

# Bioavailability of sedimentary metals from a contaminated bay

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**ABSTRACT:** Sediments are considered the sink for metals in aquatic environments because of their strong metal-binding capacity, but they are a potential source for metal ingestion by marine benthic animals as well. Sediment contamination is now a major environmental problem in many countries, including China. This study examines the bioavailability to a marine suspension-feeder (the clam *Mactra veneriformis*) and a deposit-feeder (the peanut worm *Sipunculus nudus*) of sediment-bound Cd, Cr and Zn along a gradient of metal contamination. To this end, metal assimilation efficiency and metal extraction by gut juice were quantified and compared. Metal concentrations in sediments collected from 4 sites were 5.40 to 43.7 mg Cd kg<sup>-1</sup>, 32.8 to 71.2 mg Cr kg<sup>-1</sup>, and 278 to 5 380 mg Zn kg<sup>-1</sup>. Metal assimilation by the clams was independent of the metal concentration in the sediment. In the deposit-feeding sipunculid, the bioavailability of sediment-bound Cd increased with increasing contamination of the sediment, as a result of the increasing partitioning of Cd into the easily exchangeable phase, and decreasing partitioning into the reducible phase. Cd assimilation also increased with increasing total organic carbon content in the sediments. The Cd assimilation efficiency was directly correlated to Cd extraction by gut juices collected *in vitro* from the peanut worm. No such relationship was found for Cr and Zn. Geochemical and biological controls on metal bioavailability therefore differ between the metals and with the extent of sediment contamination. The limitation of the radio-tracer technique to quantify metal assimilation efficiency by marine benthic invertebrates from sediments should be recognized when it is used to predict metal concentrations in target animals.

**KEY WORDS:** Metal · Bioavailability · Contamination · Sediment · Sipuncula · Clams

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## INTRODUCTION

Marine sediments are considered a sink for metals in aquatic environments, especially in estuarine and coastal waters. Anthropogenic activity (such as Zn smelting) has resulted in profound perturbation of marine ecosystems in several major Chinese rivers and bays (Chen & Zhou 1998). Sediments are a potential source for metal ingestion by marine deposit- and suspension-feeders, because they can be resuspended into the water column by tidal current action or sediment dredging (as occurs extensively in Hong Kong during land reclamation). Kinetic modeling and exper-

imental manipulation indicate that sediment ingestion can be a dominant source for metal accumulation in surface deposit-feeders (Selck et al. 1998, Wang et al. 1999, Lee et al. 2000a,b, Yan & Wang 2002). Assessment and prediction of sediment toxicity are daunting tasks, because numerous factors influence sediment toxicity to aquatic biota. A central issue in the prediction of metal toxicity is bioavailability to aquatic organisms. Over the past few decades, extensive studies have examined the geochemical controls on the bioavailability of sediment-bound metals to marine benthic invertebrates (e.g. Campbell et al. 1988, Luoma 1989, Bryan & Langston 1992, Ankley 1996, Campbell & Tessier 1996, Chapman et al. 1998, Thomas & Bendell-Young 1998, Lee et al. 2000a).

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Trace metal bioavailability from sediments has been studied in recent years by 2 approaches: (1) the measurement of metal assimilation efficiency (AE) as a first-order physiological parameter, and (2) the extraction of metals by gut juices prepared *in vitro* from deposit-feeding animals. The first parameter quantifies the actual fraction of ingested metal that is incorporated across the gut lining of the animals (Decho & Luoma 1994, Selck et al. 1999, Wang & Fisher 1999, Wang et al. 1999); this is generally quantified using the radiotracer technique. The second parameter quantifies the potentially bioavailable metal within the animal's gut following metal solubilization by gut juice (Mayer et al. 1996, 2001). In this approach, the metal fraction extracted by gut juice collected *in vitro* from deposit-feeding invertebrates represents the maximum level of metal bioavailability from the sediment. Nevertheless, the extent to which the extracted metals are indeed incorporated into the animal's tissues remains to be quantified further.

Many studies have indicated that both, geochemical species and physiological processes, are critical in controlling metal assimilation from sediments (Decho & Luoma 1994, 1996, Griscom et al. 2000, Lee et al. 2000, Fan & Wang 2001). The bioavailability of sediment-bound metals to both suspension- and deposit-feeding invertebrates from contaminated sediments has seldom been studied. In this study, we have used assimilation measurement and gut juice extraction to compare the bioavailability to a marine deposit-feeding sipunculid and a suspension-feeding clam of sediment-bound metals from an extremely contaminated bay. Geochemical measurements of the sediment were concurrently carried out in our study. We examined a suspension-feeding clam, *Macrura veneriformis*, collected from the same contaminated bay, and a deposit-feeding sipunculid, *Sipunculus nudus*, collected from a relatively clean site in Hong Kong. In a previous study, we quantified metal AE by peanut worms feeding on natural sediments of different grain sizes after different durations of sediment radiolabeling (Yan & Wang 2002). We demonstrated that sediment is a dominant source for metal accumulation by the sipunculids (Yan & Wang 2002), and that there was a direct coupling between gut juice extraction of Cd and Cd assimilation. In this study, we extend our previous work and focus on the metal geochemistry of sediments collected from a severely contaminated bay and its controls on metal bioavailability to the sipunculids and clams.

## MATERIALS AND METHODS

**Sediment and animal sampling.** The oxic (surface) and anoxic (5 cm below the surface) sediments were

collected in May 2001 from 4 stations in Bohai Bay in northern China along a gradient of contamination. Samples were collected at low tide using a plastic shovel, immediately sealed in plastic bottles, transported to the laboratory, and stored at  $-80^{\circ}\text{C}$  before radiolabeling and chemical analysis. No organisms were found at 2 stations close to the point source of the metal input, whereas bivalves such as the clam *Macrura veneriformis* and scavenging gastropods were found at the 2 stations with lower metal concentrations. The clams (3 cm in shell length) were collected by hand and transported to the laboratory, where they were maintained in recirculating seawater and fed the diatom *Thalassiosira pseudonana* for about 2 wk before the AE measurements. The sipunculid *Sipunculus nudus* (body length of 5 to 6 cm) was collected from Tin Kowk, Tolo Harbor, Hong Kong, during the same period and held under laboratory conditions in clean (EPD 2001) sediments from the same location for about 2 wk before the AE measurements. Some sipunculids were immediately dissected when they were brought to the laboratory. The worms' guts, containing sediment and the gut juices, were centrifuged in small centrifuge tubes at  $8000 \times g$  for 30 min. The resulting gut juices were immediately stored at  $-80^{\circ}\text{C}$  before the gut juice extraction experiment (within 1 wk after gut juice collection). The gut pH of the sipunculid, measured by a pH microelectrode, was  $7.02 \pm 0.04$ .

