Influence of acid volatile sulfides and simultaneously extracted metals on the bioavailability and toxicity of a mixture of sediment-associated Cd, Ni, and Zn to polychaetes Neanthes arenaceodentata

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Abstract

Laboratory microcosm experiments were conducted to investigate the influence of acid volatile sulfides (AVS) and simultaneously extracted metals (SEM) in sediments on the bioavailability and toxicity of Cd, Ni, and Zn in sediments to polychaete worms Neanthes arenaceodentata. Cohorts of juvenile N. arenaceodentata were exposed to sediments spiked with metal mixtures containing Cd, Ni, and Zn (0.5–15 μmol·g⁻¹ of total SEM) with Low- (~1 μmol·g⁻¹), Medium- (~5 μmol·g⁻¹), and High-AVS concentrations (~10 μmol·g⁻¹) for 20 days to determine mortality, growth rate, and metal bioaccumulation. Tissue Cd and Zn concentrations at the end of the exposure were significantly higher in sediments with the low-AVS concentration at a given SEM concentration due to the increased dissolved metal concentrations in overlying water (OW). However, tissue Ni concentrations were not related to dissolved Ni in the OW. AVS concentrations also influenced the toxicity of metals to the worms. Significant mortality was observed only at the highest SEM treatments at Low-AVS series. Most individuals survived at the highest SEM treatments at Medium- and High-AVS series. Similarly, the growth rates of worms were reduced in treatments having higher molar differences between SEM and AVS ([SEM−AVS]). Overall, the bioavailability and toxicity of metals in sediments was not well predicted by sediment metal concentrations only, but considering the influence of geochemical factors (AVS) on the metal bioavailability improved the prediction of toxicity. Also, the relationship between tissue metal concentration and toxicity was used to determine which contaminant was most responsible for the observed toxicity of the metal mixture.

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1. Introduction

Sediments provide a concentrated reservoir of trace metals introduced via natural geochemical processes and anthropogenic activities in most aquatic environments. Once associated with sediments, metals undergo various geochemical transformations during diagenetic reactions along redox gradients (Morse et al., 1987). Since there are various metal-binding phases in sediments, geochemical forms within contaminated sediments and processes affecting chemical partitioning between sediments and interstitial or overlying water can be particularly important for determining the fate and bioavailability of sediment-associated metals (Tessier et al., 1984; Luoma, 1989).

Predicting the bioavailability and toxicity of sediment-associated metals based on the equilibrium partitioning to binding phases in sediments is a critical issue for the development of metal sediment quality criteria (SQC) (Ankley et al., 1996; Lee et al., 2000a). Di Toro et al. (1990) suggested that the dissolved metal concentrations in porewater (PW) could be largely controlled by the molar ratio of reactive metals (or simultaneously extracted metal with acid volatile sulfides; SEM) to acid volatile sulfides (AVS) in sediments through the formation of insoluble metal sulfide complexes. The influence of AVS on the metal partitioning between interstitial water and sediment particles and also on the acute toxicity of divalent metals in sediment has been demonstrated in several laboratory studies using both freshwater and marine organisms (Ankley et al., 1991; Carlson et al., 1991; Casas and Crecelius, 1994; Kemble et al., 1994; Pesch et al., 1995; Hansen et al., 1996a; Berry et al., 1996, 1999; De Witt et al., 1999; Lee et al., 2000b). Further, the effects of AVS on the chronic sublethal toxicity (growth, reproduction and community level response) of metals has also been evaluated (Hare et al., 1994; Ingersoll et al., 1994; Besser et al., 1996; Hansen et al., 1996b; De Witt et al., 1996; Liber et al., 1996; Sibley et al., 1996; Peterson et al., 1996). These studies concluded that there was no evidence of toxicity when sediments had excess AVS over SEM (e.g. SEM/AVS <1), especially for acute exposures (Ankley et al., 1996).

There is some research which disagrees with the above finding and shows significant bioaccumulation from sediments with SEM/AVS less than 1 (Ingersoll et al., 1994; Hare et al., 1994; Peterson et al., 1996; Warren et al., 1998; Wang et al., 1999; Lee et al., 2000c, 2001) and were usually inconsistent with the toxicity results from the previous AVS-normalization studies (Pesch et al., 1995; Carlson et al., 1991). Some recently conducted studies suggest that the AVS-normalization approach may not be applicable for moderately contaminated sediments with long equilibration times (e.g. field contaminated sediments). This occurs because the partitioning of metals between porewater and sediment particles could be considerably influenced by the equilibration period between spiked-metals and sediments and also by metal contamination level (Lee et al., 2000b, 2004). Therefore, AVS-normalized metal concentrations, as well as total metal concentration criteria for sediments, might have limitations for predicting the metal toxicity in assessing contaminated sediments.

