

Effect of Dissolved Organic Matter on the Uptake of Trace Metals by American Oysters

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To examine the effects of dissolved organic matter on metal bioavailability, uptake of trace metals (Cd, Co, Hg, Cr, Ag, Zn) by American oysters (*Crassostrea virginica*) was compared between treatments with different dissolved organic carbon (DOC) concentrations and contrasting low molecular weight (LMW, 1 kDa) and high molecular weight (HMW, 1 kDa–0.2 μ m) DOC fractions, using radiotracer techniques and short-term exposure experiments. Uptake rate constants ($\text{mL g}^{-1} \text{h}^{-1}$) of metals, in general, increased with increasing DOC concentrations, with an initial decrease at lower DOC concentrations. Oyster dry weight concentration factors (DCF, mL g^{-1}), determined at the end of exposure experiments (8 h), also increased for Cd, Co, Cr, Ag, and Zn, but decreased for Hg, with increasing DOC concentrations. Changes of metal uptake rate constants and DCF values with DOC concentration suggest that metal uptake pathways by American oysters vary from predominantly uptake (by diffusion of neutral) of free ionic, inorganically complexed, and LMW organic ligand complexed metals at very low DOC concentration to direct ingestion and digestion of HMW or colloiddally complexed metals at higher DOC concentrations. Measured partition coefficients (K_c) between dissolved and colloidal phases were comparable between metals, ranging from $10^{5.12}$ to $10^{5.75} \text{ mL g}^{-1}$. However, DCF values and uptake rate constants differed considerably between metals, with the highest DCF values and uptake rate constants found for B-type metals, e.g., Ag, Hg, Zn, and Cd, and the lowest ones for several intermediate-type metals (e.g., Co, Cr). Metal types and thus the interaction of metals with organic ligands, such as strong complexation of B-type metals with S-containing organic ligands, may play an important role in the bioavailability and toxicity of metals to aquatic organisms. Differences in metal uptake in contrasting LMW and HMW DOC treatments suggest a generally depressed bioavailability of colloiddally complexed metals at low DOC concentration (0.5 ppm) but a generally enhanced uptake at higher DOC concentrations.

Introduction

The effect of natural organic matter (NOM) on the bioavailability and toxicity of metals to aquatic organisms has long been recognized in environmental research (1–6). A reduction in metal toxicity was usually found in the presence of

NOM, which was largely ascribed to the presence of organic ligands that bind metals and reduce the concentration of free ionic metals in the aquatic environment. The observation that bioavailability and toxicity of trace metals to aquatic organisms decrease in the presence of NOM and with decreasing free metal ion concentration has been used to support the free ion activity model (6, 7, and references therein). However, many of the studies supporting the free ion activity model are based on organisms (phytoplankton, zooplankton, and invertebrates) other than bivalve molluscs (8, 9) and have involved model complexing ligands (5). The effects of NOM on metal bioavailability to bivalves have rarely been studied (10, 11) and are not well understood (5, 12–14).

Marine bivalves, which are filter feeding animals, are capable of concentrating metals and organic pollutants from the large volumes of seawater they filter. Therefore, marine bivalves, especially oysters and mussels, have been extensively used as model organisms in environmental studies (15–20) and as pollution indicator organisms in environmental assessment and monitoring programs (21–24). To better use bivalves as a biomonitor, pathways and mechanisms of metal uptake by bivalves need to be better understood.

Recent studies have shown that portions of dissolved organic carbon (DOC) could be directly taken up by bivalves, in addition to particulate organic matter (25, 26). Since trace metals are often associated with potentially nutritious DOC (27–29), it is possible that the uptake of DOC by bivalves may also enhance the uptake of trace metals and organic pollutants. Indeed, increased bioconcentration of organic chemicals in aquatic organisms as a consequence of DOC uptake has been reported (30). Enhanced metal uptake by bivalves and other organisms in the presence of high molecular weight DOC has also been reported (26, 31–33). However, the effects of DOC on the uptake of metals by bivalves remain poorly known (26, 33). Clearly, there is much to be learnt about the role of DOM in governing the bioavailability of metals to marine bivalves (11).

The objective of this study was to examine the effects of DOC on the accumulation of selected metals (Cd, Co, Hg, Cr, Ag, Zn) by American oysters using radiotracer techniques. Short-term exposure experiments were carried out under different DOC concentrations. Metal uptake by oysters was also compared between low molecular weight (LMW) and high molecular weight (HMW) DOC treatments to examine whether metal complexation by HMW DOC enhances or depresses the absorption of trace metals by American oysters.

Materials and Methods

Oysters. American oysters (*Crassostrea virginica*) collected from Galveston Bay, Texas, in March 2000, were used for controlled laboratory uptake experiments. Junior oysters were well cleaned and then acclimated in natural seawater for 2 days. Before the uptake experiments, oysters were cleaned again and kept in <1 kDa seawater for 6–8 h. Oysters with similar body shape and weight ranging from 20 to 25 g were selected for subsequent uptake experiments.

Metals. Radioactive metals ^{109}Cd , ^{57}Co , ^{203}Hg , $^{51}\text{Cr(VI)}$, $^{110\text{m}}\text{Ag}$, and ^{65}Zn (Amersham, NJ or Isotope Products, CA)

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TABLE 1. Typical Dissolved Organic Carbon (DOC) Concentrations (ppm) and Percentages of Colloidal Organic Carbon (COC, 1 kDa-0.2 μ m) in Different Coastal and Estuarine Waters of the Gulf of Mexico and in Different Treatments

location or treatment	DOC (ppm)	COC/DOC (%)	reference
Gulf of Mexico	0.54–1.03	31–42	48
Mississippi delta	0.76–3.0	27–45	49
Galveston Bay (TX)	1.7–5.6	59–63	41
Sabine-Neches estuary (TX)	4.4–20.9	— ^a	50
Ochlockonee estuary (FL)	2.3–18.8	37–88	42
treatment-1 ^b	0	— ^a	this work
treatment-2	0.5	52–60	this work
treatment-3	5	71–80	this work
treatment-4	10	70–85	this work

^a Not available. ^b Treatment-1 was the UV irradiated ultrafiltrate (<1 kDa) seawater, which contained only negligible DOC concentration.

were selected for the uptake experiments. These metals range from transition (or intermediate-type) metals to B-type metals, with different preferences for binding with different organic ligands (34). For example, B-type metals, such as Ag, Hg, and Zn, can preferentially form complexes with S-containing ligands while Cr and other A-type metals prefer to bind with O-containing or N-containing ligands in natural organic matter (35, 36).