**Sediment metal concentration and speciation measurements.** Sediment metal concentrations were determined as described by Langston & Spence (1994). The sediment samples were dried at  $50^{\circ}\text{C}$  for 3 d and sieved through a  $63 \mu\text{m}$  mesh. The sediments were then digested in concentrated  $\text{HNO}_3$  (70%) and  $\text{HClO}_4$  (60%). Sediment standard (SRM 1646A, estuarine sediment) was used in concurrent sediment digestion and measurements. The recovery efficiency of metals after  $\text{HNO}_3$ - $\text{HClO}_4$  digestion was  $>80\%$  compared to the standards. Total metal concentrations of the digested solution were measured using Inductively Coupled Plasma—Atomic Emission Spectrometry (ICP-AES, Perkin Elmer 3300 DV).

Speciation of the metals in the sediment was operationally quantified using a method modified from Tessier et al. (1979), although the limitations of sequential extraction are recognized (Niral & Morel 1990, Bordas & Bourg 1998). Five geochemical fractions were separated in this study: easily exchangeable (after extraction with  $\text{MgCl}_2$ ), carbonate (after extraction with  $\text{HOAc}$ ), reducible (after extraction with  $\text{NH}_2\text{OH} \cdot \text{HCl}$  in 25%  $\text{HOAc}$ ), organic (after extraction with  $\text{HNO}_3$ ,  $\text{H}_2\text{O}_2$  and  $\text{NH}_4\text{OAc}$ ), and finally the residual fraction (after digestion with concentrated  $\text{HNO}_3$  and  $\text{HClO}_4$ ). The extraction was carried out progressively on an initial weight of 1000 mg of sediment. Following each

extraction, the mixtures were centrifuged at  $800 \times g$  for 30 min at room temperature. The metal concentrations of the extracted solution were measured using ICP-AES.

**Radiolabeling of sediments and assimilation efficiency measurements.** Surface oxic sediments (grain size  $<63 \mu\text{m}$ , after sieving) were divided into 2 subsamples; one subsample was radiolabeled with  $^{109}\text{Cd}$ ,  $^{51}\text{Cr(III)}$ , and  $^{65}\text{Zn}$  for 2 wk, and the other for 2 mo. About 1 g of oxic sediments was added to 2 ml of seawater and spiked with radioisotopes (37 kBq for  $^{109}\text{Cd}$ , 74 kBq for  $^{51}\text{Cr(III)}$ , and 74 kBq for  $^{65}\text{Zn}$ ). The sediments were swirled 1 to 2 times each day. The anoxic sediments ( $<63 \mu\text{m}$ , after sieving in anoxic environment) were radiolabeled with radiotracers for 1 mo by placing them in a sealed centrifuge tube maintained within a large chamber that was continuously flushed with  $\text{N}_2$ . After the radiolabeling, the radiolabeled sediments were rinsed and centrifuged twice to remove any unbound radiotracers. The speciation of radiotracers in different geochemical phases was also quantified as described above.

Eight clams were placed in 800 ml of filtered seawater in a beaker. When the clams fully opened their siphons, sediment radiolabeled for 2 wk was added into a food suspension at a particle concentration of about  $2 \text{ mg l}^{-1}$ . The particles were homogenized by a magnetic stirrer. At 10 and 20 min after the start of the radioactive feeding, radiolabeled sediments were added into the feeding suspension again to maintain the particle concentration. After 30 min of feeding, the clams were removed from the feeding beakers, rinsed with filtered seawater, and their radioactivity was immediately measured for 1.5 min, using a Canberra NaI gamma detector. Six individuals from each treatment were each placed in individual chambers of a recirculating closed aquarium (20 l seawater). The 2 remaining individuals from each treatment were dissected to examine the distribution of the radiotracers in the soft tissue and the shells. The clams were allowed to depurate their ingested radiolabeled materials. Non-radioactive food particles (a mixture of diatoms and sediment) were fed to the clams continuously during the depuration period. Any feces produced by the clams were pipetted and counted for their radioactivity. During the 3 d depuration period, the radioactivity remaining in the clams was measured every few hours.

Before the radioactive feeding, the sipunculids were placed on a tray and allowed to defecate for 1 d. When the animals extended their pharynx, the radiolabeled sediments were immediately injected into their mouths using a

pipette, and the animals were then returned to the tray. The animals were allowed to process their injected sediments (i.e. including ejection of unwanted sediment) for 20 to 30 min and were subsequently measured for their radioactivity. For the sipunculids feeding on the radiolabeled anoxic sediments, all radioactive feeding and manipulation of the radiolabeled sediments were performed within an  $\text{N}_2$  bag. Individual worms were then placed in a 1 l beaker with 4 cm of nonradiolabeled oxic sediments on the bottom. No attempt was made to depurate the worms under anoxic conditions in this experiment. The radioactivity remaining in the animals was measured at time intervals of 6 to 24 h for a total of 4 to 6 d. The water was changed and the sediment was replaced during each radioactivity measurement. In these experiments, we found that it was difficult to distinguish and completely recover the sipunculids' fecal pellets from the sediments, and we did not quantitatively analyze the radioactivity in the pellets.

To quantify metal extraction by gut juice action, about 10 mg of radiolabeled sediments was added into a centrifuge tube containing 0.4 ml of gut juices. There were 3 replicates for each sediment treatment. The radioactivity of the radiolabeled sediment was first quantified and the tubes were then placed on a shaking table (145 rpm) at  $18^\circ\text{C}$ . After 4, 8, 12, 18 and 24 h the sediment was centrifuged at  $800 \times g$  for 30 min and then 0.2 ml of the supernatants were immediately measured for their radioactivity. The supernatant was then returned to the sediment.

