Environmental Science & Technology

pubs.acs.org/est

Article

Application of a Multi-Metal Stable-Isotope-Enriched Bioassay to Assess Changes to Metal Bioavailability in Suspended Sediments

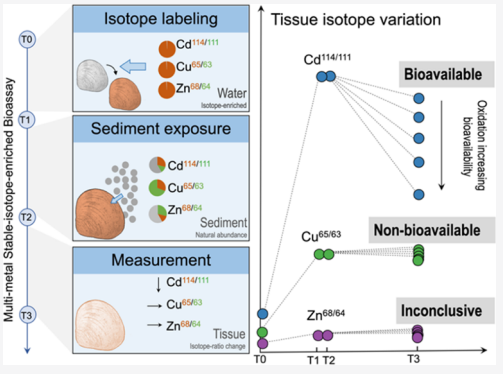
Qiuling Wu,[‖] Tianying Zheng,[‖] Stuart L. Simpson, Qiao-Guo Tan, Rong Chen, and Minwei Xie*

Cite This: *Environ. Sci. Technol.* 2021, 55, 13005−13013

Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information

ABSTRACT: The direct measurement of particulate contaminant bioavailability is a challenging aspect for the environmental risk assessment of contaminated sites. Here, we demonstrated a multi-metal stable-isotope-enriched bioassay to simultaneously measure the bioavailability of Cd, Cu, and Zn in naturally contaminated sediments following differing periods of resuspension treatment. Freshwater filter-feeding clams were pre-labeled with the isotopes ^{114}Cd, ^{65}Cu, and ^{68}Zn to elevate isotope abundances in their tissues and then exposed to metal-contaminated suspended sediments. The assimilation of sediment-associated metals by clams would decrease the isotope ratios ($Cd^{114/111}$, $Cu^{65/63}$, and $Zn^{68/64}$) in tissues, providing a direct measurement of metal bioavailability. For the sediments tested here, the method revealed bioavailable cadmium and non-bioavailable copper in sediments but was inconclusive for zinc. With a longer resuspension time, the bioavailability of particulate cadmium increased, but that of copper was unaffected. Metal bioavailability predicted using traditional wet-chemical extraction methods was inconsistent with these findings. The study indicated that multi-metal stable-isotope-enriched bioassay provides a new tool for directly assessing metal bioavailability in sediments, and this method is amenable for use in in situ assessments.



KEYWORDS: *metal bioaccumulation, metal stable isotope, oxidation of copper sulfide, Corbicula fluminea*

1. INTRODUCTION

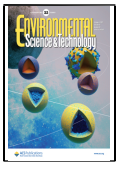
There is increasing recognition that differences in sediment contaminant bioavailability influence assessment outcomes and remediation strategies.[1−3] The assessment of contaminant bioavailability may use either chemical measurements to predict bioavailability to organisms or biological approaches that directly measure bioavailability.[4−8]

For sediment metals, the most common chemical approaches involve wet-chemical extraction methods, including analysis of acid volatile sulfide and simultaneously extracted metals (AVS and SEM)[6,9,10] or a sequential extraction method (e.g., BCR extraction),[7] to provide information on the binding strength between metals and solid phases, which are used to predict the bioavailable metal fraction.[11,12] However, these methods are criticized for not consistently discriminating bioavailable metals, resulting in data that may not improve risk assessment.[13,14] Recent progress with passive sampling techniques, such as diffusive gradients in thin-films, has offered some improvements in prediction effectiveness,[15−18] but the relatively long equilibration period (>24 h) of this technique precludes its application for measuring rapid changes in metal bioavailability.

Direct assessment of particulate metal bioavailability using organisms is also frequently hampered by method inadequacies that constrain the quality of information provided for assessments. The most common methods measure metals bioaccumulated following a period of continuously exposing organisms to contaminated sediments (bedded or suspended).[19] However, the time scale required to yield detectable accumulation is frequently several weeks to months for many benthic organisms,[20,21] and during this time period, considerable changes may occur to metal bioavailability and to the organisms' physiology.[22] A more rapid approach uses metal isotopes (either radioactive or stable) to label particles fed to organisms and determines the amount of tracers being absorbed.[23,24] However, the complexity of association between metals and sediments in the natural setting is often poorly represented using this approach. Neither of these conventional biological methods is suitable for assessing the relatively fast changes in metal bioavailability.

Recently, Croteau et al. proposed an innovative technique to directly measure bioavailable copper and zinc on natural particles in waters.[25,26] Instead of conventional labeling of the

Received: June 10, 2021
Published: September 14, 2021



https://doi.org/10.1021/acs.est.1c03827
Environ. Sci. Technol. 2021, 55, 13005−13013

metal exposure "source" (i.e., aquatic particles), the method first reverse-labels the "receptor" (i.e., an organism; freshwater snail used) with metal stable isotopes. The organisms were then exposed to natural particles, and the isotope composition in organisms was traced to determine the particulate metal bioavailability. Particles with greater concentrations of bioavailable metals resulted in greater exchange with the pre-accumulated metal isotope in the organisms and indicated a higher level of metal bioavailability. This isotopically enriched organism bioassay requires a shorter exposure time with the particles compared with conventional bioaccumulation bioassays and may also be effective for assessing dynamic changes in metal bioavailability.

While bedded sediments are an important repository for metal contaminants in aquatic ecosystems,[27−29] they may be destabilized and resuspended by many processes, including natural hydrological (e.g., storms and currents), biotic (e.g., bioturbation), and anthropogenic activities (e.g., dredging).[30,31] During resuspension, metals initially stabilized in low-bioavailability forms in anoxic environments may readily undergo fast changes in metal speciation during resuspension owing to shifts from anoxic to oxic conditions.[30,32] Oxidation of reduced metal sulfide mineral phases produces metal species with greater solubility and mobility[33,34] and potentially alters the metal bioavailability.

Here, we adapted the isotopically enriched organism bioassay to simultaneously label a filter-feeding clam with three metal stable isotopes (^{114}Cd, ^{65}Cu, and ^{68}Zn). We demonstrated the method's application to assessing metal bioavailability change associated with resuspension-induced metal sulfide oxidation. We hypothesized that resuspension of the anoxic sediment would promote oxidation of metal sulfide phases and increase the bioavailability of particulate metals to the clam. To assist with an understanding of changes occurring to the sediments, we accompanied the experiments with measurements of metals released to the dissolved phase and wet-chemical extractions to characterize changes in solid-phase metal speciation.

2. MATERIALS AND METHODS

2.1. General Methods. To minimize metal contamination, all plastic materials used, including polypropylene, polymethylpentene, and polyethylene containers, vials, and Teflon sheeting for organism dissection, were soaked in 5% HNO_3 at least overnight and rinsed thoroughly with deionized water (18.2 MΩ·cm) and then air-dried in a clean environment before use.

2.2. Sediment, Water, and the Tested Organism. Metal-contaminated sediment was collected from a heavily contaminated intertidal river at 22° 45′17″ N, 113° 46′55″ E from Shenzhen, China. Sediment from this region was fine-grained and highly contaminated by multiple metals, including Cd, Cr, Cu, Ni, Pb, and Zn.[35] Surficial sediment (<10 cm depth) was shoveled into a polypropylene bucket, transported to the laboratory, homogenized, stored sealed and undisturbed at room temperature (26 ± 1 °C), and used for experiments within 3 months.

