EXTRACTION OF SPIKED METALS FROM CONTAMINATED COASTAL SEDIMENTS: A COMPARISON OF DIFFERENT METHODS

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(Received 23 September 2002; Accepted 21 March 2003)

Abstract—Various extraction methods have been developed to assess metal bioavailability from sediments. In this study, we compared the extraction of Cd, Cr, and Zn from contaminated sediments using different extractants (normal seawater, acidic seawater of pH = 5, seawater with 1% sodium dodecyl sulfate [SDS], and gut digestive fluids collected in vitro from deposit-feeding peanut worm Sipunculus nudus) coupled with concurrent metal speciation measurements. The influences of sediment aging on metal extraction were also examined using radiotracer-spiked techniques. Sediments aging up to 100 d did not significantly affect the partitioning of spiked Cd and Cr in different geochemical phases, but the spiked Zn was partitioned more into the reducible fraction and less into the carbonate phase with increasing sediment aging. There was a major difference in the partitioning into different geochemical phases between the spiked metals and the native metals within the 100-d sediment aging. The difference between the spiked and native Cd and Zn extraction using gut juices was somewhat smaller than the strong geochemical contrast. Metals bound with the anoxic sediments were hardly extracted by different extractants. There was a significant relationship between the extraction of spiked Cd and its distribution in the exchangeable phase (positive correlation) or in the reducible phase (negative correlation). For Cr and Zn, extraction was not correlated with their partitioning in any of the geochemical phases. Further, extraction of all three metals by digestive gut fluids was not correlated with the concentrations of simultaneously extractable metals (SEM), nor with the difference between SEM and acid volatile sulfide (AVS). Our study suggests that there were large differences in extraction among metals using different extractants and only Cd extraction was significantly related to its geochemical speciation in sediments.

Keywords—Metals Digestive fluid extraction Sediment aging Bioavailability

INTRODUCTION

Coastal sediments are often the sinks of metal contaminants resulting from anthropogenic activity in aquatic systems, but they are a potential source for metal accumulation in marine benthic invertebrates [1–5]. Considering the persistence of metals in the sediments, there is a substantial need to assess the bioavailability of metals from these contaminated sediments to benthic invertebrates even though stricter regulation in recent years has markedly reduced the amount of metals released into the coastal environments. Numerous studies have attempted to quantify and predict trace-metal bioavailability from pristine or contaminated sediments to benthic animals [1,6–8], but such attempts are often impeded by the complicated metal geochemistry in sediments. Such complexity can be further confounded by animal physiology (e.g., particle ingestion and selectivity, digestive plasticity, gut passage time) that can considerably influence the intake of metals from these sediments [3,9].

Whereas classical bioaccumulation studies quantifying metal concentrations in exposed animals can be used to examine the biological availability of sediment-bound metals to benthic invertebrates [10–12], a simpler and direct approach is to quantify the bioavailable fraction of metals from bulk sediments. Currently, two approaches are employed to quantify this bioavailable fraction, including the direct assimilation efficiency measurements [13,14] and the gut-juice extraction method developed by Mayer et al. [15]. The first approach quantifies the fraction directly assimilated across the gut linings of animals and the latter approach quantifies the bulk desorbed fraction resulted from digestive gut-fluid attack. Both approaches have received increasing attention in recent years in the study of trace-metal bioavailability from sediments [3,16–18]. Recent efforts using the digestive gut fluid of peanut worm Sipunculus nudus demonstrated a significant correlation between these two methods for labile metals such as Cd, whereas for other more particle-reactive metals like Zn, the fraction of metals assimilated across the gut linings is often unrelated to the bulk fraction desorbed within guts of animals due to action of the gut juice [4,19].

Assimilation efficiency measurement, however, is often constrained by the use of radiotracer-spiked techniques. The radiotracer technique has been extensively used to measure metal assimilation in marine herbivores from ingested phytoplankton due to the relative ease in the equilibration between radiotracers and unicellular algae. It should be recognized, however, that the application of radiotracers is based on the assumption that radioisotopes behave exactly the same way as stable metals. For some inorganic particles, particularly particles composed of diverse geochemical components, such as sediments, application of the spiked technique may be limited. Once in contact with the sediments, radioisotopes typically display a multiphasic sorption pattern, involving a rapid adsorption onto the particle surfaces, followed by slower binding with the sediment matrix [20]. Such a slow equilibrium presents a great obstacle to the use of radiotracer-spiked techniques for realistic quantification of metal assimilation from natural sediments. Several recent studies have in fact cautioned against the extrapolation of laboratory-determined metal assimilation to field situations [4,21], although this technique can still be powerful for diagnosing the geochemical and physi-
iological controls on metal bioavailability from sediment. Thus, sediment aging may significantly affect metal assimilation efficiency (AE) in marine bivalves ingesting sediments [16,22]. Given such constraints, there exist few reports on the differences in metal partitioning in different geochemical fractions of sediments between native metals and spiked metals as a function of sediment aging.

