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Influence of natural dissolved organic carbon on the bioavailability of mercury to a freshwater alga

P.R. Gorski [a,b,*], D.E. Armstrong [a,b], J.P. Hurley [b,c], D.P. Krabbenhoft [d]

[a] Limnology and Marine Sciences Program, University of Wisconsin-Madison, Madison, WI, USA
[b] Environmental Chemistry and Technology Program, University of Wisconsin-Madison, Water Science and Engineering Laboratory, 660 North Park St., Madison, WI 53706, USA
[c] University of Wisconsin-Madison, Aquatic Sciences Center, 1975 Willow Drive, Madison, WI 53706, USA
[d] United States Geological Survey, 8505 Research Way, Middleton, WI 53562, USA

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Bioavailability of mercury to an alga was greatest at low concentrations of natural dissolved organic carbon and inhibited at high concentrations of natural dissolved organic carbon.

Abstract

Bioavailability of mercury (Hg) to *Selenastrum capricornutum* was assessed in bioassays containing field-collected freshwater of varying dissolved organic carbon (DOC) concentrations. Bioconcentration factor (BCF) was measured using stable isotopes of methylmercury (MeHg) and inorganic Hg(II). BCFs for MeHg in low-DOC lake water were significantly larger than those in mixtures of lake water and high-DOC river water. The BCF for MeHg in rainwater (lowest DOC) was the largest of any treatment. Rainwater and lake water also had larger BCFs for Hg(II) than river water. Moreover, in freshwater collected from several US and Canadian field sites, BCFs for Hg(II) and MeHg were low when DOC concentrations were >5 mg L^{-1}. These results suggest high concentrations of DOC inhibit bioavailability, while low concentrations may provide optimal conditions for algal uptake of Hg. However, variability of BCFs at low DOC indicates that DOC composition or other ligands may determine site-specific bioavailability of Hg.
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Keywords: Algae; Mercury; Methylmercury; Bioavailability; Dissolved organic carbon; Isotopes

1. Introduction

The bioavailability of monomethylmercury (MeHg) and inorganic mercury in the +2 oxidation state (Hg(II)) to aquatic organisms is influenced by speciation, i.e., the chemical forms of MeHg and Hg(II) in water. While the bioavailability of metals often involves active uptake of the ''free metal ion'' (Morel, 1983; Campbell, 1995; Di Toro et al., 2001), the uptake of Hg(II) and MeHg apparently involves the passive (Mason et al., 1996; Morel et al., 1998), facilitated, or active (Moye et al., 2002) transport of neutral, lipophilic complexes, such as CH_3HgCl, into the cell. Natural ''dissolved organic matter'' (DOM), often represented as ''dissolved organic carbon'' (DOC), plays an important role in controlling bioavailability because MeHg and Hg(II) form strong ''complexes'' with DOC, thereby influencing their speciation in natural waters. In fact, measurements of conditional stability constants (Hintelmann et al., 1997; Amirbahman et al., 2002; Haitzer et al., 2003; Karlsson and Skyllberg, 2003) and chemical modeling (Mason et al., 1996; Gorski et al., 2006) indicate that DOC should dominate the speciation of MeHg and Hg(II) in oxic (sulfide-free) waters when DOC concentrations are sufficiently high, reducing the bioavailability of both forms

* Corresponding author present address: Wisconsin State Laboratory of Hygiene, 2601 Agriculture Drive, Madison, WI 53718, USA. Tel.: +1 608 224 6294; fax: +1 608 224 6213.
E-mail addresses: gorskipr@slh.wisc.edu (P.R. Gorski), armstron@engr.wisc.edu (D.E. Armstrong), hurley@aqua.wisc.edu (J.P. Hurley), dpkrabbe@usgs.gov (D.P. Krabbenhoft).

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of Hg. While both Hg(II) and MeHg are bioconcentrated from water into the base of the foodweb by phytoplankton, the transfer to higher trophic levels is much greater for MeHg than Hg(II), apparently because MeHg accumulates in the cytoplasm of phytoplankton cells, while Hg(II) binds to cell membranes (Mason et al., 1996). However, evidence from field measurements and laboratory experiments indicates the influence of DOC on MeHg concentrations in aquatic foodwebs is complex.

Field measurements show that concentrations of Hg(II) and MeHg in water generally increase with increasing DOC concentrations (Driscoll et al., 1995; Babiarz et al., 2001), reflecting in part the importance of DOC in transport of MeHg and Hg(II) from uplands and wetlands into rivers and lakes (Hurley et al., 1995; Babiarz et al., 1998). While termed ''dissolved'', typically the material passing through a 0.4 μm filter, association of MeHg with DOM actually occurs in part with colloidal material in the filtrate (Babiarz et al., 2001). Concentrations of MeHg in biota also tend to increase with increasing DOC concentrations (Driscoll et al., 1995; Cleckner et al., 2003), perhaps because concentrations of MeHg in biota are related to MeHg concentrations in water. However, the bioconcentration factor, calculated as the ratio of concentrations in biota and water, tends to decrease with increasing DOC, indicative of an inhibitory effect of DOM on MeHg bioavailability (Driscoll et al., 1995).

