Bioavailability of Sedimentary Contaminants Subject to Deposit-Feeder Digestion

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Contaminants in sediments are less available than their concentrations might imply, but measures of this availability have been generally lacking. Sediments ingested by benthic animals can be expected to undergo a unique chemical environment controlled by the digestive chemistry of the organism. We measured solubilization of sedimentary contaminants—Cu, Pb, and polycyclic aromatic hydrocarbons (PAH)—by digestive fluids extracted from marine invertebrates. Bioavailability of these contaminants, thus measured, is a small fraction of total contaminant loading—typically 1−10%. The amounts of metals solubilized by digestive fluids were orders of magnitude greater than would be predicted from water−solid partitioning with clean seawater, although they correlated well with solubilization by seawater. Digestive fluids from two different animal species solubilized different amounts of metals, indicating that bioavailability varies among species even under constant mode of uptake. High concentrations of solubilizing agents, such as amino acids for metals and surfactants for PAH, in the digestive fluids can explain the enhanced solubilization. This biomimetic approach to contaminant measurement provides the basis for more accurate mechanistic and routine assessments of environmental impact.

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Introduction

The bioavailability of contaminants is usually influenced by association with solids. In aquatic systems, many contaminants are strongly adsorbed onto sediments, which can reduce their dispersion and bioavailability. The high reworking rates of coastal sediments by deposit-feeders (*1*) subject these sediments to frequent exposure to deposit-feeder digestive attack. For some species, it is becoming increasingly clear that digestive solubilization is a principal route of exposure to some sedimentary contaminants (e.g., refs 2−4). Bioavailability of sedimentary contaminants will therefore depend upon solubilization under digestive biochemical conditions (*5*), a collateral result of the animals' attempt to solubilize natural foods. Animals have evolved various biochemical approaches to the solubilization of food, resulting in a variety of environmental conditions in guts. Because these environments can differ markedly from those of ambient sediment, the cycling of contaminants may be strongly influenced by gut passage.

Assays of the bioavailable fraction of contaminants often rely on solubilization by relatively weak chemical attacks on the matrix. These approaches improve correlations between analyses of loading and biotic impacts (e.g., toxicity, bioaccumulation) relative to correlations with concentrations measured by harsher chemical attacks—strong acid for metals and nonpolar solvents for hydrophobic organic contaminants (*6, 7*). However, none of these approaches simulate digestive systems. We have demonstrated an assessment of the bioavailability of nutritional materials in sediments to heterotrophs by a biomimetic approach, using *in vitro* extractions with biochemicals (e.g., enzymes) that mimic those found in digestive systems (*8*). This biomimetic approach can both provide greater mechanistic understanding of the bioavailable pools and their interaction with organisms and serve as a basis for more accurate, routine measurement methodologies. In this paper, we report a similar but more direct approach, applied to contaminants, in which we directly measured the solubilization of coastal sedimentary contaminants by digestive fluids obtained from deposit-feeders.

Materials and Methods

Digestive fluids were extracted from the gut lumens of healthy adult individuals of two species of marine deposit-feeders (a polychaete—*Arenicola marina*, lugworm; and a holothuroid—*Parastichopus californicus*, sea cucumber). Animals were collected with care to avoid injury. *Arenicola* individuals were held on a flowing seawater table for no more than 1−2 days before dissection; longer holding times result in a change in digestive agents. *Parastichopus* individuals were maintained for extended periods of time in the laboratory with no apparent effect on their digestive agents. To remove digestive fluids, the guts were excised from the animals and opened, and the contents were removed to a centrifuge tube. Solids were removed by centrifugation at 8000*g* for 30 min. Digestive fluids were then frozen at −80 °C until use.

Sediments to study metal release were collected from sites in Passamaquoddy Bay (an unpolluted site that was also the site of *Arenicola* collection) or Boothbay Harbor (Maine), Portsmouth Harbor (New Hampshire), and San

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Diego Harbor (California). In each of these three regions, we collected both uncontaminated and contaminated sediments. Sediments to study the release of polycyclic aromatic hydrocarbons (PAH) were collected from San Diego Harbor and Eagle Harbor (Puget Sound, Washington). Sediments were collected either in intertidal areas at low tide or from shipboard in deeper water areas using a grab. Only the oxic, top few millimeters of sediment were sampled, and only if the surface layer had been preserved in the grab. Samples were put into either Ziploc bags or scintillation vials and stored in a refrigerator until shipment. The one exception to this generalization is the sediments from Eagle Harbor, used for PAH experiments; in this case, samples were collected from a subsurface, anoxic zone in which high PAH loading was known to occur. After overnight shipment to our lab in Maine, sediments were refrigerated until use.

