

HETEROGENEITY IN PHYSICOCHEMICAL PROPERTIES EXPLAINS DIFFERENCES
IN SILVER TOXICITY AMELIORATION BY NATURAL ORGANIC MATTER
TO *DAPHNIA MAGNA*CHRIS N. GLOVER,* SONIA K. SHARMA, and CHRIS M. WOOD
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Abstract—Recently collected data have shown that natural organic matter (NOM) source is an important parameter influencing the toxicity of silver to the freshwater crustacean *Daphnia magna*. The present study attempted to correlate the physicochemical properties of 11 naturally isolated and commercially available NOM sources with their ameliorative effects. The protection offered by these samples was standardized to the protective effect of Aldrich humic acid using geochemical modeling approaches that accounted for associated changes in water chemistry and, consequently, silver speciation. The protective ability of NOM was not correlated with reactive sulfide or nitrogen content, which are considered to be strong silver-binding ligands. Color (specific absorbance coefficient) was positively correlated with protection but narrowly eluded statistical significance. The peak wavelength of emission fluorescence following excitation at 370 nm and the fluorescence index values of NOM samples were significantly correlated with protective effects, suggesting that aromatic carbon content may govern the ameliorative actions of NOM. Simple optical properties may therefore act as a suitable indicator for the ability of a given NOM to protect against waterborne silver toxicity to *D. magna* as long as changes in water chemistry and, thus, silver speciation, are considered.

Keywords—Biotic ligand model Dissolved organic matter Humate Fulvate Natural organic matter source

INTRODUCTION

As our understanding of the physiological mechanisms responsible for toxicological effects of environmental contaminants has improved, so too has our ability to predict toxicological thresholds and biological impacts. Physiological characterization of silver (Ag) toxicity to aquatic animals, for example, has shown that only the free ionic form of the metal (Ag^+) is toxic [1,2]. This effect can be explained by passage of the ion across uptake epithelia as a sodium mimic [3], with subsequent inhibition of sodium metabolic pathways [4,5].

Incorporation of this mechanistic information with enhanced geochemical characterization of water bodies has led to important advances in our understanding of environmental Ag toxicity. Natural organic matter (NOM) is a component of all natural waters; has a high affinity for metals, such as Ag ($\log K = 9.0\text{--}9.2$ [6]); and as such, acts to prevent interaction of the toxic Ag ion with the processes responsible for sodium influx. Thus, the exclusion of NOM from laboratory tests probably has overestimated the biological risk posed by Ag in natural environments [7].

The merging of physiological studies with geochemical analyses has facilitated the creation of site-specific risk assessment tools, such as the biotic ligand model (BLM) [8–11]. This approach utilizes information regarding the complexation capacities and competitive interactions of toxic metals, such as Ag, with water-chemistry components. For Ag, the major complexing components of natural waters are reduced sulfides, chloride, and NOM, whereas sodium ions compete with Ag uptake and thus ameliorate toxicity by virtue of their shared uptake pathway. Using this information, derived from geochemical constants and physiological mechanisms, the toxicity

of Ag can be predicted on a site-specific basis. Until now, however, modeling approaches have generally assumed that all NOMs have equivalent protective abilities. The results of several recent studies now argue against this. Toxicity of both copper [12] and Ag [13] toward the highly sensitive freshwater cladoceran *Daphnia magna*, for example, is highly dependent on both the concentration and the origin of the NOM that is present. The qualities of NOM responsible for this differential protection are yet to be established, but indications suggest that optical properties may correlate with ameliorative ability [12], at least for copper.

The ability to attribute heterogeneous protection with a simply measured parameter of the NOM would provide an excellent surrogate for determining the potential risk that a given body of water may pose to aquatic life if contaminated with toxic metals (see, e.g., [12]). Using the toxicological information in the companion study [13], the differential protection against Ag toxicity to *D. magna* offered by 11 commercially available and naturally isolated NOM samples was correlated to various physicochemical properties of the NOMs. The aim of the present study was to discern a simple surrogate measure for incorporation into modeling approaches that will refine these models and increase their ability to accurately predict the environmental risk of aquatic contaminants such as Ag.

