



## Bioavailability of sediment-bound Cd, Cr and Zn to the green mussel *Perna viridis* and the Manila clam *Ruditapes philippinarum*

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### Abstract

We assessed the degree to which Cd, Cr and Zn bound with sediment were assimilated by the green mussel *Perna viridis* and the Manila clam *Ruditapes philippinarum*. The influences of the metal concentration in the sediment, the presence of phytoplankton, and the oxidation condition of the sediment on metal assimilation were examined. No major difference was found for metal assimilation efficiency (AE) in sediment with different metal concentrations, except for Cd in the green mussels, in which the AE increased by  $1.7 \times$  when the Cd concentration in sediment was elevated to  $15 \times$  the natural background level. The higher assimilation of Cd with increasing Cd load in ingested sediment may be due to the higher desorption of Cd in the acidic gut of the bivalves. Both mussels and clams assimilated metals at a higher efficiency from the diatom diet (*Thalassiosira pseudonana*) than from inorganic sediment particles. The presence of algal particles had little influence on metal assimilation from ingested sediment, and conversely, the presence of sedimentary particles had little effect on metal assimilation from ingested diatom (except for Cd in the mussels). In the mussels, AEs were higher from oxic sediment than from anoxic sediment by  $3.1 \times$  for Cd,  $2.0 \times$  for Cr, and  $1.4 \times$  for Zn, and in the clams AEs were higher from oxic sediment by  $2.8 \times$  for Cd,  $2.0 \times$  for Cr, and  $2.0 \times$  for Zn. Our study suggested that metals associated with anoxic sediment can be potentially available to marine bivalves, and that metal AEs determined for a single diet were probably not affected by the presence of other food particles. © 2000 Elsevier Science B.V. All rights reserved.

**Keywords:** Heavy metals; Green mussel; Manila clam; Assimilation efficiency

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## 1. Introduction

In aquatic environments, heavy metals discharged from industrial or sewage effluents or from atmospheric deposition may be rapidly removed from the water column and transported to bottom sediment (Förstner and Wittmann, 1981; Fung and Lo, 1997). Consequently, metal concentrations in sediment are often several orders of magnitude higher than those in ambient seawater (Luoma, 1990). There has been increasing awareness that sediment may serve as an important source for metal bioaccumulation in aquatic animals (Luoma, 1989). For example, Cd associated with the sediment can contribute up to 60–100% of the total Cd burden in sediment dwelling chironomidae (Bendell-Young, 1999) or polychaete (Selck et al., 1998, 1999; Wang et al., 1999). However, quantitative study on metal bioaccumulation from sediment is still limited (Luoma, 1989; Campbell et al., 1988; Luoma and Fisher, 1997). Although a few studies have shown that sediment ingestion can be an important source of metal uptake, the geochemical controls of sediment on metal bioavailability are not yet fully understood (e.g. organic matter; Jenne and Luoma, 1977; Luoma, 1989; Newman and Jagoe, 1994; Lin and Chen, 1998; Selck et al., 1999).

It has been generally conceived that quantification of metal bioavailability from contaminated sediment is a difficult task due to the complex metal geochemistry in sediment, animal physiology and ecology. One important physiological parameter quantifying metal bioavailability from ingested food particles is the metal assimilation efficiency (AE), but this parameter remains largely unquantified for most sediment-ingesting invertebrates (Gagnon and Fisher, 1997; Wang et al., 1998, 1999; Griscom et al., 2000; Arifin and Bendell-Young, 2000). Metal AEs from ingested sediment tended to be lower than those from ingested phytoplankton in the clam *Macoma balthica* and the mussel *Mytilus edulis* (Gagnon and Fisher, 1997; Wang et al., 1997; Lee and Luoma, 1998). Cr was however absorbed by the clams at a much higher efficiency when it was associated with bacteria rather than phytoplankton (Decho and Luoma, 1991). Schlekot et al. (1999) demonstrated that Cd AE in the amphipod *Leptocheirus plumulosus* decreased with increasing Cd concentration in bacterial exopolymers in the sediment.

Recently, metal AE from the ingested food source has been extensively quantified in a few marine invertebrates (Wang and Fisher, 1999). Most of these studies however quantified metal AEs in animals feeding on single diets. There are few studies which investigate metal assimilation in a mixture of diets. Whether the presence of other food particles affects the assimilation of metals from a particular particle type is unknown for most animals.

Development of sediment quality criteria must be based on rigorous study on metal bioavailability from contaminated sediment. Currently, it is generally accepted that acid volatile sulfide (AVS) is the key geochemical component in anoxic sediment for determining metal bioavailability and toxicity to aquatic benthic invertebrates (Di Toro et al., 1993; Ankley, 1996). Recent evidence, however, indicated that metals bound with AVS can also be bioavailable to deposit-feeding invertebrates (Lee et al., 2000). In marine polychaetes and bivalves, it was found that metals associated with anoxic sediment were directly bioavailable to these animals (Wang et al., 1999; Griscom et al., 2000). Clearly there is a need to investigate whether metals associated with anoxic

sediment, many of which are in sulfide pools, may be available for biological uptake in diverse benthic invertebrates.

In this study, we quantified the assimilation of sediment-bound Cd, Cr, and Zn in two marine bivalves (the green mussel *Perna viridis* and the Manila clam *Ruditapes philippinarum*) from Hong Kong coastal waters. These two bivalves are epifaunal (*P. viridis*) and infaunal (*R. philippinarum*) suspension feeders. In their natural environments, they primarily feed on suspended materials, including phytoplankton and inorganic seston, many of which may originate from resuspended sediment, especially during the typhoon season. Sediment dredging currently practised extensively in Hong Kong coastal waters or tidal currents can result in the resuspension of deposited sediment into the water column (Burgess and Scott, 1992), which may be a potential source for metal accumulation in bivalves. We have recently quantified the metal AE in these two bivalves feeding on diverse algal particles (Chong and Wang, 2000). We considered the influence of metal concentration and the oxidation conditions of sediment on metal assimilation. The presence of phytoplankton on metal assimilation from ingested sediment was also examined. No previous study has assessed the influence of metal concentration in sediment and the presence of phytoplankton on metal assimilation from sediment.

## 2. Materials and methods

### 2.1. Bivalves and metals

The green mussel *Perna viridis* and the Manila clam *Ruditapes philippinarum*, both of which were used as biomonitors in tropical and subtropical waters (Rainbow and Phillips, 1993), were used in this study. The green mussels *P. viridis* (shell length of 3.0–4.2 cm, dry weight of 0.25–0.4 g) were collected from Tung Chung on Lantau Island, Hong Kong during the summer of 1999. The clams *R. philippinarum* (shell length of 3–4 cm, dry weight of 0.3–0.4 g) were collected from Tolo Harbor in Hong Kong during the summer of 1999. After being brought back to the laboratory, both bivalves were cleaned of epibionts and acclimated at room temperature (about 23°C) and 30 ppt for 14 days before the experiments. During the acclimation period, the bivalves were fed with the diatom *T. pseudonana* (clone 3H). One week prior to the experiment, bivalves were fed with a mixture of sediment and diatoms to ensure that the digestive enzymes were acclimated to the sediment.

The three metals Cd, Cr and Zn are considered in this study because of concern for their potential contamination of Hong Kong coastal waters (Rainbow, 1993). Radiotracer technique was employed to follow the behavior of stable metals in the bivalves. The radioisotopes  $^{109}\text{Cd}$ ,  $^{51}\text{Cr(III)}$  and  $^{65}\text{Zn}$  were obtained from New England Nuclear.  $^{51}\text{Cr(III)}$  was used in this study primarily due to its dominance in the particulate phase compared with Cr(VI) (Wang et al., 1997).

