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High concentrations of complexed metals in the guts of deposit feeders

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Abstract

Sediment particles passing through the guts of deposit feeders are subject to an environment unusually rich in dissolved organic material, especially proteinaceous materials, capable of binding metals. Concentrations of many heavy metals are high in gut fluids of various deposit feeders from pristine environments. Concentrations of Cu and Cd show strong correlations with total acid-hydrolyzed amino acids (TAHAA) in gut fluids in a cross-phyletic survey of 35 deposit feeder species. Similar correspondence with TAHAA obtains among individuals, along longitudinal gut sections, and among molecular weight fractions. Multiple incubations of sediments with a commercial protein solution having similar TAHAA concentration as gut fluid solubilized many metals, showing convergence toward similar composition as gut fluid. Adherence of gut-dissolved metal concentrations to the Irving–Williams order suggests that the rich milieu of soft ligands contained in gut fluids provides a mechanism for mobilization of softer metals in sediments.

Dense populations of marine deposit-feeding invertebrates often process large amounts of sediment. As an example, individuals of the lugworm (*Arenicola marina,* Polychaeta), a common nonselective deposit-feeder species in sandy intertidal zones (Flach and Beukema 1994), can ingest up to 20× their body weight of wet sediments per day (Cadée 1976). Studies on the effects of bioturbation on geochemistry of metals in sediment often emphasize physical movement of particles caused by feeding and burrowing of this species (e.g., Rasmussen et al. 1998), while the impact of digestive processes occurring in the guts of these animals has been largely ignored.

Functionally, the tubular guts of deposit feeders often resemble a plug-flow reactor (Penry and Jumars 1987) where transiting sediments are processed. These animals secrete a wide range of digestive agents (e.g., enzymes and surfactants) to hydrolyze and solubilize nutrients from bulk sedimentary matrices (Mayer et al. 1997). The gut lumen is not a somatic part of the organism but is rather an extracellular environment differing from the ambient sediments in that it contains high concentrations of dissolved organic matter (DOM). The DOM is especially rich in proteinaceous materials that usually account for about half of total DOM. These proteinaceous materials are in the forms of secreted enzymes and hydrolyzed food and consist of monomers and polymers of amino acids (Mayer et al. 1997). Many forms of DOM are capable of complexing metals, but proteinaceous materials are especially rich in ligand groups (e.g., amine, thiol, carboxyl) capable of complexing softer metals. Thus, sedimentary metals passing through guts experience very different biogeochemical conditions than in ambient sediment, and new equilibria should be established between the fluid and solid phases. This re-equilibration should not be only a function of individual metal geochemical behavior and biogeochemical conditions in guts but also be controlled by gut residence time of sediments. Relatively soft metals that are amenable to covalent association with DOM should be primarily affected by this re-equilibration process.

Previous work involving in vitro incubations of metal-contaminated sediments with lugworm gut fluid showed marked dissolution of metals from sediments as a result of complexation with gut proteinaceous materials due to excess solid-phase metal concentrations (Mayer et al. 1996; Chen and Mayer 1998). The extent of the dissolution is typically two to three orders of magnitude higher with incubation of the same sediments in seawater. However, whether this di-

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Table 1. Information on organisms used in this study.

Species	ID	Phylum/Class	Feeding mode	Location	Date	Gu Sections	Replicates
Arenicola brasiliensis	AB	Polychaeta	DPF	Half Moon Bay, CA	5 Jul 97	Midgut	5(1)
Amphitrite johnstoni	AJ	Polychaeta	DPF	Sheepscot Estuary, ME	5 Aug 97	Midgut	1(20)
Arenicola marina	AMA	Polychaeta	DPF	Passamaquoddy Bay, ME	1 Sep 97	Midgut	4(20)
Abarenicola pacifica	AP	Polychaeta	DPF	False Bay, WA	17 Aug 97	Midgut	1(14)
Abarenicola vagabunda	AV	Polychaeta	DPF	Eagle Cove, WA	17 Aug 98	Midgut	5(1)
Cirratulus cirratus	CC	Polychaeta	DPF	Damariscotta Estuary, ME	2 Sep 98	Whole gut	1(20)
Clymenella torquata	CT	Polychaeta	DPF	Damariscotta Estuary, ME	13 Aug 98	Whole gut	1(20)
Eupolymnia heterobranchiata	EH	Polychaeta	DPF	Little Tutka Bay, AK	17 Oct 97	Whole gut	1(7)
Nereis diversicolor	ND	Polychaeta	DPF/SF	Booth Bay, ME	21 Jul 98	Whole gut	1(20)
Nicolea venustula	NIV	Polychaeta	DPF	Pleasant Bay, ME	5 Sep 98	Whole gut	1(1)
Schizobranchia insignis	SCI	Polychaeta	SF	False Bay, WA	1 Jul 94	Midgut	1(5)
Spio setosa	SS	Polychaeta	DPF	Damariscotta Estuary, ME	11 Aug 98	Whole gut	1(20)
Travisia foetida	TF	Polychaeta	DPF	Puget Sound, WA	16 Aug 97	Whole gut	2(1)
Myxicola infundibulum	MYI	Polychaeta	SF	Pleasant Bay, ME	5 Sep 98	Whole gut	1(2)
Nereis virens	NEV	Polychaeta	O	Sheepscot Estuary, ME	20 Jul 96	Whole gut	1(20)
Astarte castanea	AC	Bivalvia	SF	Pleasant Bay, ME	5 Sep 98	Stomach	1(6)
Crassostrea virginica	CV	Bivalvia	SF	Damariscotta Estuary, ME	22 Jul 98	Stomach	4(1)
Ensis directus	ED	Bivalvia	SF	Damariscotta Estuary, ME	22 Jul 98	Stomach	3(1)
Hiatella arctica	HA	Bivalvia	SF	Pleasant Bay, ME	5 Sep 98	Stomach	1(1)
Mya arenaria	MA	Bivalvia	SF	Damariscotta Estuary, ME	22 Jul 98	Stomach	3(20)
Modiolus demissus	MD	Bivalvia	SF	Narragansett Bay, RI	27 Aug 98	Stomach	4(1)
Modiolus modiolus	MM	Bivalvia	SF	Damariscotta Estuary, ME	22 Jul 98	Stomach	2(2)
Brisaster latifrons	BL	Echinoidea	DPF	Puget Sound, WA	16 Aug 97	Whole gut	2(1)
Echinarachnius parma	EP	Echinoidea	DPF	Damariscotta Estuary, ME	26 Aug 98	Foregut	5(5)
Cucumaria frondosa	CF	Holothuroidea	SF	Damariscotta Estuary, ME	20 Jul 96	Whole gut	2(20)
Chiridota laevis	CL	Holothuroidea	DPF	Pemaquid Point, ME	20 Aug 98	Whole gut	1(1)
Chirdota sp.	CS	Holothuroidea	DPF	Jakolof Bay, AK	16 Oct 97	Whole gut	1(4)
Eupentacta quinquesemita	EQ	Holothuroidea	DPF	Jakolof Bay, AK	16 Oct 97	Whole gut	2(10)
Molpadia intermedia	MOI	Holothuroidea	DPF	Puget Sound, WA	16 Aug 97	Whole gut	5(5)
Parastichopus californicus	PC	Holothuroidea	DPF	Puget Sound, WA	1 Jul 96	Midgut	5(5)
Echiurus echiurus	EE	Echiura	DPF	Little Tutka Bay, AK	17 Oct 97	Midgut	4(1)
Urechis caupo	UC	Echiura	SF	Bodega Bay, CA	24 Jun 97	Midgut	4(1)
Saccoglossus bromophenolosus	SB	Hemichordata	DPF	Damariscotta Estuary, ME	12 Aug 98	Midgut	1(10)
Protoglossus graveolens	PG	Hemichordata	DPF	Damariscotta Estuary, ME	20 Aug 98	Midgut	1(1)
Siphonosoma ingens	SI	Sipuncula	DPF	Bodega Bay, CA	24 Jun 97	Whole gut	4(1)

