# Influence of Acid Volatile Sulfide and Metal Concentrations on Metal Bioavailability to Marine Invertebrates in Contaminated Sediments

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An 18-day microcosm study was conducted to evaluate the influence of acid volatile sulfides (AVS) and metal additions on bioaccumulation from sediments of Cd, Ni, and Zn in two clams (Macoma balthica and Potamocorbula amurensis) and three marine polychaetes (Neanthes arenaceodentata, Heteromastus filiformis, and Spiophanes missionensis). Manipulation of AVS by oxidation of naturally anoxic sediments allowed use of metal concentrations typical of nature and evaluation of processes important to chronic metal exposure. A vertical sediment column similar to that often found in nature was used to facilitate realistic biological behavior. Results showed that AVS or porewater (PW) metals controlled bioaccumulation in only 2 of 15 metal-animal combinations. Bioaccumulation of all three metals by the bivalves was related significantly to metal concentrations extracted from sediments (SEM) but not to [SEM - AVS]or PW metals. SEM predominantly influenced bioaccumulation of Ni and Zn in N. arenaceodentata, but Cd bioaccumulation followed PW Cd concentrations. SEM controlled tissue concentrations of all three metals in H. filiformis and S. missionensis, with minor influences from metal-sulfide chemistry. Significant bioaccumulation occurred when SEM was only a small fraction of AVS in several treatments. Three factors appeared to contribute to the differences between these bioaccumulation results and the results from toxicity tests reported previously: differences in experimental design, dietary uptake, and biological attributes of the species, including mode and depth of feeding.

# Introduction

Understanding factors controlling the bioavailability of metals in sediments requires mechanistic knowledge of sediment geochemistry and organism-mediated exposure pathways. Numerous studies have emphasized that the geochemical nature of metal associations in sediments influences metal bioavailability (1-5) and that ecological and physiological differences can modify the extent to which organisms are exposed to metals (6-9).

The free-ion activity model effectively explains some of the geochemical influences on biological exposures (bioavailability) and effects of metals, for at least some aquatic organisms (10, 11). These concepts have been applied to sediments using equilibrium partitioning theory (12). Sulfides in anoxic sediments, especially acid volatile sulfides (AVS), can react with cationic metals to make insoluble metal sulfides (13-15) and can thereby control porewater (PW) metal concentrations (16, 17). Porewater metal concentrations are used to predict toxicity to benthic animals. In these studies AVS and simultaneously extracted metals (SEM) are operationally defined by solubility in cold weak HCl. It is asserted that if [SEM - AVS] < 0, then the PW metal concentrations should be low, and no toxicity of metals to sediment dwelling organisms should be observed (16-18). Acute toxicity is a common endpoint in studies concerned with AVS criteria (12, 16, 18-22). Some longer-term studies considered more complicated effects (23-26), but most studies employed high concentrations of metals. Thus acute responses are the best known (e.g., refs 23, 24, and 26), although bioaccumulation was considered in a few of studies cited above (19, 21, 27-29).

A number of factors that affect metal bioavailability from sediments have not been directly addressed in earlier studies of the [SEM - AVS] concept. By design, most laboratorybased studies controlled the relative concentrations of SEM and AVS by spiking sediments with metals. This resulted in covariance of SEM and [SEM - AVS]; direct influences of the two variables are not completely separated. Furthermore, high concentrations of metals are often needed in this design approach to achieve a range of [SEM - AVS] because initial [AVS] are usually high in the sediments. More moderate metal concentrations might better test chronic toxicity or questions about alternative exposure routes such as diet. Metal effects are also often tested in homogenized bulk sediments. In nature, complex, dynamic, three-dimensional oxidation gradients characterize natural sediments (30, 31). Benthic biota interact with those gradients in a variety of ways to obtain food and oxygen (31, 32). In addition, epifaunal and infaunal animals in anoxic sediments generally maintain an oxic microenvironment in their burrows and feeding zones either for O<sub>2</sub> respiration or to avoid sulfide toxicity.

