Influences of Dietary Uptake and Reactive Sulfides on Metal Bioavailability from Aquatic Sediments

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Understanding how animals are exposed to the large repository of metal pollutants in aquatic sediments is complicated and is important in regulatory decisions. Experiments with four types of invertebrates showed that feeding behavior and dietary uptake control bioaccumulation of cadmium, silver, nickel, and zinc. Metal concentrations in animal tissue correlated with metal concentrations extracted from sediments, but not with metal in porewater, across a range of reactive sulfide concentrations, from 0.5 to 30 micromoles per gram. These results contradict the notion that metal bioavailability in sediments is controlled by geochemical equilibration of metals between porewater and reactive sulfides, a proposed basis for regulatory criteria for metals.

A central problem in biogeochemistry and environmental management is understanding the uptake and transfer into food chains of aquatic contaminants, including heavy metals. Toxic metals introduced into aquatic environments by human activities typically accumulate in sediments. A common notion is that the association of metals with reactive sulfides controls toxicity in sediments by controlling porewater metal concentrations. An acid-extractable fraction of (iron) sulfides, called AVS (acid-volatile sulfide), can form thermodynamically stable metal sulfide precipitates in sediments, and thereby governs the behavior of divalent metals such as Cd, Cu, Ni, Pb, and Zn (*1*, *2*). Acute metal toxicity to benthic animals did not occur in experiments when there was sufficient AVS in the sediment to sequester all of the simultaneously extracted metal [termed SEM or EM (*3*)]; that is, when [EM − AVS] < 0 or EM/AVS < 1 (*2*, *4*). These experiments typically used conditions that increased the likelihood that porewater metal concentrations were high and that porewater controlled metal bioavailability. To evaluate bioavailability more realistically, we studied bioaccumulation of environmentally relevant concentrations of metals (Cd, Ni, and Zn), and we used a sediment column with vertical stratification of oxygen concentrations, which is typical of natural conditions. The ratios of porewater to sediment metal concentrations in our experiments were in the range of those commonly observed in nature, and experimental conditions were such that the animals were likely to feed on the sediments.

We examined Cd, Ni, and Zn accumulation in four types of benthic invertebrates: the filter-feeding clam *Potamocorbula amurensis,* the facultative deposit-feeding clam *Macoma balthica,* the surface deposit-feeding worm (polychaete) *Neanthes arenaceodentata,* and the head-down deep deposit–feeding polychaete *Heteromastus filiformis.* All are common in estuaries. Sulfide-rich anoxic sediment was obtained from a tidal mudflat in San Francisco Bay and was held under anoxic conditions (mixed with deaerated filtered seawater and kept under a N_2 atmosphere). In order to vary AVS, an aliquot of this sediment was mixed with 0.45-μm filtered seawater (salinity 25) and oxidized by bubbling continuously with air for 3 days. The AVS was readily oxidized and declined from 30 to 0.5 μmol g^{-1}. Oxidized and anoxic sediments were mixed at varying ratios to produce four levels of AVS (0.5, 7.5, 15, and 30 μmol g^{-1}). In one series of experiments, sediments containing a single nominal AVS concentration of 7.5 μmol g^{-1} were enriched with four levels of a Cd-Ni-Zn mixture (*5*). Unenriched sediment (AVS = 7.5 μmol g^{-1}) was used as a control. In a second series, the four nominal AVS treatments each received one concentration of the metal mixture (*5*). Before the introduction of test animals, a vertical redox gradient was established in each experimental microcosm by 1 week of equilibration with an oxidized water column (*6*). AVS, EM, and porewater metal concentrations were determined at the beginning and end of the 18-day bioaccumulation experiment (*7*). Animals were allowed to defecate their gut contents before tissue metal analysis.

Results for Cd were illustrative of those for all three metals (Fig. 1). Both clams accumulated significantly more Cd in all treatments than did controls, although the molar concentrations of extractable Cd in sediment (0.02 to 0.2 μmol g^{-1}) were substantially lower than the AVS concentrations (0.5 to 30 μmol g^{-1}). In all treatments, [EM − AVS] was less than zero, and concentrations of Cd in porewater were low (Fig. 1). Tissue Cd concentrations in *M. balthica* and *P. amurensis* increased linearly with increasing extractable Cd in sediment (Fig. 2; slope ± SE for *M. balthica* = 0.14 ± 0.02, $P < 0.001$). Tissue Cd followed Cd in porewater only when the metal concentration in sediment was varied, but not when only AVS was varied (Fig. 1); nor did Cd uptake increase when AVS decreased. No significant association ($P > 0.05$) was found between tissue Cd concentrations and Cd in porewater or [EM − AVS] (Fig. 2). Thus, extractable Cd in sediment, not AVS, controlled Cd bioaccumulation in our experiments. Similarly, bioaccumulation of Ni and Zn by the clams was also best related with extractable metal concentrations ($P < 0.001$). AVS and porewater metals had no apparent relation to the bioaccumulation of Ni and Zn when the confounding influence of sediment metal concentration was eliminated by varying only AVS.