## RESULTS

### Metal geochemistry in contaminated sediments

Metal concentrations in the 4 sediments collected from the contaminated bay are shown in Table 1. Concentrations were highest in the sediments collected at locations close to the smelting factory and decreased at increasing distances from the factory (e.g. Stn C). There was a marked difference between stations with regard to metal speciation in the sediments. An in-

Table 1. Stable metal concentrations in sediments collected from different sites of a contaminated bay in China

Site	Cd ( $\text{mg kg}^{-1}$ )	Cr ( $\text{mg kg}^{-1}$ )	Zn ( $\text{mg kg}^{-1}$ )	Fe content (%)	Total organic carbon (%)
A	$43.7 \pm 3.10$	$71.2 \pm 2.40$	$5380 \pm 39.2$	3.1	1.74
B	$23.6 \pm 2.60$	$50.1 \pm 2.20$	$1350 \pm 8.20$	2.2	1.45
C	$5.00 \pm 0.60$	$31.6 \pm 2.80$	$251 \pm 38.1$	1.5	0.64
D	$5.40 \pm 0.10$	$32.8 \pm 1.20$	$278 \pm 9.80$	1.6	0.85

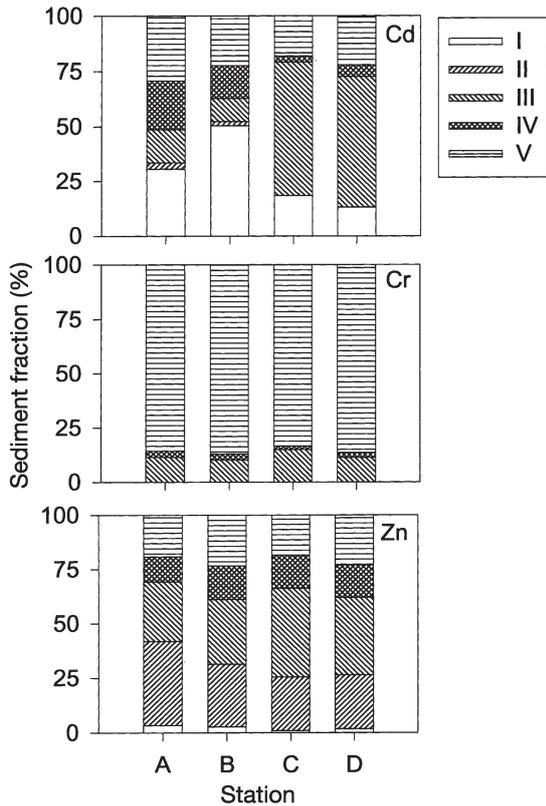


Fig. 1. Speciation of stable Cd, Cr, and Zn in sediments from a contaminated bay. A–D refer to sediment sites in Table 1. I: easily exchangeable phase; II: carbonate phase; III: reducible phase; IV: organic phase; V: residual phase

crease in Cd concentration was coupled with a higher partitioning into the easily exchangeable phases (30 to 50%), and a lower partitioning in the reducible phase (Fig. 1). Most of the Cr (83 to 87%) was associated with the residual fraction irrespective of Cr concentration. For Zn, speciation appeared to be less affected by the concentration. A very small percentage of Zn was found in the exchangeable phase (<3%), despite its exceedingly high concentrations. Partitioning of Zn increased in the carbonate phase and decreased in the reducible phase with increasing Zn concentrations in the sediments.

Table 2. *Macraa veneriformis*. Assimilation efficiency (AE, %) and gut passage time (GPT, h) of Cd, Cr, and Zn in clams after feeding on radiolabeled contaminated sediments. Sediment sites are characterized in Table 1

Loca- tion	Cd		Cr		Zn	
	AE (%)	GPT (h)	AE (%)	GPT (h)	AE (%)	GPT (h)
A	21.8 ± 6.1	4.0 ± 2.6	6.1 ± 2.4	2.6 ± 0.8	25.2 ± 4.1	3.8 ± 1.6
B	21.4 ± 3.4	3.0 ± 1.1	5.2 ± 1.9	2.1 ± 0.8	22.5 ± 2.2	3.3 ± 1.0
C	23.3 ± 4.9	5.2 ± 3.3	5.2 ± 2.9	3.2 ± 1.0	22.0 ± 4.3	3.7 ± 0.7
D	25.4 ± 6.8	3.9 ± 2.2	7.4 ± 2.4	2.4 ± 0.7	22.5 ± 2.6	2.9 ± 0.7

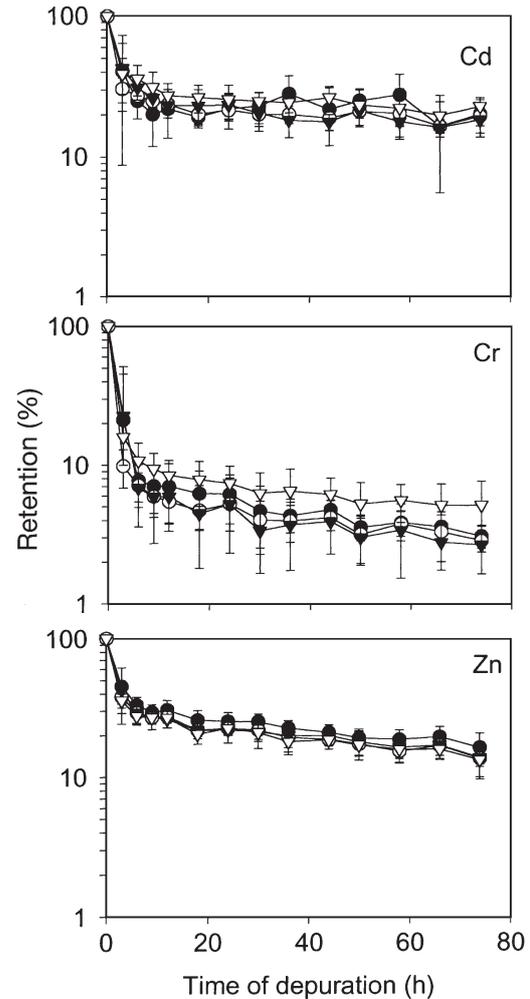


Fig. 2. *Macraa veneriformis*. Retention of ingested Cd, Cr, and Zn after feeding on radiolabeled sediments from a contaminated bay. A–D refer to the sites in Table 1. Mean ± SD (n = 5)

#### Metal assimilation by the clams

Depuration of Cd, Cr, and Zn by the clams is shown in Fig. 2. Depuration of the ingested metals was rapid within the first 12 h, and slower afterwards. Radioactivity measurements indicated that metal egestion in the feces was negligible after 24 h of depuration (data not shown). Over 90% of the loss of unassimilated metals was recovered in the feces during the depuration period. Assimilation and digestion of ingested metals were thus assumed to be complete within 24 h and the AE was calculated as the fraction of ingested metals retained in the clams after 24 h of depuration (Table 2). In general, there was no significant difference in AE between