In this study, we simulate environmentally realistic metal, AVS, and geochemical gradients in sediments and evaluate effects on bioavailability. Bioavailability is defined as the metal available from environmental media for accumulation into an organism. Bioaccumulation is used here to determine bioavailability because it is a biological process directly responsive to metal transport into the organism, which determines the metal dose the organism receives. If bioaccumulation is a general indicator of metal dose, it should have linkages to chronic toxicity. Understanding whether bioaccumulation can be predicted using AVS-based criteria is therefore important. An 18-d laboratory bioaccumulation study employs five test species: two bivalves (the filterfeeding clam Potamocorbula amurensis, the facultative suspension/deposit-feeding clam Macoma balthica) and three polychaetes (the surface deposit feeder Neanthes arenaceodentata, the head-down deposit feeder Heteromastus filiformis, deposit feeder Spiophanes missionensis). These species were chosen with consideration for their diversity in feeding behavior, feeding zone, burrowing mode, and direct contact with sediment. [SEM – AVS] was controlled in some treatments by manipulating [SEM], but in another set of

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treatments it was controlled by manipulating [AVS]. This design aided testing effects of AVS with environmentally realistic metal concentrations. A detailed geochemical characterization within distinct sediment boundaries was included to better approximate the exposure of animals in appropriate zones of the sediments. Geochemical aspects are reported in the preceding paper (*33*).

## **Experimental Design**

Two series of experiments were designed to evaluate the effects of metal concentration and AVS on bioaccumulation. In the first series [SEM – AVS] was manipulated by spiking four concentrations of a Cd–Ni–Zn mixture to sediments having 7.5  $\mu$ mol/g AVS (variable SEM series). In the second series [AVS] was manipulated to vary [SEM – AVS] while keeping the [SEM] constant (variable AVS series). Sediment with 7.5  $\mu$ mol/g AVS and no spiked metals was used as a control treatment.

Sediment Manipulation. Detailed protocols for sediment treatments and analysis are described elsewhere (33). Briefly, sulfide-rich sediment was collected from a tidal mudflat near Palo Alto, San Francisco Bay. Half of the collected sediments were mixed 1:1 with 0.22-µm filtered seawater (salinity 25 ppt) and was oxidized by bubbling with air for 3 d. The remaining half was mixed 1:1 with deaerated seawater and kept under a N2 atmosphere. The [AVS] after 3-d mixing in oxidized and anoxic sediments were 0.5 and 30  $\mu$ mol/g, respectively. The oxidized and anoxic sediments were mixed to achieve [AVS] of 0.5, 7.5, 15 and 30  $\mu$ mol/g, and then all were spiked with 0.06  $\mu$ mol/g Cd, 2.4  $\mu$ mol/g Ni, and 7  $\mu$ mol/g Zn (variable AVS series). [SEM] was varied in four sediments containing [AVS] = 7.5  $\mu$ mol/g, by spiking 0.02  $\mu$ mol Cd/g, 0.6  $\mu$ mol Ni/g, and 2  $\mu$ mol Zn/g in one sediment and 3, 5, or 7 times these concentrations in three other sediments, respectively (variable SEM series). Summed SEM was applied in [SEM – AVS] calculations (17, 33). When the [SEM] of a certain element was calculated for [SEM - AVS], all the metals with greater affinity for AVS than the metal of concern were considered together as SEM (see ref 33 for details).

**Experimental Protocol.** The experimental sediments were equilibrated for 4 d under a  $N_2$  atmosphere and mixed vigorously several times daily. The sediments were settled for 2 d, and then 4 L was transferred to duplicate 6-L polycarbonate containers. The transferred sediments were aerated and allowed to consolidate for 1 wk. Finally, the overlying water was replaced 3 times with new batch of salinity-25 ppt seawater over a period of 3 d. Overall, spiked metals were equilibrated with sediments for 16 d prior to animal introduction. Preliminary studies showed that partitioning of metals to porewater stabilized following this period.

After acclimation to experimental salinity (25 ppt) and temperature ( $15 \pm 1$  °C) over a 1 wk period (see Preparation of Experimental Animals, Supporing Information), the test animals (10 *M. balthica*, 10 *P. amurensis*, 14 *N. arenaceodentata*, 20 *H. filiformis*, and 20 *S. missionensis* per container) were introduced to the experimental sediments. The polychaetes *S. missionensis* and *H. filiformis* were introduced only to the variable AVS series. The density of test animals (see also ref *33*). Most treatments had two replicates. Half of the overlying water was changed every other day and continuously aerated with 0.45  $\mu$ m-filtered air during the incubation.

