

Photochemical control of copper complexation by dissolved organic matter in Rocky Mountain streams, Colorado

Marjorie L. Brooks¹

Department of Fishery and Wildlife Biology, Colorado State University, Fort Collins, Colorado 80523; National Center for Ecological Analysis and Synthesis, 735 State Street, Suite 300, Santa Barbara, California 93101

Diane M. McKnight

Institute of Arctic and Alpine Research, 1560 30th Street, University of Colorado, Boulder, Colorado 80309

William H. Clements

Department of Fish, Wildlife and Conservation Biology, Colorado State University, Fort Collins, Colorado 80523

Abstract

We investigated photochemical, seasonal, and source effects on copper (Cu) complexation by dissolved organic matter (DOM). Cu-DOM complexation regulates Cu toxicity by decreasing the activity of the cupric ion ($\{Cu^{2+}\}$), the most bioavailable Cu species. However, DOM is photochemically unstable under solar insolation. We analyzed Cu-DOM complexation before and after photooxidation of DOM collected from six rivers during spring runoff and late summer ($n = 12$ DOM samples). After irradiation of DOM for 24 h in a solar simulator (~ 4 d of ambient insolation), we analyzed Cu-DOM complexation during potentiometric titrations of Cu into dissolved organic carbon concentrations of 5 mg L^{-1} . In 10 DOM samples across the range of titrations (Cu, 7.8×10^{-8} to $8.7 \times 10^{-6} \text{ mol L}^{-1}$), photooxidation of DOM decreased Cu complexation, increasing $\{Cu^{2+}\}$ by an average of $156\% \pm 28\%$ ($\bar{x} \pm \text{SE}$). In one DOM sample, irradiation had no net effect on $\{Cu^{2+}\}$ ($6\% \pm 12\%$), whereas in another Cu complexation was enhanced ($30\% \pm 4\%$). Cu complexation that was indistinguishable before irradiation decreased significantly more during photooxidation of DOM in spring ($185\% \pm 25\%$) than in summer ($74\% \pm 14\%$). The specific ultraviolet absorption coefficient at 254 nm explained $\sim 60\%$ of the variation in conditional stability constants of Cu-DOM complexes regardless of DOM source, season, or extent of photooxidation. During a simulated contaminant event where $1.5 \times 10^{-6} \text{ mol L}^{-1}$ Cu was added to site waters, water chemistry reduced bioavailability in 6 of 12 cases to below the $\{Cu^{2+}\}$ expected to cause 50% mortality ($\{Cu^{2+}\}_{LC50}$) in larval fish. However, after 6 d of photooxidation, none of the site waters remained below $\{Cu^{2+}\}_{LC50}$.

Whether a given copper (Cu) concentration is toxic to aquatic biota depends mainly on the activity of the free cupric ion ($\{Cu^{2+}\}$) (MacRae et al. 1999; Luider et al. 2004). In natural waters, dissolved organic matter (DOM) is key to determining $\{Cu^{2+}\}$ because it complexes Cu and diminishes its ionic concentration. In recognition of this important relationship, dissolved organic carbon concentration (DOC), a component that typically comprises half of DOM) has been incorporated into regulatory criteria for Cu (U.S. Environmental Protection Agency 2003; 2003 draft update of ambient water quality criteria for copper. EPA 822-R-03-026, available from: <http://www.epa.gov/waterscience/criteria/copper>). However, those water-quality algorithms assume that all DOM has the same Cu

complexation characteristics despite the breadth of variation in DOM composition between sources. Compositional differences in DOM can occur because allochthonous DOM is derived from the decomposition of terrestrial plants, which contain high proportions of lignin. In contrast, autochthonous DOM has a lower C:N ratio, higher lipid content, and lower aromaticity because of considerable contributions from algae and bacteria that lack lignin (McKnight and Aiken 1998). This source-dependent heterogeneity has the potential to contribute to variations in the binding-site densities and conditional stability constants of metal-DOM complexes (i.e., binding affinities) as reported in several studies (McKnight et al. 1983; Breault et al. 1996; Brooks et al. in press). Moreover, DOM is photochemically unstable and sunlight alters its Cu complexation—a factor that further complicates our understanding of the bioavailability of metals in ecosystems.

In natural organic matter (Moffett and Zika 1987; Gao and Zepp 1998) and commercial organics such as nitrilotriacetic and amino acids (Langford et al. 1973; Moffett et al. 1990; Hayase and Zepp 1991), irradiation prompts ligand-to-metal charge transfer that releases metals in their reduced forms. Moreover, Cu may be released from irreversible photolytic cleavage of the acidic substituents

¹ Present address: National Center for Ecological Analysis and Synthesis, 735 State Street, Suite 300, Santa Barbara, California 93101.

Acknowledgments

We thank Marcella Appel, Walter Johnston, Donna Kashien, Katherine Mitchell, and Richard Thorp for field assistance. This work was funded by a U. S. Environmental Protection Agency STAR research grant R829640. We thank two anonymous reviewers for helpful comments that greatly improved this manuscript.

that bind metals (Langford et al. 1973; Moffett et al. 1990; Gao and Zepp 1998). Field studies under ambient insolation show that this transformation of DOM can diminish binding-site density for Cu significantly in seawater (Moffett et al. 1990) and in estuarine waters (Shank et al. 2006). Photooxidative loss of binding sites likely explains observed increases in $\{Cu^{2+}\}$ in the euphotic zone of seawater, particularly during periods of stratification (Moffett 1995). Controlled laboratory experiments also demonstrate that binding-site concentrations on the strong Cu ligands in photooxidized DOM decrease significantly by 20% (Sander et al. 2005) to 90% (Shank et al. 2006). Effects of photooxidation of DOM on Cu complexation can vary tremendously between sources. For example, before irradiation, DOM from both a river and a wetland bound equal amounts of Cu (Brooks et al. in press). After photooxidation, binding affinities became virtually identical; however, wetland DOM bound 150% more Cu than riverine DOM per unit DOC because irradiation doubled binding-site densities in wetland DOM, but halved them in riverine DOM.

Sunlight has other profound effects on DOM. Photo-mineralization directly removes as much as 50% of DOC in less than 11 d of natural or equivalent artificial insolation (Molot and Dillon 1997; Xie et al. 2004). Approximately ~15% more DOC is indirectly removed by cleavage of DOM macromolecules into low-molecular-weight organic acids that are then respired by bacteria (Mopper et al. 1991; Miller et al. 2002). Residual DOM is photochemically altered as indicated by photobleaching—the decrease in the absorption coefficient ($a_{\text{wavelength}}$, $2.303 \times A_{\text{wavelength}}$ pathlength⁻¹, reported in m⁻¹, where A is absorbance). Photobleaching decreases a_{350} (a proxy for the photoreactive organic pool) by 15% to 30% in the residual DOM during exposures equivalent to 30 h of sunlight (Moran et al. 2000, Xie et al. 2004). Evidence is emerging that DOM from pure algal cultures photoreacts quite differently than mixtures of autochthonous and allochthonous DOM. Such mixtures produce low-molecular-weight organic acids (Miller et al. 2002), but pure cultures of algal-derived DOM do not (Tranvik and Kokalj 1998; Obernosterer et al. 2001), nor are they photomineralized (Obernosterer and Benner 2004).

Spectroscopic methods have long been used to gain biogeochemical information about DOM. For example, low “specific absorbance coefficients” at 340 or 350 nm (so-called SAC; $2.303 \times A_{350} \div \text{DOC}$, mg cm⁻³ with pathlength in cm, reported as cm² mg⁻¹) in natural waters were associated with high metal bioaccumulation on the gill (Luider et al. 2004) or toxicity of metals to juvenile rainbow trout (*Oncorhynchus mykiss*) (Richards et al. 2001). Apparently, DOM with lower aromaticity from autochthonous sources bound less metal per unit DOC. In this study, we tracked changes in the fluorescence index (excitation 370 nm, emission 450 : 500 nm) and the specific ultraviolet (UV) absorption ($\text{SUV}\alpha = 2.303 \times A_{254\text{nm}}$ pathlength⁻¹ [DOC]⁻¹ and reported as m⁻¹ L mg⁻¹) to determine if they were predictive of changes in Cu-DOM binding properties. The fluorescence index is a measure of the chemical characteristics of quinone moieties in the

humic fraction that can indicate allochthonous versus autochthonous sources of DOM (McKnight et al. 2001). $\text{SUV}\alpha$ provides information about bulk chemical characteristics of DOM and strongly correlates with DOM aromaticity (Weishaar et al. 2003). Because the atmosphere blocks wavelengths shorter than 280 nm, compounds that absorb electromagnetic radiation below that wavelength are indirectly affected by photochemical processes, probably through production of reactive oxygen species such as the superoxide and hydroxyl radical (Voelker et al. 2000).