MATERIALS AND METHODS

Determination of NOM source and concentration

Details of the NOM samples are given in the companion study [13]. Nordic Reservoir NOM, Nordic Reservoir fulvic acid, Nordic Reservoir humic acid (NRH), and Suwannee River NOM were all obtained as freeze-dried powders from the International Humic Substances Society (St. Paul, MN, USA). Aldrich humic acid (AHA; sodium salt) was also obtained from

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a commercial source (Sigma-Aldrich, St. Louis, MO, USA) as a freeze-dried powder. Black Creek NOM (BCN; ON, Canada), Grand River NOM (GRN; ON, Canada), Dundas Wastewater Treatment Plant NOM (DSN; ON, Canada), Luther Marsh NOM (LMN; ON, Canada), Rochester Wastewater Treatment Plant (R(I) and R(II); NY, USA) were all collected directly from these water bodies via reverse osmosis [13]. The R(I) and R(II) samples were collected from the same body of water at different times.

The companion study [13] also describes the method of determining NOM concentration. Briefly, dilution series from each commercial NOM stock and from LMN were created, and total organic carbon concentration was measured (Shimadzu TOC analyzer 5050A; Mandel Scientific, Guelph, ON, Canada). The absorbance (300 nm) was measured in an identical duplicate series, and a standard curve was created. Subsequent organic carbon concentrations were determined by measuring absorbance in each NOM sample. The concentration of other NOM samples (BCN, GRN, DSN, R(I), and R(II)) was determined by the known dilution of a measured stock solution.

Ion analysis

Calcium, magnesium, and sodium levels were determined in NOM stocks via flame atomic absorption spectrometry (220FS; Varian, Palo Alto, CA, USA). Chloride was determined spectrophotometrically according to the method described by Zall et al. [14]. Because of interference caused by the color of NOM stocks, care was taken to dilute stocks for chloride analysis as much as possible and to apply a suitable blank before addition of the color substrate.

Toxicological modeling

The toxicological data set used for this analysis was taken from the accompanying study [13], analyzing the differential protective effects of NOM samples on Ag toxicity to *D. magna*. Two different geochemical speciation models were used: MINEQL⁺ (Ver 4.5 [15]) and the BLM (Ver AP08 [8]). The BLM approach utilizes the Chemical Equilibria in Soils and Solutions (CHESS) program incorporating the Windermere Humic Aqueous Model (WHAM), a geochemical speciation model that distinguishes between humic and fulvic content, and it treats these components differently. When the humic content is unknown, a default setting of 10% humic content is recommended. This recommendation was applied herein. The model was run in speciation mode. Modeling via MINEQL⁺ used a binding constant (log *K*) of 7.5 for the interaction between Ag and the humic substance, as derived for *D. magna* by Bury et al. [11]. It was assumed that 35 nmol of Ag-binding sites exist per milligram of NOM carbon content [6,9]. Other modeling constants were those that came standard with each program.

To obtain an indication of the protective ability of each NOM sample relative to AHA, a relative protective unit concept was applied. The Ag concentration and the water-chemistry conditions at the median lethal concentration (LC50) of each tested NOM source and level were used to model the toxic fraction of Ag that resulted in 50% mortality. For the BLM approach, the toxic fraction was considered as the Ag-gill component, a measure that accounts for competition effects (sodium) as well as complexation effects. The possibility that AgCl-gill is toxic to *D. magna* has not been adequately investigated; thus, the gill Ag chloride output was not included

as part of the toxic fraction. Indeed, preliminary calculations showed that its inclusion into the toxic fraction produced only minor changes in relative protective units (data not shown). For the MINEQL⁺ approach, the toxic fraction was considered to be the free Ag (Ag⁺). Consequently, MINEQL⁺ modeling accounted only for the complexation effects.

The equivalent toxic fraction was then obtained for AHA at that NOM concentration. This was derived from the regression analysis of the AHA data, which provided a concentration of Ag at the LC50 value for the NOM level of interest. This Ag concentration was then substituted into the models. The ratio of the NOM to AHA toxic fraction resulted in an indication of the relative protective power of a given NOM sample. Samples with a ratio greater than 1 offer more protective effect per milligram of carbon compared to that afforded by AHA, whereas NOM samples with a ratio less than 1 offer less protection than AHA at an equivalent concentration. When more than one concentration of an NOM sample was examined, the ratios were averaged. Final ratios were always within twofold of the individual ratio values.

Nitrogen and sulfide analysis

Samples were analyzed for total nitrogen content using a total nitrogen analyzer (7000V; Antek Industries, Houston, TX, USA). To account for the possible contribution of bacterially produced ammonia in stock samples, the nitrogen analysis was repeated after samples had been purged of ammonia. Purging was facilitated by the addition of 1 N KOH to NOM stocks (raising pH to ~11 and favoring gaseous ammonia formation), which were bubbled with air for 8 h to drive off gaseous ammonia [16].