Artificial fresh water (AFW) was prepared by dissolving 40 mg/L $CaSO_4$, 50 mg/L $CaCl_2$, 15 mg/L $MgSO_4$, 96 mg/L $NaHCO_3$, and 4 mg/L KCl in deionized water. The AFW was used throughout the experiments for clam cultivation, metal isotope labeling, sediment resuspension, and clam depuration.

The Asian clam (*Corbicula fluminea*) was collected at 23°28′40″ N, 113°49′46″ E from Guangdong Province, China. Individuals of shell lengths ranging from 1.2 to 1.5 cm were used for experiments. The clams were acclimated under laboratory conditions for at least a week prior to use, during which time they were fed the green algae *Chlorella* sp. (3.5 mg/clam) daily before a water renewal.

2.3. Resuspension Experiments. Batch sediment resuspension experiments (to prepare the sediments with differing degrees of oxidation used for bioassay sediment exposures in Section 2.4) were conducted in 5 L plastic beakers (polypropylene or polymethylpentene, Nalgene) where 20 g of wet anoxic sediments in 4 L of AFW was continuously stirred for 14 days. In brief, from the equilibrated anoxic sediment, a sediment core (∼7 cm depth) was taken using a 300 mL syringe with the end removed (5 cm in diameter and 18.5 cm in length), and ∼100 g of anoxic sediment was extruded into a clean beaker. This sediment was rapidly homogenized, and then in triplicate, portions of this sediment (20 g wet weight, ca. 7 g dry weight) were immediately dispersed in 50 mL of AFW by shaking and then added into 5 L plastic beakers containing 4 L of AFW (total volume).

During the 14-day resuspension, sediment and water samples were taken at time periods of 0.2 h (10 min following addition into beaker), 1.5, 3, 4.5, and 8 h and then 1, 3, 5, 7, 9, 11, and 14 d (for all three replicates). From these samples, measurements were made of pH, alkalinity, dissolved metals, dissolved sulfate, total recoverable metals (TRM), AVS, and SEM. At each designated time, 50 mL of slurry was centrifuged at 2095*g* for 3 min and the supernatant was filtered (0.2 μm, PES membrane). The filtrate was split into two aliquots. One aliquot was acidified with concentrated nitric acid (70%, trace metal grade, Sigma) to pH < 2 and stored for metal analysis; the other aliquot was used to measure pH, alkalinity, and dissolved sulfate concentration. The sediments remaining in the centrifuge tubes were dried in an oven at 80 °C to constant weight and used for TRM analyses. At the same time, another 50 mL of sediment slurry was also sampled for the AVS and SEM analysis.

2.4. Isotopically Enriched Clam Bioassay. The isotopically enriched organism bioassay contained two stages: labeling the organisms with metal stable isotopes (i.e., labeling stage) and then a suspended-sediment exposure stage (using the sediment prepared as described in Section 2.3). The isotope composition in the organism was determined following exposure to sediments subjected to differing degrees of resuspension.

2.4.1. Labeling Stage. During an initial 14-day labeling stage, clams were cultured in water spiked with a mixture of metal stable isotopes (1 μg L^{-1} of ^{114}Cd, 10 μg L^{-1} of ^{65}Cu and ^{68}Zn) (99.07% of ^{114}Cd, 99.6% of ^{65}Cu, and 99.26% of ^{68}Zn, ISOFLEX, San Francisco, California, USA) in a flow-through microcosm. The flow-through exposure system (Figure S1, Supporting Information) ensured that the clams were labeled in constant concentrations of metal isotopes. During this labeling period, clams were fed daily with 3.5 mg/clam of green algae *Chlorella* sp. for 1 h in separate containers and then rinsed with deionized water to remove attached debris before being placed back to the flow-through microcosm.

To track changes in metal-isotope concentrations in clam tissues, at least 10 clams were sampled every other day from the microcosm, immersed immediately into 1 mM ethylenediaminetetraacetic acid (EDTA) solution for ca. 1 min to

https://doi.org/10.1021/acs.est.1c03827
Environ. Sci. Technol. 2021, 55, 13005−13013

terminate metal uptake, and then dissected using a stainless-steel scalpel. The soft tissue was rinsed with the EDTA solution and then with deionized water twice and then freeze-dried before being digested for metal analysis. At the same time, a control group of clams were cultured under the same conditions without exposing to metal isotopes. To monitor whether the metal-isotope labeling significantly influenced the growth of clams, 10 clams were also sampled every other day from the control batch and dissected to compare their dry tissue weight with those from the labeling batch. Dry tissue samples of the control group at the beginning and end of the labeling stage were also measured for background tissue metal-isotope concentrations.

2.4.2. Suspended-Sediment Exposure Stage. The suspended-sediment exposure stage involved feeding the metal-isotope-enriched clams for 1 h with sediments that had been prepared as treatments corresponding to different resuspension times (described earlier). Five sediment treatments were used with resuspension times of 0.1 h, 1, 3, 7, and 14 days, with each test treatment conducted in duplicate. One day before sediment feeding, the clams were starved to maximize the ingestion of sediments. An hour before the sediment exposure, 10 clams were introduced into a beaker containing 1 L of clean AFW. The water was continuously stirred, and clams were allowed to adjust to the conditions for 1 h. At the commencement, 50 mL of suspension slurry was retrieved from the resuspension beaker and centrifuged at 2095*g* for 3 min. The supernatant was discarded, and the sediments at the bottom were immediately dispersed in 50 mL of AFW by shaking and then introduced to exposure beakers to create the sediment treatment, with water continuously stirred (i.e., this step occurred at each resuspension time point to commence a new test).

Because the organisms' physiology is easily affected by changing conditions, the variability caused by using organisms at different times may obscure the true difference in sediment bioavailability. To minimize these artifacts, the biological exposure to the different sediment treatments (different resuspension times) was carefully prepared to occur simultaneously. This required resuspension of sediments in advance using independent batches but following the same operational protocols as described above (Section 2.3). The concentration of suspended particles was environmentally realistic (ca. 40 mg/L) and enough to sustain the filtration by the clams during exposure (<50% loss of suspended particles). To minimize metals being released from suspended particles during exposure and being absorbed via the aqueous exposure pathway, the duration of sediment exposure was 1 h.

At the end of 1 h sediment exposure, the clams were taken out of the exposure chambers, rinsed with deionized water to remove attached particles, and placed in clean water for depuration. The depuration period was 2 days, and new AFW was renewed every 3 h on the 1st day and every 6 to 12 h on the 2nd day to minimize feces potentially releasing metals that could re-enter the organisms. After the depuration period, all clams from each treatment were dissected and metals analyzed as described above.

2.5. Analysis of Water, Sediment, and Organism Tissues. Filtered water samples from the resuspension experiments were analyzed for pH, alkalinity, and dissolved sulfate concentrations (Supporting Information Note S1). Sediment samples were characterized for particle size distribution and analyzed for TRM. AVS and SEM were determined following the purge-and-trap method (Supporting Information Note S1).[9] Metal concentrations in the clam tissue were determined by digesting the dried clam tissue in 65% HNO_3 at 80 °C for 8 h and analyzing metal concentrations in the digest.