We hypothesized that: (1) photooxidation of DOM decreases its Cu complexation; (2) photooxidation has greater affect on DOM collected in spring than summer because snow and ice cover on the streams and the turbidity and greater stream depths during snowmelt protect DOM from insolation; and (3) spectral qualities are good predictors of Cu-DOM complexation because they covary with the extent of photooxidation of the components responsible for Cu complexation.

Materials and methods

DOM collection—We collected natural DOM from six sites during peak flow in spring (24 May to 24 June 2003) and base flow in summer (16 to 20 August 2003). Sites encompassed a range of altitudes, DOC concentrations, and metals contamination in the mineralized belt of the Rocky Mountains in Colorado. DOM was collected from streams with low trace metal concentrations: Homestake Creek (Home; 39°29'N, 106°22'W, 2,463 m), Deer Creek (Deer; 39°56'N, 105°86'W, 3,250 m), East Fork of the Arkansas River (EFAR; 39°27'N, 106°22'W, 3,021 m), and a braided reach of the East Fork of the Arkansas River (EFAB; 39°27'N, 106°31'W, 3,021 m). In addition, we collected DOM from rivers containing moderate and high transition metals concentrations, the Eagle River (Eagle; 39°56'N, 106°40'W, 2,463 m) and the Snake River (Snake; 39°58'N, 105°87'W, 3,165 m), respectively. To calculate the transit period for each stream, we calculated average channel volume from directly measured channel width and depth at all field sites and obtained discharge rates for Eagle, EFAR, and Snake River from nearby water monitoring stations (U.S. Geological Survey 2003; National Water Information System: <http://waterdata.usgs.gov/co/nwis/inventory>). These transit rates were used to estimate rates for nearby sites (Home, EFAB, and Deer). In situ tracer studies are the optimal means of determining downstream flow, which we did not perform. Thus, downstream transit rates are approximations only meant to illustrate relative differences.

After filtration through prerinsed 10- μm and 5- μm Hytrec II (Osmonics), 1- μm (Corning Costar), and 0.2- μm filters (Filterite, US Filtration & Separations), natural waters were passed through a H⁺ cation-exchange resin that removed $\geq 95\%$ of all metals (Ca²⁺, Mg²⁺, Na⁺, K⁺, Cd, Cu, Fe, Ni, Pb, and Zn). Processing for approximately 8 h through a portable reverse osmosis system (PROS/2S, RealSoft) increased initial DOC concentrations approximately 40-fold. Because of existing low pH levels in Snake River, we added NaHCO₃ as necessary to DOM concen-

trates to maintain circumneutral pH. Previous comparisons of natural organic matter from raw filtered water, sorption onto hydrophobic resin, and reverse osmosis show that reverse osmosis does not alter DOM structure or elemental composition (Maurice et al. 2002). Concentrates were stored in the dark at 4°C in acid-washed, ashed (500°C for 2 h) borosilicate bottles at circumneutral pH. Storage pH is extremely important because natural organics rapidly undergo spontaneous hydrolysis if stored at pH near or below 2. Monitoring of storage effects over the span of several months indicated no change in DOC concentration or the rate of DOC loss during irradiation.

Geochemical analyses—Unless otherwise designated, all reagents were analytical grade except HCl (trace-metal grade; Spectrum) and HNO₃ (OPTIMA, Seastar Chemicals). The higher-grade acids were used for pH adjustment during titrations and geochemical analyses. All glassware and labware were soaked in 18% analytical grade HNO₃ (v/v) for at least 2 h and rinsed five times before use with 18-MO Ω m water (Milli-Q, Millipore Corporation). The 18-MO Ω m water was used for analytical dilutions and analytical blanks.

We analyzed filtered field site waters (Table 1) and DOM solutions used in titrations for DOC, major anions, cations, transition metals, pH, alkalinity, and hardness. We also measured fluorescence and the absorption coefficient of UV radiation in DOM solutions. We analyzed DOC by catalytic combustion (Shimadzu, model 5000A). Anions (F⁻, Cl⁻, NO₃⁻, PO₄³⁻, SO₄²⁻) were analyzed by ion chromatography (Dionex, model DX-100). Cations (Ca²⁺, Mg²⁺, Na⁺, K⁺), Ni, and Zn were analyzed by flame atomic absorption spectrophotometry (Perkin-Elmer, Perkin-Elmer model 372). We determined transition metal concentrations aside from Ni and Zn by graphite furnace atomic absorption spectrophotometry with Zeeman background correction (Varian, model SpectrAA-600). pH was measured with a Corning model 340 meter and a Corning flat-surface combination pH electrode. We determined alkalinity of water samples by titration to pH 4.5 with H₂SO₄. We analyzed the controls and irradiated DOM for changes in fluorescence using a FluoroMax-2 Spectrofluorometer (Horiba Jobin Yvon). We measured the absorbances for SUV α and a_{350} with a diode array spectrophotometer (Hewlett-Packard 8452A).

Irradiation of DOM—We conducted photodegradation experiments at intensities simulating natural sunlight. We normalized photoreactivity by diluting DOM concentrates with 18 MO Ω m water to a standard absorbance of 0.33 at 350 nm in a 1-cm cell. We irradiated triplicate samples of absorbance-standardized DOM solutions in tightly capped, quartz Hungate tubes (14.6 \times 1.9 cm) placed in a solar simulator (Atlas Suntest CPS+) under a full-spectrum 1.0-kW xenon lamp for 24 h in a continuous-circulation cooling bath (25.4°C \pm 1.3°C, mean \pm SD). This exposure duration lowers oxygen by about 30%, but does not deplete it (Xie et al. 2004). Radiation intensities, measured with an International Light radiometer (model 1400), were compared to noontime cloudless conditions recorded at the

field sites on 21 June 2003. Intensities within the solar simulator were 1.4 \times for UV-B (detector: 265–332 nm), 1.8 \times for UV-A (subtraction of UV-B from total UV detector: 250–400 nm), and equivalent to photosynthetically active radiation (detector: 400–1000 nm). In one day, surface water theoretically receives the equivalent of 7.6 h of noon sun (=12 h \times 2/ π ; Miller and Zepp 1995) at solar equinox (i.e., 21 September or 31 March). Therefore, we estimated conservatively that 24 h of irradiation in the solar simulator equaled \sim 4.4 d ($[24 \times 1.4] \div 7.6$) of UV-B at the field sites. The cuprous ion (Cu⁺) is reoxidized to Cu²⁺ rapidly in \sim 100 μ s following photooxidation (Voelker et al. 2000). Nonetheless, we performed Cu titrations more than 2 and less than 24 h after irradiation of DOM to allow completion of any indirect photochemical changes associated with longer-lived reactive oxygen species.

Cupric ion measures and data analysis—We quantified changes in Cu-DOM complexation by analyzing results from potentiometric titrations of Cu(NO₃)₂ into two to three separately irradiated solutions of natural DOM for controls and each exposure period. We chose potentiometric titration as our analytical technique because of its demonstrated reliability in freshwater analyses of Cu-organic complexation across the range of total Cu concentrations of interest to us (Christl et al. 2005). Titrations were conducted in a 200-mL double-wall reaction vessel with light shielding and constant-temperature circulating water bath (25°C \pm 0.8°C, mean \pm SD) with a stirrer, automated microburette, mV meter, and MTS Software (Smart Burette System, software version 2.1, McIntosh Analytical Systems). We recorded {Cu²⁺} with an ion-selective electrode (Cu-ISE; Orion Research, model 9429) and a double-junction Ag/AgCl reference electrode (Orion Research, model 09-02) in DOC solutions standardized to 5 mg L⁻¹ with an ionic strength of 0.01 mol L⁻¹ (as NaNO₃) at pH 5.985 \pm 0.025. Because DOC concentrations decreased during the 24 h of photodegradation, increased percentages of photodegraded DOM maintained the 5 mg L⁻¹ DOC. We sparged DOM solutions with ultrahigh-purity nitrogen for 30 min before and throughout titrations. Sparging with nitrogen eliminates any potential Cu complexation with carbonates and creates an anoxic environment that minimizes possible oxidation of DOM by atmospheric oxygen. We calibrated the Cu-ISE by titrating ethylenediamine (EN) into 5 \times 10⁻⁵ mol L⁻¹ Cu(NO₃)₂ over a pCu²⁺ concentration range of 4.3 to 14 (pCu²⁺ = $-\log_{10}$ Cu²⁺), then calculated pH-specific {Cu²⁺} at each EN concentration with the geochemical speciation program MINTQA2 (U. S. Environmental Protection Agency 2006. <http://www.epa.gov/ceampubl/mmedia/minteq/index.htm>). We used the Davies equation to account for differences in ionic strength between the calibration solution (0.1 mol L⁻¹ with NaNO₃) and DOM solutions (Benedetti et al. 1995). Stability constants for complexes were log K = 10.5 for Cu²⁺ + EN \leftrightarrow Cu(EN) and log K = 19.6 for Cu²⁺ + 2EN \leftrightarrow Cu(EN)₂. The Nernstian response of the calibration for Cu(EN) was 28.17 mV/pCu. This was slightly less than the theoretical slope of 29.5 mV pCu⁻¹, but it was consistent

Table 1. Composition of field site waters and DOM standardized to 5 mg L⁻¹ DOC. Coefficients of variation for three analytical aliquots were <10% in most instances. Units are $\mu\text{mol L}^{-1}$ unless otherwise specified. Detection limits in $\mu\text{mol L}^{-1}$ for these analytes were: Cd (0.001), Cu (0.005), F⁻ (0.74), Fe (0.02), Mn (0.002), Ni (0.54), NO₃⁻ (0.05), Pb (0.002), PO₄³⁻ (2.48), and Zn (0.062). Alk = alkalinity. Below detection limits = BDL. We do not report individual concentrations in site waters of Ca²⁺ (mean \pm SD from all sites combined, 383 \pm 215), Mg²⁺ (199 \pm 128), and K⁺ (17 \pm 4), or DOM for Ca²⁺ (208 \pm 154), Mg²⁺ (142 \pm 125), and K⁺ (17 \pm 20), which we excluded from MINTEQA2 calculations for all DOM solutions.