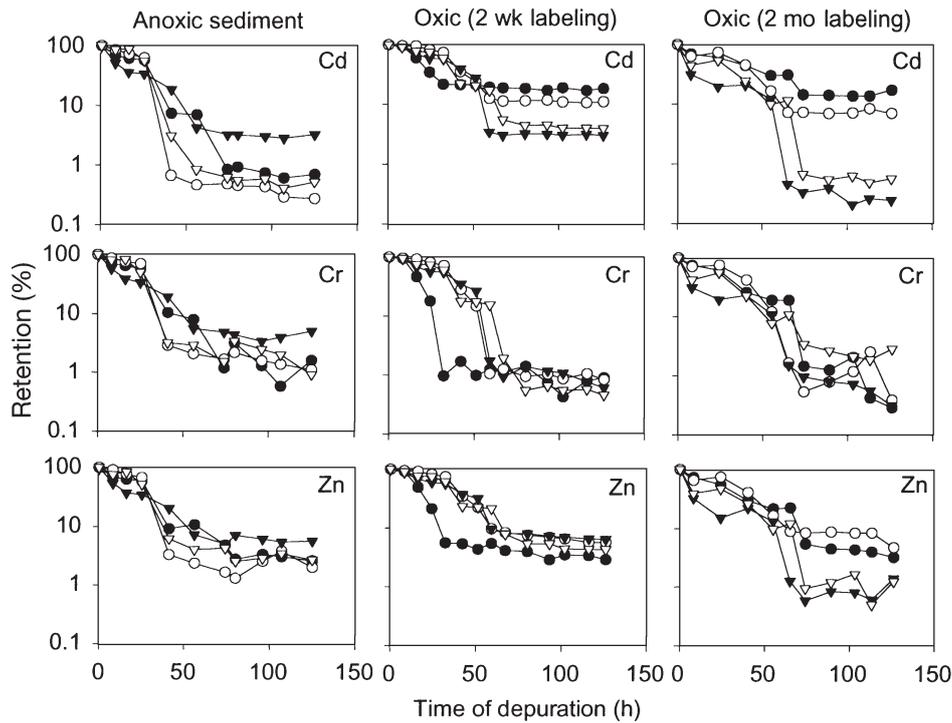


Fig. 3. *Sipunculus nudus*. Retention of ingested Cd, Cr, and Zn after feeding on radiolabeled anoxic and oxic sediments from a contaminated bay. Oxic sediments were radiolabeled for 2 wk and for 2 mo. A–D refer to the sites in Table 1. Mean  $\pm$  SD (n = 6)

sediments. The AEs of Cd and Zn were comparable, whereas Cr was barely assimilated by the clams ( $\leq 7.4\%$ ). The gut passage time (GPT) of the metals, defined as the time at which 90% of unassimilated metals were egested via the feces (based on the assumption that 100% of unassimilated metal was eliminated in 24 h), was rather short, ranging from 2.1 to 6.8 h (Table 2). There was no significant correlation between AE and GPT.

#### Metal assimilation by the sipunculids

The sipunculids retained the ingested metals for 1 d before a notable egestion of unassimilated metals occurred (Fig. 3). Feces were not collected for radioactivity measurements, so that the precise time at which digestion was completed was not determined from the depuration pattern. In general, depuration appeared to level off after 72 h. We thus operationally defined the metal AE as the percentage of metals retained in the sipunculids at 72 h of depuration (Fig. 4). Among the oxic sediments, there was a significant difference between sites in metal AE for Cd ( $p < 0.05$ , 1-way ANOVA), whereas no significant difference was found for Cr and Zn. Cd associated with the oxic sediments was assimilated with greater efficiency from strongly contaminated oxic sediments (7 to 19%) than from weakly contaminated oxic sediment (0.3 to 4%), whereas Cd from anoxic sediment was assimilated by the sipunculids at comparably low efficiency in the

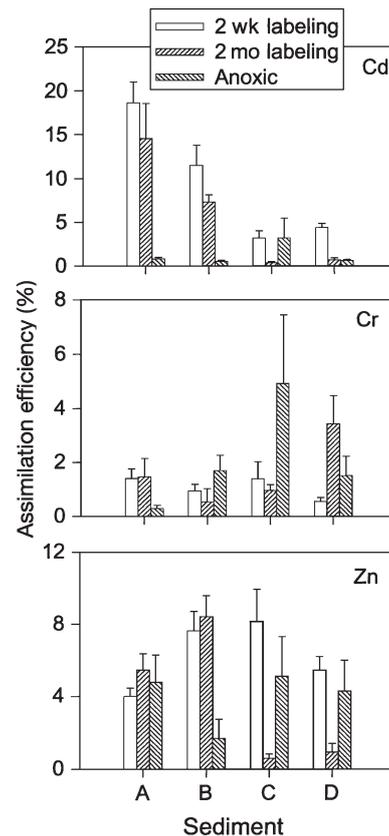


Fig. 4. *Sipunculus nudus*. Metal assimilation efficiency after feeding on radiolabeled sediments from a contaminated bay. Oxic sediments were radiolabeled for 2 wk and for 2 mo. A–D refer to sediment sites in Table 1. Mean  $\pm$  SD (n = 6)