Metal concentrations in the aqueous solution, including water and the acid digest, were determined using a combination of inductively coupled plasma optical emission spectrometry (ICP−OES, Agilent 5110) and inductively coupled plasma mass spectrometry (ICP−MS, PerkinElmer NexION 2000) depending on their concentration levels and analysis purpose. Concentrations of Fe, Mn, Cu, and Zn were measured by ICP−OES. Cadmium concentrations in all samples and metal isotope concentrations (^{111}Cd, ^{114}Cd, ^{63}Cu, ^{65}Cu, ^{64}Zn, and ^{68}Zn) in water and the organism tissue digest were analyzed by ICP−MS. Quality control procedures include using internal reference standards (^{103}Rh and ^{74}Ge, 5 μg L^{-1}), running regular blank samples, measuring quality control standards (10 μg L^{-1}) for every 20 samples, and determining metal concentrations in certified reference materials (CRMs: MESS-4 sediment and the SRM 2976 mussel tissue). The recoveries for the CRMs were 91 ± 3, 120 ± 8, and 107 ± 2% for TRM−Cd, −Cu, and −Zn in MESS-4 and 89 ± 1, 102 ± 1, and 92 ± 1% for tissue Cd, Cu, and Zn in SRM 2976, respectively.

2.6. Data Analysis. Using ^{114}Cd, ^{65}Cu, and ^{68}Zn as tracers, we are able to determine the concentration of these isotopes newly accumulated into clams and also track the change in isotope ratios of $Cd^{114/111}$, $Cu^{65/63}$, and $Zn^{68/64}$.

During the labeling phase, because clams were exposed to only spiked metal isotopes (^{114}Cd, ^{65}Cu, and ^{68}Zn), the newly accumulated ^{114}Cd, ^{65}Cu, and ^{68}Zn in clam tissues were determined by subtracting the background concentrations from the total concentrations of each metal isotope, as detailed in Supporting Information Note S2 and Figure S2[36]

$$[^{114}Cd]_{new} = [^{114}Cd]_{instru} \times 28.75\% - [^{111}Cd]_{instru} \times 28.75\% \quad (1)$$

$$[^{65}Cu]_{new} = [^{65}Cu]_{instru} \times 30.85\% - [^{63}Cu]_{instru} \times 30.85\% \quad (2)$$

$$[^{68}Zn]_{new} = [^{68}Zn]_{instru} \times 18.50\% - [^{64}Zn]_{instru} \times 18.50\% \quad (3)$$

where $[^{114}Cd]_{new}$, $[^{65}Cu]_{new}$, and $[^{68}Zn]_{new}$ are the newly accumulated metal isotope concentrations in the clam; $[^{114}Cd]_{instru}$, $[^{111}Cd]_{instru}$, $[^{65}Cu]_{instru}$, $[^{63}Cu]_{instru}$, $[^{68}Zn]_{instru}$, and $[^{64}Zn]_{instru}$ are the total metal concentrations reported by the ICP−MS instrument, which are derived from the measured signal of each isotope assuming that each element has a natural abundance; and the numbers 28.75, 30.85, and 18.5% are the natural isotope abundances of ^{114}Cd, ^{65}Cu, and ^{68}Zn, respectively.

The isotope ratio of each metal ($Cd^{114/111}$, $Cu^{65/63}$, and $Zn^{68/64}$) was calculated as

$$Cd^{114/111} = \frac{[^{114}Cd]_{instru} \times 28.75\%}{[^{111}Cd]_{instru} \times 12.80\%} \quad (4)$$

$$Cu^{65/63} = \frac{[^{65}Cu]_{instru} \times 30.85\%}{[^{63}Cu]_{instru} \times 69.15\%} \quad (5)$$

https://doi.org/10.1021/acs.est.1c03827
Environ. Sci. Technol. 2021, 55, 13005−13013

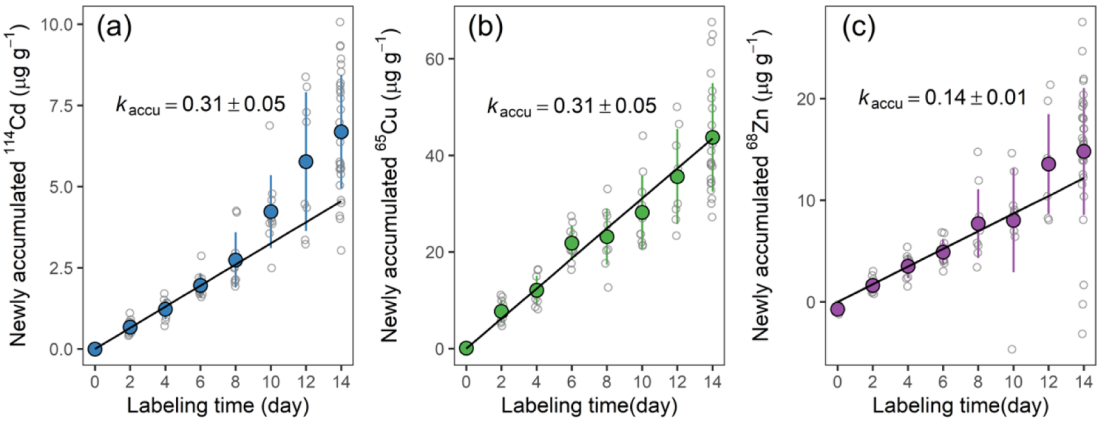


Figure 1. Newly accumulated ^{114}Cd, ^{65}Cu, and ^{68}Zn in the clam tissue during the labeling phase. The spiked metal concentrations in water were 1, 10, and 10 μg L^{-1} for ^{114}Cd, ^{65}Cu, and ^{68}Zn, respectively. The net uptake rate constant of metals from water k_{accu} (L g^{-1} d^{-1}) was calculated from the rate of change in newly accumulated metals in the clam tissue, obtained by linear least-squares fitting of newly accumulated metal concentrations (mean concentrations) over 14 days normalized with metal isotope concentrations in ambient water. Each open circle represents the isotopic ratio in the tissue of an individual clam, the solid points represent the mean isotopic ratio of the group, and the error bar represents standard deviation.