gestively enhanced dissolution occurs in natural, uncontaminated environments is unknown.

High background levels of Cu, As, Pb, and Cd concentrations were reported in gut fluids of lugworm and a deposit-feeding sea cucumber from unpolluted environments (Mayer et al. 1996). These background levels imply that sedimentary metals were solubilized as a result of gut ligand complexation during long-term, deposit-feeding activities and that equilibria between gut fluid and the animal's clean home sediment may exist. The primary objective of this paper is to test these possibilities by (1) analyzing background levels of a wide range of metals in the gut fluid of *A. marina*; (2) analyzing background levels of selected metals in a wide variety of other benthic invertebrates; (3) assessing the potential role of gut proteinaceous materials as soft ligands in maintaining these high metal concentrations by examining correlations between metals and proteinaceous materials interphyletically, intraphyletically, along gut sections, and among molecular weight separates; and (4) assessing the extent of sedimentary metal solubilization from clean sediment by in vitro incubation approaches with protein solutions of similar concentration as gut fluids.

Materials and Methods

Collection of organisms—Organisms were collected from various locations in the United States (Table 1). The habitats of these organisms were at least 14 km from known pollution sources, and their relatively pristine conditions were corroborated by low sedimentary metal concentrations (unpubl. data) and/or observations of high species diversity. After collection, these organisms were held without sediments in a flowing seawater table for 0–48 h until gut fluids were sampled. The composition of gut fluids in some species was unlikely affected by this procedure as indicated by relatively stable enzymatic activities in the lugworm following a prolonged gut evacuation period. In other species there may have been changes in gut chemistry, but for the relationships described here we believe that these effects are minimal (discussed further below).

Sampling of gut fluids—Animals were dissected to collect gut fluids. During dissection, body walls were cut open with care to avoid rupturing guts. A thin plastic pipette tip or a clean glass needle was used to penetrate the gut wall to

sample luminal fluids of small organisms. Gut fluid of larger species, such as *Parastichopus californicus*, was directly poured into a clean beaker from an opened end of the gut. Sections of guts where fluid were collected are listed in Table 1. Sufficient volume of gut fluid from smaller organisms was accumulated by pooling from multiple individuals. Sediments were removed from gut fluids by centrifugation at 8,000 × g and 4°C for 30 min, and the fluids were stored in 1.5-ml plastic vials at −80°C until analysis. Before sampling gut fluid from a population of *A. marina*, the body width of each individual was recorded at the fifth setiger. The worms were intentionally disturbed and always became turgid before measurement, providing a good correlation ($r^2 = 0.85$, $n = 46$) between body width and dry weight.

In another experiment, gut fluids from different gut sections (foregut, midgut, mid-hindgut, hindgut) of lugworms were sampled with a glass needle. Gut fluids from 15 individuals (from Passamaquoddy Bay, Maine, 1998) were pooled to get enough volume for metal and total acid hydrolyzable amino acid (TAHAA) analysis for each gut section (three replicates).

Molecular weight distribution of metals and TAHAA in lugworm gut fluid—A pooled lugworm gut fluid was separated into four molecular weight (MW) fractions (>100 kDa, 10–100 kDa, 2–10 kDa, and <2 kDa) by sequential centrifugation through three sets of ultrafiltration units (SPECTRUM®) with molecular weight cutoffs of 100 kDa, 10 kDa, and 2 kDa, respectively. To avoid clogging the membranes, gut fluids were first diluted 10× with NANOpure water before separation and centrifuged at 4°C, according to the manufacturer's instructions, at 500 × g, 1,500 × g, and 2,000 × g for 100 kDa, 10 kDa, and 2 kDa membranes, respectively.

