

Assessment of Sedimentary Cu Availability: A Comparison of Biomimetic and AVS Approaches

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Sedimentary Cu bioavailability during deposit feeding is determined by both the digestive physiology of the organisms and the geochemistry of the sediments. We assessed the contribution of these two factors by using a biomimetic approach involving extraction of Cu with digestive fluids of two deposit feeders and one suspension feeder and a geochemical approach measuring Cu associated with acid-volatile sulfide (AVS) in sediments. Cu bioavailability determined by the biomimetic method varied among species with varying digestive physiology but all showed a marked increase when $SEM_{Cu}-AVS \geq 0$, corroborating the premise underlying the AVS method in determining sedimentary Cu bioavailability. The existence of a positive $SEM_{Cu}-AVS$ threshold ($0.4-1.5 \mu\text{mol/g-sediment}$) suggests the existence of additional Cu-binding phases or mixed Cu(I)-Cu(II) sulfides in sediments. In addition, Cu bioavailable to digestive fluids was much less than that measured as $SEM_{Cu}-AVS$, indicating that the AVS method overestimates Cu bioavailability to digestive fluid of deposit feeders. Incubation of digestive fluids with two Cu-bound model phases, goethite and sulfide, corroborated the relative unavailability of sulfide-bound Cu. Subsurface deposit feeders feeding on anoxic sediments may be exposed to less Cu than their surface-feeding counterparts in Cu-contaminated environments.

Introduction

Geochemical approaches have been frequently used to assess bioavailability of sedimentary metals since the 1980s (1 and references therein). These approaches are based on the principle that acid-volatile sulfide (AVS), organic matter, and hydroxides of Al and Fe act as solid metal-binding phases to prevent availability and toxicity of metals to organisms (2-4). For example, Fe-normalized metal concentrations in iron oxide fractions measured by a sequential extraction method positively correlated with bioaccumulation data (5), and toxicity correlated with the increasing differences between simultaneously extracted metal (SEM) and AVS (e.g., SEM/AVS or $SEM-AVS$) (6). However, inconsistent results have been observed using these approaches to assess sediment toxicity (6, 7). Thus it is more conservative to use geochemical approaches to stress the nontoxic nature of the sediments (e.g., $SEM/AVS < 1$) rather than to state the degree of toxicity (e.g., when $SEM/AVS \geq 1$) (8). Sedimentary metal bioavailability is a result of interactions between organisms and

geochemical aspects of sedimentary metals. Thus geochemical approaches address only one side of the interaction, which might have contributed to uncertainty in assessing bioavailability and hence subsequent toxicity (6-7, 9). The other side of bioavailability, deposit-feeding biology and digestive physiology (10-12), has been rarely addressed until recently.

Deposit feeders can accumulate high concentrations of heavy metals from contaminated environments via ingestion of sediments (1, 13). A biomimetic approach, which measures metals solubilized during incubation of digestive fluids of deposit feeders with contaminated sediments, has been developed to assess sedimentary metal bioavailability (12). Results show that digestive fluids enhance solubilization of metals from sediments but vary greatly among species of deposit feeder. Dissolved, histidine-containing proteins and peptides in digestive fluids, with concentrations varying among deposit feeders, were found to be responsible for releasing and complexing sedimentary Cu (14). However, our results also indicate that variations in ligand concentration alone cannot account for the observed bioavailability. Rather, the amount of metal released is determined by the ratios of gut ligand concentration to sedimentary metal loading and is a function of reaction time between sediments and digestive fluids (15), indicating the complexity of organism-sediment interaction in determining metal bioavailability.