Metals. For metal release experiments, sediments were first centrifuged to remove most of their original pore water. Wet sediments were then incubated with digestive fluids or seawater (collected from the Damariscotta Estuary, ME) by adding 250−1000 μL of fluid to 60−800 mg (dry weight) of sediment. The ratio of fluid to sediment dry weight varied about 4× over the range of experiments because of a combination of small sample size, sample heterogeneity, and the fact that the sediment had to be added in the wet state to avoid artifacts from drying. The slurries were then held for 4 h at room temperature on a shaker. Control incubations included digestive fluids without sediment. After incubation, fluids were removed from the slurries by centrifugation at 8000*g* for 30 min at room temperature.

Dissolved metals in digestive fluids or seawater were analyzed by direct injection into a graphite furnace atomic absorption spectrophotometer (Perkin-Elmer 5100ZL) or inductively coupled plasma-mass spectrometer (ICP-MS, Perkin-Elmer Elan). Standard additions were performed routinely and showed recoveries of 90−110%. Solid-phase metals were analyzed by direct injection of sediment, slurried with 0.005% Triton-X and 5% HNO_3, and put into the graphite furnace, using an ultrasonic slurry sampler to prevent settling. This method gave excellent agreement with certified values of the metals for NRC-Canada standards MESS-1 and BCSS-1.

PAH. Incubations for PAH release were performed in a similar manner as the metal release experiments, with some differences. The sediment was stored frozen at −70 °C before incubation. Wet sediment and either digestive fluid or seawater were mixed in a ratio of 1 mL:1 g wet weight, incubated in a rotary shaker at 100 rpm and 20 °C for 4 h, and then centrifuged at 11 000*g* for 20 min to remove fluids from sediment. PAH were extracted from digestive fluids and sediments using a modified, single-phase dichloromethane:methanol extraction (Fang and Findlay, submitted). The extractions were performed in 50-mL screw-capped test tubes. The single-phase extraction mixture consisted of 7.5 mL of dichloromethane, 15.0 mL of methanol, and 5.0 mL of 50 mM phosphate buffer (pH 7.4). Sediments were shaken and allowed to stand in the dark at 4 °C for 24 h. The extracts were partitioned into aqueous and organic phases by the addition of dichloromethane and water, with the PAH collected from the organic phase. Extracts were cleaned up by passage through Na_2SO_4 and silicic acid columns. Analyses were conducted using a Hewlett-Packard 5890 gas chromatograph fitted with a DB-5 fused silica column and a Hewlett-Packard 5792 mass selective detector operated in the mass selective mode. Deuterated standards added prior to extraction showed recovery efficiencies for PAH from digestive fluid to average 85−90%. This extraction method has been shown to provide excellent (88−117%) recoveries of PAH with a certified marine sediment standard (NIST 1942a). Sediment dry weights were obtained on the incubated sediments after extraction.

TABLE 1

Ranges of Concentrations (μg L^{-1}) of Dissolved Metals in the Digestive Fluids from Foregut and Midgut Regions of Individuals of Two Marine Deposit-Feeders[a]

	Arenicola marina	*Parastichopus californicus*
copper	655−1108	66−161
lead	15−79	10−25
arsenic	1578−2797	55−120
cadmium	8.4−18.7	1.7−7.7

[a] *Arenicola* was collected from Passamaquoddy Bay, Maine, and *Parastichopus* was collected from Puget Sound, Washington.

Organic Carbon. Sediment samples were analyzed for total organic carbon, after vapor-phase acidification to remove carbonates, by a Perkin-Elmer 2400-II CHN analyzer.

Results

Metal Release. The digestive fluids of these animals, before incubation with contaminated sediment, contained very high concentrations of metallic and nonmetallic elements (Table 1), despite being collected from apparently unpolluted environments. These levels are much higher than are found in seawater. Of the two animal species used for these experiments, the higher levels were found with *Arenicola*.