Natural sediment was collected from Tsim Bei Tsui in the northwest of Hong Kong. After being collected from the field, the sediment was filtered through a 20- $\mu\text{m}$  mesh, and stored at  $-4^\circ\text{C}$  before radiolabeling. The organic matter content of the sediment,

measured by a CHN analyzer, was about 2.3% of the sediment dry weight. Other geochemical characteristics of the sediments such as the metal partitioning in different geochemical fractions was not quantified in this study.

## 2.2. Metal assimilation efficiency from ingested sediment

Sediment (60 mg dry weight in 5 ml 0.2  $\mu\text{m}$  filtered seawater held in a centrifuge tube) was radiolabeled with radiotracers. Radioisotope additions were 54 kBq  $\text{l}^{-1}$  (corresponding to 6.6 nM) for  $^{109}\text{Cd}$  (in 0.1 N HCl), 92.7 kBq  $\text{l}^{-1}$  (corresponding to 0.2 nM) for  $^{51}\text{Cr}$  (in 0.1 N HCl), and 54 kBq  $\text{l}^{-1}$  (corresponding to 6.4 nM) for  $^{65}\text{Zn}$  (in 0.1 N HCl). Prior to the radioisotope additions, a microlitre quantity of 0.5 N NaOH was added to maintain the pH at 8. The radiotracers were radiolabeled (or equilibrated) onto sediment for 5 days (or 10 days in the oxic/anoxic experiment described below), and then centrifuged twice at  $800 \times g$  for 20 min and the supernatant was discarded. The sediment pellet was resuspended in 5 ml 0.2  $\mu\text{m}$  filtered seawater before being fed to the bivalves. Partitioning of the radiotracers onto the sediment was not determined in our study.

AE was determined by the pulse-chase radiolabeled feeding technique (Wang and Fisher, 1999). There were five replicate individuals for each bivalve in each treatment. An individual bivalve was placed in a polypropylene beaker containing 1 l of filtered seawater. Radiolabeled sediment was added at a density of 1.5 mg (dry weight)  $\text{l}^{-1}$  to avoid pseudofeces production by the bivalves. Particle concentration was maintained similar to ensure that the assimilation can be compared among different treatments and experiments, although it is noted that the assimilation of metals was also minimally affected by a change in particle concentration (Wang et al., 1995; Ke and Wang, in preparation). Furthermore, our unpublished data indicated that the bivalves were not able to selectively ingest particles from a particle mixture (e.g. diatom and sediment) at this low particle concentration (Ke and Wang, in preparation). After feeding on radiolabeled sediment for 30 min (or 10 min in the oxic/anoxic experiment described below), the bivalves were rinsed and their radioactivity was measured non-destructively with a gamma counter. The bivalves were then individually allowed to depurate of their ingested particles in an enclosed recirculating seawater aquarium for 78 h, as described in Wang et al. (1995). During the depuration period, the diatom *T. pseudonana* and the sediment was fed to the mussels and clams at a rate about 2% of their tissue dry weights each day. The radioactivity remaining in the bivalves was monitored every 3 h within the first day and every 6–12 h between days 2 and 3. Feces produced during the depuration period were removed frequently and the radioactivity determined. The bivalves were dissected at the end of the 78-h depuration period. Because previous study indicated that the digestion and assimilation of metals by the two bivalves were complete within 60 h (Chong and Wang, 2000), we operationally defined the AE as the percentage of metals retained in the bivalve's tissue after 60 h of depuration.

During the radioactive feeding period, mussels and clams may take up some dissolved metals due to metal desorption from the radiolabeled particles. To assess this possibility, seawater containing radiolabeled sediment or diatoms was filtered through a 0.2- $\mu\text{m}$  membrane to remove any particles. Two individual bivalves were then exposed to these

batches of seawater for 4–6 h, and the radioactivity in the body of bivalves was determined at time intervals. Our results indicated that bivalves accumulated a negligible amount of radioactivity compared with the ingestion of radiolabeled particles (< 3%) and thus the dissolved uptake due to metal desorption from particles was minimal within the short term pulse feeding period.

### 2.3. Effects of metal concentration in sediment on metal assimilation

Sediment was spiked with stable metals for 5 days at a concentration of  $200 \mu\text{g l}^{-1}$  for Cd,  $1000 \mu\text{g l}^{-1}$  for Cr, and  $4000 \mu\text{g l}^{-1}$  for Zn. Other conditions were similar as described above. Following 5 days equilibrium, concentrations of ‘amended’ metals in the sediment, calculated from the partitioning of radiotracers in the sediment, were  $16.7 \mu\text{g g}^{-1}$  for Cd,  $83.3 \mu\text{g g}^{-1}$  for Cr, and  $333 \mu\text{g g}^{-1}$  for Zn, respectively, as compared to background concentrations of  $1.2 \mu\text{g g}^{-1}$  for Cd,  $33 \mu\text{g g}^{-1}$  for Cr, and  $222 \mu\text{g g}^{-1}$  for Zn (measured by  $\text{HNO}_3$  and  $\text{H}_2\text{SO}_4$  acid digestion; Ong Che, 1999). Thus the metal concentration in the amended sediment was 15, 3.5, and  $2.5 \times$  higher than the background metal concentration for Cd, Cr, and Zn, respectively. The ‘control’ sediment was only labeled with radiotracers without being amended with stable metals.

To simulate metal desorption within the acidic gut of bivalves, an experiment was conducted to measure metal desorption at pH 5.5 (the typical gut pH in bivalves; Owens, 1974) and at pH 8.0 (control, the pH of seawater). The labeled sediment was resuspended at pH 8.0 and pH 5.5 (prepared by the addition of dilute HCl) at a concentration of  $5 \text{ mg l}^{-1}$ . There were two replicates for each experimental treatment. At time intervals (0.5, 1, 2, 3, 4, 8, 12, 20, and 28 h), sediment was collected by filtration onto 0.2- $\mu\text{m}$  polycarbonate membranes, rinsed with filtered seawater, and the radioactivity was measured. A 1-ml sample was also taken for radioactivity measurements (representing the total radioactivity of sediment and water). The partitioning of metals between the sediment and the dissolved phase was then calculated.

### 2.4. Effects of diatoms on metal assimilation from ingested sediment

Sediment was labeled as described above and fed to the bivalves at a concentration of  $1.5 \text{ mg l}^{-1}$ . In another treatment, the radiolabeled sediment was mixed with unlabeled diatoms (*T. pseudonana*) immediately before being fed to the bivalves. The diatom concentration in the feeding suspension was 10,000 cells  $\text{ml}^{-1}$  (corresponding to  $0.2 \text{ mg l}^{-1}$ ). The assimilation of sediment-bound metals with and without the presence of diatom particles was then compared.

It was likely that metals might have desorbed from the radiolabeled sediment, followed by uptake by the unlabeled diatom in the feeding suspension. These ‘new’ accumulated diatom metals may then be ingested by the bivalves, complicating the interpretation of results from the metal assimilation experiment. To assess this possibility, seawater containing the resuspended radiolabeled sediment was filtered through a 0.2- $\mu\text{m}$  membrane to remove any particles. The unlabeled diatom cells were then exposed to the filtered seawater at a density of 20,000 cells  $\text{ml}^{-1}$ . The percentage of metals accumulated in the diatom cells was then measured at time intervals for 4 h.