In this study, we compared the different extraction methods using the biomimetic approach developed by Mayer et al. [15], by incubating sediments with gut digestive fluids collected in vitro from the peanut worm *S. nudus*, normal seawater, acidic seawater (pH = 5), and surfactants. This approach has been increasingly attractive to researchers assessing pollutant bioavailability from contaminated sediments [17,23,24]. We further examined the change of metal speciation as a function of sediment aging. Extraction was also performed for sediments with different ratios of acid volatile sulfide and simultaneously extractable metals, which have been shown to be critical in controlling metal bioavailability from contaminated sediments [10,25].

**MATERIALS AND METHODS**

*Gut-juice collection and measurement*

Sipunculans (*S. nudus*) were collected from Ting Kok, Hong Kong, at low tide. Gut digestive fluids were extracted as described in Mayer et al. [15]. During the dissection, the body walls of the animals were cut open with care to avoid rupturing the guts. The animal guts were excised and opened, and the contents were removed into a centrifuge tube. Sediment particles were then removed from gut juices by centrifugation at 800 g for 30 min. The juices were frozen at −80°C and were used for metal extraction within one week of storage. Amino acid concentrations in the gut juices were measured using a HITACHI 835-50 amino acid analyzer (Tokyo, Japan). Metal concentration in the juices was measured using an inductively coupled plasma-mass spectroscopy (ICP-MS; Perkin-Elmer, Elan 6000, Norwalk, CT, USA).

*Sediment collection and measurements*

Contaminated natural sediments were collected from two stations in Bohai Bay off the east coast of China using a plastic grab and were then placed into polyethylene bottles and stored. Sample A was collected 40 km from a metal smelting factory and sample B was collected 10 km from the same factory. The oxic sediments were collected from the surface, whereas the anoxic sediments were collected from a depth below 5 cm from the surface and immediately sealed in a plastic bag. In the laboratory, the oxic sediments were wet sieved (63 μm), resuspended in 0.2-μm filtered seawater, and stored at −4°C before use. Sediment total organic carbon content (<63 μm) was measured using a CHNSO 2400 element analyzer (Perkin-Elmer, Shelton, CT, USA). Loss on ignition was measured after ashing the sediment at 500°C for 4 h. Total metal concentration in the sediment was measured using an established method [26]. Briefly, the sediments were first dried at 50°C for 3 d. A fraction of sediment (0.250 g dried sediment) was then added into a glass tube containing 4 ml of concentrated ultrapure HNO₃ (70%) and 1 ml concentrated HClO₄ (60%). The glass tubes were digested in an aluminum block using a sequential heating procedure of 50°C for 3 h, 70°C for 0.5 h, 100°C for 0.5 h, 150°C for 3 h, and finally 190°C until complete dryness. After cooling, 10 ml of HNO₃ (2%) was added into the glass tubes, which were then placed in the heating block at 70°C for 1 h. The digested dissolved solution was placed into a centrifuged tube, centrifuged at 800 g for 30 min, and the metal concentrations measured using ICP-MS.

The metal speciation of sediment was determined using the Tessier et al. [27] sequential extraction procedure, still the best available method to operationally quantify the partitioning of metals in different geochemical fractions [28]. Details of the experimental conditions are given in Fan et al. [26]. Following each extraction, the mixtures were centrifuged at 800 g for 30 min at room temperature. To the aliquots of the extracted solution from each step, 1 ml of HNO₃ (70%) was added. The solution was heated to dryness on an aluminum heating block at 140°C [29]. The remaining material was then digested in 10 ml of HNO₃ (2%) at 70°C for 1 h. Metal concentrations of the extracted fractions were finally quantified using ICP-MS.