Metal and TAHAA analysis—Analyses for 17 metals were carried out on samples of lugworm gut fluids (each pooled from ≥10 individuals) and on a seawater sample (S ≈ 32, Damariscotta Estuary, Maine). Mo, Li, Rb, Sr, and Ba concentrations were measured by an inductively coupled plasma-mass spectrometer (ICP-MS, Perkin-Elmer Elan 5000), while the others (Cr, Mn, Fe, Co, Ni, Cu, Zn, Cd, Pb, As, Se, and Al) were determined by a graphite furnace atomic absorption spectrophotometer (GFAAS, Perkin-Elmer 5100ZL). These metals were chosen on the basis of environmental significance and analytical feasibility. Coefficient of variation on GFAAS measurements on gut fluids was typically <5%, while that of ICP-MS analysis ranged up to 10–20% except for Ba (20–30%). Standard additions were performed on each batch of samples to ensure recoveries of 90–110%. High concentrations of organic matter and salts in gut fluid can cause poor recoveries, but dilution of gut fluid with NANOpure water brought recoveries to 100 ± 10%. Interferences from the seawater matrix caused large standard deviations for samples near the detection limits of our GFAAS method (direct injection) that may result in overestimation of metal concentrations in seawater. Nevertheless, these potential errors are not sufficient to interfere with our conclusions (*see below*). Error bars in graphs and tables reflect combined standard deviations from analytical and experimental replicates, unless stated otherwise. Only Cu and Cd in gut fluids of organisms other than the lugworm were analyzed due to low sample volume in many small organisms. The wide range of animal species was chosen to corroborate metal–ligand relationships found in lugworms.

Dissolved TAHAA in gut fluid were analyzed according to Mayer et al. (1995). The gut AAs were first hydrolyzed in 6 N HCl at 110°C for 22 h, and the hydrolyzed samples were derivatized with orthophthaldialdehyde followed by fluorometric detection. TAHAA were examined because of their high concentrations in gut fluid (Mayer et al. 1997), their known potential complexation with a variety of metals (Martell and Smith, 1982), and their demonstrated involvement in binding Cu and Pb in gut fluids (Chen and Mayer 1998, 1999).

Tissue metal concentrations were compared to those in gut fluid of lugworms. Nongut body parts of 15 lugworms from the same population were saved for metal analysis, because the large surface area in guts may be contaminated with sediments and to avoid sampling metal-rich granules likely associated with gut tissues (Brown 1982). The tissues were freeze-dried, ground, and digested with 70% HNO_3 in a microwave sample preparation system (CEM MSP1000). The digestion protocol was adapted from that for fish tissues provided by the manufacturer. After digestion, sample volumes were brought up to 10 ml with NANOpure water, and the metals were measured by GFAAS. A standard material (bovine liver, NBS 1577) was similarly analyzed, and its metal concentrations were within one standard deviation of the certified values.

Incubation of gut fluid and bovine serum albumin (BSA) solution with multiple aliquots of clean sediment—Gut fluid in some invertebrates (including the lugworm) appears to remain relatively immobile while the sediment transits (Mayer et al. 1997; Weston and Mayer 1998). To mimic continuous feeding events, therefore, one aliquot of gut fluid or BSA solution was incubated sequentially with seven aliquots of noncontaminated, surface sediments from the worms' habitat. The sediment was collected in June 1998 for a previous project and stored at 4°C for about 6 months before use. Incubation of lugworm gut fluid with this sediment allows testing if metal concentrations in gut fluid have reached a constant level vis-a-vis this sediment. Gut fluids were sampled from the midgut section and pooled from more than 100 large lugworms collected in November 1997 and then stored at −80°C until use in this experiment. The TAHAA concentration of this gut fluid was approximately 90 mM.

BSA was chosen because, at similar TAHAA concentrations as gut fluids, it releases a similar amount of metals from contaminated sediments in batch (Cu; Chen and Mayer 1998) and time-course experiments (Cu, Cd, and Pb; Chen and Mayer 1999). BSA was used to test if (1) metals could be remobilized from clean sediments when a mimic of a low metal-concentration gut fluid is encountered and (2) if the pattern of metal concentration becomes similar to that of real gut fluid. BSA solution was prepared by diluting a concentrated BSA stock solution (Sigma P5304), prepared with sea-

Table 2. Concentrations and enrichment factors (EF) of borderline (BL), class A (A), and oxyanionic (OX) metals in gut fluid of lugworms (μmol L^{-1}), tissues of lugworms (μmol g^{-1} dwt, $n = 19$), seawater (μmol L^{-1}), and the Earth crust (μmol g^{-1}). ND = not determined. EF_{GF} = enrichment factors in gut fluid, EF_{ts} = enrichment factors in tissues. Average metal concentrations in the crust from Martin and Whitfield (1983). Each sample of gut fluid was pooled from midguts of >10 individuals. (n = number of pooled samples analyzed.

	Gut fluid		Tissue		Seawater	Crust
Metal	Concentration	EF_{GF}	Concentration	EF_{TS}	concentration	concentration
Cr(BL)	0.13(5)	2.29×10^{1}	0.03±0.01	1.59×10^{1}	0.01±0.01	1.37×10^{0}
Mn(BL)	4.65(5)	8.52×10^{1}	0.29±0.09	1.49×10^{1}	0.076±0.006	1.31×10^{1}
Fe(BL)	269(4)	1.00×10^{2}	4.9±0.4	5.13×10^{0}	0.01±0.02	6.43×10^{2}
Co(BL)	3.20(4)	3.49×10^{3}	0.019±0.003	5.79×10^{1}	0.010±0.002	2.20×10^{1}
Ni(BL)	13.5(4)	3.88×10^{3}	0.012±0.002	9.77×10^{0}	0.041±0.002	8.35×10^{1}
Cu(BL)	11.7(7)	5.58×10^{3}	0.112±0.005	1.50×10^{2}	0.03±0.03	5.04×10^{1}
Zn(BL)	32.0(4)	3.95×10^{3}	1.23±0.05	4.23×10^{2}	0.028±0.008	1.94×10^{0}
Cd(BL)	0.12(8)	1.62×10^{4}	0.012±0.003	4.39×10^{3}	0.008±0.003	1.78×10^{3}
Pb(BL)	0.26(9)	8.08×10^{2}	0.005±0.001	4.40×10^{1}	0.01±0.01	7.72×10^{2}
Mo(OX)	2.24(2)	3.03×10^{4}	ND	ND	0.10±0.02	1.77×10^{2}
As(OX)	36.6(9)	8.33×10^{4}	0.27±0.01	1.70×10^{3}	0.10±0.03	1.05×10^{1}
Se(OX)	3.26(3)	1.24×10^{6}	0.24±0.03	2.54×10^{5}	0.01±0.05	6.33×10^{4}
Li(A)	26(2)	1.03×10^{3}	ND	ND	27±4	6.05×10^{0}
Rb(A)	1.87(2)	3.43×10^{2}	ND	ND	1.35±0.06	1.31×10^{0}
Sr(A)	88(1)	6.66×10^{3}	ND	ND	73±7	3.17×10^{0}
Ba(A)	0.19(1)	1.41×10^{1}	ND	ND	0.14±0.09	3.24×10^{0}
Al(A)	10.7(4)	1.00×10^{0}	3.8±0.5	1.00×10^{0}	0.15±0.02	2.57×10^{3}

