Vol. 276: 125–136, 2004 | MARINE ECOLOGY PROGRESS SERIES
Mar Ecol Prog Ser | Published August 2

Uptake of Hg(II) and methylmercury by the green mussel *Perna viridis* under different organic carbon conditions

Jin-Fen Pan, Wen-Xiong Wang*

Department of Biology, The Hong Kong University of Science and Technology (HKUST), Clear Water Bay, Kowloon, Hong Kong

ABSTRACT: The bioavailability and toxicity of mercury to aquatic organisms are greatly dependent on its chemical species, such as inorganic and methylated mercury. We examined the influences of various physico-chemical parameters [metal concentration, salinity, dissolved organic carbon (DOC) and colloidal organic carbon (COC) on the bioavailability of inorganic mercury (Hg(II)] and monomethylmercury (MeHg) to the green mussel *Perna viridis*. The uptake rate constant of MeHg was about 8.5x higher than that of Hg(II). The uptake increased by 75% for Hg(II) and 117% for MeHg with decreasing salinity from 30 psu to 15 psu. Biogenic DOC derived from diatom decomposition decreased the uptake of Hg(II), consistent with the results of DOC originating from natural seawater. In contrast, the uptake of Hg(II) was enhanced considerably in the presence of humic acid. The bioavailability of MeHg was only weakly influenced by differences in DOC quality or quantity, but it was significantly decreased by estuarine DOC. The influence of COC on Hg bioavailability was also dependent on the colloidal structural properties and the colloid-metal complexation. Binding with colloidal nanoparticles increased the uptake of Hg(II) by 3.3 to 7.3 times compared to low molecular weight-complexed Hg, but decreased the uptake of MeHg by 42 to 73%. For both Hg(II) and MeHg, the uptake of the 9 d aged radiolabeled metals was lower than the uptake of the 2 d aged radiolabeled forms. Our study suggests that various mechanisms, such as facilitated transport and direct colloidal ingestion, are involved in the uptake of Hg(II) and MeHg under different dissolved and colloidal organic carbon conditions. COC and DOC need to be separately considered, given their different influences on mercury uptake.

KEY WORDS: Mercury · Methylmercury · Dissolved organic carbon · Colloidal organic carbon · Influx

INTRODUCTION

Mercury can be toxic to aquatic organisms at very low environmental concentrations. Its environmental toxicity has been extensively studied over the past decades (Jensen & Jernelov 1969, Rabenstein 1978, Choi 1990). Mercury in the aquatic environment is predominated by 2 major chemical forms, inorganic (Hg(II)) and methylated mercury (MeHg). Inorganic mercury can be transformed either biologically or chemically into highly toxic methylmercury (Wood et al. 1968, Jensen & Jernelov 1969, Nagase et al. 1982). Differences between Hg(II) and MeHg bioaccumulation in many aquatic species has been documented (Riisgard & Famme 1986, Watras & Bloom 1992, Rouleau et al. 1993, Laporte et al. 2002). MeHg is more toxic and readily bioconcentrated and biomagnified along food chains, resulting in irreversible and, most likely, cumulative damage to the animals' central nervous systems (Wobeser 1975, Choi 1990). MeHg pollution is of great environmental concern in many developed and developing countries, including Hong Kong (Dickman et al. 1999, Parsons 1999).

Numerous studies have identified dissolved organic carbon (DOC) as an important variable influencing the bioavailability of trace metals to aquatic organisms (e.g. Parent et al. 1996, Sjoblom et al. 2000, Wright & Mason 2000, Guo et al. 2001). Observations of the interactions

*Corresponding author. Email: wwang@ust.hk

between metals and DOC are, however, conflicting. In some studies, a reduction in metal toxicity was found in the presence of natural organic matter, which was largely ascribed to the complexation of organic ligands with metals and, thus, a reduction in the concentration of available free ionic metals (Campbell 1995, Absil et al. 1996). Choi et al. (1998) observed a decrease in MeHg uptake by the Sacramento blackfish *Orthodon microlepidotus*, as well as in the MeHg level in the gills, with increasing DOC concentrations. Enhanced metal bioavailability or toxicity for bivalves and other organisms in the presence of high molecular weight DOC has also been reported (Winner 1986, Roditi et al. 2000, Guo et al. 2001). Such inconsistent results may largely reflect the complex geochemical behavior of DOC and the functional physiology of organisms in accumulating metals from DOC environments.

A great fraction of bulk DOC (20 to 60%) is indeed present in the colloidal phase (Guo et al. 1994), which is operationally defined as the size fraction of 1 kDa to 0.22 µm (Guo & Santschi 1997). A few studies have shown that 40 to 60% of the dissolved Hg(II) and MeHg is actually in the colloid-bound fraction (Choe & Gill 2003, Choe et al. 2003), but the influence of colloidal complexation on mercury bioavailability or toxicity remains essentially unknown at present. The important role of colloids in affecting the bioavailability of some metals to aquatic organisms has been recently recognized (Carvalho et al. 1999, Wang & Guo 2000, Chen & Wang 2001, Guo et al. 2002, Pan & Wang 2002). Roditi et al. (2000) found that the uptake of Hg, Ag and Cd by zebra mussels increased by 3.6×, 8.7× and 32×, respectively, in the presence of high molecular weight DOC (>1 kDa). Wang & Guo (2000), however, found that colloids had negligible influence on Cd uptake by the green mussel *Perna viridis* compared to the low molecular weight (LMW, <1 kDa) bound metals. Pan & Wang (2002) reported increased Cr and Fe bioavailability to green mussels through colloidal binding. In contrast to these studies on filter-feeding bivalves, Chen & Wang (2001) showed that colloidal Fe bioavailability to marine diatoms was substantially lower than that of LMW-bound Fe. Thus, different groups of aquatic organisms may have different mechanisms for the uptake of colloidally bound metals.

In the present study, we employed a radiotracer technique to examine the effects of DOC, COC, and other environmental factors (metal concentration and salinity) on the uptake of Hg(II) and MeHg by the green mussel *Perna viridis*. We compared the differences between the 2 mercury species in their bioavailability to the mussels. The influence of DOC on Hg uptake was studied using DOC originating from phytoplankton decomposition and humic acids (HA) as a model compound. Humic acids are among the ubiquitous dissolved organic materials in marine environments, and account for 10 to 30% of total DOC in seawater (Millero & Sohn 1992). Binding to humic acid may potentially render metals unavailable for absorption through biological membranes (McCarthy 1989), but other studies have also reported that dissolved HA increases or does not affect metal uptake (e.g. mussel *Mytilus edulis*, Huang 1982, *Daphina magna*, Winner 1984, 1986).

MATERIALS AND METHODS

Mussels and Hg. Green mussels *Perna viridis*, with a shell length of 3.0 to 4.0 cm and a dry tissue weight of 0.09 to 0.21 g, were collected from Ma On Shan, Tolo Harbor, Hong Kong. The mussels were usually acclimated in the laboratory for 1 to 2 wk at 20°C and 28 psu prior to the experiments. They were continuously fed with the diatom *Thalassiosira pseudonana* (clone 3H) at a ratio of 2% dry tissue weight each day. Before the uptake experiments, the mussels were starved overnight to avoid any production of feces during the exposure period. After exposure, the mussels were dissected into shells and different tissues, including gills, digestive gland and remaining soft tissues. All experiments were conducted at a temperature of 20°C.