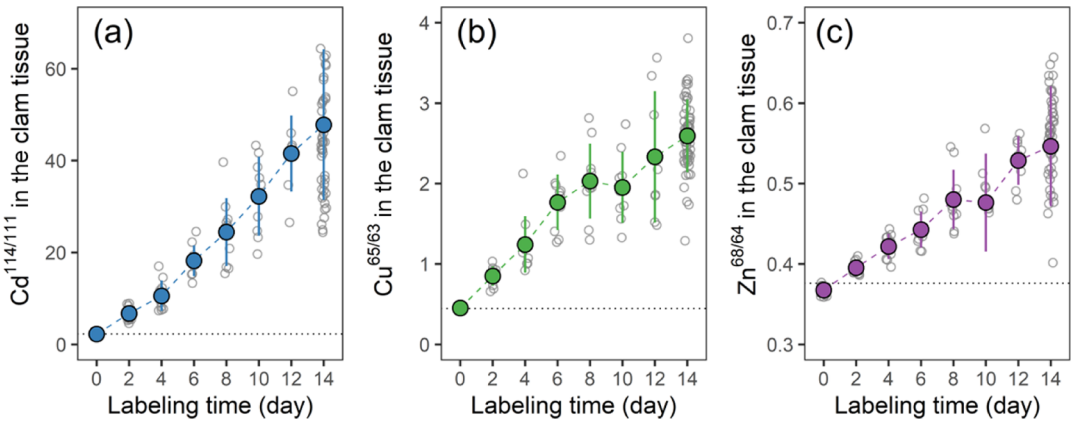


Figure 2. Isotopic ratio of $Cd^{114/111}$, $Cu^{65/63}$, and $Zn^{68/64}$ increased during the 2-week labeling phase. Each open circle represents the isotopic ratio in the tissue of an individual clam, the solid points represent the mean isotopic ratio of the group, and the error bar represents standard deviation.

$$Zn^{68/64} = \frac{[^{68}Zn]_{instru} \times 18.50\%}{[^{64}Zn]_{instru} \times 49.20\%} \quad (6)$$

where the numbers 12.80, 69.15, and 49.20% are the natural isotope abundances of ^{111}Cd, ^{63}Cu, and ^{64}Zn, respectively.

3. RESULTS AND DISCUSSION

3.1. Characterization of Sediments and Organisms. The sediment was fine-grained (Supporting Information Figure S3), with 90% of the particles less than 100 μm and a median diameter of 7.8 μm. Sediments contained very high concentrations of Cd, Cu, and Zn, with TRM concentrations of 2.3 ± 0.1, 1900 ± 100, and 1200 ± 100 mg kg^{-1}, respectively. All these metals exceeded the corresponding sediment quality guideline values.[37] The anoxic sediment was very dark in color. The AVS concentration in the equilibrated sediment was 45 ± 13 μmol g^{-1} dry weight, but no free sulfide (<10 μg L^{-1}) was detected in porewater, suggesting that all sulfide was associated in the solid phase.

The background metal concentrations in the clam tissue were 1.1 ± 0.3, 26 ± 7, and 170 ± 30 μg g^{-1} for Cd, Cu, and Zn, respectively, comparable to the metal concentrations of this species in other regions.[38] Labeling of metal stable isotopes did not influence the growth of clams, during which the clams showed comparable growth rates with the clams reared in clean water (Supporting Information Figure S4).

3.2. Labeling of Clams with Spiked Isotopes. During the 2-week labeling of clams with dissolved metal isotopes, there were substantially increased isotope abundances in the clam tissue (Figures 1 and 2). The newly accumulated ^{114}Cd, ^{65}Cu, and ^{68}Zn in the clam tissue steadily increased from 0 to 5.8 ± 1.6, 45 ± 13, and 16 ± 7 μg g^{-1} (Figure 1), corresponding to net uptake rate constants of 0.31, 0.31, and 0.14 L g^{-1} d^{-1}, respectively. The isotope accumulation resulted in isotope ratios in clam tissues increasing from 2.3 ± 0.0 to 44 ± 14 for $Cd^{114/111}$ (1800% increase), from 0.45 ± 0.01 to 2.5 ± 0.5 for $Cu^{65/63}$ (456% increase), and from 0.38 ± 0.00 to 0.56 ± 0.07 for $Zn^{68/64}$ (47% increase) over the 14-day labeling period (Figure 2). The magnitudes of increase in isotope ratio were influenced by the background concentrations of metals in organisms before exposure (the background tissue concentrations are in the order of Zn > Cu > Cd).

3.3. Sediment Metal Bioavailability Assessment Using the Isotopically Enriched Clam Bioassay. On the field-contaminated sediment particles, the isotope ratios of $Cd^{114/111}$ (2.3), $Cu^{65/63}$ (0.45), and $Zn^{68/64}$ (0.38) were lower than those in the isotopically enriched clams. Because there

https://doi.org/10.1021/acs.est.1c03827
Environ. Sci. Technol. 2021, 55, 13005−13013

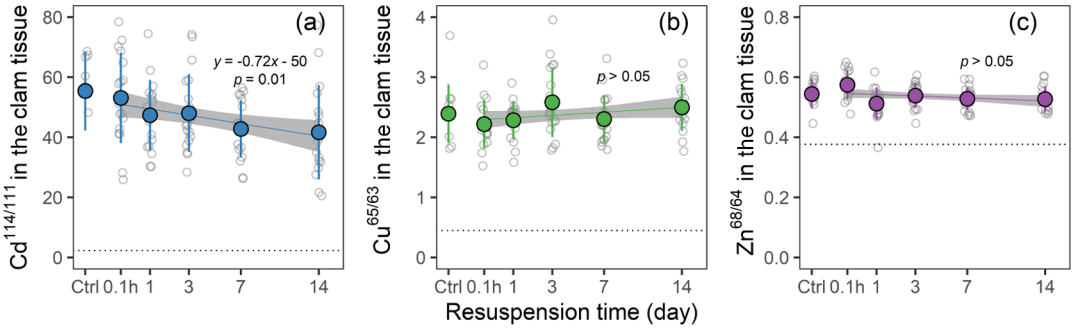


Figure 3. Isotopic ratio of $Cd^{114/111}$, $Cu^{65/63}$, and $Zn^{68/64}$ in the clam tissue following dietary uptake of suspended sediments subjected to differing resuspension treatments. Each open circle represents the isotopic ratio in the tissue of an individual clam, the solid points represent the mean isotopic ratio of the group, and the error bar represents standard deviation. The control (Ctrl) group was not fed with suspended sediments. The horizontal dotted lines represent the isotopic ratio of each metal in its natural abundance. The linear regression lines were fitted using the metal isotope ratios in individual clams at different treatments.

