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Complexation between Hg(II) and dissolved organic matter in stream waters: an application of fluorescence spectroscopy

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Abstract. Complexation between Hg(II) and dissolved organic matter (DOM) collected from streams in Ontario, Canada, was studied using three-dimensional excitation emission matrix (3DEEM) fluorescence spectroscopy. The results show that DOM reacted with Hg(II) rapidly, and the complexation reached pseudo-equilibrium within 20 s. Maximum excitation/emission (Ex/Em) wavelengths shifted towards the longer wavelengths, indicating that DOM structure changed during its interaction with Hg(II). Using fluorescence quenching titrations, complexing parameters, conditional stability constants and the percentage of fluorophores participating in the complexation, were estimated by the modified Stern–Volmer equation. The experimental and field survey results suggest that the Hg–DOM complexation in various streams was related to water quality parameters, e.g. DOC, Cl^-, and cation concentrations, and was strongly affected by UV irradiation.

Introduction

In aquatic environments, the complexation between trace metals and natural DOM has been widely investigated since DOM has a high affinity with these metals. In many cases, DOM complexation controls the speciation, toxicity, bioavailability and ultimate fate of the metals (Bidoglio and Stumm 1994). Molecular fluorescence spectroscopy, particularly three-dimensional excitation emission matrix (3DEEM) fluorescence spectroscopy, has been used often to investigate the complexation between DOM and metals, such as copper and aluminum, due to its simplicity and sensitivity (Ryan and Weber 1982; Smith and Kramer 1999; Wu et al. 2001; Wu and Tanoue 2001a, b). Fluorescence intensity of DOM is either enhanced or decreased when it is bound to metals; this is dependent on metal speciation, binding sites or ligands of fluorophores and several other factors, e.g. pH, ionic strength, anions and cations (Ryan and Weber 1982; Cabaniss 1992; Esteves da Silva et al. 1998; Lu and Jaffe 2001; Wu et al. 2001; Wu and Tanoue 2001a, b).

In many areas, mercury (Hg) contamination in fish is a major concern, resulting in the issuing of advisories or the closing of lake fisheries in North America. Consequently, many investigations have focused on the fate and biogeochemical cycling of Hg in natural environments, and results indicate that DOM, particularly humic substances, plays a key role in its accumulation and transport in aquatic environments in general (Lee and Iverfeldt 1991; Fjeld and Rognerud 1993; Leermarkers et al. 1995; Watras et al. 1995; Yin et al. 1997). In lakes, a strong inverse relationship between fish Hg and DOC concentrations was reported by Fjeld and Rognerud (1993) and Watras et al. (1995). While these studies demonstrated the importance of DOM in mercury biogeochemistry, few have focused on the chemical complexation process and factors that influence the complexation. Lu and Jaffe (2001) investigated the interaction between Hg(II) and DOM collected from estuarine water samples of the Florida Everglades using emission and synchronous fluorescence methods, and experimentally examined the effect of pH, Cl^-, Ca^{2+}, Mg^{2+}, and $CaCO_3(s)$ on the complexation. The objective of the present study was to investigate the Hg complexation of DOM collected in headwater streams in Ontario using 3DEEM fluorescence spectroscopy. Both steady-state and kinetic fluorescence measurements were performed to evaluate the quenching of DOM and structural changes caused by the addition of Hg(II). Conditional stability constants and binding capacities were calculated using the modified Stern–Volmer equation (Esteves da Silva et al. 1998). Finally, field survey and UV exposure experiments were carried out to examine the possible effect of water quality parameters, e.g. cation and DOC concentrations, and UV irradiation on the complexation.

Experimental

Study site

The study sites used for the fluorescence quenching experiments are located in the District of Muskoka and Haliburton County on the southern edge of the Precambrian Shield of South-central Ontario, Canada. Water samples were collected from 13 headwater streams of five lakes (Dickie, Harp, Plastic, Blue Chalk and Red Chalk) between April and May 2002. The catchments ranged in area from 10 to 456 ha. The study sites are the focus of long-term studies on the impacts of long range transport of atmospheric pollutants (acids and trace metals), climate change, and shoreline development on water quality in forested catchments and lakes, and have been described in detail by Dillon et al. (1991), Dillon and Molot (1997) and Molot and Dillon (1997). For the kinetic and UV irradiation experiments, the water samples were collected from Miller Creek of Peterborough Country in Ontario between May and September 2002. All samples were filtered using 0.45 μm Whatman glass-fiber filters before analyses or further treatment.

