

Toxicity of copper to larval *Pimephales promelas* in the presence of photodegraded natural dissolved organic matter

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Abstract: Copper (Cu) complexation with natural dissolved organic matter (DOM) mitigates Cu bioavailability to aquatic biota by decreasing the activity of the cupric ion (Cu^{2+}). However, DOM is photochemically unstable. In a previous study, we demonstrated that irradiation (~13 days sunlight) of DOM collected from a river decreased its binding-site density for Cu by 45%, but increased binding-site density in wetland DOM by 147%. Binding-site densities correlated positively with ketones and aldehydes (C-II groups). Herein, we determined the mortality of larval fathead minnows (FHM; *Pimephales promelas*) as a function of Cu^{2+} in 96 h static-renewal toxicity tests without DOM. Next, we calculated Cu^{2+} in control and photooxidized DOM and then predicted mortality of larval FHM in toxicity tests. Observed mortalities agreed with predictions ($r^2 \approx 0.96$) in treatments with lowest binding-site densities and proportions of C-II groups (highest Cu^{2+}). However, treatments with ~50% lower Cu^{2+} , containing higher proportions of C-II groups, had equally high mortalities and poorer fits with predictions ($r^2 \approx 0.75$), possibly indicating that Cu bound to C-II groups are bioavailable. To our knowledge, this study is the first to predict and then directly test the effects of DOM photooxidation on Cu toxicity.

Résumé : La formation de complexes entre le cuivre (Cu) et la matière organique dissoute (DOM) naturelle atténue la biodisponibilité du Cu aux organismes aquatiques en réduisant l'activité de l'ion cuprique (Cu^{2+}). Cependant, la DOM est photochimiquement instable. Dans une étude antérieure, nous avons démontré qu'une irradiation (~13 jours d'ensoleillement) de la DOM récoltée dans une rivière réduit la densité de ses sites de liaison avec le Cu de 45 %, alors que l'irradiation augmente la densité des sites de liaison de la DOM de terres humides de 147 %. Il y a une corrélation positive entre la densité des sites de liaison et les cétones et les aldéhydes (groupes C-II). Dans la présente étude, nous déterminons la mortalité de larves de têtes-de-boule (FHM; *Pimephales promelas*) en fonction de Cu^{2+} après 96 h dans des tests de toxicité à renouvellement statique en l'absence de DOM. Nous avons ensuite calculé Cu^{2+} dans des conditions témoins et en présence de DOM photooxydée et prédit la mortalité des larves de FHM dans les tests de toxicité. Les mortalités observées s'accordent aux prédictions ($r^2 \approx 0,96$) dans les situations expérimentales correspondant aux densités les plus faibles de sites de liaison et de proportions de groupes C-II (Cu^{2+} les plus fortes). Cependant, dans les conditions expérimentales avec Cu^{2+} ~50 % inférieures et de plus fortes proportions de groupes C-II, les mortalités sont également élevées et correspondent moins bien aux prédictions ($r^2 \approx 0,75$), ce qui indique peut-être que Cu lié aux groupes C-II demeure biodisponible. À notre connaissance, notre étude est la première à prédire les effets de la photooxydation de la DOM sur la toxicité du Cu et à les vérifier directement.

[Traduit par la Rédaction]

Introduction

Complexation with dissolved organic matter (DOM) in aquatic ecosystems can greatly decrease the toxicity of metals. For copper (Cu), such binding decreases the chemical activity of the cupric ion (Cu^{2+}), which is the most toxic Cu species (Playle et al. 1993; Erickson et al. 1996; MacRae et al. 1999). DOM originates from autochthonous (aquatic

algae, bacteria, and macrophytes) and allochthonous (vascular, terrestrial plants) sources. With concentrations of 2–10 mg·L⁻¹ dissolved organic carbon (DOC, which comprises about half of DOM; Thurman 1985), DOM typically comprises the largest pool of organic or inorganic ligands for Cu in natural waters (McKnight and Aiken 1998). However, the binding affinity of DOM for Cu varies by orders of magnitude depending on the DOM source (McKnight et al. 1983;

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Cabaniss and Shuman 1988; Breault et al. 1996). Consequently, at the same concentration of DOC, mortality of aquatic biota exposed to Cu can vary by a factor of two among DOM sources (Richards et al. 2001; De Schampheleere et al. 2003; Ryan et al. 2004).

In addition to apparent source-related differences, DOM is photochemically unstable. Photooxidation by sunlight can alter DOM in several important ways, such as photomineralization, photobleaching, and production of low molecular weight (LMW) organics. Photomineralization converts organic carbon to CO and dissolved inorganic carbon (defined as $\text{CO}_2 + \text{H}_2\text{CO}_3 + \text{HCO}_3^- + \text{CO}_3^{2-}$; Miller and Zepp 1995; Xie et al. 2004). Sunlight can remove 15%–50% of DOC from natural waters in the span of 2–11 days (Molot and Dillon 1997; Moran et al. 2000; Brooks et al. 2007). Photobleaching (loss of absorbance of ultraviolet and visible radiation) is an indirect measure of chemical transformation in the DOM remaining after photomineralization. For example, photobleaching of DOM across the ultraviolet spectrum (UV, 280–400 nm) normalized by DOC concentration can indicate loss of sp^2 hybridized bonds, (i.e., double bonds in aromatics and olefins; Weishaar et al. 2003).

The photochemistry of DOM can differ considerably between DOM leached from algal cultures and allochthonous DOM, particularly with regard to production of LMW organics. Irradiation of allochthonous DOM cleaves its large macromolecules into LMW organics, such as carboxylic acids that are microbially labile (biologically available carbon sources; Moran and Zepp 1997; Bertilsson and Tranvik 2000; Miller et al. 2002). DOM from algal cultures is photobleached, indicating chemical transformation (Obernosterer and Benner 2004). However, irradiation of algal DOM does not produce labile compounds (Tranvik and Kokalj 1998) nor is it photomineralized (Obernosterer and Benner 2004). Differences in photoreactivity due to algal input are attenuated in natural waters because lakes and streams receive a mixture of allochthonous DOM from surface runoff in addition to autochthonous inputs. Thus, is variation in Cu–DOM complexation due to source-related differences in precursor organics or to DOM photodegradation, or both?

Little is known about effects of photooxidation of natural DOM on its complexation of Cu. In a previous study, irradiation of the same wetland and riverine DOM used in our current study had unexpectedly opposite effects on Cu–DOM complexation (Brooks et al. 2007). Irradiation of DOM collected from a wetland increased its binding-site density for Cu by 147%, but decreased binding-site density in DOM from a river by 45%. Binding affinities of the two DOM types were virtually identical after photooxidation. The net outcome of photochemical changes was that the wetland DOM bound 148% more Cu per milligram DOC than the riverine DOM. Because photooxidation of DOM profoundly altered Cu complexation and the $\{\text{Cu}^{2+}\}$ in solution, we predicted that irradiation of riverine and wetland DOM would also alter Cu toxicity. The specific objectives of this study were to (i) quantify how photooxidation and source of DOM alter Cu toxicity to larval fathead minnows (FHM, *Pimephales promelas*) and (ii) examine the relationship between $\{\text{Cu}^{2+}\}$ and Cu toxicity in the presence of DOM.