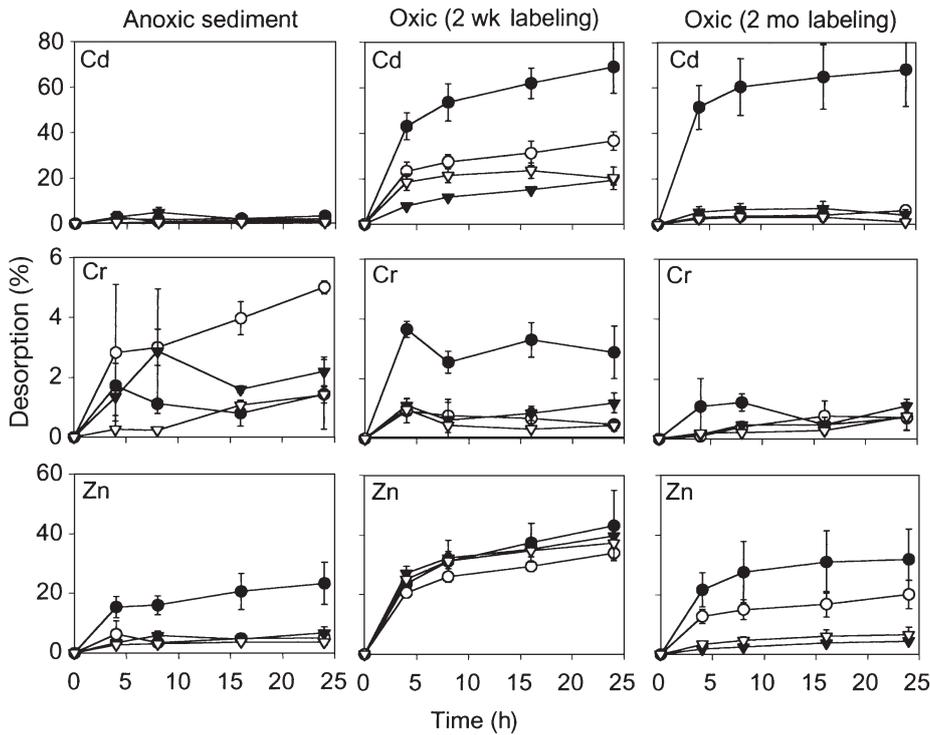


Fig. 5. *In vitro* extraction by gut juices from *Sipunculus nudus* of Cd, Cr, and Zn bound to radiolabeled sediments from a contaminated bay. Oxidic sediments were radiolabeled for 2 wk and for 2 mo. A–D refer to sediment sites in Table 1. Mean  $\pm$  SD (n = 3)

case of all 4 sediments; at the 2 highest Cd concentrations, AEs from anoxic sediment were significantly lower than from oxidic sediment ( $p < 0.01$ ,  $t$ -test). The AE of Cd generally decreased with increasing radiolabeling time, although the difference was not statistically significant. For Cr, there was no statistically significant difference in AEs among the 4 sediments ( $p > 0.05$ , 1-way ANOVA), and the AE were generally low (0.3 to 5%). The AEs of Zn were also low (0.6 to 8%), and there was no major difference among sediments, except for the sediments radiolabeled for 2 mo, in which Zn was assimilated at a higher efficiency at the 2 highest Zn contaminations.

The radiolabeled sediments were extracted by gut juices prepared *in vitro* from the sipunculids (gut pH was nearly neutral, 7.02). The desorption of radiolabeled metals from different sediments is shown in Fig. 5. Cr was the least extractable metal, with  $< 5\%$  desorption within 24 h. Cd and Zn from anoxic sediment were also barely desorbed. Gut juice extraction of Cd and Zn increased with increasing sediment contamination in the oxidic sediment. Metals were rapidly extracted within the first 3 h of contact with the gut juice, after which desorption became slower, and appeared to attain a steady-state after 24 h. When the 3 radiolabeling experiments were considered together, the AE for Cd was significantly correlated with its extraction by gut juice, whereas no such relationship was evident for Cr and Zn (Fig. 6). When the 3 metals were further considered together, there was a signifi-

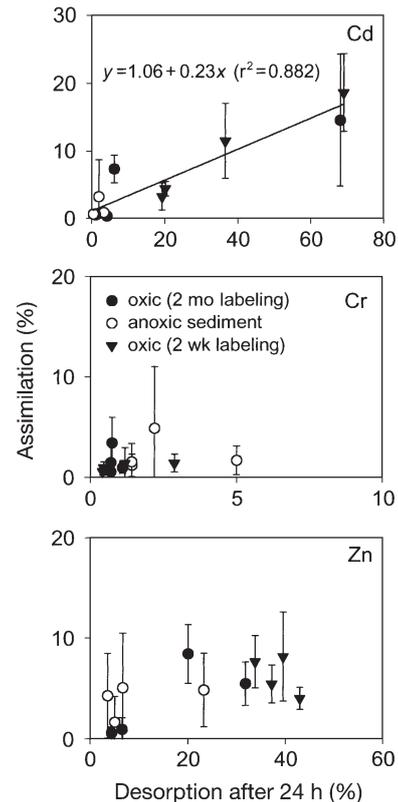


Fig. 6. *Sipunculus nudus*. Relationship between metal assimilation efficiency (mean  $\pm$  SD, n = 6) and *in vitro* metal extraction by gut juices. Oxidic sediments were radiolabeled for 2 wk and for 2 mo

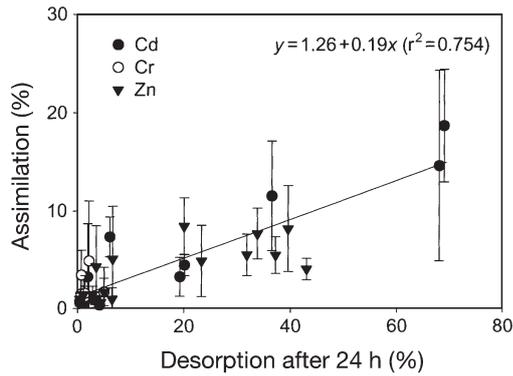


Fig. 7. *Sipunculus nudus*. Relationship between metal assimilation efficiency (mean  $\pm$  SD,  $n = 6$ ) and *in vitro* metal extraction by gut juices based on pooled data for Cd, Cr, and Zn

cant correlation between the metal AE and the metal extraction (Fig. 7), indicating that the difference in metal AE among the 3 metals was probably partly due to their differential extraction by gut juices.