Laboratory experiments demonstrate that strong ligands such as DOM can reduce the bioavailability of Hg(II) and MeHg to phytoplankton (Mason et al., 1996; Moye et al., 2002; Gorski et al., 2006), apparently because the complexes of Hg(II) and MeHg with DOC are too large or hydrophilic to pass through the cell membrane. However, DOC may enhance uptake (Pickhardt and Fisher, 2007), perhaps through association of DOM with cell surfaces which may facilitate adsorption of Hg species to the cells and/or promote internalization by influencing the permeability of the cell membrane (Vigneault et al., 2000; Boullemant et al., 2004).

We define bioavailable Hg as the amount that associates with the algal cells and do not distinguish between abiotic adsorption to the cell surface and internalization. In either case, the cell-associated Hg has the potential for transfer into food webs through consumption of the alga by organisms higher in the food chain, bioaccumulating mainly in the form of MeHg (Morel et al., 1998).

The primary goal of this investigation was to examine whether natural DOC limits the bioavailability of both Hg(II) and MeHg to algae. By using a bioassay approach described previously to assess bioavailability (Gorski, 2004; Gorski et al., 2004, 2006), we examined bioavailability of Hg to test alga in various field-collected waters of different DOC concentrations, and presumably, compositions. Size-fractionation into colloidal and dissolved fractions, using filtration and ultrafiltration was used to compare the influences of the materials in the colloidal and ''truly dissolved'' phases on the bioavailability of Hg to algae. Changes in bioavailability of Hg across the mixing zone of a tributary as it enters a large lake were also assessed to test the hypothesis that Hg is more bioavailable in lake water than river water. In addition, we investigated the bioavailability of Hg in rainwater and how bioavailability would change as rainwater is mixed with different receiving waters. Finally, samples of a wide variety of natural waters collected across the USA and Canada ($n = 27$), representing differing sources and concentrations of DOC from various ecological settings and aqueous matrices, were investigated to extend our understanding of the influence of DOC on the bioavailability of Hg in natural waters.

2. Materials and methods

2.1. Test organism

Batch cultures of the alga *Selenastrum capricornutum* (UTEX 1648) were used to assess Hg bioavailability to freshwater aquatic organisms. Algal cultures were maintained in Fraquil medium (Morel et al., 1978), using the nutrient modifications described by Price et al. (1989) and following protocols described previously (Gorski et al., 2004, 2006). The ratio of non-radioactive (stable) isotope concentrations in the medium (ng L^{-1}) and organisms (ag $cell^{-1}$) after a 24 h incubation period was used as the endpoint for calculating bioavailability. See Sections 2.6 or 2.7 for further specifics.

2.2. Bioassay design

For quality control purposes, each bioassay consisted of sets of Fraquil control flasks, which were at least duplicated (four sets, eight flasks total). The Fraquil used in this study was deficient in Cu, Zn and EDTA to prevent confounding ligand competition (see Gorski et al., 2006). The sets consisted of: Fraquil only; Fraquil and algae; Fraquil and Hg additions; and Fraquil, algae and Hg. These controls enabled us to monitor the behavior of the Fraquil, algae and mercury to identify and separate effects associated with field-collected water in treatment flasks.

Aliquots (450 mL) of field-collected water were poured into acid-leached 500 mL polycarbonate culture flasks. Note that recovery of Hg was previously shown to be excellent from acid-leached polycarbonate flasks (Gorski et al., 2004). Each site or water treatment (depending on experiment) consisted of a treatment control flask (no Hg additions) and a treatment spike flask. If enough volume was available, each treatment was replicated (two controls and two spike flasks per treatment). Each spike flask received ^{201}Hg(II) and ^{199}MeHg, and all flasks, spikes and controls, received 4000 cells mL^{-1} of *Selenastrum.* Flasks were incubated for 24 h, and measurements were taken of temperature and also cell density using a Coulter® EPICS XL™ flow cytometer (Beckman Coulter, Miami, FL, USA). A subsample (approximately 175 mL) was filtered separately for HgT and MeHg through ashed QFF filters held in Teflon filter towers, and filtrate was collected directly into Teflon bottles. Replicate aliquots were also filtered for DOC (about 30 mL through 25 mm diameter ashed GF/F filters with filtrate collected in ashed glassware). Dissolved organic carbon was analyzed on a Shimadzu (Kyoto, Japan) model TOC-V CSH total organic carbon analyzer with a Shimadzu AS1-V autosampler. Four repeated measures were analyzed from each sample and the relative standard deviation (RSD) was <2%.

2.3. Field collection sites

Samples for investigation of the influence of mixing of river water and Lake Superior water on Hg bioavailability were collected in the Tahquamenon Bay–Whitefish Bay region of Lake Superior, located in the Upper Peninsula of Michigan, USA. River water was collected from the mouth of the Tahquamenon River (TAQ), where the river empties into Tahquamenon Bay. Lake Superior water was obtained at Point Iroquois, Michigan, USA. The ''plume,'' or mixed site, was a 50:50 (volume:volume) mixture of water from the two sites.

Samples for investigation of the influence of mixing of rainwater with lake water were obtained at Devils Lake State Park, WI, USA. Rainwater was

collected in bins fitted with acid-cleaned Teflon liners. The samples were collected at the air monitoring station (USA - Mercury Deposition Network site #WI31) during a rainstorm on July 31, 2003. The rainwater was sieved (35 μm) to remove any debris or insects and then frozen. Water from Devils Lake was sampled from the south shore of the lake at 0.5 m depth using a peristaltic pump–Teflon tubing sampling system. Water from the Tahquamenon River site (see above) was used to investigate the influence of mixing rainwater and river water. The mixed samples (rainwater + Devils Lake Water) and (rainwater + Tahquamenon River Water) were 50:50 (volume: volume) mixtures of water from each respective site.