Extraction of Natural Organic Matter. Seawater (with a salinity of ~30‰) was collected from Galveston Bay, where oysters were collected, and prefiltered through a 0.2 μ m Nuclepore cartridge. High molecular weight (HMW, 1 kDa-0.2 μ m) DOC was then concentrated from the prefiltered seawater using a 1 kDa Amicon S10Y1 ultrafiltration membrane (37, 38). The ultrafiltered seawater (<1 kDa) was further exposed to UV radiation to reduce the residual LMW DOC. This UV irradiated seawater (with a salinity of ~30‰ and a DOC concentration of <0.1 ppm) was further diluted with ultrapure water to result in a salinity of ~20‰ “DOC-free” seawater for uptake experiments. A salinity of 20‰ has previously been shown to be the optimum salinity for American oyster growth in Galveston Bay (39). This “DOC-free” seawater was used as a control in uptake experiments. Concentrations of DOC in all samples were quantified using a high temperature combustion TOC analyzer (40).

A total of four treatments with different DOC concentrations were set up to examine how metal bioavailability to American oysters changes with natural DOC concentrations. The DOC concentrations in these four treatments were 0 (~0.05 ppm, as a control), 0.5, 5, and 10 ppm, respectively, which were prepared by adding different volumes of concentrated HMW DOC (1 kDa-0.2 μ m) and UV irradiated “DOC-free” seawater. DOC concentrations used in the uptake experiments are within the ranges found in coastal and estuarine waters (Table 1). Proportions of dissolved (<1 kDa) and colloidal (1 kDa-0.2 μ m) organic carbon fractions changed with DOC concentrations (Table 1), ranging from mostly LMW in the low DOC treatments to mostly HMW DOC in the high DOC treatments (Table 1). Natural colloidal organic carbon (COC) in estuarine waters comprises up to 30–88% of the total DOC (41, 42). The presence of high COC concentrations in estuarine and coastal waters may significantly influence a metal’s speciation and bioavailability to bivalves (26, 33).

Uptake Experiments. Short-term exposure experiments were carried out to examine the important role of DOC in governing the accumulation of trace metals by American oysters and to compare the effects of low molecular weight (LMW) and high molecular weight (HMW) DOC on metal uptake by oysters. The short-term exposure approach used

here has been applied in many previous studies (e.g., 11, 43).

Four individual oysters were used in each treatment as replicates. Oysters were placed separately in 500 mL Teflon containers with 300 mL of solution containing four different DOC concentrations. All containers with different DOC concentrations were spiked with the same amounts of radioactive metals, ¹⁰⁹Cd, ⁵⁷Co, ²⁰³Hg, ⁵¹Cr(VI), ^{110m}Ag, and ⁶⁵Zn, before the uptake experiments, and then the mixtures were equilibrated for 2 h. Experimental preparations, tracer addition, and sample handling were carried out inside on a clean bench to prevent possible metal contaminations.

At time intervals of 2, 4, 6, and 8 h, oysters were removed, rinsed with unlabeled LMW seawater (<1 kDa), and γ counted to determine the activity of each metal. After γ counting, oysters were returned to their original Teflon jars. In addition, an aliquot of the solution phase in each container was also γ counted for the activity determination of each metal at each time interval for the calculation of the metal’s concentration factor in oysters. After the uptake experiment was terminated (i.e., after 8 h), each oyster was dissected into shell and soft tissue, which were then γ counted again to examine the oyster body partitioning of metals between shell and soft tissue.

After γ counting, preweighed soft tissues of oysters were further dried at 80 °C for 24 h. The dry weight was then used to calculate the dry weight based concentration factor (DCF), as follows

$$DCF \text{ (mL g}^{-1}\text{)} = A_{ST}/A_W \quad (1)$$

where A_{ST} is the radioactivity of metals in the soft tissues of oyster (dpm/g dried tissue), and A_W is the radioactivity of metals in the water (dpm/mL solution). Because the radioactivity of metals in the dried soft tissues was not available at each time interval (since oysters were dissected only after 8 h of exposure), a conditional DCF (CDCF) was used for the time series data points to calculate uptake rate constants of metals. CDCF was calculated from the radioactivity of metals in the whole individual oyster (A_O , dpm/g dried tissue plus shell) and in the water (A_W , dpm/mL solution) at each time interval:

$$CDCF \text{ (mL g}^{-1}\text{)} = A_O/A_W \quad (2)$$

A separate experiment contrasting LMW (<1 kDa) and HMW (1 kDa-0.2 μ m) DOC treatments was also carried out to compare the uptake of metals by the American oyster. The LMW DOC treatment was prepared from the ultrafiltrate, which is the <1 kDa seawater collected during ultrafiltration (38), while the HMW DOC treatment was prepared from the concentrated HMW DOC (1 kDa-0.2 μ m) solution, extracted by a 1 kDa ultrafilter and diluted with the UV irradiated “DOC-free” seawater. The overall DOC concentration (0.5 ppm) in these two treatments was the same. Four individual oysters were used in each treatment. Other procedures were the same as stated above.