In early experiments, we worked with fine-grained sediments to maximize contaminant signals. However, we generally found strong, net adsorption of metals from digestive fluid to the sediment as well as loss of the natural color of the digestive fluids after incubation. Both of these animal species normally ingest coarse-grained sediments in their native habitat. For the subsequent experiments reported here, we therefore obtained coarser grained sediments, even though such sediments do not normally have the highest contaminant loadings.

The sediments used for our experiments were from marine harbors. The most intensive contamination, in terms of total metal concentrations, was found with Cu (Figure 1). All sediments were taken in areas of intense recreational, naval, and fishing boat traffic, and their Cu concentrations likely represent loadings from anti-fouling paints. Significant Pb contamination (total concentration = 186 μg g^{-1}) was also found with a harbor sediment from Maine; all other sediments had total lead concentrations of <110 μg g^{-1}.

Upon incubation with harbor sediments containing high levels of Cu contamination, *Arenicola* and *Parastichopus* digestive fluids showed large increases in dissolved Cu concentration, reaching levels as high as 39.5 mg (L of digestive fluid)$^{-1}$. This high concentration is normalized per volume of digestive fluid added to the wet sediment, after correction for dilution by the pore water in the wet sediment with the conservative assumption that all metal

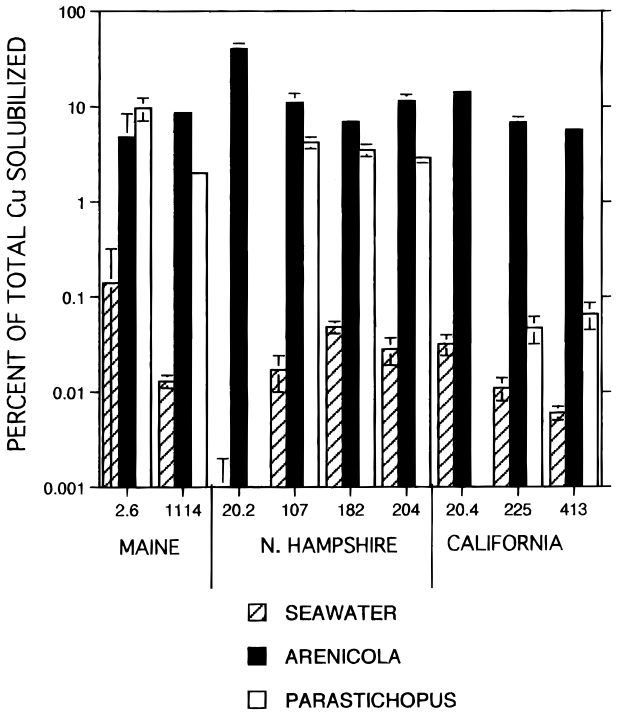

FIGURE 1. Percent release of Cu from nine sediments after incubation with seawater or digestive fluids from *Arenicola marina* or *Parastichopus californicus.* Total sedimentary concentrations of Cu (μg g^{-1}) labeled on the *x*-axis. Samples with no value plotted had no change in digestive fluid concentration, except for the uncontaminated California sediment (20.4 μg g^{-1}) onto which 75% of the Cu from the *Parastichopus* digestive fluid was adsorbed.

released in the seawater control was derived from the original pore water. Digestive fluids released 0−14% of the total sedimentary Cu from contaminated sediments (Figure 1). The uncontaminated sediment from New Hampshire yielded 40% of its Cu to *Arenicola* digestive fluid. Negligible change in Cu concentration of the digestive fluid was found for uncontaminated sediment from the animals' native habitat. *Parastichopus* virtually always released much smaller fractions of sedimentary Cu than *Arenicola*. From each sediment, digestive fluids extracted much greater amounts of Cu than seawater.

These harbor sediments had consistently high Cu loadings, but generally only minor or negligible loadings of most other metals. Other toxic metals, such as Pb, Cd, and As, accordingly showed very minor changes in concentration in digestive fluid upon incubation with sediments. The Maine sediment with significant Pb contamination caused an increase in *Arenicola* digestive fluid concentration from 15 to 275 μg L^{-1}, equivalent to a release of 0.3% of the Pb in the sediment. ICP-MS scans indicated a lack of significant concentration change for numerous elements, including Li, Co, Rb, Al, Mo, and others.