To extend our experiments to a more diverse range of aqueous matrix compositions, we acquired water samples from a variety of lakes and streams in the USA and Canada. Samples were collected by personnel from the United States Geological Survey (USGS) over a 1 week period using trace-metal clean techniques (Olson and DeWild, 1999). Sampling sites included national and provincial parks in the US and Canada, estuarine waters in San Francisco Bay, clear water streams in Oregon, dark water streams in Florida, and two locations in the Everglades (Table 1). All samples were filtered on site, shipped by express mail to the USGS Mercury Research Lab in Middleton, WI, USA, and refrigerated (approximately 1 week) until bioassay experiments were initiated.

2.4. Ultrafiltration

Water samples collected from the Tahquamenon River and Lake Superior and the river water–lake water mixture (“plume”) were subjected to ultrafiltration using clean techniques as described by Babiarz et al. (1997). The procedure provided three types of sample. The “filtrate” (<0.45 μm) contains all material passing through a 0.45 μm filter. The functionally named “concentrate” contains “colloidal” material in the 0.45 μm to 10 kDa size range (10 kDa is about 0.0015 μm). The “permeate” contains “truly dissolved” material (<10 kDa). The filtrate fraction represents the traditional “dissolved” fraction, which actually contains both dissolved and colloidal material. The concentrate fraction was diluted back to the ambient field concentration of DOC with deficient Fraquil medium before use in the bioassay experiments.

Table 1
Water types or sites represented in the multi-site experiment

DOC (mg L^{-1})	Water type	Designation	State or province	Country
1.2	657	San. Fran. Bay Delta	CA	USA
1.2	Fraquil	Lab Control Medium	–	–
1.3	Cutoff Slough	San. Fran. Bay Delta	CA	USA
1.3	Beaverton Creek	NAWQA (USGS site)	OR	USA
2.3	FT I17	San. Fran. Bay Delta	CA	USA
2.7	E.F. Dairy Creek	NAWQA (USGS site)	OR	USA
2.8	Lookout Creek	NAWQA (USGS site)	OR	USA
3.2	Little Trout Lake	Voyageurs Nat. Park	MN	USA
4.3	Oak Creek	NAWQA (USGS site)	WI	USA
4.7	Winnange Lake	ELA	Ontario	Canada
5.2	Mukooda Lake	Voyageurs Nat. Park	MN	USA
5.3	Pike River	NAWQA (USGS site)	WI	USA
8.2	Brown Lake	Voyageurs Nat. Park	MN	USA
8.3	L239	ELA	Ontario	Canada
8.5	L115	ELA	Ontario	Canada
9.0	Oslo Lake	Voyageurs Nat. Park	MN	USA
9.5	St. Mary’s River	NAWQA (USGS site)	FL	USA
10.0	Peary Lake	Voyageurs Nat. Park	MN	USA
10.3	L979Q	ELA	Ontario	Canada
10.8	Tooth Lake	Voyageurs Nat. Park	MN	USA
11.2	Ryan Lake	Voyageurs Nat. Park	MN	USA
12.2	Scummy Outflow	ELA	Ontario	Canada
12.8	Crane Lake	Voyageurs Nat. Park	MN	USA
13.9	3A15	Everglades	FL	USA
20.0	Agnes	Voyageurs Nat. Park	MN	USA
20.1	Shoepack Lake	Voyageurs Nat. Park	MN	USA
22.1	Quarterline Lake	Voyageurs Nat. Park	MN	USA
38.3	F1	Everglades	FL	USA

NAWQA, National Water Quality Assessment; USGS, United States Geological Survey; ELA, Experimental Lakes Area.

2.5. Hg processing and analysis

Methylmercury determinations were made using the now widely adopted distillation and ethylation procedure that minimizes matrix interference effects of DOC in natural samples (Horvat et al., 1993; Olson et al., 1997). Briefly, an 80 mL portion of the acidified, filtered sample is first poured into a 125 mL Teflon distillation bottle that contains 1.0 mL of 1 M $CuSO_4$ (20% w/v). In the case of algal cells collected on QFF filters, the samples were placed in bottles with approximately 80 mL of high purity Milli-Q water, 0.5 mL KCl (20% w/v), 1.0 mL H_2SO_4 (50% v/v) and 1.0 mL of 1 M $CuSO_4$. Distillation was conducted using standard techniques, including blanks, duplicates and spiked samples. Spike recoveries were between 75 and 125%.

For total Hg (HgT) determination, filtrate samples were first oxidized with BrCl and placed in a 40 °C oven overnight. Algal cells on QFF filters and blank filters were placed in 125 mL Teflon bottles with 100 mL MQ and 4 mL BrCl and also placed in an oven overnight.