The bioaccumulation of contaminants has been suggested as a more accurate end point for assessing the bioavailability (Ankley, 1996) and a more reliable indicator of the toxicity potential than whole sediment contaminant concentrations (Borgmann et al., 1993, Landrum et al., 1994). Developing a quantitative relationship between the metal bioaccumulation and toxicity in contaminated sediments could further extend our understanding of the complex mechanisms of metal bioavailability and permit bioaccumulation data to serve as a useful indicator of the potential metal toxicity in moderately contaminated sediments. However, few studies have investigated the influence of geochemical factors on bioaccumulation, the toxicity of metals, and simultaneously tried to relate tissue metal concentrations to the observed toxic responses to improve prediction (Borgmann, 2000).

In 20-day laboratory microcosm experiments, we evaluated the influence of AVS and dissolved/particulate metal concentrations in sediments on the bioavailability to the marine polychaetes *Neanthes arenaceodentata*, and we also examined lethal and sublethal (growth inhibition) toxicity of Cd, Ni and Zn. The results of the metal bioaccumulation and the toxicity to the test animals were compared with various geochemical parameters such as SEM, [SEM−AVS] and dissolved metal concentrations in overlying water. The growth inhibition data was compared with tissue metal concentrations to evaluate the tissue metal accumulation as a predicting indicator.
for the observed toxic responses in test species exposed to metal mixture.

2. Experimental methods

Sediment bioassays were conducted to evaluate the influence of AVS and metal concentrations on the bioavailability and toxicity (mortality and growth inhibition) of a mixture of Cd, Ni, and Zn to juvenile N. arenaceodentata. Test animals were exposed to experimental sediments containing three different AVS concentrations (1–10 μmol·g⁻¹; Low-, Medium- and High-AVS levels) and five different sediment metal levels (0.5–14 μmol·g⁻¹ of total SEM; M0–M4) for 20 days.

2.1. Sediment preparation

The experimental sediment was obtained from a muddy tidal flat of Euhang-Ri, Taean-gun on the west coast of Korea (36°48'N; 126°11'E). Collected sediment, which contained ~15 μmol·g⁻¹ of natural AVS, was screened through 1-mm nylon mesh at the site, covered, and transferred directly to laboratory, where a portion of the collected sediment was manipulated to achieve three nominal AVS concentrations (1, 5, and 10 μmol·g⁻¹ as Low-, Medium- and High-AVS levels, respectively). The sediment was mixed with an equivalent volume of deoxygenated saline water (30 psu) and divided into two batches. One batch of the sediment was kept in a closed container and purged with N₂ gas periodically (anoxic sediment). The remaining sediment was oxidized by aerating continuously for a week (oxidized sediment). The AVS concentrations in sediments were manipulated to Low-AVS (~1 μmol·g⁻¹), Medium-AVS (~5 μmol·g⁻¹) and High-AVS level (~10 μmol·g⁻¹) by mixing the oxidized and anoxic sediment in appropriate ratios.

Following the AVS-manipulation, sediments were spiked with a stock mixture of Cd, Ni, and Zn to yield four nominal metal levels (1×, 2×, 4× and 8× [0.02 μmol Cd·g⁻¹+0.3 μmol Ni·g⁻¹+1.2 μmol Zn·g⁻¹] as M1, M2, M3 and M4, respectively). The nominal concentrations of metals (Table 1) were chosen to reflect the range of metal-contamination levels in field sediments from Korean coastal areas. Unspiked sediment (M0) was used as Control for each AVS level. Sediment-metal slurries were mixed vigorously by an electric mixer for ~1 h and shaken several times a day for 10 days.

Following the metal spiking process, the 200 ml of the sediment slurry was distributed to each exper-