Radioactivity Measurements by γ Counting. Radioactivities of ¹⁰⁹Cd, ⁵⁷Co, ²⁰³Hg, ⁵¹Cr, ^{110m}Ag, and ⁶⁵Zn were measured on a Canberra ultrahigh purity Ge well detector (31, 38). The γ emission of ¹⁰⁹Cd was detected at 88 keV, ⁵⁷Co at 122 keV, ²⁰³Hg at 279 keV, ⁵¹Cr at 320 keV, ^{110m}Ag at 657 keV, and ⁶⁵Zn at 1115 keV. Counting times were adjusted to result in a propagated error of <5% or better for all samples depending on metals. Decay corrections were applied to correct to the initial time of the experiment whenever necessary. One σ standard errors reported here are those after error propagation.

Results and Discussion

Variations of Metal Uptake with DOC Concentration. Figure 1 shows the measured dry weight concentration factor (DCF)

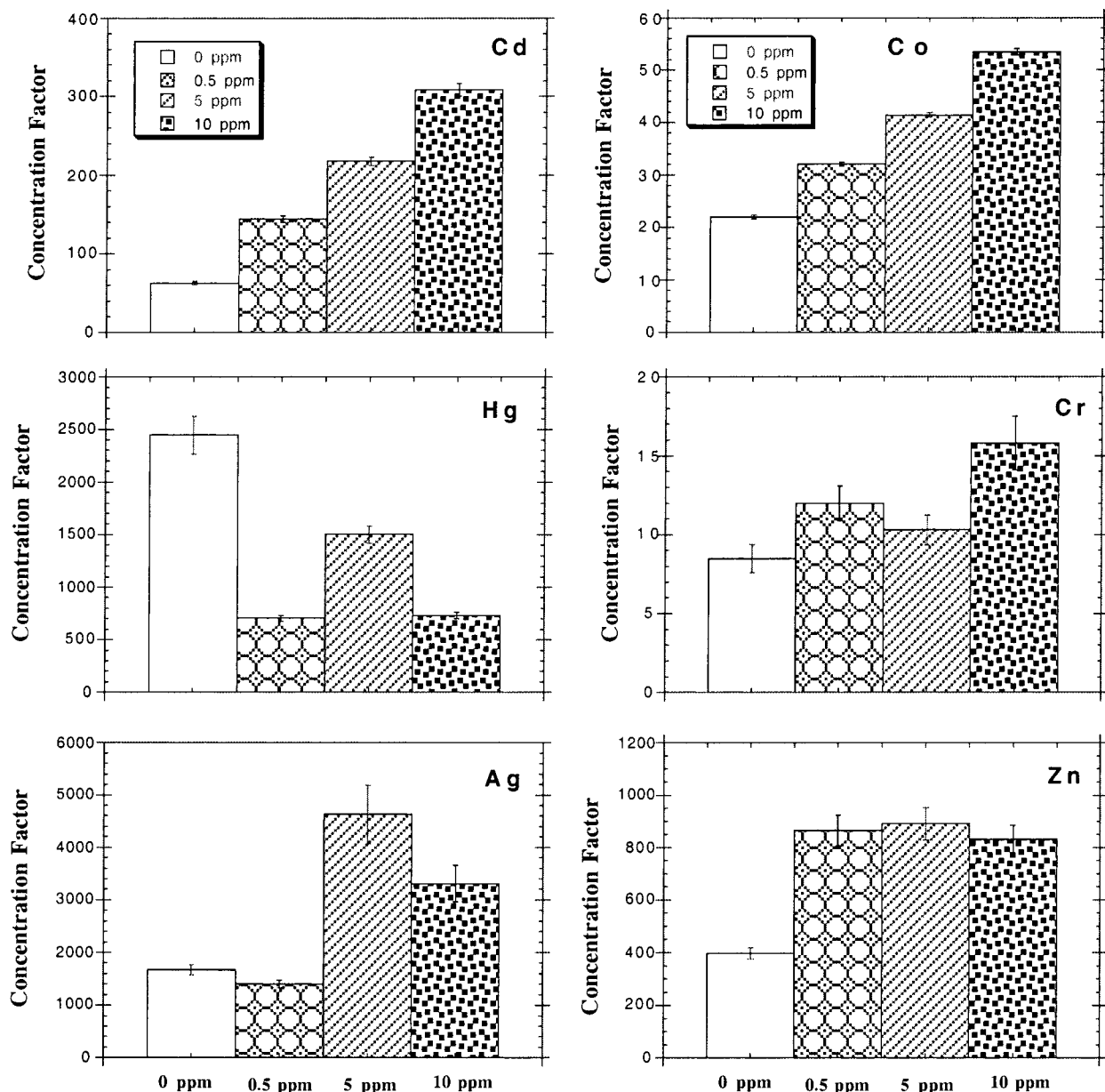


FIGURE 1. Measured dry weight based concentration factor (DCF, mL g^{-1}) of metals between oyster tissue and ambient water at different dissolved organic carbon (DOC) concentrations (0, 0.5, 5, and 10 ppm).

TABLE 2. Measured Average Uptake Rate Constants for Different Metals in Different DOC Treatments for American Oysters (*C. virginica*) as Well as a Comparison with Marine Mussels (*M. edulis*)

metal	uptake rate constant ($\text{mL g}^{-1} \text{h}^{-1}$)				mussels ^a ($\text{mL g}^{-1} \text{h}^{-1}$)
	DOC (0 ppm)	DOC (0.5 ppm)	DOC (5 ppm)	DOC (10 ppm)	
Cd(II)	11.7 ± 0.6	9.13 ± 0.46	10.7 ± 0.53	27.6 ± 1.4	15.2
Co(II)	18.1 ± 0.9	10.2 ± 0.5	10.4 ± 0.52	20.9 ± 1.01	5.2
Hg(II)	68.2 ± 3.4	18.2 ± 0.9	45.2 ± 2.3	73.3 ± 3.7	N/A
Cr(VI)	1.44 ± 0.07	1.46 ± 0.07	0.84 ± 0.04	1.60 ± 0.08	4.2
Ag(I)	67.1 ± 3.4	45.0 ± 2.2	261 ± 13	426 ± 22	74.8
Zn(II)	32.6 ± 0.2	57.3 ± 2.9	66.0 ± 3.3	109 ± 6	43.5

