

Metal partitioning between colloidal and dissolved phases and its relation with bioavailability to American oysters

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Abstract

Kinetics and the extent of metal partitioning between colloidal and dissolved phases and coagulation of metals associated with colloids were examined to determine their effects on the bioavailability of selected metals (Cd, Co, Hg, Ag, Fe, and Zn) to American oysters (*Crassostrea virginica*) using radiotracer and short term exposure experiments. After dispersion of radiolabeled colloids into low molecular weight (LMW, <1 kDa) seawater, metal partitioning between dissolved (<1 kDa) and colloidal (1 kDa–0.2 μ m) phases resulted in a consistent pattern, with a relatively constant percentage in the colloidal phase for each metal. On average, about 90% of Hg and Fe, ~60% of Ag and ~40% of Zn, Co, and Cd were measured in the colloidal fraction during a short term exposure experiment, consistent with their partitioning in natural waters. Controlled laboratory experiments carried out in parallel using radioactively tagged colloids showed that coagulation of colloidal species, quantified as the fraction retained by a 0.2 μ m filter, was insignificant for most metals under the conditions and time periods of the uptake experiments.

The bioavailability of colloiddally complexed metals, measured in terms of dry weight concentration factor (DCF, ml g⁻¹) and uptake rate constant (ml g⁻¹ h⁻¹), was somewhat depressed compared with their counterpart in the LMW treatment, but could be well predicted from the results of the LMW treatment and metal partitioning. Both DCF values and uptake rate constants were higher in the LMW treatment than in the colloidal treatment. In addition, B-type metals, such as Ag, Hg, and Zn, all had higher values of DCF and uptake rate constants, regardless of treatments, except for Cd which had a lower DCF

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and uptake rate constant. In contrast, Co and Fe had significantly lower DCF values and uptake rate constants. Most of Hg and Ag (60–80%) were measured in the soft tissue of oysters in both LMW and colloidal treatments. In contrast, 80% of Fe, 75% of Co, and ~60% of Cd were observed on the shell, while Zn was found evenly distributed between shell and soft tissue of oysters. These results agree well with the variation pattern of both DCF value and uptake rate constant for these two groups of metals. © 2002 Elsevier Science Ltd. All rights reserved.

Keywords: American oysters; Bioavailability; Metals; Colloids; Dissolved organic carbon; Bivalves

1. Introduction

Marine bivalves, especially oysters and mussels, have long been used as model organisms in environmental studies (Engel, 1999; Luoma, Johns, Fisher, Steinberg, Oremland, & Reinfelder, 1992; Reinfelder, Wang, Luoma, & Fisher, 1998; Roditi & Fisher, 1999; Wang, & Fisher, 1997, 1999) and as pollution indicator organisms in environmental assessment and monitoring programs (Goldberg, Koide, Hodge, Flegal, & Martin, 1983; O'Connor, 1996; Rainbow & Phillips, 1993). Therefore, metal bioavailability to marine bivalves has received extensive attention over the past several decades (e.g. Hamelink, Landrum, Bergman, & Benson, 1994; Roditi & Fisher, 1999; Tessier & Turner, 1995; Wang & Fisher, 1997). However, uptake pathways and mechanisms of metals by marine bivalves are not well understood (Guo, Hunt, Santschi, & Ray, 2001; Roditi, Fisher, & Sanudo-Wilhelmy, 2000).

Most previous studies examined the bioavailability of metals to marine bivalves with respect to either “dissolved” or particulate forms (Fisher, Teysse, Fowler, & Wang, 1996; Tessier & Turner, 1995; Wang & Fisher, 1997, and references therein). However, the traditionally defined “dissolved” phase contains not just dissolved species (defined as the <1 kilo-Dalton, kDa, low molecular weight, LMW, fraction) but also colloidal (1 kDa–0.2 μm) materials (Guo & Santschi, 1997; Guo, Santschi, & Warnken, 2000), which include microparticles and macromolecules as well as molecular assemblies such as micelles (Buffle & Leppard, 1995). Since the behavior of colloids can be significantly different from those of dissolved and particulate phases (e.g. Gustafsson & Gschwend, 1997), the question whether colloiddally complexed metals are bioavailable to aquatic organisms needs increased attention. Indeed, recent studies have shown that colloidal species of a number of trace metals can be bioavailable to penaeid shrimp (e.g. Carvalho, Benfield, & Santschi, 1999), mussels (e.g. Roditi et al., 2000; Wang & Guo, 2000b), marine plankton (e.g. Wang & Guo, 2000a), and oysters (e.g. Guo et al., 2001), even though different pathways within the organisms were indicated. However, metal partitioning between colloidal and dissolved phases in previous studies was not well defined. How metal’s bioavailability to aquatic organisms is modified by the partitioning of metals to colloidal macromolecular organic matter is still largely unknown. Our ability to predict metal concentration of bivalves requires a full understanding of the relationship between bioavailability of metals and their speciation or association with different chemical moieties. While marine colloids are mostly composed of macromolecular organic matter (Guo & Santschi,

1997, and references therein), the complexation of metals to different functional groups in biomolecules is not well explored.

