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Bioavailability of arsenic, cadmium and chromium for the deposit-feeding polychaete *Nereis succinea*

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Abstract of the Dissertation

Bioavailability of arsenic, cadmium and chromium for the deposit-feeding polychaete *Nereis succinea*

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My dissertation addressed the issue of metal bioaccumulation in marine deposit-feeding polychaetes that may serve as a conduit for metals between sediments and benthic predators. The ubiquitous polychaete *Nereis succinea* was used as a model species to study bioaccumulation patterns and processes of arsenic (V), cadmium and chromium (III). Sediments used in my study were collected from three different sites – two in Chesapeake Bay and one in San Francisco Bay. Specific objectives were to understand the relative significance of metal uptake route (water vs. diet) contributing to the total metal body burden accumulated by the worm, and secondly to identify those factors most influencing bioavailability of ingested metal. Hypothesized factors affecting metal bioavailability included the distribution of metal in food, including the geochemical partitioning of metals in various inorganic and organic sedimentary fractions and the metal's subcellular partitioning in algal cells, and the chemical composition of *N. succinea*'s gut fluid composition. Experimentation largely relied on application of radioisotopes (^{73}As, ^{109}Cd, ^{51}Cr) to follow biological processes such as metal uptake by worms

from water, assimilation from ingested food and its loss from tissues, and abiotic processes such as binding with various sedimentary fractions. Kinetic parameters such as metal assimilation efficiencies (AE) and loss rate constants (k_e) that were generated from aqueous exposure and pulse-chase feeding experiments using live organisms were then used to predict metal accumulation for deposit-feeding polychaetes residing in sediments at two different estuaries – i.e., Chesapeake Bay and San Francisco Bay. Modeling showed that in nearly all cases As, Cd and Cr are accumulated by worms primarily from ingested diet, and secondly that metals loosely associated with sediment particles, represented by the operational fraction "carbonex" combining *exchangeable* and *carbonate* fractions, can be used to predict metal bioaccumulation in *N. succinea*. Furthermore, multiple regression analysis showed positive relationships between metal AEs and *exchangeable* and *carbonate* fractions independently and when combined, whereas other operationally defined fractions, also determined by the sequential extraction procedure (including *Fe/Mn oxides*, both *organic* fractions, *pyrite* and non-extracted *residue* were found as non-available. Algal As was highly available (AE: 72%), but the bioavailability of As sorbed to goethite was very low (AE: < 3%) suggesting that As bound to labile organic matter is more important than As in abiotic particles as a source for polychaetes. *N. succinea* showed no difference in the assimilation patterns for Cd associated with algal cells or goethite, but Cr showed the opposite pattern to As, where it was more bioavailable from goethite (34%) than from algae (3%). Bioavailability of sediment-bound As decreased with its exposure time for sediments. Results of experiments investigating the influence of gut fluid on As release from particles showed that while the percentages of particulate As released into gut fluid were higher or similar to AEs, this release was an essential but not sufficient factor for assimilation.

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Chapter I: *Introduction*

In coastal systems, especially nearby industrial or metropolitan centers, sediments are commonly greatly enriched with a wide variety of contaminants, especially particle-reactive metals and organic compounds. Anthropogenic inputs of these contaminants are of special concern and can cause significant increases of these contaminants above background levels (Schropp et al. 1990 and references therein). Traditionally, levels of metal contamination have been determined by analyzing metal concentrations in water, sediments, biota or all of them together (Bryan et al. 1985). Perhaps, relating metal concentrations to background levels in seawater or sediment could be used as one criterion for assessing whether a particular location is contaminated. For example, As concentrations in the open ocean range from 0.01 to 0.03 µM (Bryan et al. 1985; Chen et al. 2000), therefore water contamination could be assumed at levels above this range. Similarly for sediments, metal concentrations in the Earth's crust can be assumed as background. According to Martin and Whitfield (1983), the average concentration of As in the crust is 10.5 µmol g^{-1}, Cd is 1.78×10^{3} µmol g^{-1} and Cr is 1.37 µmol g^{-1}. Contaminants can enter coastal waters through atmospheric delivery (e.g., Hg, As, dioxins as a byproduct of coal combustion), fluvial input (pesticide and fertilizer runoff from agriculture), shipyard activities and boating (e.g., Cr, As, Cd, Pb, Cu), and sewage and dumping activities (e.g., Cr, Cu, Zn, Ni) (da Silva Oliveira et al. 2007; Worms et al. 2010).