Sulfide content of NOM samples was determined according to the chromium reducible sulfur method [17]. Because of limited substrate availability, only the reverse-osmosis isolates were subjected to this analysis.

Specific absorbance coefficient

The relative color of the NOM samples was ascertained by application of the specific absorbance coefficient (SAC) [18,19]:

$$\text{SAC}_{300} \text{ (cm}^2 \text{ mg/C)} = \frac{(2.303 \cdot \text{Abs}_{300}) / (\text{path length})}{[\text{NOM}] / 1,000 \text{ cm}^3}$$

The wavelength of 300 nm was the wavelength at which peak absorbance occurred. The NOM concentration was expressed in terms of mg C/L. Color determinations were performed using an ultraviolet/visible spectrophotometer (Ultrspec 4054; LKB, Bromma, Sweden).

370-nm emission scans

This protocol was adapted from that described by McKnight et al. [20]. The NOM stocks were diluted to 10 mg C/L and adjusted to pH 10 using 0.1 N KOH. Samples were scanned using a luminescence spectrometer (LS 50B; PerkinElmer, Norwalk, CT, USA) at a scanning speed of 120 nm/min. Emission was monitored over a wavelength range of 400 to 650 nm following excitation at 370 nm. Blank spectra (deionized water, >17.5 MΩ-cm, pH 10; Barnstead Nanopure II, Dubuque, IA, USA) were subtracted from all NOM-sample scans to account for matrix effects. These experiments were repeated with adjustment of samples to pH 2 to eliminate the possibility that the effects at pH 10 were influenced by fluorescence

Table 1. Ion content of natural organic mater (NOM) stock solutions

	Ion content ($\mu\text{mol}/\text{mg C}$)			
	Ca ²⁺	Cl ⁻	Mg ²⁺	Na ⁺
Black Creek NOM ^a	31	91	17	24
Green River NOM ^a	61	765	111	199
Rochester Wastewater Treatment Plant (I) ^b	48	674	46	219
Rochester Wastewater Treatment Plant (II) ^b	43	1,092	73	348
Dundas Wastewater Treatment Plant ^a	6	1,005	7	110
Luther Marsh NOM ^a	0	29	0	0
Aldrich humic acid ^c	2	11	1	17
Nordic Reservoir fulvic acid ^d	0	5	0	0
Nordic Reservoir humic acid ^d	0	16	0	0
Nordic Reservoir NOM ^d	0	54	0	26
Suwannee River NOM ^d	0	24	0	1

^a Reverse-osmosis isolate collected from Ontario, Canada.

^b Reverse-osmosis isolate collected from New York, USA.

^c Freeze-dried sample obtained from Sigma-Aldrich (St. Louis, MO, USA).

^d Freeze-dried sample obtained from International Humic Substances Society (St. Paul, MN, USA).

quenching resulting from metals associated with each sample [21].

RESULTS

Toxicity data obtained in the companion study [13] were subjected to a geochemical modeling exercise. Because only Ag⁺ is considered to be toxic, the speciation of Ag in each test was of considerable importance. Consequently, an analysis of the major inorganic ions influencing Ag speciation and Ag uptake was performed (Table 1). Of particular note was the high chloride content of some reverse osmosis-isolated stocks. The R(II) and DSN samples, for example, had chloride levels greater than 1 mmol/mg C. By accounting for the high chloride levels and the resulting increase in protection caused by the formation of nontoxic Ag chloride species, the relative protective capacity of DSN was reduced from a value approximately fivefold greater [13] to one approximately equivalent to that offered by AHA (Fig. 1). Similarly, the protective effect of R(II) was reduced by half, from 130 to 65% of the protection offered by AHA at an equivalent organic carbon concentration.

Accounting for the water-chemistry contributions of each NOM sample, significant protective advantages were still discerned (Fig. 1). A commercially available NOM, NRH, offered a fivefold greater protection than AHA, whereas a naturally isolated NOM, LMN, protected *D. magna* from Ag toxicity more than 3.5-fold better than AHA. Four samples (three reverse-osmosis isolates and one commercially available sample) had protective effects that were less than that of AHA, with the GRN sample protecting only half as well.