The objectives of this study were to examine: (1) metal partitioning between colloidal and dissolved phases and coagulation of metals associated with colloids during short term exposure experiments; and (2) metal bioavailability to American oyster (*Crassostrea virginica*) under low molecular weight (LMW) and high molecular weight (HMW) treatments. Partitioning and coagulation are two important factors likely affecting the interpretation of metal bioavailability studies when colloids are used in exposure experiments.

2. Materials and methods

2.1. Oysters

American oysters (*Crassostrea virginica*) were collected from Galveston Bay, Texas in October 1999. Junior oysters with similar body weights, ranging from 20 to 25 g, and body shapes, ranging from 3 to 5 cm, were used in short term exposure experiments. Oysters were well cleaned and were acclimated for <1 day without being fed before the uptake experiments. The low molecular weight (<1 kDa) fraction of ultrafiltered seawater, with a salinity of 20‰ and a water temperature of 24 °C, was used for the acclimation.

2.2. Metals

Radioactive metals, ^{109}Cd , ^{57}Co , ^{203}Hg , $^{110\text{m}}\text{Ag}$, ^{59}Fe , and ^{65}Zn (Amersham, NJ and Isotope Products, CA) were used for the partitioning and uptake experiments. These metals are either transition metals (e.g. Fe, Cd, Co, and Zn) or type-B metals (e.g. Ag and Hg; Stumm & Morgan, 1995). It has been shown that different types of metals can have distinct complexing and transport behavior with different types of organic compounds (e.g. Santschi, Tang, Wen, & Gill, 1997) and thus, could have very different uptake pathways and bioavailabilities. Therefore, these metals show a wide range in the extent of partitioning between colloidal and dissolved phases and will allow us to investigate the processes which control the bioavailability of metals associated with macromolecular organic matter to American oysters.

2.3. Isolation of colloidal organic matter and radiolabeling

Seawater (with a salinity of ~20‰ and a DOC concentration of 260 μM) was collected from the same place where the oysters were collected in Galveston Bay, and prefiltered through a 0.2 μm Nuclepore cartridge. Colloids were then isolated from the prefiltered seawater using a DC-10 ultrafiltration system equipped with two 1 kDa (Amicon S10Y1) cross-flow ultrafiltration cartridges (Guo & Santschi, 1996; Guo, Wen, Tang, & Santschi, 2000). The LMW (<1 kDa) permeate, with a DOC concentration of 102 μM , was used as a control in uptake and coagulation experiments.

The concentrated colloidal organic matter (COM) was spiked with radioisotopes, ^{109}Cd , ^{57}Co , ^{203}Hg , $^{110\text{m}}\text{Ag}$, ^{59}Fe , and ^{65}Zn . The spiked COM was allowed to equilibrate overnight (~15 h). After that time the radiolabeled colloids were concentrated and separated from those uncomplexed and the LMW-complexed radioactive metals by ultrafiltration. For the control experiments with free ionic or LMW forms, a fraction of the <1 kDa permeate was labeled right before the uptake experiments with the same isotopes. The radiolabeled permeate (LMW forms) and colloids (high molecular weight, HMW, forms) were then immediately used for the uptake and coagulation experiments. Partitioning of metals between colloidal and dissolved phases was monitored during the exposure experiments.

2.4. Partitioning of metals between dissolved and colloidal phases

A stirred cell ultrafiltration unit with 1 kDa membranes (Amicon YM1) was used to separate colloidal and the 1 kDa membrane-passing permeate phases during exposure experiments. Radioactivities of each metal in both permeate and retentate fractions were measured to monitor the partitioning of metals between colloidal (> 1 kDa) and the <1 kDa fraction in each treatment of the uptake experiments. The percentages of colloidal metals were quantified at 0.5, 4 and 8 h, respectively, during the exposure experiments.

2.5. Coagulation of radiolabeled colloids

It is possible that colloids may coagulate and form new particulate phases (Storðal, Santschi, & Gill, 1996; Wen, Santschi, & Tang, 1997), which could facilitate uptake by oysters through filter feeding activity and causing artifacts for the uptake of dissolved metals. In order to examine this possibility, coagulation experiments were carried out in parallel to assess the extent of colloidal coagulation under our experimental conditions. Radiolabeled colloids were dispersed into unspiked permeate seawater to result in a total volume of 250 ml and a colloidal organic carbon (COC) concentration of 208 μM (Table 1). For the control experiment, the spiked permeate was used. At 0, 2, 4, 6, 8 h time intervals, an aliquot of the bulk solution was filtered through a 0.2 μm filter (polycarbonate, 25 mm) to quantify the fraction of each metal retained by the filter. Possible sorption of radioactive metals onto filter membranes during filtration was monitored by measuring isotopic activities of a second filter placed beneath the first membrane. The activities of

Table 1

Concentrations of dissolved (DOC) and colloidal organic carbon (COC) in the LMW and HMW treatments for uptake and coagulation experiments and for metal partitioning assessments

Exp. ID	Description	Background DOC (μM)	COC concentration (μM)
LMW	LMW treatment	102	0
HMW	HMW treatment	102	208

radioactive metals on the second filter were usually negligible compared to those on the first filter.