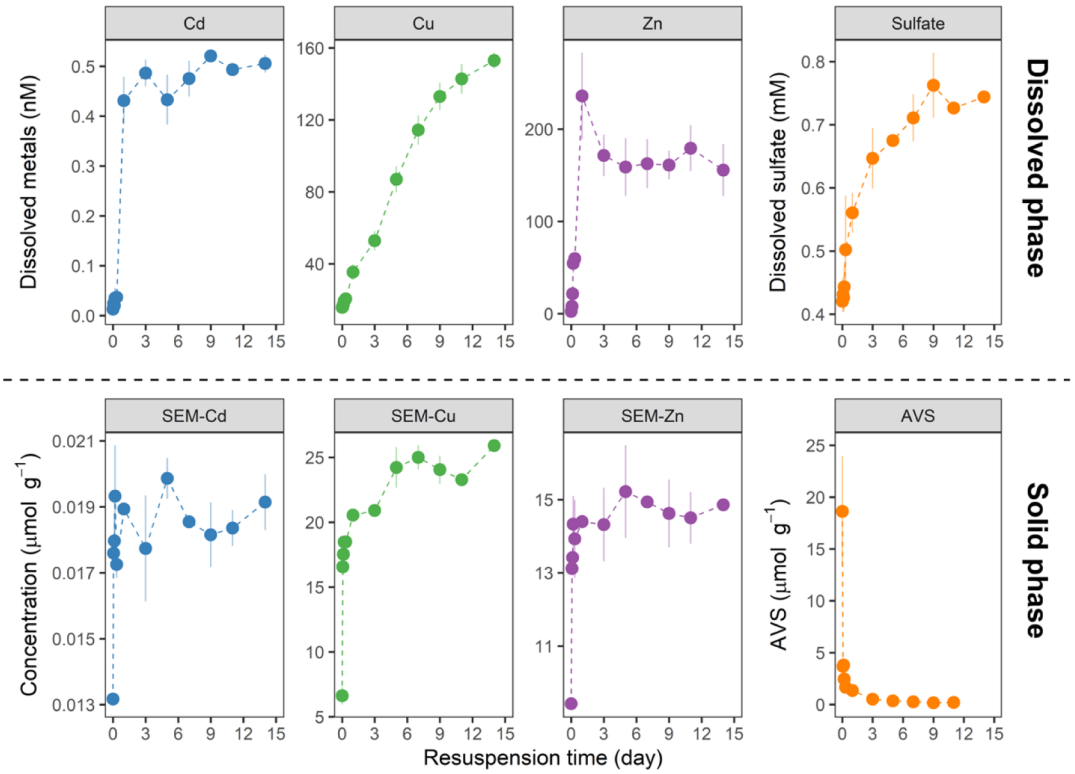


Figure 4. Release of dissolved constituents and changes in metal speciation during the resuspension. Top: temporal release of dissolved Cd, Cu, Zn, and sulfate. Bottom: temporal changes in AVS and SEM−Cd, −Cu, and −Zn. The symbols in the figure represent the mean of the triplicate experiments, while the error bars represent standard deviation from triplicate experiment results.

was no observable release of dissolved Cd, Cu, and Zn over the 1 h suspended-sediment exposure, decreases in the isotope ratios ($Cd^{114/111}$, $Cu^{65/63}$, and $Zn^{68/64}$) in tissues following suspended-sediment exposure would suggest assimilation of sediment-associated metals. Therefore, the decrease in isotope ratio of cadmium ($Cd^{114/111}$) in the clam indicated the presence of bioavailable particulate cadmium (Figure 3). However, the isotope ratio of copper remained the same, indicating non-bioavailable particulate copper in sediments. In the case of zinc, the difference of $Zn^{68/64}$ between the sediment and the clam was not high (ca. 0.17); this reduces the sensitivity of the method, and thus, the bioavailability of sediment-bound zinc was inconclusive.

The sensitivity of this approach depends on various factors. For the three targeted metals, we estimated that the clams assimilated ca. 2.0 ng of cadmium during the suspended sediment exposure based on the decrease of the $Cd^{114/111}$ ratio by 10%; in contrast, owing to smaller differences in Cu- and Zn-isotope ratios between the labeled clam tissues and the natural sediments, assimilation of 26 ng of bioavailable copper and 1300 ng of bioavailable zinc would have been required to cause a similar level of decrease in $Cu^{65/63}$ and $Zn^{68/64}$ ratios (Supporting Information Note S3). Thus, the sensitivity of the isotopically enriched organism approach is influenced by the final isotopic ratio at the end of the organism labeling period, which depends on the accumulation kinetics and background metal-isotope concentrations in the organism tissue. Here, the clams accumulated the cadmium and copper isotope labels rapidly from the aqueous solution, with a similar net uptake rate constant at 0.31 L g^{-1} d^{-1}. The average background

https://doi.org/10.1021/acs.est.1c03827
Environ. Sci. Technol. 2021, 55, 13005−13013

concentration of cadmium was 1 order of magnitude lower than that of copper (Cd^{114}: 0.32 $\mu g\ g^{-1}$, Cd^{111}: 0.14 $\mu g\ g^{-1}$, Cu^{65}: 8.0 $\mu g\ g^{-1}$, Cu^{63}: 18 $\mu g\ g^{-1}$). In contrast, the clams accumulated the zinc isotope label slowly from the water (0.14 $L\ g^{-1}\ d^{-1}$) and also had a high background zinc concentration (Zn^{68}: 31 $\mu g\ g^{-1}$, Zn^{64}: 84 $\mu g\ g^{-1}$). Collectively, the higher net uptake rate constant and the lower background concentration make this organism suitable for measuring cadmium and copper bioavailability but less appropriate for zinc.

To improve the sensitivity of this approach, changes could be made to elevate the final isotopic ratio at the end of the organism labeling period, including increasing the concentrations of bioavailable metal stable isotopes at the labeling stage, extending the labeling time, and using rarer metal stable isotopes and organisms with lower natural background metal concentrations. In an attempt to enhance the uptake of the metal isotope, Croteau and Luoma used a very (moderately) soft synthetic water of weakly acidic pH (~6) to maximize the fraction of bioavailable ^{65}Cu in exposure solution.[25,39] The labeling period was also extended to ca. 8 weeks to increase the abundance of ^{65}Cu and ^{67}Zn in a freshwater snail *Lymnaea stagnalis*.[25,26] However, the health of organisms (growth or survivorship) should be continuously monitored when implementing these actions to ensure that the labeling stage did not impair the physiological functions of the isotope-enriched bioassay. Selection of more appropriate stable isotopes (i.e., lower natural abundance, e.g., ^{67}Zn instead of ^{68}Zn) and organisms with lower background metals may further benefit the labeling effectiveness,[26] but the practicability and cost-effectiveness should be evaluated in advance.

The results also indicated that longer resuspension durations enhanced the cadmium isotope exchange from particles to clams, and the multi-metal isotopically enriched organism bioassay was effective for detecting this change. The cadmium assimilated steadily increased from 1.4 ng of sediments resuspended for 0.1 h to 7.1 ng of sediments resuspended for 14 d (slope = −0.72 ± 0.28, p = 0.01, linear regression, Figure 3a), suggesting that processes occurring during the resuspension enhanced the bioavailability of sediment-associated cadmium.

3.4. Temporal Variation in Dissolved Metals, Sulfate, AVS, and SEM. Release of dissolved constituents and variation in AVS and SEM concentrations provide some indication of changes in metal speciation during the resuspension assisted with an understanding of processes occurring to the sediments that influence the mobility and bioavailability of metals (Figure 4).

Over the 14-day sediment resuspension period, metals and sulfate were released into water and the release-removal patterns were different (Figure 4 and Supporting Information Figure S5). The release of copper occurred throughout the resuspension period, with dissolved copper steadily increasing from 16 ± 2 to 150 ± 4 nM over 14 days. Releases of zinc and cadmium were different, with both metals displaying a rapid initial release and then a plateau. The dissolved zinc concentration rapidly increased from 2.7 ± 0.7 to 240 ± 50 nM within the first day, then decreased slightly to 170 ± 20 nM on the following day, and then remained relatively constant over the next 11 days. The dissolved cadmium concentration also increased rapidly from 0.013 ± 0.006 to 0.43 ± 0.05 nM for the first day and then varied within the range of 0.40−0.52 nM, but not decreasing or clearly increasing. Dissolved manganese was initially high (ca. 500 nM), indicating direct release of porewater containing manganese, and then increased a little further to 690 nM before then decreasing over the next 8 days to <10 nM (Supporting Information Figure S5). Dissolved iron was initially at 490 ± 90 nM but decreased rapidly to <25 nM within 1.5 h (Supporting Information Figure S5). The oxidation rate of dissolved manganese(II) is slow compared to that of iron(II),[40] and the decrease in dissolved manganese concentration over the 1−8 day period is consistent with this. The precipitation of Fe/Mn oxyhydroxides due to the oxidation of Fe(II) and Mn(II) in porewater is expected to contribute to immobilization of the dissolved metals Cd, Cu, and Zn due to adsorption reactions.[41]

The sulfate concentration increased from 0.42 ± 0.01 to 0.50 ± 0.09 mM for the first 8 h and then further to 0.76 ± 0.05 mM on Day 9 and was then constant to Day 14 (Figure 4). This resulted in a net release of 1.32 mmol sulfate over 14 days of resuspension. A portion of the increasing sulfate concentrations may be attributed to metal sulfide oxidation, as discussed in detail in Supporting Information Note S4.