PAH Release. Both of the sediments used had high loadings of PAH, with total analyzed levels of 33 μg g^{-1} for the San Diego samples and 156 μg g^{-1} for the Eagle Harbor samples. The total organic carbon levels (TOC) values were high−4 and 4.6% for the San Diego and Eagle Harbor samples, respectively. Initial concentrations of PAH in the *Arenicola* digestive fluids, before incubation with contaminated sediment, were negligible, consistent with the uncontaminated nature of the animals' native habitat. After incubation, the total PAH concentrations in the digestive fluids increased to 0.83 and 2.1 μg mL^{-1} for the San Diego and Eagle Harbor samples, respectively. Freezing of the sediments for storage may have influenced extractability; by analogy with bioavailable amino acids, this freezing likely increased extractability (*8*). Analyses of digestive fluid and sediments after incubation showed excellent recoveries, averaging 93% of that initially present in the sediment. Analogous recoveries from the seawater incubation controls were 65% for the Eagle Harbor sample and 99% for the San Diego sample.

Digestive fluids solubilized from 0 to <10% of the total PAH loadings (Figure 2), proportions similar to the Cu release. As with metals, digestive fluids solubilized considerably greater amounts than seawater controls, which released a maximum of 0.32% of the total PAH. Patterns of PAH release differed between the two sediments. In the Eagle Harbor sample, about 2% of each compound was solubilized, except for pyrene with 8% release. In the San Diego Harbor sample, decreasing release with increasing molecular weight was observed, ranging from 9% for phenanthrene to undetectable release for indeno[1,2,3-*cd*]-pyrene and benzo[*ghi*]pyrene.

Discussion

Only minor fractions of the total contaminant loadings of Cu and PAH of a variety of sediments were found here to be solubilized by the digestive fluids of these marine invertebrates. These results corroborate bioassay studies indicating that generally only small fractions of freshly spiked contaminants are bioaccumulated (*5, 9*). The fractions of total loadings that are bioavailable tend to be lower in our study than those studies using contaminant spikes, likely because bioavailability appears to decrease with aging of the contaminant in the sediment (*10, 11*). The contaminants in our study sediments were almost certainly in place much longer than for most laboratory studies.

Comparison to Seawater Release. Estimates of contaminant impact are increasingly based on partitioning of contaminant between water and the solid phase (*12−14*). Our results for Cu show highly significant correlations ($p < 0.01$) between the amounts solubilized by clean seawater attack vs extraction by digestive fluid from each of the two species of animal used in this study (Figure 3). This finding is consistent with the good correlations found between toxicity and pore water concentrations (*14, 15*), in that each measure may provide predictive value regarding which sediments have relatively high bioavailable concentrations.

Solubilization of contaminants during digestive attack is however considerably greater than predicted from seawater−solid partitioning, so that the correlations of Figure 3 implicate only the rank order of sediments. The actual exposure to Cu during digestive attack is much greater than can be explained by pore water concentrations. Enrichment factors, defined as ratios of the amount solubilized by digestive fluid relative to that by seawater, ranged from 9 to 235 for PAH experiments in which measurable release was found for the seawater incubations. Even greater factors are implied for samples with PAH levels below detection in the seawater incubations. For Cu release from contaminated sediments, enrichment factors ranged from 5 to 2389, showing that digestive fluids solubilize much more metal than does seawater. Seawater−solid partitioning models, therefore, would underestimate absolute bioavailability under digestive conditions, a conclusion consistent with previous studies (*9, 25*). Furthermore, the high variability in enrichment factors reduces the predictive