### 2.5. Effects of sediment on metal assimilation from ingested diatoms

The diatom *T. pseudonana* was radiolabeled as described in Wang et al. (1996). Briefly, diatoms in the late exponential phase were filtered and resuspended in 0.2- $\mu\text{m}$  water containing f/2 levels of N, P, Si, and vitamins, and f/2 levels of trace metals without additions of EDTA, Cu, and Zn. Radioisotope additions were 37 kBq l<sup>-1</sup> for <sup>109</sup>Cd, 55.6 kBq l<sup>-1</sup> for <sup>51</sup>Cr, and 37 kBq l<sup>-1</sup> for <sup>65</sup>Zn. After 4 days of growth, the diatom cells were collected by 3- $\mu\text{m}$  polycarbonate membranes before resuspension in 30 ml 0.2  $\mu\text{m}$  of filtered seawater. Radiolabeled diatoms were then fed to the bivalves at a concentration of 10,000 cells ml<sup>-1</sup>. In another treatment, unlabeled sediment (1.5 mg l<sup>-1</sup>) was added to the radiolabeled diatom suspension (10,000 cells ml<sup>-1</sup>). The assimilation of diatom-bound metals with and without the presence of sedimentary particles was then compared.

### 2.6. Effects of oxidation conditions of sediment on metal assimilation

The oxic sediment was radiolabeled as described above. Anoxic sediment was collected from a 4–5-cm depth of sediment (filtered through a 20- $\mu\text{m}$  mesh) and resuspended in anoxic seawater. The anoxic seawater was prepared by the addition of sodium sulphite (100 mg l<sup>-1</sup>). The tube containing the anoxic seawater was placed in a chamber continuously flushed with nitrogen gas. Each day, the oxygen level in the water was monitored by an oxygen probe (Strathkelvin instruments oxygen interface model 928). Sodium sulphite was added to remove oxygen if required. Both the oxic and anoxic sediment was radiolabeled for 10 days. During the pulse-feeding period, anoxic seawater was prepared by the addition of sodium sulphite, thus the feeding of bivalves on the radiolabeled anoxic sediment was performed under the anoxic conditions. The feeding time was reduced to 10 min to minimize any reoxidation of the anoxic sediment in the seawater. AEs were then determined as described above.

### 2.7. Gamma radioactivity counting

The radioactivity of <sup>109</sup>Cd, <sup>51</sup>Cr and <sup>65</sup>Zn in the bivalves was measured non-invasively by a Canberra NaI (Tl) gamma detector. The radioactivity of sediment and algae were measured by a Wallac 1480 NaI (Tl) gamma detector. The gamma emission of <sup>109</sup>Cd was detected at 22 keV, of <sup>51</sup>Cr at 320 keV, and of <sup>65</sup>Zn at 1115 keV. The radioactivity was calibrated for the radioactive decay, spillover from a higher energy window to a lower window, and counting efficiency. The counting time was adjusted to produce a propagated counting error < 5%.

## 3. Results

The depuration patterns of sediment-bound metals in the mussels and the clams following radiolabeled pulse feeding are shown in Figs. 1–3. The depuration pattern for

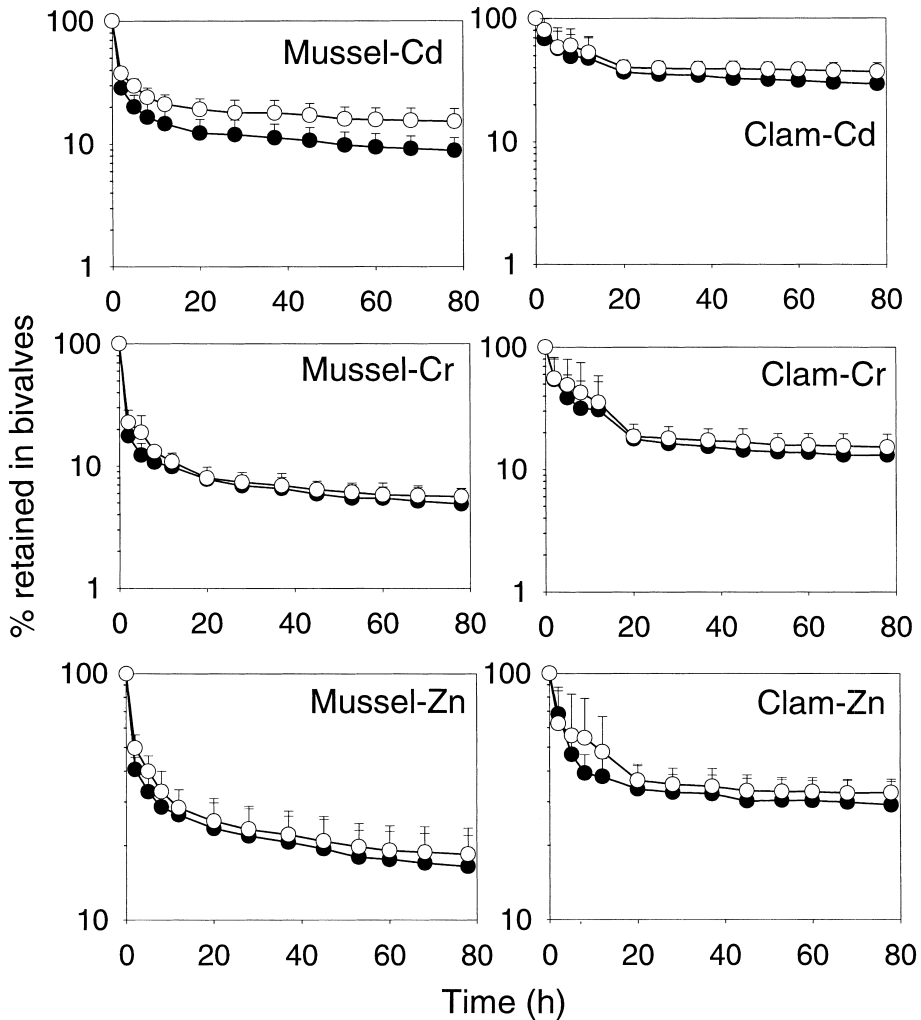


Fig. 1. The depuration of Cd, Cr, and Zn in the green mussel *Perna viridis* and the clam *Ruditapes philippinarum* following a pulse feeding on radiolabeled sediment. (●) Sediment without a stable metal spike, the background concentration was  $1.2 \mu\text{g g}^{-1}$  for Cd,  $33 \mu\text{g g}^{-1}$  for Cr, and  $222 \mu\text{g g}^{-1}$  for Zn. (○) Sediment amended with  $16.7 \mu\text{g g}^{-1}$  of Cd,  $83.3 \mu\text{g g}^{-1}$  of Cr, and  $333 \mu\text{g g}^{-1}$  of Zn. Mean + S.D. ( $n = 5$ ).

each metal was generally similar in sediment with different geochemical properties. Metals were lost rapidly within the first 1 day, followed by a much slower loss. The AE, calculated as the % of metals retained after 60 h of depuration, was highest for Zn (ranging from 17 to 50% in the mussels and from 11 to 53% in the clams) (Table 1). Cr was the least assimilated metal, with AE ranging from 5 to 20% in the mussels and from 4 to 20% in the clams. Assimilation of Cd was intermediate between Cr and Zn. There was however notable variation of metal AE determined among different experiments.