Table 1

Mean SEM concentrations (μmol·g⁻¹) of Cd, Ni, Zn and total divalent metals (Cd+Cu+Ni+Pb+Zn), AVS concentrations (μmol·g⁻¹), and molar difference (μmol·g⁻¹) between SEM and AVS ([SEM−AVS]) for Cd, Ni, and Zn in experimental sediments at the beginning (t=0 days) and end (t=20 days) of the exposure, time-averaged (t=0 days and t=20 days) concentration (μM) of the dissolved metal in overlying water (OW)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>SEM</th>
<th>AVS</th>
<th>OW concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M0</td>
<td>0.0004</td>
<td>0.1</td>
<td>0.2</td>
</tr>
<tr>
<td>M1</td>
<td>0.012</td>
<td>0.4</td>
<td>1.4</td>
</tr>
<tr>
<td>M2</td>
<td>0.040</td>
<td>0.7</td>
<td>2.9</td>
</tr>
<tr>
<td>M3</td>
<td>0.091</td>
<td>1.5</td>
<td>5.4</td>
</tr>
<tr>
<td>M4</td>
<td>0.174</td>
<td>2.7</td>
<td>10.1</td>
</tr>
<tr>
<td>Medium</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M0</td>
<td>0.0004</td>
<td>0.1</td>
<td>0.2</td>
</tr>
<tr>
<td>M1</td>
<td>0.011</td>
<td>0.4</td>
<td>1.3</td>
</tr>
<tr>
<td>M2</td>
<td>0.038</td>
<td>0.6</td>
<td>2.7</td>
</tr>
<tr>
<td>M3</td>
<td>0.079</td>
<td>1.4</td>
<td>5.1</td>
</tr>
<tr>
<td>M4</td>
<td>0.154</td>
<td>2.6</td>
<td>9.9</td>
</tr>
<tr>
<td>High</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M0</td>
<td>0.0004</td>
<td>0.1</td>
<td>0.2</td>
</tr>
<tr>
<td>M1</td>
<td>0.010</td>
<td>0.4</td>
<td>1.2</td>
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<tr>
<td>M2</td>
<td>0.037</td>
<td>0.8</td>
<td>2.7</td>
</tr>
<tr>
<td>M3</td>
<td>0.069</td>
<td>1.3</td>
<td>5.1</td>
</tr>
<tr>
<td>M4</td>
<td>0.133</td>
<td>2.5</td>
<td>9.2</td>
</tr>
</tbody>
</table>
imental beaker and allowed to stand for 1 week. The overlying water was then changed and equilibrated with sediment for another 1 day until prior to adding test animals. A sediment sample was collected from experimental beakers to determine the sediment organic carbon content and grain size distribution. The remaining material was preserved in a deoxygenated glass bottle with lid for the later metal and AVS analysis. The total organic carbon (TOC) content of the experimental sediments for all treatments was not significantly different. Mean TOC content (±standard deviation; n=9) determined on a carbon analyzer after acid pretreatment was 0.52±0.07%.

Particle size was analyzed by pipetting and dry sieve method of Folk (1954). The mean particle size (n=2) of the experimental sediment was 6.5 μm and mean sand, silt and clay contents were 8.5%, 63.3%, and 28.2%, respectively.

2.2. Bioassay procedure

Laboratory cultured juvenile polychaetes, N. arenaceodentata (2 weeks, post-emergence) were obtained from DJ Reish, California State University, Long Beach, USA and were acclimated gradually over 1 week to the experimental temperature (16±0.8 °C) and salinity (30±0.3 psu). Culturing and testing protocols for lethal and sublethal toxicity tests followed Reish (1985) and Dillon et al. (1993). Following acclimation, the test animals were randomly divided into groups of 10 individuals and introduced into each of five replicate 1-l beakers (four for biological samples and one for chemical analysis). Each beaker contained sediments (200 ml), and equilibrated with overlying water (800 ml). The experimental water was 0.45-μm filtered natural seawater (~30 psu) and the exposure was conducted at 16 °C. During the 20-day exposure, the overlying water was aerated continuously and half was changed every other day. Overlying water (OW) was sampled (20 ml) from 5 cm above sediment surface at 1, 4, 9, 12, 15 and 19 days after the initiation of incubation, filtered (0.45 μm), and preserved by acidification for later metal analysis. Porewater was not sampled due to logistical limitations. A previous study using a similar protocol to the present study showed that the metal concentrations in the OW near sediment surface were equilibrated quickly with PW (Lee et al., 2001). Therefore, we assumed that comparing OW metals to biological data should not be substantially different from using PW metals. Test chambers were illuminated with a light/dark cycle of 14:10 h. During the exposure period, worms were fed with finely ground TetraMin® (8 mg ind.⁻¹) twice a week. Overlying water qualities including temperature (16±0.2 °C), salinity (30±0.3 psu), pH (7.8±0.3) and dissolved oxygen (>90%) were determined three times a week to ensure the consistency of the physical properties of water media.