Water	Site/Season	DOC (mg L ⁻¹)	pH	Alk (meq)	Na ⁺	F ⁻	Cl ⁻	NO ₃ ⁻	SO ₄ ²⁻	Cd	Cu	Fe	Mn	
Site waters	Home/Spring	6.9	6.00	0.20	60	BDL	4.8	BDL	21	BDL	BDL	3.5	0.4	
	Summer	2.8	6.42	0.36	65	BDL	15.6	12.8	28	0.001	0.010	19.0	BDL	
	Eagle/Spring	2.9	8.28	1.20	52	BDL	13.0	4.5	74	BDL	BDL	BDL	0.5	
	Summer	2.0	7.61	1.20	77	BDL	22.0	7.3	98	0.001	0.030	8.4	BDL	
	EFAB/Spring	1.4	8.16	1.04	71	BDL	30.3	7.8	148	0.001	0.012	BDL	0.9	
	Summer	1.1	7.36	1.48	148	BDL	62.8	10.4	358	0.003	0.008	BDL	BDL	
	EFAR/Spring	1.8	8.12	1.00	55	BDL	36.0	7.2	101	0.001	BDL	BDL	0.1	
	Summer	0.9	7.41	1.52	163	BDL	49.5	11.0	379	BDL	0.009	BDL	BDL	
	Deer/Spring	1.8	7.18	0.32	36	BDL	6.2	8.8	8	BDL	BDL	BDL	BDL	
	Summer	1.2	6.95	0.52	69	BDL	12.6	12.0	120	BDL	0.031	BDL	BDL	
	Snake/Spring	0.6	6.17	0.16	60	BDL	9.01	7.5	335	0.007	BDL	1.8	4.5	
	Summer	0.9	4.94	0.02	97	BDL	20.0	11.5	644	0.012	0.192	4.3	BDL	
	Titration DOM	Home/Spring	5.0	5.99	0.31	28	4.6	6.9	BDL	14	BDL	BDL	2.5	BDL
		Summer	5.0	5.97	0.30	28	0.4	7.4	2.5	21	BDL	BDL	1.8	BDL
		Eagle/Spring	4.9	5.98	0.30	33	2.4	15.0	1.1	86	BDL	BDL	0.9	BDL
		Summer	5.0	5.98	0.31	214	2.2	1.8	0.3	380	BDL	BDL	11.3	BDL
EFAB/Spring		5.0	5.98	0.29	169	3.4	19.0	1.2	799	BDL	BDL	5.3	BDL	
Summer		5.1	5.99	0.30	1,074	BDL	3.3	0.7	1943	BDL	BDL	9.0	BDL	
EFAR/Spring		5.1	5.99	0.31	122	2.3	1.01	1.0	140	BDL	BDL	6.7	BDL	
Summer		5.0	5.98	0.30	1,275	BDL	3.9	2.2	2174	BDL	BDL	10.1	BDL	
Deer/Spring		4.9	5.991	0.30	57	1.0	0.21	0.4	222	BDL	BDL	4.0	BDL	
Summer		5.0	5.98	0.31	886	BDL	7.7	1.6	471	BDL	0.012	8.2	BDL	
Snake/Spring		5.0	5.98	0.31	2,326	9.5	1.1	0.5	1732	0.05	BDL	4.9	BDL	
Summer		4.9	5.98	0.30	15,187	BDL	30.2	0.5	2724	0.07	0.04	7.8	BDL	

and reproducible. Daily electrode function was assessed by titration of Cu(NO₃)₂ into ionic strength-adjusted 18-MOHm water (0.01 mol L⁻¹ NaNO₃) without EN buffer over a range of total pCu from 5 to 7.4. This produced a linear slope of 30.8 \pm 0.44 mV/pCu_T (mean \pm SD, n = 22), which was also consistent and reproducible. The detection limit for ISEs is the concentration at which the log-linear relationship of Cu with the electromotive force becomes nonlinear as indicated by deviation greater than 1 standard error. Because the slope of the electrode function remained linear in both calibration and sample titrations, our instrument detection limit was a concentration <pCu 14, and the method detection limit in our test matrix was <pCu 7.4. Depending upon the solution, each injection was allowed to equilibrate for 2 to 30 min until the rate of change was <0.2 mV min⁻¹.

We based Cu-DOM complexation on two classes of ligands using a nonlinear least-squares fit in the geochemical optimization program FITEQL (version 4.0; Herbelin and Westall 1999). The high-affinity ligand had a high conditional stability constant (K_1) and low binding-site concentration ($[L_1]$). The weaker ligand with low affinity (K_2) had 10 \times greater binding-site density ($[L_2]$) than $[L_1]$. Because Cu can be toxic to aquatic organisms at low concentrations, the high-affinity-low-concentration binding sites can substantially mitigate metal toxicity. Goodness of fit was evaluated by the weighted sum of squares divided by the degrees of

freedom (WSOS df⁻¹). Our WSOS df⁻¹ values ranged from 3 \times 10⁻⁴ to 0.02, consistent with those of FITEQL examples given by Herbelin and Westall (1999), which range from 3 \times 10⁻⁸ to 8.7. Moreover, we evaluated the fit of our model by comparing observed and predicted (calculated by MINTEQA2) {Cu²⁺} for exposure times 0 and 24 h. For those calculations, we input conditional stability constants and ligand densities of Cu-DOM complexes and most water chemistry (Table 1). However, we excluded cation competition by Ca²⁺, Mg²⁺, and K⁺ from calculations because humic substances have a preferential affinity for Cu versus alkali and alkaline earth metals (Hering and Morel 1988), which greatly minimizes competition with Cu²⁺ for binding to humic substances (Tipping 2002). Our calculated {Cu²⁺} was within 0.5% to 33% (mean \pm SD; 18 \pm 9.0) of observed {Cu²⁺} in DOM titration solutions (Fig. 1). In addition, we performed MINTEQA2 calculations to determine {Cu²⁺} for all site waters with direct measures of DOM binding parameters and site water chemistries. Conditional stability constants in calculations were adjusted by one log₁₀ unit as appropriate per each unit deviation from pH 6 (Cabaniss and Shuman 1986; Lu and Allen 2002).

Site-specific rates of photochemical release of {Cu²⁺}—We averaged the photooxidative release rate of {Cu²⁺} across the range of titrations for DOC-standardized DOM. Based on the demonstrated relation between

photoreactivity and light absorption, we then adjusted the photooxidative release rate of $\{\text{Cu}^{2+}\}$ by the exposure-averaged specific absorption coefficient ($a_{350\text{-Exp}} \div \text{DOC-Exp} = a_{350\text{-Exp}}^*$) of the absorbance-standardized DOM used in irradiation experiments. We determined the exposure-averaged specific absorption coefficient from changes in exposure-averaged absorption ($a_{350\text{-Exp}}$) and DOC concentrations (DOC_{Exp}) after 24 h of DOM irradiation. We adjusted the $\{\text{Cu}^{2+}\}$ release rate of DOM for the lesser intensity of ambient sunlight versus the solar simulator ($\times 0.714$). We applied this rate to field-site waters by multiplying the $\{\text{Cu}^{2+}\}$ release rate by the a_{350} of site waters ($a_{350\text{-SW}}$), then dividing this rate per unit site-water DOC in $\mu\text{mol L}^{-1}$. Finally, we converted the rate per hour to per day (7.6 h in solar simulator d^{-1} ; Miller and Zepp 1995). This model applies only to surface waters; however, stream depths are shallow, 0.39 ± 0.15 m (mean \pm SD).

In addition to calculating and reporting observed $\{\text{Cu}^{2+}\}$ as described above, we performed MINTEQA2 calculations of $\{\text{Cu}^{2+}\}$ in site waters during a hypothetical contamination event in which $1.5 \mu\text{mol L}^{-1}$ Cu was introduced at field sites. From that initial $\{\text{Cu}^{2+}\}$ on site and the release rate of $\{\text{Cu}^{2+}\}$ in site waters, we followed the plume downstream to investigate how $\{\text{Cu}^{2+}\}$ would be released in downstream waters after photooxidation for 1, 3, and 6 d.