The partitioning of radiotracers in different geochemical species of sediments over the 2 wk and the 2 mo radiolabeling period is indicated in Fig. 8. In

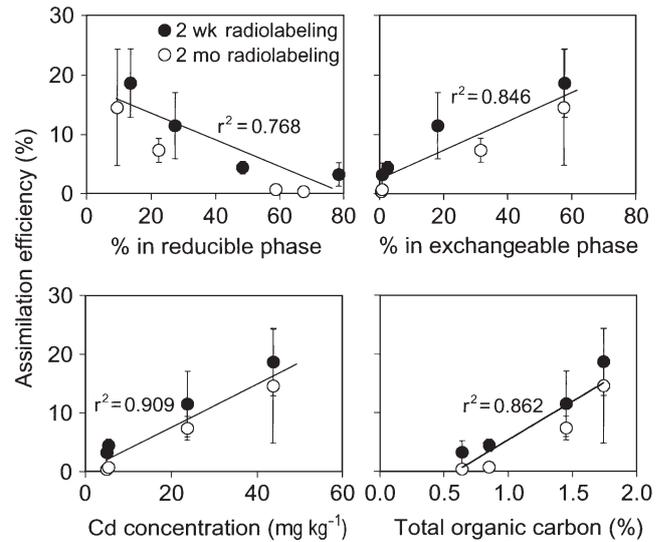


Fig. 9. *Sipunculus nudus*. Relationship between the  $^{109}\text{Cd}$  assimilation efficiency (mean  $\pm$  SD,  $n = 6$ ), and Cd partitioning into the reducible and the easily exchangeable phases (top left and right, respectively) and the Cd concentrations and organic carbon content in sediment (bottom left and right)

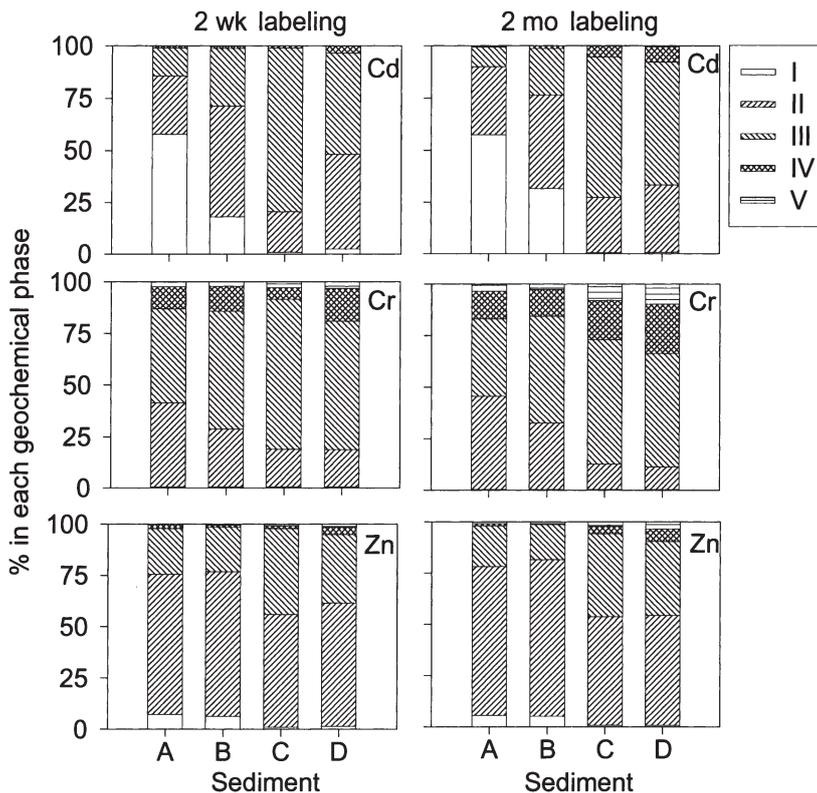


Fig. 8. Speciation of radiotracer  $^{109}\text{Cd}$ ,  $^{51}\text{Cr}$ , and  $^{65}\text{Zn}$  in oxic sediments collected from the contaminated bay after 2 wk and 2 mo radiolabeling. A–D refer to sediment sites in Table 1. I: easily exchangeable phase; II: carbonate phase; III: reducible phase; IV: organic phase; V: residual phase

general, very little of the radioisotopes was partitioned into the residual phase within the 2 mo radiolabeling. Cd partitioning was considerably influenced by Cd concentrations in sediments. A much higher fraction of Cd was found in the easily exchangeable phase at the 2 highest Cd concentrations. Most of the Cr and Zn was distributed in the carbonate and reducible phases. Partitioning of these 2 metals in the carbonate phase was also higher at the 2 higher metal concentrations. Metal AEs were further correlated with the partitioning of radiotracers in the different geochemical phases of the sediment (Fig. 9). There was a significant positive correlation between the AE of Cd and the Cd partitioning into the easily exchangeable phase, and a negative correlation between the AE of Cd and the Cd partitioning into the reducible phase (Fig. 9). Thus, these 2 geochemical species were critical in affecting Cd bioavailability from contaminated sediment. A significant positive relationship between the Cd AE and the Cd concentrations or the total organic carbon contents in the sediments was also found (Fig. 9). There was no significant correlation between the AEs and the partitioning of Cr or Zn in different geochemical fractions or their concentrations in sediment.

## DISCUSSION

The study area was seriously contaminated with Cd and Zn due to the unregulated input of industrial wastes. Cr contamination appeared to be of less concern in this bay area, and its concentration was somewhat comparable to concentrations measured in other regions (e.g. Hong Kong, Lam et al. 1997). At the 'cleanest' sites (Stns C and D), Cd and Zn concentrations were still 30× and 5× higher, respectively, than the concentrations quantified in pristine coastal sediments (Bryan & Langston 1992). Among the 4 sediments, a much larger fraction of Cd and Zn was partitioned in the reducible phase at lower concentrations, thus reducible oxides appeared to be the main binding site for these metals. Partitioning in the easily exchangeable and carbonate phases increased with increasing Cd and Zn concentrations in the sediments, indicating that anthropogenic input contributed to the sediment contamination (due to the relatively short history of exposure). In one of the major Chinese estuaries (the Pearl River Estuary), Li et al. (2001) recently found that the residual fraction was the most important phase for Zn, followed by the reducible fraction. Lam et al. (1997) indicated that the speciation of Cr in the sediments collected from Hong Kong coasts showed a distinct spatial pattern as a result of differences in the sediment sulfide content.

Our study demonstrated that there was a marked difference in assimilation among the different metals by benthic invertebrates in response to sediment contamination. Both Cd and Zn were appreciably assimilated by the clam *Mactra veneriformis*. There was no clear difference in the measured AE and the metal gut passage time among the 4 different types of sediments, indicating that metal concentrations in these sediments have little influence on metal digestion and assimilation by the clams. In contrast, we have recently demonstrated that a much higher Cd concentration had a significant influence on Cd assimilation by the mussel *Perna viridis* and the clam *Ruditapes philippinarum* collected from the clean areas in Hong Kong (Fan et al. unpubl.). For example, when these animals ingested sediments containing 400 mg kg<sup>-1</sup> Cd and 9300 mg kg<sup>-1</sup> Zn, the Cd AE was as low as 1%. We think that the low AE at these extraordinarily high Cd concentrations is due to the saturation of the binding sites within the digestive diverticula and the competitive interaction with a very high Zn concentration associated with these sediments. The insensitivity of the clams *M. veneriformis* to contaminated sediments may also be caused by their acclimation to the metal contamination in the bay (the Cd concentration in the sediment where the clams were collected was as high as 5 mg kg<sup>-1</sup>). In fact, it was rather remarkable that these

clams were able to grow in such a contaminated area, as no organisms were found at the 2 most contaminated stations (Stns A and B).