Materials and methods

Study sites, DOM collection, and irradiation of DOM

We collected DOM during spring runoff (16 and 17 June 1999) from two surface waters with contrasting hydrology: Chimney Park wetland (41.05°N, 106.37°W, 2715 m altitude; designated CPWDOM) and the Laramie River (41.10°N, 106.19°W, 2280 m; designated LRDOM) in the Rocky Mountains of Wyoming, USA. Chimney Park, a palustrine wetland, receives a mixture of organic input from the adjacent lodgepole pine (*Pinus contorta*) forest, willows (*Salix* spp.), emergent sedges (*Carex* sp.), and submersed vascular macrophytes (*Myriophyllum* sp.) colonized by epiphytic microbial communities. The Laramie River is a third-order, cobble-bottom river that drains a mixed *Poaceae* grassland – lodgepole pine watershed.

After filtration through prerinsed 10, 5 (Hytrex II, Osmotics Incorporated), 1 (Corning Costar Corporation), and 0.2 μm filters (Corning Costar Corporation), natural waters were passed through a H^+ cation-exchange resin that removed $\geq 95\%$ of all metals (Ca, K, Mg, Na, Cd, Cu, Fe, Ni, Pb, and Zn). Processing for ~ 8 h through a portable reverse osmosis system (PROS/2S, RealSoft; Serkiz and Perdue 1990) increased initial DOC concentrations ([DOC]) from 14.8 to 606 $\text{mg DOC}\cdot\text{L}^{-1}$ (CPWDOM) and from 7.5 to 353 $\text{mg DOC}\cdot\text{L}^{-1}$ (LRDOM). Mass balance calculations showed that 95% of DOC was recovered by reverse osmosis. Maurice et al. (2002) demonstrated that reverse osmosis does not alter DOM structure or elemental composition from that of raw water. Concentrates were stored in the dark at 4 °C in acid-washed, ashed (500 °C for 2 h) borosilicate bottles at pH 6. Storage pH is extremely important because natural organics rapidly undergo spontaneous hydrolysis if stored at pH near or below 2 (Schwarzenbach et al. 1993). Monitoring of storage effects over the span of several months indicated no change in [DOC] or the rate of DOC loss during irradiation.

We photodegraded DOM for toxicity tests at radiation intensities approximating natural sunlight. Prior to irradiation, we normalized photoreactivity by diluting DOM concentrates to a standard absorbance of 0.33 ± 0.02 absorbance units (a.u.) (mean \pm standard deviation (SD)) at 350 nm in a 1 cm cell, resulting in [DOC] of ~ 40 $\text{mg C}\cdot\text{L}^{-1}$ (CPWDOM) and ~ 30 $\text{mg C}\cdot\text{L}^{-1}$ (LRDOM). We irradiated DOM solutions in tightly capped, quartz Hungate tubes (14.6 cm \times 1.9 cm) in a solar simulator (Atlas Suntest CPS+) under a full-spectrum 1.0 kW xenon lamp for 72 h in a continuous-circulation cooling bath (25.4 ± 1.3 °C; mean \pm SD). To obtain adequate volumes of irradiated DOM, solutions were irradiated during several separate sessions in the solar simulator, then pooled to standardize any potential variation among irradiation sessions. Radiation intensities were measured with an International Light radiometer (model 1400). Compared with noontime cloudless conditions recorded at the field sites on 21 June 2003, intensities within the solar simulator were 1.4 times greater for UV-B (detector: 265–332 nm), 1.8 times for UV-A (subtraction of UV-B from total UV detector: 250–400 nm), or equal to photosynthetically active radiation (detector: 400–1000 nm). In 1 day, surface water theoretically receives the equivalent of 7.6 h of noon sun ($= 12 \text{ h} \times 2 \times \pi^{-1}$) (Miller and Zepp 1995). Therefore, 72 h of irradiation in the solar simulator at 1.4 times field in-

tensities of ambient UV-B radiation theoretically equaled 13.2 days of UV-B at our field sites. Thus, 13.2 days of full spectrum exposure is an environmentally reasonable period of ambient solar exposure in a region that receives 75% of cloudless summer sunlight (Curtis and Grimes 2004).

Water chemistry

Unless otherwise designated, all reagents were analytical grade, except HCl (trace-metal grade, Spectrum) and HNO₃ (OPTIMA, Seastar Chemicals). We used the higher-grade acids 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 with 18 MOhm water (Milli-Q, Millipore Corporation). The 18 MOhm water was used for analytical dilutions and analytical blanks. Dionized water was used to dilute well water for toxicity tests.

Chemistries of DOM solutions for toxicity tests were directly measured by a variety of analytical methods. 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, model 372). Concentrations of other trace metals were determined by graphite furnace atomic absorption spectrophotometry with Zeeman background correction (Varian, model SpectrAA-600). [DOC] were analyzed by catalytic combustion on a total organic carbon analyzer (Shimadzu, model 5000A) in samples acidified to pH < 3 and sparged for 8 min with hydrocarbon-free air using regular-sensitivity catalyst. pH was measured with a meter (Corning model 340) and a Corning flat-surface combination pH electrode. Alkalinity of water samples was determined by titration to pH 4.5 with H₂SO₄ according to standard methods (APHA 1995).

Toxicity tests

Larval FHM for all bioaccumulation experiments came from a resident brood population of FHM at the University of Wyoming. When fully “eyed-up” and nearing hatch (~5 days after spawn), tiles containing FHM eggs were placed in laboratory incubators containing test matrix water that was free of added Cu or DOM (Table 1). After hatching, we collected larval fish for each test within 24 h and immediately started a test. Larvae were not fed during the tests.

Carboys and 150 mL test chambers were acid-washed by soaking in 18% HNO₃ (v/v) for at least 2 h, then rinsed five times before use with 18 MOhm water. Carboys and test chambers were preconditioned for each test by filling with the appropriate test solution, allowing potential adsorption to occur for >24 h, and refilling with fresh test solution. We were unable to detect any loss of Cu in preconditioned carboys and test chambers. Test solutions were mixed >24 h prior to the start of each test to allow sufficient time for maximal Cu–DOM complexation (Ma et al. 1999). Copper was added as CuCl₂. Any solutions containing DOM were stored at 4 °C in the dark until ~3 h before introducing fish, during which time the solutions warmed to the test temperature of 25 °C. We measured pH, alkalinity, and hardness in each exposure chamber at the onset and the end of each toxicity test.

Table 1. Chemistry of matrix water solutions used in toxicity tests without added dissolved organic carbon (DOC; tests NODOC 1 and NODOC 2) and in toxicity tests (TOX 1 and TOX 2) using dissolved organic matter (DOM) collected from Chimney Park wetland (CPW) and Laramie River (LR) in southeast Wyoming, USA.