**Metal extraction of sediments by four extractants**

The radioisotopes ¹⁰⁹Cd (in 0.1 N HCl), ⁵¹Cr(III) (in 0.1 N HCl), and ⁶⁵Zn (in 0.1 N HCl) were obtained from New England Nuclear (Boston, MA, USA). The three isotopes were studied in a mixture. The oxic and anoxic sediments from two sites were radiolabeled for 7, 20, 50, and 100 d, respectively. Radioisotope additions were 17.5 kBq for all three radioisotopes. Radiolabeling was conducted at different times such that metal extraction was conducted using the same batch of extractants at the same time. The anoxic sediments were radiolabeled in a sealed plastic tube placed within a chamber that was continuously flushed with N₂. All operations were performed in an N₂ glove bag. Experiments were then performed to quantify metal extraction from radiolabeled oxic and anoxic sediments by four extractants, i.e., sipunculan gut juices, normal seawater, seawater adjusted to pH = 5.0 using hydrochloric acid (hereafter referred to as pH = 5 seawater), seawater with 1% synthetic surfactant sodium dodecyl sulfate (SDS) added to it (hereafter referred to as 1% SDS seawater) [30]. Approximately 10 mg of radiolabeled sediments were centrifuged twice at 800 g for 30 min at 4°C, and the supernatant was discarded. Four hundred microliters of extractant were added into the radiolabeled sediments and the mixtures were then incubated at 16°C on a shaker (125 rpm). After 4, 8, and 12 h, the sediment mixture was centrifuged and 200 μl of supernatants were removed and quantified for radioactivity. The percentage of the radiotracers released into the extractant was then calculated. The supernatants were then returned to the sediments. Each experimental treatment (i.e., sediment aged for four different periods and extracted with four different extractants) had two replicates.

Experiments were also conducted to quantify the release of native metals from the oxic and anoxic natural sediments by gut-juice action; again, two replicates were done for each treatment. Approximately 10 mg of natural contaminated sediments were centrifuged twice at 800 g for 30 min, and the supernatant was discarded. About 400 μl of gut juices were added into the sediment. The mixtures were then incubated at 16°C for 4 h on a shaker; afterwards, 200 μl of gut juices were removed and the metal concentration was measured by the ICP-MS.

**Metal extraction from sediments with different concentrations of acid volatile sulfide and simultaneously extractable metals**

Oxic and anoxic radiolabeled sediments were mixed at different ratios to produce different acid volatile sulfide (AVS)
and simultaneously extractable metals (SEM) concentrations, which were quantified as described in Allen et al. [31]. All transferring procedures were performed in an N₂ bag to avoid oxidation of the sediment. Briefly, 1 to 2 g (wet wt) of sediment was added into a sealed reaction flask containing 100 ml deoxygenated distilled water that had been previously purged with N₂ for 0.5 h, followed by a slow addition of 20 ml of 6 M cold deoxygenated HCl. The system was bubbled with N₂ for 40 min. H₂S released from the sediment was then trapped in a 0.5 M NaOH solution and quantified by the colorimetric method. The mixture in the reaction flask was filtered through a 0.2-μm membrane, and metal concentrations were measured by ICP-MS. The total SEM concentration was the sum of the molar concentrations of five metals (Cd, Cu, Pb, Ni, and Zn). The extractions of Cd, Cr, and Zn by the gut juices (4 h) were then performed as described above.

Radioactivity measurement

Radioactivity was measured with a Wallac 1480 NaI (TI) gamma detector (Turku, Finland). All measurements were appropriately standardized and calibrated with spillover. The gamma emission of ¹⁰⁹Cd was determined at 88 keV, of ⁵¹Cr at 264 keV, and of ⁶⁵Zn at 1115 keV. Counting times were adjusted to yield a propagated counting error of <5%.

RESULTS

Metal concentrations and speciation in contaminated sediments and gut juices

Geochemical measurements of the contaminated sediments are shown in Table 1. Both sediment samples (A and B) were highly contaminated with Cd and Zn, but less so with Cr. Metal and amino acid concentrations in the digestive gut fluids of S. nudus are shown in Table 2. Concentrations of trace metals were high for some (Zn > Pb > Cu > Cd) and relatively low for others. Concentrations of Cr in the gut juices were not determined in this study. Among the amino acids quantified in this study, glycine was the most concentrated (6.13 mM).