The rapid (<1 d) decrease in sediment AVS concentrations (Figure 4) was consistent with FeS oxidation, while the ongoing decrease in AVS and continued sulfate release is consistent with the oxidation of other less labile metal sulfide phases.[42−44] The SEM measurements can provide some indication of the oxidation of metal monosulfide phases (MeS); however, because the 1 M HCl extraction also dissolved non-sulfide phases, for most metals, the SEM data are not easy to use for predicting changes in metal bioavailability. Cadmium and zinc fall into this category, where the rapid increase and then a plateau of SEM−Cd and SEM−Zn concentrations cannot simply be attributed to CdS or ZnS oxidation (Figure 4). The SEM−Cu concentration increased rapidly and then steadily with an increased resuspension duration up to 9 days when it approached the TRM−Cu concentration (Supporting Information Table S1). For copper, the data were consistent with CuS and Cu_2S oxidation (Supporting Information Note S4).

3.5. Wet-Chemical Measurement Prediction of Changes in Particulate Bioavailability. The chemical measurements provided an indication of the oxidation processes occurring during resuspension but were less informative of the change in particulate metal bioavailability. The release of dissolved metals and AVS−SEM extraction results revealed that the particulate metal lability was different between metals over the course of sediment resuspension: SEM−Cu suggested that a gradual increase in copper bioavailability may be occurring with suspension time (i.e., less associated copper sulfide phases), whereas SEM−Zn and SEM−Cd were invariant after 1.5 h.

However, the bioavailability results determined by the multi-metal isotopically enriched organism bioassay were inconsistent with the bioavailability changes that may be predicted from the AVS−SEM analyses for copper and cadmium. The bioavailability of sediment-bound copper to the clam was low (i.e., negligible isotope exchange between sediments and clams) and did not increase due to resuspension-induced shift from sulfide to non-sulfide forms, as indicated by the chemical measurements. In contrast, the bioavailability of sediment-bound cadmium to the clam increased with resuspension time, but this change was not evident when predicted from the dissolved cadmium or AVS−SEM measurements.

https://doi.org/10.1021/acs.est.1c03827
Environ. Sci. Technol. 2021, 55, 13005−13013

The low particulate copper bioavailability following the 14-day oxidation period may be attributed to the high affinity of copper for non-sulfide solid phases [i.e., binding to Fe and Mn (hydro)oxides and organic matter].[11,31,45] The partition coefficient for copper log K_d (L kg^{-1})[46] gradually decreased from 6.3 at Day 0 to 5.2 at Day 14. These log K_d values were consistent with the commonly reported copper partition coefficients between suspended sediments and water and were the higher range of values typically reported (e.g., log K_d 3.1−6.1 with a median of 4.7),[46] indicating the strong binding strengths between copper and the solid phase even when its chemical form was transformed by resuspension-induced oxidation. Thus, the strong binding between copper and these phases may preclude its assimilation following ingestion.

The increased bioavailability of particulate cadmium as a result of sediment resuspension may be due in part to CdS oxidation. For initially anoxic sediments, the cadmium was predicted to be in the CdS form because of its solubility product (K_{sp}) being 10 orders of magnitude lower than that of FeS.[6] This was consistent with the high log K_d of 6.2 for cadmium in sediments on Day 0, suggesting the low bioavailability of sediment-associated cadmium at the commencement of the resuspension experiment. The log K_d of cadmium was 4.7 on Day 2 and varied between 4.8 and 4.5 for the remaining days. The 2 orders of magnitude decrease in partition coefficient for cadmium suggests that particulate cadmium may have changed to more labile and bioavailable forms (e.g., bound to Fe/Mn oxyhydroxides or organic matter) by Day 2 of resuspension. However, changes to the bioavailability of particulate cadmium during the resuspension process could not be revealed by the bulk chemical characterization method.

3.6. Implications for Assessment of Contaminated Sediments. The intended advantage of the multi-metal isotopically enriched organism bioassay over other methods is that the organism directly provides the bioavailability assessment of the sediment-associated metals in a relatively short period of time. Chemical extraction methods can only be used for predicting bioavailability. Other laboratory-based approaches with organisms suffer from long durations required to detect changes compared to background tissue concentrations or in the case of field-collected organisms a lack of knowledge of exposure history and weak correlations with many environmental factors. Although this study was conducted in the laboratory, the method developed here could be readily applied for the in situ measurement of contaminant bioavailability in environmental setting by transplanting isotopically enriched organisms into the field. In the present study, we aimed to capture the rapid changes in particulate metal bioavailability by minimizing the sediment exposure time to a very short period (1 h). While this measure effectively limited the release of dissolved metals to prevent unrealistically high exposure to dissolved metals,[47] it may also lower the sensitivity of the approach to measure particulate metal bioavailability, particularly in the case of copper and zinc. When applying this approach to the field to measure the bioavailability of metals associated with either suspended sediments or bedded sediments, the exposure durations of hours to days could be used to suit the assessment needs. However, in such settings, both aqueous and dietary exposure routes may contribute to the overall change of the isotopic composition in the bioassay. The contributions through different exposure pathways could be differentiated by estimating the contribution from aqueous metals using the measured dissolved metal concentrations and biokinetic parameters (e.g., uptake and efflux rate constant).[21,48]

The present study also reaffirmed the inadequacies of the wet-chemical extraction methods for predicting metal bioavailability. Complementary chemistry and biology lines of evidence should be used in conjunction to improve the assessment effectiveness.

Overall, we have demonstrated that exposing multi-metal isotopically enriched clams to suspended sediments was able to reveal bioavailable cadmium and non-bioavailable copper in the tested sediments but was inconclusive for zinc. This direct bioavailability assessment method was able to identify that the bioavailability of particulate cadmium increased with sediment resuspension time, and that of copper remained unaffected. Measurements of dissolved metals, AVS, and SEM revealed that changes in copper speciation occurred more slowly than cadmium and zinc and were attributed to the much slower oxidation rates of the copper sulfide mineral phase. However, these chemical measurements were less informative on the bioavailability of sediment-associated metals.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.est.1c03827.