Chemical analysis

The pH value of the water samples was determined using a pH meter (Accumet®, Model 825MP, Fisher Scientific). DOC concentrations were measured by a high-temperature catalytic oxidation method (TOC 5000, Shimadzu Co. Ltd, Japan) using potassium hydrogen phthalate as a standard. Cl^-, Ca^{2+} and Mg^{2+} concentrations were analyzed by ion chromatography.

Kinetics of the Hg–DOM complexation, and fluorescence quenching titrations

A spectrofluorometer (SPEX, Jobin Yvon, Instruments S.A. Inc.) was used for all fluorescence measurements. The spectrofluorometer was equipped with a 150-W Xenon short arc lamp and a multichannel charge coupled device (CCD) detector. It can be optimized to allow for a full Ex/Em 300/500 nm spectral acquisition in less than 1 s, thus making both kinetic and steady-state measurements possible. Ex wavelength was set from 250 to 550 nm, and Em from 250 to 700 nm, applying a bandwidth of 2 nm for both. A 0.25 mm slit width was chosen for both Ex and Em. Matlab™ software was used to obtain the 3DEEM surface and contour plots in which the maximum Ex/Em wavelengths can be identified.

In order to study the kinetics of the Hg–DOM complexation, kinetic binding runs were performed with a stopped-flow apparatus designed in the laboratory. The water sample and a 100 μM mercuric ion solution were pumped into a mixing chamber and then directly into the 25 μL quartz flow-cell of the spectrofluorometer for observation. The Hg–DOM complexation was monitored by measuring the 3DEEM fluorescence spectra every 7 s. The spectra over time were collected and manipulated using Grams/32c™ software. Two parameters, total fluorescence intensity and maximum Ex/Em wavelengths, were used to examine changes in the fluorescence spectra versus time in the complexation process. The total fluorescence intensity was measured at Ex/Em 350/440 nm, and maximum Ex/Em wavelengths were determined in each spectra.

Fluorescence quenching titrations were performed to characterize binding properties of the water samples with Hg(II). A mercuric nitrate solution was added incrementally to achieve a final concentration of approximately 150 μM. For a 1:1 stoichiometric model of Hg(II) complexation by DOM, the modified Stern–Volmer equation was used to evaluate the complexing parameters, i.e. conditional stability constants and binding capacities (Esteves da Silva et al. 1998; Lu and Jaffe 2001):

$$F_0/\Delta F = 1/(fK[\mathrm{Hg}]) + 1/f \tag{1}$$

where F and F_0 are the fluorescence intensities of the sample with and without the addition of Hg(II), respectively. $\Delta F = F_0 - F$, f is the fraction of the initial fluorescence that corresponds to the binding fluorophores, and K is the conditional stability constant. If the plot of $F_0/\Delta F$ versus 1/[Hg] is linear, K and f values can be estimated from the slope $[(1/(fK)]$ and the intercept $(1/f)$ (Esteves da Silva et al. 1998).

UV irradiation

A UV chamber was assembled utilizing Q-Panel lamps [4 × (UV-A 340) and 6 × (UV-B 313)] rated at 40 W. The chamber was enclosed within an environmental laboratory held at a constant temperature of 10 °C. UV-transparent 530 mL sterile polyethylene sample bags (Fisherbrand) were used to hold 100 mL filtered water samples, which had been collected from Miller Creek. The bags were completely transparent above 300 nm and had 90% transparency below 300 nm. While in the chamber, the bags were laid on their sides to maximize the exposure to UV radiation. Thus, the exposed surface of each filled bag was 14 × 7.5 cm with water a thickness of 0.8 cm. The samples were irradiated with 20.0 ± 7.3 W/m^2 of UV-A and 0.99 ± 0.26 W/m^2 of UV-B radiation. The incubation period was up to 8 days. Bags containing samples wrapped with foil were not under UV exposure, and were used as dark controls. The exposed and control water samples were then subjected to further fluorescence quenching experiments.