The partitioning of Cd, Cr, and Zn in spiked sediments as a function of aging in different operationally defined geochemical species is shown in Figure 1. Stable metal speciation in sediments is also shown for comparison. The sum of metal speciation in the exchangeable, carbonate, reducible, organic matter, and residual phases was normalized to 100%. Spiked Cd was mostly partitioned in the exchangeable and carbonate phases and hardly in the organic and residual phases. In contrast, native Cd partitioned evenly in different geochemical phases except for the exchangeable in sediment B. The fraction of both spiked and native Cd in the exchangeable phase of sediment was somewhat higher in the more contaminated sediment B. The radioisotope ⁵¹Cr was mostly partitioned in the carbonate, reducible, and organic phases. In contrast, native Cr was mainly distributed in the residual phase, with the remaining partitioned in the reducible and organic phases. Most of the spiked Zn was fractioned in the carbonate and reducible phases, whereas the bulk of native Zn was distributed in the carbonate, reducible, and organic phases. Among the three metals, speciation of ¹⁰⁹Cd and ⁵¹Cr was scarcely influenced by sediment aging (within 100 d). For ⁶⁵Zn, fraction in the carbonate phase gradually decreased but increased in the reducible phase as sediment aging increased. Other geochemical fractions were not much influenced by sediment-metal aging. The abrupt change at 25 d aging for sediment A remains unexplained.

Metal extraction from contaminated sediments by four extractants

Figures 2 through 4 show the metal extraction from oxic natural sediments by the four extractants, i.e., gut juices, normal seawater, pH = 5 seawater, and 1% SDS seawater. Both Cd and Zn were quickly extracted within the first 4 h, followed by slower extraction. Among the three metals, Cd was the most extractable metal and Cr the least, with extraction efficiency consistently below 3%. Relative rates of extraction by the four extractants were gut juices > pH = 5 seawater > 1% SDS seawater > normal seawater. The fractions of extracted metals in the two sediment samples were somewhat comparable within 4 h. The relationship among the four extractants in metal release is shown in Table 3. There was a significant

<table>
<thead>
<tr>
<th>Sediment</th>
<th>TOC (%)</th>
<th>LOI (%)</th>
<th>Fe (%)</th>
<th>Mn (%)</th>
<th>Cd (mg/kg)</th>
<th>Cr (mg/kg)</th>
<th>Zn (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.59</td>
<td>3.31</td>
<td>1.50</td>
<td>0.032</td>
<td>7.50 ± 0.85</td>
<td>27.1 ± 4.50</td>
<td>321 ± 45.1</td>
</tr>
<tr>
<td>B</td>
<td>1.59</td>
<td>7.93</td>
<td>3.01</td>
<td>0.063</td>
<td>37.9 ± 1.40</td>
<td>40.5 ± 5.70</td>
<td>4270 ± 132</td>
</tr>
</tbody>
</table>

Table 2. Concentrations of metals and amino acids in the gut juice of sipunculan (Sipunculus nudus) collected from Ting Kok, Hong Kong. Mean ± standard deviation (n = 3)

<table>
<thead>
<tr>
<th>Metals</th>
<th>Conc. (mM)</th>
<th>Amino acids</th>
<th>Conc. (mM)</th>
<th>Amino acids</th>
<th>Conc. (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cd (μM)</td>
<td>0.142 ± 0.059</td>
<td>Alanine</td>
<td>3.14</td>
<td>Methionine</td>
<td>0.47</td>
</tr>
<tr>
<td>Cu (μM)</td>
<td>1.436 ± 0.095</td>
<td>Arginine</td>
<td>1.49</td>
<td>Phenylalanine</td>
<td>1.09</td>
</tr>
<tr>
<td>Ni (μM)</td>
<td>0.881 ± 0.016</td>
<td>Aspartic acid</td>
<td>1.43</td>
<td>Proline</td>
<td>0.78</td>
</tr>
<tr>
<td>Pb (μM)</td>
<td>1.564 ± 0.028</td>
<td>Cysteine</td>
<td>0.66</td>
<td>Serine</td>
<td>1.14</td>
</tr>
<tr>
<td>Zn (μM)</td>
<td>10.47 ± 0.075</td>
<td>Glutamic acid</td>
<td>2.11</td>
<td>Threonine</td>
<td>1.76</td>
</tr>
<tr>
<td>Al (mM)</td>
<td>8.89 ± 0.37</td>
<td>Glycine</td>
<td>6.13</td>
<td>Tryptophan</td>
<td>0.10</td>
</tr>
<tr>
<td>Ca (mM)</td>
<td>580 ± 6.00</td>
<td>Histidine</td>
<td>0.39</td>
<td>Tyrosine</td>
<td>0.72</td>
</tr>
<tr>
<td>Fe (mM)</td>
<td>48.6 ± 0.00</td>
<td>Isoleucine</td>
<td>0.69</td>
<td>Valine</td>
<td>1.45</td>
</tr>
<tr>
<td>Mg (mM)</td>
<td>1960 ± 3.75</td>
<td>Leucine</td>
<td>1.37</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mn (mM)</td>
<td>4.36 ± 0.00</td>
<td>Lysine</td>
<td>1.44</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
positive relationship in Cd extraction efficiency among the four extractants. For Cr, there was no significant correlation among the extractants; but for Zn, significant correlation was found between gut-juice extraction and normal seawater or 1% SDS extraction.