^a Uptake rate constants for marine mussels are taken from Wang et al. (17) for Cd, Co, Ag, and Zn and from Wang et al. (18) for Cr(VI).

in oysters for different metals (Cd, Co, Hg, Cr, Ag, and Zn) under different dissolved organic carbon (DOC) concentrations. Values of measured DCF exhibited a general increase with increasing DOC concentrations for Cd, Co, Cr, Zn, and Ag (Figure 1). For example, DCF values of Cd after 8 h exposure increased from $\sim 63 \pm 2 \text{ mL g}^{-1}$ in the "DOC-free" treatment

to $140\text{--}300 \text{ mL g}^{-1}$ in treatments with added DOC. In contrast to these metals, Hg was somewhat depressed in its bio-availability to oysters as DOC concentration increased (Figure 1), with DCF values decreasing from $\sim 2400 \text{ mL g}^{-1}$ in the "DOC-free" treatment to $700\text{--}1500 \text{ mL g}^{-1}$ in treatments with added DOC.

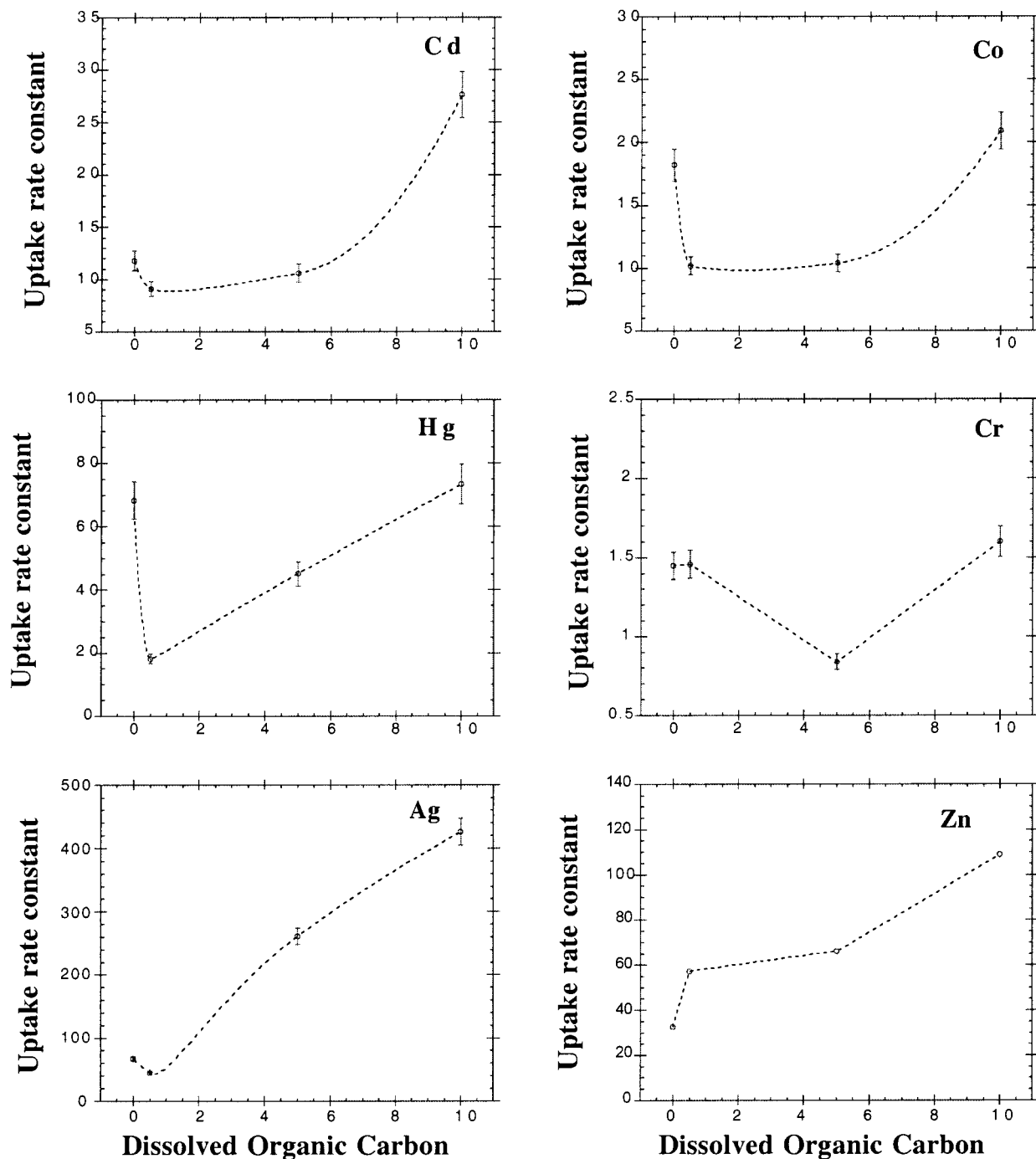


FIGURE 2. Variations of metal uptake rate constants ($\text{mL g}^{-1} \text{h}^{-1}$) with dissolved organic carbon concentrations.

Many previous studies have shown that bioavailability and toxicity of metals to aquatic organisms can be reduced as DOC concentration increases (2, 6), a trend that can be predicted using a free ion activity model (7). Our results showing reduced uptake of Hg by American oysters with increasing DOC concentrations seem broadly consistent with the general trend predicted by the free ion activity model. Nevertheless, our observations of increased uptake of Zn, Cd, and Ag (and less for Co and Cr), caused by further increasing DOC concentrations, seem to be contradictory to the free ion activity model. However, the free ion activity model is largely based on plankton (or alga) testing and/or surrogate ligands. In addition, it has been found that the free ion activity model fails for some elements and algae (5). Lee and Luoma (14) reported increased bioavailability of metals to estuarine bivalves due to the addition of macroalgae (i.e.,

organic matter). Most recently, Roditi et al. (26) also found that uptake of dissolved Cd, Ag, and Hg by zebra mussels increased significantly in the presence of HMW DOC, ranging from 4 to 32 times depending on metals. Thus, our results reported here are consistent with these previous findings and further elucidate new pathways of metal uptake by marine bivalves, i.e., metals can be bioavailable to America oysters not only from free ionic forms but also from HMW DOC complexed species at higher DOC concentrations. This is important for a better use of bivalves as pollution monitors in environmental assessment and monitoring programs (21, 24).