Alternatively, the level of metal contamination can be determined based on concentration normalized to aluminum. In this approach, the metal of interest is regressed against aluminum and the upper 95% prediction limit of the regression determines the threshold between the non-contaminant (below) and contaminant (above) metal concentrations (Schropp et al. 1990). Aluminum serves as a good reference metal for determining metal contamination, first because of its crustal abundance (i.e., second most abundant element), second, because its sedimentary concentration is not anthropogenically impacted, and lastly because aluminum to metal ratios are constant in the Earth's crust (Schropp 1990 and references therein). One shortcoming of this approach is the need for a large amount of data to draw a meaningful regression; a second is that metal concentrations appearing above the 95% prediction limit may be due to measurement error

during sample analysis (sample contamination) or local sediment enrichment with metal at non-impacted sites (Schropp et al. 1990).

Metals and metalloid contaminants in waters and sediments can pose health risks for organisms and humans, due to their potential to bioaccumulate in marine organisms followed by trophic transfer in aquatic food chains (Järup 2003; Luoma and Presser 2009). Animals inhabiting contaminated environments can accumulate metals directly from water via sorption and by drinking, or by absorbing them in their guts from ingested food. Once absorbed metals stored in animal tissues they can be transferred to predators (Fisher and Reinfelder 1995). Assimilated metals can become toxic and impair the physiology of animals if tissue concentrations are sufficiently high. Increased levels of toxic metals are thought to lead to ecological shifts in marine communities including in the benthos, resulting in lower species diversity (Hansen et al. 1996). For example, Dauer (1993) reports that ecological parameters - community biomass, species richness, and biomass made of deep-dwelling and equilibrium species generally decline, which the presence of opportunistic species typically increases in contaminated compared to uncontaminated sediments of Chesapeake Bay. Depending on the metal and the route via which organisms can be exposed to metal, the toxic effects can vary. Assessments by Long et al. (1995) of adverse effects induced by metal contaminants in marine sediments are widely used by the US Environmental Protection Agency.

SIGNIFICANCE OF DEPOSIT-FEEDING POLYCHAETES IN BENTHIC FOOD-CHAINS AND IN ELEMENTAL CYCLING

Deposit-feeding polychaetes are an important component of the benthic food web because they are the link in energy flow from sediments to animals that prey on them (e.g., crabs, fish, birds etc.). Deposit-feeding polychaetes ingest large volumes of sediments enriched with detritus and microbes. Without the deposit-feeding invertebrate communities much of the organic matter deposited onto the seabed would not be used, and a more complex community structure could not prevail (Pace et al. 1984). In addition, feeding and other activities of deposit-feeders have a significant impact on elemental cycling. These activities include construction of burrows leading to rearrangement of sediment particles and irrigation leading to changes in oxygen conditions in burrows that are sculpted in the sediment (Aller 1978). Mn and Fe minerals, such as Fe and Mn oxides or Fe sulfides, are directly impacted by oxygen regime shifts

(Aller 1978). Iron and Mn are present in nature in several species (e.g., Fe^{+2} or Fe^{+3}), which are determined by redox conditions. Other elements such as Cu, Zn, As, and Cd that associate with Fe and Mn minerals are also sensitive to oxygen such that, when they are bound to Fe oxides and the environment becomes more reducing, Fe and elements bound to Fe oxides are released into solution (Cullen and Reimer 1989).

NEREIS SUCCINEA

As with other nereid polychaetes, *Nereis succinea* is a ubiquitous species found on the east and west coasts of the United States (Fauchald and Jumars 1979), where it that inhabits organic-rich sediments in the intertidal zones of estuaries (Kristensen 1983). Flax Pond on Long Island (40°57'48.63"N, 73°09'00.50" W) exemplifies the type of muddy habitat where *N. succinea* resides. Individuals live for up to 2-3 years and reach body sizes of 15 cm for adults (Ahrens 2000). The mudflat in Flax Pond is occupied by approximately 1500 individuals of *N. succinea* per square meter, similar to its sister species *N. diversicolor* in other locations (Hansen and Kristensen 1998). Burrows of *N. diversicolor* are 5-6 cm deep in sandy sediments, 10-12 cm deep in muddy sediments, but shallower (4 and 6-8 cm, respectively) when sediments are enriched with fresh algal organic matter (Hansen and Kristensen 1998). At Flax Pond, during spring and summer *N. succinea* can be observed at depths similar to those for *N. diversicolor*, while in fall and early spring worms are found deeper (personal observation).