DOC source	Experiment	DOC (mg·L ⁻¹)	pH	Alkalinity (mequiv·L ⁻¹)	Concentration (µmol·L ⁻¹)								
					Ca ²⁺	Mg ²⁺	Na ⁺	K ⁺	Cl ⁻	NO ₃ ⁻	SO ₄ ²⁻	Cu	Fe
CPW	NODOC 1,	<0.10	7.1	0.64	109	150	52	5.7	22	15	29	Varied ^a	<0.02
	NODOC 2												
LR	TOX 1	Varied ^b	7.0	0.60	116	122	122	2.7	68	108	42	1.5±0.07	0.17
	TOX 2	Varied ^b	6.8	0.61	216	164	146	6.7	138	34	68	1.4±0.06	0.13
CPW	TOX 1	Varied ^b	6.8	0.48	137	126	175	6.9	44	124	55	1.3±0.07	0.65
	TOX 2	Varied ^b	6.9	0.60	184	163	155	7.1	3.9	51	46	1.8±0.01	0.56

Note: The matrix water of all tests was a mixture of deionized and well waters. pH, alkalinity, hardness, and Cu encompass values from all treatments of varying DOC concentration (*n* = 10). Values for anions, cations, and Fe were taken from analyses of the exposure solutions irradiated for 72 h and diluted to ~2.5 mg DOC·L⁻¹ in each toxicity test. Cu values are mean ± standard deviation (SD). The following analytes were below detection limits (detection limits in µmol·L⁻¹ in parentheses): Cd (0.002), F⁻ (0.74), Fe (0.02), Ni (0.54), Pb (0.002), PO₄³⁻ (2.48), and Zn (0.092).

^aCu concentrations ranged 0.01–2.9 µmol·L⁻¹ (for specific values see Brooks 2003).

^bFor DOC concentrations see Table 2.

Table 2. Measured dissolved organic carbon (DOC) concentrations (mean \pm standard deviation (SD)) and cupric ion activity ($\{Cu^{2+}\}$) determined by MINTEQA2 calculations in unirradiated (0 h) and irradiated (72 h) dissolved organic matter (DOM) from Chimney Park wetland (CPWDOM) and the Laramie River (LRDOM) using average DOC concentrations and other chemistries in Table 1 for toxicity tests TOX 1 and TOX 2.

Experiment	DOC (mg·L ⁻¹)				$\{Cu^{2+}\}$ ($\mu\text{mol}\cdot\text{L}^{-1}$)			
	CPWDOM		LRDOM		CPWDOM		LRDOM	
	0 h	72 h	0 h	72 h	0 h	72 h	0 h	72 h
TOX 1	0.8	1.1	0.7	0.7	0.23	0.13	0.21	0.26
	1.5	1.3	1.0	1.1	0.14	0.10	0.16	0.19
	1.7	1.7	1.6	1.3	0.12	0.07	0.08	0.17
	2.3	2.0	1.8	1.7	0.08	0.06	0.06	0.13
	3.0	2.6	2.2	2.0	0.05	0.04	0.05	0.10
TOX 2	0.7 \pm 0.67	0.7 \pm 0.62	0.6 \pm 0.01	0.4 \pm 0.03	0.32	0.19	0.36	0.47
	1.4 \pm 0.42	1.5 \pm 0.08	1.2 \pm 0.02	1.2 \pm 0.05	0.16	0.08	0.22	0.31
	2.4 \pm 0.78	2.5 \pm 0.20	2.3 \pm 0.07	2.5 \pm 0.03	0.09	0.04	0.08	0.17
	3.6 \pm 0.41	3.4 \pm 0.35	3.3 \pm 0.03	3.6 \pm 0.16	0.05	0.03	0.05	0.09
	4.7 \pm 0.48	4.7 \pm 0.38	4.5 \pm 0.06	4.8 \pm 0.04	0.03	0.01	0.03	0.06

Note: For toxicity test TOX 1, $n = 1$ analytical replicates; for toxicity test TOX 2, $n = 2$ analytical replicates.

We performed two different types of 96 h static-renewal toxicity tests. In one type (TOX 1 and TOX 2), we evaluated the protective effect of DOM before and after photodegradation in treatments standardized to the same series of DOC concentrations. In the other type of test, which lacked added DOC (NODOC A and NODOC B), we determined the concentration–response relationship between $\{Cu^{2+}\}$ and mortality of larval FHM.

In TOX 1 and TOX 2 tests, larval FHM were exposed to five DOC concentrations from each of the two sources of irradiated and unirradiated DOM with [Cu] held constant (Table 1). TOX 1 was a preliminary test of [DOC] ranging from 0 to 3 mg·L⁻¹. In TOX 2, we increased the range of [DOC] to 0–5 mg·L⁻¹ (Table 2). Irradiation of DOM generally photomineralizes 10%–20% of organic carbon to inorganic carbon as CO₂ and CO (Miller and Zepp 1995; Brooks et al. 2007; see also Results). Thus, in the tests with irradiated DOM, we added higher percentages of DOM to test solutions to compensate for loss of DOC due to photomineralization. This protocol standardized irradiated solutions to the same [DOC] as contained in solutions of unirradiated DOM. At each [DOC], three replicate chambers contained 10 fish in 100 mL of toxicity test solution. Before daily replacement, we warmed fresh solutions of test waters to 25 °C to avoid thermal shock to the fish.

No DOM was added to NODOC A and NODOC B tests, which contained a low background [DOC] of 0.1 mg·L⁻¹ in the same matrix water used in TOX 1 and TOX 2 tests (Table 1). We exposed fish to a series of five [Cu] ranging from 0.01 to 1.6 $\mu\text{mol}\cdot\text{L}^{-1}$ in NODOC A and from 0.01 to 2.9 $\mu\text{mol}\cdot\text{L}^{-1}$ in NODOC B (see Brooks 2003 for measured [Cu]). Each exposure level had three replicate chambers containing 10 fish per 100 mL solution. From the combined concentration–response relationships of NODOC A and NODOC B, we calculated the expected mortality as a function of $\{Cu^{2+}\}$.

For all toxicity tests, we calculated $\{Cu^{2+}\}$ with the geochemical speciation program MINTEQA2 (Version 3.1; Allison et al. 1991). In these MINTEQA2 calculations, we entered measured water chemistries (Table 1) and the conditional stability constants (K_1 and K_2) and binding-site densities ($[L_1]$ and $[L_2]$) for 2-ligand models of Cu–DOM complexation in control and irradiated solutions of the same CPWDOM and LRDOM (Table 3), which were determined in a previous study (Brooks et al. 2007). We did not directly measure $\{Cu^{2+}\}$ in toxicity solutions because reliable readings with a cupric ion selective electrode required the addition of 0.01 mol·L⁻¹ NaNO₃ for ionic strength adjustment to stabilize the electrode (Saar and Weber 1982; Christl et al. 2005). Such measures taken at a higher ionic strength would not constitute direct measures.