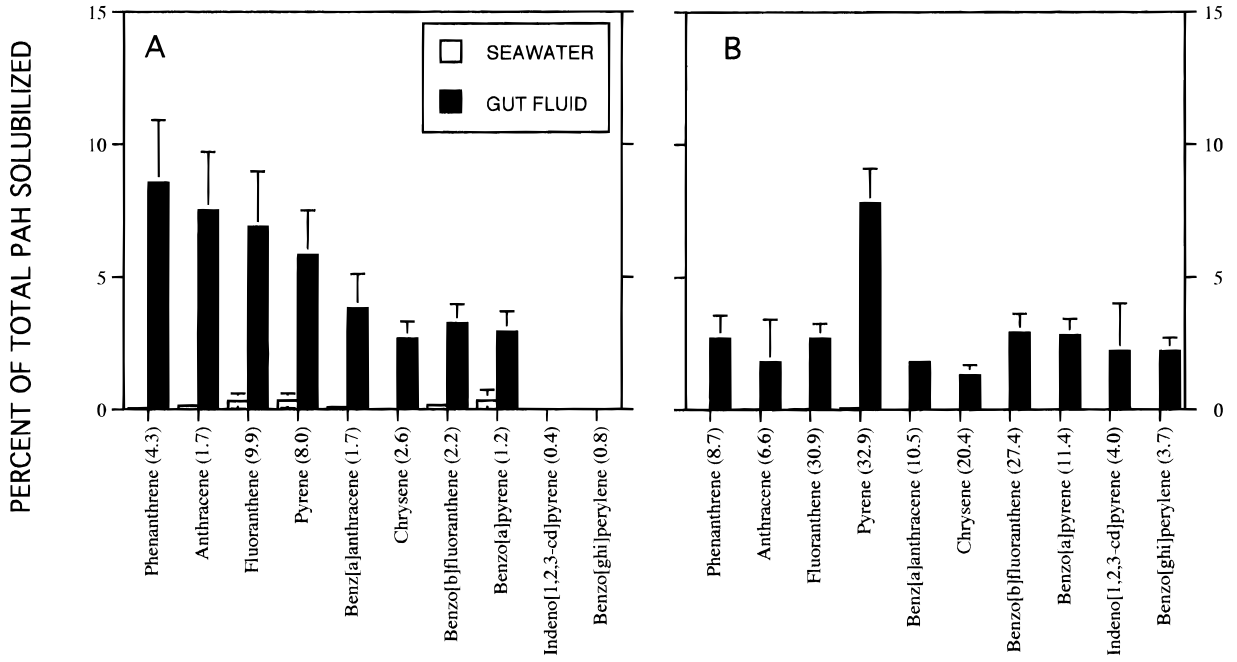

FIGURE 2. Percent release of PAH from sediments sampled in (A) San Diego Harbor, California, and (B) Eagle Harbor, Washington. Total concentrations of individual PAH (μg g^{-1}) are given in parentheses. Seawater values are too small to be seen on these plots for many samples and were below detection for about half of the samples.

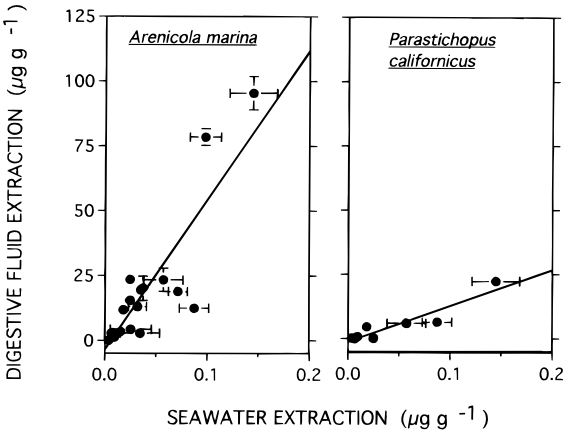

FIGURE 3. Concentrations of Cu extracted by seawater, *Arenicola* digestive fluid, and *Parastichopus* digestive fluid normalized to sediment weight (μg g^{-1}). Linear correlations are highly significant ($p < 0.001$) for each species' digestive fluid vs seawater. Lines represent linear regressions. More experiments were run with *Arenicola* than with *Parastichopus*.

power of water–solid partitioning data or models for patterns of relative contaminant impact.

Solubilizing Agents. The enhanced solubilization of contaminants relative to seawater is likely due to solubilizing agents secreted by animals into their digestive lumens. We have found digestive fluids to be typically colored, often with yellow to dark brown hues. This coloring suggests high organic matter concentrations. Dissolved amino acid concentrations were measured and found to be extremely high, with average levels in the digestively active gut sections of 0.4 M for *Arenicola* and 0.01 M for *Parastichopus* (Mayer et al., unpublished). These amino acids represent a combination of secreted enzymes and hydrolyzed food waiting to be absorbed. Amino acids in monomeric and polymeric forms are strong complexing agents for Cu (*17*) and represent one possible mechanism for metal complexation. The greater solubilization, and hence enrichment factors, in *Arenicola* relative to *Parastichopus* may result from the higher amino acid or perhaps other unexamined compound class concentrations. Changes in E_h/pH may also affect contaminant solubilization, but the small differences found in deposit-feeder guts relative to ambient sediment (*18*) do not imply a significant role in metal solubilization.