Recently, direct evidence for the accumulation of natural DOC on the surfaces of living cells has been documented (5, 13). Campbell et al. (13) also found that the negative surface charge of cell surfaces can increase in the presence of DOC.

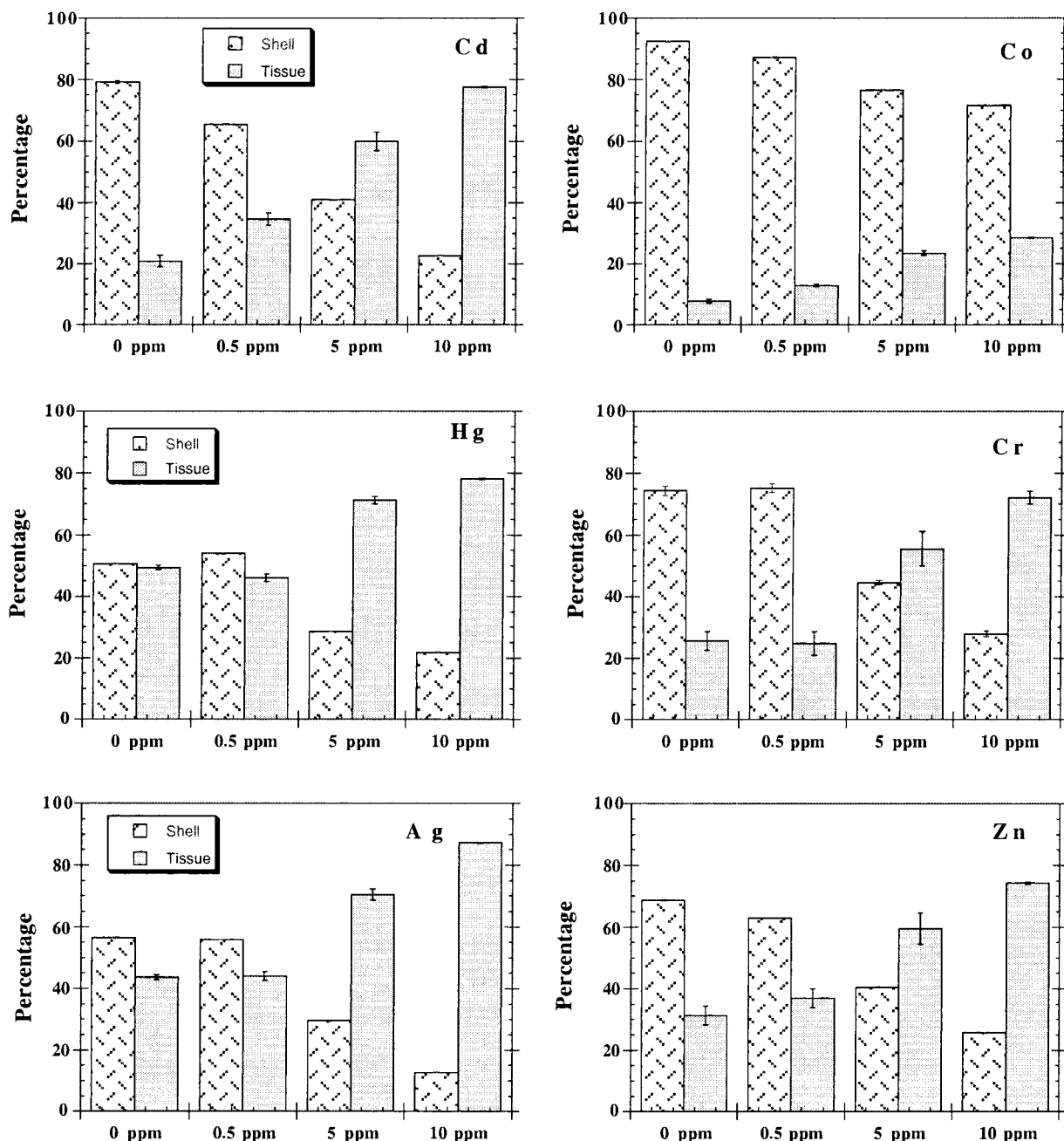


FIGURE 3. Body distribution of metals between oyster shell and soft tissue after 8 h exposure.

The increased negative charge on the surfaces of living cells could also enhance the direct interactions of metal ions with organisms. In addition, it is also possible that metals complexed with DOC can be directly taken up through absorption of natural DOC by marine bivalves (14, 26, 33). Thus, our observed increased uptake of metals by American oysters caused by increasing DOC concentrations can be reconciled by assuming direct absorption of natural DOC (25, 26) or through the increased interactions of metals with charged organism surfaces (13).

Routes or pathways of metal uptake by American oysters at low DOC concentrations could be largely from the absorption of LMW "dissolved" metals, including those free ionic, inorganically, and organically complexed forms, via carrier molecules, direct diffusion of neutral complexes, or ATP-requiring channels (7, 10, 11, 44). As DOC concentrations increased, uptake of metals became slightly depressed, such as in the case of Ag and Hg (Figure 1). However, as DOC concentrations continued to increase, the direct uptake of

organically complexed metals, likely through the ingestion and digestion of colloiddally bound metals (14), or pinocytosis in gills, could have caused the enhanced metal uptake. More studies are needed to distinguish these different pathways.