The gamma radioisotope ^{203}Hg(II) (in the form of $HgCl_2$, with a specific activity of 26.1 GBq Hg g^{-1}) was purchased from the Riso National Laboratory, Denmark. Radioactive monomethylmercury was synthesized by reacting $^{203}HgCl_2$ with tetramethyltin (Rouleau & Block 1997). The final product, MeHg (with a purity of 98%, analyzed by paper chromatography), was dissolved in Na_2CO_3 solution (0.05 M). The radioactivity of isotopes in the tissues and water samples was assayed using a Wallac 1480 NaI gamma detector (Wallac Turku, Finland) at 279 keV.

Measurement of Hg uptake by the mussels. The uptake of Hg(II) and MeHg by the mussels was quantified using a short-term exposure approach (0.5 h), which has been extensively employed to quantify metal uptake by marine bivalves (Wang et al. 1996, Luoma & Fisher 1997, Lee et al. 1998). Short-term exposure was used in order to minimize the decrease in ambient mercury concentration and the possible coagulation of colloidal particles during the exposure period (Stordal et al. 1996a, Guo et al. 2002). Earlier experimental studies demonstrated that metal uptake by green mussels proceeds in a linear pattern over exposure time with minimal initial surface adsorption, thus metal uptake determined at a specific time of exposure reflects the true uptake by the mussels (Chong & Wang 2001). The mussels were individually (n = 8) placed into 200 ml of experimental medium held

in a polymethylpentene (PMP) beaker. Exposure started when the mussels opened their shell valves. After exposure, the mussels were removed, rinsed with nonradioactive seawater and dissected, and the radioactivity in soft tissues was counted. A 20 ml aliquot of the exposure water was removed at the beginning and end of exposure for radioactivity measurements. The losses of both Hg species from the water (including sorption onto the glass wall and evaporation) within the 0.5 h period were <6% for Hg(II) and <0.6% for MeHg in the absence of mussels. The fraction of Hg(II) and MeHg in the particulate phase (>0.2 µm) was also determined by filtering a 10 ml aliquot through a 0.2 µm polycarbonate membrane. Results confirmed that a negligible fraction of mercury (<2.4%) was associated with the particulate phase during the short exposure period. After the radioactivity measurements, the tissues were finally dried at 80°C for 24 h and the dry weights were measured.

The influx rate of Hg into the mussels was calculated by:

$$I = A_{tissue}/(SA \times t) \quad (1)$$

where I is the influx rate of Hg into the mussels (ng g^{-1} h^{-1}), A_{tissue} is the radioactivity in the mussel soft tissues (corrected counts per min (ccpm) g^{-1} tissue dry weight), SA is the specific activity of the Hg isotope (ccpm ng^{-1}), and t is the exposure duration (h). Alternatively, the uptake of Hg by the mussels can be quantified by the dry weight concentration factor (DCF), calculated by:

$$DCF = A_{tissue}/A_{water} \quad (2)$$

where A_{water} is the radioactivity in the water (ccpm l^{-1}), calculated as the mean of the measurements taken at the beginning and end of exposure. Combining Eqs. (1) & (2), the influx rate I can be calculated as:

$$I = DCF \times C_w/t = k_u \times C_w \quad (3)$$

where C_w is the dissolved Hg concentration, and k_u is the uptake rate constant. DCF provides a direct comparison between the uptake of Hg and MeHg, since the dissolved concentrations used in the experiments were different. Comparison of the DCF assumed that the k_u of mercury is independent of C_w, which was confirmed in our first experiment quantifying the uptake rates at different C_w (see Results).

Mercury uptake at different dissolved concentrations and salinity. The uptake of Hg(II) and MeHg by the green mussels at 4 different dissolved concentrations was measured (7.2, 10.5, 129, and 250 ng l^{-1} for Hg(II), and 12.3, 24.7, 117, and 222 ng l^{-1} for MeHg). The different metal concentrations were obtained by spiking the seawater with specific measures of isotope radioactivities. Background total dissolved Hg concentrations were 0.08 to 0.8 ng l^{-1}, and were considered negligible compared to the radioactive spike. Radioisotopes were equilibrated with the seawater (filtered through a 0.2 µm filter) overnight before the uptake experiments. Eight replicated individuals were used for each concentration. At the end of exposure, the mussels were dissected and the soft tissues were radioassayed. The influx rate at each concentration was calculated using Eq. (1), based on 0.5 h exposure.

The influence of salinity on mercury uptake was also examined. The green mussels were collected from a salinity of 27 psu and were acclimated at 30, 25, 20, 15 psu for 2 wk. The salinity was reduced in a stepwise manner (5 psu every 5 d) to allow the mussels to gradually acclimate. The lower salinity seawater was obtained by diluting the 34 psu Clear Water Bay (CWB) seawater with Nanopure distilled water. The uptake of Hg(II) and MeHg by the mussels at each salinity was then determined, as described above.

Influence of dissolved organic carbon on mercury uptake. Three different experiments using different sources of DOC were conducted to examine the influence of DOC on the uptake of Hg(II) (91 ng Hg l^{-1}) and MeHg (17 ng Hg l^{-1}) by the mussels. In the first experiment, which was repeated twice at different times, the DOC was obtained from diatom decomposition (phyto-DOC). Late log phase diatom *Thalassiosira pseudonana* (clone 3H) was collected, resuspended into 0.22 µm filtered Clear Water Bay seawater, and allowed to decompose for 4 wk (Wang & Guo 2001). Afterwards, the decomposing medium was filtered through 0.22 µm membranes and the filtrates (phyto-DOC) were diluted with ultrafiltered seawater (<1 kDa, see below) at different ratios to result in different biogenic DOC concentrations. The seawater with different DOC concentrations was then spiked with ^{203}Hg(II) or Me^{203}Hg. After 10 to 12 h of equilibration, their uptake by the mussels was determined.

In the second experiment, seawater was collected from different coastal (Tolo Harbor, TH, Clear Water Bay, CWB: 30 psu) and estuarine (Yuen Long, YL, 21 psu) regions in Hong Kong. Clear Water Bay is under the influence of oceanic currents, and Tolo Harbor is a coastal semi-enclosed bay. In contrast, Yuen Long is under the influence of the Pearl River Delta, with significant freshwater input from the river (Pan & Wang 2004). Background DOC concentrations are shown in Table 1. The background total dissolved Hg concentrations in Hong Kong coastal waters are typically 0.08 to 0.8 ng l^{-1} (W. X. Wang unpubl. data). The seawater from different origins was collected and filtered through 0.22 µm Millipore Millipak 100 filters. Each filtered seawater sample was then photo-oxidized (UV) for 48 h with an ACE UV lamp (Model 7480). The UV oxidation removed close to 100% of the coastal DOC (CWB and TH: to non-detectable level), and 94% of the estuarine DOC (YL: from 387 to 24 µM

Table 1. Organic carbon concentrations used in experiments on the uptake of mercury bound with colloids of different sources by green mussels. COC: colloidal organic carbon (1 kDa to 0.22 µm). DOC: total dissolved organic carbon (<0.22 µm). nd: not determined. The experimental LMW organic carbon concentration was 95.5 µM. Background total Hg concentrations were 0.08 to 0.8 ng l^{-1} (W. X. Wang unpubl. data)

	Clear Water Bay	Tolo Harbor	Yuen Long	Phyto-DOC
COC (µM)	22.4	28.0	27.4	nd
DOC (µM)	80.8	132	387	1420
Salinity (psu)	30	30	21	30
Spiked Hg(II) concentration (ng l^{-1})	91	91	91	91
Spiked MeHg concentration (ng l^{-1})	17	17	17	17

after UV oxidation). DOC concentration before and after UV oxidation was measured as described in Guo et al. (1994). The control (without UV oxidation) and the UV-oxidized seawater were spiked with ^{203}Hg(II) or Me^{203}Hg. After 10 to 12 h equilibration, the green mussels were exposed to the radioactive seawater for 0.5 h and the uptake was quantified. Since the salinity of the estuarine YL treatment was only 21 psu at the time of collection, the mussels were acclimated to this salinity for 2 wk before the uptake measurements.