Details on the procedures for analysis of water, sediment, and organisms; calculation of newly accumulated metal isotope concentrations; calculation of approach sensitivity; and interpretation of metal sulfide oxidation from sulfate release and changes in AVS and SEM (PDF)

■ AUTHOR INFORMATION

Corresponding Author

Minwei Xie − *State Key Laboratory of Marine Environmental Science, Key Laboratory of the Ministry of Education for Coastal and Wetland Ecosystem, College of the Environment and Ecology, Xiamen University, Xiamen, Fujian 361102, China;* orcid.org/0000-0003-4359-8738; Email: minweixie@xmu.edu.cn

Authors

Qiuling Wu − *State Key Laboratory of Marine Environmental Science, Key Laboratory of the Ministry of Education for Coastal and Wetland Ecosystem, College of the Environment and Ecology, Xiamen University, Xiamen, Fujian 361102, China*

Tianying Zheng − *State Key Laboratory of Marine Environmental Science, Key Laboratory of the Ministry of Education for Coastal and Wetland Ecosystem, College of the Environment and Ecology, Xiamen University, Xiamen, Fujian 361102, China*

Stuart L. Simpson − *Centre for Environmental Contaminants Research, CSIRO Land and Water, Sydney, New South Wales 2334, Australia; Hong Kong Branch of Southern Marine Science and Engineering Guangdong Laboratory, Hong Kong University of Science and Technology, Kowloon 999077 Hong Kong, China*

Qiao-Guo Tan − *State Key Laboratory of Marine Environmental Science, Key Laboratory of the Ministry of Education for Coastal and Wetland Ecosystem, College of the*

Environment and Ecology, Xiamen University, Xiamen, Fujian 361102, China; orcid.org/0000-0001-9692-6622

Rong Chen − *State Key Laboratory of Marine Environmental Science, Key Laboratory of the Ministry of Education for Coastal and Wetland Ecosystem, College of the Environment and Ecology, Xiamen University, Xiamen, Fujian 361102, China*

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.est.1c03827

Author Contributions

‖Q.W. and T.Z. contribute equally to the work.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The authors would like to thank Dr. Graeme Batley and three anonymous reviewers for valuable comments to the manuscript, and Dr. Jinghe Peng for ion chromatography analysis. This study was supported by the National Natural Science Foundation of China (grant nos. 42077372 and 21707113 to M.X.), the Fundamental Research Funds for the Central Universities in China (grant no. 20720200113 to M.X.), and the Hong Kong Branch of Southern Marine Science and Engineering Guangdong Laboratory (Guangzhou) (grant no. SMSEGL20SC02 to S.L.S.).

■ REFERENCES

(1) Sorell, T.; McEvoy, K. Incorporating bioavailability considerations into the evaluation of contaminated sediment sites. *Remed. J.* **2013**, *23*, 63−72.

(2) Burgess, R. M.; Berry, W. J.; Mount, D. R.; Di Toro, D. M. Mechanistic sediment quality guidelines based on contaminant bioavailability: Equilibrium partitioning sediment benchmarks. *Environ. Toxicol. Chem.* **2013**, *32*, 102−114.

(3) Maruya, K. A.; Landrum, P. F.; Burgess, R. M.; Shine, J. P. Incorporating contaminant bioavailability into sediment quality assessment frameworks. *Integrated Environ. Assess. Manag.* **2012**, *8*, 659−673.

(4) Simpson, S. L.; Spadaro, D. A.; O'Brien, D. Incorporating bioavailability into management limits for copper in sediments contaminated by antifouling paint used in aquaculture. *Chemosphere* **2013**, *93*, 2499−2506.

(5) Schlekat, C. E.; Garman, E. R.; Vangheluwe, M. L.; Burton, G. A., Jr Development of a bioavailability-based risk assessment approach for nickel in freshwater sediments. *Integrated Environ. Assess. Manag.* **2016**, *12*, 735−746.

(6) Di Toro, D. M.; Mahony, J. D.; Hansen, D. J.; Scott, K. J.; Carlson, A. R.; Ankley, G. T. Acid volatile sulfide predicts the acute toxicity of cadmium and nickel in sediments. *Environ. Sci. Technol.* **1992**, *26*, 96−101.

(7) Tan, Q.-G.; Ke, C.; Wang, W.-X. Rapid assessments of metal bioavailability in marine sediments using coelomic fluid of sipunculan worms. *Environ. Sci. Technol.* **2013**, *47*, 7499−7505.

(8) Landrum, P. F.; Robinson, S. D.; Gossiaux, D. C.; You, J.; Lydy, M. J.; Mitra, S.; Ten Hulscher, T. E. M. Predicting Bioavailability of Sediment-Associated Organic Contaminants forDiporeiaspp. and Oligochaetes. *Environ. Sci. Technol.* **2007**, *41*, 6442−6447.

(9) Allen, H.; Fu, G.; Boothman, W.; Di Toro, D.; Mahony, J. *Determination of Acid Volatile Sulfide and Selected Simultaneously Extractable Metals in Sediment*; Office of Water Regulations and Standards: US Environmental Protection Agency: Washington, DC, 1991; pp 1−22.

(10) Fetters, K. J.; Costello, D. M.; Hammerschmidt, C. R.; Burton, G. A. Toxicological effects of short-term resuspension of metal-contaminated freshwater and marine sediments. *Environ. Toxicol. Chem.* **2016**, *35*, 676−686.

(11) Chapman, P. M.; Wang, F.; Janssen, C.; Persoone, G.; Allen, H. E. Ecotoxicology of metals in aquatic sediments: binding and release, bioavailability, risk assessment, and remediation. *Can. J. Fish. Aquat. Sci.* **1998**, *55*, 2221−2243.

(12) Ankley, G. T.; Di Toro, D. M.; Hansen, D. J.; Berry, W. J. Technical basis and proposal for deriving sediment quality criteria for metals. *Environ. Toxicol. Chem.* **1996**, *15*, 2056−2066.

(13) DeForest, D. K.; Toll, J. E.; Judd, N. L.; Shaw, A.; McPeek, K.; Tobiason, K.; Santore, R. C. Sediment Toxicity Data and Excess Simultaneously Extracted Metals from Field-Collected Samples: Comparison to United States Environmental Protection Agency Benchmarks. *Integrated Environ. Assess. Manag.* **2021**, 1−13.

(14) Simpson, S. L.; Batley, G. E. Predicting metal toxicity in sediments: a critique of current approaches. *Integrated Environ. Assess. Manag.* **2007**, *3*, 18−31.

(15) Zhang, H.; Davison, W. Use of diffusive gradients in thin-films for studies of chemical speciation and bioavailability. *Environ. Chem.* **2015**, *12*, 85−101.

(16) Amato, E. D.; Simpson, S. L.; Jarolimek, C. V.; Jolley, D. F. Diffusive gradients in thin films technique provide robust prediction of metal bioavailability and toxicity in estuarine sediments. *Environ. Sci. Technol.* **2014**, *48*, 4485−4494.

(17) Gillmore, M. L.; Price, G. A. V.; Golding, L. A.; Stauber, J. L.; Adams, M. S.; Simpson, S. L.; Smith, R. E. W.; Jolley, D. J. The Diffusive Gradients in Thin Films Technique Predicts Sediment Nickel Toxicity to the Amphipod Melita plumulosa. *Environ. Toxicol. Chem.* **2021**, *40*, 1266−1278.

(18) Xie, M.; Simpson, S. L.; Huang, J.; Teasdale, P. R.; Wang, W.-X. In Situ DGT Sensing of Bioavailable Metal Fluxes to Improve Toxicity Predictions for Sediments. *Environ. Sci. Technol.* **2021**, *55*, 7355−7364.