water (S ≈ 32) from the batch analyzed above, to a similar TAHAA concentration as in the gut fluid above.

To start each incubation cycle, duplicates of about 5 g wet weight of the sediment were incubated with 10 ml gut fluid or BSA solution in acid-cleaned 15-ml centrifuge tubes on a shaker for 1 h at 20°C. The 1-h incubation period mimics the typical gut residence time of 20–60 min for the lugworm. After incubation, sediments were removed by centrifugation at 20,000 × g for 30 min at 4°C and 100 μl of the supernates was subsampled for measuring concentrations of TAHAA and metals. Another aliquot of sediment was then added to these tubes containing gut fluid or BSA for the subsequent incubation, and the process repeated. The high-speed centrifugation was necessary to prevent carryover of Al-rich mineral colloids to the subsequent incubations. The weight of the sediment was proportionally reduced in each incubation to ensure a constant fluid : sediment ratio of 2 : 1 ml g^{-1}. Controls, including gut fluid and BSA without sediment incubation, were performed at the same experimental conditions.

Results

Metal concentrations in gut fluid and tissue of the lugworm—Class A metal (Li, Rb, Sr, Ba) concentrations in gut fluid of *A. marina* were similar to seawater, except Al (Table 2). Al concentrations higher than those in seawater suggest the presence of mineral colloids, as Al is enriched in mineral materials. Concentrations ranging from one to four orders of magnitude greater than seawater were found for borderline metals (Fe, Co, Ni, Cu, Zn, Cd, and Pb). Mo, Se, and As that normally exist as oxyanions in seawater were also significantly enriched in gut fluids. The average concentrations of 12 metals in tissues of *A. marina* (Table 2) are consistent with a previous report on this species in the Netherlands (Jenner and Bowmer 1990), except for Ni concentrations which were about one order of magnitude lower in our study.

TAHAA and metal relationships—The relationships between TAHAA and metals in gut fluid were examined at four levels: interphyletic (35 species of polychaetes, echinoderms, mollusks, and others), intraspecies (among individuals of the lugworm), along gut sections (lugworm), and among MW fractions (lugworm).

TAHAA concentrations in gut fluids of the 35 species varied more than two orders of magnitude, including one order of magnitude variation among lugworms (Fig. 1). Two groups of organisms formed the end members of the TAHAA concentration spectrum, with polychaetes typically at the higher and echinoderms at the lower ends of the concentration range. Mollusks and other taxa (Echiura, Hemichordata, Sipuncula) bridge the gap between polychaetes and echinoderms.

Interphyletically, Cu concentrations correlated positively with gut TAHAA concentrations ($P < 0.01$, Fig. 1a). The relationship between Cd and TAHAA was also significant but not as strong ($P < 0.05$, Fig. 1b) with large variations among species having high TAHAA concentrations (≥50 mM). As with TAHAA, polychaetes generally had higher metal concentrations than echinoderms.

Intraphyletically, gut fluid Cu and Cd concentrations correlated strongly with TAHAA ($P < 0.01$ for both Cu and Cd, Fig. 1), and each correlated inversely with body size of *A. marina* ($P < 0.01$ for Cu and $P < 0.05$ for Cd, Fig. 2), with TAHAA ranging from about 100 mM for the larger to 900 mM for the smaller individuals. The ratios of Cu or Cd to TAHAA, however, did not correlate significantly with body size.

TAHAA and metal concentrations showed similar rank patterns along the gut sections of lugworms (Fig. 3, $P <$