The relationship between Cd extraction and its speciation in two geochemical fractions in sediments is shown Figure 5. The release of $^{109}\text{Cd}$ by the four extractants was correlated positively with the $^{109}\text{Cd}$ fraction in the exchangeable phase, but was negatively correlated with the fraction of Cd in the reducible phase. For Cr and Zn, no significant correlation was found between gut-juice extraction and their partitioning in any of the geochemical species.

The three metals were extracted from the anoxic sediment at a very low rate (Fig. 6). For Cd, the percentage extracted was consistently lower than 1.1%. Zinc extraction was somewhat higher than the extraction of Cd, but the percentage was also low (<3% in most cases). Similar to the extraction of Cr from oxic sediment, Cr was extracted very slowly from the anoxic sediments. The amount of metal extracted by different extractants was so small that no consistent pattern can be discerned regarding the differences among extractants, sediment aging, or between the two sediment types.

Metal extracted from anoxic sediment with different SEM/AVS concentrations

The efficiencies of gut juices in extracting Cd, Cr, and Zn from sediments with different SEM and AVS concentrations are shown in Figure 7. Different concentrations of AVS and SEM were achieved by mixing the two sediments with different ratios. No statistically significant relationship was found between the extraction of the three metals and the concentrations of SEM or (SEM - AVS) in sediments. However, there was a trend that both the upper limit and the variability of the percentage of extraction decreased as the concentrations of AVS increased.

**DISCUSSION**

Animal guts represent a reaction zone in which the animals must quickly solubilize food into an absorbable form and extract it from the indigestible residues [32]. Such rapid solubilization is mediated by gut biochemical reactions due to actions by enzymes and surfactants or changes in acidity and redox potential. In this study, we compared the extractability of three metals by gut juices with that by three extractants (normal seawater, seawater adjusted to pH = 5, and 1% SDS seawater). In general, digestive fluids extracted the largest fractions of metals among the four extractants examined in this study. There was a significant relationship for Cd among the four extractants. Mayer et al. [15] showed a significant correlation between the Cu solubilization by clean seawater and gut juices collected from polychaetes. The significant correlation in extraction by gut juice, seawater, lowered pH seawater, and SDS may indicate that Cd uptake (or desorption) may not necessarily be controlled by surfactant activity or digestive-enzyme activity. It appears that Cd uptake by the
animals may have been simply controlled by the physical-chemical desorption process. In fact, recent comparative studies also demonstrated a significant correlation between the bulk desorption and assimilation efficiency of Cd measured as the fraction taken up across the animal gut tissues [4,19]. Several studies indicated that Cd desorption within the digestive gut may control its assimilation by a variety of marine invertebrates (bivalves, sipunculans) [4,19,33].

The extraction of Cr and Zn was more complicated than that of Cd. For Zn, significant correlation was observed only between gut juices and normal seawater or 1% SDS extractions, but it was weaker than that for Cd. Cr was essentially unavailable for extraction, indicating that there was little bioavailable to the animals from the sedimentary source. This is confirmed by the direct Cr AE measurement (<7%) in S. nudus [4,19]. Consistently, extractions of Cr and Zn were decoupled from the direct assimilation measurements, suggesting that absorption of these two metals across the gut lining was not controlled by the amount of metals desorbed within the digestive tract. One likely explanation may be the different chemical behavior (e.g., chemical speciation, sorption, and desorption) of these two metals from Cd. Similar to findings of earlier studies [17,32], decreasing pH increased metal release from the sediment when compared with simple extraction by seawater. Addition of surfactants, however, did not enhance metal release from sediments.