Analysis followed established analysis protocols for MeHg (Horvat et al., 1993; Liang et al., 1994; Olson et al., 1997) and HgT (Gill and Fitzgerald, 1987; USEPA, 2002) that convert the Hg to gaseous forms. In short, the analysis of MeHg involved ethylation and carbon trap amalgamation followed by gas chromatograph separation, and HgT analyses were made using $SnCl_2$ reduction and dual gold trap amalgamation. In order to quantify both the specific Hg isotope used in the assays and the ambient Hg, detection was accomplished using quadra-pole inductively-coupled plasma mass spectrometry (ICP-MS; Perkin-Elmer Model Elan 6100, Perkin-Elmer, MA, USA) rather than traditional cold vapor atomic fluorescence spectroscopy (CVAFS). These procedures have been previously described by Hintelmann and Evans (1997) and Branfireun et al. (2005). Briefly, after completing the purge and trap steps, samples are introduced into the ICP-MS by thermal desorption from carbo traps (MeHg samples) or gold traps (HgT samples) into a mercury-free argon stream. Aqueous standards and blanks were assayed for proper initial calibration, as well as bracketing standards throughout the analyses after every eighth (MeHg) or sixth (HgT) sample to assess accuracy during each analysis.

2.6. Calculation of bioconcentration factor

The bioconcentration factor (BCF) was calculated as:

$$\mathrm{BCF} = \frac{C_A}{C_W} \quad (\mathrm{pL\ cell^{-1}})$$

where C_A is the concentration of Hg isotope measured in the algae (ag $cell^{-1}$), and C_W is the concentration of Hg isotope measured in the medium (ag pL^{-1}). Note that all isotope concentrations refer to concentrations in excess of the background (ambient) isotope concentration. We expressed C_A on a cellular basis (ag $cell^{-1}$), rather than mass (ng kg^{-1}), because cell density measurements were more accurate than mass measurements (Gorski, 2004). The BCF can be converted to the approximate value of the corresponding volume concentration factor (volume/volume concentration factor) or VCF (Pickhardt and Fisher, 2007) by dividing by the cell volume, which is approximately 4 pL.

$$\mathrm{VCF} \cong \frac{\mathrm{BCF}}{4}$$

Concentrations of C_A and C_W used in BCF calculations and cell volume estimates can be found in Gorski (2004).

2.7. Stable isotope additions

For the river–lake water mixing experiment, each treatment flask received ^{201}Hg(II) at 0.21 ng L^{-1} and ^{199}MeHg at 0.67 ng L^{-1}. In the rainwater experiment, each treatment flask received ^{201}Hg(II) at 1 ng L^{-1} and ^{199}MeHg at 1 ng L^{-1}. For the multi-site experiment, each spike concentration was doubled from the rainwater experiment. Since each flask was spiked with both Hg(II) and MeHg, the two forms of Hg could be measured simultaneously. The Hg(II) isotope standards were acquired from the US Department of Energy, Oak Ridge National Laboratory, TN, USA, and the MeHg isotope was methylated from Hg(II) standards (H. Manolopoulos, University of Wisconsin-Madison, personal communication).

Isotope purity was measured and accounted for during subsequent calculations. Isotope concentrations were calculated using matrix algebra in an Excel spreadsheet format (Hintelmann and Evans, 1997). The calculations took into account the natural distribution of the five isotopes of Hg, the purity of the added standards, blank corrections and background concentrations. Isotopes were reported as excess concentration (or "spike"), which was the concentration of the added isotope above background Hg concentrations. The background Hg concentration was measured from either the concentration of ^{198}Hg or the means of the concentrations of ^{198}Hg, ^{200}Hg and ^{202}Hg. Concentrations of excess isotope were considered detectable if they were >3% of the background Hg concentration. The 3% criterion was the average daily relative standard deviation (RSD) of routinely analyzed quality control standards. The excess isotope concentrations for C_A and C_W were used to calculate BCF (see Section 2.6).

2.8. Statistics

Data were first tested for normality and constant variance (Kolmogorov–Smirnov test). Some ANOVA analyses required log transformation of data. Analyses (*t*-test and ANOVA) were completed with SAS version 8.02 software (SAS Institute, Inc.).

3. Results

3.1. River–lake water mixing experiment

In this bioassay experiment, both filtered and ultrafiltered waters were investigated. DOC concentrations were much higher in the Tahquamenon River than in Lake Superior or simulated "plume" (lake water–river water mixture) waters (Fig. 1A). The concentration of DOC in the Fraquil medium was much lower than in the natural waters. Similar concentrations of colloidal and dissolved DOC were found in the Tahquamenon River, plume, and Lake Superior water due in part to the degree to which the concentrate (colloidal fraction) was diluted following ultrafiltration.

A general trend of decreasing BCF with increasing DOC concentration was observed (Fig. 1A and B), i.e., the BCF for river water and plume water < lake and Fraquil. When comparing the BCFs for MeHg in Fraquil and lake water samples, as a group, to those from the river and plume treatments, the Fraquil and lake samples had significantly higher BCFs (ANOVA, $p < 0.01$, $F = 23.3$, $df = 15$). When comparing the BCFs for MeHg in filtrate fractions (i.e., filtrate, colloidal, and dissolved) between the lake water and plume water, the lake water had consistently higher mean BCF for each fraction, although high variability, and the colloidal fraction had the highest mean BCF within each water type. Unfortunately, missing samples and variability precludes us from definitively identifying significant differences associated with ultrafiltration fractions for Hg(II).