In the third experiment, we used humic acid (HA, Aldrich) as the standard DOC to examine mercury uptake, using seawater collected from Clear Water Bay. The HA stock solution (173.7 mg C l^{-1}, quantified by a Shimadzu TOC-5000A Total Organic Carbon Analyzer) was prepared by dissolving the HA in 0.1 N NaOH. HA concentrations from 20 to 2500 µMC were used in the 2 repeated experiments. The control treatment received no HA. HA typically makes up 10 to 30% of total DOC in seawater (Millero & Sohn 1992, Benner 2002), and thus the lower HA concentrations examined in this study were environmentally relevant. At the end of the exposure, the metal distribution in the gills, the digestive gland, and the remaining soft tissues was also evaluated.

Influence of colloidal organic carbon (COC) on mercury uptake. Colloidal nanoparticles were collected as described in Pan & Wang (2002, 2004). Natural coastal seawater (TH and CWB) and estuarine seawater (YL) was collected and first filtered through a 0.22 µm membrane to collect the natural colloids. In addition, diatoms were decomposed and the collected filtrates (<0.22 µm) were used to isolate phyto-colloids. The 0.22 µm filtrate of natural seawater or decomposing-diatom medium (described above for DOC experiments) were then ultrafiltrated using a spiral-wound cross-flow ultrafiltration cartridge, with a specific molecular weight cutoff of 1 kDa (Amicon S10Y1 Spiral Cartridge), to isolate colloids in the size range of 1 kDa to 0.2 µm. A concentration factor of 40 (i.e. 1 l of colloids was isolated from 40 l of 0.2 µm filtered seawater) was applied during the ultrafiltration. The isolated colloids and ultrafiltered seawater (low molecular weight, LMW, <1 kDa) were kept at 4°C before the experiments. Concentrations of COC were quantified using methods described in Guo et al. (1994) and are shown in Table 1. The colloids were radiolabeled with ^{203}Hg(II) or Me^{203}Hg separately in a Teflon bottle for 3 d. After radiolabeling, the colloids were first passed through 0.22 µm polycarbonate membranes to remove any coagulated particles, and were ultrafiltered again using an Amicon stirred cell with a 1 kDa ultrafiltration membrane, at a concentration factor of 8. The retentate (colloids) and the ultrafiltrated water were immediately used in the uptake experiments. The experimental Hg(II) and MeHg concentrations were 90 ng Hg l^{-1} and 10 ng Hg l^{-1}, respectively.

We further examined the uptake of ^{203}Hg(II) or Me^{203}Hg bound with colloidal particles over different durations. The control treatment was ultrafiltered water spiked with ^{203}Hg(II) or Me^{203}Hg directly and equilibrated for 4 h. The colloids were obtained from decomposing diatoms, as described in Wang & Guo (2001) and Pan & Wang (2004). The diatoms *Thalassiosira pseudonana* were inoculated into 0.22 µm filtered medium containing f/2 levels of macronutrients and vitamins, and f/20 levels of Fe, Mn and Co, without addition of other trace metals and EDTA. After 6 d of growth, the diatoms were collected onto 3 µm polycarbonate membranes and rinsed with nonradioactive 0.22 µm filtered seawater, then resuspended into <1 kDa ultrafiltered water and decomposed for 1 mo. The medium was subsequently filtered through 0.22 µm polycarbonate membranes, and the filtrates were further ultrafiltered using an Amicon stirred cell with a 1 kDa ultrafiltration membrane, at a concentration factor of 8. Aliquots of the retentate, which contained phyto-colloids, were subsequently spiked with isotopes (^{203}Hg(II) or Me^{203}Hg), and aged for 2 d and 9 d, respectively, in a Teflon jar. After radiolabeling, they were again filtered through a 0.22 µm polycarbonate membrane to remove coagulated particles. The filtrates were ultrafiltered as described above using a stirred cell with a 1 kDa ultrafiltration membrane, and the radiolabeled colloids were immediately used in uptake experiments (using ultrafiltrated seawater) to minimize potential repartitioning of the colloidal metals.

At the end of the exposure period, the repartitioning of ^{203}Hg(II) or Me^{203}Hg in the medium among the particulate (>0.22 µm), colloidal (1 kDa to 0.22 µm), and LMW phases was quantified. A 40 ml aliquot was sampled and ultrafiltered through a 1 kDa ultrafiltration membrane using a stirred cell (Amicon) under 30 psi N_2 pressure. The ultrafiltrate was defined as the LMW phase.

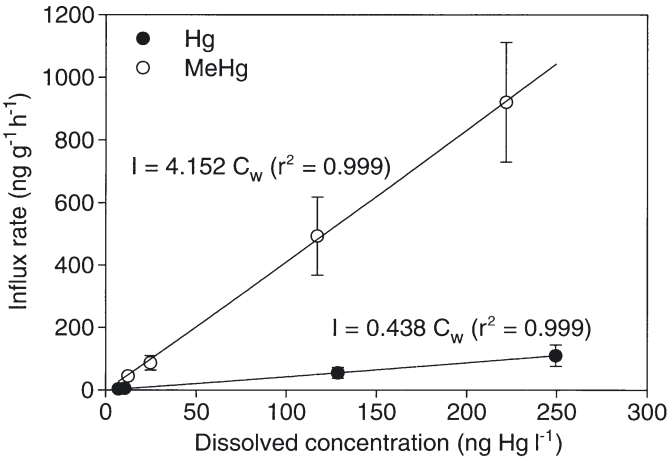

Fig. 1. *Perna viridis*. Influx of Hg(II) and MeHg at different dissolved mercury concentrations. Mean ± SD (n= 8)

RESULTS

Mercury uptake at different concentrations and salinity

The influx rates of Hg(II) and MeHg into the green mussels at different dissolved mercury concentrations are shown in Fig. 1. There was a positive linear relationship between the influx rate of each Hg species and its dissolved concentration, thus the influx rate was directly proportional to mercury concentration in the water. The uptake rate constant, calculated from the slope of the linear regression between the influx rate and the dissolved metal concentration (Eq. 3), was 0.438 and 4.152 l g^{-1} h^{-1} for Hg(II) and MeHg, respectively. Comparing the 2 mercury species, the influx rate of MeHg was about 8.5× higher than that of Hg(II) at the same concentration.

Uptake of the 2 mercury species at different salinities is shown in Fig. 2. With a decrease in salinity from 30 psu to 15 psu, the influx rate increased by 75% for Hg(II) and by 117% for MeHg. Statistical analysis indicated that a change in salinity significantly affected the uptake of both Hg species, especially MeHg ($p < 0.05$, 1-way ANOVA). However, the major change in the uptake of both Hg species occurred between 25 psu and 30 psu, and there was no significant difference in their uptake between 15 psu and 25 psu.