Bioaccumulation from porewater governed Cd bioaccumulation in *N. arenaceodentata* (Fig. 1). This result was most obvious in the sediment containing the lowest AVS and highest Cd in porewater. Tissue Cd concentrations in *N. arenaceodentata* increased with porewater concentrations ($P < 0.001$) but not with extractable Cd ($P > 0.05$). However, Ni and Zn bioaccumulation by *N. arenaceodentata* did not follow porewater concentrations or the predicted influences of AVS. As with the clams, Ni and Zn uptake increased with concentrations of extractable metals (slope ± SE for Ni = 0.18 ± 0.02 and for Zn = 0.30 ± 0.06, $P < 0.001$). The uptake of Cd predominantly from porewater in *N. arenaceodentata* contradicted biokinetic model predictions that dietary uptake accounts for >98% of Cd bioaccumulation in the polychaete *Nereis succinea* (*8*).

No significant bioaccumulation of Cd was found for the head-down deposit-feeding worm *H. filiformis*, which was exposed only to variable AVS. Because *H. filiformis* inhabits deep sediments and does not actively aerate its burrow, Cd availability to this species may be strongly influenced by the high AVS in its microhabitat. *H. filiformis* did accumulate Ni (at a level ~11 times that of the

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control) and Zn (at a level of ~2 times that of the control) in proportion to metal concentration in sediments and not to EM/AVS.

A poor association between porewater metals and bioaccumulation of metals by animals was observed in 10 of the 12 treatments (four species times three metals). One explanation is that organisms usually accumulated metals mainly through direct ingestion of sediment, regardless of AVS content. A radiotracer experiment was conducted to directly test the bioavailability of dietary Cd and Ag sulfide. We also compared metal sulfide bioavailability to that of metals on oxidized particles. The latter may constitute an important particle type eaten by benthic animals. Radioactive ^{109}Cd and ^{110m}Ag were coprecipitated with FeS onto glass beads (10 μm in diameter) and cured for 18 hours according to an established method (*9*). An aliquot of the metal sulfide was reoxidized by bubbling with filtered air for 18 hours. The sulfide-coated and reoxidized particles were suspended separately in seawater (salinity 30) and fed to *M. balthica* and the mussel *Mytilus edulis* for 5 min. The assimilation efficiency of ingested ^{109}Cd and ^{110m}Ag was determined by a pulse-chase protocol (*10*).

Both bivalves assimilated 14 to 19% Cd from metal sulfide particles, which is comparable to assimilation from reoxidized particles (10 to 20%) (Table 1). Deposit-feeding *M. balthica* also assimilated Ag from metal sulfide particles with an efficiency of 15 to 28%; assimilation from reoxidized particles was 16 to 21%. The filter-feeding mussel *Mytilus edulis* assimilated Ag from both particle types with lower efficiency than did *M. balthica*, but metal availability from sulfide precipitates was not lower than from reoxidized particles in either bivalve. Ingested particulate metal sulfides could be solubilized if gut fluids were mildly acidic, by retention for more than a day in a mildly oxidized gut (*11*), or by association with the high concentrations of organic ligands that are typical of invertebrate gut fluids (*12*). Additionally, bivalves allocate ingested food to the digestive gland, where intensive intracellular digestion occurs, which could further enhance metal assimilation (*13*). Whatever the mechanism, it is apparent that the AVS-independent correlation of tissue and sediment metal concentrations could be caused by direct metal accumulation from diet, even if most metals in that diet were in sulfide form. Others (*14*) have also shown the importance of dietary metal uptake in benthic invertebrates.

If porewater metal concentrations were solely responsible for metal bioavailability, organisms living in sediments should be protected from metal effects when AVS is in excess of extractable metals. We applied this concept to Ag bioavailability as observed in ~200 different samplings between 1977 and 1998 from a South San Francisco Bay mudflat (*15*). The sediments from this mudflat contained molar concentrations of extractable Ag (extracted with 0.4 N HCl) of ≤0.02 μmol g^{-1}, and total Ag rarely exceeded 0.03 μmol g^{-1} (*15*, *16*).