Statistical analyses—To evaluate photochemical changes in Cu-DOM complexation, statistical analyses were performed using SPSS (Ver. 11.5). One-way and factorial analyses of variance (ANOVA) (three-way) were used to test for effects of irradiation, source, and season of DOM collection on various parameters of Cu-DOM complexation. Conditional stability constants were \log_{10} transformed and binding-site densities were squared to satisfy the assumptions of ANOVA. *t*-tests were used to evaluate miscellaneous differences as noted in the text. Regression analysis was used to evaluate how well $\text{SUV}\alpha$, the fluorescence index, and DOC concentrations predicted variation in slope and various parameters of Cu-DOM complexation. Alpha was 0.05 for all significance tests.

Results

Geochemistry of field site waters—Before processing by cation exchange and reverse osmosis, ambient water composition varied tremendously among sampling sites (Table 1). In general, pH was lower in summer than in spring, but remained circumneutral with the exception of Snake, which dropped to pH 4.9 in August. DOC concentrations in site waters ranged from 6.9 mg L^{-1} in Home to 0.6 mg L^{-1} in Snake and were typically highest in the spring when overland snowmelt flushed organics into the waterways. Conversely, cation and anion concentrations were lowest in the spring, consistent with dilution by high runoff discharge. Concentrations of these dissolved ions varied by three orders of magnitude among sites and seasons. Most sites had low (near or below detection limit) transition metal concentrations with the exception of Home and Snake, which had moderate ($<0.01 \mu\text{mol L}^{-1}$) to high

($>0.01 \mu\text{mol L}^{-1}$) metal concentrations depending on season. Except for Mn, metal concentrations were generally higher during base flow in summer than during spring runoff.

Geochemistry of titration solutions and irradiation experiments—The geochemistry of DOM solutions used in titration treatments was relatively similar in composition among sources after processing (Table 1). Cation exchange removed $\geq 95\%$ of transition metals, most of which remained below detection limits with the exception of $\mu\text{mol L}^{-1}$ concentrations of Fe in all titration solutions and lower (nmol L^{-1}) concentrations of Cd or Cu in DOM collected in summer from Snake and Deer. DOM titration solutions contained $\mu\text{mol L}^{-1}$ concentrations of the major cations and anions with the exception of Na^+ and SO_4^{2-} in summer Snake DOM. Na^+ concentration increased because of the addition of NaHCO_3 to buffer H^+ increases during processing by reverse osmosis. Acid mine drainage that lowered ambient pH to 4.94 also increased SO_4^{2-} concentrations. The geochemistry of absorbance-standardized DOM solutions can easily be calculated from those presented in Table 1 for DOC-standardized DOM solutions used in titrations by multiplying those concentrations by the ratio of DOC in absorbance-standardized DOM (Table 2) to 5 mg of DOC.

Photochemical and seasonal effects on Cu complexation—In $\sim 85\%$ of cases, irradiation of DOM decreased Cu-DOM complexation, causing more than twofold increases in $\{\text{Cu}^{2+}\}$ in potentiometric titrations (Table 3, Fig. 1). Across the range of titrations (7.8×10^{-8} to $8.7 \times 10^{-6} \text{ mol Cu L}^{-1}$), photooxidation of DOM decreased Cu complexation in 10 DOM types, increasing their $\{\text{Cu}^{2+}\}$ by an average of $156\% \pm 28\%$ (mean of percentage difference between each titration point \pm SE; Table 3). In contrast, $\{\text{Cu}^{2+}\}$ in two DOM types collected in summer were unchanged (Deer, $6\% \pm 12\%$) or decreased by $30\% \pm 4\%$ (Snake). Changes in $\{\text{Cu}^{2+}\}$ due to photooxidation of summer DOM from Deer and Snake were significantly different from other DOM types (*t*-test = 5.31, $p = 0.001$).

Photooxidative changes in DOM increased $\{\text{Cu}^{2+}\}$ in spring by an average of $185\% \pm 25\%$ (mean \pm SE), whereas DOM collected in summer increased $\{\text{Cu}^{2+}\}$ by $74\% \pm 14\%$ ($117\% \pm 18\%$ if Deer and Snake DOM were excluded) (Fig. 1, Table 3). In addition to comparing photochemical changes across the entire range of Cu-ISE titrations, we investigated differences at total Cu concentrations of $0.79 \mu\text{mol L}^{-1}$, which bracketed the $\{\text{Cu}^{2+}\}$ of $0.035 \mu\text{mol L}^{-1}$ under these conditions. This $\{\text{Cu}^{2+}\}$ value corresponded with 50% mortality ($\{\text{Cu}^{2+}\}_{\text{LC50}}$) in larval fathead minnows (*Pimephales promelas*) (Erickson et al. 1996). Table 3 shows that in all but one instance (Deer summer DOM), irradiation increased $\{\text{Cu}^{2+}\}$ from below this toxic level to above it.

Regarding individual parameters of Cu-DOM complexation, binding-site densities increased in 8 ($[\text{L}_1]$) and 6 ($[\text{L}_2]$) of the 12 DOM solutions (Fig. 2) after treatment. Overall, photooxidation decreased DOM binding affinity for Cu, significantly lowering K_1 by $68\% \pm 15\%$ (mean \pm SE) and

Table 2. Calculation of photooxidative rate of $\{Cu^{2+}\}$ release in DOM averaged across the range of titrations and adjusted to each individual field-site water in Fig. 1. The $\{Cu^{2+}\}$ release rate of DOM was adjusted by the specific absorption coefficient from averages of photobleaching and photomineralization of DOM during irradiation exposures, $a_{350-Exp}^*$ (from the exposure-averaged $a_{350-Exp}$ and DOC_{Exp}), then corrected for the greater intensity of the solar simulator ($\times 0.714$). To apply this rate to site waters, we multiplied the DOM $\{Cu^{2+}\}$ release rate ($rate_{DOM}$) by each site-water absorption (a_{350-sw}), and divided by site-water DOC concentrations in $\mu mol L^{-1}$ to adjust rates to field conditions. We then converted the rate per hour to rate per day (7.6 h in solar simulator d^{-1}). Observed $\{Cu^{2+}\}$ in site waters were determined with MINTEQA2 using the direct measures of individual stream geochemistry in Table 1 and Cu-DOM complexation parameters in Fig. 2. Downstream flow rates are approximations meant to illustrate relative differences.

	Home		Eagle		EFAB		EFAR		Deer		Snake	
	Spring	Summer	Spring	Summer	Spring	Summer	Spring	Summer	Spring	Summer	Spring	Summer
DOM: Avg. $\{Cu^{2+}\}$ release rate across titration ($\mu mol \{Cu^{2+}\} mg C^{-1} h^{-1}$)	0.008	0.034	0.035	0.018	0.031	0.030	0.025	0.010	0.028	-0.004	0.027	-0.022
DOM: Time 0, $a_{350-Exp}$ (m^{-1})	67.4	64.88	60.78	61.7	62.43	63.9	65.28	60.58	64.35	63.53	85.55	55.33
DOM: Exp-Avg. $a_{350-Exp}$ (m^{-1})	56.2	61.9	61.1	56.4	54.6	58.0	57.3	57.0	55.2	56.9	62.5	42.9
DOM: Time 0, [DOC] ($mg L^{-1}$)	32	25.5	29.3	47	33.9	43.3	34.3	40.7	30.6	50.4	72.1	54
DOM: Exp-Avg. DOC_{Exp} ($mg L^{-1}$)	23.9	30.3	28.3	44.6	32.3	41.6	33.7	41.8	28.7	43.9	59.7	50.5
DOM: Exp-Avg. $a_{350-Exp}^*$ ($L mg^{-1} m^{-1}$)	2.3	2.0	2.2	1.3	1.7	1.4	1.7	1.4	1.9	1.3	1.0	0.8
DOM: Rate _{DOM} by $a_{350-Exp}^*$ ($\mu mol L^{-1} h^{-1} m$)	0.003	0.017	0.016	0.014	0.018	0.021	0.015	0.008	0.014	-0.003	0.026	-0.026
Solar simulator adjustment (0.714)	0.002	0.012	0.012	0.010	0.013	0.015	0.011	0.005	0.010	-0.002	0.019	-0.019
Site waters: a_{350-sw} (m^{-1})	1.47	7.45	5.22	5.31	2.70	1.71	1.55	4.55	3.36	1.05	4.38	3.56
Site waters: $a_{350-adjusted}$ rate, ($a_{350} \times DOM \{Cu^{2+}\}$ rate) ($\mu mol L^{-1} h^{-1}$)	0.003	0.088	0.060	0.053	0.035	0.026	0.016	0.024	0.035	-0.002	0.081	-0.066
Site water: DOC-adjusted rate (h^{-1}) ($\{Cu^{2+}\}$ release rate $\div DOC$)	3.1×10^{-5}	2.0×10^{-3}	1.3×10^{-3}	1.7×10^{-3}	1.2×10^{-3}	1.7×10^{-3}	7.3×10^{-3}	1.4×10^{-3}	1.2×10^{-3}	-1.1×10^{-3}	8.9×10^{-3}	-4.8×10^{-3}
$\{Cu^{2+}\}$ release rate per day (7.6 h d^{-1}) (d^{-1})	0.0002	0.0151	0.0100	0.0130	0.0095	0.0132	0.0055	0.0108	0.0094	-0.0008	0.0675	-0.0362
Obs. $\{Cu^{2+}\}$ ($\mu mol L^{-1}$) Note Cu_T (Table 1)	0	9.2×10^{-5}	0	4.1×10^{-5}	3.0×10^{-5}	1.6×10^{-5}	0	2.5×10^{-5}	0	2.6×10^{-4}	0	0.151
Downstream flow rate ($km d^{-1}$)	59	19	98	36	16	5	52	17	20	12	52	17