2.6. Uptake of colloiddally complexed metals by oysters

Four individual oysters were used as replicates in each treatment. Oysters were placed each in a 500 ml Teflon container with 300 ml solution containing either the spiked permeate or the spiked colloidal solution during short term exposure experiments. At each time interval (2, 4, 6, and 8 h), oysters were removed, rinsed with unlabeled permeate seawater, and gamma counted to determine their metal activity. After gamma counting, oysters were returned to their original Teflon beaker. An aliquot of the solution phase in each beaker was also gamma counted to determine the activity of each metal after each time interval. At the end of the uptake experiment, each oyster was dissected into shell and soft tissue, which were then gamma counted separately to examine the body distribution of metals between oyster shell and soft tissue.

After final gamma counting, soft tissues of oysters were then dried at 80 °C for 24 h to determine the dry weight that was needed to calculate the dry weight based concentration factor (DCF), as $DCF = A_{ST}/A_w$. Where A_{ST} is the radioactivity of the metals in the dried soft tissue of oyster (cpm/g dried tissue) and A_w is the radioactivity of metals in the water (cpm/ml solution). For time series data, a conditional DCF (CDCF) value was calculated as $CDCF = A_O/A_w$ for the estimate of uptake rate constants, where A_O is the activity in the whole oyster (in cpm/g dried oyster, tissue plus shell) and A_w is the activity in solution, in cpm/ml solution (Guo et al., 2001).

2.7. Radioactivity measurements by gamma counting

Radioactivities of ^{109}Cd , ^{57}Co , ^{203}Hg , $^{110\text{m}}\text{Ag}$, ^{59}Fe , and ^{65}Zn were measured on a Canberra ultra high purity Ge well detector (Guo et al., 2001). The gamma emission of ^{109}Cd was determined at 88 keV, ^{57}Co at 122 keV, ^{203}Hg at 279 keV, $^{110\text{m}}\text{Ag}$ at 657 keV, ^{59}Fe at 1099 keV, and ^{65}Zn at 1115 keV. Counting times were adjusted to result in a propagated error of <2–10% or better for soft tissues and solution phase, depending on metals. Decay corrections were applied to correct to the initial time of the experiment for short lived isotopes. Errors reported here were one sigma standard errors.

3. Results and discussion

3.1. Partitioning of colloiddal metals during short term exposure experiments

A central issue when examining the bioavailability of colloiddally bound metals to aquatic organisms using radiotracer techniques is whether metals can remain associated with colloids after radioactively tagged colloids had been dispersed into the

experimental systems. While colloids may coagulate and form new particulate phase, it is also possible that radioactive metals associated with the colloids re-partition into the <1 kDa dissolved phase and thus decrease the amounts of metals in the defined colloidal phase during uptake experiments. Such questions, however, had not been addressed in previous studies on colloidal bioavailability (e.g. Carvalho et al., 1999; Guo et al., 2001; Roditi et al., 2000).

Variations of the colloidal fraction of metals during a short term exposure experiment are shown in Fig. 1. As becomes evident, not all metals remained associated with