After 18 d, sediment cores and test animals were collected from the experimental sediments. Sediment cores were sectioned at 5 depth intervals (0-0.5, 0.5-1.5, 1.5-3, 3-4.5,and 4.5-7.5 cm, from surface to bottom), and each section was subjected to AVS, SEM and PW metal analysis (*33*). Test animals were removed, counted, and transferred to chambers

#### Clam M. Balthica



FIGURE 1. Mean tissue Cd, Ni, and Zn (bar) in the clam *Macoma* balthica as related to metal SEM (solid line) and [SEM – AVS] ( $\mu$ mol/g) at the end of 18-d incubation. Error bars represent standard deviation around the mean. In the variable AVS series, [AVS] increase from S1 (0.5  $\mu$ mol/g) to S4 (30  $\mu$ mol/g), and in the variable SEM series metal concentrations increase from control (Con) to M4.

that contained filtered seawater at a salinity of 25 ppt, to allow animals to depurate gut contents. Following 3-d depuration (no particles were apparent in the animals by this time), soft-tissues were removed from shells, freezedried, transferred to borosilicate glass vials, and digested with trace metal grade concentrated nitric acid (Ultrex) following established procedures (*34*). The detailed analytical procedure is given in Tissue Metal Analysis (see Supporting Information).

#### Results

Geochemical conditions in the different treatments were explained in detail by Lee et al. (*33*) and are summarized in Table 1 (see Supporting Information). Tissue metal concentrations of test animals were compared to the geochemistry of the horizon where the animals most actively fed and where redox conditions approximated their microhabitats. The two clams (*P. amurensis, M. balthica*) and the worm *N. arenaceodentata* ingest primarily surface sediments or maintain oxygenated conditions in their burrows (*31*), so their bioaccumulation was compared to surface sediments (0–1.5 cm). The remaining two deposit feeding worms ingest deep anoxic sediments and do not require strictly oxygenated conditions (*35*), so bioaccumulation in *H. filiformis* and *S. missionensis* was compared to deep sediments (3–7.5 cm).

**Metal Accumulation in the Bivalves.** Data from *M. balthica* are used to illustrate the change in bioaccumulation with extracted metal concentrations or [SEM – AVS] (Figure 1); regressions between key variables are shown for both bivalves in Figures 2 and 3. In the variable SEM series, bioaccumulation of Cd, Ni, and Zn in the two bivalves increased linearly with [SEM], PW metal concentrations (Figures 1–3), and with [SEM – AVS] (Figure 4 exemplified for Ni in *M. balthica*). All the relationships were statistically

Clam M. balthica



FIGURE 2. Relationships of mean tissue metal concentrations in the clam *M. balthica* with SEM and PW metals in the control (**I**), the variable SEM series (**O**), and the variable AVS series (**O**) of treatments. Error bars represent standard deviation around the mean. The dotted regression line is shown when the relationship is significant (p < 0.05). (\*\*: p < 0.01; \*\*\*: p < 0.001.)

significant (Figures 2 and 3). Causation in these relationships is unclear because all three independent variables covaried.

In the variable AVS series, it is important to note that covariation of [SEM - AVS] (or PW metal concentrations) with [SEM] was eliminated by varying [AVS] and holding [SEM] constant. In this series, both species of clams accumulated significantly more metals than the controls in all sediments. The degree of bioaccumulation was the same (not significantly different) as the uptake observed at the corresponding metal exposure in the variable SEM series. Tissue metal concentrations varied little with [SEM - AVS] or PW metal concentrations (Figures 1-4). For example, negative [SEM - AVS] values and very low PW metals were observed at the highest AVS concentration (S4), but both clams still accumulated significantly more Ni and Zn than the control clams. It is also notable that Cd bioaccumulation occurred when [SEM - AVS] < 0 in most treatments. P. amurensis accumulated slightly more Ni and Zn from the sediments with lower [AVS] (S1 and S2) than from sediments with higher [AVS] (S3 and S4) (see Table 1, Supporting Information). But the difference was a small fraction of the overall bioaccumulation.

When data were combined from both manipulation approaches (i.e., variable SEM and variable AVS series), tissue metal concentrations in both clams showed a significant positive relationship with [SEM] (p < 0.001) (Figures 2 and 3). Some significant relationships also occurred between bioaccumulation and PW metals or [SEM – AVS] in the combined data, but these relationships were driven by the variable SEM series (data not shown). The variable AVS series showed that this relationship was not the result of influences from PW metals or [SEM – AVS] (Figure 4).