Table 3. Photochemical changes in Cu complexation by DOM collected from six sources during spring peak flow (Sp) and summer base flow (Sum) after DOM irradiation for 24 h. We present average changes in cupric ion activity (mean Δ {Cu²⁺}). Values are the mean of percentage difference between each titration point \pm SE across the range of total Cu titrated into 5 mg L⁻¹ DOC (shown in Fig. 1). In addition, we report the absolute changes in the titration point bracketing 0.035 μ mol L⁻¹ {Cu²⁺}, the activity that causes 50% mortality (LC50) in larval fathead minnows (Erickson et al. 1996; {Cu²⁺} at LC50). In DOM from Snake (summer), irradiation increased Cu complexation denoted by negative {Cu²⁺}. * = $p \leq 0.05$ in t -tests comparing control to irradiated treatments.

Time (h)	Homestake Creek		Eagle River		EFAB		EF5 River		Deer Creek		Snake River		Averages	
	Sp	Sum	Sp	Sum	Sp	Sum	Sp	Sum	Sp	Sum	Sp	Sum	Sp	Sum
Mean Δ {Cu ²⁺ } (%)	53 (17)	149 (25)	311 (62)	63 (7)	231 (56)	190 (25)	129 (13)	68 (16)	284 (55)	6 (12)	79 (6)	-30 (4)	185 (25)	74 (14)
{Cu ²⁺ } at LC50 (μ mol L ⁻¹)	0.025 (0.003)	0.019 (0.006)	0.011 (0.009)	0.033 (0.020)	0.018 (0.006)	0.018 (0.007)	0.022 (0.008)	0.021 (0.008)	0.009 (0.006)	0.035 (0.037)	0.047 (0.006)	0.095 (0.006)	0.022 (0.006)	0.037 (0.0003)
	0.045 (0.026)	0.048 (0.009)*	0.065 (0.003)	0.060 (0.031)	0.081 (0.031)	0.061 (0.008)	0.051 (0.009)	0.049 (0.001)	0.058 (0.053)	0.033 (0.017)	0.093 (0.001)*	0.065 (0.029)	0.054 (0.008)	0.053 (0.003)

K_2 by 21% \pm 25% (ANOVA, K_1 , $p < 0.001$; K_2 , $p = 0.04$). Despite increased K_2 , corresponding binding-site losses yielded a net loss of Cu complexation in Home (spring and summer) and Snake (spring) DOM. In contrast, photooxidation of Snake DOM collected in summer increased Cu complexation because of minimal effects on K_1 , K_2 , or [L₁], but near doubling of [L₂] (Fig. 2). For DOM collected from Deer (summer), increased [L₁] and [L₂] compensated for decreased K_1 and K_2 , causing an overall increase in Cu complexation. Individual parameters of Cu-DOM complexation did not differ seasonally (ANOVA, $p \geq 0.20$). In addition, irradiation did not alter Cu complexation between seasons of collection (interaction photooxidation \times season, ANOVA, $p \geq 0.14$).

Photochemical and seasonal effects on DOM composition—Although irradiation treatments photomineralized, photobleached, and decreased the fluorescence index in all DOM solutions, the magnitude of results varied (Fig. 3). Comparing irradiated to unirradiated DOM solutions, DOC losses ranged from 10% to 20% (13% \pm 1%, mean \pm SE) and were highly significant ($p < 0.001$). SUV α decreased by 21% \pm 2% ($p < 0.001$) and the fluorescence index decreased by 12% \pm 1% (ANOVA, $p = 0.01$). Most likely, as indicated by low fluorescence index values and high SUV α values, sources of DOM collected in both spring and summer originated from terrestrial sources with relatively little algal input.

Predicting Cu-DOM complexation— K_1 and K_2 were positively related to SUV α , with no significant difference between irradiated and unirradiated treatments (t -tests of slopes, $p \geq 0.37$). Because SUV α did not consistently explain the variation in binding-site densities of both ligands, we do not consider it a general predictor of complexation capacity. We excluded three spring DOM treatments from regression analyses because they fell outside of 95% prediction intervals: unirradiated and irradiated Home DOM and irradiated Eagle DOM. Photochemical changes in SUV α were not uniform (Fig. 4B). The greatest changes occurred in DOM solutions with low initial SUV α values (i.e., less UV absorption per milligram of DOC), indicating greater relative losses of aromaticity. The fluorescence index explained only the binding affinity of the high-affinity ligand, K_1 . Thus, we do not consider it a general predictor of Cu-DOM complexation.

In stream waters, {Cu²⁺} varied because of total Cu concentration, Cu-inorganic complexation, Cu-DOM complexation, DOC concentration, conditional stability constants, and binding-site densities (Table 2). With the exception of the Snake River in summer, {Cu²⁺} in all site waters were below toxic levels. To explore how a contaminant event might affect Cu bioavailability as a plume moves downstream, we calculated the {Cu²⁺} for all site waters if we introduced 1.5 μ mol L⁻¹ total Cu (Fig. 5). Under this hypothetical situation, in 6 of 12 cases, on-site chemistry mitigated {Cu²⁺} to below {Cu²⁺}_{LC50} (shown as a dashed line in Fig. 5). After 1 d of photooxidative release, downstream waters mitigated {Cu²⁺} to below {Cu²⁺}_{LC50}