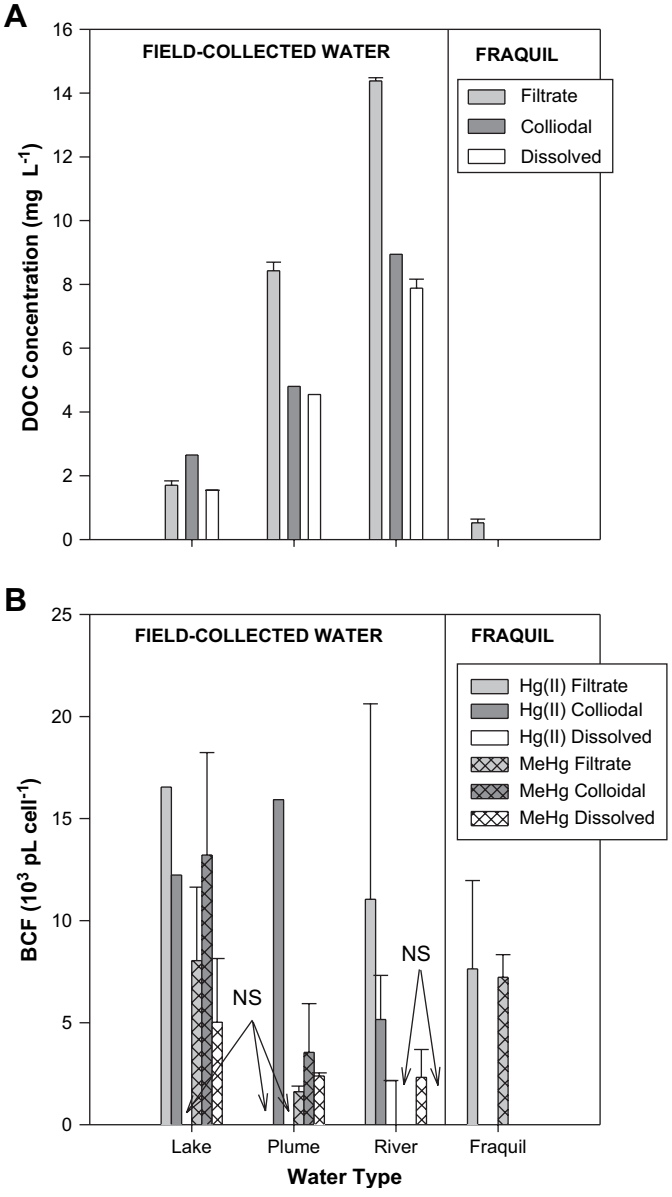

Fig. 1. Comparison of mean DOC concentrations and mean bioconcentration factors for a river and lake water. (A) Total, dissolved and colloidal dissolved organic carbon (DOC) concentrations in three waters and the control growth medium Fraquil:Tahquamenon River water (River), Lake Superior water (Lake), and a 50:50 volumetric mix of river and lake waters (Plume). The three DOC size fractions are filtrate or "total" DOC (<0.45 μm), colloidal (0.45 μm–10 kDa) and dissolved (<10 kDa). Fraquil medium was not ultrafiltered. (B) Bioconcentration of Hg(II) and MeHg from each water by *Selanastrum capricornumum* after 24 h, represented by the bioconcentration factor (BCF). NS, no sample. Error bars (mean ± 1 standard deviation (SD)) shown on replicated measurements.

3.2. Rainwater mixing experiment

The DOC concentrations were similar in rainwater and Devils Lake water, but over 10 times higher in Tahquamenon River water (Fig. 2A). The BCFs reflected this trend. The