A few studies have examined the assimilation of sediment-bound metals by marine bivalves (Decho & Luoma 1994, Gagnon & Fisher 1997, Chong & Wang 2000, Griscom et al. 2000, Lee et al. 2000a, Fan & Wang 2001). Various factors affecting metal assimilation from sediments have been considered in these previous studies, including sediment type, metal concentration in sediment, metal speciation, metal-metal interaction, and metal desorption. Assimilation of sediment-bound metals by bivalve species was generally lower than the assimilation from phytoplankton. The quantified metal AEs in *Mactra veneriformis* were similar to those measured in other clam species (*Macoma balthica*: Decho & Luoma 1994, Griscom et al. 2000; *Ruditapes philippinarum*: Chong & Wang 2000, Fan & Wang 2001). Generally, the assimilation of metals such as Cd and Zn is higher in clams than in mussels (Reinfelder et al. 1997, Chong & Wang 2000). In our study, metals were rapidly processed by the clams, with a typical gut passage time <5 h. Such a short gut passage time may also partially account for the insensitivity of metal AEs to the different degrees of sediment contamination. Difference in metal gut passage has been implied to partially account for the variability of metal assimilation from different food particles (Wang & Fisher 1996a).

In our study, ingested metals were retained in the gut of deposit-feeding *Sipunculus nudus* for about 1 d before a significant egestion occurred, whereas the first radioactive bolus in the clams occurred within the first 2 h of gut evacuation and a complete assimilation of metals occurred after 24 h of depuration. One reason for the longer retention of the ingested metals and sediments in the peanut worm is the large body size of the animals as well as the 'peculiarity' of its digestive system. The anus and the mouth are on the same side of the body, and the gut length is at least 1.3× longer than the whole body length. Because of the longer retention of metals and sediment within the gut, we generally observed a large individual variability of the metal retention in the sipunculids. The calculated standard deviation was even greater than the mean AE in some treatments. A comparison of the metal digestive strategy between the clam and worm may be of limited value because of the different functional physiologies of these 2 species. Furthermore, the clams were collected from the same sites where the contaminated sediments were collected, whereas the worms were collected from the clean sites. Whether there is any physiological and biochemical adaptation for the clams to handle the metal contamination remains to be studied further.

The AEs for Cd, Cr, and Zn in the sipunculids were similar or lower than in our previous measurements

using relatively clean sediments (Yan & Wang 2002). The AEs were 7 to 21%, 0.5 to 7%, and 6 to 8% for Cd, Cr, and Zn, respectively, in sipunculids fed clean sediments of different sizes and durations of radiolabeling. When the animals fed on contaminated sediments (Cd: 2.6 to 9.0 mg kg<sup>-1</sup>; Zn: 180 to 428 mg kg<sup>-1</sup>), the AEs of Cd and Zn were 21 to 30% and 7 to 15%, respectively (Yan & Wang 2002). Similar to the finding in our previous study, we did not find a statistically significant influence of the radiolabeling duration (2 wk vs 2 mo) on the metal assimilation by the sipunculids, although the AEs determined after 2 mo of radiolabeling were generally lower than those determined after 2 wk of radiolabeling.

Cd associated with the anoxic sediments was assimilated at a much lower efficiency than Cd associated with the oxic sediments, whereas there was no apparent difference between the oxic and anoxic sediments for Cr and Zn. The anoxic sediment was radiolabeled with radiotracers for 1 mo before the AE measurement, and it was assumed that the radiotracers may have bound with the sulfide fraction of the sediment, although this was not verified in this study. Among the 3 metals, Cd has a higher binding affinity for sulfide than does Zn, whereas the affinity of Cr binding with sulfide in the sediment remains unknown. Generally, several metals bound with anoxic sediments or sulfide phase were assumed to have a low bioavailability to benthic invertebrates due to their strong affinity with the sulfide ligands (Ankley 1996, Wang et al. 1999). In a recent study in which we artificially prepared the acid volatile sulfide (AVS), we found that the Cd AE by the clam *Ruditapes philippinarum* was significantly reduced when bound with sulfide, whereas the Cr AE was not affected by the sulfide or was even higher due to the rapid remobilization from the AVS particles (Fan & Wang 2001). The bioavailability of metals bound with the anoxic sediment to the benthic invertebrates differs between metals and animal species and depends on the affinity of these metals in binding with the sulfide. Lee et al. (2000a) speculated that the re-oxidation of metal-sulfide complex within the invertebrate gut can lead to an even higher assimilation from the AVS particles. In another study, Lee et al. (2000b) demonstrated a significant relationship between the metal accumulation in polychaetes and clams, and the simultaneously extractable metal concentration in the sediment (SEM), but there was no relationship between the metal accumulation and the [AVS-SEM].

Our study demonstrates that sedimentary geochemical speciation greatly affects Cd assimilation by the deposit-feeding sipunculids. Higher bioavailability of Cd with increasing partitioning in the easily exchangeable phase indicates that Cd assimilation is probably controlled by desorption within the animal gut. Consis-

tent with findings in other studies (Tessier et al. 1984, 1993, Bryan & Langston 1992, Thomas & Bendell-Young 1998), binding with the reducible phase inhibits Cd bioavailability to the animals, likely caused by its competitive binding with Fe for ligands available in the gut. The influence of sediment geochemistry on Cr and Zn assimilation by the sipunculids remains unclear. We did not observe any significant correlation between the AEs of Cr and Zn and their speciation in sediments. Because most Cr was bound with the geochemically inert phase (residual fraction), which was difficult to extract by gut juice, any geochemical control is difficult to distinguish. Furthermore, the much lower AE of Cr in the sipunculids results in a great difficulty in studying the geochemical controls on Cr bioavailability. Presumably, redox chemistry [Cr(III) and Cr(VI)] in the sediment plays a far more important role in controlling Cr bioavailability to benthic invertebrates. For Zn, the AEs were also relatively low (<8%) and it was thus difficult to differentiate the sediment geochemical controls on its assimilation. For these 2 metals, the physiological influence may also overshadow the influence of the sediment geochemistry. The interaction between metal geochemistry and animal physiology thus determine the differences in the bioavailability among the toxic metals. In a recent study, Thomas & Bendell-Young (1998) indicated that sediment geochemistry accounted for 31% of the variability of Cd and 39% of the variability of Zn concentrations in the clam *Macoma balthica*.