Because those Cu–DOM binding parameters were determined at pH 6.0 but the toxicity tests were conducted at pH ~ 6.9, we increased the conditional stability constants by 0.9 log₁₀ units in MINTEQA2 calculations, consistent with a one log₁₀ unit increase in conditional stability constants of Cu–DOM complexes per pH unit from pH 4 to 8.5 (Cabaniss and Shuman 1986; Lu and Allen 2002). The 1:1 ratio of the log K and pH of metal–organic complexation is consistent for many metals and a variety of natural organic compounds: Cu–fulvic acid (Cabaniss and Shuman 1988; Christl et al. 2005); Cu – natural DOM (Lu and Allen 2002); Cu –, Cd –, Al – natural DOM complexes (Kinniburgh et al. 1996); and Al – fulvic acid (Brown and Driscoll 1993; see review in Tipping 2002).

Some variation exists in this 1:1 ratio at high Cu concentrations (total [Cu] > 0.1 mmol·L⁻¹) because Cu–DOM complexation changes the electrostatic properties of unoccupied DOM sites. High pH values (pH > 8.4) also alter the ratio (Cabaniss and Shuman 1988; Lu and Allen 2002) by dissociation of protons from phenolic binding sites on DOM (Lu and Allen 2002) or increases in monovalent metal

Table 3. Photochemical effects for controls (0 h) and irradiated (72 h) dissolved organic matter (DOM) collected from Chimney Park wetland (CPWDOM) and Laramie River (LRDOM) (all data from Brooks et al. 2007).

DOM source	Irradiation time (h)	Ligand densities ($\mu\text{mol}\cdot\text{mg DOC}^{-1}$)		$\log_{10} K_1$	$\log_{10} K_2$	[LMWOA] ($\text{mmol}\cdot\text{L}^{-1}$)	C-II (%)	AR/AL-I
		$[L_1]$	$[L_2]$					
CPWDOM	0	0.13±0.01	0.70±0.08	8.0±0.1	6.3±0.1	1.3±0.01	2.11	0.13
	72	0.22±0.03	1.83±0.36	7.6±0.2	5.9±0.1	1.4±0.02	5.60	0.08
LRDOM	0	0.17±0.02	1.60±0.42	7.6±0.1	5.8±0.1	0.94±0.06	4.0	0.53
	72	0.10±0.01	0.88±0.16	7.6±0.1	5.9±0.1	1.60±0.02	2.88	0.23

Note: Parameter descriptions are as follows: copper-binding site concentrations ($[L_1]$, $[O_2]$) and conditional stability constants (K_1 , K_2) in a two-ligand model of Cu–DOM complexation; summed concentrations of six low molecular weight organic acids ([LMWOA]: acetic, citric, lactic, malic, oxalic, and succinic); the percentage of carbon in carbonyl groups of ketone and aldehyde substituents (C-II region of ^{13}C NMR spectra); and the ratio of aromatic rings and olefins (AR region) to aliphatic carbohydrate carbons, ester carbons, and amines (AL-I region). Parameters are conditional for pH 6.0, 25 °C, and 0.01 mol·L $^{-1}$ NaNO $_3$. Values for Cu–DOM complexation parameters and [LMWOA] were determined from three separate irradiation replicates of a single dilution of DOM concentrate from each field site. DOM solutions from several irradiation sessions were pooled to collect an adequate mass for ^{13}C NMR spectral analysis.

hydroxyl species able to complex with monodentate DOM ligands (Browne and Driscoll 1993). These caveats do not apply to the conditions of our study.

In addition, we input the conditional stability constants ($\log_{10} K_{\text{Cu-FHM}} = 6.6$) and binding-site densities ($[L_{\text{Cu-FHM}}] = 4.9 \times 10^{-11} \text{ mol}\cdot\text{fish}^{-1}$) of larval FHM from bioaccumulation experiments (Brooks et al. 2006). In MINTEQA2 calculations, we did not include acid dissociation constants for the DOM or competition by Ca^{2+} , Mg^{2+} , and K^+ . The Cu–DOM conditional stability constants and their pH-dependent adjustments implicitly included Cu^{2+} competition with H^+ for binding to DOM, thus obviating the need to incorporate H^+ competition into the MINTEQA2 calculations. Any potential competition by Ca^{2+} , Mg^{2+} , and K^+ with Cu for DOM ligands was implicitly included in the toxicity tests because concentrations in test matrices (Table 1) were similar or virtually identical to those present in the DOM solutions used to develop Cu–DOM binding parameters (Brooks et al. 2007). Moreover, we excluded Ca^{2+} , Mg^{2+} , and K^+ from speciation calculations because several studies have demonstrated their minimal competition with Cu for binding sites on DOM (Hering and Morel 1988; Alberts et al. 1992; Breault et al. 1996). Including such competition would have underestimated Cu complexation by DOM.

After calculating the $\{\text{Cu}^{2+}\}$ for each [Cu] in NODOC A and NODOC B tests, the logit transform of mortality was calculated as $\ln[M/(1-M)]$, where M is the average proportion of mortality (number of dead fish per number of total fish at each $\{\text{Cu}^{2+}\}$). We then regressed the logit of mortality versus $\ln\{\text{Cu}^{2+}\}$:

$$(1) \quad \ln[M/(1-M)] = a \ln\{\text{Cu}^{2+}\} + b$$

Rearranging eq. 1 to solve for mortality, we used the regression slope (a) and the ordinate intercept (b) to predict the percent mortality (m) in TOX 1 and TOX 2 tests as

$$(2) \quad m = [\exp(a \ln\{\text{Cu}^{2+}\} + b) \times \{1 + [\exp(a \ln\{\text{Cu}^{2+}\} + b)]\}^{-1} \times 100$$

where $\{\text{Cu}^{2+}\}$ was calculated at each [DOC] using MINTEQA2. Finally, we compared the expected mortality

with the observed mortality at each [DOC] in TOX 1 and TOX 2.

Statistical analyses

The $\{\text{Cu}^{2+}\}$ associated with 50% lethality (i.e., $\{\text{Cu}^{2+}\}_{\text{LC50}}$) for all toxicity tests (TOX 1 and TOX 2; NODOC A and NODOC B) were calculated using the Trimmed Spearman–Kärber LC50 procedure in TOXSTAT (Version 3.5; WEST, Inc. and Gulley 1996). For TOX 1 and TOX 2 tests, which contained DOM, we first calculated the $[\text{DOC}]_{\text{LC50}}$ using the same procedure in TOXSTAT, then calculated the $\{\text{Cu}^{2+}\}_{\text{LC50}}$ at that [DOC] in MINTEQA2. In all hypothesis testing, we set $\alpha = 0.05$. t tests were used to evaluate miscellaneous differences as noted in the text.

Results

Photochemical changes in DOM

In photooxidation experiments of DOM, photomineralization removed 15%–20% of DOC from CPWDOM and LRDOM after 72 h of irradiation. The extent of photomineralization for the current study did not differ significantly from earlier irradiation of DOM in the solar simulator (Brooks et al. 2007). After exposure, we diluted the irradiated DOM for toxicity tests to the [DOC] listed in Table 2.