Figure 1 indicates that few differences existed between the two modeling approaches used. The BLM approach utilizes a more complex calculation for assessing the effect of NOM on Ag complexation and, under the conditions used here, also incorporated a factor accounting for Ag-sodium competition for uptake. The BLM approach resulted in protective unit values that averaged 95% of those derived by the MINEQL⁺ modeling. Values reported throughout the text are those obtained from MINEQL⁺ modeling. Likewise, MINEQL⁺ values were used for all correlation analyses described herein. The BLM-derived values were quantitatively similar and gave results for statistical significance identical to those of MINEQL⁺ values.

The modeling approach took the dissolved (Ag⁺) values at the LC50 for each concentration of each sample. If the ob-

served changes in toxicity amelioration were simply the result of altered water chemistry, then it would be expected that an identical free Ag ion (Ag⁺) activity would have resulted from the modeling of each NOM value. However, this was not the case (Fig. 2), suggesting that the observed heterogeneity in NOM samples was not a consequence of inorganic ion alteration of free Ag⁺ (i.e., chloride complexation) but, rather, a function of the organic matter itself. Similar results (not shown) were achieved when the toxic fraction derived from BLM modeling (Ag-gill) was plotted against Ag concentration at the LC50. These results indicate that the different protective properties result not from differences in water chemistry, but

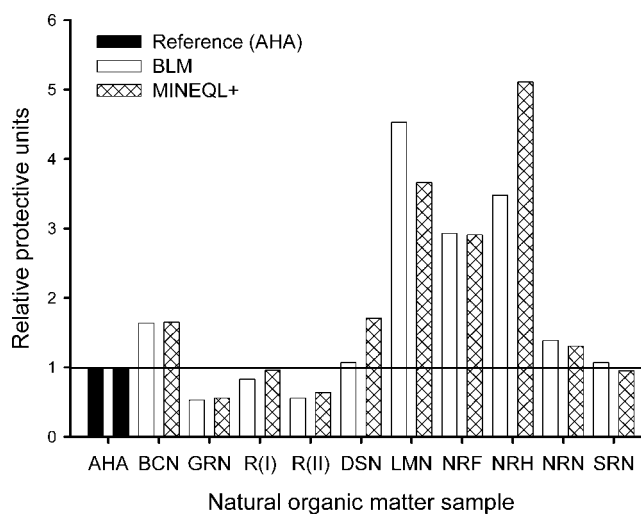


Fig. 1. Relative protection against Ag toxicity to *Daphnia magna* offered by 11 commercial and naturally isolated natural organic matter sources as determined by geochemical modeling approaches using the biotic ligand model (BLM; white bars) or MINEQL⁺ (Environmental Research Software, Hallowell, ME, USA; hatched bars) relative to the protection offered by Aldrich humic acid (AHA; black bars). The modeling approach is detailed in *Materials and Methods*. Natural organic matter (NOM) sources with bars greater than one (reference line) are more protective than AHA, while those with bars less than one are less protective. BCN = Black Creek NOM; GRN = Grand River NOM; R(I) and R(II) = Rochester Wastewater Treatment Plant NOM; DSN = Dundas Wastewater Treatment Plant NOM; LMN = Luther Marsh NOM; NRF = Nordic Reservoir fulvic acid; NRH = Nordic Reservoir humic acid; NRN = Nordic Reservoir NOM; SRN = Suwannee River NOM.

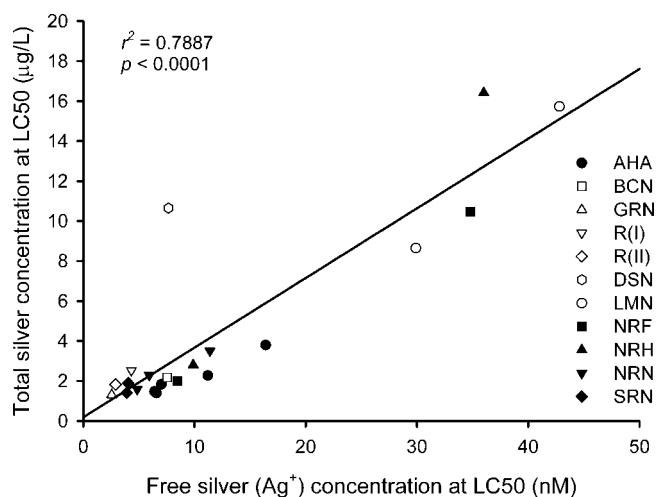


Fig. 2. Apparent relationship between the amount of free silver ion (Ag^+) and total Ag ($\mu\text{g/L}$) at the *Daphnia magna* 48-h median lethal concentration (LC_{50}) for 11 commercial and naturally isolated natural organic matter sources. The Ag^+ concentration was calculated via MINEQL⁺ (Environmental Research Software, Hallowell, ME, USA). Significance and r^2 values were obtained directly from correlation analyses using SigmaPlot (Ver 8.02; SPSS, Chicago, IL, USA). See Figure 1 for acronyms used.

rather, from differences in the Ag^+ -binding properties of the various NOMs.