The objectives of this study are to investigate the relationship between sulfide-binding and digestive solubilization of Cu and to make a quantitative comparison between bioavailable fractions measured by the AVS and biomimetic approaches. Specifically, we test whether sedimentary sulfide plays an important role in limiting Cu solubilization by the digestive fluid of deposit feeders, considering sulfide as a strong Cu-binding phase preventing Cu toxicity (3). We focus on Cu bioavailability because Cu is one of the most common contaminants in coastal environments where deposit feeders are abundant. We measured the amounts of Cu released by digestive fluids of three species of organisms from sediments having various $SEM_{Cu}-AVS$ values, which correlate with the degree of Cu solubility. We then investigated whether digestive fluids can solubilize Cu from synthesized sulfide and goethite ($\alpha\text{-FeOOH}$) phases.

Experimental Section

In vitro Digestion. Surface sediments (Table 1) were collected with acid-cleaned polyethylene spoons and stored in Ziploc bags at 4 °C for less than a week before used in experiments. Sediments with a wide range of Cu and AVS concentrations were sampled from estuarine sites in ME, U.S.A. including sites influenced by shipyard and tanker activities.

Digestive fluids of three species, the polychaete *Arenicola marina* and the holothuroids *Parastichopus californicus* and *Cucumaria frondosa*, were collected and stored as before (14). In vitro digestion was started by adding 0.4 mL of digestive fluid to 0.2 g of wet sediment (three replicates), and then the mixtures were incubated at room temperature for 4 h on a shaker. The experiments were stopped by centrifuging the slurry for 30 min at 8000g and 4 °C, and the fluids were analyzed for Cu, Pb, Cd, Zn, and Ni. Controls included digestive fluids without sediment and seawater incubation with sediments. Metals in fluids and sediments were measured by a graphite furnace atomic absorption spectrophotometer (12).

Acid-Volatile Sulfide. AVS was measured with a modification of a cold HCl method (16). Wet sediments (2-7 g)

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TABLE 1. Cu Released by 1 N HCl, Gut Fluids of *Arenicola marina*, *Parastichopus californicus*, *Cucumaria frondosa*, and Seawater from the Sediments of Boothbay Harbor (BBH), Portland Harbor (PLH), Kennebec Estuary (BIW) and Lubec (SO), ME, U.S.A. as Well as Total Sedimentary Cu and Acid-Volatile Sulfide (AVS)

| sample ID | 1 N HCl | <i>A. marina</i> | <i>P. californicus</i> | <i>C. frondosa</i> | seawater | total Cu | AVS |
|------------------------|-------------|------------------|------------------------|--------------------|---------------|-------------|-------------|
| BBH-1 (I, sand) | 7.01 ± 0.30 | 1.65 ± 0.43 | 0.78 ± 0.37 | 0.19 ± 0.05 | 0.006 ± 0.001 | 8.59 ± 0.08 | 0.48 ± 0.08 |
| BBH-2 (I, coarse sand) | 1.19 ± 0.02 | 0.18 ± 0.01 | 0.16 ± 0.01 | 0.04 ± 0.00 | 0.001 ± 0.000 | 1.20 ± 0.01 | 0.02 ± 0.01 |
| BBH-3 (I, sand) | 3.66 ± 0.09 | 1.34 ± 0.24 | 0.22 ± 0.13 | 0.14 ± 0.03 | 0.006 ± 0.002 | 6.00 ± 0.14 | 0.84 ± 0.21 |
| BBH-4 (I, sandy silt) | 5.50 ± 0.09 | 1.74 ± 0.64 | 0.59 ± 0.07 | 0.07 ± 0.01 | 0.020 ± 0.002 | 9.56 ± 0.34 | 0.19 ± 0.02 |
| BBH-5 (I, silt) | 0.70 ± 0.00 | 0.09 ± 0.02 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.000 ± 0.000 | 1.79 ± 0.02 | 0.34 ± 0.05 |
| BBH-6 (I, clay) | 1.57 ± 0.06 | 0.44 ± 0.04 | 0.08 ± 0.01 | 0.00 ± 0.00 | 0.001 ± 0.000 | 3.31 ± 0.16 | 0.12 ± 0.02 |
| BBH-7 (I, coarse sand) | 0.03 ± 0.00 | 0.01 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.000 ± 0.000 | 0.06 ± 0.00 | 0.39 ± 0.12 |
| BBH-8 (S, silty clay) | 0.28 ± 0.01 | 0.07 ± 0.01 | 0.02 ± 0.01 | 0.00 ± 0.00 | 0.000 ± 0.000 | 0.28 ± 0.00 | 0.16 ± 0.04 |
| PLH-F (I, silt) | 0.40 ± 0.01 | 0.05 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.000 ± 0.000 | 0.48 ± 0.01 | 2.24 ± 0.39 |
| PLH-B (S, silt) | 0.18 ± 0.01 | 0.03 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.000 ± 0.000 | 0.18 ± 0.00 | 1.27 ± 0.08 |
| BIW-1 (S, silt) | 0.15 ± 0.00 | 0.02 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.000 ± 0.000 | 0.50 ± 0.01 | 0.09 ± 0.01 |
| BIW-2 (S, silt) | 0.14 ± 0.01 | 0.01 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.000 ± 0.000 | 0.30 ± 0.01 | 0.07 ± 0.00 |
| BIW-3 (S, silt) | 0.10 ± 0.00 | 0.01 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.000 ± 0.000 | 0.30 ± 0.01 | 0.09 ± 0.03 |
| SO (I, sand) | 0.01 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.000 ± 0.000 | 0.04 ± 0.00 | 0.03 ± 0.01 |