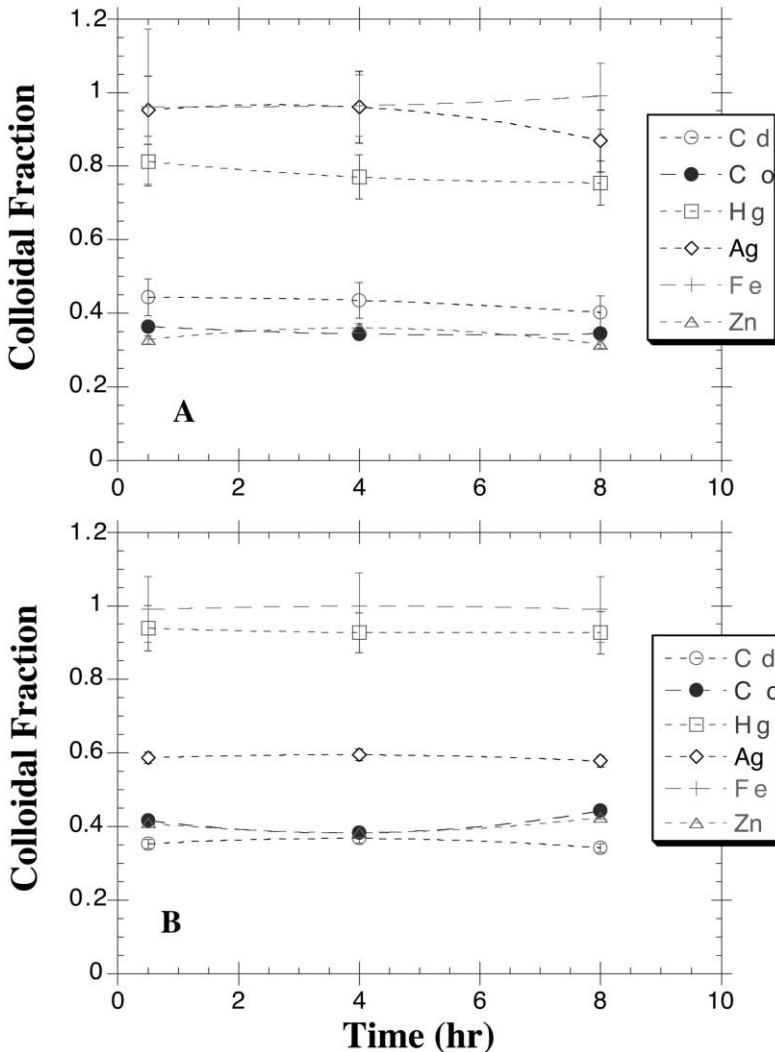


Fig. 1. Variations of colloidal fractions with time, after radiolabeled colloids had been dispersed into low molecular weight (<1 kDa) seawater with a final COC concentration of 42 μM (A) and 208 μM (B).

colloids after having been dispersed into LMW (<1 kDa) seawater. Metal partitioning between dissolved and colloidal phases occurred to a different extent among different radioactive metals (Fig. 1). On average, >90% of Hg and Fe, ~60% of Ag, and ~40% of Co, Zn and Cd were measured in the >1 kDa colloidal fraction in the high molecular weight (HMW) treatment (Table 2). These results are consistent with recent reports on metal phase speciation or metal colloidal fractions for Galveston Bay waters (e.g. Stordal, Gill, Wen, & Santschi, 1996 for Hg; Wen et al., 1999 for Fe, Co, Cd, and Zn; Wen, Santschi, Gill, Paternostro, & Lehman, 1997 for Ag). Different colloidal fractions observed for different metals indicate that the partitioning of metals between dissolved and colloidal phases is largely controlled by the metal's chemical properties, such as affinity for COM, and the surface activity of the macromolecular chelate, resulting in different partition coefficients (K_c) between dissolved and colloidal phases for each metal (Table 2). Thus, it is obvious that not all radiolabeled metals remained in the colloidal phase.

The kinetics of metal partitioning, after radioactively labeled colloids dispersed into the experimental system, appears to be fairly fast (Fig. 1). The percentage of colloidal metals remained almost constant during the time period of the experiment, from 0.5 to 8 h, although the colloidal fraction varied for different metals. Even though the repartitioning of colloidal metals appears to be inevitable, the quasi-constant colloidal fraction under the experimental conditions should allow one to examine the relative importance of colloiddally complexed metals to the bioavailability to aquatic organisms.

3.2. Coagulation of radioactively tagged colloidal organic matter

Coagulation of radioactively tagged colloids, determined from the fraction retained by a 0.2 μm filter, were examined in a controlled laboratory experiment carried out in parallel to the oyster uptake experiments. In the absence of preformed

Table 2

Values of $\log K_c$, percentages of colloidal fraction (1 kDa–0.2 μm), and average uptake rate constants measured for each metal^a

Metal	Log[K_c (ml/g)]	% in colloidal fraction HMW treatment	Uptake rate constant (ml g ⁻¹ h ⁻¹)	
			LMW treatment	HMW treatment
Cd	4.3–6.6	33±3	7.38	6.09
Co	4.6–7.0	42±1	5.91	5.05
Hg	5.4–7.0	94±2	19.5	2.11
Ag	4.4–6.8	75±6	38.8	7.25
Fe	4.6–6.4	99±5	8.72	3.06
Zn	4.5–6.7	40±3	24.1	11.5

^a K_c was calculated as $[\text{Me}]_c / \{[\text{Me}]_d \times C_c\}$, where $[\text{Me}]_c$ is the metal's activity concentrations in the colloidal (>1 kDa, dpm/liter) and $[\text{Me}]_d$ in the <1 kDa dissolved phases (dpm/l), and C_c is the mass concentration (g/ml) of colloidal organic matter (COM) converted from COC concentration using a factor of 2.5 between COM and COC.

suspended particulate matter, coagulation of colloids during uptake experiments was minimal for most metals examined, except for Hg and Ag (Fig. 2). There were no detectable amounts of Cd, Co, and Zn retained by the 0.2 μm filter during the 8 h time period, whereas the 0.2 μm filter-retained fractions of Ag, Fe, and Hg ranged from <5 to 20% (Fig. 2). Negligible amounts of Cd, Co and Zn retained by the 0.2 μm filter are consistent with the lower colloidal fraction observed for these metals. Furthermore, the filter-retained fractions remained approximately constant for all metals during the coagulation experiment. There were no significant differences in the filter-retained fractions of metals between the LMW and colloidal treatments during the 8 h uptake period, except for Fe in the LMW treatment, indicating that the overall effect of coagulation on the metal uptake by oysters should be small in short term exposure experiments.