MINTEQA2 calculations of $\{\text{Cu}^{2+}\}$ in our toxicity test solutions TOX 1 and TOX 2 indicated that photochemical changes in Cu–DOM complexation produced an average of 31%–85% greater calculated $\{\text{Cu}^{2+}\}$ per unit DOC in the DOM solutions with lower Cu binding-site densities (i.e., irradiated LRDOM and unirradiated CPWDOM) than in the corresponding treatments with higher Cu-ligand density (i.e., unirradiated LRDOM and irradiated CPWDOM; Table 2). The inclusion of $K_{\text{Cu-FHM}}$ and $[L_{\text{Cu-FHM}}]$ for biotic ligands (the larval fish) in MINTEQA2 calculations (parameters from Brooks et al. 2006) had little influence on aqueous Cu speciation in the test solutions because the small mass of fish in the exposure chambers bound $\leq 0.2\%$ of total Cu (0.000 12 g dry weight·fish $^{-1}$ with 10 fish·chamber $^{-1}$).

Table 4. Photochemical changes in the concentration of dissolved organic carbon ([DOC]) in unirradiated and irradiated solutions of dissolved organic matter (DOM) from Chimney Park wetland (CPWDOM) and Laramie River (LRDOM).

DOM source	Irradiation time (h)	[DOC] ($\mu\text{mol}\cdot\text{L}^{-1}$)	
		TOX 1	TOX 2
CPWDOM	0	41±0.9	41±1.1
	72	35±0.9*	33±0.6*
Decrease (%)		15	20
LRDOM	0	30±0.1	32±0.6
	72	25±0.8*	25±0.4*
Decrease (%)		19	20

Note: Data from three separate irradiation replicates of a single dilution of DOM concentrate from each field site. Neither the extent nor rate of photomineralization differed significantly between this study and an earlier study (Brooks et al. 2007). These DOM solutions were subsequently used in toxicity tests TOX 1 and TOX 2.

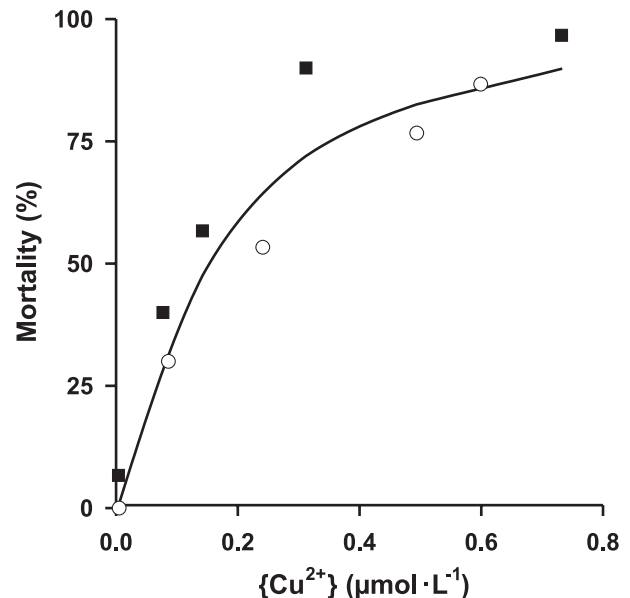
* $p \leq 0.05$.

Toxicity tests

Using combined data from NODOC A and NODOC B tests (Fig. 1), the regression relationship between logit mortality and $\ln\{\text{Cu}^{2+}\}$ in the absence of DOC had a slope of 1.326 with an intercept of -3.017 (eq. 1). Observed mortalities varied by 1%–34% from the composite concentration–response curve. Based on that regression relationship, we predicted mortality as a function of MINTEQA2-calculated $\{\text{Cu}^{2+}\}$ for each [DOC] in TOX 1 and TOX 2 tests using eq. 2 (Fig. 2). The observed mortalities at a given [DOC] did not differ considerably between the irradiated and unirradiated DOM treatments (Fig. 2). Measuring mortality as a function of $\{\text{Cu}^{2+}\}$, with the exception of TOX 1 conducted in CPWDOM, the $\{\text{Cu}^{2+}\}_{\text{LC50}}$ differed significantly between irradiated and unirradiated treatments in all TOX 1 and TOX 2 tests (Table 5) based on lack of overlap in confidence intervals and t test results (CPWDOM t test = 2.56 and $p = 0.047$; LRDOM TOX 1 t test = 5.72 and $p = 0.004$; LRDOM TOX 2 t test = 3.36 and $p = 0.021$). Measuring mortality as a function of DOC, the $[\text{DOC}]_{\text{LC50}}$ in the presence of the same total [Cu] were virtually identical between irradiated and control treatments regardless of source.

We compared expected with observed results (relative difference = (predicted – observed)/observed $\times 100\%$) for TOX 2 because of its greater range of DOC. In DOM treatments with low binding-site density (i.e., higher $\{\text{Cu}^{2+}\}$); expected to follow topmost curves in Figs. 2c, 2d), the observed mortalities were within an average of $9\% \pm 7\%$ (mean \pm SD; irradiated LRDOM, $r^2 = 0.95$) to $22\% \pm 6\%$ (control CPWDOM, $r^2 = 0.97$) of our predictions based on $\{\text{Cu}^{2+}\}$. These residuals fall within the 1%–34% range of relative residuals in DOM-free NODOC A and NODOC B tests (Fig. 1). In contrast, treatments with higher binding-site densities for which we estimated lower mortality because of their 30%–50% lower $\{\text{Cu}^{2+}\}$, our predictions (lower curves in Figs. 2c, 2d) underestimated observed mortality by $43\% \pm 11\%$ (mean \pm SD; control LRDOM, $r^2 = 0.66$) to $51\% \pm 9\%$

Fig. 1. Composite concentration–response curve for fathead minnow (*Pimephales promelas*) larvae exposed to Cu in toxicity tests NODOC A (■) and NODOC B (○), which were conducted in matrix waters to which dissolved organic matter was not added. The solid line is the modeled concentration–response curve predicted from logit transformations of the mortality data regressed on the natural logarithm of the cupric ion activity ($\{\text{Cu}^{2+}\}$) (eq. 2: $m = [\exp(a \ln\{\text{Cu}^{2+}\} + b)]\{1 + [\exp(a \ln\{\text{Cu}^{2+}\} + b)]\}^{-1} \times 100$), where $a = 1.52034$, and $b = 2.94985$.



(irradiated CPWDOM, $r^2 = 0.83$). Moreover, the $\{\text{Cu}^{2+}\}_{\text{LC50}}$ of these two treatments are significantly lower than that calculated for the average $\{\text{Cu}^{2+}\}_{\text{LC50}}$ determined for larval FHM in toxicity tests without DOC (NODOC A and NODOC B; Table 5), as demonstrated by the lack of overlap in the 95% confidence intervals and results of t tests (irradiated CPWDOM t test = 3.36 and $p = 0.021$; control LRDOM t test = 3.01 and $p = 0.032$).