^a All data as mean ± 1 SD ($n \geq 2$) with a unit of $\mu\text{mol/g-sed}$. I = intertidal, S = subtidal.

were digested in a system previously flushed with nitrogen by using deaerated HCl adjusted to 1 N final concentration. The evolved H_2S was carried away by a steady flow of nitrogen and collected in a deaerated solution of 0.03 M zinc acetate plus 0.012 M sodium acetate and precipitated as ZnS. The precipitated sulfide was then measured colorimetrically (17, 18) with calibration curves made with freshly prepared Na_2S solution standardized against thiosulfate. The nitrogen flow was adjusted so that recovery of Na_2S standards was better than 90%. The extraction slurry was centrifuged at 5000g for 20 min, and the supernatant was used for analysis of simultaneously extracted Cu, Pb, Cd, Zn, and Ni (SEM).

Cu Bound on Goethite. Goethite-coated glass beads with a mass content of 0.3% Fe were synthesized following a modification on Atkinson et al. (19), by aging the acid-washed microbeads (20–27 μm , Ferro Co. Cleveland) with 0.17 M $\text{Fe}(\text{NO}_3)_3$ and 1.02 M KOH solutions for 36 h in a 70 °C oven. The coated beads were washed 10 times with MilliQ water and stored as wet sediments in a polyethylene tube at 4 °C until use. The procedure yields a red-brown sand with surface area of about 50 m^2/g (19). Cu^{2+} was adsorbed onto these synthesized beads (two replicates) by spiking 50 μL of CuSO_4 solution containing $3.84 \times 10^{-2} \mu\text{mol}$ Cu into a centrifuge tube containing about 0.3 g of wet beads slurried with 0.2 mL of pH 7 MOPS (4-morpholinepropanesulfonic acid) buffer (0.01 M MOPS and 0.01 M NaNO_3), so that the Fe:Cu ratio in each tube was about 360:1 with a sedimentary Cu concentration of about 12 ppm which is typical for in situ sediments with 0.3% Fe (20). The mixtures were vortexed and incubated at room temperature for 24 h. The adsorption of Cu onto goethite was close to 100%, because no Cu was detected in the supernate after the incubation. The apparently strong adsorption of Cu on goethite is consistent with surface complexation models (21). An aliquot of 0.4 mL of gut fluids or seawater then was added to the tube to initiate in vitro digestion experiments. Controls included beads without spiked Cu^{2+} and seawater with Cu-bound beads.