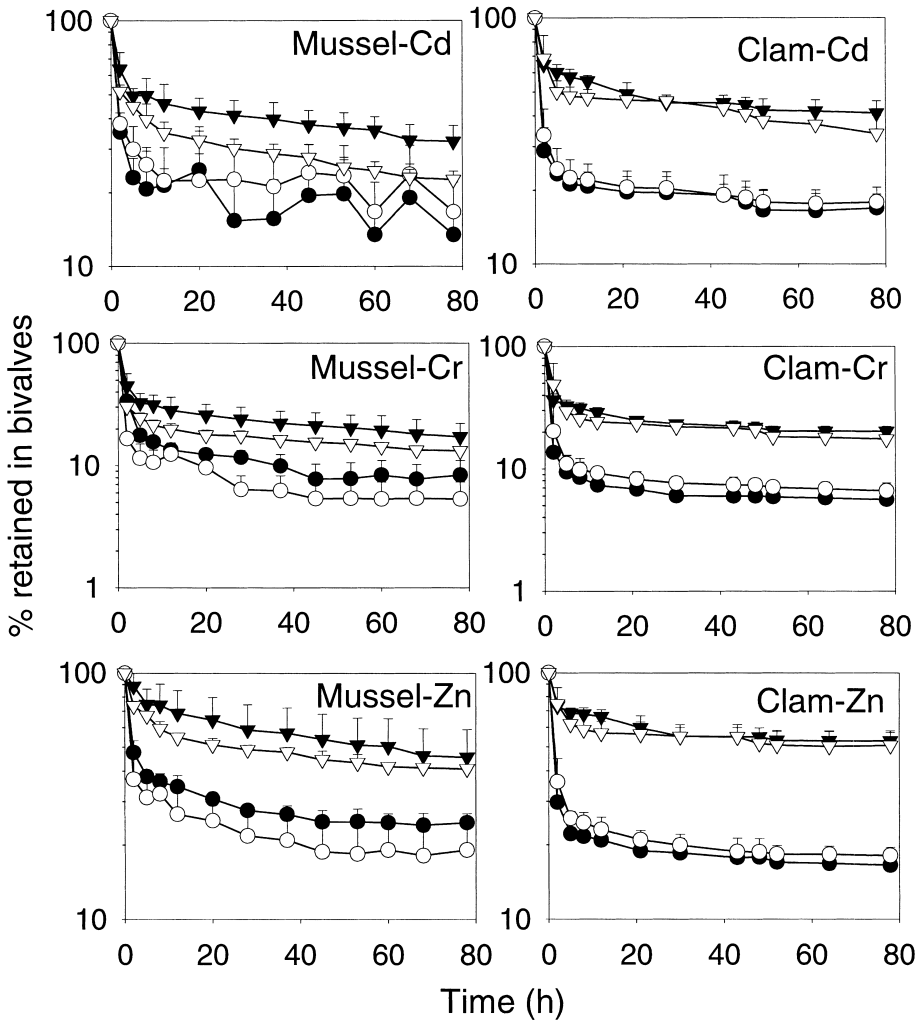


Fig. 2. The depuration of Cd, Cr, and Zn in the green mussel *Perna viridis* and the clam *Ruditapes philippinarum* following a pulse feeding on radiolabeled sediment or diatoms. (●) Radiolabeled sediment with the presence of unlabeled diatom *Thalassiosira pseudonana*; (○) radiolabeled sediment only; (▼) radiolabeled diatoms only; (▽) radiolabeled diatoms with the presence of unlabeled sediment. Mean + S.D. ( $n = 5$ ).

Metal concentration in the sediment did not significantly influence the metal AEs in either bivalves ( $P > 0.05$ ,  $t$ -test), except for Cd in the mussels (Fig. 1, Table 1). The Cd AE increased by  $1.7 \times$  when the sediment was amended with Cd  $15 \times$  higher than the background Cd concentration ( $P < 0.05$ ,  $t$ -test). Between the two bivalve species, metal AEs from 'amended' sediment were higher in clams than in mussels by about  $2.4 \times$  for Cd,  $2.7 \times$  for Cr and  $1.7 \times$  for Zn.



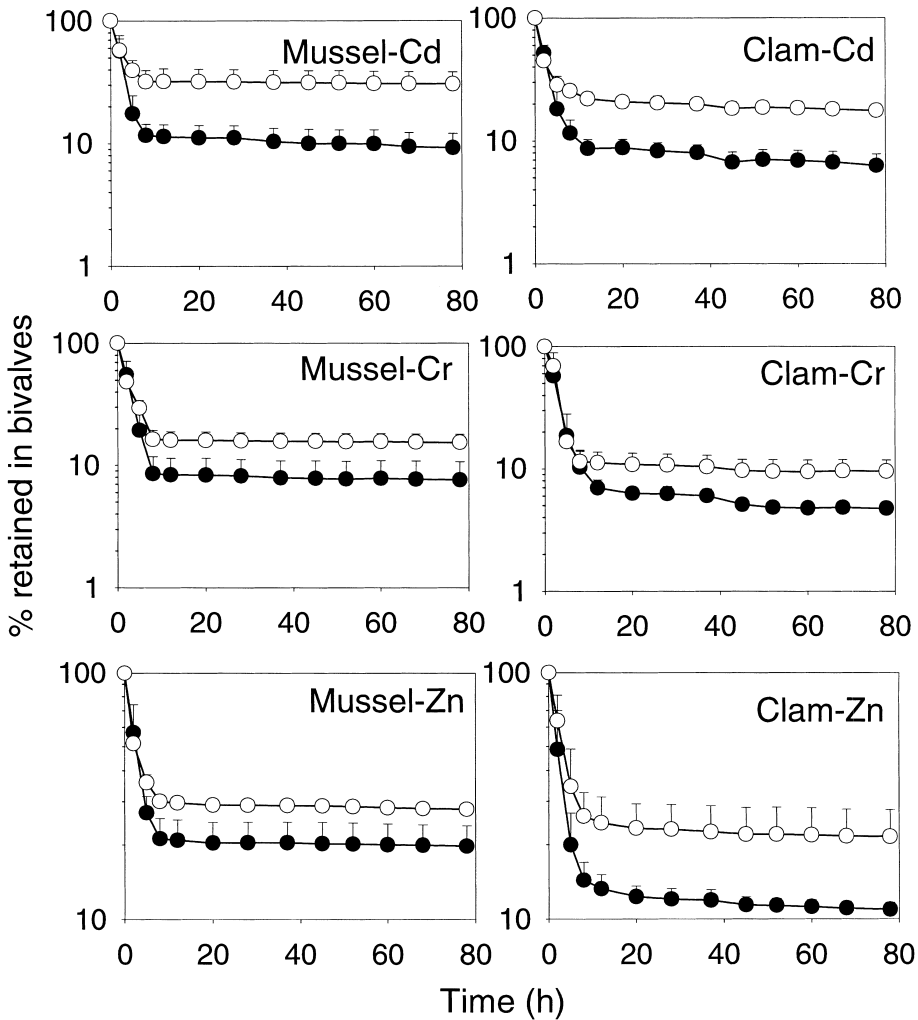


Fig. 3. The depuration of Cd, Cr, and Zn in the green mussel *Perna viridis* and the clam *Ruditapes philippinarum* following a pulse feeding on radiolabeled sediment. (●) Radiolabeled anoxic sediment; (○) radiolabeled oxic sediment. Mean + S.D. ( $n = 5$ ).