Both Ag and Hg showed slightly higher filter-retained fractions in the HMW (or colloidal) than in the LMW treatments (Fig. 2). However, the difference in the filter retaining fractions between HMW and LMW treatments was variable and was, for

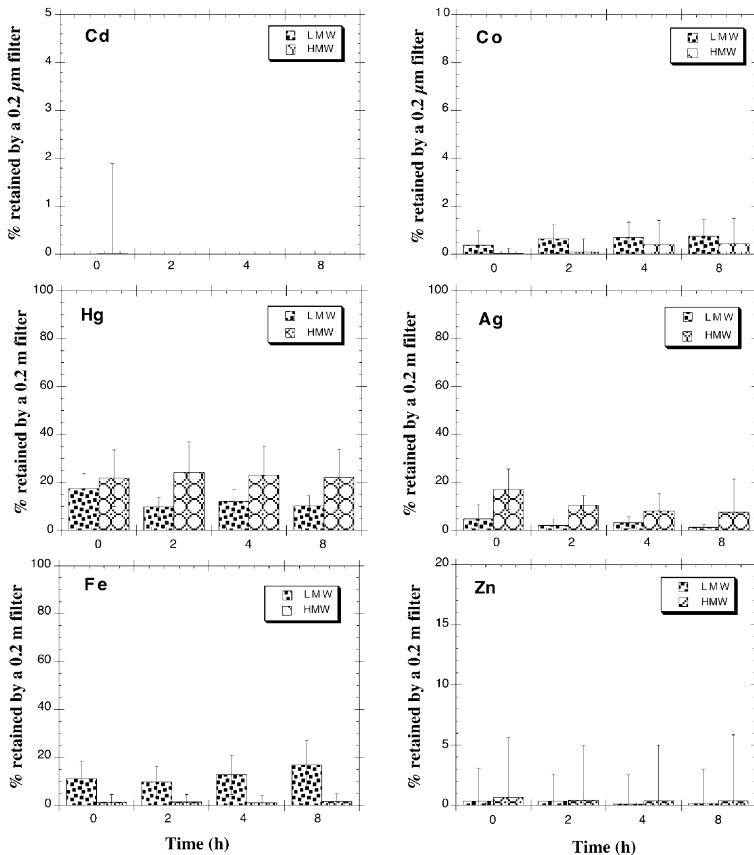


Fig. 2. Variations of the percentage of radioactive metals retained by a 0.2 μm filter during a coagulation experiment using radioactively tagged colloidal organic matter.

most of the samples, insignificant for Ag and Hg (Fig. 2). In contrast to Ag and Hg, coagulation of Fe in the LMW treatment was somewhat higher than that in the HMW treatments (~10% in the LMW treatment vs. < 5% in the HMW treatment). The relatively higher filter-retained fraction for the LMW Fe could be due to the presence of higher amounts of inorganic Fe hydroxides or surface-active substances, which could coagulate or sorb onto the filter surface during the filtration process (e.g. Buffle, Perret, & Newman, 1992; Honeyman & Santschi, 1989; Honeyman & Santschi, 1991; Quigley, Santschi, Guo, & Honeyman, 2001). However, compared with the generally low fraction of LMW metals retained by the 0.2 μm filter, coagulation of these radioactively tagged colloids was considered to be negligible.

Recent laboratory evidence has shown that a significant fraction of estuarine and marine colloids could coagulate relatively rapidly onto filterable particles (Chin, Orellana, & Verdugo, 1998; Quigley et al., 2001; Stordal, Santschi, & Gill, 1996; Wen, Santschi, & Tang, 1997). However, many of these studies were carried out using larger pore size pre-filters (0.7 μm in Chin et al., 1998; 0.4 μm in Stordal, Santschi, & Gill, 1996; Wen, Santschi, & Tang, 1997) or whole water samples in the presence of natural suspended particulate matter, or newly formed particles through river water and seawater mixing (Stordal, Santschi, & Gill, 1996; Wen, Santschi, & Tang, 1997). The presence of these particles and larger size colloids (1 kDa to 0.7 μm or 0.4 μm) could have caused an enhancement in the coagulation rate of colloids. Alternatively, colloids used in our experiments had a different composition from those previously used. Differences in colloidal compositions are poorly known between seasons and locations. If coagulation of colloids during the uptake experiments would have been significant, the newly formed particles through coagulation would have been an artifact in the testing procedures of the bioavailability of colloiddally complexed metals to bivalves, since bivalves are capable of retaining particles through filter feeding. These results from controlled laboratory experiments using small colloids (1 kDa–0.2 μm) demonstrate that coagulation of radioactively labeled colloidal organic matter was minimal under our experimental conditions. Without adding the > 0.2 μm particles, coagulation of small colloids was quite slow. These results are consistent with the constant colloidal metal fractions observed in our partitioning experiments (Fig. 1). Therefore, enhancement of bioavailability resulting from colloidal coagulation is less likely during such short-term exposure experiments.