Cu Bound on Sulfides. This experiment was processed in a N_2 glovebox except for centrifugation which was under continuous N_2 flow. A mixture of CuS and FeS (two replicates) was freshly made by spiking 50 μL of CuSO_4 solution containing $3.84 \times 10^{-2} \mu\text{mol}$ of Cu into a centrifuge tube containing 0.2 g of dry acid-cleaned microbeads and 200 μL of Na_2S solution containing 0.5 μmol of S^{2-} and then adding 200 μL of $\text{Fe}_2(\text{NH}_4)_2\text{SO}_4$ solution containing 0.5 μmol of Fe^{2+} , so that the Fe:Cu ratio was 26:1. This procedure yields a sediment with 2.5 μmol of FeS/g-sed which matches the highest AVS level in the in situ sediments (Table 1) but keeping the same Cu and gut ligand concentrations as in the goethite

experiment. The mixture was allowed to sit in a N_2 glovebox for 1 h, which is sufficiently long for the formation of CuS (22), followed by in vitro digestion with deaerated digestive fluids. Controls included FeS precipitates without spiked Cu and the CuS/FeS mixture incubated with seawater. Black precipitates of FeS were observed in the synthesized sediments throughout the digestion experiment, suggesting that bulk chemistry remained anoxic.

Results and Discussion

Metals Released by Digestive Fluids and 1 N HCl (SEM). 1 N HCl released an average of $62 \pm 26\%$ of the total Cu (Table 1) from contaminated sediments, which is much more than those by digestive fluids of *Arenicola marina* ($12 \pm 8\%$), *Parastichopus californicus* (~5%), and *Cucumaria frondosa* (~2%) or by seawater (~0.13%). These results indicate that extraction by 1 N HCl, one of the traditional methods for assessing sedimentary metal bioavailability (1), solubilizes more Cu than digestive fluids and also corroborates our earlier findings on the rank of Cu solubilization by the digestive fluids from these species (15). Enhanced Cu solubilization by HCl may be due to the acidic pH which represents a much harsher attack on sediments, in contrast to neutral pH in the guts of marine deposit feeders which maintain active exchange with ambient environment via high volumes of sediment throughput (23). Similarly, digestive fluids released insignificant fractions of sedimentary Pb, Zn, Cd, and Ni (<2%) in comparison to Cu, although Pb and Zn can account for a significant fraction of total SEM in the 1 N HCl extraction (up to ~50%, data not shown).

Cu Bioavailability Determined by Biomimetic and AVS Methods. The biomimetic approach defines Cu bioavailability as the amount solubilized by digestive fluids, in comparison to $\text{SEM}_{\text{Cu-AVS}}$ which represents the minimum amount of Cu bound to non-AVS phases (Figure 1). Among the sediments tested, bioavailable Cu to digestive fluid of *Arenicola marina* was markedly higher for those with $\text{SEM}_{\text{Cu-AVS}} > 0$. In comparison, $\text{SEM}_{\text{Cu-AVS}}$ values of 0.4 and 1.5 $\mu\text{mol/g-sed}$ for the holothuroid (*Parastichopus californicus* and *Cucumaria frondosa*) gut fluids, respectively, were necessary before a significant increase in Cu solubilization was observed. These increases suggest that sedimentary AVS limits Cu bioavailability to deposit feeding. Positive $\text{SEM}_{\text{Cu-AVS}}$ thresholds in the holothuroid experiments imply that additional solid binding phases besides AVS, such as organic matter (13, 24), can compete with dissolved gut ligands from these animals. These positive thresholds may also result from the presence of Cu(I) sulfide in addition to Cu(II) sulfide in these sediments,