Following the 20-day exposure, three subsamples of sediment were collected from the beaker dedicated to chemical analysis for measurement of SEM and AVS. Sediments in the biological replicates were sieved through 300-μm sieve to collect surviving worms. Worms were allowed to depurate undigested diets for 1 day in clean seawater. The number of surviving worms was recorded, then pooled individuals from each beaker were placed into the pre-weighed glass vials and freeze-dried. Following freeze-drying, vials were weighed to determine individual dry weight.

Separate 96-h water-only toxicity tests for Cd and Zn were conducted with N. arenaceodentata to confirm the sensitivity of test animals used in the present study. The 96-h LC50 (95% CI) values for Cd and Zn determined by the Spearman-Karber method were 71 (55, 83) and 22 (18, 25) μM, respectively, and were comparable to previous studies (Reish, 1985).

2.3. Analytical procedure

Sediment AVS and SEM, and overlying water metal analyses were performed within 1 week of sample collection. The collected sediment was kept in a tightly sealed jar at 4 °C until AVS and SEM analysis. AVS concentrations were determined by the cold-acid (1 N HCl) purge and trap technique described by Boothman and Helmstetter (1992). The sulfide concentrations were quantified with a sulfide ion-specific electrode. System performance was verified by determining the recoveries of sulfide spiked to sediment. The recoveries of spiked sulfides (mean±S.D.) were always in the range of 80% to 120%. All AVS determinations were duplicated for each sediment sample. The 1 N HCl extract (SEM) of sediment was filtered through a 0.45-μm syringe filter and metal concentration were determined in the filtrate by flame atomic absorption
spectroscopy (AAS). Metals in OW were determined, following 10× dilution with Milli-Q water, by GF-AAS with the standard addition method.

Freeze-dried tissue samples were digested in borosilicate glass vials with concentrated nitric acid following the method of Brown and Luoma (1995). Each vial was covered with a reflux glass bulb and placed on a hot plate at 80 °C. The samples were digested until solution became clear. Following the digestion, samples were evaporated and reconstituted in 1% nitric acid. The aliquots were filtered at 0.45 μmol with a syringe filter and analyzed for Cd, Ni and Zn by flame AAS and/or graphite furnace AAS (GF-AAS).

Procedure blanks and standard reference materials (TORT-1, NRC; oyster tissue (1566a), NIST) were analyzed to ensure quality control and assurance. Mean recoveries for Cd, Ni, and Zn were 95±3%, 93±3%, and 97±2%, respectively. The experimental containers and glassware used for sediment handling, chemical analysis, and sample storage were acid washed, followed by soaking in deoxygenated Milli-Q water for 1 week. The sediment samples were handled under a glove bag filled with N2 gas.

2.4. Data analysis

Molar difference between SEM and AVS ([SEM–AVS]) was separately calculated for each metal Cd, Ni and Zn. Since solubility products of metal sulfides increase in order of Ag>Cu>Pb>Cd>Ni (Di Toro et al., 1990), when multiple metals are employed in experiments, the SEM used for [SEM–AVS] evaluation should include the molar concentrations of the metals whose solubilities are lower than the metal of interest (Lee et al., 2000b). For example, determination of the influence of AVS on Cd via [SEM–AVS] should include the sum of the molar concentrations of Ag, Cu, Pb and Cd. Similarly, sum of molar concentrations of Ag, Cu, Pb, Cd, Zn, and Ni SEM were included for calculating [SEM–AVS] for Ni. Since the AVS concentrations changed over time, [SEM–AVS] was calculated using time-averaged AVS concentration.

Statistical significance was set at α=0.05, otherwise noted. Statistica® was used for all statistical analysis including ANOVA and regression analysis. Dry weight based concentrations were used for all tissue data. The geochemical data used for comparison with tissue metal data were averaged values determined from sediments sampled at the beginning and end of the exposure.

3. Results

3.1. Sediment geochemistry

Mean SEM concentrations of Cd, Ni, and Zn were significantly different among different metal treatments (M0–M4) in each AVS series (Table 1, p<0.001). AVS levels and sampling time did not significantly affect the SEM levels in sediments (p>0.05 for both cases). Differences of Cd, Ni, and Zn SEM concentrations in sediments spiked at the same metal concentration were less than 20% among different AVS series and sampling times. The proportion of each metal SEM to the sum of SEM (total SEM) of Cd, Ni, and Zn in the sediments increased in the order of Cd (0.6–0.8%)<Ni (25–30%)<Zn (~70%). Sum of Ag, Cu and Pb SEM concentrations was consistent among treatments (~0.2 μmol·g⁻¹).