Due to anoxic conditions just a few millimeters below the sediment surface worms need to periodically irrigate their burrows with oxygenated overlying water to supply oxygen. When oxic overlying water is not available - e.g. at low tide, worms can switch to anaerobic metabolism to cope with anoxia (Kristensen 1983 and references therein). During high tide, oxygen in the walls of irrigated burrows is higher than in non-irrigated burrows. Oxygen in irrigated burrows penetrates from 1 to 4 mm into the burrow wall (Hansen and Kristensen 1998). Oxygen presence is indicated by a rusty color of iron oxide on the burrow walls. Dynamic burrow conditions - e.g., oscillating oxygen levels - can impact some elemental cycles. This in turn can have implications for determining the speciation and concentration of redox elements such as As and Cr and non-redox elements such as Cd, which associates with oxygen sensitive minerals such as iron oxides.

N. succinea, which pumps oxygenated water into the burrow by movements of its body, has been identified as a more efficient regulator of burrow O_2 tension (O_2 tension in burrow: 30 – 140 mm Hg) than other nereids (*N. virens* and *N. diversicolor*: 30 - 90 and 30 – 70 mm Hg, respectively) (Kristensen 1983). Because worms exchange gases via parapodia, which are organs that are in direct contact with the burrow environment, they experience elevated stress at times of anoxia due to increased levels of toxic metals, sulfide and ammonium. Concentrations of these chemicals increase in non-irrigated burrows. For example, metals associated with iron oxides are released as oxides become reduced. Ammonium that is excreted by animals cannot be diluted at low tide due to the lack of overlying water supply, therefore its concentration also increases in the burrow.

N. succinea display several characteristic features in their reproduction. As observed in the field and laboratory during reproduction individuals of *N. succinea* have a milky color and they do not feed (personal observation). During reproduction male and female worms undergo metamorphosis to heteronereids. In nature, reproduction of *N. succinea* is synchronized with moon cycles and influenced by ambient temperature (Hardege et al. 1990). Before and during swarming in the water, worms release pheromones and their spawning ends with death resulting in many decomposing bodies in the sediment (Hardege et al. 2004; personal observation).

Except for the non-feeding reproductive life stage, *N. succinea* is considered non-selective deposit-feeder. According to Cammen (1980a) its diet contains detrital organic material and microbes - including algae that are mixed with sediment. When feeding, *N. succinea* scrapes the surface of the sediment, after which it retracts back into u-shaped burrow (Fauchald and Jumars 1979). As reported by Cammen (1980b) adult worms at an average body mass of 5.8 mg dry weight feed in plug-flow manner at rates of ~20 mg of sediment d^{-1} (temp. = 15°C). When ingested sediment transits the gut in 1-12 hours (gut transit time in the field is shorter than in the lab at 22°C) (Ahrens 2000; Cammen 1980a; Penry and Jumars 1990; personal observation).

ARSENIC, CADMIUM AND CHROMIUM AND THEIR ACCUMULATION IN DEPOSIT-FEEDERS

In this thesis, I describe experiments conducted with three elements, As, Cd, and Cr, all of which can pose environmental health risks. Baseline levels for elemental contamination could be related to their crustal concentrations. For example, average concentrations of As, Cd, and Cr in the crust are 10.5, 1.78×10^3 and 1.37(μmol g^{-1}), respectively. Arsenic is a metalloid that is

widely present in nature both in terrestrial and aquatic systems. Its concentrations can vary spatially due to the types of local sedimentary rock that predominate in a region (Onishi and Sandell 1955; Vaughan 2006) or the presence of anthropogenic activities that release As (Cullen and Reimer 1989). Estuarine sediments tend to be more enriched with anthropogenically introduced As than sediments underlying open ocean waters (Bruland and Lohan 2004; Cullen and Reimer 1989). Levels of human introduced As are above the background levels particularly in areas close to urban, agricultural and industrial centers. Anthropogenic activities leading to As contamination include agriculture, where As is used as a pesticide, industry, where it is used as a paint component and a wood preservative, and mining, smelting and coal burning (Vaughan 2006; Wang and Mulligan 2006).