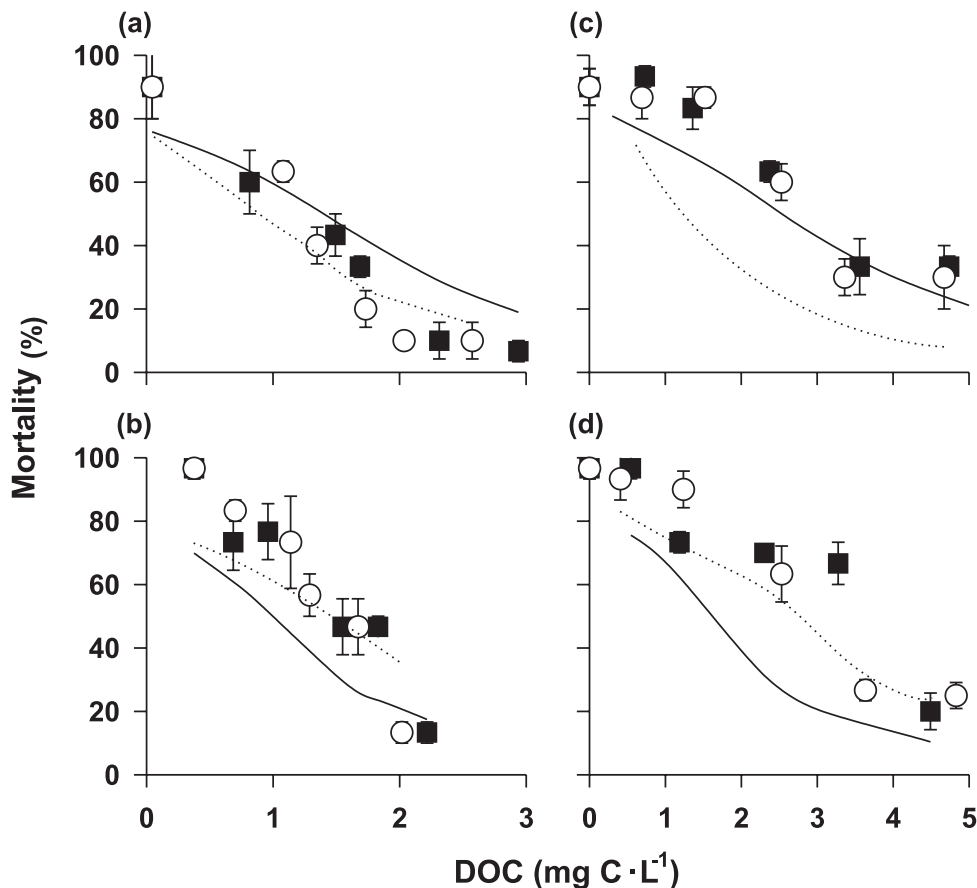
Discussion

Photochemical effects on Cu bioavailability

Because Cu toxicity is dominated by Cu^{2+} (e.g., Chakoumakos et al. 1979; Laurén and McDonald 1986; Erickson et al. 1996), we expected mortality of larval FHM would increase as $\{\text{Cu}^{2+}\}$ increased in the otherwise constant water quality of the toxicity tests containing DOM (TOX 1 and TOX 2). Toxicity tests conducted in DOM treatments with low binding-site density and higher $\{\text{Cu}^{2+}\}$ coincided with predictions. However, our predictions were an average of ~45% below observed mortality in treatments with higher binding-site densities and 30%–50% lower $\{\text{Cu}^{2+}\}$.

These results present an interesting quandary. Regardless of source, the DOM treatments that bound the most Cu (i.e., having the lowest $\{\text{Cu}^{2+}\}$) were no more protective against Cu toxicity than the DOM treatments with lower binding-site densities that had higher $\{\text{Cu}^{2+}\}$. This quandary is illustrated by virtually identical $[\text{DOC}]_{\text{LC50}}$ values among treatments within the same test despite significant differences in their corresponding $\{\text{Cu}^{2+}\}_{\text{LC50}}$ values. Possible explanations

Fig. 2. Concentration–response curves from toxicity test TOX 1 for (a) dissolved organic matter (DOM) from Chimney Park wetland (CPWDOM) and (b) DOM from Laramie River (LRDOM), and from toxicity test TOX 2 for (c) CPWDOM and (d) LRDOM, comparing the lethality of a constant [Cu] in the presence of unirradiated DOM (■) or in the presence of DOM that was irradiated for 72 h in a solar simulator (○). Water chemistry for these toxicity tests are listed in Tables 1 and 4. Calculated LC50s are shown in Table 5. The curves are estimated mortalities using eq. 2 with $\{Cu^{2+}\}$ calculated in MINTEQA2 for each concentration of dissolved organic carbon (DOC) in irradiated DOM (broken line) and unirradiated DOM (solid line). Error bars are \pm SE.



for this inconsistency are (i) lack of a good fit between actual $\{Cu^{2+}\}$ and those predicted by MINTEQA2 modeling, (ii) random variability within or among toxicity tests, and (iii) Cu species other than $\{Cu^{2+}\}$ that contributed to toxicity. The first explanation is unlikely, because in both irradiated and unirradiated treatments of CPWDOM and LRDOM, MINTEQA2-estimated $\{Cu^{2+}\}$ were within $9\% \pm 1\%$ (mean \pm SE; range 0.2%–17%) of actual $\{Cu^{2+}\}$ measured with an ion-selective electrode across a Cu titration range of 1.6×10^{-7} to $1.7 \times 10^{-5} \mu\text{mol}\cdot\text{L}^{-1}$ into $5 \text{ mg DOC}\cdot\text{L}^{-1}$ (Brooks et al. 2007). Regarding random variability in toxicity tests, toxicity data commonly differ by twofold between predicted and observed values (e.g., fig. 5 in Santore et al. 2001). However, our calculations consistently underpredicted observed mortality in treatments with low $\{Cu^{2+}\}$ (i.e., irradiated CPWDOM and unirradiated LRDOM), suggesting a systematic rather than random difference.

In addition to Cu^{2+} , Cu–inorganic complexes might have contributed to Cu toxicity. A number of researchers have reported that $CuCO_3^0$ (Shaw and Brown 1974) and $Cu(OH)_x$ species appear to contribute to toxicity (e.g., Chakoumakos et al. 1979; Laurén and McDonald 1986; Meador 1991). However, it is unlikely that $Cu(CO_3)_x$ or $Cu(OH)_x$ species contributed substantially to toxicity in our study for several reasons.