AVS concentrations among different AVS series were significantly different both at the beginning (t=0 day) and end (t=20 days) of the exposure (Table 1, p<0.001). Exposure time and metal level also influenced the AVS concentrations in sediments (p<0.01 for both cases, Table 1). AVS concentrations at t=0 from Low-, Medium- and High-AVS series ranged from 0.7 to 1.4, 4.3 to 5.1 and 7.2 to 10.5 μmol·g⁻¹, respectively and decreased to about half of the initial values by the end of the exposure. The AVS concentrations in M4 were 2.7 to 9.0 times higher than those in the control (M0) at the end of the exposure (Table 1).

The [SEM–AVS] for each metal increased with metal concentration and decreased with AVS concentration (Table 1). The [SEM–AVS] for Cd in the experimental sediments were all negative. The [SEM–AVS] for Ni and Zn were positive in all sediments from the Low-AVS series and the M2 to M4 sediments from the Medium-AVS series. Most [SEM–AVS] from the High-AVS series were negative except for the M4 sediments.

Dissolved metal concentrations in OW were clearly controlled by [SEM–AVS] for each metal,
showing the influence of AVS on metal partitioning (Fig. 1). Cadmium concentrations in OW were mostly below the detection limit (0.003 μM) for all sediments from the High-AVS series, while most OW Cd in metal-spiked sediments with lower AVS levels were detected up to 0.027 μM, even though [SEM/AVS] for Cd were all negative (Table 1). The dissolved Ni concentrations in OW were similar for the Medium- and Low-AVS series, although the [SEM–AVS] for Ni were higher in Low-AVS series. Dissolved Ni concentrations were better related to Ni SEM than to the [SEM/AVS] for Ni because most of the AVS was bound to other metals with lower solubility product for the metal sulfide than Ni (e.g. Zn). However, OW Zn concentrations were higher in Low-AVS series, since more Zn was bound to sulfide in the Medium-AVS series compared to the Low-AVS series.

3.2. Bioaccumulation of metals

Bioaccumulation of metals in *N. arenaceodentata* mostly increased linearly with spiked metal concentration in sediments for each AVS series (Table 2,  p<0.001). However, tissue concentrations of Cd and Zn were significantly higher in sediments with the lowest AVS concentrations at a given nominal metal level (Table 2). Therefore, the relationships between [SEM] and tissue concentrations of Cd and Zn were significantly different among the different AVS series; the slopes of the relationship of Cd and Zn [SEM] versus tissue metal in the Low-AVS series were 19 and 4 times higher than those in the other two AVS series, respectively (Fig. 2a,c). Tissue concentrations of Cd and Zn increased sharply when the [SEM/AVS] increased to close to or greater than 0 (Fig. 2d,f). Tissue Ni concentrations, however, increased linearly with Ni [SEM] and were not significantly related to the [SEM–AVS] for Ni.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mortality (%)</th>
<th>Growth rate</th>
<th>Tissue concentrations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SEM Mean</td>
<td>S.D. Mean</td>
<td>SEM Mean S.D.</td>
</tr>
<tr>
<td>Low</td>
<td>M0 2.5</td>
<td>4.3</td>
<td>0.21 0.05</td>
</tr>
<tr>
<td></td>
<td>M1 5.0</td>
<td>5.0</td>
<td>0.18 0.02</td>
</tr>
<tr>
<td></td>
<td>M2 2.5</td>
<td>4.3</td>
<td>0.13 0.02</td>
</tr>
<tr>
<td></td>
<td>M3 27.5</td>
<td>37.0</td>
<td>0.09 0.04</td>
</tr>
<tr>
<td></td>
<td>M4 95.0</td>
<td>5.0</td>
<td>N.A. a</td>
</tr>
<tr>
<td>Medium</td>
<td>M0 2.5</td>
<td>4.3</td>
<td>0.19 0.03</td>
</tr>
<tr>
<td></td>
<td>M1 7.5</td>
<td>4.3</td>
<td>0.18 0.06</td>
</tr>
<tr>
<td></td>
<td>M2 2.5</td>
<td>4.3</td>
<td>0.20 0.02</td>
</tr>
<tr>
<td></td>
<td>M3 5.0</td>
<td>5.0</td>
<td>0.19 0.04</td>
</tr>
<tr>
<td></td>
<td>M4 2.5</td>
<td>4.3</td>
<td>0.11 0.01</td>
</tr>
<tr>
<td>High</td>
<td>M0 2.5</td>
<td>4.3</td>
<td>0.18 0.02</td>
</tr>
<tr>
<td></td>
<td>M1 5.0</td>
<td>8.7</td>
<td>0.19 0.03</td>
</tr>
<tr>
<td></td>
<td>M2 5.0</td>
<td>5.0</td>
<td>0.21 0.08</td>
</tr>
<tr>
<td></td>
<td>M3 10.0</td>
<td>7.1</td>
<td>0.18 0.05</td>
</tr>
<tr>
<td></td>
<td>M4 2.5</td>
<td>4.3</td>
<td>0.20 0.03</td>
</tr>
</tbody>
</table>