**Bioaccumulation in the Polychaetes.** Bioaccumulation of all three metals in *N. arenaceodentata* increased with [SEM]

when variable SEM controlled [SEM – AVS] and among all data (Figure 5). Unambiguous designation of causation was not possible from the positive relationship between tissue and PW Ni and Zn in the variable SEM series, because of the covariance of PW metal and spiked metal levels. When variable AVS was used to control [SEM – AVS], the polychaete accumulated the same concentrations of Ni and Zn (~10  $\times$  more Ni and 2  $\times$  more Zn than control worms) in all four treatments. Bioaccumulation was similar to that observed at that metal exposure in the variable SEM series. Bioaccumulation of Ni and Zn did not change as [SEM – AVS] or metal concentrations in PW changed, when the latter were manipulated by varying AVS (Figures 4 and 5). Uptake of Ni and Zn in the worm followed SEM in this series, but not PW Ni and Zn.

*N. arenaceodentata* accumulated high concentrations of Cd from sediments containing the least [AVS] (S1) compared to S2–S4 treatments. The best explanation for this result is that the PW source was important for Cd accumulation in this species. The Cd uptake in *N. arenaceodentata* related significantly (p < 0.001) with PW metals in the variable AVS series, largely as a result of the S1 treatment.

Notable mortality was observed for *N. arenaceodentata* and seemed to correspond to the PW metal concentrations. Mortality of *N. arenaceodentata* increased from 10% in control, M1 and S4 treatment to 23% in S3 and to 58-96% in the rest of treatments.

Bioaccumulation by *H. filiformis* and *S. missionensis* was studied only by varying AVS (Figure 6). The significance of treatment effects could not be evaluated statistically because there was only one sample of 20 pooled individuals for each treatment. No obvious bioaccumulation of Cd in these two worms was found, although they were exposed to much greater Cd in sediments than the control worms. Cadmium



FIGURE 3. Relationships of mean tissue metal concentrations in the clam *P. amurensis* with SEM and PW metals in the control (**I**), the variable SEM series (**O**), and the variable AVS series (**O**) of treatments. Error bars represent standard deviation around the mean. The dotted regression line is shown when the relationship is significant (p < 0.05). (\*: p < 0.005; \*\*\*: p < 0.001.)

associated with sediments was not apparently bioavailable to these worms. In contrast, Zn concentration in these two worms were ~2 × Zn in control worms. Ni in *H. filiformis* was ~11 × that of Ni in control worms and was related neither to PW metals nor to [SEM – AVS]. Uptake of both Ni and Zn by *H. filiformis* was similar among the different [AVS], which was consistent with (the similar) spiked metal concentration among treatments. However, Ni in *S. missionensis* from S1 was 2–3 × that found in the other treatments and 8 × that from control. This result suggests that *S. missionensis* accumulated Ni from both PW and other (dietary) sources.

**Comparison of Metal Bioavailability among Species.** The slopes of the regression between tissue metals and SEM for each metal could represent overall bioavailability from sediment (bioaccumulation factor), when a significant relationship existed between the two variables (Figures 2, 3, and 5). The slopes for the three metals increased in the order *P. amurensis* > *N. arenaceodentata* > *M. balthica.* Compared to *M. balthica, P. amurensis* had much higher Cd and Ni in the control group and responded much greater to spiked sediments. *M. balthica* accumulated ~190  $\mu$ g/g Zn in the control group (3 × the Zn in *P. amurensis* for control group) but responded to sediment Zn ~4 × less than did *P. amurensis.* Among three worm species, generally *N. arenaceodentata* bioaccumulated the three metals most and *H. filiformis* least.

#### Discussion

The equilibrium partitioning-based AVS normalization approach predicts that PW metals should be low when SEM < AVS (16, 17). Consistent with this idea, we found that concentrations of PW Cd, Ni, and Zn in the experimental sediments used for this study were controlled by AVS concentration. Toxic effects were eliminated in most earlier



FIGURE 4. Relationships of mean tissue Ni concentrations in the clam *M. balthica* and the polychaete *N. arenaceodentata* with [SEM – AVS] ( $\mu$ mol/g) in the control (**II**), the variable SEM series (**O**), and the variable AVS series (**O**) of treatments. The vertical lines represent [SEM – AVS] = 0. Error bars represent standard deviation around the mean. (\*\*\*: p < 0.001.)