These digestive fluids exhibit surfactant activity as well, indicated by frothiness during pipetting. More quantitative indication was provided by measuring contact angles of droplets of digestive fluids, from the digestively active gut sections, placed on Parafilm (Mayer et al., unpublished). We have found deposit-feeder contact angles to be quite low, with *Arenicola* having many values in the 30–40° range. These values are similar to ones obtained with commercial surfactants. Compared to the normal contact angles of >100° for clean seawater on Parafilm, these angles indicate high surfactant activity in the digestive fluids. Furthermore, these fluids show evidence for surfactant micelles in their digestive fluids, based on titrations of the digestive fluids with clean seawater. Surfactants, especially at levels that lead to micellization, are well-known solubilizers for PAH and other lipophilic contaminants (*19, 20*).

Only two experiments examined PAH release and showed distinctly different patterns of release of the various PAH. The experiment with decreasing release with increasing molecular weight, and hence increasing octanol–water partition coefficient, provides a pattern of release (Figure 2) similar to desorption into polar solvents, indicating that the sediment has a higher affinity for the hydrophobic PAH than the digestive fluid. The experiment with similar release percentages for all PAH but pyrene cannot be explained without further data. It is possible that freezing affected these comparative results.

Differences in bioavailability of contaminants to different species of benthic animals have been inferred from bioaccumulation experiments and suggested to be due to different modes of exposure (*16*). Our results show that a

similar mode of exposure—digestion in this case—can result in markedly different bioavailability for different species. The digestive fluid from *Arenicola* was always able to solubilize much more Cu than that from *Parastichopus* (Figure 1). However, the amounts extracted by the digestive fluids of each species showed highly significant correlation ($p < 0.01$) with one another, indicating that this approach may provide relative estimation of bioavailability among sediments. More work with other sediments and animal species will be needed to corroborate this finding.

Caveats. Our early experiments showed that incubation with finer grained sediments resulted in net adsorption of color and metals from the digestive fluid onto the sediment. This analytical approach may therefore be limited in its practical application. For example, routine measurement of bioavailable contaminants in survey mode might be problematic because of the wide range of grain sizes typically encountered. However, the approach can be highly informative regarding effects on endemic species.

Our analyses estimate only solubilization and ignore the toxicity of the dissolved product or its absorption across gut walls. Some of the solubilized contaminant may be excreted without absorption. The high, pre-existing metal concentrations in digestive fluids hint at lack of absorption. However, we find even higher concentrations of amino acids in low molecular weight form that can be absorbed (*21*). Concentration alone is not evidence for or against absorption, and the fate of these solubilized contaminants within organisms needs further investigation. Nevertheless, even if solubilized contaminants are excreted instead of absorbed, the geochemical impact of solubilization will be significant, because solubilized contaminants will be more susceptible to other fates such as physical transport, chemical reactions, or uptake by other organisms.

Other caveats apply to these results, or any measure of bioavailability. First, human analysts cannot select sediment particles in the same fashion as the animals (*22*), leading to sampling bias relative to an animal's ability to access contaminants (*23*). Second, the data presented here represent batch experiments with 4-h incubations. This time period was selected as an intermediate value for many deposit-feeders; it is too long for *Arenicola* and too short for *Parastichopus* (unpublished data). Kinetics experiments indicate that the extent of solubilization for metals does not reach equilibrium in 4 h (unpublished data), so that solubilization kinetics, relative to gut residence times of the animals, will influence the extent of bioavailability (*5*). Evidence for this time dependence was inferred from an inverse relationship between bioaccumulation and gut residence time by Klump et al. (*24*).

However, none of these caveats invalidates the principal conclusions of low fractions of bioavailability under digestive conditions, its species-specificity, and its relative but not absolute agreement with results from water—sediment partitioning. The high concentrations of potential solubilizing agents for contaminants found in these guts, such as amino acids and surfactants, imply that deposit-feeder digestive systems are potentially important zones for the environmental cycling of contaminants. The great range of digestive conditions in deposit-feeders will necessitate greater appreciation of digestive physiology to assess the impact of gut passage on contaminated sediments. These results underscore the importance of a biomimetic approach to *in vitro* measurement of bioavailability (*8*), in order to study the nature of the bioavailable pool and the means by which it is made available. Mechanistic understanding of solubilization by digestive fluids will also provide a basis for the design of analytical approaches that are capable of measuring bioavailable concentrations of contaminants in ecosystems. This biomimetic approach may bridge the gap between chemically and biologically based assays in environmental decision making.

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