First, in the presence of high [DOM] and circumneutral pH, dissolved inorganic ligands bound relatively little of the Cu. For example, MINTEQA2 calculations showed that inorganics complexed less than 2% of Cu ($\sim 0.03 \mu\text{mol}\cdot\text{L}^{-1}$) at the highest [DOC] having the greatest binding-site density and the lowest $\{Cu^{2+}\}$ in each toxicity test (i.e., the irradiated CPWDOM and unirradiated LRDOM). In the two highest [DOC] exposure solutions, mortality tended to remain constant at $\sim 10\%$ (TOX 1) and $\sim 15\%$ (TOX 2). Thus, constant mortality in the presence of increasing concentrations of organics and decreasing concentrations of $Cu(CO_3)_x$ and $Cu(OH)_x$ species, which lowered $\{Cu^{2+}\}$, indicates that some fraction of Cu complexed by DOM was either directly toxic or enhanced the uptake of Cu^{2+} .

This conclusion is consistent with findings that $\{Cu^{2+}\}$ alone is not a precise indicator of toxicity in the presence of Aldrich humic acid (a commercially available organic matter of terrestrial origin, Erickson et al. 1996). In that study, the $\{Cu^{2+}\}_{LC50}$ decreased linearly as [DOC] increased, indicating that a constant percentage of Cu bound to Aldrich humic acid was bioavailable.

Copper–organic complexes might enhance Cu bioavailability via several mechanisms: (i) alleviation of diffusion limitation of Cu^{2+} from the exposure water to the biotic

Table 5. Concentrations of dissolved organic carbon ($[\text{DOC}]_{\text{LC50}}$) and cupric ion activity ($\{\text{Cu}^{2+}\}_{\text{LC50}}$) at 50% mortality (means with 95% confidence intervals) for 96 h static-renewal toxicity tests (TOX 1 and TOX 2), containing unirradiated or irradiated dissolved organic matter (DOM) from Chimney Park wetland (CPWDOM) or Laramie River (LRDOM).

DOM source	Experiment	Irradiation time (h)	$[\text{DOC}]_{\text{LC50}}$ ($\text{mg}\cdot\text{L}^{-1}$)	$\{\text{Cu}^{2+}\}_{\text{LC50}}$ ($\mu\text{mol}\cdot\text{L}^{-1}$)
CPWDOM	TOX 1	0	1.45 (1.26–1.63)	0.22 (0.17–0.27)
		72	1.44 (1.29–1.58)	0.13 (0.10–0.17)
	TOX 2	0	2.42 (1.92–2.92)	0.12 (0.09–0.18)
		72	2.98 (2.38–3.58)	0.05 (0.04–0.07)* [†]
LRDOM	TOX 1	0	1.10 (0.78–1.41)	0.10 (0.09–0.12)
		72	1.12 (0.88–1.36)	0.17 (0.16–0.19)*
	TOX 2	0	3.08 (2.61–3.55)	0.06 (0.04–0.08) [†]
		72	2.79 (2.39–3.22)	0.13 (0.10–0.16)*
Matrix water	NODOC A	NA	NA	0.13 (0.10–0.18)
	NODOC B	NA	NA	0.26 (0.18–0.34)
	Mean NODOC	NA	NA	0.17 (0.10–0.24)

Note: Total [Cu] was constant in TOX 1 and TOX 2 (Table 1), whereas [Cu] varied in NODOC A and NODOC B (range: 0.01–2.9 $\mu\text{mol}\cdot\text{L}^{-1}$; Brooks 2003). $[\text{DOC}]_{\text{LC50}}$ was calculated with TOXSTAT. From the resulting average [DOC] and confidence intervals, we calculated corresponding $\{\text{Cu}^{2+}\}$ in MINTEQA2. For toxicity tests without DOC (NODOC A and NODOC B and the average of both), $[\text{DOC}]_{\text{LC50}}$ was not applicable (NA). In those tests, we calculated $\{\text{Cu}^{2+}\}$ at each total [Cu] (Table 2) in MINTEQA2 and then determined the $\{\text{Cu}^{2+}\}_{\text{LC50}}$ with TOXSTAT.

* $p < 0.05$; compares control (0 h) with irradiated treatments (72 h).

[†] $p < 0.05$; compares the absolute difference between $\{\text{Cu}^{2+}\}_{\text{LC50}}$ in TOX 1 or TOX 2 ($\{\text{Cu}^{2+}\}_{\text{LC50-TOX 1 or 2}}$) versus the mean $\{\text{Cu}^{2+}\}_{\text{LC50}}$ for mean NODOC in bottom row.

membrane via direct exchange of Cu between the Cu–organic complexes and binding sites on the membrane, (ii) alteration of permeability of the biotic membrane, and (iii) passage of intact Cu–organic moieties across cell membranes. Regarding the first mechanism, most bioaccumulation studies indicate that diffusion delivers aqueous Cu^{2+} and other Cu species to the surface of the biotic ligands more quickly than the maximal potential rate of biologic uptake (Campbell 1995; Grosell and Wood 2002). A disjunctive pathway could overcome this bottleneck. By this pathway, Cu–organic complexes diffuse rapidly into the boundary layer, then Cu dissociates from the Cu–DOM complex to equalize the chemical gradient in $\{\text{Cu}^{2+}\}$ induced by rapid biological internalization of Cu^{2+} at the biotic surface. Thus, the direct movement of exchangeable Cu from a Cu–organic complex to a binding site on the membrane surface could enhance the rate of Cu supply. In a supply-limited transport system, any increase in the rate of Cu supply could greatly increase the rate of Cu uptake. If this mechanism applied to all Cu–organic complexes, then metal uptake would be insensitive to Cu speciation. Our results indicate that few, if any, Cu–organic complexes contributed to Cu toxicity in experiments with high $\{\text{Cu}^{2+}\}$ and low carbonyl concentrations (i.e., unirradiated CPWDOM and irradiated LRDOM). However, in experiments with low $\{\text{Cu}^{2+}\}$, the diffusion limitation was potentially alleviated by Cu dissociation from a limited fraction of Cu–organics. Given that acidification of the gill boundary layer has been shown to alter ion exchange (Wilson et al. 1994), it is possible that acidic moieties on DOM alter the boundary layer and allow direct Cu passage.

Another possible explanation for greater bioavailability of Cu in some treatments is alteration of the biotic ligands via interaction between organic matter and the biological recep-

tor. For example, increased metal uptake occurs in the presence of natural organics that alter the negative surface charge of algae (*Chlorella pyrenoidosa*, *Synechococcus leopoliensis*) and isolated fish gill cells (*Salmo salar*; Campbell et al. 1997) or increases membrane permeability of an alga *C. pyrenoidosa* (Parent et al. 1996). Although our data do not preclude them, these mechanisms have not been demonstrated with in vivo studies in fish.

Finally, passage through membranes of metals bound to commercial organics as metal–lipophilic complexes occurs in rainbow trout (*Oncorhynchus mykiss*; Block et al. 1991) and several algal, invertebrate, and fish species (reviewed in Campbell 1995). We did not test this possibility with our natural DOM, and thus cannot reject it.