#### Polychaete N. arenaceodentata



FIGURE 5. Relationships of mean tissue metal concentrations in the polychaetes *N. arenaceodentata* with SEM or PW metals in the control (**■**), the variable SEM series (**●**), and the variable AVS series (**○**) of treatments. Error bars represent standard deviation around the mean. The dotted regression line is shown when the correlation coefficient is significant (p < 0.05). (\*\*\*: p < 0.001.)



FIGURE 6. Tissue Cd, Ni, and Zn (bar) in the polychaetes *H. filiformis* and *S. missionensis* as related to metal SEM (solid line) and [SEM - AVS] ( $\mu$ mol/g) at the end of 18-d incubation.

studies when SEM  $\leq$  AVS (*17, 18, 26*). But in the present study organisms bioaccumulated significant amounts of metals from sediments when SEM was a only small fraction

of AVS (i.e. SEM  $\ll$  AVS). Bioavailability increased linearly with the sediment metal concentrations irrespective of AVS or PW metal concentrations. Other studies (*21, 26, 36, 37*) that showed benthic animals can bioaccumulate metals when SEM < AVS alluded to ingestion of contaminated food, heterogeneity of geochemistry around animals' microhabitats, adsorption to surface membranes, or differences in behavior of animals as potential mechanisms. Those factors, along with aspects of experimental design, may help explain the differences in interpretation of bioavailability between the present study and the earlier body of work.

It is also possible that another reason for the differences is the choice of the biological response used to study bioavailability. AVS-based criteria are currently being considered as sediment quality guidelines by the regulatory community (38) and so have been developed to directly address lack of toxicity in metal contaminated sediments (16, 17). Bioaccumulation is not a direct measure of adverse effect but only an indicator for the potential for metal toxicity (39, 40) because it is a surrogate measure of dose (6, 41, 42). But generic relationships between bioaccumulation and toxicity of metals are not yet well understood. Some sitespecific instances are documented where elevated bioaccumulation is accompanied by chronic adverse metal effects (40). Broad linkages between bioaccumulation and toxicity may be complicated. For example, animal species differ in abilities to detoxify bioaccumulated metals (e.g., via metallothionein or granule induction) or develop tolerance (41). There is also emerging evidence that metals bioaccumulated from dietary source could have novel, chronic adverse effects on aquatic organisms (42-45).

The protocol used to manipulate the relative concentrations of metals and AVS can have important influences on interpretations of sediment bioassays. The series of treatments that varied metal concentrations with a constant [AVS] simultaneously varies two variables, so causation is ambiguous. Clearly separating the variables by varying [AVS] and holding [SEM] constant demonstrated that bioavailability followed changes in particulate metal concentrations alone, in 12 of 15 metal-species combinations. This does not mean that metal concentration will always predict bioavailability in nature, but it does illustrate the importance of experimental design in defining geochemical influences on bioavailability. Most previous studies also used high metal concentrations and relatively short metal-sediment equilibration times. The combination of high concentrations and short equilibration time could shift the partitioning of metals preferentially to PW (6, 33). Varying [AVS] allowed use of environmentally relevant concentrations of metals, manipulation of [SEM -AVS] without changing the metal concentrations of sediment, and it minimized distortion of particle/water partitioning.

The results of the present study are best explained if dietary uptake from ingested sediments was often a dominant route of Cd, Ni, and Zn bioaccumulation by the five benthic invertebrates. Dietary uptake would explain why metal bioaccumulation was better related to extractable, sedimentassociated metals than to PW metals. In a radiotracer experiment, Lee et al. (46) directly demonstrated that *M. balthica* and *Mytilus edulis* could assimilate sulfide-associated Ag and Cd. The assimilation efficiencies from metal sulfide particles were similar to or exceeded those from metals associated with oxidized particles. Others also showed that metals associated with anoxic sediments were available for assimilation by the deposit feeding polychaete *Neanthes* (*Nereis*) succinea (47) and bivalves *M. balthica* and *M. edulis* (48).

Microcosm studies and biokinetic models (6, 7, 37, 45– 49) report that dietary uptake is often largely responsible for metal bioaccumulation in benthic animals. For example, Lee et al. (37) concluded that bioaccumulation of Ag, Cd, and Zn in *N. arenaceodentata* occurred predominantly from ingestion of contaminated sediments and contaminated supplementary food over a range of [SEM – AVS]. Wang et al. (47) estimated that >98% of Cd, Co, Se, and Zn, and >65% of Ag bioaccumulation in the worm *Nereis succinea* were accounted for by ingestion of contaminated sediments. Munger and Hare (49) reported that bioaccumulation of Cd in the freshwater insect *Chaoborus punctipennis* was predominantly taken up from ingested food.