Arsenic has a high affinity for sulfur. This metalloid displays concentrations in estuarine sediments typically in the range of 0.13– 13.3 mmol kg^{-1} (Bryan and Langston 1992). In seawater, concentrations of As are generally about 0.01 - 0.03 μmol L^{-1} (Chen et al. 2000; Donat and Bruland 1995), although coastal waters can display concentrations of about 0.11 μmol L^{-1} (Chen et al. 2000). In seawater, As speciates largely as $HAsO_4^{2-}$ (Bruland 1983). In sediments, arsenic is commonly found associated with sulfur minerals and with iron oxides (Greenwood and Earnshaw 1984).

In human history, arsenic has been most known for being a deadly poison. In many Asian countries such as Taiwan, Bangladesh, Burma, and parts of India, its presence at high levels in groundwater used for drinking leads to serious human health problems including various forms of cancer (Mead 2005). Also, in some regions of the US, such as New Hampshire and Michigan, natural ground water can be sufficiently enriched (<1 to >100 000 μg L^{-1}) (Nordstrom 2002) for As to pose health problems for people who drink it (Peters et al. 1999). The World Health Organization recommends that safe to drink water should have As levels lower than 10 μg L^{-1} (0.13 μmol L^{-1}). High levels of arsenic in ground waters of Asia and North America generally have a natural source and result from the erosion of arsenic-enriched rocks. Problems involving human health issues have become the main motivation for research on the geochemical cycling and bioaccumulation of As.

In both terrestrial and marine environments, plants and animals have been shown to accumulate As (Fattorini et al. 2005; Ferguson and Gavis 1972; Riedel et al. 1989; Waring et al.

2005). Reported As levels in marine polychaetes range widely from <0.01 to 33.3(3) mmol kg^{-1} (Chen et al. 2000; Fattorini et al. 2005). Fish and other seafood can also be As-enriched, however, human consumption of As-containing diet has generally not been identified as a health risk because most As found in seafood is in organic forms (e.g., arsenobetaine) that are considered non-toxic (Buchet et al. 1994).

Because of the potential biological impacts there is interest in the rate and extent to which As can build up in aquatic food chains, and the extent to which it can be mobilized by aquatic organisms in these systems. The mechanisms responsible for As entry into organisms are also of interest. For example, due to chemical similarities between P and As, As can enter into metabolic pathways and associate with organisms as a P analog, particularly when P is a limiting nutrient (Andreae and Klumpp 1979). The recent discovery by Wolf-Simon et al. (2010) of a bacterial strain using As as a substitute for P led to speculation about alien life forms on other planets, where P may be absent but As is present.

Cadmium is an element used by some phytoplankton, while it acts as a potent toxicant to animals living in Cd contaminated aquatic systems (DeWitt et al. 1996). Cd can be used in diatoms as a cofactor in carbonic anhydrase when Zn is unavailable (Xu et al. 2006). Cadmium concentrations in open ocean waters average about 0.003 µmol L^{-1} and in the Earth's crust 1.73 mol kg^{-1} (Chen et al. 2000; Martin and Whitfield 1983). Its particle reactivity is salinity-dependent and Cd desorption from particles is facilitated by its chloro-complexation in estuaries (Zwolsman et al. 1997). The geochemical behavior of Cd tends to vary due to the geochemistry and physics of a given estuarine basin. In the open ocean Cd shows regeneration patterns similar to those of nutrient elements such as P (Bruland 1983). In anoxic sediments it typically associates with iron sulfides and pyrite, and in oxic sediments it binds to manganese oxides (Guo et al. 1997; Morse and Luther 1999; Rosenthal et al. 1995). Cadmium levels in surface sediment sampled from the open ocean and from contaminated harbors can range from <0.1 to 3 µg g^{-1} (Bargagli et al. 1996; Holmes et al. 1974; Rosenthal et al. 1995; Tam and Wong 2000; Windom et al. 1989). Cadmium is also bioaccumulated by benthic invertebrates and its concentrations can range from 1.2 to 17 µg g^{-1} (dry wt) or 0.01–140 µg g^{-1} (wet wt) in exposed bivalves (Ng et al. 2008; Wang et al. 1999). Cadmium in other marine invertebrates including polychaetes can

range from <0.03 to 13 µg g^{-1} dry wt (Bargagli et al. 1996; Brown and Luoma 1995; Frazier 1979).