Mercury uptake influenced by total dissolved organic carbon

Mercury uptake was first examined using DOC originating from diatom decomposition (phyto-DOC) in 2 repeated experiments (Fig. 3). In both experiments, DOC concentration significantly affected the uptake of Hg(II) ($p < 0.01$, 1-way ANOVA). With increasing DOC concentration (from 250 to 1039 µM in Expt. 1, and

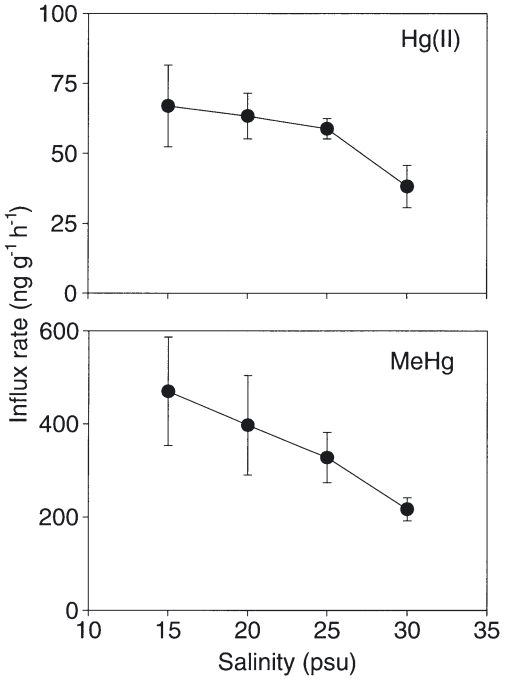

Fig. 2. *Perna viridis*. Influx of Hg(II) and MeHg at different salinities. Mean ± SD (n= 8)

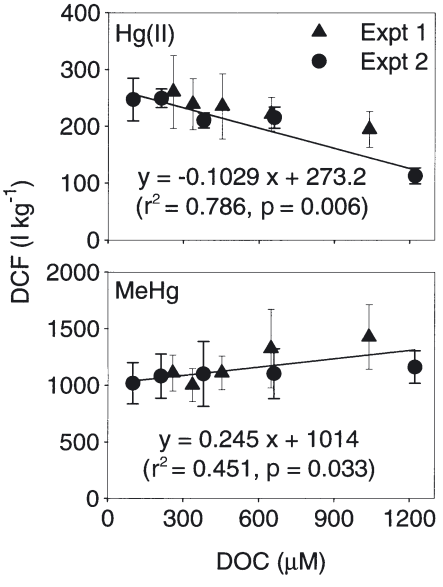

Fig. 3. *Perna viridis*. Relationship between the dry weight concentration factor (DCF) of Hg(II) and MeHg and the concentration of dissolved organic carbon (DOC) originating from decomposition of the marine diatom *Thalassiosira pseudonana*. Data from 2 repeated experiments (Expt. 1 and Expt. 2) are shown. Mean ± SD (n= 8)

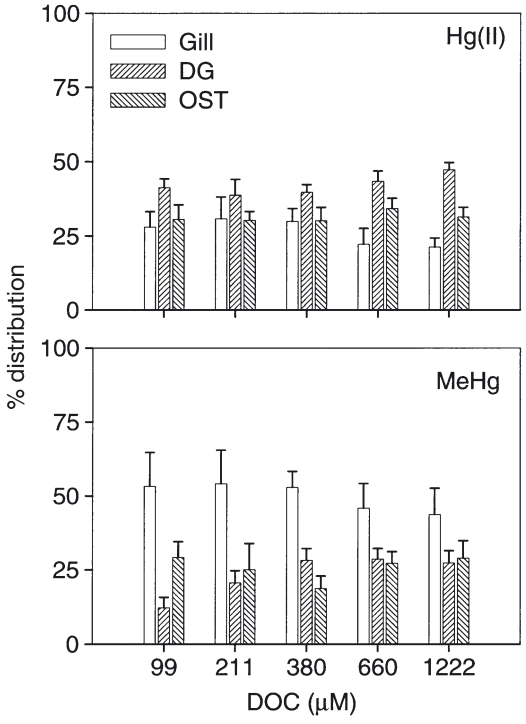

Fig. 4. *Perna viridis*. Percentage distribution of Hg(II) and MeHg after exposure to different concentrations of biogenic dissolved organic carbon. Phyto-DOC was obtained by diatom decomposition. Mean + SD (n = 5). Gill: mussel gills, DG: digestive gland, OST: remaining soft tissues

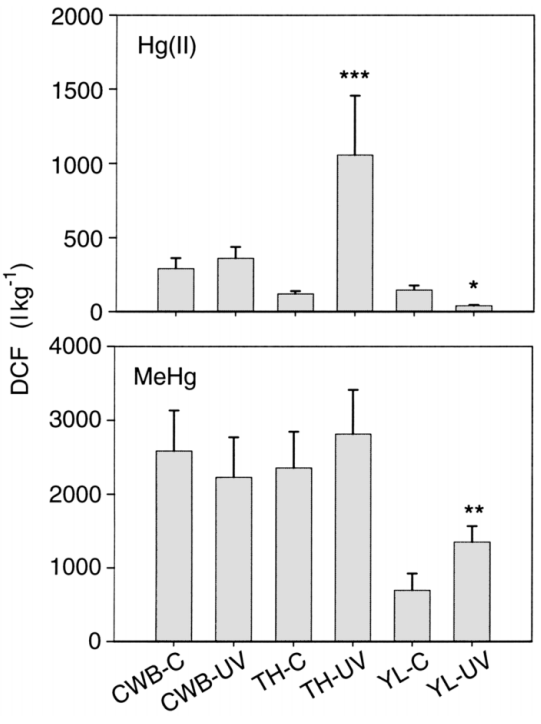

Fig. 5. *Perna viridis*. Dry weight concentration factor (DCF) of Hg(II) and MeHg in seawater collected from 3 different locations in Hong Kong before (Control: C) and after UV oxidation (UV). Mean + SD (n = 8). CWB: Clear Water Bay, TH: Tolo Harbor, YL: Yuen Long. Statistically significant differences between the control and the UV-oxidized treatments in each group are denoted by $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$

from 99 to 1222 µM in Expt. 2), Hg(II) uptake decreased by 25 to 54% in both repeated experiments. A positive linear relationship was observed for Hg(II) ($p < 0.01$) when the 2 experiments were considered together (Fig. 3). For MeHg, no significant influence of DOC concentration on its uptake was observed in either of the 2 repeated experiments ($p > 0.05$). However, when the results of the 2 experiments were pooled together, MeHg uptake was significantly and positively affected by DOC concentration ($p = 0.03$, Fig. 3). The metal distribution in mussel soft tissues were similar in the 2 experiments, and the result from Expt. 2 is shown in Fig. 4. For Hg(II), about 17 to 31% was associated with the gills and 38 to 50% with the digestive gland, whereas a higher fraction of MeHg (43 to 54%) was in the gills, and the remaining fraction was nearly evenly distributed between the digestive gland (12 to 28%) and the other soft tissues (18 to 29%). There was no obvious difference in the distribution of both Hg(II) and MeHg in different mussel tissues at low DOC concentrations (<380 µM). At the 2 highest DOC concentrations (660 and 1222 µM), the Hg(II) distribution in the gills decreased (from 30 to 21%) and that in the digestive gland increased (from 41 to 47%), but this was not statistically significant. For MeHg, its distribution in the gills decreased from 53 to 44% when DOC increased from the lowest to the highest concentration. In contrast, the distribution in the digestive gland was significantly higher at the 3 high DOC concentrations (380 to 1222 µM) than at the 2 low DOC concentrations (99 and 211 µM).

Uptake of Hg(II) and MeHg by mussels in seawater collected from different sources (coastal: TH and CWB, and estuarine: YL) with or without UV oxidation is shown in Fig. 5. Mercury uptake was conducted at the respective salinity of each location (i.e. 30 psu for TH and CWB, and 21 psu for YL). In general, the uptake of both mercury species from YL water was significantly lower than that from the coastal waters of TH and CWB. UV oxidation significantly increased Hg(II) uptake from coastal waters, especially for TH (a 7.8× increase after UV oxidation, $p < 0.001$, *t*-test). In contrast, uptake from YL seawater decreased by 73% after UV oxidation ($p < 0.05$, *t*-test). For MeHg, its uptake remained comparable after the coastal seawater was UV oxidized, but increased by 94% in YL seawater after UV oxidation ($p < 0.01$, *t*-test).