Metal Uptake Rate Constants for American Oysters. On the basis of the time series data from treatments with different DOC concentrations, the average uptake rate constants ($\text{mL g}^{-1} \text{h}^{-1}$) of metals could be calculated from the slope of plots of conditional dry weight concentration factor (CDCF) vs time. Results of calculations of uptake rate constants are listed in Table 2 and depicted in Figure 2. In general, average uptake rate constants of metals increased as DOC concentration increased. For most metals, uptake rates were lowest when the DOC concentration was 0.5 ppm. Uptake of Zn was consistently enhanced by increased DOC concentrations. However, the uptake rate constants for Hg in treatments with elevated DOC concentrations did not get higher than that in the "DOC-free" treatment until the DOC concentration was 10 ppm, even though Hg also showed an increase in

TABLE 3. Comparison of log K_c and Dry Weight Concentration Factor (DCF)^a

metal	metal type	log K_c (l kg ⁻¹)	DCF (mL g ⁻¹)	
			DOM-free treatment	treatments with DOM
Cd(II)	B-type	5.12 ± 0.19	63 ± 2.3	144–308
Co(II)	intermediate-type	5.27 ± 0.05	22 ± 0.34	32–54
Hg(II)	B-type	5.75 ± 0.26	2447 ± 178	704–1502
Cr(VI)	intermediate-type	5.15 ± 0.18	8.5 ± 0.9	10–16
Ag(I)	B-type	5.13 ± 0.14	1667 ± 97	1400–4629
Zn(II)	B-type	5.32 ± 0.28	398 ± 22	831–891

^a Values of DCF are those measured after 8 h exposure. K_c is defined here as the partition coefficient of metals between colloidal (1 kDa–0.2 μm) and 1 kDa ultrafilter-passing phases.

uptake rate constants with increasing DOC after the initial decrease. Cr (and Fe, not shown) showed no obvious increase in the uptake rate constants, with the initial decrease extending to a DOC concentration of 5 ppm.

The variations of metal uptake rate constants with different DOC concentrations, which show an initial drop at lower DOC concentration and an increase at higher DOC concentrations (Figure 2), further support our proposed uptake pathways as discussed in the previous section. That is, without DOC or at very low DOC concentrations, metal uptake by oysters is largely from direct diffusion or absorption of free ionic, inorganically, and/or organically (LMW ligands) complexed metals. As DOC concentration increases, metal uptake by oysters initially decreases due to the complexation of metals with organic matter. However, when DOC concentration gets high enough, metal uptake pathways by oysters shift from uptake of ionic forms (or direct diffusion of neutral forms) of metals to direct ingestion or digestion of organically complexed metals, causing metal uptake rate constants to increase again with increasing DOC concentrations. This indicates that colloidally complexed metals could also be directly bioavailable when DOC concentrations are high enough.

To the best of our knowledge, there are very few other data available for comparison with our observations of the effects of DOC on the uptake rate constants of metals in American oysters. However, values of average uptake rate constants reported here (Table 2) are comparable with those observed for marine mussels under natural environmental conditions (17, 18). Among the observed uptake rate constants of the different metals, Ag, Hg, and Zn had the highest values, followed by those of Cd, Co, and Cr (Table 2). It is quite evident that metals that tend to complex with S-containing ligands (e.g., Ag, Hg, and Zn) have the highest uptake rate constants in American oysters, while metals that are intermediate-type or A-type elements (e.g., Co, Cr) have lower uptake rate constants. Similar trends have been reported for marine mussels (17, 18). It is interesting that Cd, one of the B-type metals, also had relatively lower uptake rate constants compared to other B metals (Table 2), likely due to its relatively low level of complexation with HMW DOC (27, 29).

Variations of DCF between Metals. Figure 1 shows that measured DCF values of metals were generally higher for Cd, Co, Cr, Ag, and Zn as DOC concentrations increased, while DCF values of Hg in treatments with elevated DOC concentrations were somewhat depressed. Organic ligands appear to depress oyster uptake of Hg, but they appear to enhance it for Ag, Zn, Cd, Cr, and Co. When we further compare the DCF values of different metals, Ag and Hg showed highest DCF values (700–4500 mL g⁻¹), followed by Zn (400–900 mL g⁻¹), Cd (63–310 mL g⁻¹), Co (22–54 mL g⁻¹), and Cr (8–16 mL g⁻¹) (Table 3).

Values of DCF between metals did not follow the pattern of metal partition coefficients (K_c) between colloidal and 1

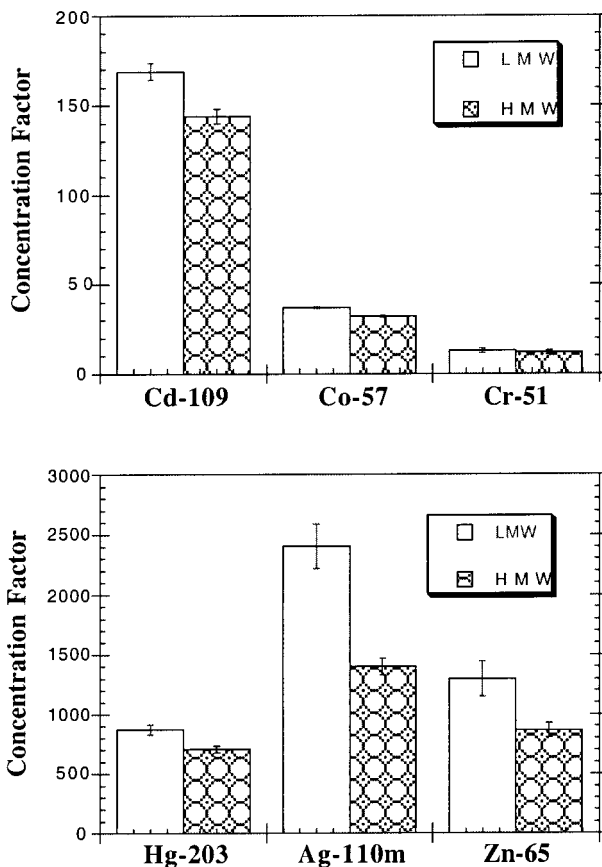


FIGURE 4. Measured dry weight concentration factor (DCF, mL g⁻¹) of metals in contrasting LMW and HMW DOC treatments (all at 0.5 ppm).