Experimental design may also influence whether animals are exposed to metals via diet. Animals exposed to high PW metal concentrations may not burrow into sediment (21). Similarly, animals may avoid ingestion of extremely contaminated sediments (8). High metal concentrations could also contribute to the onset of toxicity before more chronic, dietary effects are manifested or organisms may die before they begin ingesting sediments. A vertical redox gradient with a surface oxidized layer may be critical to normal behavior and, perhaps, ingestion of sediment. The oxidized characteristics of surface sediments may not be the only important factor in conditioned sediments, but it could be a surrogate for oxygenated microhabitats such as burrows (31).

Comparison of several species of test animals with different biological attributes has significant advantages for understanding the biological and geochemical influences on metal bioaccumulation. Feeding mode, burrowing depth, feeding depth, and irrigation habits all can have an important influence on metal exposure (*3, 6, 26, 50, 51*). For example, the head-down deposit feeder *H. filiformis* inhabits deep sediments, ingests sulfide-rich sediments, and does not actively irrigate their burrows with oxic overlying water. This animal was most likely exposed to a microenvironment where metals were predominantly in metal sulfide precipitates and

where concentrations of PW metals were minimal. *H. filiformis* did not bioaccumulate Cd, but it did bioaccumulate Ni and Zn (although less than other species). Consistent with this, field studies showed that *H. filiformis* accumulated negligible amounts of Cd, Ni, and Zn from a metal contaminated mud flat (*52*) and survived in sediments highly contaminated with Zn and Pb (16700 and 5270  $\mu$ g/g, respectively) (*53*).

The bivalves, in contrast, can filter particles from the water-sediment interface and retain these particles within their digestive tracts for long periods of time (gut residence is 24-72 h (54)). Sulfide chemistry and PW metal concentration had the least impact on metal exposure to bivalves. It is notable that PW Ni and Zn had a small influence on tissue concentrations in P. amurensis but not in M. balthica. M. balthica is primarily a deposit feeder. It has some capability of filter-feeding, but its filtration rate (55), feeding rate, and gut passage time (56) are slow compared to P. amurensis, which primarily feeds by filtering large volumes of suspended particles (e.g., filtration rate is  $10 \times$  or more that of *M*. balthica (55)). The greater filtration rate of P. amurensis could accentuate uptake from dissolved routes. The greater feeding rate could also increase overall bioaccumulation. Consistent with this, Lee et al. (55) reported that influx rates of Cd, Cr, and Zn in *P. amurensis* from a dissolved source were  $4-5 \times$ those in M. balthica.

Neanthes (or Nereis) is an active surface burrowing worm. Irrigation of its burrow with oxidized water might enhance exposure to PW metals through gills or surface membranes. Additionally, these worms ingest large amounts of sediments. Cammen (57) reported that Neanthes (Nereis) succinea daily ingests  $2-9 \times$  more sediment than their body dry weight. The high ingestion rate might explain the predominant role of metal uptake from ingestion of contaminated sediments predicted by Wang et al. (47) in Neanthes (Nereis) succinea from biokinetic modeling and indicated by our observations for Ni and Zn. Similarly, Lee et al. (37) reported that contributions of PW to the metal bioaccumulation in N. arenaceodentata exposed to a variable AVS sediment series for 25 d were up to 8% for Ag, 30% for Cd, and 20% for Zn, even in the most oxidized sediments. The reasons why PW Cd had a significant influence on bioaccumulation by N. arenaceodentata in the present study are not apparent, however.

Development of sediment quality criteria will require better understanding the significance of less than extreme sediment contamination. In such circumstances, exposure of organisms may occur via ingestion of particles and effects on populations may be manifested via chronic toxicity. Field efforts or laboratory investigations that employ protocols capable of simulating naturally occurring contaminated sediments are the next step in advancing such understanding, including more fully evaluating how broadly the AVSnormalized approaches should be applied. Consideration of chronic responses and mechanisms that link toxicity and bioaccumulation is also an important next step, particularly with regard to understanding links between long-term dietary exposures to metals and adverse effects.

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### Supporting Information Available

Preparation of experimental animals, tissue metal analysis, and Table 1. This material is available free of charge via the Internet at http://pubs.acs.org.

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