The influence of HA on mercury uptake was examined in 2 repeated experiments with different concentration

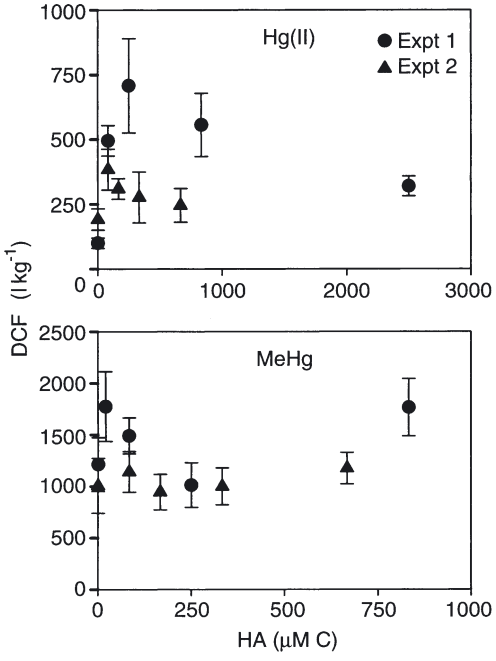

Fig. 6. *Perna viridis*. Dry weight concentration factor (DCF) of Hg(II) and MeHg at different humic acid (HA) concentrations. Two repeated experiments were conducted. Mean + SD (n = 8)

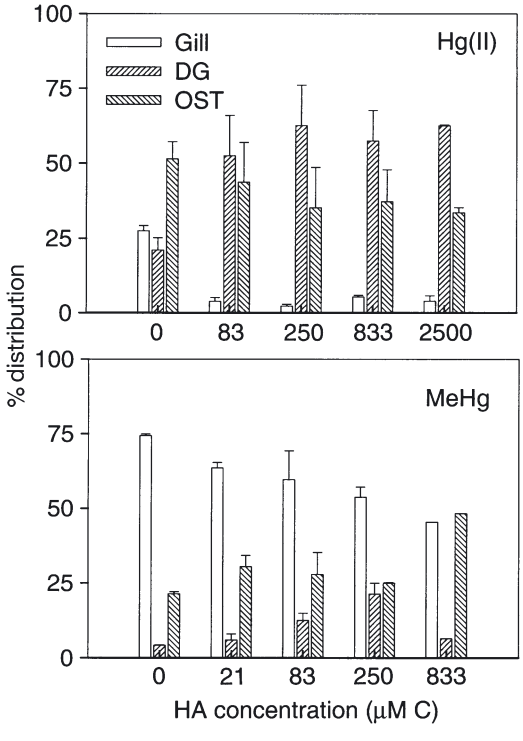

Fig. 7. *Perna viridis*. Percentage distribution of Hg(II) and MeHg after exposure at different concentrations of humic acid. Mean + SD (n = 5). Gill: mussel gills, DG: digestive gland, OST: remaining soft tissues

ranges (Fig. 6). Generally, the presence of HA significantly increased Hg(II) uptake ($p < 0.05$, 1-way ANOVA), especially at a lower HA concentration. In Expt. 1, Hg(II) uptake increased by 3.9 to 6.1 times ($p < 0.001$, *t*-test) at HA concentrations of 83 to 250 µMC. Further increasing the HA concentration decreased Hg(II) uptake, but this was also significantly higher than the uptake in the absence of HA. In Expt. 2, the extent to which the Hg(II) uptake was influenced by HA was less pronounced. In contrast to Hg(II), the presence of HA did not significantly influence the uptake of MeHg in the 2 repeated experiments ($p > 0.05$, 1-way ANOVA).

The distribution of Hg(II) and MeHg in different mussel tissues at the end of exposure to HA-containing seawater in Expt. 1 is shown in Fig. 7. In the presence of HA, a significantly higher fraction of Hg(II) (53 to 63%) was detected in the digestive gland, whereas the fraction of Hg(II) in the gills was significantly lower ($p < 0.05$), compared to the control treatment without HA addition. As much as 63% of Hg(II) was found in the digestive gland and only 2% in the gills, at a HA concentration of 250 µMC, compared to 21% in the digestive gland and 28% in the gills in the control treatment (without HA). In contrast to Hg(II), a much higher fraction of MeHg (45 to 75%) was associated with the gills after exposure. With increasing HA concentration, the fraction of MeHg in the gills decreased significantly, whereas the fraction in the digestive gland generally increased until the HA concentration reached 833 µMC.

Mercury uptake influenced by colloidal organic carbon

When Hg(II) was bound to colloids isolated from coastal seawater (CWB and TH) and phyto-colloids, its uptake was significantly enhanced compared to the uptake of LMW-bound Hg(II) (Fig. 8). Binding of Hg(II) to colloids of estuarine origin, however, did not significantly influence its uptake ($p > 0.05$, *t*-test). The quantified DCF from the estuarine treatment was the lowest among the 4 types of colloids tested. In contrast to Hg(II), the uptake of MeHg was significantly depressed when bound with the colloidal particles (Fig. 8). In this experiment, the DCF of MeHg was reduced by 34, 24, 45, and 29%, respectively, for colloids isolated from CWB, TH, YL, and biogenic sources. Similar to Hg(II), the estuarine-bound MeHg had the lowest bioavailability among the 4 types of colloids examined. The partitioning of both species of mercury by the end of exposure was also examined. About 70 to 80% of Hg(II) was still associated with the colloids, compared to only 40 to 50% of MeHg. The distribution of mercury in different mussel tissues was not quantified in this experiment.

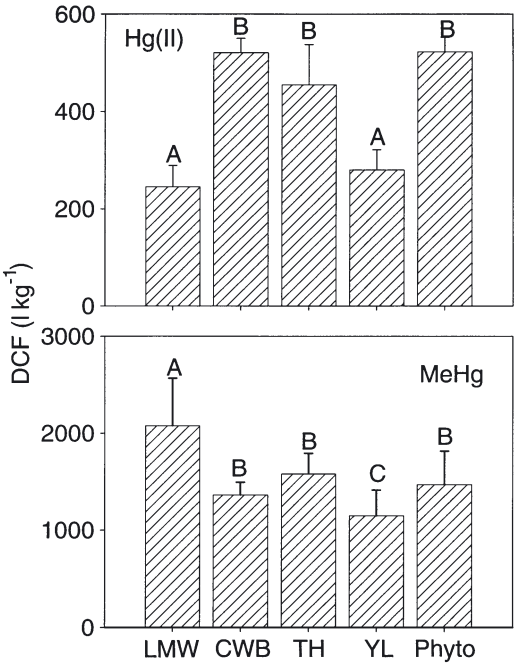

Fig. 8. *Perna viridis*. Dry weight concentration factor (DCF) of Hg(II) and MeHg following exposure to low molecular weight-complexed (LMW, as control) and radiolabeled colloidally complexed metals. CWB: colloids from Clear Water Bay, TH: colloids from Tolo Harbor, YL: colloids from Yuen Long, Phyto: colloids from diatom decomposition. Mean + SD (n = 8). Means not sharing the same letters indicate a significant difference ($p < 0.05$)