Correlation analyses compared the relative protective ability calculated from the MINEQL⁺ modeling exercise to the raw values obtained for the various physicochemical assays performed. These analyses showed that the protective ability of NOM samples was not correlated to the nitrogen (11 sources, $r^2 = 0.1925$, $p = 0.1770$) or sulfide (six sources, $r^2 = 0.1046$, $p = 0.5517$) content of the sample (not shown). Results from a subsequent experiment in which NOM samples were purged of ammonia before nitrogen analysis revealed a similar trend, indicating that binding effects of the NOM samples themselves were not masked by dissolved ammonia concentrations. In fact, samples that had been purged of dissolved ammonia exhibited values that were statistically indistinguishable from those of nonpurged samples, indicating negligible dissolved ammonia content. Likewise, a follow-up sulfide experiment was performed to ensure the results from the reported experiment were not compromised by delays between the sulfide and toxicity analyses. Again, no correlation between protective effect and sulfide content was observed.

In contrast, the specific absorbance coefficient (color) of NOM samples exhibited a positive linear correlation with protective effect (Fig. 3). This relationship was not, however, significant ($p = 0.0789$). However, exclusion of the AHA data point (see *Discussion*) greatly improved both the fit ($r^2 = 0.6790$) and the significance ($p = 0.0034$) of the correlation.

Emission scans of the NOM samples yielded a number of different patterns (Fig. 4A and B). Similar patterns were observed for all naturally isolated samples, with the exception of LMN, which exhibited a smaller emission peak shifted to a longer wavelength. Of the commercially available samples, AHA and NRH displayed flat, broad curves, with the NRH curve being offset to the right. The wavelength at which the peak fluorescence intensity occurred was correlated with protective ability (Fig. 4C). This relationship was positive and significant ($p < 0.05$), with an increasing peak wavelength corresponding to an increased ability to protect against Ag

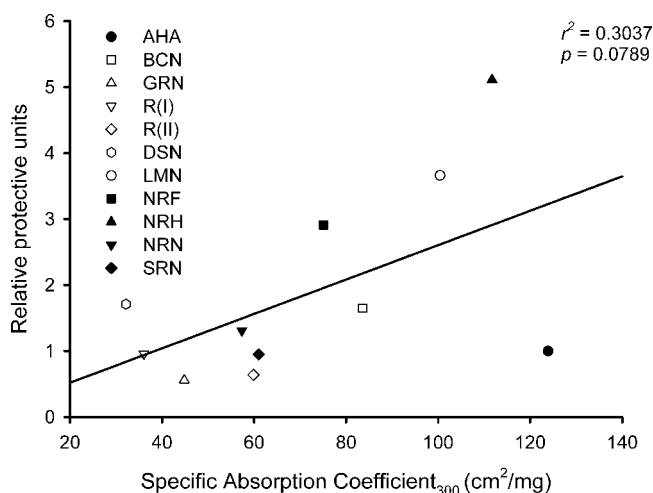


Fig. 3. Relationship between specific absorbance coefficient at 300 nm (SAC_{300}) and relative protective ability of 11 commercial and naturally isolated natural organic matter sources against Ag toxicity to *Daphnia magna*. The relative protective units and the SAC_{300} were calculated as described in *Materials and Methods*. Significance and r^2 values were obtained directly from correlation analyses using SigmaPlot (Ver 8.02; SPSS, Chicago, IL, USA). See Figure 1 for acronyms used.

toxicity to *D. magna*. Similar patterns were observed when samples were analyzed at pH 2 (data not shown), suggesting that the observed effects were independent of pH and metal quenching.

Results from the emission scans also were used to determine the fluorescence index of each NOM sample [20]. This was calculated as the ratio of fluorescent intensity at wavelengths of 450 and 500 nm. The fluorescence index was significantly negatively correlated with protection ($p < 0.05$) (Fig. 5). Increasing fluorescence index values correlated with decreased ameliorative effects on Ag toxicity to *D. magna*.