Trace metals are bound with different geochemical phases in the sediment. More Cd was partitioned into the exchangeable phase and more Zn into the carbonate phase in the more contaminated sediment, suggesting that metals were primarily of anthropogenic origins. Metal concentrations in these two sediments were at least one order of magnitude higher than the typical metal concentrations in pristine marine environments [7]. There was a marked difference in metal speciation between the native metals and the spiked metals. This is especially pronounced for Cr, in which about 73 to 81% of native Cr was found in the residual phase, whereas <5% of spiked Cr was distributed in the residual phase after 100 d of sediment aging. There was very little association of the spiked metals with the residual geochemical phase for all three metals examined in this study, whereas residual was an important phase for native metals in the sediments.

There are also significant differences in the ability and mechanism of metal sorption onto sediment geochemical phases among different metals. Zinc tends to have a stronger affinity with Fe-Mn oxide, clay, and organic matter than Cd [34]. Cadmium tends to bind with the exchangeable site of sediment but not the inner crystal lattices of minerals, whereas Zn can
Table 3. Correlation coefficients of the relationships in metals extraction from contaminated sediments among four extractants (gut juices, normal seawater, seawater adjusted to pH = 5, and 1% sodium dodecyl sulfate [SDS] seawater) after 4 h of extraction. Correlation was carried out for two sediments aged for different durations (n = 8).

<table>
<thead>
<tr>
<th>Metal/extractants</th>
<th>Gut juices</th>
<th>Normal seawater</th>
<th>pH = 5 seawater</th>
<th>1% SDS seawater</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cd</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gut juices</td>
<td>1.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal seawater</td>
<td>0.710**</td>
<td>1.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH = 5 seawater</td>
<td>0.686*</td>
<td>0.964**</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>1% SDS seawater</td>
<td>0.500*</td>
<td>0.801**</td>
<td>0.722**</td>
<td>1.0</td>
</tr>
<tr>
<td>Cr</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gut juices</td>
<td>1.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal seawater</td>
<td>0.081**</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH = 5 seawater</td>
<td>0.118**</td>
<td>0.280**</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>1% SDS seawater</td>
<td>0.033**</td>
<td>0.116**</td>
<td>0.036**</td>
<td>1.0</td>
</tr>
<tr>
<td>Zn</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gut juices</td>
<td>1.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal seawater</td>
<td>0.528*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH = 5 seawater</td>
<td>0.113**</td>
<td>0.328**</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>1% SDS seawater</td>
<td>0.505*</td>
<td>0.125**</td>
<td>0.014**</td>
<td>1.0</td>
</tr>
</tbody>
</table>

* p < 0.05.  
** p < 0.01.  
*** p < 0.001.

diffuse into the inner crystal lattices of minerals. Sediment aging was thus more important for Zn partitioning in different geochemical phases than for Cd. Our study demonstrated that there was insignificant change in the speciation of spiked metals within the 100-d aging period, except for Zn, in which a certain level of decrease in carbonate fraction and an increase in reducible fraction were observed. The time required for newly spiked metals to reach equilibrium with the native metals in the sediments thus remains unknown. Several studies have cautioned that the introduction of new metals to sediments and the subsequent partitioning among the sediment components may not necessarily reflect those observed in the actual environment [4,21]. Such limitation in fact has presented a great challenge to the study of sediment bioavailability and toxicity using spiked metals.

Fig. 5. The relationships between the extraction of spiked Cd by four extractants (normal seawater, pH = 5 seawater, 1% sodium dodecyl sulfate [SDS] seawater, and gut juices) after 4 h and spiked-Cd distribution in the easily exchangeable fraction and in the reducible fraction in sediments.

Fig. 6. Extraction of spiked Cd, Cr, and Zn from anoxic sediments by normal seawater, pH = 5 seawater, 1% sodium dodecyl sulfate seawater, and gut juices extracted from sipunculans after 12 h. Mean ± semirange (n = 2).