3.3. *Dry weight concentration factors between LMW and HMW treatments*

In general, soft tissue based DCF values measured after 8 h of exposure decreased from LMW treatment to HMW treatment (Fig. 3). Higher DCF values in the oysters for metals in the LMW treatment indicate that LMW (including free ionic) species are the most available forms to oysters in marine environments. Significantly lower DCF values of metals in the HMW treatments compared to the LMW treatments all point to a lower bioavailability for colloiddally complexed metals. In other words, bioavailability of colloiddally complexed metals can be somewhat depressed under our experimental conditions. These results are consistent with our previous

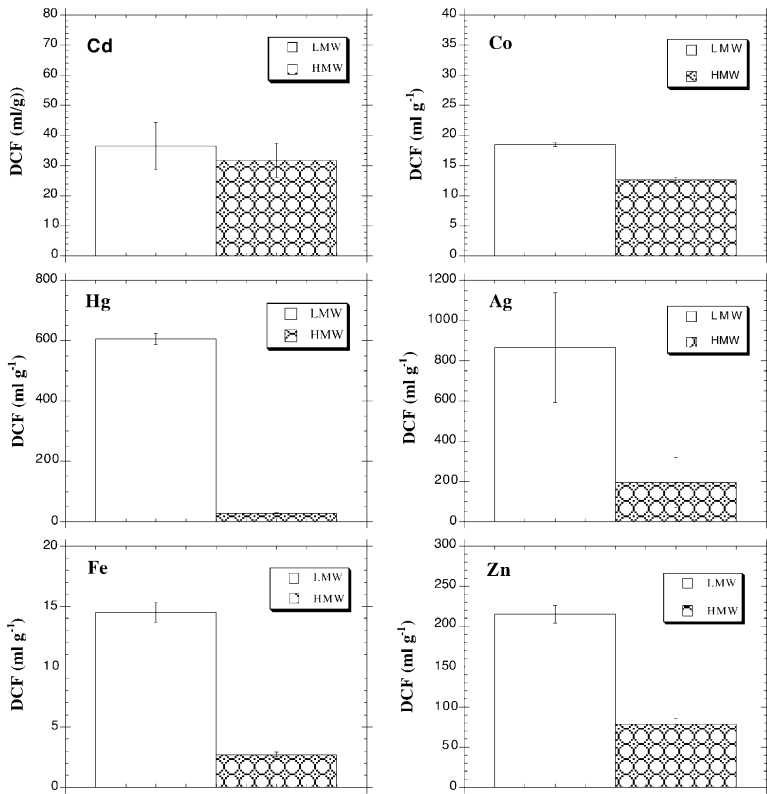


Fig. 3. Measured dried weight concentration factor (DCF, ml g⁻¹) of metals in the soft tissues of oysters after 8 h exposure. The low molecular weight (LMW) treatment is the control with permeate spiked with radioactive metal ions and high molecular weight (HMW) is the colloidal treatment with a COC concentration of 208 μM.

report on depressed bioavailability on HMW complexed metals under conditions of low DOC concentrations, 0.5 mg-C/l (Guo et al., 2001). However, our results are different from recent studies that show enhanced uptake for some colloiddally complexed metals to other organisms (e.g. Carvalho et al., 1999; Roditi et al., 2000).

Low bioavailability of colloiddally complexed metals is broadly consistent with the free ion activity model (Campbell, 1995; Van Ginneken, Chowdhury, & Blust, 1999). If we assume that only the free ionic and LMW complexed metals are available for uptake by oysters (Campbell, 1995; Sunda & Lewis, 1978), then a predicted DCF value can be calculated from the measured DCF values in the LMW treatment, multiplied by the metal's LMW fraction in the colloiddal treatment. Indeed, a linear correlation between measured DCF in colloiddal treatment and predicted DCF values from the LMW metal fraction present in the colloiddal treatment is observed (Fig. 4). Although the correlation in Fig. 4 is weak, data points below the 1:1 solid line provide evidence of bioavailability of colloiddal metals. This result is quite

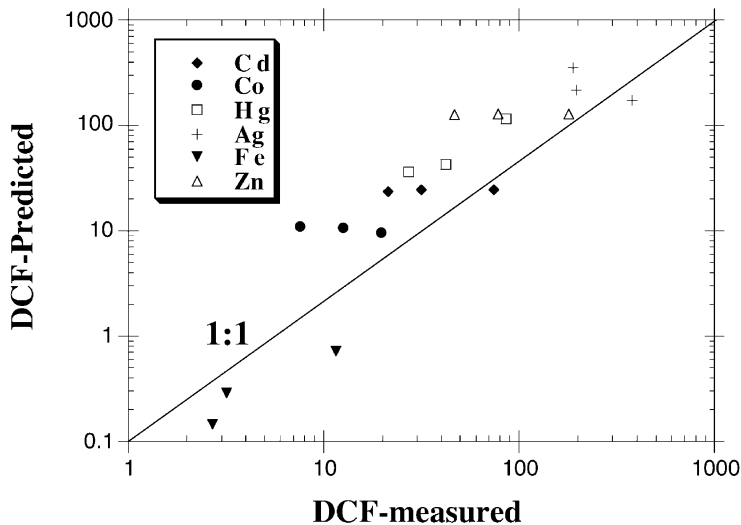


Fig. 4. Relationship between the predicted DCF (ml g^{-1}) and measured soft tissue DCF (ml g^{-1}) of metals in colloidal treatments compared with a 1:1 relationship shown as a solid line. Predicted-DCF values were estimated from the value of the DCF measured in the LMW treatment multiplied by the fraction of metals in the LMW phase, assuming that free ionic or LMW complexed metals are the only species bioavailable to the oysters.