DISCUSSION

Correlation of toxicity amelioration with optical properties of NOM

The heterogeneity of the ameliorative effects of NOM on Ag toxicity to *D. magna* was governed by the optical properties of the NOM. Protective ability was positively correlated with the peak wavelength of emission following excitation at 370 nm and was inversely correlated with fluorescence index. These findings suggest that these optical properties could be used as surrogate measures for predicting the protective effect of a NOM toward Ag toxicity to aquatic organisms.

The optical properties that exhibited the strongest correlation with protective abilities were those indices that reflected the aromaticity of the NOM sample. The peak wavelength of fluorescence emission following excitation at 370 nm is likely characteristic of aromaticity [22], whereas McKnight et al. [20] showed a strong, inverse correlation between fluorescence index and aromaticity. This suggests that the relative protective abilities of NOM samples may be a function of aromatic content.

Results of some studies suggest that both color and aromaticity are higher with increased molecular mass of NOM fractions [23], suggesting that this may be a determining factor accounting for the observed patterns. This is consistent with evidence that Ag associates preferentially with higher-molecular-weight fractions of dissolved organic material [24]. These

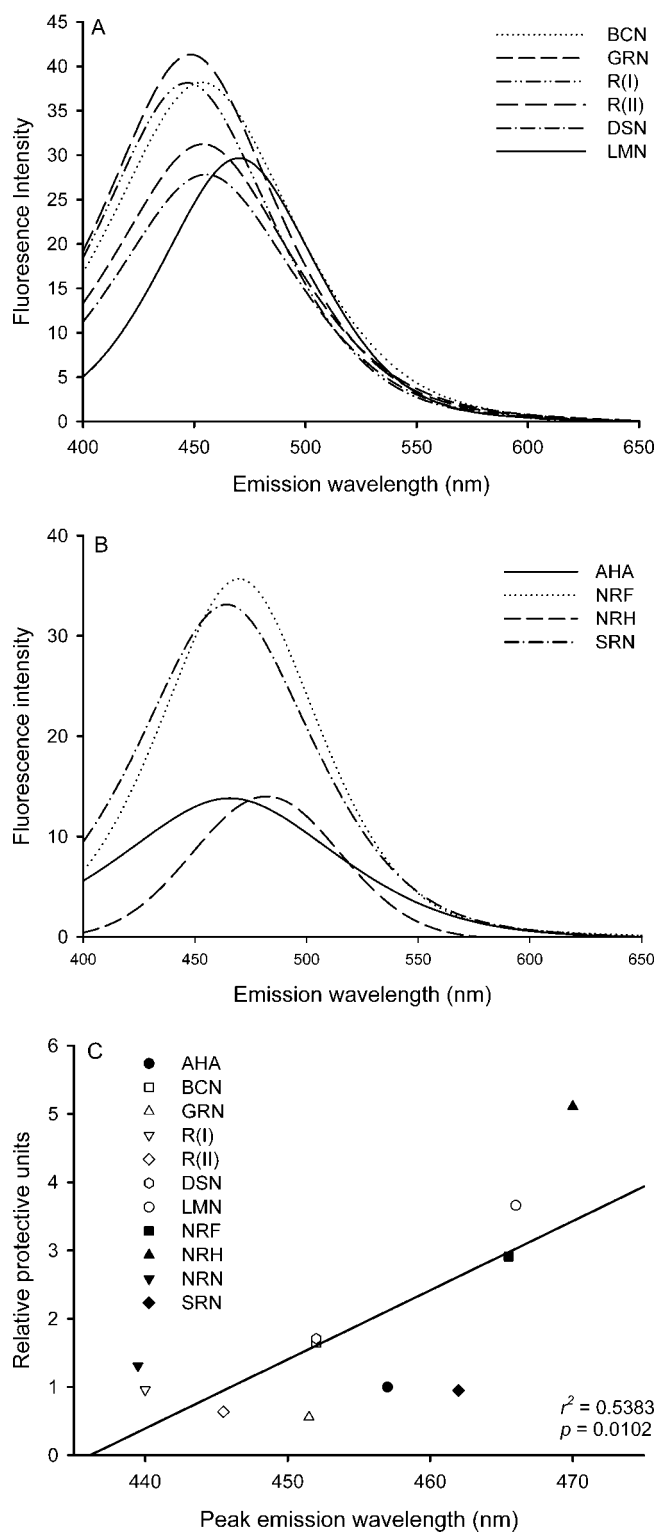


Fig. 4. Emission scans (400–650 nm) of (A) naturally isolated and (B) commercial natural organic matter sources following excitation at 370 nm and (C) the relationship between the wavelength of peak emission following excitation at 370 nm and relative protective ability of these natural organic matter sources against Ag toxicity to *Daphnia magna*. Significance and r^2 values were obtained directly from correlation analyses performed using SigmaPlot (Ver 8.02; SPSS, Chicago, IL, USA). See *Materials and Methods* for more details, see Figure 1 for acronyms used.