The % of metals desorbed from radiolabeled sediment at pH 5.5 and pH 8.0 is shown in Fig. 4. Significant desorption of metals was observed at both pH 5.5 and pH 8.0. Desorption was greater at pH 5.5 than at pH 8.0. For example, within 0.5 h of resuspension, about 19% of the Cd and about 33–40% of Zn were desorbed at pH 8.0, compared with 60–73% of the Cd and 74–83% of the Zn desorption at pH 5.5. Most desorption occurred within the first 2 h, followed by slower desorption. Cd was however desorbed continuously from the sediment. Among the three metals, Cr desorbed the

Table 1

Assimilation efficiencies (AEs) of Cd, Cr and Zn in the green mussel *Perna viridis* and the Manila clam *Ruditapes philippinarum* feeding on sediment with different geochemical properties (mean±S.D.,  $n = 5$ )<sup>a</sup>

Sediment type	Mussels			Clams		
	Cd	Cr	Zn	Cd	Cr	Zn
Expt. 1						
'Unamended' sediment	9.5±2.7*	5.4±1.8	17.6±5.2	31.3±6.9	13.7±3.3	30.3±7.3
'Amended' sediment	15.8±4.0*	5.8±1.0	19.1±4.9	38.3±5.8	15.8±3.9	32.9±3.8
Expt. 2						
Labeled diatom	35.8±4.9**	19.5±6.2	50.4±15.0	41.7±4.7	20.4±2.0	53.0±5.2
Labeled diatom + sediment	24.5±2.2**	14.3±1.1	41.7±1.1	36.8±4.3	18.1±3.1	50.2±7.4
Expt. 3						
Diatom + labeled sediment	13.5±4.1	8.4±2.7	24.7±2.1	16.4±3.6	5.8±1.6	16.8±2.9
Labeled sediment	16.7±5.3	5.3±2.0	19.1±5.8	17.6±1.8	6.9±1.0	18.3±0.4
Expt. 4						
Anoxic sediment	9.9±3.0**	7.8±3.1*	20.0±4.3*	6.9±1.5***	4.7±0.6*	11.2±0.8*
Oxic sediment	30.8±8.0**	15.6±2.6*	28.4±1.6*	18.5±2.6***	9.4±2.3*	21.9±6.3*

<sup>a</sup> Statistical significant difference between two treatments is indicated by \* $P < 0.05$ , \*\* $P < 0.01$ , or \*\*\* $P < 0.001$ .

least, while the desorption of Cd and Zn was comparable. Metal concentration in the sediment appeared to have little effect on metal desorption at pH 8.0. More metals were however desorbed within the first few hours when the sediment was amended with metals. For example, 73% of Cd was desorbed within the first 0.5 h in sediment amended with Cd, compared with 61% desorption in 'unamended' sediment.

In the experiments testing the influence of particle mixture on metal assimilation, there was no major difference in metal AEs between the mussels and the clams (Fig. 2, Table 1). However, metal AEs were higher for both bivalves feeding on the radiolabeled diatoms than on the radiolabeled sediment by  $\sim 2.3 \times$  for Cd,  $3.4 \times$  for Cr and  $2.7 \times$  for Zn. In clams, the presence of unlabeled diatoms or sediment did not affect the AEs of radiolabeled sediment or diatoms. In mussels, the addition of sediment significantly reduced the assimilation of Cd from ingested diatom (Table 1). Assimilation of Cr and Zn was also slightly reduced but the difference was not statistically significant. Assimilation of sediment bound Cr and Zn was also slightly increased with the addition of nonradiolabeled diatoms.

Uptake of desorbed metals (from radiolabeled sediment) by unlabeled diatom *T. pseudonana* within the 4-h exposure period is shown in Fig. 5. Very small amounts (less than 5%) of the desorbed metals were accumulated by the diatoms within the exposure period. We did not measure the uptake of desorbed metals (from radiolabeled diatoms) by the sediment in this study.

In the experiment testing the influence of an anoxic condition on metal assimilation,

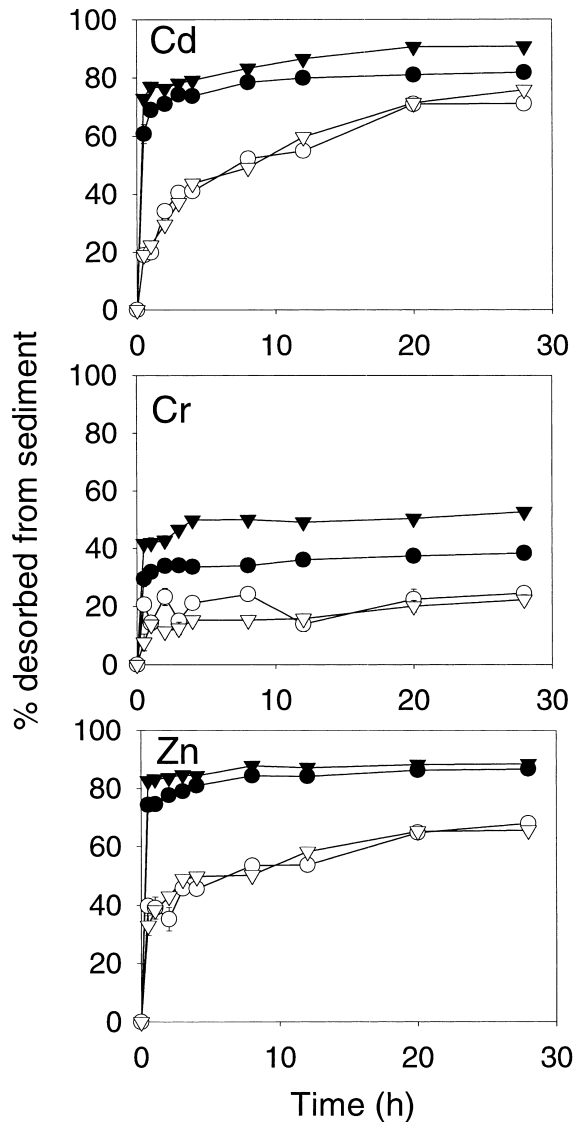


Fig. 4. The percentage of Cd, Cr and Zn desorbed from the radiolabeled sediment following resuspension at pH 5.5 and pH 8.0. (●) Unamended sediment at pH 5.5; (○) unamended sediment at pH 8.0; (▼) amended sediment at pH 5.5; (▽) amended sediment at pH 8.0. Mean  $\pm$  S.D. ( $n = 2$ ).

there was very little loss of metals from the mussel's body during the 2–3-day period of depuration (Fig. 3), in contrast to other experiments (Figs. 1 and 2). When animals fed on oxic sediment rather than on anoxic sediment, their AEs increased by  $3.1 \times$  for Cd,

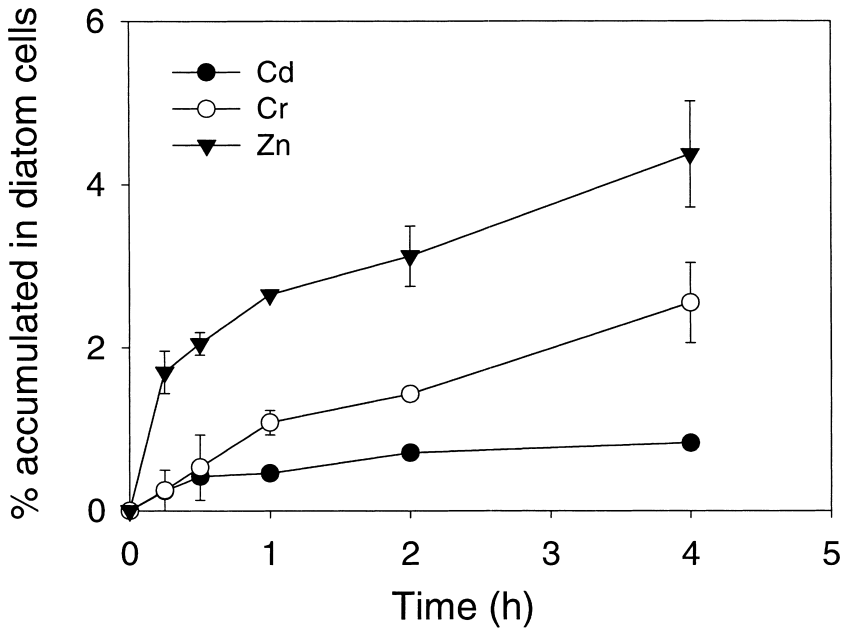


Fig. 5. The % of desorbed Cd, Cr, and Zn accumulated in diatoms *Thalassiosira pseudonana*. Mean  $\pm$  S.D. ( $n = 2$ ).