*Not available.*
Fig. 2. Tissue Cd, Ni, and Zn concentrations in *N. arenaceodentata* exposed to Low-(□), Medium- (△) and High-AVS (■) series treatments as a function of geochemical parameters including SEM, [SEM–AVS], or dissolved concentrations in overlying water (OW) for each metal. Error bars represent standard deviation around the mean. The vertical lines in (e) and (f) represent [SEM–AVS] is 0 and the vertical line in (g) represents the limit of detection (LOD). Solid lines in (a) and (c) are regression lines using data from Low-AVS treatments, dotted lines in the same panels represent the other AVS treatments, and solid lines in (b), (g), (h) and (i) represent all treatments. The asterisks represent significant difference of slopes from 0 (NS: not significant or *p* > 0.05; *p* < 0.05; **p** < 0.01; ***p*** < 0.001).
Fig. 3. Mortality (%) of *N. arenaceodentata* as a function of geochemical parameters including total SEM concentrations and the molar difference between SEM and AVS ([SEM−AVS]) in sediments, and dissolved Ni and Zn concentrations in overlying waters.

Fig. 4. The individual growth rate of *N. arenaceodentata* exposed to Low- (□), Medium- (△) and High-AVS (■) series treatments as a function of molar difference between SEM and AVS ([SEM−AVS], µmol·g⁻¹) in sediments, and dissolved Ni and Zn concentrations in overlying water. The linear regression line was drawn only when [SEM−AVS] was positive.
Tissue metal concentrations significantly increased with dissolved metal concentrations in OW (Fig. 2g–i).

3.3. Lethal toxicity of metals to benthic animals

Mortality of *N. arenaceodentata* was mostly $<5\%$ in both control and metal-spiked sediments, even in sediments with highest metal concentrations from the Medium- and High-AVS series (Table 2). However, significant mortality of *N. arenaceodentata* was observed in the M3 and M4 treatment compared to control in the Low-AVS series, possibly due to the increased bioavailable metal concentrations (Table 2). Worm mortality was better explained by the dissolved Zn concentrations in OW than the other geochemical variables such as total [SEM], total [SEM/C0 AVS] or OW Ni (Fig. 3).

3.4. Sublethal toxicity of metals to *N. arenaceodentata*

The mean growth rate on a tissue dry weight basis was $0.19 \pm 0.01$ (mg·day$^{-1}$·ind.$^{-1}$) for *N. arenaceodentata* exposed to the control sediments from all AVS series (Table 2). A significantly reduced tissue growth rate (mg·day$^{-1}$·ind.$^{-1}$; ± standard deviation) of *N. arenaceodentata* was observed in sediments with elevated [SEM–AVS] and dissolved metal concentrations in OW, such as M2 (0.13±0.02) and M3 (0.09±0.04) from the Low-AVS and M4 (0.11±0.01) from the Medium-AVS series (Table 2). The growth rates of worms were generally comparable when the [SEM–AVS] was $<2$. However, growth rates were significantly decreased for both Ni and Zn when the [SEM–AVS] was $>2$ (Fig. 4). Growth rates were significantly correlated with OW Ni and Zn (Fig. 4), but not with OW Cd (data not shown). Inhibition of growth was best explained by Zn concentrations in water and sediment, followed by Ni and Cd.

Growth rates of *N. arenaceodentata* were significantly correlated with tissue concentrations of Cd ($r^2=0.56$, $p<0.01$) and Zn ($r^2=0.71$, $p<0.001$), but not with tissue Ni concentrations (Fig. 5). The reduced growth rates were observed when the tissue Zn in *N. arenaceodentata* was approximately 6 μmol·g$^{-1}$ or greater (Fig. 5).