Although other researchers report enhanced uptake of Cu bound to LMW organics such as amino acids, nitrilotriacetic acid, citrate, or ethylenediamine (Campbell 1995; Errecalde et al. 1998), this study did not correlate mortality with photoproduction of the LMW organic acids (acetic, citric, lactic, malic, oxalic, and succinic). In CPWDOM, constant concentrations of LMW organic acids during irradiation indicated that they did not contribute to increased Cu bioavailability in toxicity tests with irradiated CPWDOM. In LRDOM, irradiation increased the concentration of LMW organic acids by 70%. However, the unirradiated LRDOM treatments with low concentrations of these LMW organic acids apparently had the highest concentrations of bioavailable Cu–organic complexes. Thus, it appears that at radiation intensities approximating those of ambient sunlight, photoproduction of the LMW organic acids neither correlated with nor contributed to Cu bioavailability. Our findings are consistent with those of Playle et al. (1993), who showed no apparent transport of Cu by LMW organics in adult FHM

Table 6. Ratio of dissolved organic carbon concentration in all binding sites ([DOC] of $[L_{TOT}]$) to [DOC] concentration in C-II region of ^{13}C NMR spectra ([DOC] of C-II) in unirradiated (0 h) and irradiated (72 h) solutions of dissolved organic matter (DOM) from Chimney Park wetland (CPWDOM) and Laramie River (LRDOM) (data from Brooks et al. 2007).

DOM source	Irradiation time (h)	[DOC] of $[L_{TOT}]$ ($\mu\text{mol C}\cdot\text{L}^{-1}$)	[DOC] of C-II ($\mu\text{mol C}\cdot\text{L}^{-1}$)	C-II/ $[L_{TOT}]$
CPWDOM	0	36	76	2.1
	72	72	165	2.3
LRDOM	0	57	107	1.9
	72	25	60	2.4

at environmentally relevant concentrations of ethylenediaminetetraacetic acid, nitrilotriacetic acid, ethylenediamine, citrate, or oxalic acid.

Possible photochemical mechanism for bioavailable Cu-organic complexes

Based on ^{13}C nuclear magnetic resonance (^{13}C NMR) spectra collected during an earlier study of photochemical effects on CPWDOM and LRDOM (Brooks et al. 2007), the proportions of carbonyls on ketone and aldehyde carbons (C-II groups) in both DOM types were positively correlated with both L_1 ($r^2 = 0.83$, $p = 0.10$, $n = 4$) and L_2 ($r^2 = 0.92$, $p = 0.02$, $n = 4$). In CPWDOM, photooxidation apparently increased Cu complexation by producing new binding sites on C-II groups, whereas photolysis apparently removed this class of ligands in LRDOM. Although absolute changes in the fraction of C associated with ketones and aldehydes were small, twice as many C atoms occurred in C-II groups as the density of Cu-binding sites. Assuming a conservative average of ≤ 6 C atoms per binding site (Leenheer et al. 1998), they were adequate to account for at least one-third of the change in density of Cu-binding sites, (Table 6). This class of oxy-functional ligands is located in the most downfield, unshielded region of ^{13}C NMR spectra, representing moieties that are highly electronegative, probably because of their association with aromatic groups. Therefore, it is logical that small changes in the relative proportions of these ligands would impact the Cu binding-site densities of DOM. Because mortality did not differ between irradiated and unirradiated DOM in TOX 1 and TOX 2 tests despite significant differences in $\{\text{Cu}^{2+}\}$, we conclude that Cu-ketone or Cu-aldehyde complexes might act as Cu transporters by altering the microenvironment of biologic receptors, directly exchanging Cu with binding sites on membranes, or by passing through membranes as intact Cu-organic complexes. For example, Campbell et al. (1997) showed that binding of Al-DOM complexes to algal cells affected membrane permeability and postulated that direct exchange could occur. In addition, Errécalde et al. (1998) demonstrated active uptake of Cd- and Zn-citrate complexes by citrate transporters. Although plausible, this conclusion is tenuous because we base it on Cu toxicity in the presence of DOM from only two sources before and after irradiation.

Metal-binding sites are oxygen-rich groups on substituents of aromatics, such as ethers, esters, ketones, and aldehydes (Leenheer et al. 1995, 1998; Brooks et al. 2007). Different photochemical mechanisms related to the initial chemical composition of DOM from allochthonous versus

autochthonous sources might explain the production (wetland DOM) and loss (riverine DOM) of DOM ligands that could act as Cu transporters. Compared with allochthonous DOM, autochthonous DOM derived from algae, macrophytes, and bacteria contains more proteins and lipids, resulting in lower ratios of aromatic to aliphatic components (McKnight and Aiken 1998). Consistent with higher lipid fractions, Brooks et al. (2007) report that CPWDOM had a much lower aromatic to aliphatic ratio than that of LRDOM (Table 3, 0.13 versus 0.53 prior to irradiation). In CPWDOM, we propose that irradiation might have created bioavailable Cu-DOM complexes by polymerization and photooxidation of polyunsaturated lipids that produces aldehydes and is the proposed mechanism for production of humic substances in marine environments (Harvey et al. 1983; Kieber et al. 1997).

Allochthonous DOM contains lignin, which is only produced by vascular plants and is not found in algae or bacteria. Degradation of allochthonous plants releases low amounts of proteins and lipids compared with relatively high carbohydrate concentrations. Dissolved lignin in allochthonous DOM contains a variety of oxygen functional substituents (McKnight and Aiken 1998) on an array of phenolic compounds such as syringyl and vanillyl phenols with oxygen-rich links (Castellan et al. 1987; Opsahl and Benner 1995). Photodegradation of allochthonous DOM cleaves ester and ether linkages, removes ketones and aldehydes, and photomineralizes the majority of the lignin (Castellan et al. 1987; Opsahl and Benner 1993, 1998), thus decreasing aromaticity and removing oxy-functional substituents (Brooks et al. 2007). We propose that photodegradation destroyed the apparently bioavailable Cu-DOM complexes in LRDOM, possibly by cleaving ketones and aldehydes (C-II groups) from the lignin-rich, allochthonous DOM. Photochemical cleavage of oxy-functional groups coincided with, and might explain, the 70% increase in LMW organic acids in the riverine DOM.

In conclusion, whether sunlight interacts with DOM to create or deplete metal-binding sites varies among sources of DOM. In addition, the apparent photochemical production or loss of DOM fractions that act as Cu-organic transporters is source dependent. At coarse resolution, $\{\text{Cu}^{2+}\}$ alone is a general predictor of Cu bioavailability. However, at a finer level of resolution, it appears that some Cu-organic complexes — possibly carbonyls in ketones and aldehydes in DOM — appear to facilitate transport of Cu across cell membranes, which explains why some Cu-DOM complexes apparently contribute to toxicity. Additional research is

needed to (i) clarify the mechanisms by which Cu bio-availability increases, particularly to larval life stages, (ii) improve predictions of Cu toxicity in the presence of DOM from different sources with varying histories of photooxidation, and (iii) to examine the long-term effects of intensifying photooxidation of DOM due to global increases in ultraviolet radiation.

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