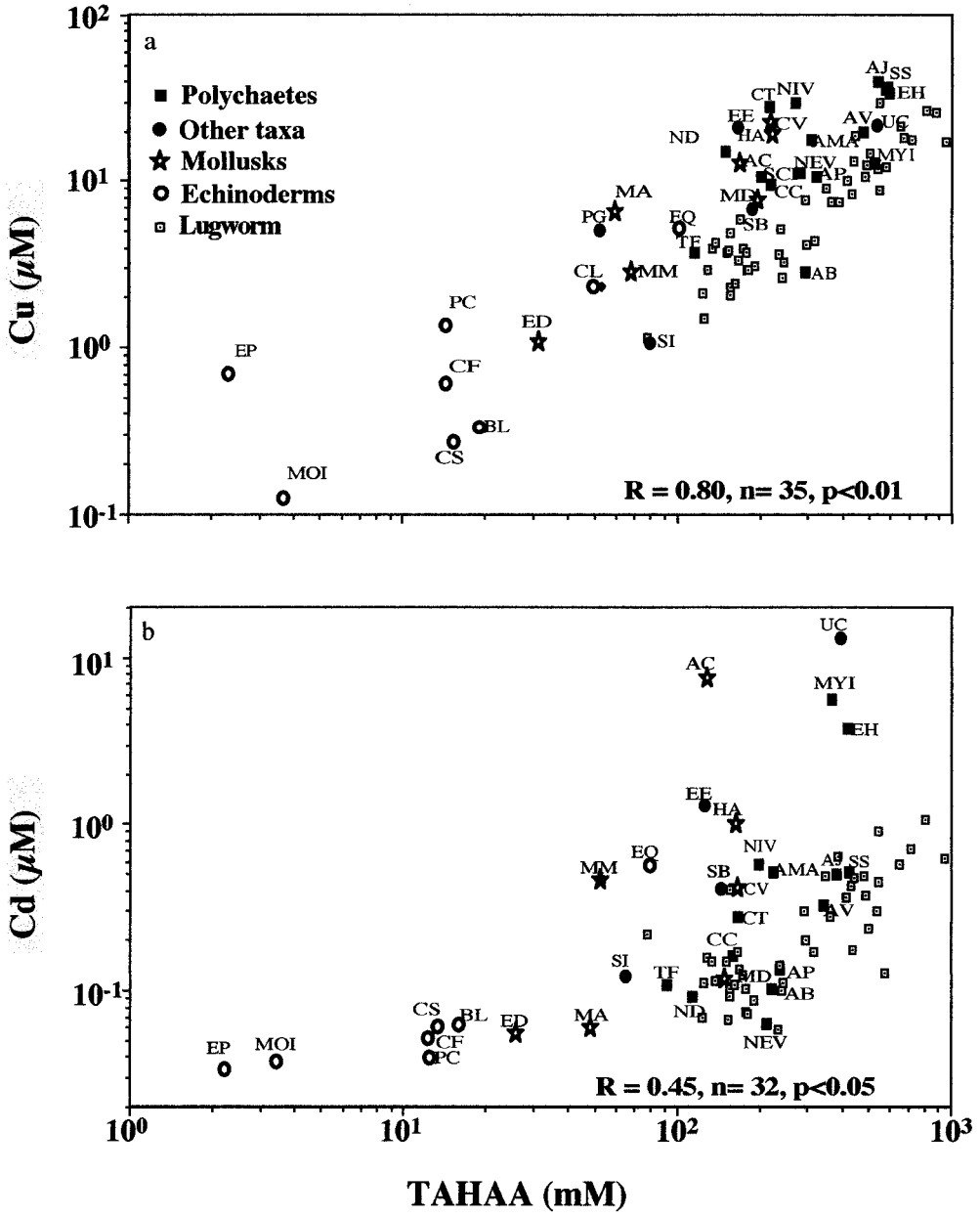

Fig. 1. (a) Cu and (b) Cd versus TAHAA in gut fluids of 35 species of organisms. See Table 1 for key to various species. Data for the individuals of lugworm are not included in the calculation of the correlation coefficients (R). Concentrations were averaged when replicates were available (see Table 1).

0.01, Kendall's concordance W-test; Daniel 1990). TAHAA and metal concentrations were low in foregut sections, highest in the midgut section that normally has the highest digestive intensity as indicated by enzyme activities and surfactancy (Mayer et al. 1997), and reduced to lowest levels in the hindgut. This pattern of TAHAA distribution along the guts is consistent with the digestive biology of many deposit-feeding polychaetes, in which the hydrolyzed TAHAA from sediments and extracellular enzymes appear to be retained selectively in the midgut and decoupled from the transiting sediments (Mayer et al. 1997).

TAHAA and metals in the midgut fluid of the lugworm appeared in all four MW fractions (Fig. 4), with the distribution of Cu and Cd again following similar patterns as TAHAA ($P < 0.025$, Kendall's concordance W-test; Daniel 1990). The appearance of metals in higher molecular weight fractions implies some form of complexation. Among these separates, the Cu : TAHAA concentration ratio was significantly higher in the 2–10-kDa fraction. This fraction may contain metal-rich, metallothionein-like proteins (that have molecular weights in this range) but contains only a minor portion of the total dissolved Cu. TAHAA in the high MW fractions (>10 kDa) that presumably represent digestive enzymes secreted by organisms (Mayer et al. 1997) accounted

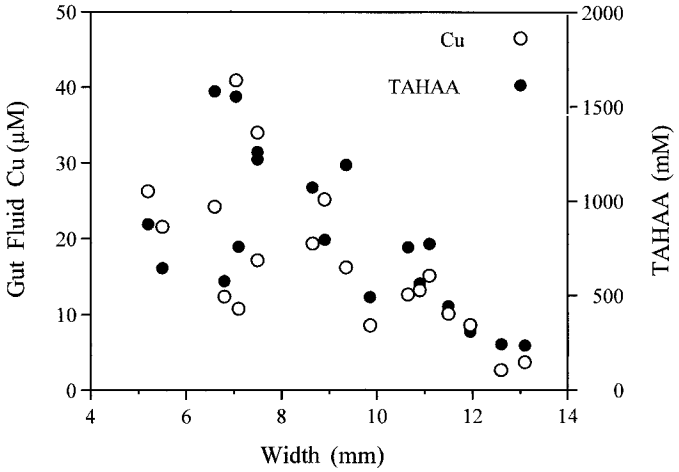

Fig. 2. Concentrations of Cu and TAHAA in lugworm gut fluids versus body width at the fifth setiger of the individuals.

for 80% of the total TAHAA, while that of the low MW fraction (<10 kDa) that are likely dominated by hydrolyzed food items only accounted for 20%. Almost all of the low MW TAHAA was smaller than 2 kDa, with a negligible amount in the 2–10-kDa fraction.

Repeat incubation experiments—In the incubation experiments with gut fluid, TAHAA and the softer metals (Ni, Cu, Co, and to a lesser extent Zn) showed relatively small changes in concentrations, especially in the first several cycles (Fig. 5d–g). The minimal concentration changes of the softer metals suggest that most of these metals were in steady state with this sediment; it also suggests that the buildup or decline of gut metals is a slow process with small increment or decrement with each passage of sediments. Larger changes were evident with the redox-sensitive elements Fe and Mn and with Al (Fig. 5b–c,h). The declining Mn and Fe concentrations may be due to reoxidation and subsequently adsorption of the existing, dissolved Mn and Fe in gut fluid once it encountered oxic sediments during incubation. Cr was present at very low levels and showed erratic behavior.