TABLE 4. Comparison of Uptake Rate Constants (mL g⁻¹ h⁻¹) between Low Molecular Weight (LMW) and High Molecular Weight (HMW) DOC Treatments and Their Ratios in HMW to LMW DOC Fractions^a

metal	uptake rate constant (mL g ⁻¹ h ⁻¹)		ratio of k_{HMW}/k_{LMW}
	LMW treatment	HMW treatment	
Cd	17.2	9.1	0.53
Co	13.7	10.2	0.74
Hg	74.1	18.2	0.25
Cr	1.81	1.46	0.81
Ag	303	44.9	0.15
Zn	145	57.3	0.40

^a Both treatments had a DOC concentration of 0.5 ppm.

kDa ultrafilter-passing phases (Table 3). This indicates that uptake of metals by American oysters is not simply a process controlled by the metal's ability to sorb on surfaces. In other words, oysters discriminated (or selectively accumulated) organic components and metals during DOC absorption and metal uptake. For example, measured partition coefficients (K_c) were comparable between all metals, ranging from 10^{5.12} to 10^{5.75} mL g⁻¹ (Table 3). However, values of DCF were considerably different between metals, varying from up to 1000–4000 mL g⁻¹ for Ag and Hg to <16 mL g⁻¹ for Cr. As shown in Tables 2 and 3, metals with high DCF values and uptake rate constants are all B-type metals, such as Hg, Ag, Cd, and Zn (34, 35). This agrees with other experimental results by Reinfelder et al. (19), who also observed higher assimilation efficiencies in oysters for B-type metals compared to transition (or intermediate-type) metals (e.g., Co). It is possible that the absorption and accumulation of these

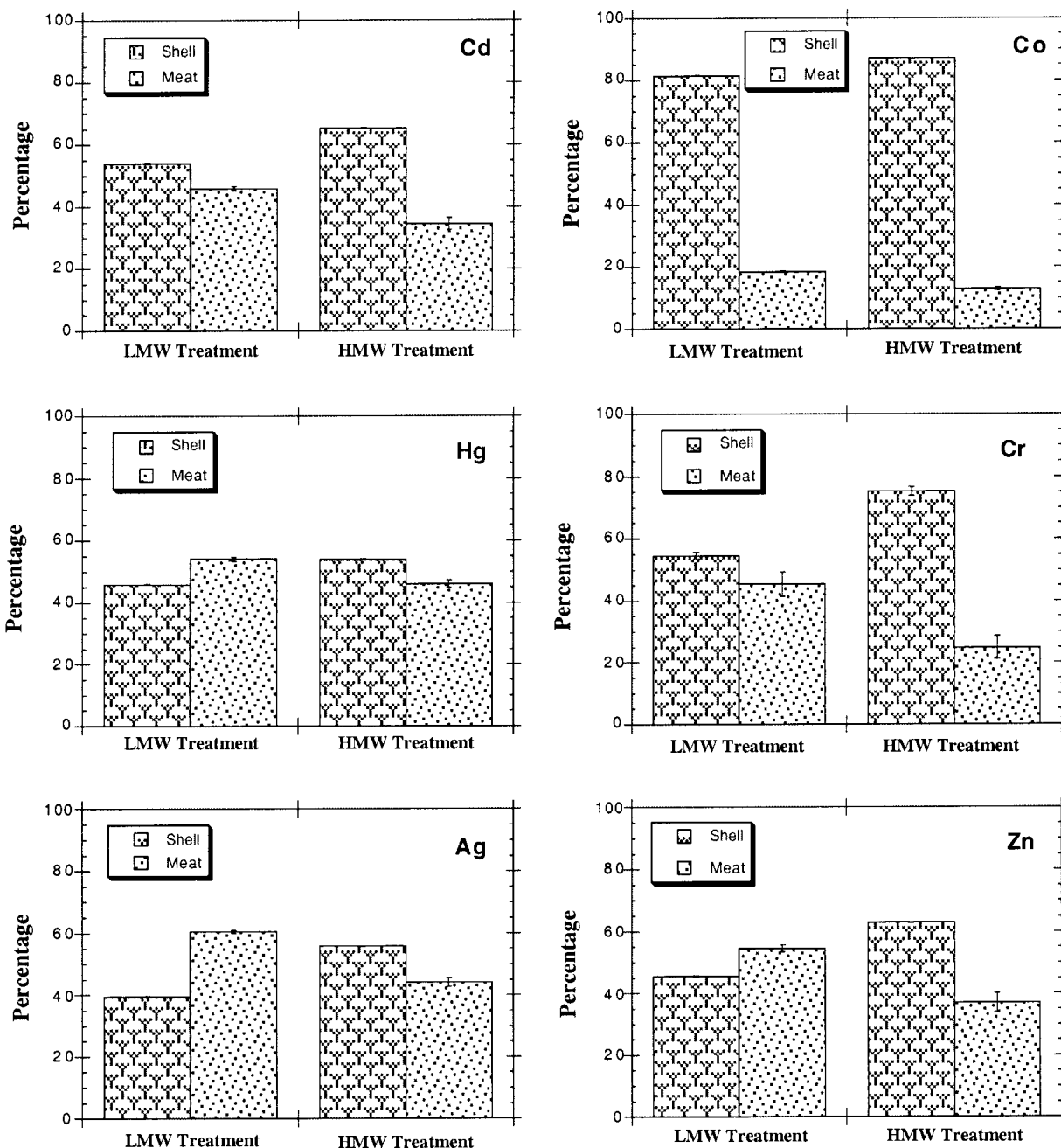


FIGURE 5. Metal partitioning between oyster shell and soft tissue in contrasting LMW and HMW DOC treatments (all at 0.5 ppm).

B-type or soft metals in oysters is mainly through preferential binding with protein or sulfur-containing ligands (36, 45), followed by penetration through cell membranes (11, 44). In contrast, those metals that are intermediate-type elements but have chemical properties more similar to those of A-type metals (e.g., Cr and Co) had the lowest DCF values and uptake rate constants. This was the case even though these metals have K_c values similar to those of B-type metals (Table 3). Therefore, the uptake and accumulation of metals by oysters are largely controlled by the chemical properties of metals and their interactions with organic matter in seawater (12).