Results and discussion

Fluorescence properties of DOM of stream waters

Fluorescence spectroscopy is a useful tool to characterize DOM chemical properties in natural waters (Senesi 1990). 3DEEM fluorescence spectroscopy has been used successfully to probe the chemical structure of DOM because of its ability to distinguish among different classes of DOM of different origins (Mopper and Schultz 1993; Coble 1996; Wu et al. 2001; Wu and Tanoue 2001a, b). The fluorescence patterns of DOM samples in this study were similar, and exhibited one major fluorescence peak at Ex/Em 340–355/430–455 nm. These peaks are commonly seen for natural DOM samples, and are considered to originate from humic substances (Mopper and Schultz 1993; Coble 1996; Wu et al. 2001; Wu and Tanoue 2001a, b).

Table 1 illustrates the water quality parameters and fluorescence of 13 streams. There was a strong negative relationship between maximum fluorescence intensity and pH ($R^2 = -0.61$, $p < 0.001$), and a positive relationship with DOC ($R^2 = 0.80$, $p < 0.001$). The strong relationship between fluorescence and DOC has been reported previously in natural waters (Smart et al. 1976; Chen et al. 2002). For DOC, there was a strong negative correlation with pH ($R^2 = -0.55$, $p < 0.001$). This result is not totally consistent with that of Neary et al. (1990), who reported that high pH lakes tended to associate with

Table 1. pH values and concentrations of DOC, Cl, Mg and Ca, fluorescence and calculated binding parameters in streams

Samples[a]	pH	DOC ($\mathrm{mg\ L^{-1}}$)	Mg ($\mathrm{mg\ L^{-1}}$)	Ca ($\mathrm{mg\ L^{-1}}$)	Cl ($\mathrm{mg\ L^{-1}}$)	Fluorescence intensity (arb)	Log K	f (%)
HP3A	6.48	3.11	0.29	1.19	0.47	18,778	5.18	30.42
HP4	6.18	6.24	0.69	1.83	0.85	27,150	4.97	30.83
HP5	5.47	8.81	0.76	2.03	0.85	31,592	4.48	45.74
HP0	6.3	8.81	0.79	2.17	2.13	22,715	4.45	60.46
DE8	5.01	9.46	0.59	1.89	3.31	35,883	4.36	53.64
DE10	4.58	10.50	0.68	2.54	4.65	32,558	4.35	68.92
DE0	5.93	8.52	0.47	1.55	0.46	30,578	4.65	57.13
PC0L	5.41	3.30	0.65	1.43	1.20	9249	4.34	32.55
PC1-03	4.37	9.73	0.58	1.97	0.47	40,823	4.62	81.75
BC1	6.38	3.52	0.56	1.65	0.40	16,749	4.43	42.10
BCOF	7.01	2.99	0.51	1.59	0.35	5977	5.2	22.64
RC1	5.76	4.07	0.54	1.59	0.34	22,241	4.99	57.44
RC2	4.43	11.44	0.56	1.49	0.37	40,769	5.06	62.77

As shown in Equation 1, log K is the stability constant, and f is the fraction of the initial fluorescence that corresponds to the binding fluorophores.

[a] HP = Harp, DE = Dickie, PC = Plastic, BC = Blue Chalk, and RC = Red Chalk.

low DOC, while low pH varied widely in DOC in 777 lakes in Ontario. This is probably because the DOM in lakes has already received extensive UV exposure and has been greatly altered because of the relatively long flushing rates of lakes while the DOM in streams is relatively "new" (Molot and Dillon 1997). Thus, DOC concentrations tend to be much higher in streams, and DOM may play a more significant role in controlling the acidity of the samples. The observed correlation between DOC and pH in stream waters suggests that the acidity was probably due to various acidic functional groups such as carboxyl or due to humic substances. It has been reported that the fluorescence at Ex 300–400 nm and Em 400–500 nm is mainly associated with conjugated bond systems containing functional groups such as carboxyl groups (Senesi 1990). Therefore, the results here also suggest that humic substances and their acidic functional groups are mainly responsible for DOM fluorescence in the streams in the Dorset area.