In the BSA experiment, concentrations of the softer metals (Ni, Cu, Zn) increased steadily during each incubation cycle. Mn increased to a peak in the fourth cycle, followed by steady concentrations. Co showed similar behavior to Mn in the first four cycles; these two metals commonly behave in parallel fashion in sedimentary environments (Shaw et al. 1990). Cr was initially present in BSA at a high concentration that declined slightly during the incubations. Fe and Al showed rapid increase to a peak in the second cycle followed by steady decreases. This similarity suggests mineral colloid peptization followed by losses to the sedimentary phase. Thus, the gut fluid and BSA solutions tended to converge in terms of metal concentrations and TAHAA : metal ratios, in most cases to within one order of magnitude of each other at the end of the repeat incubations (Fig. 5).

Discussion

High concentrations of borderline metals in gut fluids as a result of complexation by proteinaceous materials—Sub-

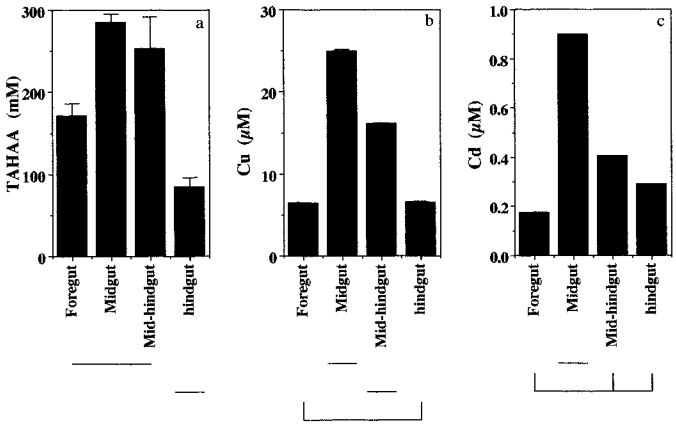

Fig. 3. Concentrations of TAHAA (a), Cu (b), and Cd (c) along gut sections of lugworms. Error bars = ±1 standard deviation. Bars under the graph are results of multiple comparisons among pairs of means with the T-method (Sokal and Rohlf 1995). Rank patterns among the gut sections are consistent for TAHAA, Cu, and Cd ($P < 0.01$, Kendall's concordance W; Daniel 1990).

stantial evidence indicates that lugworm gut fluid is seawater-based. For example, the dissolved, class A metal (Li, Rb, Sr, Ba) concentrations are nearly identical between gut fluid and seawater (Table 2), as are conductivity and pH measurements (unpubl. data). However, gut fluids are enriched relative to seawater in materials such as DOM (Mayer et al. 1997) and borderline metals and metalloids (Table 2). Two possible explanations for the elevated heavy metal concentrations are mineral colloids, whose presence is indicated by elevated Al concentrations, and/or DOM in gut fluid.

The possible role of mineral colloids in gut fluid can be assessed with enrichment factors. The enrichment factor (EF) of a metal M (Chester 1990) is defined as:

$$EF_M = ([M]/[Al])_{gut\ fluid}/([M]/[Al])_{crust},$$

which is the ratio of Al-normalized M concentration in gut

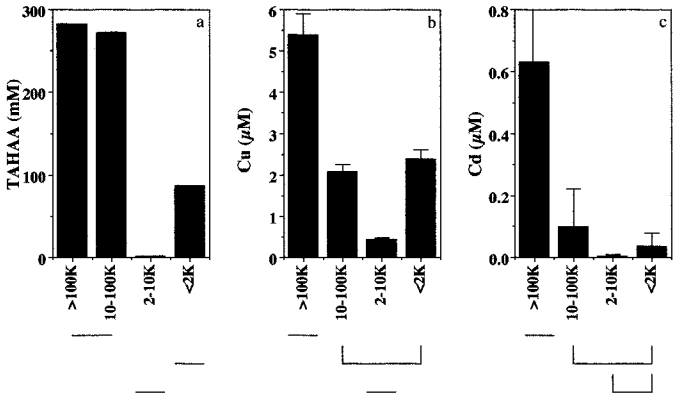

Fig. 4. Concentrations of TAHAA (a), Cu (b), and Cd (c) among different molecular weight fractions of lugworm gut fluid. Error bars = ±1 standard deviation. Bars under the graph are results of multiple comparisons among pairs of means with the T-method (Sokal and Rohlf 1995). Rank patterns among the molecular weight fractions are consistent for TAHAA, Cu, and Cd ($P < 0.025$, Kendall's concordance W; Daniel 1990).