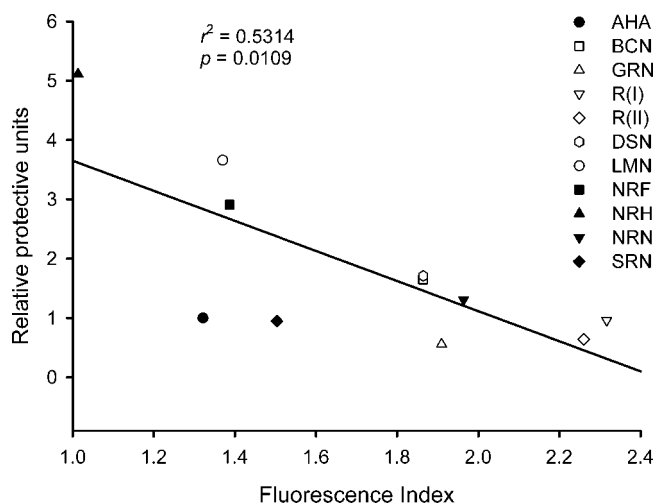


Fig. 5. Relationship between the fluorescence index and relative protective ability of 11 commercial and naturally isolated natural organic matter sources against Ag toxicity to *Daphnia magna*. The relative protective units and fluorescence index were calculated as described in *Materials and Methods*. Significance and r^2 values were obtained directly from correlation analyses using SigmaPlot (Ver 8.02; SPSS, Chicago, IL, USA). See Figure 1 for acronyms used.

observations suggest that the higher aromaticity of molecular-weight fractions may explain their greater capacity for binding Ag and, thus, their ability to prevent the free ion (Ag^+) from interacting with the site of toxic action, and they are in line with the evidence presented here.

A strong relationship exists between these optical properties and toxicity amelioration, but the possibility exists that this relationship may not be causal. Instead, optical properties and protective properties could covary with another mitigating factor [25]. This is unlikely in the present study, because other potential ameliorating agents in NOM samples were investigated (sulfide, nitrogen, and chloride content).

Previous investigations of the effect of NOM source

The finding that optical properties correlate well with toxicity amelioration is not without precedent. A study investigating the influence of NOM source on the toxicity of metals to rainbow trout [26] noted that the fluorescence and absorbance properties associated with aromaticity were strong candidates for predicting the ability of a given NOM to prevent gill metal binding, a surrogate measure of toxicity. This finding was recently supported by another study from the same laboratory [19]. Similarly, Haitzer et al. [27] positively correlated the aromaticity of NOM samples with their ability to reduce polycyclic aromatic hydrocarbon bioconcentration, whereas copper accumulation rates in mussels were inversely correlated with carbon content, an indirect indicator of aromaticity [28]. These studies all examined surrogate toxicity measures (toxicant binding/accumulation) with NOM properties. A recent paper by De Schamphelaere et al. [12] correlated the optical properties of NOM samples directly with copper toxicity measurements and determined a significant relationship between ultraviolet absorbance and copper-complexing capacity. The present results also are based directly on toxicity and encompassed a range of differing NOM samples, which was large enough to establish significant correlations. It is intriguing to note that although copper (phenolic groups [29]) and Ag (nitrogen and/or sulfide groups [30,31]) are considered to bind

to different functional groups, optical properties may predict the protective effects of NOM regarding both metals.

The considerable heterogeneity in physicochemical properties between the different samples used here, and an approach that accounted for altered water chemistry, may explain the distinct, source-dependent amelioration reported in the present study versus the weaker effect of NOM source reported in a recent study investigating Ag toxicity to fathead minnows [32]. Results also likely depend on the sensitivity of the organism used and, therefore, on the concentration range of Ag used in the tests, with much stronger NOM-source effects being noted in studies involving the highly sensitive invertebrate *D. magna* (present study and [12]) as opposed to the less marked influences on studied fish species [26,32,33]. Nevertheless, most investigations indicate that optical properties may be useful for determining the protective effect of a given NOM. This assumes that the potential caveats to these approaches, such as altered water chemistry and low sensitivity at low NOM concentrations, are duly considered [34].