Chromium is a metal that shows redox chemistry. Its particle reactivity depends on its oxidation state such that Cr(VI), which is the oxidized Cr species, is more soluble than the reduced Cr(III), which is much more particle-reactive. Cr (III) is found in reducing environments such as anoxic sediments as a product of microbial reduction from Cr (VI) (Tebo and Obraztsova 1998). Cr(VI), which is present in the aqueous environment as CrO_4^{2-} and $HCrO_4^-$, is considered highly toxic, and therefore Cr presence in the natural environment is of great concern (Nieboer and Jusys 1988). The greater toxicity of anionic Cr (VI) than cationic Cr (III) is due to its higher solubility, oxidizing power, and cell membrane permeability (Walsh and O'Halloran 1998). However, one study showed that mussels and other invertebrates collected near a tannery discharging Cr (III)-enriched effluent accumulated chromium at high concentrations (Walsh and O'Halloran 1998). Experimental work has shown that some organic Cr (III) species are bioavailable, explaining the observed high Cr (III) bioaccumulation levels (Walsh and O'Halloran 1998). Sedimentary Cr found in the lattices of clay minerals is largely not available for uptake. Its inert behavior has been therefore used as a tracer of gut transit of ingested food (Wang et al. 1997). Some studies have also shown that Cr bioavailability depends on the type of particles with which it is associated (e.g., mineral particles vs. algae or bacteria) (Decho and Luoma 1994).

Chromium can enter coastal ocean and estuaries via rivers that receive wastewaters from mining activities or other industries (e.g., plating and finishing of metal products, textiles and leather tanning) (Fishbein 1981; Lewis et al. 2000; Walsh and O'Halloran 1996). It reaches levels on the order of 1-10 µg L^{-1} in rivers and in estuarine and coastal sediments on the order of mg g^{-1}, especially nearby tanneries (Graham et al. 2009; Walsh and O'Halloran 1996; Windom et al. 1989). While Cr concentrations measured for invertebrates feeding on contaminated sediments are <2 µg g^{-1} (wet wt), fish that have been captured in Cr contaminated estuaries had on average 4.0 µg g^{-1} of Cr (Chen et al. 2000; Roling et al. 2007; Weston and Maruya 2002).

In summary, the three elements chosen for study differ in their geochemical behaviors such that Cd is not a redox element, and As and Cr are. Arsenic in oxic environments is primarily present as As +5, and in anoxic environments it can be reduced to As +3, whereas Cr in oxic

environments is present in its hexavalent state (Cr +6) and in the absence of oxygen can be reduced to Cr +3. All three elements can be found in sediments in association with iron oxides, although Cr has an additional high affinity for organic matter. As and Cd can associate with sulfides if these are present.

BIOAVAILABILITY OF METALS

A universal definition of bioavailability is still debated in the literature, partly because toxicologists tend to use different definitions than biogeochemists. According to Semple et al. (2004) "a bioavailable compound is that, which is freely available to cross an organism's cellular membrane from the medium the organism inhabits at a given time. Once transfer across the membrane has occurred, storage, transformation, assimilation, or degradation can take place within the organism". It is important to keep in mind that not all levels of bioavailable chemicals need to lead to a toxic effect, and the entire pool of bioavailable chemical need not be retained in the tissues, because much of it can be metabolized or excreted.

Figure 1 illustrates diverse uptake routes and effects of contaminants in aquatic organisms. Significant sources of bioavailable metal for animals can include dissolved metal and metal associated with diet. Bioaccumulated metal can be chronically or acutely toxic when taken up either from water or from diet, largely as a function of the internal dose of metal. Finally, there have been several approaches such as biomimetic, Biolotic Ligand Model (BLM) or Free Ion Activity Model (FIAM) that predict the concentrations and/or pools of metal that are biologically available for uptake from the aqueous phase.