$2 \times$  for Cr and  $1.4 \times$  for Zn in the mussels, and  $2.7 \times$  for Cd,  $2 \times$  for Cr, and  $2 \times$  for Zn in the clams, suggesting that metals associated with anoxic sediment had a lower bioavailability to the bivalves.

#### 4. Discussion

Sediment can be considered an important source of metal accumulation in many benthic invertebrates, but the extent to which sediment-bound metals are bioavailable has not been quantified extensively (Gagnon and Fisher, 1997; Wang et al., 1998, 1999; Griscom et al., 2000). Recent studies have strongly indicated that sediment ingestion can often account for up to 100% of the metal body burden in several deposit feeding or facultative feeding invertebrates (Selck et al., 1998; Wang et al., 1999; Stecko and Bendell-Young, 2000). Geochemical properties of sediment have been shown to be critical in affecting metal bioavailability (Luoma, 1989; Wang and Fisher, 1999), but the controls they place on metal bioavailability are not fully known (Griscom et al., 2000). Both organic carbon and acid volatile sulfide are found to be critical in affecting metal bioavailability from contaminated sediment (Ankley, 1996; Chapman et al., 1998). Few studies however considered the influence of animal ecology and physiology on metal bioavailability from sediment. In this study, we quantified metal bioavailability from ingested sediment in the bivalves by measuring metal AE as a physiological probe. Our

study demonstrated that sediment-bound metals can be appreciably assimilated by the bivalves, although the AE was somewhat lower than the AEs from unialgal diets. For comparison, AEs for natural seston collected from coastal waters were 10% for Cd, 9.5% for Cr, and 27% for Zn in the green mussels *P. viridis*, and 22% for Cd, 11% for Cr, and 31% for Zn in the clams *R. philippinarum* (Chong and Wang, 2000). Lower assimilation of metals from ingested sediment than from ingested phytoplankton food has been observed in several bivalves (Wang et al., 1997; Lee and Luoma, 1998).

Although the two bivalves examined in this study are benthic suspension feeders, sedimentary particles can be a potentially important source for metal uptake by the animals due to sediment resuspension as a result of tidal currents or typhoons, particularly in shallow regions. Furthermore, sediment dredging may contribute substantially to sediment resuspension in the water column. Sediment often contains high concentrations of metals and once ingested by animals, may then make these metals bioavailable to the animals.

In our experiments we radiolabeled sediment for 5–10 days (as in most previous studies). We did not quantify the geochemical binding of radiotracers with the sediment, which may appreciably affect metal AE. It is likely that the AE determined by the radiotracer technique may have been different from the ‘true’ AE of metals that have been equilibrated with the sediment for an extended period of time. Thus, the AEs that we measured may represent the AEs from the ‘new’ introduced metals in the sediment. Recently, Griscom et al. (2000) demonstrated that metal AEs were a function of the radiolabeling period in the sediment. With an increase in the radiolabeling period from 1 day to 35 days, the AEs of Cd in the mussel *M. edulis* decreased from 21.7% to 8.5%, and those of Zn decreased from 41.7% to 23.4%. The duration of radiolabeling was however found to have no major influence on metal AE in the marine polychaete *Nereis succinea* (Wang et al., 1999). In our study, we were mainly concerned with the influence of different chemical (e.g. metal concentration in ingested sediment, and oxic/anoxic condition of sediment) and ecological conditions (presence of other food particles) on metal assimilation from ingested sediment.

In our previous study (Chong and Wang, 2000), we found that the assimilation of metals from phytoplankton was generally higher in the clams than in the mussels. There was however no clear trend revealed by our different experiments that the assimilation of metals from sediment was higher in the clams than in the mussels. In addition, differences in AEs among different experiments was also apparent from our study. For example, AEs varied by up to  $3 \times$  in the two experiments examining the influence of metal concentration and oxidation conditions in sediment on metal assimilation. AEs determined from the anoxic sediment were lower than the AEs for oxic sediment in the same experiment, but were comparable to AEs determined for oxic sediment in other batches of experiments. Our study therefore highlights the difficulty of comparing metal AEs determined in different batches of experiments. Differences in animal’s physiological conditions may greatly affect metal assimilation (Wang and Fisher, 1997).

Under most circumstances, metal concentration in sediment had little effect on the assimilation of sediment-bound metals in the mussels and the clams. It appeared that bivalves were not able to differentiate the difference in metal concentrations in ingested sediment. In our study, difference in Cr and Zn concentrations in ‘amended’ and

'unamended' sediment was only  $2.5\text{--}3.5 \times$ . Consequently, metal influx in bivalves due to the ingestion of sediment will increase proportionately with an increase in metal concentration in ingested sediment. There was no evidence that the bivalves can regulate their metal uptake in response to a change in metal concentration in the food particles. In contrast, Wang and Fisher (1996b) demonstrated that the mussel *M. edulis* reduced its AEs of Zn when the diatom food was more contaminated with Zn, implying the possibility of regulating Zn influx from ingested diatom.

Among the three metals examined, it appeared that only Cd assimilation in the mussels was affected by an increase in the metal's concentration in ingested sediment (by  $15 \times$ ). The mechanism underlying the influence of Cd concentration on Cd AE remains to be further explored. Wang and Fisher (1996b) also found that Cd assimilation in the mussel *M. edulis* increased with an increase in Cd concentration in the ingested diatom. One underlying mechanism may be the higher desorption of Cd with increasing Cd concentration in the ingested diatom under the acidic gut pH. For example, more Cd was found to desorb with an increase in its concentration in the diatom at a low pH (Wang and Fisher, 1996b). Similarly, in this study we found that the degree of metal desorption at pH 5.5 was higher in sediment amended with metals, especially during the first few hours of sediment resuspension. Digestion was most intensive within the first few hours of particle ingestion. However, no difference in the AEs of Cr and Zn was found, despite their higher desorption from 'amended' sediment. We also did not observe any influence of Cd concentration in sediment on Cd AE in the clam *R. philippinarum*. Gagnon and Fisher (1997) demonstrated that metal desorption (Ag, Cd, Co) was critical in influencing metal assimilation in the mussel *M. edulis*. Metal desorption from sediment within the gut was the first step in controlling metal bioavailability to deposit-feeding invertebrates (Mayer et al., 1996).