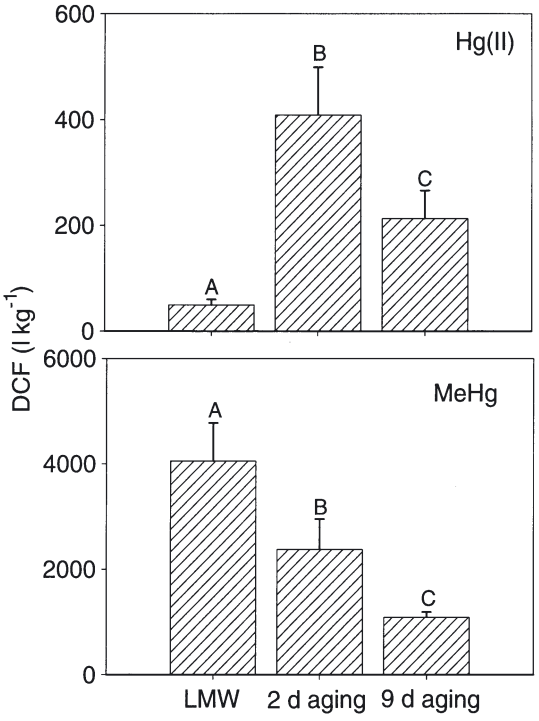

Fig. 9. *Perna viridis*. Dry weight concentration factor (DCF) of Hg(II) and MeHg following exposure to metals complexed with low molecular weight (LMW, as control), and phyto-colloids radiolabeled for 2 and 9 d, respectively. Mean + SD (n = 8). Means not sharing the same letters indicate a significant difference ($p < 0.05$)

A separate experiment examined the uptake of Hg(II) and MeHg bound to phyto-colloids of different ages (Fig. 9). The uptake of colloidal Hg(II) of different ages was significantly enhanced compared with that of the LMW treatment (by 3.3 to 7.3×), while the uptake of colloidal MeHg was greatly depressed (by 42 to 73%) ($p < 0.001$, *t*-test). Moreover, the uptake of 2 d aged radiolabeled colloidal Hg(II) and MeHg was 48% and 54% higher, respectively, than that of the 9 d aged colloidal Hg. In this experiment, about 70 to 80% of Hg(II) and 50 to 60% of MeHg was associated with the colloidal particles by the end of exposure.

DISCUSSION

Mercury uptake at different concentrations and salinities

Our results indicate that mussels have a much faster uptake of MeHg than of Hg(II), consistent with previous studies on marine invertebrates and fish (Saouter et al. 1993, Odin et al. 1996). The higher potential of MeHg bioaccumulation is often attributed to the lipophilicity of this organic compound (Boudou et al. 1991). In our study, the influx rates of both Hg(II) and MeHg were directly proportional to their ambient dissolved concentrations, thus both species of mercury were probably taken up by passive diffusion or facilitated transport mechanisms. Laporte et al. (2002) suggested that the accumulation of both chemical species across the gills and intestine of the blue crab *Callinectes sapidus* was dominated by ligand exchange or facilitated transport. Golding et al. (2002) concluded that the uptake of Hg(II) by 2 facultatively anaerobic bacteria species, *Vibrio anguillarum* and *Escherichia coli*, was by facilitated rather than by passive diffusion.

In our study, a higher uptake of both Hg(II) and MeHg was observed at a lowered salinity, consistent with many experimental results for other trace metals (Wang et al. 1996, Blackmore & Wang 2003). Because the lower salinity seawater was prepared by diluting with Nanopure distilled water, the resulting DOC concentration was lower than at higher salinity. However, such a potential difference (over the range of 40 to 80 µM) is unlikely to contribute to the observed differences in Hg accumulation at different salinities. The most commonly accepted explanation for the increasing bioavailability of metals at lowered salinity is the increasing free metal ion (the most bioavailable

form) concentration because of the reduced chlorocomplexation. For example, Chong & Wang (2001) demonstrated that the uptake of Cd, Cr and Zn by green mussels increased by 1.6 to 1.8× with decreasing salinity from 30 psu to 15 psu. For these metals, speciation can be significantly impacted by a change in salinity, thus the activity (or concentration) of free ion species may partially account for the enhanced uptake at a lowered salinity. The speciation of MeHg is presumably little influenced by a change in salinity, but the neutral form $HgCl_2$ and the anionic form $HgCl_4^{2-}$ are dominant at low and high salinity, respectively (Laporte et al. 1997). Blackmore & Wang (2003) suggested that a change in membrane permeability in green mussels may potentially explain the differences in metal uptake at various salinities.

In our experiments, the DCFs of both mercury species in YL seawater (with a salinity of 21 psu) were much lower than those measured in coastal waters (with typical salinity of 27 to 30 psu). These results are in contrast to those obtained in the salinity experiments, showing increased uptake at lowered salinity. However, substantial differences in the DOC quantity and quality were found in these different sources of seawater, thus any influences of salinity in these experimental seawaters may have been confounded by this inherent variation. It would therefore be interesting to further investigate the influence of the interaction between salinity and DOC on mercury uptake by the mussels.

Mercury uptake influenced by total dissolved organic carbon

Because of its high binding and buffering capacities, natural DOC plays a significant role in the bioavailability of trace metals (Santschi et al. 1999). Our results showed that any influence of DOC on the uptake of Hg(II) and MeHg was greatly dependent on the origins of DOC (i.e. quality) and there was a strong contrast between the 2 Hg species. Using phyto-DOC obtained from diatom decomposition, we demonstrated that Hg(II) uptake decreased at a higher concentration of phyto-DOC. Similarly, Hg(II) uptake from Yuen Long, which contained the highest DOC concentration, was also reduced compared to the coastal waters containing relatively low DOC concentrations. Furthermore, when the coastal seawater was UV oxidized to remove the DOC, Hg(II) uptake was increased. Thus, both the phyto-DOC and natural DOC experiments were consistent in showing reduced uptake of Hg(II) by the green mussels with increasing DOC concentration.

Humic acids are ubiquitous in natural environments with a typical average molecular weight of 2000 to 3000 Da (Matthews et al. 1995) and are effective complexing agents in natural seawater (Weber 1988). In contrast to phyto-DOC and natural DOC, addition of humic acid significantly enhanced the uptake of Hg(II) by the mussels. This was confirmed in 2 repeated experiments, although the ranges of HA concentration used in these 2 experiments were somewhat different. With a further increase in HA concentration to 250 µMC, the uptake of Hg(II) declined. The Hg(II) distribution in the various mussel tissues may point to the potential mechanism of the enhanced uptake in the presence of HA. In the presence of HA, the distribution of Hg(II) in the digestive gland increased, whereas its distribution in the gills decreased. Therefore, some of the Hg(II) may have been bound with humic substances and indeed accumulated through the ingestion process, directly into the digestive gland.

The uptake of MeHg by the mussels in the presence of DOC from different origins was in marked contrast to that of Hg(II). In general, its uptake was not greatly affected by the source and the concentration of DOC (including biogenic and HA). With the removal of DOC from the 2 coastal waters (CWB and TH), for example, the flux of MeHg remained rather constant. Our results from the marine mussels are thus different from other previous studies. For example, Choi et al. (1998) found that DOC reduced the uptake of MeHg by the blackfish *Orthodon microlepidotus*. The distribution of MeHg in different mussel tissues also contrasted strongly with that of Hg(II). A large fraction (48 to 74%) of MeHg was associated with the gills, whereas only a small fraction was in the digestive gland (4 to 21%), suggesting that the gills were the primary sites for MeHg uptake in the mussels. With increasing HA concentration, the fraction of MeHg in the digestive gland increased, but the majority of MeHg was still distributed in the gills and in the other soft tissues. The increased ingestion of MeHg into the digestive gland at a higher HA concentration was not sufficient to result in a much greater uptake.