The higher AE in sipunculids with increasing Cd concentration in the sediment was at least due to its greater partitioning in the easily exchangeable phase and the smaller partitioning in the reducible phase. Because of the dependence of Cd AE on its concentration in the sediment, the uptake from the dietary exposure pathway will increase disproportionately with increasing Cd concentration, leading to a higher Cd dose and body burden in the animals. Several recent studies also demonstrated that Cd assimilation in marine bivalves increased with increasing Cd concentrations in ingested food (Wang & Fisher 1996b, Chong & Wang 2000). One mechanism underlying the greater bioavailability with increasing Cd concentration is the greater mobility of Cd because more Cd is associated with the easily exchangeable and carbonate phases. However, acidity-induced desorption within the gut was presumably insignificant given the neutral gut pH in the sipunculids. Although a significant correlation between the Cd AE and the sediment TOC was observed in this study, it cannot be concluded that TOC was the sole factor that affected Cd assimilation because TOC also co-varied with the metal concentrations in these contaminated sediments. Previously, Wang et al. (1999) found that the TOC in sediment

did not significantly affect the metal assimilation in a deposit-feeding polychaete, *Nereis succinea*.

Comparison of metal AE and metal extraction by gut juice suggested that the difference in AEs among Cd, Cr, and Zn may be partially caused by the difference in their extraction. Our study attempted to compare the validity of the 2 methods (AE and gut juice extraction measurements) in assessing metal bioavailability from contaminated sediments. The chemical components in the gut may vary with different locations, seasons or physiological conditions such as feeding rates. This was not quantified in our study, and it is unknown which particular molecular compounds (e.g. digestive enzymes, surfactants, or simply the gut liquid) are responsible for the metal extraction. Chen et al. (2000) demonstrated a strong correlation of Cu and Cd concentrations and the total acid-hydrolyzed amino acids (TAHAA) in gut fluids in a cross-phyletic survey of 35 deposit feeders, suggesting that the dissolved organic material, especially proteinaceous materials, may be critical in binding metals.

Cr was the least extractable metal and its AE was consistently the lowest among the 3 metals studied. For Cd, it is possible to use the gut juice extraction to compare the potential bioavailabilities from different types of marine sediments. Comparisons of metal bioavailability for Cr and Zn among the different types of sediment using gut juice extraction may be of limited value because there is no significant correlation between their AEs and their extraction. In our previous study, we also found that the AE measurement and the gut juice extraction appear to couple only for Cd, whereas there is no relationship between the AE and the gut juice extraction for Cr or Zn (Yan & Wang 2002). In addition, the much lower extraction of metals bound with anoxic sediments by gut juices is consistent with the finding for Cu by Chen & Mayer (1999). Extensive studies have demonstrated the significant correlations between metal concentrations in benthic invertebrates (mainly clams and polychaetes) and metal concentrations in sediments measured by weak acid extraction and corrected for Fe oxide or organic matter content (Luoma & Bryan 1978, Bryan & Langston 1992, Luoma & Fisher 1997). In fact, the correlation between Cd AE and Cd fraction in the easily exchangeable phase was also highly significant in our study. Such a strong relationship, however, does not necessarily guarantee a significant relationship between metal concentrations in the animals and metal distribution in the easily extractable fraction (as shown in many field studies), because metal influx is a function of metal AE, metal concentration in the sediment, as well as ingestion rate of the animals. The last parameter has to be considered when interpreting the relationship between metal concentration in animals and

metal concentration in sediment across a wide geographical area.

In our study, the sediments were radiolabeled with the radiotracers for 2 wk to 2 mo before the AE experiments. The speciation of these radiotracers in the 4 sediment types was, however, in marked contrast with the stable metal speciation when quantified by the Tessier et al. (1979) extraction method. In general, radiotracers were partitioned more into the easily exchangeable and carbonate phases, with little distribution into the residual phase, whereas a significant fraction of the stable metals was distributed in the residual phase (especially in the case of Cr). It is unknown whether the radiotracers would be in full equilibrium with the stable metals if they were radiolabeled for an extended period of time. Extrapolation of the metal AE determined by the radiotracer technique to the field conditions should therefore be treated with caution. It is thus necessary to only consider metals in the labile phases in predicting metal concentration in deposit-feeding invertebrates when using the radiotracer technique. This can be a particular problem for metals such as Cr that are mostly associated with the residual phase. When the total metal concentrations (e.g. quantified by total acid digestion) are considered, the predicted metal concentrations in the animals may be overestimated (Wang et al. 1997). Thus, many studies have demonstrated that the total metal concentration in the sediment is not a useful predictor of metal concentrations in the animals, whereas metals in the extractable fraction are a much better predictor of metal concentration, especially when calibrated with the Fe or organic matter content (Bryan & Langston 1992, Luoma & Fisher 1997, Lee et al. 2000b, Stecko & Bendell-Young 2000). Stecko & Bendell-Young (2000) further cautioned that the metal speciation achieved in laboratory conditions using radiotracer labeling technique should be applicable to that observed in the field in studying the role of complex sediment geochemistry in influencing metal bioavailability to sediment-ingesting animals.

Kinetic modeling using the experimentally quantified AE indicates that sediment ingestion can represent a dominant source for metal accumulation by marine deposit-feeding animals, at least for those surface deposit-feeders that feed primarily on the oxic sediment (Selek et al. 1998, Wang et al. 1999). Marine deposit-feeders are known to ingest a large amount of sediments in order to meet their nutritional requirements (e.g. about 2 times their tissue body weight each day; Cammen 1980). Such a high ingestion rate, coupled with a high metal concentration in sediments, exceeds the metal uptake from the aqueous phase (porewater and overlying water) and contributes to the dominance of sediment as a direct source for metal

accumulation in these deposit-feeding animals. Environmental regulation of contaminant loading in sediments should thus directly consider sediments as an important source for metal uptake and accumulation.

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