Previous studies indicated that the chemical composition of the sediment could affect AEs of sediment-bound metals (Luoma and Fisher, 1997; Griscom et al., 2000). A few studies demonstrated that metal AEs were generally lower when the metals were associated with inorganic particles rather than organic particles. For example, AEs of Cd, Co and Zn associated with inorganic Fe oxide, which is one of the main components in sediment, were reduced by  $2 \times$  compared with AEs from ingested algal cells in the marine copepod *Acartia tonsa* and *Temora longicornis* (Wang et al., 1996). Similarly, organic coating of the sediment significantly enhanced the metal AEs in the clam *M. balthica* (Harvey and Luoma, 1985). Although the feeding physiology of bivalves on a mixture of natural sediment and phytoplankton has been extensively studied (Bayne et al., 1987, 1993), the influence of living particle assemblage on metal assimilation from sediment remained largely unknown. Lee and Luoma (1998) demonstrated that the clams (*Potamocorbula amurensis* and *M. balthica*) assimilated Cd, Cr, and Zn at higher efficiencies from food particles enriched with algal particles than from food particles poor in living microalgal biomass. Our study suggested that metal AEs were higher from living diatom cells than from sediment, and the presence of other particles did not affect metal assimilation from ingested phytoplankton or sediment. Thus, metal assimilation measured in the presence of a single diet should reflect its assimilation from that particular type of particle in the mixture of food materials.

It was likely that different particles may have competed for metals within the

bivalve's guts, and due to their differential gut passage (Wang and Fisher, 1996a), may be assimilated differently. For example, a metal bound with sediment may desorb within the gut and re-absorb onto the phytoplankton debris, which may then be channeled further for intracellular digestion (Decho and Luoma, 1991; Wang et al., 1995), leading to a higher assimilation. Conversely, a metal bound with phytoplankton may desorb within the gut and re-absorb by the inorganic particles, leading to a lower assimilation because of the particle's rapid passage through the gut (Wang and Fisher, 1996a). In our study, we only found that Cd bound with diatoms was assimilated at a significantly lower efficiency with the presence of sedimentary particles. No statistically significant difference was found for other metals in both species. Selectivity within the gut appeared to be much smaller in the clams than in the mussels.

Bioavailability of metals from oxic sediment was generally higher than from anoxic sediment (Förstner, 1989; Wollast, 1989; Simpson et al., 1998; Wang et al., 1999). Our results on the two bivalves were consistent with these previous studies. Metals associated with anoxic sediment were assimilated at a much lower efficiency than metals from oxic sediment, similar to the finding in the clam *M. balthica* (Griscom et al., 2000). Nevertheless, Griscom et al. (2000) also showed that the AEs of metals (except Ag) were higher from anoxic sediment than from oxic sediment in the mussel *M. edulis*. In our study, the anoxic conditions of the sediment were carefully controlled by daily measurements of oxygen levels in the sediment and by the addition of sodium sulphite. The feeding time was also reduced to 10 min to minimize reoxidation of sediment in anoxic seawater. We found that the feeding rate was reduced to about 50% of the feeding rate in animals maintained in oxic waters within the 10-min feeding period. In this study we did not measure the acid volatile sulphide fraction, which has been demonstrated to be critical in controlling metal toxicity from anoxic sediment (Ankley, 1996; Chapman et al., 1998).

Several recent studies have consistently demonstrated that metals associated with anoxic sediment were in fact bioavailable to benthic bivalves and polychaetes (Wang et al., 1998, 1999; Griscom et al., 2000; Lee et al., 2000). Metals bound with sulfide (the main binding ligand for anoxic sediment) may have a low solubility product, leading to rapid gut passage and thus a lower assimilation. Our results nevertheless suggested that metals in anoxic sediment should be treated cautiously. It is likely that some metals of this sulfide-binding pool may get re-oxidized during the gut passage in the bivalves. Further studies are needed to examine the controls of different geochemical ligands on metal assimilation in the bivalves.

In summary, our study demonstrated that both the green mussel and the Manila clam were able to assimilate metal from resuspended sediment particles. Metal concentration in the sediment did not appreciably affect the assimilation of sediment-bound metals, although Cd assimilation increased with increasing Cd concentration in sediment presumably due to the increased desorption under an acidic condition. Metal assimilation from ingested diatom was higher than assimilation from ingested natural sediment. The presence of other particles did not significantly affect metal assimilation from a particular type of food particles. Cd assimilation from diatom was however reduced with the presence of sediment. Furthermore, metals were more bioavailable from oxic sediment than from the anoxic sediment. Understanding metal bioavailability from

sediment with different geochemical properties is important in assessing metal toxicity from contaminated sediment. Our study highlights the importance of sediment as a potentially important source of metal accumulation in the two bivalves common in subtropical and tropical waters.

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## References

- Arifin, Z., Bendell-Young, L.I., 2000. Influence of a selective feeding behaviour by the blue mussel *Mytilus trossulus* on the assimilation of Cd-109 from environmentally relevant seston matrices. *Mar. Ecol. Prog. Ser.* 192, 181–193.
- Ankley, G.T., 1996. Evaluation of metal/acid-volatile sulfide relationships in the prediction of metal bioaccumulation by benthic macroinvertebrates. *Environ. Toxicol. Chem.* 15, 2138–2146.
- Bayne, B.L., Hawkins, A.J.S., Navarro, E., 1987. Feeding and digestion by the mussel *Mytilus edulis* L. in mixture of silt and algal cells at low concentrations. *J. Exp. Mar. Biol. Ecol.* 111, 1–22.
- Bayne, B.L., Iglesias, J.I.P., Hawkins, A.J.S., Navarro, E. et al., 1993. Feeding behaviour of the mussel, *Mytilus edulis*: response to variation in quantity and organic content of the seston. *J. Mar. Biol. Assoc. UK* 73, 813–829.
- Bendell-Young, L.I., 1999. Application of a kinetic model of bioaccumulation across a pH and salinity gradient for the prediction of Cd uptake by the sediment dwelling chironomidae. *Environ. Sci. Technol.* 33, 1501–1508.
- Burgess, M.R., Scott, K.J., 1992. The significance of in-place contaminated marine sediment on the water column: process and effects. In: Burton, G.A. (Ed.), *Sediment Toxicity Assessment*. Lewis Publishers, London, pp. 221–240.
- Campbell, P.G.C., Lewis, A.G., Chapman, P.M., Crowder, A.A., Fletcher, W.K., Imber, B., Luoma, S.N., Stokes, P.M., Winfrey, M., 1988. *Biologically Available Metals in Sediment*. National Research Council of Canada, Ottawa, Publ no. NRCC 27694.
- Chapman, P.M., Wang, F., Janssen, C., Persoone, G., Allen, H.E., 1998. Ecotoxicology of metals in aquatic sediment: binding and release, bioavailability, risk assessment, and remediation. *Can. J. Fish. Aquat. Sci.* 55, 2221–2243.
- Chong, K., Wang, W.-X., 2000. Assimilation of Cd, Cr and Zn by the green mussel *Perna viridis* and the clam *Ruditapes philippinarum*. *Environ. Toxicol. Chem.* 19, 1660–1667.
- Decho, A.W., Luoma, S.N., 1991. Time course in the retention of food material in the bivalves *Potamocorbula amurensis* and *Macoama balthica*: significance to the absorption of carbon and chromium. *Mar. Ecol. Prog. Ser.* 78, 303–314.
- Förstner, U., 1989. Environmental factors controlling the uptake and release of organic and inorganic pollutant burden. In: *Proceedings of the International Seminar on the Environmental Aspects of Dredging Activities*, Nantes, France, pp. 33–47.
- Förstner, U., Wittmann, G.T.W., 1981. *Metal Pollution in the Aquatic Environment*. Springer-Verlag, Berlin.
- Fung, Y.S., Lo, C.K., 1997. Determination of heavy metal profiles in dated sediment cores from Sai Kung Bay, Hong Kong. *Environ. Int.* 23, 317–335.
- Gagnon, C., Fisher, N.S., 1997. The bioavailability of sediment-bound Cd, Co, and Ag to the mussel *Mytilus edulis*. *Can. J. Fish. Aquat. Sci.* 54, 147–156.