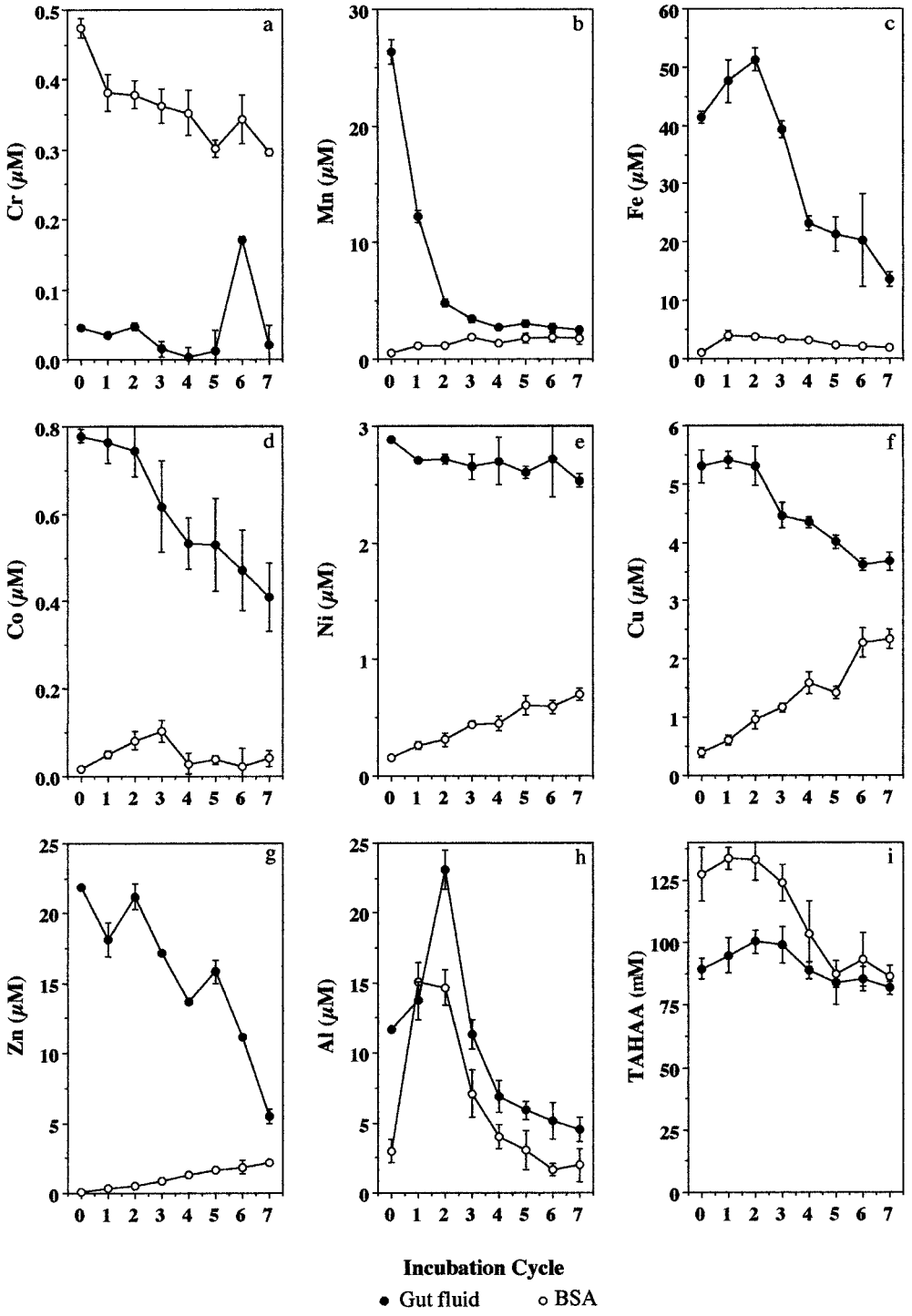

Fig. 5. Multiple incubation experiment. Concentrations of Cr, Mn, Fe, Co, Ni, Cu, Zn, Al (all μM), and TAHAA (mM) in lugworm gut fluid and BSA solution before and after sequential incubation with seven aliquots of clean sediment from the lugworm's habitat. Error bars = ± 1 standard deviation.

fluid to that of the Earth's crust. An enrichment factor of 1 means that the enrichment is exactly that to be expected from the appearance of mineral material in the gut fluid, while higher values indicate processes enriching concentrations beyond those to be expected from suspensions of uncontaminated minerals. The enrichment factors of all metals other than Al in the gut fluid are $\gg 1$ (Table 1), precluding minerals as the reason for their elevated concentrations. For metals such as Rb and Sr, these elevated enrichment factors are due to high solubility in seawater, but for those metals with

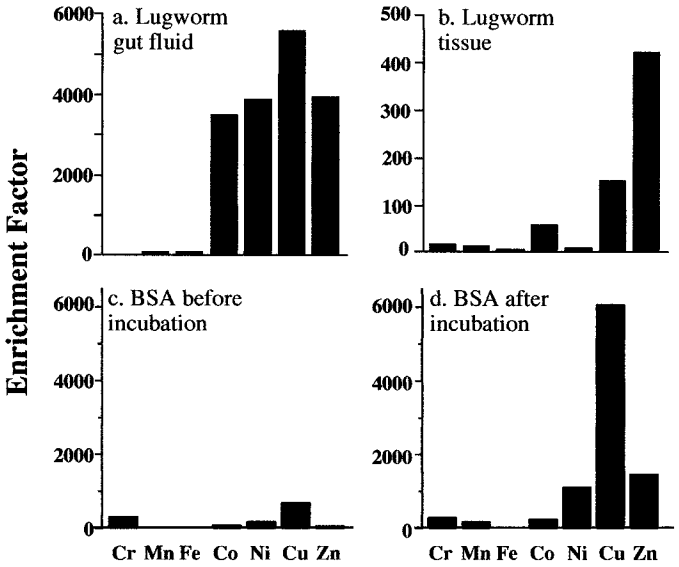

Fig. 6. Enrichment factors of the first-row transition metals in gut fluid and tissue of lugworm (a,b), and in BSA solution before and after eight cycles of incubation (c,d).

concentrations higher than in seawater some other enrichment mechanism(s) must be present.

The soft ligands associated with the elevated DOM provide such a mechanism. The extensive correlations between TAHAA and Cu and Cd concentrations among species, gut sections, and molecular weight fractions (Figs. 1–4) suggest proteinaceous materials as solubilizing agents for softer metals, though other ligands are also possible. Trends among enrichment factors of different elements are also consistent with the role of soft ligands. The relative enrichment factors for Fe, Co, Ni, Cu, and Zn follow the well-known Irving–Williams sequence of Fe < Co < Ni < Cu > Zn (Stumm and Morgan 1996), to be expected for soft ligand–metal interactions (Fig. 6a). This correlational evidence is not definitive, as can be obtained from direct speciation experiments (e.g., Chen and Mayer 1998), but complexation by soft ligands is the most reasonable inference from the data available.

Enrichment factors in gut fluid are higher than those of tissue (Table 2). There is a much higher ratio of Al to organic matter in the tissue than in the gut fluid, implying more mineral contamination in the tissue material that could explain the lower enrichment factors.