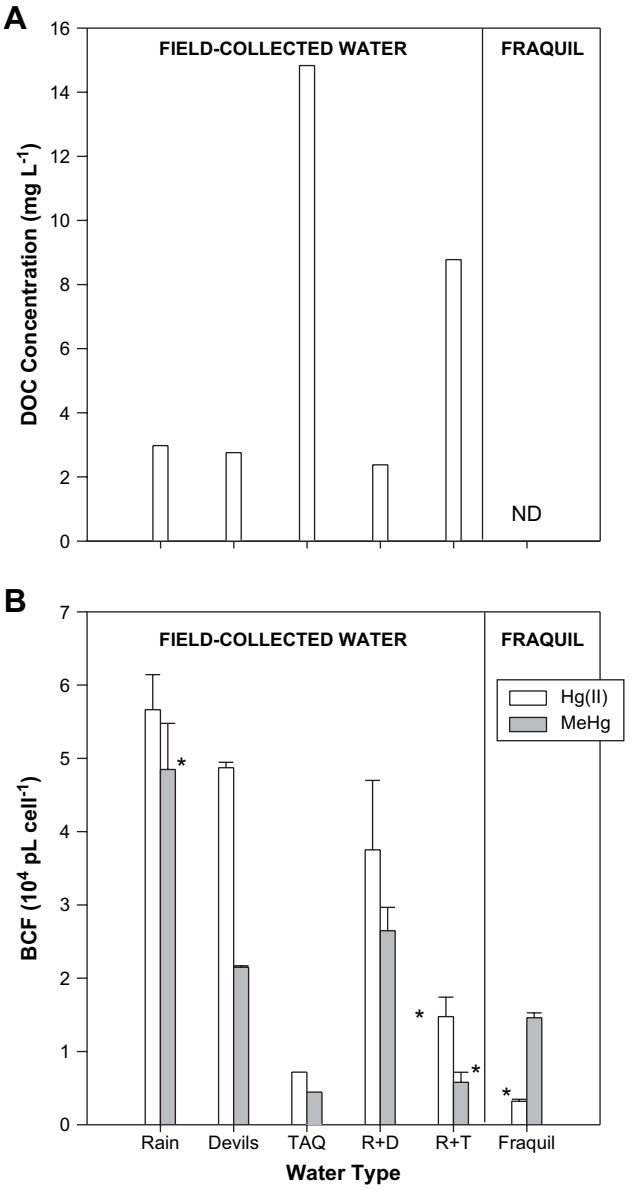

Fig. 2. Comparison of DOC concentrations and mean bioconcentration factors for rain, lake and river waters. (A) Dissolved organic carbon (DOC) concentrations in rainwater (Rain), Devils Lake water (Devils), Tahquamenon River water (TAQ), and 50:50 mixtures of rainwater and Devils Lake water (R + D) and rainwater and Tahquamenon River water (R + T). Fraquil is the growth medium (control). ND, not detectable. (B) Bioconcentration factors (BCF) for uptake of Hg(II) and MeHg from the waters by *Selanastrum capricornumum*. *Significant difference at $p < 0.05$. Error bars (mean ± 1 standard deviation (SD)) shown on replicated measurements.

BCFs for both Hg(II) and MeHg were high in the rain and lake waters and low in the Tahquamenon River water, while BCFs for the mixtures (rain + Devils Lake or rain + Tahquamenon River waters) fell between the values for the respective sources (Fig. 2B). The BCFs were higher for Hg(II) than MeHg in each water or mixture. Conversely, the BCF was higher for MeHg than Hg(II) in Fraquil. There were significant differences in log transformed BCFs for Hg(II) (ANOVA, $p < 0.01$, $F = 132.1$, df = 11). The BCFs for Hg(II) in the rainwater, Devils Lake water and the rainwater + Devils Lake water mixture were statistically similar. The BCF for Hg(II) in rainwater + Tahquamenon River water was significantly different from other water types (*t*-test, $p < 0.01$, $t = 6.8$, df = 7) as was the BCF for Fraquil (*t*-test, $p < 0.01$, $t = 5.9$, df = 9). Significant differences were also observed in log transformed BCFs for MeHg (ANOVA, $p < 0.01$, $F = 40.3$, df = 11). The BCFs for MeHg in Devils Lake water, rainwater + Devils Lake water and Fraquil were statistically similar. The BCF for MeHg was significantly higher in rainwater than other water types (*t*-test, $p < 0.05$, $t = 3.01$, df = 10), while the BCF in Tahquamenon River water and Rainwater + Tahquamenon River water (when grouped together) were significantly lower (t-test, $p < 0.01$, $t = 5.43$, df = 10). Since the Tahquamenon River water was represented by only one flask, it was not analyzed statistically, but BCFs for both Hg(II) and MeHg were very low at this site.

3.3. Multi-site experiment

As observed in the rainwater and river water mixing experiments, the BCFs for both Hg(II) and MeHg were low when the DOC concentrations were high (Figs. 3 and 4). However, BCFs were highly variable at low (<5 mg L^{-1}) DOC concentrations (Fig. 3). The BCFs for Hg(II) were generally higher than those observed for MeHg, and the BCFs for Hg(II) exhibited higher variability. Often a high BCF for Hg(II) did not correspond to a high BCF for MeHg. For example, in the Fraquil control, the BCF for Hg(II) was 2.4×10^4, but the BCF for MeHg was 2.0×10^2; the Beaverton site showed an opposite trend. The BCFs for Hg(II) and MeHg appeared to converge at higher DOC concentrations. It is interesting to note that for water collected from site F1 located in the Florida Everglades, which had the highest DOC concentration (38 mg L^{-1}), observed BCFs for Hg(II) and MeHg were very similar and low, 1.3×10^2 and 2.1×10^2, respectively.

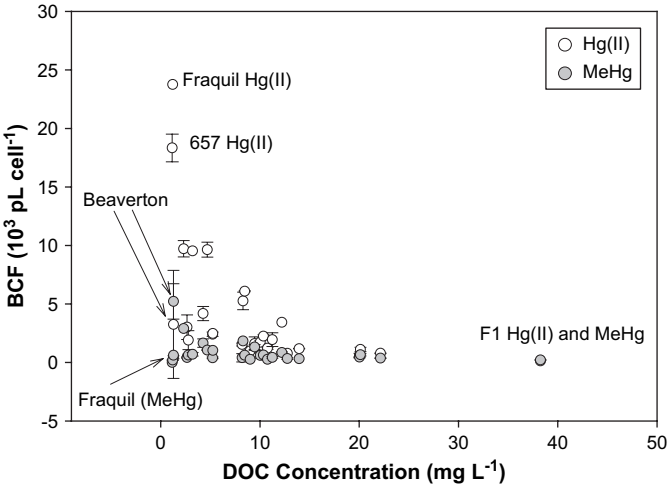

Fig. 3. Mean bioconcentration factor (BCF) versus dissolved organic carbon (DOC) concentration for uptake of Hg(II) and MeHg by *Selanastrum capricornumum* from the natural waters sampled in the multi-site investigation. Error bars (mean ± 1 standard deviation (SD)) shown on replicated measurements.