interesting. However, more measurements are needed to establish a more quantitative relationship between predicted and measured DCF values, since DCF values could be significantly affected by experimental conditions, such as DOC concentration and organic composition (e.g. Guo et al., 2001), as well as the physiological status of oysters.

Values of measured DCF in oysters also varied from metal to metal (Fig. 3). On average, Ag had the highest DCF values ($200\text{--}800 \text{ ml g}^{-1}$), followed by Hg ($<100\text{--}600 \text{ ml g}^{-1}$), Zn ($75\text{--}215 \text{ ml g}^{-1}$), Cd ($30\text{--}35 \text{ ml g}^{-1}$), Co ($12\text{--}18 \text{ ml g}^{-1}$), and Fe ($3\text{--}15 \text{ ml g}^{-1}$). High values of DCF after 8 h of exposure for Ag, Hg and Zn are consistent with their high uptake rate constants (see discussion below) compared to other metals (Cd, Co, Fe). Low DCF values measured for Fe indicate low bioavailability of colloidal Fe to the oysters and are consistent with the high percentage of Fe partitioned to the shell (see Section 3.4).

Higher DCF values of Zn in the oysters agree well with the fact that Zn is one of the most biologically essential elements. The high partitioning coefficients (K_d or K_c) between dissolved, colloidal (K_c) and particulate (K_d) phases for metals (Table 2 and Stordal, Gill, 1996; Wen, Santschi, Gill et al., 1997), is likely the cause for the lowered DCF values in the HMW treatment, as they, when colloiddally complexed, were not as exchangeable as the other metals. Ag, a B-type metal, has been shown to be strongly complexed by organic ligands, especially reduced sulfur-containing functional groups, such as thiols (Gekeler, Grill, Winnacker, & Zenk, 1988; Santschi et al., 1997), which may have been the cause for its high oyster concentration factor

observed in the LMW treatments. Uptake of colloidal Ag and Hg by oysters through direct ingestion was likely not a major process under our experimental conditions. Since dissolved (i.e. filter-passing) Fe in seawater is mostly in the colloidal form (Table 2 and Power, Landing, & Bauer, 1996; Wen, Santschi, Gill, & Paternostro, 1999), colloidal Fe should also have had a similar DCF value as Ag and Hg, if uptake of colloidal metals is mainly by direct ingestion. Elevated DCF values of colloidal Ag and Hg compared to other metals in the HMW treatments could have also resulted from the direct transport of macromolecular organic complexed metals through lipid layer penetration (Simkiss & Taylor, 1995).

Using the time series data, metal uptake rate constants were estimated for each metal in both LMW and HMW treatments (Guo et al., 2001) and are listed in Table 2. Similar to the lower DCF values observed for the HMW treatment, average uptake rate constants in the HMW treatment also had lower values for all metals. For example, the uptake rate constant for Hg was $19.5 \text{ ml g}^{-1} \text{ h}^{-1}$ in the LMW treatment compared to $2.1 \text{ ml g}^{-1} \text{ h}^{-1}$ in the HMW treatment, $38.8 \text{ ml g}^{-1} \text{ h}^{-1}$ vs. $7.525 \text{ ml g}^{-1} \text{ h}^{-1}$ for Ag, $24.1 \text{ ml g}^{-1} \text{ h}^{-1}$ vs. $11.5 \text{ ml g}^{-1} \text{ h}^{-1}$ for Zn, and $7.4 \text{ ml g}^{-1} \text{ h}^{-1}$ vs. $6.1 \text{ ml g}^{-1} \text{ h}^{-1}$ for Cd (Table 2). While B-type metals had relatively higher uptake rate constants, except for Cd, uptake rate constants for Co and Fe were significantly lower in both HMW and LMW treatments, $5\text{--}5.9 \text{ ml g}^{-1} \text{ h}^{-1}$ for Co and $3\text{--}8.7 \text{ ml g}^{-1} \text{ h}^{-1}$ for Fe. Both uptake rate constant and dry weight concentration factor of metals between LMW and HMW treatments support a depressed bioavailability of colloiddally complexed metals to American oysters. Decreased bioavailability in colloiddally complexed metals observed here has important implications for environmental assessment and research. For example, it implies that the LMW metal species may mostly account for the toxicity and bioavailability of potentially toxic metals to aquatic organisms. Consequently, the traditional approaches using the total dissolved metal concentrations to evaluate the water quality might be over predictive for certain metals, such as Hg and Ag, which have the majority of their total dissolved fraction in the colloidal phase. Therefore, the metals' phase speciation, along with its chemical speciation, is critical for water pollution and water quality assessments.