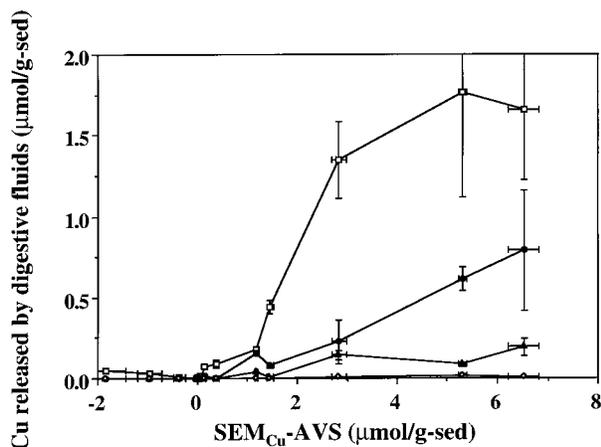


FIGURE 1. Cu bioavailability ($\mu\text{mol per g-sediment} \pm 1 \text{ SD}$) determined by biomimetic (Y-axis) and $\text{SEM}_{\text{Cu-AVS}}$ (X-axis) methods from sediments listed in Table 1. Seawater ($-\diamond-$) and digestive fluids of *Arenicola marina* ($-\square-$), *Parastichopus californicus* ($-\bullet-$), and *Cucumaria frondosa* ($-\blacktriangle-$) were used for the biomimetic method.

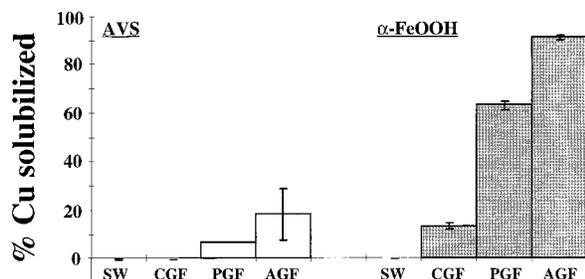


FIGURE 2. Cu (% of the total, $\pm 1 \text{ SD}$) solubilized from AVS (open bar) and goethite (closed bar) phases by seawater (SW) and digestive fluids of *Cucumaria frondosa* (CGF), *Parastichopus californicus* (PGF), and *Arenicola marina* (AGF). Total Cu concentrations in both experiments are $3.84 \times 10^{-2} \mu\text{mol}$ with Fe:Cu mole ratio of 26 for AVS and 360 for goethite phases.

because it takes 2 mol of Cu(I) to form Cu_2S in comparison to 1 mol of Cu(II) to form CuS (25).

The amount of Cu available to digestive fluids was much less than that measured as $\text{SEM}_{\text{Cu-AVS}}$. Thus only a fraction of the HCl-soluble, non-AVS-bound Cu will be solubilized during the digestive process, and this fraction decreases in the order *Arenicola* > *Parastichopus* > *Cucumaria* (Figure 1). This decreasing order is consistent with the decreasing concentrations of the strongest Cu-binding ligands (e.g., histidine) among the digestive fluids of these organisms (14). In addition, these results indicate that sedimentary Cu availability is not a single value that could be observed by extraction with a chemical approach such as SEM-AVS. Rather, it varies according to the digestive physiology of organisms.

Sulfide and Goethite as Solid Cu-Binding Phases. A much smaller fraction of Cu was released from the sulfide than from the $\alpha\text{-FeOOH}$ phases by digestive fluids, corroborating that sulfide is a stronger Cu-binding phase in sediments (Figure 2). This comparison is consistent with the difference between Cu-binding constants of sulfide ($\log K_{\text{CuS}} = 36.1$) and hydroxyl group ($\log K_{\text{CuOH}} = 6.5$) and the results from field-collected sediments with varying sulfide concentrations (Figure 1). In situ sediments may contain mixtures of $\alpha\text{-FeOOH}$ and sulfides of a wide range of concentrations. The increasing Cu bioavailability from an $\alpha\text{-FeOOH}$ -

dominated phase compared to a sulfide-dominated phase (Figure 2) corroborates observations made on Cu accumulation in *Chironomus tentans* from nitrogen- and air-treated sediments (26). These results suggest that sedimentary redox state, in addition to the species of organisms (Figure 2), could have profound influence on Cu bioavailability from sediments of given total Cu concentrations. Thus, deposit feeders feeding on subsurface anoxic sediments may be less exposed to Cu toxicity than those feeding on surface oxic sediments.

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