If proteinaceous materials in the gut fluid are the metal ligands, then they are far from saturated, as indicated by TAHAA : metal ratios of $>10^4$: 1. Because each of the protein amino acids usually makes up at least 1% of the total TAHAA (Mayer et al. 1997), it is clear that no individual amino acid ligand is saturated in gut fluid. Indeed, in contaminated sediments these proteinaceous materials are able to complex significantly higher levels of metals (Mayer et al. 1996; Chen and Mayer 1999).

Origins of gut metals—The extraordinarily high DOM concentrations in the gut fluids of deposit feeders provide a complexation capacity that can accumulate metals dissolved from sediments during feeding. Few, if any, other environments in nature expose sediments or soils to solutions containing 0.01–1 M dissolved TAHAA. It is well known that DOM can mobilize sedimentary metals or metalloids by direct complexation and/or by reductive attack of metal-binding Mn/Fe oxides followed by complexation (Rashid 1985; Weber 1988; Godtfredsen and Stone 1994). Both of these mechanisms could lead to metal accumulation in deposit-feeder guts upon ingestion of sediments, as evidenced by the increasing Ni, Cu, Zn concentrations, and elevated Mn in the BSA incubation experiment (Fig. 5). Although the metals found dissolved in gut fluids may also result from secretion by the animal, the combination of the Irving–Williams pattern and the BSA solubilization data imply that most metals derive from direct dissolution from sediment during gut passage.

Previous work on the kinetics of metal solubilization by gut fluids showed that gut metal concentrations result from a competition between ligands dissolved in the gut and those on sediment surfaces, and that metals can be transferred in either direction (Chen and Mayer 1999). Adding a metal-deficient source of protein—BSA—shows net movement into solution. Exposing normal gut fluids to sediment contaminated with excess metals shows similar net movement into solution (Mayer et al. 1996; Chen and Mayer 1999). However, net movement from gut ligands to sediment can certainly occur, such as we observed in our repeat incubation experiments (e.g., Fe in Fig. 5c). We have also observed readsorption of Cd and Hg, after initial release, during time courses (Chen and Mayer 1999; Lawrence et al. 1999) that may explain some of the variance in the Cd versus TAHAA data (Fig. 1b). The causes of these transfers to sediment are not understood but may result from adsorption of metal–ligand complexes or redox processes such as formation of metal oxyhydroxides or incorporation into sulfide phases. Given the variable nature of sediment ingestion and possibly gut fluid flow dynamics (Mayer et al. 1997), it seems quite possible that metal levels fluctuate in vivo—at least within the bounds of the within-species scatter in Fig. 1. Metal absorption at the gut epithelium may further complicate these dynamics in vivo and was not addressed in our experiments.

Equilibration with tissues?—Metal concentrations in whole body tissues, rather than in gut fluids, are often found to correlate with ambient metal levels in contaminated areas (Packer et al. 1980; Luoma and Bryan 1982). We therefore checked for similarity of borderline metal : TAHAA ratios between gut fluids and tissues of *A. marina.* We calculated metal : TAHAA ratios in tissues by assuming a protein content in worm tissue of 75% (Dall et al. 1991; Chen and Mayer unpubl. data) and an average gut fluid TAHAA concentration of 500 mM. This calculation showed that most metal : TAHAA ratios agreed, to within one order of magnitude, between tissues and gut fluid, except for Ni (Fig. 7). In the case of Cu, for which we have the most compelling evidence for proteinaceous materials as the relevant metal ligands in gut fluid (Chen and Mayer 1998), the Cu : TAHAA ratios of tissues and gut fluids were within a factor of two of one another throughout the size spectrum of individuals. Thus, the intriguing possibility arises that gut fluid serves as

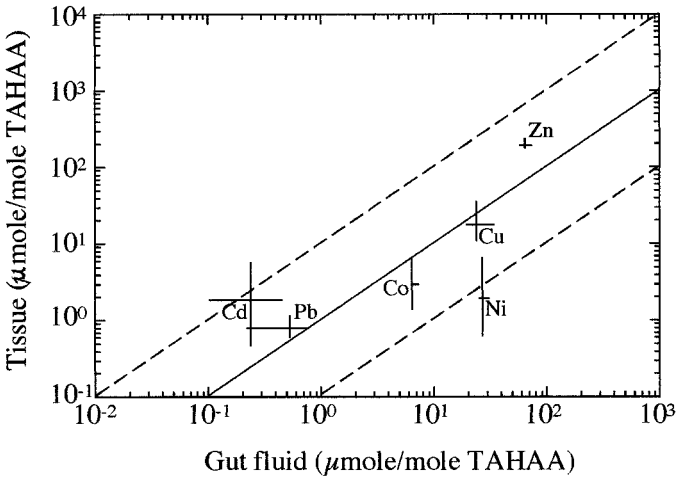

Fig. 7. Metal : TAHAA ratios (μmole-metal : mole-TAHAA) in gut fluid and tissue of lugworms. Y error bars = ± 1 standard deviation and are asymmetrical due to log-scales; X-error bars = data range. Solid diagonal line = 1 : 1 ratio; dashed = $\pm 10\times$ range.

an intermediate reservoir, equilibrating with sediments and to some extent transferring this loading to tissues.

Simple equilibration would clearly be an oversimplification, because animals have stronger regulatory control over tissue metal compositions (Rainbow and Dallinger 1993). Tissue enrichment factors do not follow the Irving–Williams order (Fig. 6b). Zn's high tissue EF is consistent with its ubiquity in enzymes and structural proteins. The tissue Ni : TAHAA ratio falls significantly below the 1 : 1 ratio (Fig. 7), which may represent the exclusion of this element in multicellular organismal biochemistry (Fraústo da Silva and Williams 1991).

Gut fluid, on the other hand, seems to accumulate borderline metals more passively from the environment according to simple ligand–metal interactions that result in the Irving–Williams order. Better understanding of the role of gut ligands as intermediaries in the equilibration of tissue metal concentrations to sediments will require further research into the mechanisms of absorption of solubilized metals, as well as regulation within tissues.

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