3.4. *Body distribution of metals between shell and soft tissue*

Body distributions of metals between shell and soft tissue of the oysters after 8 h of exposure demonstrate different distribution patterns for different metals (Fig. 5). On average, most radioactivity of Fe, Co, and Cd was found in the shell of the oysters, with $>80\%$ of Fe, $70\text{--}80\%$ of Co, and $55\text{--}70\%$ of Cd in the shell. In contrast to Fe and Co, most activity of Ag and Hg was measured in the soft tissues of oysters, ranging from 70 to 80% in the soft tissue for Ag and 55 to 85% for Hg. Zinc was almost evenly distributed between shell and soft tissue (Fig. 5). Overall, higher percentages of Hg and Ag in the soft tissues are consistent with their higher uptake rate constants and higher DCF values measured on soft tissue following 8 h exposure. Similarly, lower percentages of Fe, Co and Cd found in the oyster's soft tissues also correspond to their lower uptake rate constants and lower DCF values.

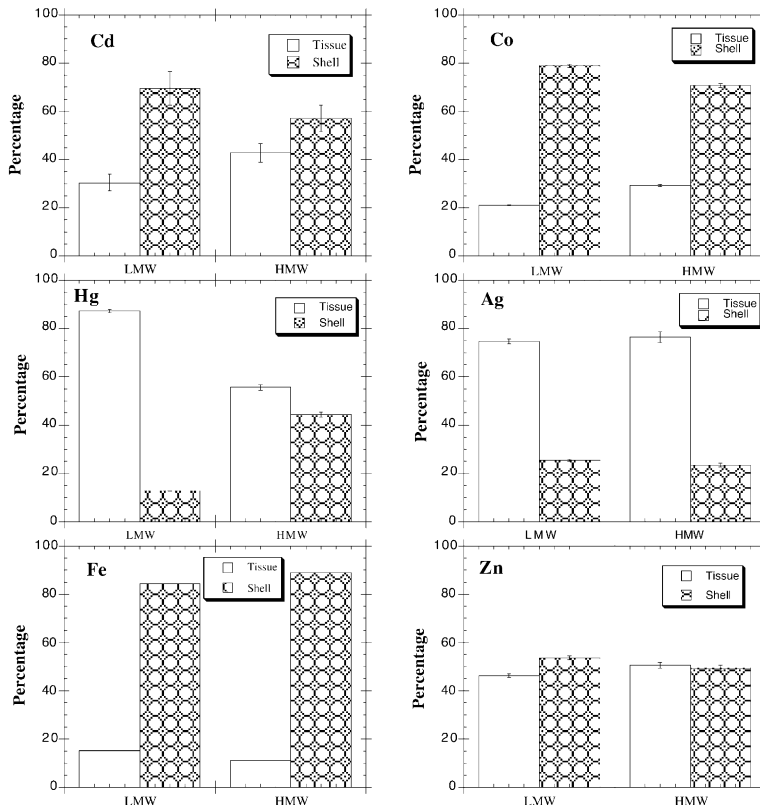


Fig. 5. Partitioning of metals (%) in oyster body parts (shell and soft tissues) in LMW and HMW treatments. Note that errors for Hg and Zn are extremely small and can hardly be seen. The majority of Cd, Co, and Fe were sorbed onto oyster shells whereas Hg and Ag were mostly associated with the soft tissues of oysters, and Zn was almost evenly distributed between shell and soft tissues.

4. Conclusions

Results from short term exposure experiments show that the bioavailability of colloiddally complexed metals to American oysters (*Crassostrea virginica*) was somewhat depressed compared with that of LMW complexed metals. Both uptake rate constants and DCF values were higher for each metal in the LMW treatment compared with those in the HMW treatment. Measured dry weight concentration factors in the oysters were highest for Ag, followed by Hg and Zn, with the lowest DCF value found for Fe and Co. Similarly, uptake rate constants were also higher for Ag, Hg, and Zn, compared with those of Cd, Co, and Fe.

A lower bioavailability of colloiddally complexed metals to oysters under our experimental conditions suggests that macromolecular organic matter may ameliorate the bioavailability and toxicity for certain heavy metals, such as Hg and Ag. The body distribution of metals showed that Fe, Co and Cd were mostly partitioned

onto the oyster shell whereas Hg and Ag were mostly found in the soft tissues of oysters. However, Zn was almost evenly distributed between shell and soft tissues.

Since colloidal metals were generally less bioavailable, we surmise that the concentration of total dissolved metals is not an accurate predictor for oyster bioavailability and water quality and toxicity assessments of metals in coastal marine environments. Therefore, chemical and phase speciation of metals should be considered in environmental monitoring and assessments.

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