported that studies which use commercially available HAs found consistently larger reductions in bioavailability than similar studies which used either unaltered surface water or organic matter extract from natural sources (soil or water).

Evidence that TOC mitigated Zn toxicity was observed in community respiration measures. On the basis of the results from this study, the mechanism driving interactions between Zn and TOC appeared to be linked to TOC adsorption to cell surfaces. We feel this scenario best explains the increase in Zn concentrations associated with the periphyton and lower respiration rates in the presence of HA. HA has a high molecular weight making passive or active transported across cell walls unlikely. Therefore, elevated Zn concentrations associated with the periphyton were likely adsorbed to cell surfaces. This suggested mechanism is in agreement with several studies, which have demonstrated an affinity for TOC with bound contaminants to adsorb to cell surfaces (*23*, *31*, *46*). The high concentrations of Zn associated with periphyton in treatments with HA may also explain the lower respiration rates in these treatments due to increased toxicity to respiring microbes. This mechanism remains unknown.

Our results showed an inverse relationship between community respiration and Zn concentrations associated with the periphyton. Periphyton accumulated significantly more Zn in TOC-treated streams, and respiration in these streams was significantly less than in controls. We suggest that the high Zn concentrations associated with the periphyton were a direct result of the addition of HA. Several recent studies have demonstrated that humic and fulvic acids adsorb to biological surfaces, potentially altering the permeability of cells (*31*, *46*, *47*). In particular, Parent et al. (*31*) noted aluminum concentrations associated with the microalga *Chlorella* increased in the presence of fulvic acid. Metal adsorption may have been greater in our microcosms treated with TOC because HAs have a greater adsorption affinity for biological surfaces than fulvic acids (*47*). *A. grandis* did not show a similar pattern of increased Zn levels with increased TOC, suggesting a different mode of Zn uptake. We suggest that the greater bioaccumulation in periphyton from treatments with TOC resulted from adsorption of humic acids to periphyton and increased uptake of Zn.

Community respiration varied significantly among treatments, but there was no indication that TOC mitigated Zn toxicity or that UV-B interacted with Zn (Figure 3). In fact, in contrast to our predictions, respiration was significantly lower in treatments with TOC. This response was unlikely to be a result of direct effects of HA on respiration but was probably caused by interactions between TOC and Zn. Previous research has demonstrated that HA at the concentration tested in this study (TOC = 4.7 mg/L) had no impacts on respiration (Kashian, unpublished data).

Estimating the relative contribution of microbial, periphyton, or macroinvertebrate respiration was not possible because respiration was measured using entire benthic communities. It is unlikely that reduced respiration was attributable to macroinvertebrates because abundance of these organisms showed little variation among treatments. Although the pattern of reduced respiration in streams treated with TOC mirrors the increase in Zn concentrations observed in periphyton, periphyton biomass did not differ among treatments. Alterations in periphyton quality or changes in the microbial community may account for the declines in respiration in the TOC-treated streams.

Although we did not investigate direct effects of UV-B alone, it is unlikely that reduced respiration was a result of UV-B exposure. Hill et al. (*15*) reported that photosynthetic rates were not directly affected by UV-B exposure; however, significant reductions in periphyton community respiration were attributable to Zn at concentrations lower than those tested in this study (*48*). In our study, Zn alone did not cause significant declines in respiration; however, our study exposed entire benthic communities rather than just periphyton communities used in the Hill et al. (*49*) study.

These data have important implications for uptake and transfer of metals in aquatic foodwebs. Because periphyton accumulates metals at concentrations much greater than those in water, grazing macroinvertebrates are often exposed to very high levels of Zn (*22*, *29*). We do suspect that a substantial amount of Zn was not adsorbed to TOC or the

periphyton resulting in decreased abundances of sensitive taxa. In our experiments, reduced abundances of Heptageniidae occurred in all Zn treated streams. These results suggest that the influence of humic materials on metal bioavailability and toxicity are complex and that the presence of organic matter may not necessarily reduce metal exposure as predicted.

The potential impacts of metals and UV-B radiation on stream communities are likely to vary considerably because of natural variation in habitat, physicochemical characteristics, and organism behavior and sensitivity. This research demonstrated the importance of considering metal-complexing properties of organic matter when investigating the effects of metals on stream communities. Although Zn may bind to TOC, metal bioavailability to periphyton may not necessarily be reduced. The binding affinity of TOC to algal cells may actually increase metal availability and exposure.

Ecosystems are seldom impacted by a single perturbation; therefore, it is critical that we improve our understanding of the complex interactions among multiple stressors. Multifactor studies that simultaneously manipulate several variables are particularly important for assessing stressor impacts and interactions when estimating acceptable levels of contamination for protection of ecological integrity. By examining the effects of multiple perturbations on ecological end points at several levels of biological organization (individual, population, community, ecosystem), we were able to detect impacts that may have been otherwise missed. In a controlled microcosm experiment, we demonstrated that metals interact with natural environmental variables to influence exposure and ecological effects. These measures differ in sensitivity and provide insight into potential mechanisms, thus giving a more comprehensive understanding of total impacts**.** These experiments were limited in spatial and temporal scale however, and comprehensive field experiments are necessary to more fully understand the direct and indirect effects of UV-B, TOC, and metals on natural communities.

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