(19) ASTM. *Standard Guide for Determination of the Bioaccumulation of Sediment Associated Contaminants by Benthic Invertebrates*; ASTM International: West Conshohocken, PA, 2019.

(20) Weng, N.; Wang, W.-X. Seasonal fluctuations of metal bioaccumulation and reproductive health of local oyster populations in a large contaminated estuary. *Environ. Pollut.* **2019**, *250*, 175−185.

(21) Tan, Q.-G.; Zhou, W.; Wang, W.-X. Modeling the Toxicokinetics of Multiple Metals in the Oyster Crassostrea hongkongensis in a Dynamic Estuarine Environment. *Environ. Sci. Technol.* **2018**, *52*, 484−492.

(22) Mubiana, V. K.; Vercauteren, K.; Blust, R. The influence of body size, condition index and tidal exposure on the variability in metal bioaccumulation in Mytilus edulis. *Environ. Pollut.* **2006**, *144*, 272−279.

(23) Wang, W.; Stupakoff, I.; Fisher, N. Bioavailability of dissolved and sediment-bound metals to a marine deposit-feeding polychaete. *Mar. Ecol. Prog. Ser.* **1999**, *178*, 281−293.

(24) Luoma, S. N.; Rainbow, P. S. *Metal Contamination in Aquatic Environments: Science and Lateral Management*; Cambridge University Press, 2008.

(25) Croteau, M.-N.; Cain, D. J.; Fuller, C. C. Novel and nontraditional use of stable isotope tracers to study metal bioavailability from natural particles. *Environ. Sci. Technol.* **2013**, *47*, 3424−3431.

(26) Croteau, M.-N.; Cain, D. J.; Fuller, C. C. Assessing the dietary bioavailability of metals associated with natural particles: extending the use of the reverse labeling approach to zinc. *Environ. Sci. Technol.* **2017**, *51*, 2803−2810.

(27) Burton, G. A. Metal bioavailability and toxicity in sediments. *Crit. Rev. Environ. Sci. Technol.* **2010**, *40*, 852−907.

(28) Pan, K.; Wang, W.-X. Trace metal contamination in estuarine and coastal environments in China. *Sci. Total Environ.* **2012**, *421*−*422*, 3.

(29) Wang, W.-X.; Pan, K.; Tan, Q.; Guo, L.; Simpson, S. L. Estuarine pollution of metals in China: science and mitigation. *Environ. Sci. Technol.* **2014**, *48*, 9975−9976.

(30) Cantwell, M. G.; Burgess, R. M.; Kester, D. R. Release and phase partitioning of metals from anoxic estuarine sediments during periods of simulated resuspension. *Environ. Sci. Technol.* **2002**, *36*, 5328−5334.
(31) Eggleton, J.; Thomas, K. V. A review of factors affecting the release and bioavailability of contaminants during sediment disturbance events. *Environ. Int.* **2004**, *30*, 973−980.
(32) Simpson, S. L.; Apte, S. C.; Batley, G. E. Effect of short-term resuspension events on the oxidation of cadmium, lead, and zinc sulfide phases in anoxic estuarine sediments. *Environ. Sci. Technol.* **2000**, *34*, 4533−4537.
(33) Xie, M.; Alsina, M. A.; Yuen, J.; Packman, A. I.; Gaillard, J.-F. Effects of resuspension on the mobility and chemical speciation of zinc in contaminated sediments. *J. Hazard. Mater.* **2019**, *364*, 300−308.
(34) Simpson, S. L.; Ward, D.; Strom, D.; Jolley, D. F. Oxidation of acid-volatile sulfide in surface sediments increases the release and toxicity of copper to the benthic amphipod Melita plumulosa. *Chemosphere* **2012**, *88*, 953−961.
(35) Xie, M.; Simpson, S. L.; Wang, W.-X. Bioturbation effects on metal release from contaminated sediments are metal-dependent. *Environ. Pollut.* **2019**, *250*, 87−96.
(36) Tan, Q.-G.; Lu, S.; Chen, R.; Peng, J. Making Acute Tests More Ecologically Relevant: Cadmium Bioaccumulation and Toxicity in an Estuarine Clam under Various Salinities Modeled in a Toxicokinetic-Toxicodynamic Framework. *Environ. Sci. Technol.* **2019**, *53*, 2873−2880.
(37) Simpson, S.; Batley, G. *Sediment Quality Assessment: A Practical Guide*; CSIRO Publishing: Collingwood, Vic, Australia, 2016; pp 1−346.
(38) Bilos, C.; Colombo, J. C.; Presa, M. J. Trace metals in suspended particles, sediments and Asiatic clams (Corbicula fluminea) of the Río de la Plata Estuary, Argentina. *Environ. Pollut.* **1998**, *99*, 1−11.
(39) Croteau, M.-N.; Luoma, S. N. Delineating copper accumulation pathways for the freshwater bivalve Corbicula using stable copper isotopes. *Environ. Toxicol. Chem.* **2005**, *24*, 2871−2878.
(40) Stumm, W.; Morgan, J. J. *Aquatic Chemistry: Chemical Equilibria and Rates in Natural Waters*; John Wiley & Sons, 2012; Vol. *126*.
(41) Simpson, S. L.; Batley, G. E. Disturbances to metal partitioning during toxicity testing of iron(II)-rich estuarine pore waters and whole sediments. *Environ. Toxicol. Chem.* **2003**, *22*, 424−432.
(42) Di Toro, D. M.; Mahony, J. D.; Gonzalez, A. M. Particle oxidation model of synthetic FeS and sediment acid-volatile sulfide. *Environ. Toxicol. Chem.* **1996**, *15*, 2156−2167.
(43) Simpson, S. L.; Apte, S. C.; Batley, G. E. Effect of short-term resuspension events on trace metal speciation in polluted anoxic sediments. *Environ. Sci. Technol.* **1998**, *32*, 620−625.
(44) Cooper, D. C.; Morse, J. W. Biogeochemical controls on trace metal cycling in anoxic marine sediments. *Environ. Sci. Technol.* **1998**, *32*, 327−330.
(45) Simpson, S. L.; Rosner, J.; Ellis, J. Competitive displacement reactions of cadmium, copper, and zinc added to a polluted, sulfidic estuarine sediment. *Environ. Toxicol. Chem.* **2000**, *19*, 1992−1999.
(46) Allison, J. D.; Allison, T. L. *Partition Coefficients for Metals in Surface Water, Soil, and Waste*. Report EPA/600/R-05, 2005; p 74.
(47) Mann, R. M.; Hyne, R. V.; Simandjuntak, D. L.; Simpson, S. L. A rapid amphipod reproduction test for sediment quality assessment: in situ bioassays do not replicate laboratory bioassays. *Environ. Toxicol. Chem.* **2010**, *29*, 2566−2574.
(48) Wang, W.-X.; Tan, Q.-G. Applications of dynamic models in predicting the bioaccumulation, transport and toxicity of trace metals in aquatic organisms. *Environ. Pollut.* **2019**, *252*, 1561−1573.

https://doi.org/10.1021/acs.est.1c03827
Environ. Sci. Technol. 2021, 55, 13005−13013