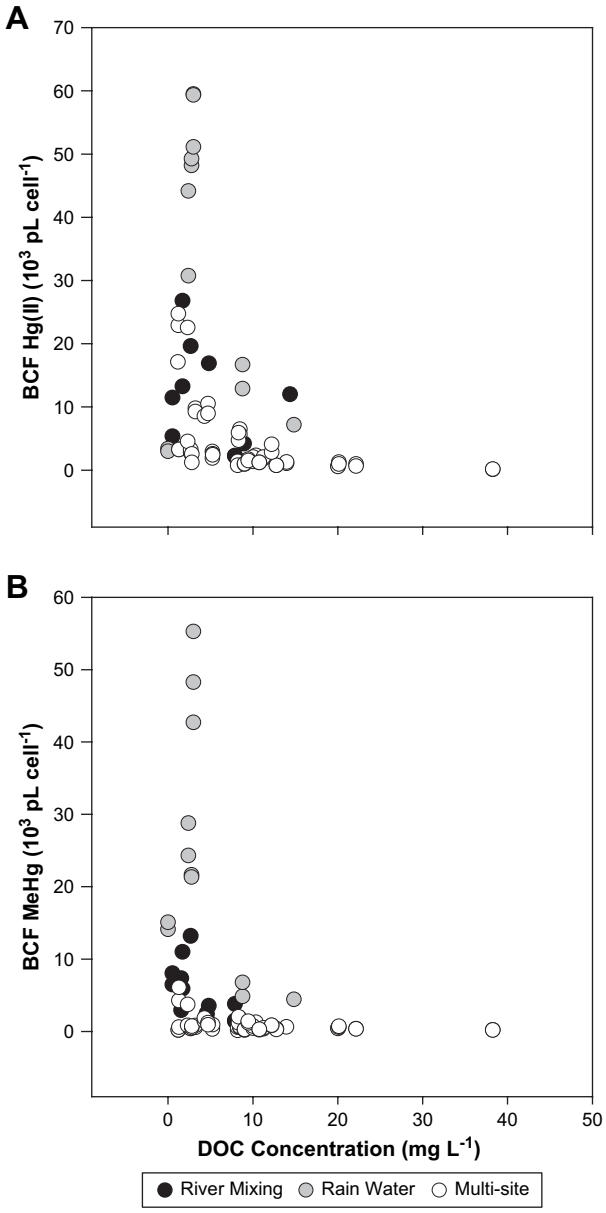

Fig. 4. Bioconcentration factor (BCF) versus dissolved organic carbon (DOC) concentration, combining data from the three experiments shown in Figs. 1, 2, and 3. (A) Hg(II) B) MeHg. Each point represents an individual culture flask.

4. Discussion

This investigation was conducted to determine whether constituents in natural waters influence the bioavailability of Hg(II) and MeHg to algae. The concentration of DOC was emphasized because both Hg(II) and MeHg form strong complexes with DOC (Amirbahman et al., 2002; Haitzer et al., 2003), and we hypothesized that binding of Hg to DOC would reduce bioavailability. DOC is also a readily measured and important water chemistry variable. However, if binding to DOC is dominated by high affinity organic ligands present at low concentration (Han et al., 2006), the influence of DOC on BCF may not be reflected in bulk DOC concentration. We note that other factors could also result in variability among different systems in uptake of Hg(II) and MeHg by phytoplankton, such as other strong ligands, differences in sorption or internalization of complexed Hg by algae, concentrations of Hg(II) or MeHg, as well as growth and physiology of the alga.

Algal densities are also important in mercury bioaccumulation (Pickhardt et al., 2002). To control for this factor, each flask was inoculated with the same starting algal density. The alga grew similarly among treatments within each bioassay experiment, and no correlation was found between ending algal densities and BCF (Gorski, 2004). If extreme water quality conditions were to be tested, algal growth and associated uptake or sorption could be altered by substances in the field water.

Our results indicate that high concentrations of dissolved organic matter can reduce the bioavailability of Hg(II) and MeHg to freshwater phytoplankton (Figs. 1–4). These finding are consistent with results from other investigations showing that bioconcentration factors tend to decrease with increasing concentrations of DOM (Driscoll et al., 1995) and that the uptake of MeHg by algae can be inhibited by humic substances (Moye et al., 2002). Bioconcentration of Hg(II) and MeHg from river water (Tahquamenon River) containing a high concentration of DOC (approximately 15 mg L^{-1}) was low and increased when DOC concentrations were diluted by mixing with rainwater or lake water (Fig. 2). Similar results were obtained for MeHg when Tahquamenon River water was mixed with Lake Superior water, although the influence on bioconcentration of Hg(II) was not significant (Fig. 1). The low level of Hg(II) addition (0.2 ng L^{-1}) used in this experiment may have influenced the ability to detect differences in bioconcentration. However, results from the multi-site investigation further confirmed that bioconcentration was low when DOC was high (Fig. 3). Similarly, Driscoll et al. (1995) observed decreased concentrations of MeHg in fish when DOC concentrations exceeded approximately 8 mg L^{-1}. Taken together, our results indicate bioconcentration by algae of both Hg(II) and MeHg is inhibited when DOC concentrations exceed approximately 5 mg L^{-1}.