Kinetics of the Hg–DOM complexation

Figure 1a exhibits the changes in total fluorescence intensity after mixing the sample with a mercuric ion solution using the stopped-flow apparatus. The fluorescence intensity decreased rapidly as the Hg–DOM interaction proceeded. The reaction reached pseudo-equilibrium after approximately 20 s. Previous studies have demonstrated that Rayleigh scattering can be used to monitor precipitate formation during metal titration as it is related to the number of particles in the solution (Ryan and Weber 1982). Rayleigh scattering measured at Ex = 450 nm simultaneously with the fluorescence intensity was constant (Figure 1a) over the time, indicating that no coagulation or precipitation formation was occurring. Therefore, it can be concluded that the fluorescence decrease was due solely to fluorescence quenching by Hg(II). It has been reported that the reaction between Hg(II) and DOM under static conditions is relatively slow because of the slow process of the replacement of Ca^{2+} bound to DOM by Hg(II) and of OH^- ligands by the organic ligand (Lu and Jaffe 2001). However, the results here (Figure 1) indicate that the interaction was more rapid than previously reported. Although mercury is considered to be a weak quencher due to its high atomic weight compared to copper (Saar and Weber 1980), the complexation between Hg(II) and DOM observed in our study was similar to that between Cu(II) and fulvic acid in a previous study. In that study, the binding of copper to fulvic acid was found to be fairly rapid, and the reaction was virtually at equilibrium after approximately 20–30 s (Lin et al. 1995).

In order to examine the possible changes in the fluorescence patterns during the Hg–DOM complexing process, differential spectra were obtained by subtracting from the spectra when pseudo-equilibrium was reached. There was only one major fluorescence peak in each differential spectra (data not shown). Figure 1b shows the changes in Ex/Em wavelengths of the fluorescence peak in the differential spectra over a period of 100 s. Both Ex and Em wavelengths increased by up to Ex/Em 40/25 nm in the first 20 s, and after that they remained stable. At present, little is known about structural changes during the Hg–DOM interaction since previously these reactions were monitored as fluorescence intensity changes of one or multi-components by emission or synchronous fluorescence spectra (Lin et al. 1995; Lu and Jaffe 2001). Fluorescence intensity gives no information regarding the structural nature. The observed changes in maximum Ex/Em wavelengths indicate the occurrence of structural transitions of DOM during its complexation with Hg. The possible structural changes include conformational changes or changes in rigidness as DOM binds to Hg.

Table 2. Changes in fluorescence and estimated complexing parameters of a Miller Creek water sample (collected in September 2002) versus UV irradiation time (days). As shown in Equation 1, log K is the stability constant, and f is the fraction of the initial fluorescence that corresponds to the binding fluorophores.

Days	f (%)	Log K	Fluorescence intensity (arb)
0	36.9	4.9	35,689
1	36.5	4.6	25,899
2	30.4	4.6	12,353
3	20.5	4.8	11,669
4	20.7	4.3	11,023
5	19.5	4.7	6113
6	9.5	4.1	5444
7	13.1	4.3	4067
8	14.3	4.1	5177

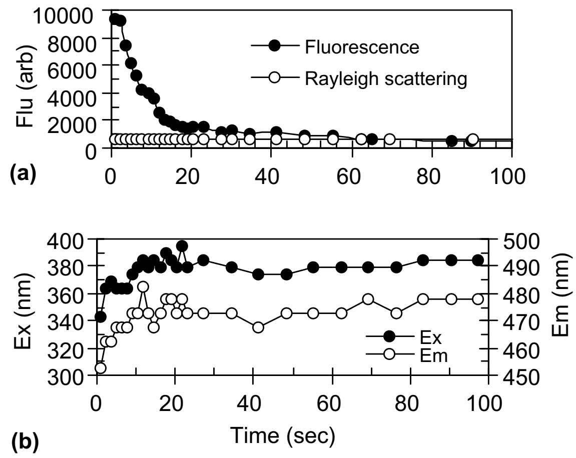

Figure 1. Fluorescence kinetics in the Hg–DOM complexation: (a) changes in fluorescence intensity at Ex/Em 350/440 nm and Rayleigh scattering at Ex = 450 nm with time; (b) changes in Ex and Em wavelengths of the fluorescence peak of the differential spectra with time. The water sample was collected from Miller Creek in June 2002.