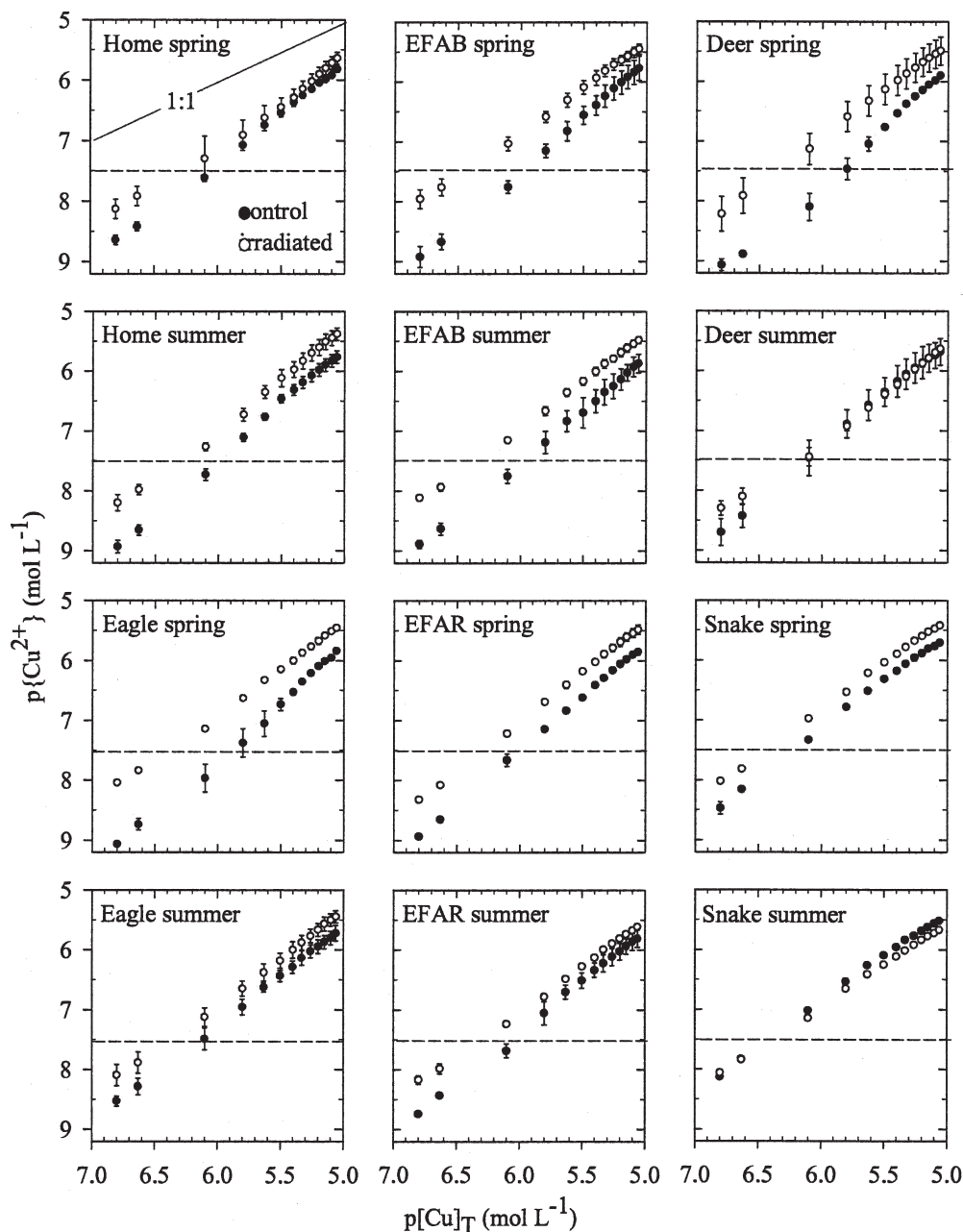


Fig. 1. Relation between negative logs of cupric ion activity ($p\{Cu^{2+}\}$) versus total Cu concentration ($p[Cu]_T$) during titration of Cu into control solutions and DOM solutions irradiated for 24 h (~ 4 d sunlight). Values are mean \pm SD at each titration point for separately irradiated analytical replicates of absorbance-standardized DOM collected in spring and summer. Dashed lines are the $\{Cu^{2+}\}_{LC50}$ reported by Erickson et al. (1996) to cause 50% mortality in larval fathead minnows. The 1:1 line in Home spring shows the theoretical relation between $p\{Cu^{2+}\}$ and $p[Cu]_T$ in the absence of DOM.

in 4 of 12 cases. After 6 d, all site waters exceeded $\{Cu^{2+}\}_{LC50}$. This model applies only to surface waters and does not take into account attenuation of UV radiation with depth.

Discussion

Photochemical effects on Cu-DOM complexation—Results from most of our experiments supported our hypothesis

that photodegradation of DOM generally decreases Cu complexation. In 10 of 12 of DOM types, irradiation increased $\{Cu^{2+}\}$ by an average of 156% across the range of Cu concentrations in potentiometric titrations (Fig. 1, Table 3). Such similarity is remarkable given that some aspects of site water geochemistry and a_{350nm} (a representative wavelength for photoreactivity) varied tremendously among the streams (Tables 1, 2). Moreover, stream chemistry shifted seasonally between the hydrologic extremes of high

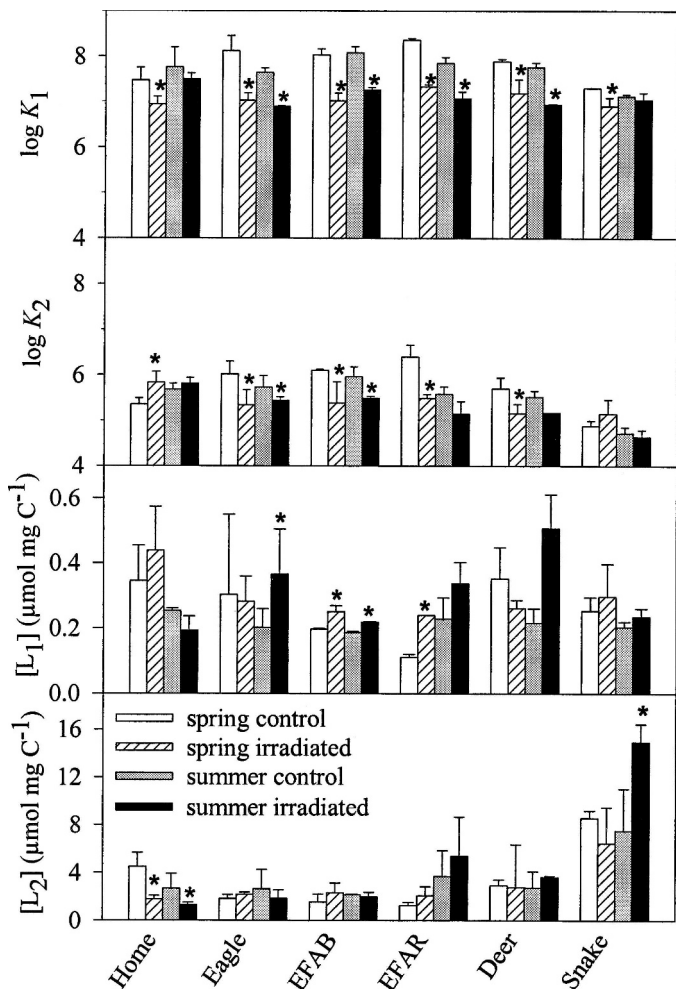


Fig. 2. Photochemical change in conditional stability constants ($\log K_1$ and $\log K_2$) and ligand concentrations ($[L_1]$ and $[L_2]$) for Cu-DOM complexes. Results for each field site are grouped by unirradiated and irradiated treatments of DOM collected in spring and summer. Error bars = SD. * = $p \leq 0.05$ in t -tests comparing control to irradiated treatments.

discharge when spring runoff directly imports terrestrial DOM versus base flow conditions that allow greater photochemical and microbial alteration of DOM (Waiser and Robarts 2004). In contrast to cases in which Cu-DOM complexation degraded, irradiation of two DOM types collected in summer either had no effect (Deer) or enhanced Cu complexation, decreasing $\{Cu^{2+}\}$ by 30% (Snake).

Photooxidation rates and prior irradiation of DOM undoubtedly produce the array of Cu-binding patterns observed in Cu-DOM complexation. Brooks et al. (in press) reported loss of binding-site densities with unchanged binding affinity in riverine DOM. We observed the same photochemical trend in Home summer DOM (Fig. 2). Similarly, Shank et al. (2006) reported this pattern for a strong Cu ligand in DOM from both the upper reach of Cape Fear estuary in North Carolina (salinity = 22) and more saline portion of the lower estuary ($S = 25$). In that study, they developed a one-ligand model with a fixed binding affinity, but acknowledged the presence of other

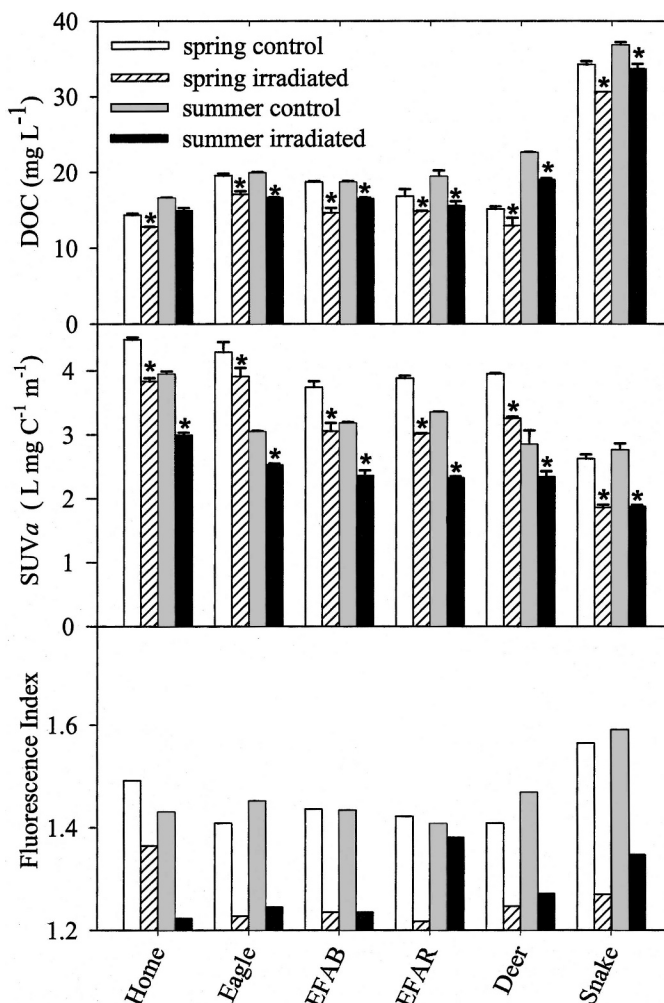


Fig. 3. Photochemical change in dissolved organic carbon (DOC) concentration, the specific UV absorption coefficient at 254 nm (SUV_{254}), and the fluorescence index. Results for each field site are grouped by unirradiated and irradiated treatments of DOM collected in spring and summer. Error bars = SD. * = $p \leq 0.05$ in t -tests comparing control to irradiated treatments.

ligands, which were above the analytical detection limit of voltammetry. During irradiation of DOM from freshwater Lake Hayes in New Zealand, the strong Cu ligand was also investigated with voltammetry. Irradiation lowered binding-site densities; however, that change was accompanied by an increase in binding affinity (Sander et al. 2005), suggesting that substituents associated with aromatics were retained while binding sites on aliphatic components were photodegraded. Just the opposite pattern was observed in a study with wetland DOM (increased $[L]$ and decreased $\log K$ values), suggesting that photodegradation can sometimes affect substituents associated with aromatics (Brooks et al. in press).