Mercury uptake influenced by colloidal organic carbon

A few recent studies have emphasized the significance of colloidal complexation for mercury in the marine environment (Stordal et al. 1996b, Choe & Gill 2003, Choe et al. 2003), but there has been essentially no data on the influence of COC on Hg uptake. In our experiments, the majority of Hg(II) remained in the colloidal phase, whereas a significant fraction of MeHg was found in the LMW phase at the end of exposure. Our results demonstrated that colloidal binding significantly influenced the mercury bioavailability. In gen-

eral, colloidal binding enhanced bioavailability of Hg(II) to the mussels, whereas it depressed that of MeHg. Furthermore, the uptake of Hg(II) and MeHg did not vary significantly for colloids isolated from coastal seawater and biogenic sources, whereas the bioavailability of Hg(II) bound with estuarine colloids was comparable to the LMW-complexed Hg(II). These results suggest that the structural properties of the colloids can have a large effect on mercury uptake by mussels. A few recent studies also found that the geochemical origin of colloids critically influences Fe bioavailability to marine phytoplankton (Chen et al. 2003, Wang & Dei 2003). Increasing bioavailability of colloid-bound metals to green mussels has been recently demonstrated for Cr and Fe (Wang & Guo 2000, Pan & Wang 2002). The uptake of colloidal-bound Zn is, however, reduced compared to LMW-bound Zn (Wang & Guo 2000). These data strongly suggest that there are tremendous differences in the mechanisms of metal uptake from the colloidal phase. Carvalho et al. (1999) examined the bioavailability of colloidal metals to a marine shrimp and concluded that uptake rates are comparable for LMW- and colloidal-complexed Ag, Cd, Fe, Hg and Mn, but are higher for LMW-complexed Zn, Ba and Sn.

The uptake of colloidal Hg(II) and MeHg aged for different durations may also indicate the mechanisms involved. Both species of Hg were radiolabeled with the diatom colloids for 2 to 9 d. The uptake of colloid-bound mercury decreased with increasing contact time with the colloids. This trend was consistent between Hg(II) and MeHg; thus, colloidal mercury is less labile and dissociation from the colloidal particles may be the necessary step in the uptake of colloidal mercury. Furthermore, a few studies have indicated that metals complexed with DOC may be directly taken up through absorption of natural DOC by bivalves (Roditi et al. 2000, Guo et al. 2001). Roditi et al. (2000) have shown that the uptake of dissolved Cd, Ag and Hg by zebra mussels increases significantly (from 4 to 32× depending on metals) in the presence of DOC. Guo et al. (2001) documented an increasing uptake of Cd, Co, Ag and Zn by the American oyster *Crassostrea virginica* with increasing DOC concentration. They attributed this increase to the direct ingestion and digestion of colloidally complexed metals at higher DOC concentrations. The increase in Hg(II) uptake by the green mussels with increasing HA concentrations can be explained by the possible direct absorption of natural DOC (Tack & Polk 1996, Roditi et al. 2000) or through the increasing interactions of metals with the charged surfaces of the animals (Campbell et al. 1997). Similarly, the increasing influx of colloidal Hg(II) compared to the LMW-Hg may also be attributed to ingestion into the mussel digestive gland.

In conclusion, our study demonstrated that DOC, and particularly COC, can substantially affect the accumulation of Hg(II) and MeHg in green mussels. Enhanced uptake of Hg(II) was documented in the presence of humic acids as standard DOC compounds, whereas reduced uptake was found in the presence of DOC obtained from diatom decomposition or from natural seawater collected from various locations in Hong Kong coastal waters. The uptake of MeHg was, however, not influenced by the concentration and origin of DOC. In contrast, COC binding of Hg(II) significantly enhanced its uptake, but the uptake of MeHg was depressed when bound with the colloidal materials. Given the contrasting influences of DOC and COC on mercury uptake, there is a need to consider DOC and COC separately in their control of trace metal bioavailability to aquatic organisms. There is also a substantial need to further study the influence of DOC on metal bioavailability, as well as on the mechanisms of metal accumulation in marine bivalves.

Acknowledgements. We thank the anonymous reviewers for their helpful comments on this work. This study was supported by a Competitive Earmarked Research Grant from the Hong Kong Research Grants Council (HKUST6118/01M) to WXW.

LITERATURE CITED

Absil MCP, Berntssen M, Gerringa LJA (1996) The influence of sediment, food and organic ligands on the uptake of copper by sediment-dwelling bivalves. Aquat Toxicol 34: 13–29

Benner R (2002) Chemical composition and reactivity. In: Hansell DA, Carlson CA (eds) Biogeochemistry of marine dissolved organic matter. Academic Press, Amsterdam, p 59–90

Blackmore G, Wang WX (2003) Inter-population differences in Cd, Cr, Se, and Zn accumulation by the green mussel *Perna viridis* acclimated at different salinities. Aquat Toxicol 62:205–218

Boudou A, Delnomdedieu M, Georgescauld D, Ribeyre F, Saouter E (1991) Fundamental roles of biological barriers in mercury accumulation and transfer in freshwater ecosystems. Water Air Soil Pollut 56:807–821

Campbell PGC (1995) A critique of the free-ion activity model. In: Turner DR (ed) Metal speciation and bioavailability in aquatic systems. John Wiley & Sons, Chichester, p 45–102

Campbell PGC, Twiss MR, Wilkinson KJ (1997) Accumulation of natural organic matter on the surfaces of living cells: implications for the interaction of toxic solutes with aquatic biota. Can J Fish Aquat Sci 54:2543–2554

Carvalho RA, Benfield MC, Santschi PH (1999) Comparative bioaccumulation studies of colloidally complexed and free-ionic heavy metals in juvenile brown shrimp *Penaeus aztecus* (Crustacea: Decapoda: Penaeidae). Limnol Oceanogr 44:403–414

Chen M, Wang WX (2001) Bioavailability of natural colloid-bound iron to marine plankton: influences of colloidal size and aging. Limnol Oceanogr 46:1956–1967