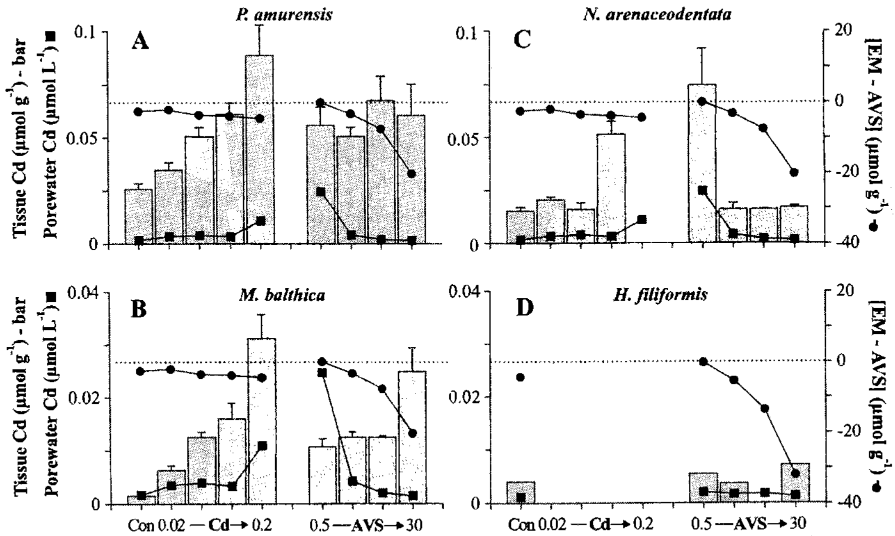

Fig. 1. Mean tissue Cd concentrations (bars) in the clams *P. amurensis* (**A**) and *M. balthica* (**B**) and the polychaetes *N. arenaceodentata* (**C**) and *H. filiformis* (**D**), related to extractable Cd, AVS [EM − AVS] (circles), and porewater Cd (squares) determined at the end of an 18-day bioassay. Error bars represent SDs around the mean (n = six replicates of three individuals for each clam; n = four replicates of one to six individuals for *N. arenaceodentata*; and n = 1 of 20 individuals for *H. filiformis*). No *N. arenaceodentata* data were available for the highest Cd treatment because of mortality. The dotted line represents [EM − AVS] = 0, below which animals should be protected from the effects of toxic metals, according to previous studies (*2*). The [EM − AVS] or porewater Cd values are from the depth where the animals feed most actively (*6*). Con, control treatment without metal enrichment (AVS = 7.5 μmol g^{-1}).

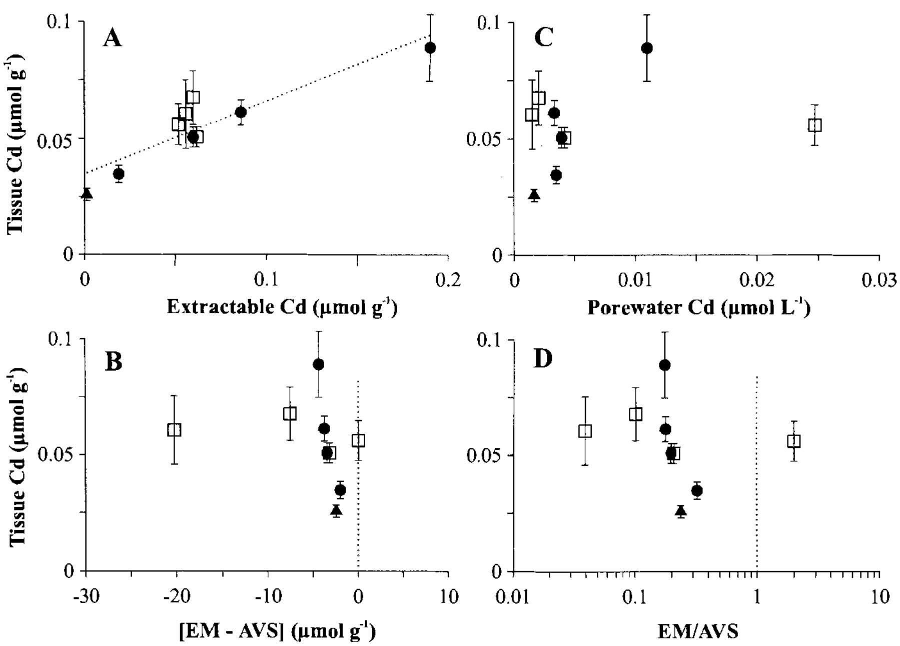

Fig. 2. Mean tissue Cd concentration (μmol g^{-1} dry weight) in *P. amurensis* when extractable Cd was varied and AVS was held constant (circles), or when AVS was varied and extractable Cd was held constant (squares). The control (triangles) was from the unenriched sediment with a nominal AVS of 7.5 μmol g^{-1}. The relationships of (**A**) tissue Cd to extractable Cd, (**B**) [EM − AVS], (**C**) porewater Cd, and (**D**) EM/AVS are shown. Error bars represent SDs around the mean (n = six replicates of three individuals). The vertical dotted line in (B) and (D) represents either [EM − AVS] = 0 or EM/AVS = 1. A significant relationship was found only between tissue Cd and extractable Cd ($y = 0.32x + 0.03$, $P < 0.001$).