Complexation of DOM by Hg(II)

3DEEM spectra of the water samples in the absence of Hg(II) and in the presence of increased Hg(II) concentrations were recorded. The fluorescence spectral patterns from the water samples were similar and all showed a decrease in fluorescence intensity as Hg(II) was added. This is consistent with fluoresence quenching of DOM by Hg(II) in a previous study (Lu and Jaffe 2001) and by other metals, e.g. Cu(II) and Pb(II) (Saar and Weber 1980).

Figure 2 exhibits the changes in fluorescence intensity as a function of Hg(II) in some water samples. When the titration data were fitted to the modified Stern–Volmer equation (Esteves da Silva et al. 1998), strong linear correlations between $F_0/\Delta F$ and 1/[Hg] were observed, as shown in Figure 3, thus conditional stability constants and binding capacities could be calculated (Table 1). The stability constants estimated for all streams were similar, ranging from 4.4 to 5.2 with an average value of 4.6 (Table 1), suggesting that the major composition of binding sites may be similar among all water samples. The constants obtained in this study are close to those (4.7) for soil-derived humic substances determined using an iodide selective electrode (Yin et al. 1997), and are also consistent with those for the natural DOM samples (4.1–4.8) and for DOM leached from periphyton, mangroves and sawgrass (4.2–5.3) in Everglades estuaries (Lu and Jaffe 2001). This indicates that fluorescence method can detect similar binding sites as other methods such as selective electrode, and Hg has a high affinity for DOM of different origins.

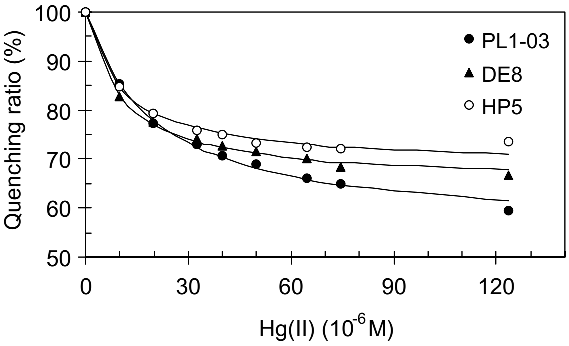

Figure 2. Mercury fluorescence quenching titration curves of some water samples in the Dorest area. DE: Dickie stream; HP: Harp stream; PL: Plastic stream.

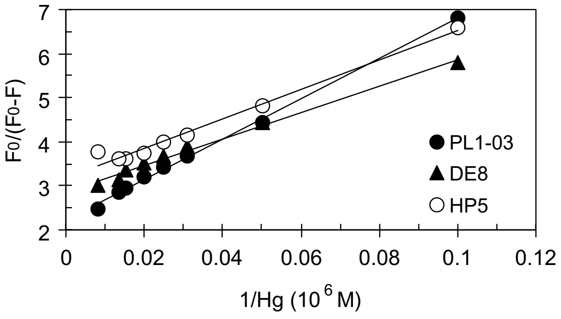

Figure 3. Plots of the modified Stern–Volmer equation. The DOM samples were the same as shown in Figure 2. *X* and *Y*-axis are referred to Equation 1.

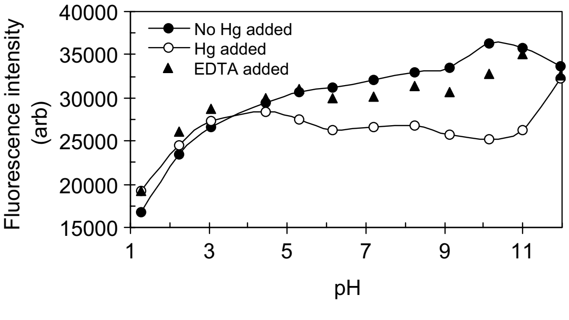

Figure 4. The influence of pH on the maximum fluorescence intensity of the water sample before and after the addition of 25 μM Hg(II). The sample was collected in Miller Creek in May 2002. 0.1 mM EDTA was added in the Hg–DOM complexes.