Fig. 7. The relationships between the extraction efficiency (%) of spiked Cd, Cr, and Zn by gut juices after 12 h and the simultaneously extractable metal (SEM) and acid volatile sulfi de (AVS) concentrations in sediments. Data are means ± semirange (n = 2).
The difference in the extractability between the native and spiked metals by the gut juices was smaller than the difference in metal speciation. For the two contaminated sediments, 38 to 46% of native Cd, 0% of Cr, and 9.1 to 10.4% of Zn were extracted by gut juices in 4 h, as compared with 42 to 56% of spiked Cd, 0.3 to 1.5% of spiked Cr, and 1.6 to 16.6% of spiked Zn under the same condition. Although sediment aging did not significantly affect the fraction of spiked Cd in different geochemical phases, the fraction of spiked Cd extracted by different extractants decreased with increasing sediment aging. There was also a significant difference in the bioavailability of Zn from spiked and native sediments, and the fraction of spiked Zn extracted by gut juices decreased with increasing sediment aging, despite that there was no major difference in Zn extraction by other extractants due to the low extraction efficiency. These results imply that geochemistry may not necessarily control metal bioavailability, and other processes such as gut physiology and biochemistry should be considered.

The release of Cd by gut juices was significantly affected by Cd partitioning in the exchangeable and the reducible phases, indicating that these two geochemical phases were most important in controlling Cd release and bioavailability. This has been consistently observed in several of our recent studies quantifying the geochemical controls on metal assimilation by marine benthic invertebrates [4,22,35]. Thus, Cd bound with the extractable fraction enhanced its absorption by the bivalves and sipunculans, whereas the reducible fraction inhibited its bioavailability. Such a relationship was less obvious for Cr and Zn. These data again imply that the geochemical controls on Cr and Zn bioavailability were much more complicated than the two geochemical species identified in this study.

There have been substantial studies on the relationship between AVS and SEM and metal toxicity to benthic invertebrates, mainly amphipods and polychaetes [8,25,36,37]. It is generally believed that metals bound with the anoxic sediment or sulfide (which is the dominant ligand bound with metals in anoxic sediment) were hardly bioavailable to benthic invertebrates, although such an assumption has been challenged recently [5]. Our study confirmed that there was very little extraction of metals bound with the anoxic sediments; thus, the bioavailability of metals bound with anoxic sediments was extremely low. Under most circumstances, <5% of metals were extracted by any of the extractants as used in our study. Cadmium tends to have a stronger binding affinity with S than Zn, and accordingly, the extraction efficiency of Cd was much lower than that of Zn. Consistently, our recent studies generally indicated that metals bound with anoxic sediments were assimilated at a negligible fraction by different groups of marine invertebrates or at a much lower efficiency than the assimilation of metals bound with the oxic sediments [3,4,19,22,38]. The gut juice of sipunculans was anoxic as confirmed by microelectrode measurements [19]. Our extraction data were thus consistent with that of Chen and Mayer [23], who also found that only a negligible fraction of Cu from the anoxic sediments was extracted by the gut juices of polychaetes. For live animals, possible reoxidation of anoxic sediments may likely occur, which may remodelize the metals bound with the anoxic sediments within the digestive tract of the animals, and thus potentially enhance metal absorption across the digestive gut linings. Lee et al. [5] have indicated that AVS-bound metals were assimilated by clams (Mactoma balitica) and mussels (Mytilus edulis) with comparable efficiency as metals bound with oxic sediments. They attributed such appreciable assimilation to the solubilization and reoxidation of metals within the gut environment. In our experiments, all treatments including sediment spike and extraction were carried out in the N2 bag to avoid oxidation of the anoxic sediments.

Our study further demonstrated that there was no statistically significant relationship between the release of the three metals and the concentrations of SEM (or SEM–AVS) in sediments. Chen and Mayer [23] examined the extraction of Cu bound with artificially synthesized AVS particles by gut juices prepared from deposit-feeding and suspension-feeding invertebrates. The extraction efficiency was highly species specific. In a recent study, we also manipulated the ratio of AVS and SEM, and experimentally quantified the metal AEs in the mussel Perna viridis [35]. We found that the difference in AVS:SEM did not significantly affect the assimilation of Cd, Cr, or Zn in the mussels. Thus, bioavailability study demonstrated that AVS:SEM cannot predict the metal bioavailability from the contaminated sediments to marine bivalves. A further challenge would be to link metal bioavailability to toxicity from contaminated sediments using the AVS/SEM approach as an initial guideline.

Acknowledgement—We are grateful to the two anonymous reviewers for their constructive comments on this work. This study was supported by a Competitive Earmarked Research Grant from the Hong Kong Research Grants Council (HKUST6113/00M) to W.-X. Wang.

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