Cr(VI) has the lowest DCF value and uptake rate constant among all metals tested (Tables 2 and 3). This is consistent with previous observations on marine bivalves (14, 18, 19), which showed that Cr had the lowest uptake rate constant among all the metals tested. Wang et al. (18) also observed different uptake rate constants for Cr(III) and Cr(VI) in marine mussels (*Mytilus edulis*), with higher values for Cr(III) than for Cr(VI). Cr(VI) was selected in our uptake experiments

because Cr(VI) is the major toxic species of dissolved Cr in aquatic environments (18, 46).

Body Distribution of Metals between Shell and Soft Tissue. After 8 h exposure, metal partitioning between shell and total soft tissue varied with DOC concentrations (Figure 3). In general, the percentage of metals partitioned on oyster shells decreased consistently with increasing DOC concentrations. For example, the percentage of Cd on the shell decreased from ~80% in the "DOC-free" treatment to ~23% at 10 ppm of DOC, while Ag had 57% on the shell in the "DOC-free" treatment and decreased to only ~13% in the treatment with 10 ppm of DOC. Conversely, the percentage of metals in soft tissues increased as the DOC concentration increased (Figure 3), consistent with the increased DCF values with increasing DOC concentration shown in Figure 1. For example, the percentage of Cd in soft tissues increased from ~21% in the "DOC-free" treatment to ~78% in the treatment with 10 ppm of DOC after 8 h exposure (Figure 3). Trends of metal partitioning shifted from predominantly in the shell

in low DOC treatments to the majority being associated with soft tissues in higher DOC treatments, except for Co. Co remained mostly partitioned to the shell rather than to soft tissues even in higher DOC treatments. These results further suggest that increased DOC concentrations can indeed increase the uptake of metals by American oysters through changing uptake pathways.

Among all metals, Hg and Ag had the highest overall percentage in soft tissues, followed by Cd, Zn, Cr, and Co (Figure 3). This trend resembles the order of DCF values, except for Cr, which had the lowest DCF value but had a percentage in soft tissue comparable to that of the other metals.

Our results show that sorption of metals on the shell can be depressed as DOC concentration becomes higher, leaving more metals in solution for oyster uptake. This is consistent with the observed increased uptake of metals caused by increased DOC concentrations in exposure experiments. Increased percentages of metals in soft tissues in higher DOC treatments for Cd, Co, Ag, and Zn are consistent with their higher dry weight concentration factors (DCF) in higher DOC concentration treatments (Figure 1). However, the relatively higher percentage of Hg in soft tissues at higher DOC concentrations is seemingly contradictory to the lowered DCF values in higher DOC treatments. Likely, the higher percentage in soft tissue is not necessarily accompanied by a higher DCF if sorption on the shell and uptake by the soft tissue are both being depressed in the higher DOC treatments due to metal complexation to specific organic ligands.

Comparative Uptake between LMW and HMW DOC Treatments. Dry weight concentration factors (DCF) of metals in oyster soft tissue after 8 h of exposure were compared under the same DOC (0.5 ppm) and radioactive metal concentrations but with contrasting LMW (<1 kDa) and HMW (1 kDa-0.2 μ m) DOC fractions (Figure 4). Values of DCF in LMW DOC treatments were consistently higher than those in HMW DOC treatments for all metals, even though values of DCF for Cr were more comparable between LMW and HMW treatments. In other words, bioavailability of metals in HMW DOC treatments was uniformly depressed compared with LMW DOC treatments at this specific DOC concentration (0.5 ppm). These results indicate that uptake of trace metals by the American oyster is mostly through the LMW complexed or free ionic metal fractions when DOC concentrations are low (47).

Furthermore, uptake rate constants of metals in the LMW DOC treatments were consistently higher than those in HMW DOC treatments (Table 4). Using the ratios of uptake rate constants between LMW and HMW DOC treatments as an index, it was found that metal uptake rates by oysters were depressed considerably for B-type metals (e.g., Ag, Hg, Zn, Cd), with ratios ranging from 0.15 to 0.53. In contrast, there was less of a decrease in the uptake rates for other metals (e.g., Cr and Co), with ratios varying from 0.74 to 0.81 (Table 4). This again demonstrates the importance of metal chemistry in controlling the bioavailability and toxicity to aquatic organisms.

The somewhat depressed bioavailability of metals in HMW DOC treatments at low DOC concentrations is also reflected in the body partitioning of metals between shell and soft tissue (Figure 5). As is evident from Figure 5, percentages of metals in soft tissues in LMW DOC treatments were consistently higher than those in HMW DOC treatments. Conversely, the percentage of metals in oyster shell was higher in the HMW DOC treatment compared with the LMW DOC treatment. Since the overall DOC concentrations (0.5 ppm) and radioactive metal concentrations were the same in both LMW and HMW DOC treatments, the higher percentage of metals on oyster shell indicates that sorption of metals associated with HMW DOC on shell surfaces can be

enhanced, and the percentage of metals in soft tissue in the HMW DOC treatment decreased at this specific DOC concentration, 0.5 ppm (Figure 5).

Recent studies have shown that colloiddally complexed metals could be bioavailable to aquatic organisms, including bivalves (26, 31-33). Our observations that HMW or colloiddally complexed metals are less bioavailable to oysters are not necessarily contradictory to the observation of increased uptake of metals in oysters with increasing DOC concentrations, considering the differences in DOC concentrations and changes in metal uptake routes. The depressed bioavailability of HMW DOC complexed metals is in general agreement with the trend predicted by the free ion activity model. However, the observation of increased uptake rates with further increasing DOC concentrations also supports a direct uptake route of DOC complexed metals by American oysters, likely through the ingestion and digestion of colloiddally bound metals. Our observations and interpretations here thus provide an alternative to the theory that metal uptake is only a function of free ion activity (2, 7).

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