Chen M, Dei R, Wang WX, Guo L (2003) Marine diatom

uptake of iron bound with natural colloids of different origins. Mar Chem 81:177–189
Choe KY, Gill GA (2003) Distribution of particulate, colloidal, and dissolved mercury in San Francisco Bay estuary. 2. Monomethyl mercury. Limnol Oceanogr 48:1547–1556
Choe KY, Gill GA, Lehman R (2003) Distribution of particulate, colloidal, and dissolved mercury in San Francisco Bay estuary. 1. Total mercury. Limnol Oceanogr 48:1535–1546
Choi BH (1990) Effects of methylmercury on the developing brain. In: Clarkson TW (ed) Advances in mercury toxicology. Plenum, New York, p 315–337
Choi MH, Cech JJ, Lagunas-Solar MC (1998) Bioavailability of methylmercury to sacramento blackfish (*Orthodon microlepidotus*): Dissolved organic carbon effects. Environ Toxicol Chem 17:695–701
Chong K, Wang WX (2001) Comparative studies on the biokinetics of Cd, Cr and Zn in the green mussel *Perna viridis* and the Manila clam *Ruditapes philippinarum*. Environ Pollut 115:107–121
Dickman MD, Leung KMC, Koo LCL (1999) Mercury in human hair and fish: is there a Hong Kong male subfertility connection? Mar Pollut Bull 39:352–356
Golding GR, Kelly CA, Sparling R, Loewen PC, Rudd JWM, Barkay T (2002) Evidence for facilitated uptake of Hg(II) by *Vibrio anguillarum* and *Escherichia coli* under anaerobic and aerobic conditions. Limnol Oceanogr 47:967–975
Guo L, Santschi PH (1997) Composition and cycling of colloids in marine environments. Rev Geophy 35:17–40
Guo L, Coleman CH, Santschi PH (1994) The distribution of colloidal and dissolved organic carbon in the Gulf of Mexico. Mar Chem 45:105–119
Guo L, Hunt BJ, Santschi PH, Ray SM (2001) Effect of dissolved organic matter on the uptake of trace metals by American oysters. Environ Sci Technol 35:885–893
Guo L, Santschi PH, Ray SM (2002) Metal partitioning between colloidal and dissolved phases and its relation with bioavailability to American oysters. Mar Environ Res 54:49–64
Huang YW (1982) Effects of temperature and chelating agents on cadmium uptake in the American oyster. Bull Environ Contam Toxicol 28:546–551
Jensen S, Jernelov A (1969) Biological methylation of mercury in aquatic organisms. Nature 223:753–754
Laporte JM, Truchot JP, Ribeyre F, Boudou A (1997) Combined effects of water pH and salinity on the bioaccumulation of inorganic mercury and methylmercury in the shore grab *Carcinus maenas*. Mar Pollut Bull 34:880–893
Laporte JM, Truchot JP, Mesmer-Dudons N, Boudou A (2002) Bioaccumulation of inorganic and methylated mercury by the gills of the shore crab *Carcinus maenas*: transepithelial fluxes and histochemical localization. Mar Ecol Prog Ser 231:215–228
Lee BG, Wallace WG, Luoma SN (1998) Uptake and loss kinetics of Cd, Cr and Zn in the bivalves *Potamocorbula amurensis* and *Macoma balthica*: effects of size and salinity. Mar Ecol Prog Ser 175:177–189
Luoma SN, Fisher NS (1997) Uncertainties in assessing contaminant exposure from sediments. In: Ingersoll CG, Dillon T, Biddinger GR (eds) Ecological risk assessment of contaminated sediments. SETAC Spec Publ Series, Pensacola, p 211–237
Matthews JC, Ekuta JE, Drake KD, Watson CF, Benson WH (1995) Nature and extent of the interactions of humic acids with a water-treatment algicide and a fungicide. Chemosphere 30:1565–1572
McCarthy JF (1989) Bioavailability and toxicity of metals and hydrophobic organic contaminants. In: Suffet IH, MacCarthy MP (ed) Aquatic humic substances: Influence of fate and treatment of pollutants. American Chemical Society, Washington, p 263–277
Millero FJ, Sohn ML (1992) Chemical oceanography. CRC Press, Boca Raton, p 353–414
Nagase H, Ose Y, Sate T, Ishikawa T (1982) Methylation of mercury by humic substances in an aquatic environment. Sci Total Environ 24:133–142
Odin M, Ribeyre F, Boudou A (1996) Temperature and pH effects on cadmium and methylmercury bioaccumulation by nymphs of the burrowing mayfly *Hexagenia rigida*, from water column or sediment source. Arch Environ Contam Toxicol 31:339–349
Pan JF, Wang WX (2002) Comparison of the bioavailability of Cr and Fe bound with natural colloids of different origins and sizes to two marine bivalves. Mar Biol 141:915–924
Pan JF, Wang WX (2004) Influences of dissolved and colloidal organic carbon on the uptake of Ag, Cd, and Cr by marine mussel *Perna viridis*. Environ Pollut 129:467–477
Parent L, Twiss MR, Campbell PGC (1996) Influences of natural dissolved organic matter on the interaction of aluminum with the microalga *Chlorella*: A test of the free-ion model of trace metal toxicity. Environ Sci Technol 30: 1713–1720
Parsons ECM (1999) Trace element concentrations in whole fish from North Lantau waters, Hong Kong. ICES J Mar Sci 56:791–794
Rabenstein DL (1978) The chemistry of methylmercury toxicology. J Chem Educ 55:292–296
Riisgard HU, Famme P (1986) Accumulation of inorganic and organic mercury in shrimp. Mar Pollut Bull 17:255–257
Roditi HA, Fisher NS, Sanudo-Wilhelmy SA (2000) Uptake of dissolved organic carbon and trace elements by zebra mussels. Nature 407:8–80
Rouleau C, Block M (1997) Fast and high-yield synthesis of radioactive $Me^{203}Hg(II)$. Appl Organomet Chem 11:751–753
Rouleau C, Pelletier E, Tjalve H (1993) The uptake and distribution of $^{203}HgCl_2$ and $Me^{203}HgCl$ in the starfish *Asterias rubens* after 24 h exposure studied by impulse counting and whole-body autoradiography. Aquat Toxicol 26:103–115
Santschi PH, Guo LD, Means JC, Ravichandran M (1999) Natural organic matter binding of trace metals and trace organic contaminants in estuaries. In: Bianchi TS, Pennock JR, Twilley RR (eds) Biogeochemistry of Gulf of Mexico estuaries. John Wiley & Sons, New York, p 347–380
Saouter E, Hare L, Campbell PGC, Boudou A, Ribeyre F (1993) Mercury accumulation in the burrowing mayfly *Hexagenia rigida* (Ephemeroptera) exposed to MeHgCl or $HgCl_2$ in water and sediment. Water Res 27:1041–1048
Sjoblom A, Meili M, Sundbom M (2000) The influence of humic substances on the speciation and bioavailability of dissolved mercury and methylmercury, measured as uptake by *Chaoborus* larvae and loss by volatilization. Sci Total Environ 261:115–124
Stordal MC, Santschi PH, Gill GA (1996a) Colloidal pumping: Evidence for the coagulation process using natural colloids tagged with Hg 203. Environ Sci Technol 30: 3335–3340
Stordal MC, Gill GA, Wen LS, Santschi PH (1996b) Mercury phase speciation in the surface waters of three Texas estuaries: Importance of colloidal forms. Limnol Oceanogr 41: 52–61
Tack JF, Polk P (1996) The uptake of colloidal melanin from seawater by marine bivalves. PSZN I: Mar Ecol 17:543–548
Wang WX, Dei RCH (2003) Bioavailability of iron bound to organic colloids by cyanobacteria *Synechococcus* and *Trichodesmium*. Aquat Microb Ecol 33:247–259.

Wang WX, Guo L (2000) Influences of natural colloids on metal bioavailability to two marine bivalves. Environ Sci Technol 34:4571–4576

Wang WX, Guo L (2001) Production of colloidal organic carbon and trace metals by phytoplankton decomposition. Limnol Oceanogr 46:278–286

Wang WX, Fisher NS, Luoma SN (1996) Kinetic determinations of trace element bioaccumulation in the mussel *Mytilus edulis*. Mar Ecol Prog Ser 140:91–113

Watras CJ, Bloom NS (1992) Mercury and methylmercury in individual zooplankton—Implications for bioaccumulation. Limnol Oceanogr 37:1313–1318

Weber J (1988) Binding and transport of metals by humic materials. In: Frimmel FH, Christman RF (eds) Humic substances and their role in the environment. Wiley, New York, p 165–178

Winner RW (1984) The toxicity and bioaccumulation of cadmium and copper as affected by humic acid. Aquat Toxicol 5:264–274

Winner RW (1986) Interactive effects of water hardness and humic acid on the chronic toxicity of cadmium to *Daphnia pulex*. Aquat Toxicol 8:281–293

Wobeser G (1975) Acute toxicity of methylmercury chloride and mercuric chloride for rainbow trout (*Sulmo gairdneri*) fry and fingerlings. J Fish Res Board Can 32:2005–2013

Wood JM, Kennedy FS, Rosen CG (1968) Synthesis of methylmercury compounds by extract of methanogenic bacteria. Nature 220:173–174

Wright DA, Mason RP (2000) Biological and chemical influences on trace metal toxicity and bioaccumulation in the marine and estuarine environment. Int J Environ Pollut 13: 226–248

Editorial responsibility: Otto Kinne (Editor), Oldendorf/Luhe, Germany

Submitted: October 31, 2003; Accepted: May 18, 2004
Proofs received from author(s): July 21, 2004