4. Discussion

This study examined the influence of reactive sulfides on the bioavailability of the divalent metals (Cd, Ni, and Zn) to sediment-dwelling polychaetes, *N. arenaceodentata* from metal-spiked sediments. AVS clearly influenced partitioning of all tested metals between the sediment and overlying water and also influenced both lethal and sublethal response of the organisms to metal contamination. These results are consistent with prediction for AVS impact on metal bioavailability and toxicity, but contradict the results from previous bioaccumulation studies suggesting the importance of dietary uptake for metal bioavailability from moderately contaminated sediments (Ankley, 1996; Berry et al., 1996; Lee et al., 2000a).
The relationship between tissue metal concentrations and sublethal effects in the present study suggested that the bioaccumulation of Zn was likely more responsible for the inhibition of growth than the other metals. Tissue bioaccumulation data was also useful for determining the relative contribution of each metal contaminant to the observed toxic responses. The relationships between metal bioaccumulation and AVS were not consistent among studies and experimental conditions. Many studies based on equilibrium partitioning theory (EqP) have shown that the toxicity of test animals was controlled by the [SEM/AVS], however, most EqP-based studies did not measure tissue bioaccumulation and even the studies measuring tissue accumulation could not show a significant relationship between bioaccumulation and [SEM/AVS]. The toxicity data were clearly related to [SEM/AVS] in the previous EqP-based studies, but bioaccumulation data seemed to be related to only [SEM] in their studies (Ankley, 1996). Some other studies from both laboratory and field experiments have shown that AVS has a limited influence on the metal bioaccumulation in sediments (Hare et al., 1994; Ingersoll et al., 1994; Besser et al., 1996; Lee et al., 2000a, 2001). The authors suggested that short-equilibration times and unnaturally high metal spiking levels in most laboratory bioassays seemed to accentuate the partitioning of metals to dissolved phase in water, making it the dominant route of exposure, while in field studies with longer equilibrium times and generally lower concentrations, dietary uptake would be more important than dissolved uptake for benthic animals (Luoma and Fisher, 1997; Lee et al., 2000c, 2001, 2004).

The influence of AVS on the tissue metal bioaccumulation in the present study was not consistent among metals. Test organisms accumulated significantly more Cd and Zn when exposed to low-AVS sediments indicating the role of AVS controlling bioavailability of those metals from sediments. However, tissue Ni accumulation was only related to the particulate Ni concentrations in sediments even though AVS significantly influenced the metal partitioning of Ni as shown by Fig. 1. The metal-sediment equilibration time in the present study was about 20 days, which was longer than equilibration times adopted in most previous acute sediment bioassay studies (mostly <7 days), but is likely much shorter than most field conditions. Thus, the present results might not be applicable to most field environments. Lee et al. (2004) showed that the toxicity decreased substantially in sediment bioassays using the amphipod, *Leptocheirus plumulosus* when the metal-sediment equilibration time increased from 5 to 95 days. The partition coefficients between the dissolved and particulate phases for Cd, Ni, and Zn from this study were lower than those from field conditions, however, they were comparable or even higher than those from most laboratory studies referred in Lee et al. (2000b), suggesting the importance of equilibration time in metal partitioning in contaminated sediments.

The relative importance of the uptake route for a metal could vary among test species, metals and/or experimental conditions. Results from this study showed that the uptake from the dissolved phase would be more responsible for Cd and Zn bioaccumulation while the uptake from particulate sediment would be more important for Ni bioaccumulation in *N. arenaceodentata*. Similarly, previous bioaccumulation studies reported the inconsistent effect of metal partitioning in contaminated sediments among different metals (Lee et al., 2000b, 2001). Bioaccumulation of Ag, Cd, and Ni was not generally related to the dissolved concentration. However, Zn bioaccumulation was influenced by dissolved concentration (Lee et al., 2001). Pesch et al. (1995) showed that significant accumulation of Cd and Ni in *N. arenaceodentata* occurred when there was an excess of AVS over SEM in sediments, but no significant mortality was found. Tissue concentrations of Cd and Ni in *N. arenaceodentata* increased with SEM level even when excess AVS over SEM concentrations existed. This result suggests the *N. arenaceodentata* could accumulate a considerable amount of Cd and Ni from particulate phase even up to similar concentration levels found for worms from sediments with excess SEM over AVS (Pesch et al., 1995). Since the toxicity of a contaminant must be closely related to the tissue concentration, it is significant that worms demonstrated substantial bioaccumulation of metals from sediment, although the biological effect caused by metals accumulated via different route might not be identical, because of toxicokinetic limitation. While bioaccumulated metals from dietary uptake might be less
responsible for acute effects, those metals may be
more related to the chronic effects from long-term
exposure found in the field conditions (Woodward
et al., 1994). Therefore, all pathways of metal uptake
especially from moderately contaminated sediments,
in which metal partitioning dynamically varied with
numerous geochemical factors such as redox poten-
tial, metal-sediment equilibration time, organic con-
tents, mineral composition, etc.