Figure 4 shows the effect of pH on the fluorescence intensity of the water sample before and after the addition of Hg(II). Above around pH = 5.0, the fluorescence intensity in the absence of Hg(II) was higher than that with the addition of Hg(II), indicating the fluorescence quenching by Hg(II). The increase of fluorescence intensity after the addition of EDTA in the Hg–DOM complexes indicates the release of Hg(II) from the complexes on to the EDTA, thus reconfirming what we observed in the Hg–DOM complexation. At approximately pH = 10, maximum fluorescence quenching occurred, indicating the strongest Hg–DOM complexation. Below pH = 4.5, the fluorescence intensity of the Hg–DOM complexes was almost the same as that of DOM in the absence of Hg(II), suggesting that no binding had occurred. This is probably because the protonation of acidic functional groups of DOM in low pH reduced its ability to complex with Hg(II) ions. DOM may be of minor importance in the Hg complexation in extreme acidic environments. These results are in agreement with two previous studies in soil and estuarine environments by Kalbitz and Wennrich (1998) and Lu and Jaffe (2001), who reported that the binding ability of humic substances and DOM to Hg(II) was greatly reduced at low pHs.

The relationship between Cl^-, cation and DOC concentrations and Hg complexation

As shown in Figure 5, log *K* dramatically increased in the DOM samples with low Cl^- concentrations. This observation can be explained by the influence of high concentrations of $Cl^-_{(aq)}$ on the formation of $HgCl^+_{(aq)}$ or $HgCl^0_{2(aq)}$, thus decreasing the binding affinity beween Hg and DOM. This result supports previous experimental studies (Lovgren and Sjoberg 1989; Lu and Jaffe 2001), and observations from estuarine environments (Leermakers et al. 1995; Lu and Jaffe 2001), which showed that Cl^- affected Hg(II) complexation with the

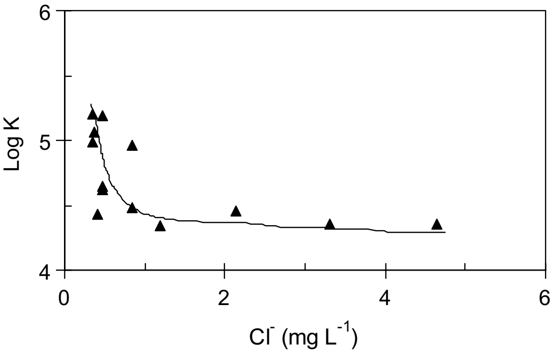

Figure 5. The relationship between log *K* value and Cl^- concentration in streams in the Dorset area.

DOM. Leermakers et al. (1995) and Lu and Jaffe (2001) reported that the binding capacity for Hg(II) decreased with incresing salinity in estuarine waters. Nevertheless, the results here suggest that the stability constant decreased with increasing Cl^- concentration even in the dilute stream headwaters.

The competing effect of cations, Ca^{2+} and Mg^{2+}, on the complexation of metal ions (Cu^{2+} and Al^{3+}) by fulvic acid has been reported (Cabaniss and Shuman 1988; Cabaniss 1992). For our 13 streams, Ca^{2+} ranged from 1.1 to 2.5 mg L^{-1}, and Mg^{2+} from 0.3 to 0.8 mg L^{-1}. High log K values appear to be related to low Ca^{2+} and Mg^{2+} concentrations (Table 1) and a negative relationship between log K and Ca and Mg concentrations was observed ($R^2 = -0.35$, $p < 0.1$ for Mg; $R^2 = -0.33$, $p < 0.1$ for Ca). This indicates that cations compete with Hg(II) ions for the binding sites on the DOM in natural streams. This field survey provides further support for previous experimental results (Lu and Jaffe 2001).

In terms of f values of the water samples, there was a strong negative relationship with pH ($R^2 = -0.57$, $p < 0.01$) and a positive relationship with DOC concentrations ($R^2 = 0.60$, $p < 0.01$). Water samples having higher f values tended to have a high DOC concentrations and low pH. Studies in European and North American lakes have shown that Hg concentration has a strong positive correlation with DOC concentration and a negative correlation with pH (Lee and Iverfeldt 1991; Watras et al. 1995). As discussed earlier, stream acidity is closely related to DOC concentration. The enhanced