- Griscom, S.B., Fisher, N.S., Luoma, S.N., 2000. Geochemical influence on assimilation of sediment-bound metals in clams and mussels. *Environ. Sci. Technol.* 34, 91–99.
- Harvey, R.W., Luoma, S.N., 1985. Effects of adherent bacteria and bacterial extracellular polymers upon assimilation by *Macoma balthica* of the sediment-bound Cd, Zn and Ag. *Mar. Ecol. Prog. Ser.* 22, 281–289.
- Jenne, E.A., Luoma, S.N., 1977. Forms of trace elements in soils, sediment, and associated waters: an overview of their determination and biological availability. In: Drucker, H., Wildung, R.E. (Eds.), *Biological Implications of Metals in the Environment*. Technical Information Center, US Energy Research and Development Administration, pp. 110–143.
- Lee, B.-G., Luoma, S.N., 1998. Influence of microalgal biomass on absorption efficiency of Cd, Cr, and Zn by the two bivalves from San Francisco Bay. *Limnol. Oceanogr.* 43, 1455–1466.
- Lee, B.-G., Griscom, S.B., Lee, J.-S., Choi, H.J., Koh, C.-H., Luoma, S.N., Fisher, N.S., 2000. Influences of dietary uptake and reactive sulfides on metal bioavailability from aquatic sediments. *Science* 287, 282–284.
- Lin, J.G., Chen, S.Y., 1998. The relationship between adsorption of heavy metal and organic matter in river sediment. *Environ. Int.* 24, 345–352.
- Luoma, S.N., 1989. Can we determine the biological availability of sediment-bound trace elements? *Hydrobiologia* 176–177, 379–396.
- Luoma, S.N., 1990. Process affecting metal concentration in estuarine and coastal marine sediment. In: Furness, R.W., Rainbow, P.S. (Eds.), *Heavy Metals in the Marine Environment*. CRC Press, Florida, pp. 52–62.
- Luoma, S.N., Fisher, N.S., 1997. Uncertainties in assessing contaminant exposure from sediment. In: Ingersoll, C.G., Dillon, T., Biddinger, G.R. (Eds.), *Ecological Risk Assessments of Contaminated Sediment*. SETAC Special Publication Series, Pensacola, pp. 211–237.
- Mayer, L.M., Chen, Z., Findly, R.H., Fang, J., Sampson, S., Self, R.L., Jumbar, P.A., Quétel, C., Donard, O.F., 1996. Bioavailability of sedimentary contaminants subject to the deposit-feeder digestion. *Environ. Sci. Technol.* 30, 2641–2645.
- Newman, M.C., Jagoe, C.H., 1994. Ligands and bioavailability of metals in aquatic environments. In: Hamelink, J., Landrum, P., Bergman, H.L., Benson, W. (Eds.), *Bioavailability, Physical, Chemical and Biological Interactions*. SETAC, Pensacola, pp. 39–61.
- Ong Che, R.G., 1999. Concentration of 7 heavy metals in sediment and mangrove root samples from Mai Po, Hong Kong. *Mar. Pollut. Bull.* 39, 269–279.
- Owens, G., 1974. Feeding and digestion in the bivalves. *Adv. Comp. Physiol. Biochem.* 5, 1–35.
- Rainbow, P.S., 1993. Biomonitoring of marine heavy metal pollution and its application in Hong Kong waters. In: Morton, B. (Ed.), *The Marine Biology of the South China Sea*. Hong Kong University Press, Hong Kong, pp. 235–250.
- Rainbow, P.S., Phillips, D.J.H., 1993. Cosmopolitan biomonitors of trace metals. *Mar. Pollut. Bull.* 26, 593–601.
- Schlekat, C.E., Decho, A.W., Chandler, G.T., 1999. Dietary assimilation of cadmium associated with bacterial exopolymer sediment coating by the estuarine amphipod *Leptocheirus plumulosus*: effects of Cd concentration and salinity. *Mar. Ecol. Prog. Ser.* 183, 205–216.
- Selck, H., Forbes, V.E., Forbes, T.L., 1998. Toxicity and toxicokinetics of cadmium in *Capitella* sp. 1. Relative importance of water and sediment as routes of cadmium uptake. *Mar. Ecol. Prog. Ser.* 164, 167–178.
- Selck, H., Decho, A.W., Forbes, V.E., 1999. Effects of chronic metal exposure and sediment organic matter on digestive absorption efficiency of cadmium by the deposit-feeding polychaete *Capitella* species I. *Environ. Toxicol. Chem.* 18, 1289–1297.
- Simpson, S.T., Apte, S.C., Batley, G.E., 1998. Effects of short-term resuspension events on the trace metal speciation in polluted anoxic sediment. *Environ. Sci. Technol.* 32, 620–625.
- Stecko, J.R.P., Bendell-Young, L.I., 2000. Uptake of <sup>109</sup>Cd from sediment by the bivalves *Macoma balthica* and *Protothaca staminea*. *Aquat. Toxicol.* 47, 147–159.
- Wang, W.-X., Fisher, N.S., 1996a. Assimilation of trace elements and carbon in the mussel *Mytilus edulis*: effect of food composition. *Limnol. Oceanogr.* 41, 197–207.
- Wang, W.-X., Fisher, N.S., 1996b. Assimilation of trace elements by the mussel *Mytilus edulis*: effects of diatom chemical composition. *Mar. Biol.* 125, 715–724.

- Wang, W.-X., Fisher, N.S., 1997. Modelling metal bioavailability for marine mussels. *Rev. Environ. Contam. Toxicol.* 151, 39–65.
- Wang, W.-X., Fisher, N.S., 1999. Assimilation efficiencies of chemical contaminants in aquatic invertebrates: a synthesis. *Environ. Toxicol. Chem.* 18, 2034–2045.
- Wang, W.-X., Fisher, N.S., Luoma, S.N., 1995. Assimilation of trace elements ingested by the mussel *Mytilus edulis*: effects of algal food abundance. *Mar. Ecol. Prog. Ser.* 129, 165–176.
- Wang, W.-X., Reinfelder, J.R., Lee, B.-G., Fisher, S.N., 1996. Assimilation and regeneration of trace elements by marine copepods. *Limnol. Oceanogr.* 41, 70–81.
- Wang, W.-X., Griscom, S.B., Fisher, N.S., 1997. Bioavailability of Cr (III) and Cr (VI) to marine mussels from solute and particulate pathways. *Environ. Sci. Technol.* 31, 603–611.
- Wang, W.-X., Stupakoff, I., Fisher, N.S., 1999. Bioavailability of dissolved and sediment-bound metals to a marine deposit-feeding polychaete. *Mar. Ecol. Prog. Ser.* 178, 281–293.
- Wang, W.-X., Stupakoff, I., Gagnon, C., Fisher, N.S., 1998. Bioavailability of inorganic and methylmercury to a marine deposit-feeding polychaete. *Environ. Sci. Technol.* 32, 2564–2571.
- Wollast, R., 1989. Physico-chemical models of marine sediment. In: *Proceedings of the International Seminar on the Environmental Aspects of Dredging Activities*, Nantes, France, pp. 191–204.