Compared to other freshwater studies fitting Cu-organic complexation to a two-ligand model, our $\log K$ values before and after irradiation (Fig. 2) are similar to those of humic substances, which range from 7.0 to 8.5 for K_1 , 5.4 to 6.6 for K_2 (McKnight et al. 1983; Breault et al. 1996).

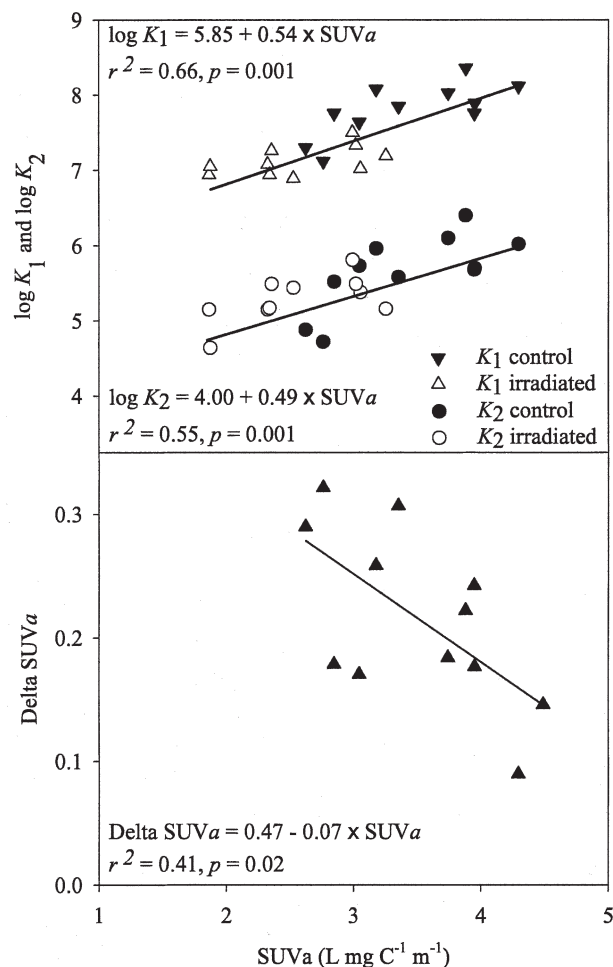


Fig. 4. (A) The specific UV absorption coefficient (SUV α) as a function of conditional stability constants ($\log K_1$ and $\log K_2$) in controls or irradiated treatments and (B) the relative change in SUV α (Δ SUV α) as a function of initial SUV α value. We excluded values for DOM collected in spring for irradiated and unirradiated Home DOM and irradiated Eagle DOM from the regression analysis because they fell outside of the 95% prediction interval.

Excluding the binding-site densities of Snake DOM that are obviously unusual, binding-site densities in our unfractionated DOM are greater than those reported for humic substances, which range from 0.12 to 0.38 $\mu\text{mol mg C}^{-1}$ for $[L_1]$ and 0.65 to 1.3 $\mu\text{mol mg C}^{-1}$ for $[L_2]$. Humic substances comprised of the hydrophobic fraction represent 40–60% of DOM (McKnight and Aiken 1998). Thus, it is logical that our binding-site densities are greater because they include hydrophilic fractions containing more nitrogenous and carboxyl groups than humic substances (Croué et al. 2003).

Seasonal variability in Cu-DOM complexation—As hypothesized, photochemical effects varied seasonally, decreasing Cu complexation to a greater extent in DOM collected in spring than in summer (Table 2). Unchanged or enhanced Cu complexation in Deer and Snake DOM had excessive influence on the seasonal variation; however,

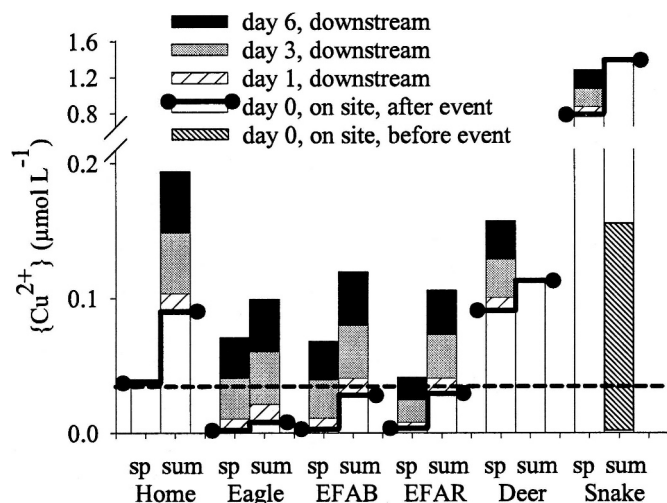


Fig. 5. Model of photochemical changes in $\{\text{Cu}^{2+}\}$ in surface waters of field sites during a hypothetical contamination event in which 1.5 $\mu\text{mol L}^{-1}$ Cu was introduced in spring (Sp) and summer (Sum) conditions. Before the contamination event, observed $\{\text{Cu}^{2+}\}$ values in streams were low, and aside from Snake Summer, do not appear at this scale (values in Table 2). Top of white bars show in-stream $\{\text{Cu}^{2+}\}$ immediately after contamination, denoted by angled barbells. Increased $\{\text{Cu}^{2+}\}$ values after 1, 3, and 6 d of transit downstream demonstrate progressive exceedance of the $\{\text{Cu}^{2+}\}$ reported by Erickson et al. (1996) to cause 50% mortality in larval fathead minnows (dashed line).

photooxidation of spring DOM remained greater even when these samples were removed from consideration. Recognizing that $\{\text{Cu}^{2+}\}$ is the virtual determinant of the bioavailability of Cu (MacRae et al. 1999; Luider et al. 2004), DOM collected in late summer may be more protective against Cu toxicity than DOM collected during spring runoff. On the other hand, slowed discharge and continual insolation exposure throughout summer may yield greater net changes in DOM.

Several processes may explain why DOM collected in summer was not as photoreactive. Because of lower stream discharge, shallower water, and longer retention times, the DOM in late summer was probably photooxidized to a greater extent than spring DOM, which had been shielded from UV radiation by winter snow, ice cover, and greater stream depths. In addition, increased growth of algae in late summer potentially altered DOM photoreactivity and Cu complexation. Autochthonous DOM from microbial communities of algae and associated bacteria has higher lipid content than allochthonous DOM (McKnight and Aiken 1998). Photooxidation and polymerization of polyunsaturated lipids in DOM can produce ester, ether, and aldehyde oxyfunctional groups (Harvey et al. 1983; Kieber et al. 1997)—electronegative moieties that complex Cu (Leenheer et al. 1998). Irradiation can enhance Cu complexation by inserting carbonyl binding sites in natural DOM (Brooks et al. in press). Except for Snake, SUV α values at all other sites were lower in summer than in spring DOM (Fig. 3), indicating prior irradiation or some algal input (Weishaar et al. 2003). If autochthonous input in

streams during late summer contributed some lipids, then it makes sense that insolation of algal-derived DOM produced Cu ligands and improved Cu complexation.

Photochemical patterns of Cu-DOM complexation were unique in DOM collected from the Snake River in summer. Cu complexation did not follow lower $SUV\alpha$ patterns. Periphyton growth in the Snake River is greatly limited by the high metal concentrations and deposition of metal oxides (McKnight et al. 1992). Thus photooxidation of algal-derived DOM is an unlikely explanation for increased binding sites during summer. Furthermore, $SUV\alpha$ in summer DOM was slightly higher than in spring (Fig. 3), suggesting less algal input in late summer. The presence of metals increases the rate and magnitude of photooxidative processes (Hayase and Zepp 1991; Gao and Zepp 1998). It is possible that Cd ($0.70 \mu\text{mol L}^{-1}$), Cu ($0.40 \mu\text{mol L}^{-1}$), and Fe oxyhydroxides ($49 \mu\text{mol L}^{-1}$) formed metal-organic complexes in the absorbance-standardized DOM solutions prepared for irradiation. The presence of metal-organics undoubtedly influenced photooxidative behavior by enhancing the production of reactive oxygen species (Voelker et al. 2000). However, the most likely reaction between DOM and reactive oxygen species is degradation of Cu complexation ability. Thus, we do not ascribe increased Cu complexation to the presence of metals alone. Possibly, metals in conjunction with unique DOM composition are responsible. The Snake River is heavily affected by acid mine drainage. As its pH approaches circumneutral levels, organic solutes sorb onto metal oxides and precipitate out of solution. This process preferentially removes more aromatic fulvic acid molecules that contain moieties that readily bind transition metals such as carboxylic acids and amino acids (McKnight et al. 1992). In late summer, acidic pH of the Snake River may have altered this fractionation effect, keeping more metal-binding, organic ligands in solution (Table 1).