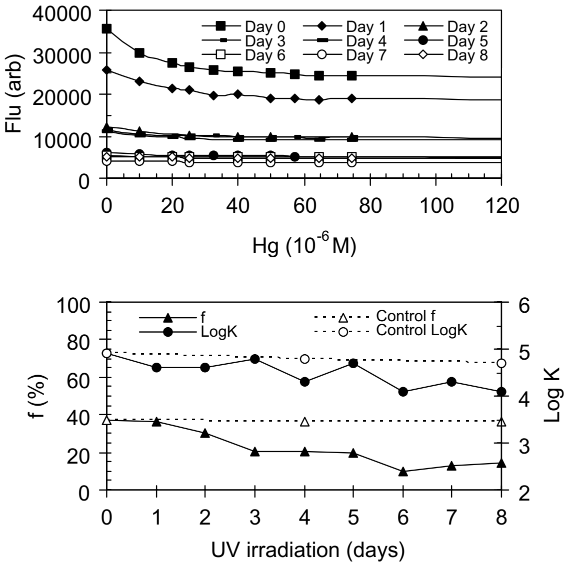

Figure 6. Influence of UV irradiation on the Hg–DOM complexation. The water sample was collected from Miller Creek in September 2002. (A) Fluorescence quenching titration curves, (B) estimated f and log K as a function of UV exposure.

Hg–DOM complexation in streams with high DOC and low pHs is considered to be due to the high proportation of functional groups or humic substances. The results in this study strongly suggest that geochemistry of Hg is dominated by its interactions with the DOM in streams in the Dorset area.

The effect of UV irradiation on the Hg–DOM complexation

Figure 6 shows the fluorescence quenching titrations and binding parameters of the water samples under different time periods of UV exposure. The fluorescence intensity and binding parameters are listed in Table 2. The results show that both K and f decreased with increasing UV irradiation; f and log K decreased from 37% and 4.9 in the original DOM sample to 14% and 4.1, respectively, after 8 days of UV irradiation, while the binding parameters remained relatively stable in the dark control samples. The significant role of UV light in the loss of and property changes of the DOM in natural aquatic environments has been previously established (Molot and Dillon 1997; Bertilsson and Tranvik, 2000). The loss in its ability to bind with Hg(II) after UV exposure may mainly be due to the breakdown of DOM molecules and/or the oxidization of functional groups. Under UV irradiation, the molecular size of DOM decreased and so its binding ability since the complexing affinity for metals has been shown to be related to the molecular size (Lin et al. 1995; Wu et al. 2002). Oxidization of the functional groups responsible for fluorescence quenching and binding for metals resulted in a decrease in the extent of quenching with increasing exposure to UV radiation (Kulovaara et al. 1996; Bertilsson and Tranvik 2000). The results of our study show that UV exposure resulted in a considerable decrease in the stability constant and the loss of binding sites.

Conclusion

This study demonstrates the usefulness of 3DEEM fluorescence spectroscopy to study the complexation between Hg(II) and DOM collected from headwaters. Results from both steady-state and kinetic fluorescence measurements indicate strong binding between Hg(II) and DOM. The fluorescence quenching titration method provides quantitative information on the complexation, while kinetic studies provide valuable information on the binding process such as equilibrium and possible structural changes.

This study also demonstrates that DOM–Hg interactions in headwaters are controlled by similar chemical parameters such as Cl^-, Ca^{2+}, Mg^{2+}, pH and DOC as reported in other very different environments (Kalbitz and Wennrich 1998; Leermakers et al. 1995; Lu and Jaffe 2001), and are strongly affected by UV radiation. However, those parameters are complicated in natural streams in that pH seems to be controlled by DOM itself and other water quality parameters may co-vary with DOM.

The results may have significant implications on the fate and transport of Hg in natural waters. The binding kinetics and chemical parameters can be potentially used for the calculation and modeling of mercury speciation and bioavailility in aquatic environments. Lakes and streams in Canadian Precambrian shield derive aquatic DOM from peat lands, and the transfer of this DOM from wetlands along the length of streams, and from streams to lakes may lead to alterations in DOM chemical and structural characteristics (Molot and Dillon 1997) and probably its binding with mercury, our results may be potentially used for the prediction of transport of Hg during these processes, and the UV irradiation experiments may indicate probable release of Hg(II) from DOM in these transits.

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