Several factors may contribute to the high variability in bioconcentration when concentrations of DOC are low (Figs. 3 and 4). High bioavailability at low DOC concentrations could reflect dominance over DOC complexes of lipophilic species such as $HgCl_2$ and CH_3HgCl, believed to bioavailable (Mason et al., 1996) in some waters. Additionally, binding of DOC to algal cells could enhance adsorption of Hg to the cells or alter the permeability of cell membranes (Vigneault et al., 2000; Boullemant et al., 2004), thereby facilitating uptake of lipophilic species. At low DOC concentrations, these factors could dominate over the inhibitory influence of Hg binding to aqueous DOC, resulting in enhancement by DOC of Hg accumulation phytoplankton in some systems (Pickhardt and Fisher, 2007). In this regard, our experiments with rainwater indicate that bioconcentration was enhanced (in comparison to the Fraquil control) in rainwater and Devils Lake water where concentrations of DOC were low (Fig. 3). The reasons for low bioavailability at low DOC concentrations in some waters are uncertain (Figs. 3 and 4).

The ''quality'' of DOC may be an important factor. High affinity organic ligands may represent a small proportion of bulk DOC (Han et al., 2006), but even at low concentration may be important in determining bioavailability. In addition, dominance of non-lipophilic inorganic complexes (e.g., sulfide clusters or biosulfide, HS^-) could reduce bioavailability. See Gorski et al. (2006) for modeling and discussion on the possible importance of low-concentration, but competitively strong inorganic ligands which reduce the binding of MeHg to algae. The variability could thus reflect the influences of substances other than DOC and may be site-specific.

Due to reduced bioavailability when DOC concentrations are high, bioconcentration of Hg by phytoplankton may respond to gradients in DOC concentration. Two examples are the regions where river waters mix with lake waters and rainwaters mix with surface waters.

4.1. River mixing zones

Concentrations of DOC are often higher in rivers than lakes. In this case, the concentration of DOC gradually decreases as the river plume mixes with the lake. Our results indicate the bioavailability of MeHg and Hg(II) would increase as DOC concentration decreases from river to lake. Within the river, the combination of high DOC concentration and strong binding of MeHg and Hg(II) to DOC (Amirbahman et al., 2002) would result in association of most of the Hg with aqueous DOC. As the river mixes with the lake, DOC is diluted, while concentrations of algae usually increase. With increasing algal abundance, the strong binding and efficient uptake of Hg by algae (Moye et al., 2002; Gorski et al., 2006; Pickhardt and Fisher, 2007) would shift the association of Hg from DOC to phytoplankton, i.e., the Hg carried by the river would become more bioavailable as the river plume is dispersed in the lake. Other outcomes might be observed depending on characteristics of the river, lake, and phytoplankton populations.

4.2. Rainwater mixing zones

The bioavailability of Hg(II) and MeHg added to rainwater (DOC = 3 mg L^{-1}) was enhanced as compared to Fraquil (DOC non-detectable), as indicated by higher BCFs (Fig. 2). Bioavailability was also enhanced in Devils Lake water (DOC = 3 mg L^{-1}) or in a mixture of rainwater and Devils Lake water. These results suggest that low concentrations of DOC may enhance bioavailability. However, bioavailability was reduced in Tahquamenon River water (DOC = 15 mg L^{-1}) or a mixture of rainwater and Tahquamenon River water (DOC = 9 mg L^{-1}), again indicating bioconcentration was inhibited by high concentrations of DOC.

Our results suggest that mixing of rainwater with surface waters enhances the bioavailability of Hg(II) and MeHg to phytoplankton, presumably because of the relatively low concentrations of DOC in rainwater. Thus, total Hg and MeHg deposited from the atmosphere in rainwater (USEPA, 1997; Hall et al., 2005) are expected to have high bioavailability (Orihel et al., 2007), and mixing of rainwater with surface water likely enhances the bioavailability to phytoplankton of Hg already present in the surface water if dilution reduces the concentration of DOC in the mixing zone to less than approximately 5 mg L^{-1}.

5. Conclusions

Bioassay experiments using *Selenastrum capricornutum* indicate the bioconcentration of both Hg(II) and MeHg to phytoplankton is low (BCF $<5 \times 10^5$ pL $cell^{-1}$) when concentrations of natural DOC are relatively high (>5–10 mg L^{-1}). However, when DOC concentrations are low (<5 mg L^{-1}), bioavailability is highly variable (BCF <5 to >50 pL $cell^{-1}$). Strong binding of Hg(II) and MeHg to DOC likely accounts for the lower bioavailability at high DOC concentrations. When DOC concentrations are low, bioconcentration factors are high in some systems, presumably due in part to limited formation of complexes of Hg(II) and MeHg with DOC. In some of these low DOC systems, bioconcentration may be enhanced by DOC. Thus, DOC concentrations in the range of a few mg L^{-1} may be optimal for bioavailability of Hg(II) and MeHg to phytoplankton, but this concentration range may depend on the nature of both the DOC and other ligands in the system. The reasons for low bioconcentration in some low DOC systems are uncertain, but may involve dominance of inorganic species which are not readily transported into algal cells. Also, the influence on bioconcentration of DOC ''molecular size'', as operationally defined by ultrafiltration, remains uncertain and may warrant further study. Finally, dilution of DOC concentrations in estuarine mixing zones may increase the bioavailability Hg and MeHg to phytoplankton.

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