It is unclear why the net Cu complexation of Deer Creek DOM from summer was fundamentally unchanged. Deer Creek is located in a drainage with more basic rocks than the Snake River, such that pH changes would not explain the diminished the photoreactivity of Deer DOM collected in summer.

Predictors of Cu-DOM complexation—Our final hypothesis that spectral qualities predict photochemical changes in Cu-DOM complexation was supported for $SUV\alpha$, but not for the fluorescence index (Figs. 3, 4). $SUV\alpha$ was a good predictor of conditional stability constants ($r^2 = 0.55$ to 0.66), tracking the strength of Cu-DOM complexation in both irradiated and unirradiated DOM from the various sources and collection seasons (Fig. 4A). $SUV\alpha$ is a strong, indirect measure of aromaticity ($r^2 = 0.97$) defined as all sp^2 hybridized molecules including phenols, quinones, and olefins (Weishaar et al. 2003). Aromatic moieties in DOM increase its electronegativity and enhance its binding affinity for positively charged metal ions (Leenheer et al. 1998; Tipping 2002). Herein, we found that unirradiated DOM types with the highest $SUV\alpha$ (highest aromaticity) changed the least during photooxidation (Fig. 4B). This means that during irradiation the greatest changes in chemical compo-

sition, particularly aromaticity, occur in low aromaticity samples. Thus, $SUV\alpha$ explains disparate DOM photoreactivity and subsequent Cu-DOM complexation. We conclude that the relative aromaticity of DOM largely determines the magnitude of DOM binding affinity for Cu, and that $SUV\alpha$ provides a good metric for Cu binding affinity. This conclusion is consistent with earlier findings that $SUV\alpha$ values were positively related to photochemical changes in binding affinity of DOM in time-series experiments (Brooks et al. in press).

Two requirements are necessary for ideal spectral characterization of photochemical changes in Cu complexation by DOM. The spectral component must: (1) reflect the general aromaticity and the presence of oxygen, nitrogen, and sulfur moieties that directly bind Cu; and (2) covary with the components of DOM that complex Cu. The fluorescence index specifically measures quinone structures (McKnight et al. 2001) and apparently always decreases when photooxidized (Moran et al. 2000; Brooks et al. in press; Fig. 3 here). Although the index clearly responds to photochemical changes, it is a specific measure of one aromatic component. These findings indicate that the index does not reflect mechanisms responsible for Cu-DOM complexation.

Biological consequences of Cu-DOM complexation—Figure 1 shows the overall importance of DOM for mitigating Cu toxicity. Photooxidation of DOM can greatly alter its protective effects with important consequences for Cu toxicity to aquatic biota. At the pH of our titration solutions (slightly below 6.0), inorganic hydroxides or carbonates complex negligible proportions of Cu ($<0.01\%$). At approximately 5 mg L^{-1} DOC, DOM decreases $\{Cu^{2+}\}$ by 90% or about an order of magnitude below the theoretical 1:1 relation of $p\{Cu^{2+}\}$ to $p[Cu]_T$ (shown in the graph of Home spring). Laboratory irradiation of most DOM types increased $\{Cu^{2+}\}$ from below the $\{Cu^{2+}\}_{LC50}$ ($0.035 \mu\text{mol L}^{-1}$, Erickson et al. 1996) to levels approximately $2\times$ higher (Fig. 1, Table 3). Summer DOM from Deer and Snake did not follow this trend and emphasize important caveats in effective Cu complexation between DOM collected from different sources in different seasons.

The simple model of a hypothetical contaminant event (Fig. 5) demonstrates the potential implications of basing Cu complexation and speciation on photochemically unstable DOM in natural systems. Estimating the bioavailability of a contaminant at the point source may completely ignore the downstream repercussions of contaminant influx. For example, immediately following the contamination event, the inorganic chemistry and DOC of half of the field sites buffered Cu bioavailability to below the $\{Cu^{2+}\}_{LC50}$. Within 1 d, after estimated downstream transit of the plume for as little as 5 km (Table 2), this remained the case at four sites. After 3 d, $\{Cu^{2+}\}$ was below the LC50 in only one site. In Home spring, Deer summer, and Snake, photooxidation of DOM had virtually no effect. Although we cite the $\{Cu^{2+}\}_{LC50}$ of larval fish as a point of reference, photooxidative release of Cu^{2+} to higher levels would affect adult life stages that have greater tolerance (Taylor et al. 2002) as well

as algal species with lower tolerance to $\{Cu^{2+}\}$. In addition, this model suggests much different seasonal implications than did the static laboratory results. Laboratory results demonstrated greater photoreactivity of DOM collected in spring. However, when placed in the dynamic geochemical environment of natural waters, this model suggests that exposure to Cu toxicity is much greater in summer, a period representing the base flow regime that exists in most of these streams for 11 months of the year.

This degree of variability in Cu speciation and photoreactivity by DOM from different sources and seasons may explain some of the variation in the biological effect of Cu complexation reported in various studies (Brooks 2003, De Schamphelaere and Janssen 2004). Comparing the protective effect of DOM from two lakes and a stream against chronic Cu toxicity, De Schamphelaere and Janssen (2004) reported no source-dependent effects on *Daphnia magna*. Contrasting results from work with rainbow trout (*Oncorhynchus mykiss*) demonstrated greater protective effects of DOM from an allochthonous source than an autochthonous source against metal toxicity (Richards et al. 2001) and metal accumulation (Luider et al. 2004). Brooks (2003) found that $\{Cu^{2+}\}$ predicted acute toxicity in ~60% of treatments, but underestimated toxicity in the remaining treatments, and did not vary between sources of DOM per unit DOC. Those findings indicate that DOC concentration is a good general predictor of Cu toxicity, if its Cu-DOM complexation parameters are known, but that source (Richards et al. 2001; Luider et al. 2004) can sometimes be a confounding factor.

Ecological implications of anthropogenic impacts on DOM—Anthropogenic alterations of natural DOM could have important implications for the bioavailability of Cu to aquatic organisms. Climate warming is expected to reduce snowpack with continuous melting throughout winter (Baron et al. 2000), eliminating an intense spring runoff. Such changes would decrease total loadings of DOC that typically increase by 100% to 500% during the peak flows of spring and early summer (Hornberger et al. 1994). Land-cover changes, and especially loss of wetlands (Dahl 2000), may alter the proportions and concentrations of DOM from autochthonous versus allochthonous sources at a landscape scale (Gergel et al. 1999). Prusha and Clements (2004) showed that percentage of forest cover was a good predictor of DOC concentration, which in turn was inversely related to body burdens of Zn and Cd in *Arctopsyche grandis*. Thus, deforestation can directly increase the bioavailability of metals to aquatic organisms by altering DOC inputs.

Incident UV radiation increased during the last 20 yr (McKenzie et al. 1999). Some climate models suggest that if all conditions (e.g., clouds, snow cover, aerosols) remain constant (an unlikely possibility), future increases in UV radiation will be negligible. However, it is generally thought that UV radiation will increase by a minimum of 10% at mid latitudes (Kerr et al. 2003). Increasing UV radiation will likely speed the rate and extent of DOM photobleaching, producing a positive feedback that drives deeper penetration of UV radiation in the water column. All of our DOM types were photobleached and photo-

mineralized in 24-h exposures (equivalent to ~4 d of ambient sunlight). DOM from pure algal cultures is not readily photomineralized (Tranvik and Kokalj 1998; Obernosterer and Benner 2004). Significant DOC losses from all DOM solutions as well as SUV α and fluorescence index values indicate that all DOM types were dominated by allochthonous sources. DOM collected during spring runoff was more susceptible to photodegradation of Cu binding capacity than DOM collected in summer. However, modeling of cupric release under dynamic stream conditions indicated greater release during the base flow conditions of summer. Photooxidation of DOM generally decreases its complexation of Cu, the net effect of which is enhanced $\{Cu^{2+}\}$ with concomitant increases in Cu toxicity. Our results suggest that global increases in UV radiation will increase Cu bioavailability in oligotrophic streams dominated by DOM from terrestrial sources. Despite terrestrial dominance of the source of DOM, variability in the structure, composition, and especially complexation of Cu show that not all DOM is alike or environmentally stable in its potential for mitigating Cu toxicity. Estimates of the effect of dynamic processes such as photochemical release of Cu^{2+} shown in Fig. 5 are a step toward developing improved models of the environmental fate of contaminants. Thus, as DOC has been included in water-quality criteria, so should measures of changes in Cu complexation due to DOM photooxidation.

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Received: 24 February 2006

Accepted: 26 September 2006

Amended: 16 November 2006