The lethal and sublethal effects observed here were
explained by the dissolved metal concentrations in
overlying water. By comparing our dissolved metal
concentration levels to the effect concentrations from
previous water-only tests, it may be possible to
determine which metal was most responsible for the
observed toxicity, with the assumption that most
biological adverse effect of metals came from the
exposure to dissolved phase. The dissolved concen-
trations of Cd (0.03 μM) and Ni (13.3 μM) in OW from
the treatment with the highest mortality (M4 in Low-
AVS series) were much lower than the 28-day median
lethal concentrations (LC50) for Cd (27 μM) and 10-
day LC50 for Ni (274 μM) from previous water-only
tests, while OW Zn (21 μM) in the same treatment was
comparable to 28-day LC50 (21 μM; Reish, 1985;
Pesch et al., 1995). Also, the mortality and growth
inhibition results were better related to OW Zn than Cd
or Ni, supporting the finding that Zn was most
responsible for the mortality of test organisms and
potentially for the sublethal effect observed.

Mortality and growth inhibition were signifi-
cantly related to tissue Cd and Zn concentrations;
however, they were not related to tissue Ni
concentrations. The insignificant relationship
between tissue Ni concentrations and growth rates
indicates that the bioaccumulation of Ni did not
reach adverse effect concentration for N. arenaceo-
dentata. Also, Cd bioaccumulation likely cause only
limited biological effects to test organisms since
some tissue Cd concentrations in growth-inhibited
worms overlapped with those in control worms.
Bioaccumulated Zn in worms as well as dissolved
Zn in water better explained the variation of growth
rate than Cd and Ni. The dissolved Zn speciation
was not varied substantially since we used seawater
and sediment from the same source and maintained
a similar experimental condition for all the treat-
ments (e.g. salinity, pH, dissolved oxygen and
temperature). However, total dissolved concen-
trations may not be a good indicator for the potential
toxicity under field conditions, and free ion activity
would a better server as an indicator accounting for
various ligand binding (Campbell, 1995). Further,
multiple pathways for metal accumulation in natural
conditions can complicate the prediction of the
potential (chronic) toxicity using dissolved metal
centration (or free ion activity) (Luoma and
Fisher, 1997). Measuring all the metal concentra-
tions in all sources (such as sediment, diets, etc.)
may not be possible. Therefore, measuring the
bioaccumulation of metals can be an alternative
indicator of potential (chronic) toxicity particularly
when measuring dissolved concentrations (or free
ion activity) is difficult or when other exposure
pathways than dissolved metal are not negligible
(Borgmann et al., 1993; Borgmann, 2000).

Most previous studies did not separate individual
metals having different solubility products when
evaluating the influence of the [SEM–AVS] on metal
bioavailability in sediments. So only total [SEM–
AVS] was used as predicting tool for metal toxicity.
However, to better understand the partitioning and
bioavailability of a specific metal in sediments, it is
appropriate to calculate [SEM–AVS] for each metal.
It is evident that all divalent metals do not behave
identically in sediments with varying [AVS]. There-
fore, it would not be appropriate to add all other
divalent metals whose solubilities as metal sulfides are
higher when calculating [SEM–AVS] to predict a
partitioning and bioavailability of a specific metal. For
example, Ni SEM concentrations may not affect the
formation of Ag2S in sediments. Since the affinity
of Ag to sulfur is much greater than Ni, only negligible
NiS will be formed until all available Ag is exhausted.
Yoo et al. (2004) showed that the partitioning and
toxicity of Ag was not related to [total SEM]–[AVS],
rather to [Ag SEM]/2–[AVS] or [Ag SEM].

Overall, the consideration about the geochemical
influences on the partitioning and bioavailability of
metals in sediments improved the prediction of lethal
and sublethal toxicity to N. arenaceodentata. Also,
tissue bioaccumulation of metals in test species was a
good indicator of metal toxicity from sediments, and
was useful to determine which element was more
responsible for the observed toxic responses. Bio-
accumulation of metals above a tolerable threshold
can be better related to the incidence of toxicity than environmental concentrations, especially under conditions having a complex media and multiple uptake pathways (Luoma and Fisher, 1997; Borgmann, 2000). Few studies have systematically evaluated the relationship between the bioaccumulation and toxicity of metals in benthic systems even though tissue concentration could reflect a real dose at target sites as well as direct measure of metal bioavailability. Further studies should be conducted to elaborate the functional relationship between bioaccumulation and toxicity of metals from sediments and define the numerous factors affecting the relationship.

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