Lack of correlation of toxicity amelioration to sulfide or nitrogen content

The lack of correlation between toxicity and strong ligand binding in the present study suggests that sulfide and nitrogen groups of the NOM samples were saturated by the levels of Ag required for toxicity. In natural waters, both organic and inorganic sulfide components will be present; consequently, the levels of Ag rarely, if ever, exceed the capacity of sulfide sequestration [35,36]. As such, the results derived here may not be representative of natural waters, in which reduced sulfides with higher Ag-binding affinity ($\log K = 12\text{--}21$ [31]) may buffer most of the Ag in the system. In such a scenario, NOM may only act to mop up Ag spillover. As a result, the strong binding ligands of the NOM (sulfide [31] and nitrogen [30]) may be better correlates of protection in these waters.

The results from the sulfide analysis should, however, be interpreted with care. The measured sulfide levels were very low, and the sample size was small ($n = 6$), resulting in a low statistical power for the correlation analysis. The importance of sulfide ligands in NOM binding of Ag requires more rigorous assessment.

Modeling considerations

Naturally isolated NOM samples offer several advantages over commercially available sources, but inorganic ions associated with these samples may distort toxicity relationships. In the case of Ag, the key contaminant is chloride. Silver has a relatively weak affinity for chloride ($\log K = 3$ [37]), but the association results in the formation of neutral Ag chloride complexes and a subsequent decrease in the toxic, free Ag⁺ level. As such, the decline in toxicity observed with NOM incorporation into test waters could be the result of Ag chloride formation and not a result of the complexation of Ag by the NOM. This was demonstrated clearly in the present study. In the companion study [13], the protection offered by DSN was approximately fivefold greater than that afforded by AHA, whereas after accounting for changes in water chemistry, virtually no difference was observed between these samples in terms of their protective capacity. In fact, the protection offered by most real-world samples in the previous paper was considerably decreased once water chemistry was considered by virtue of their generally high chloride contents (Table 1). Therefore, water chemistry must be carefully considered when

investigating differential effects of NOM source on metal toxicity.

The heterogeneity of the protective effects observed were not simply a function of altered water chemistry. Modeling Ag⁺ at the Ag concentration required to obtain 50% mortality after 48 h should result in a single value of the free ion that results in toxicity. The strong positive correlation noted in Figure 2 argues against this proposition and, therefore, indicates that the differences observed are the result of differences between the NOM samples themselves. It also suggests that modeling approaches using a single binding constant for all NOM samples may be poor predictors of Ag toxicity amelioration. The present results thus support the recommendation that a weighting factor, based on optical properties, should be incorporated into modeling scenarios [12,19,26,34].

The two modeling approaches used in the present study, BLM and MINEQL⁺, produced similar results (Fig. 1). Consequently, the more rigorous treatment of NOM-binding sites offered by the WHAM model incorporated into the BLM approach (multiple NOM-binding sites and $\log K$ values) offered little advantage over the more simplistic approach used in MINEQL⁺ (single $\log K$). Thus, use of a weighting factor accounting for optical properties likely offers the best means of incorporating the heterogeneity of NOM source into predictive models.

Commercial versus natural NOM sources

The usefulness of commercial sources of NOM as surrogates for naturally isolated samples has been questioned [38]. Under the conditions of the present study, the commercially available sources appeared to behave in a manner similar to that of the reverse-osmosis isolates, implying that these samples are adequate for use in toxicological studies.

Aldrich humic acid may be the exception. This sample usually fitted poorly to correlation analyses (see, e.g., Figs. 3 and 5), and all correlation analyses were improved by the exclusion of this data point. For example, despite somewhat similar optical properties, it provided only a fraction of the protection offered by NRH. Despite the frequent use of AHA in studies examining the toxicity of metals, such as Ag, in the aquatic environment (see, e.g., [11,39]), this NOM is thought to be terrestrial in origin [38]. Consequently, the chemical properties of this sample differ considerably from those described for aquatic NOM sources [38,40]. Therefore, results from studies that have relied on this NOM as a representative aquatic humic substance should be interpreted with caution.

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