Metal contaminants in the water column can exist in a variety of physical and chemical forms or species. They can be dissolved (in which they pass through a filter or membrane of some specified pore size) or bound to particulate matter, operationally defined as being greater in size than the pore size cutoff. The dissolved metal can speciate as the free metal ion or be bound to various inorganic and organic ligands. Complicating the picture is the association of metals with colloidal-sized particles, commonly in the 1 to 200 nm size range in natural waters (Sholkovitz 1992). The extremely small size of most colloids results in their passage through the filters commonly used to distinguish dissolved from particulate, and yet metals bound to them can behave more as particulate than as dissolved. Metals associated with larger particulate

material may also be associated with diverse ligands, and the metal may occur in the interior of the particle or bound to its surface.

Metals can associate with suspended particles including living particles such as bacterioplankton and phytoplankton, where they can passively sorb onto cell surfaces or be transported across their cell membranes, and abiotic particles via adsorption. All of these particles can aggregate (perhaps enhanced by feeding, secretory or excretory processes) and ultimately sink to the sediment below. The extent to which sinking particulate matter reaches the sediment depends on the sinking rate of the particles, the depth of the water column, and the degradation rate of the particle during its descent. In coastal systems, far more sinking particles reach the sediments than in deep ocean basins, where typically $\leq 1\%$ of particles sinking out of the euphotic zone reach the sediments. The more particle-reactive chemicals tend to have a stronger association with the particles and frequently are transported along with them to the sediment (Fowler and Knauer 1986). Examples of such elements include Pb and Pu among contaminant metals, and Th and Fe among non-contaminant metals.

Metal toxicity has been linked to its chemical speciation (Fig. 1). In a series of studies led by Sunda, it was shown that planktonic organisms responded to free metal ion concentrations rather than total metal concentration (Sunda and Guillard 1976). Biological responses to the aqueous metal bioavailability included toxic and nutritional effects. Sunda's work focused principally on Cu and, later, Cu's interactions with Zn, Cd, and Mn. An important realization from this work was that dissolved organic ligands that can complex many metals appreciably, often >90% of total dissolved metal (Bruland 1989; 1992), render a large fraction of the dissolved metal pool non-available for biological uptake. Based on realization that organisms respond primarily to the free ion activity for any given metal the so-called "Free Ion Activity Model" (or FIAM) was developed. FIAM provided explanations for many laboratory observations of metal-biota interactions. As a natural extension of the FIAM, the Biotic Ligand Model (BLM) (Paquin et al. 2002) was developed to create a better understanding of waterborne metal bioavailability that causes toxicity in invertebrates and fish.

The BLM was generated to understand "the site where metal binding results in the manifestation of a toxic effect" (Paquin et al. 2002). Both the FIAM and BLM assume only free ion bioavailability because some inorganic but mainly organic ligands in the water (FIAM) or in

the living tissues such as gills (BLM) can bind metal and hence reduce its harmful effect following its incorporation into the animal's body. DiToro et al. (1990) linked the geochemical partitioning of metal, namely its association with the acid volatile sulfides (AVS) on Cd toxicity, using toxicity indicators such as LC_{50} in benthic amphipods exposed to pore water. They concluded that reactive sulfides in the sediment protect organisms from metal toxicity by forming complexes with the metals and therefore reducing the free metal ion concentration within the pore water. This approach was simplified and it did not include the dynamic aspect of the AVS pool in sediments, which is likely to influence differences in metal toxicities observed on temporal scales due to diagenetic cycling of AVS. Also, in their study no consideration was given to metal toxicity from ingested sediment.

A number of reports point out that exceptions to the overall concept used by FIAM and BLM have been noted, including for metal uptake in aquatic protists (Campbell 1995a) and in freshwater mussels (Roditi et al. 2000a). With zebra mussels (*Dreissena polymorpha*), for example, metals such as Ag, Hg and Cd that were bound to certain components of DOC were shown as bioavailable for uptake as a nutritional source; consequently the uptake of these metals from the aqueous phase was greater (up to 32–fold for Cd) in the presence of certain organic chelators than in their absence (Roditi et al. 2000a).

As described before, bioavailable metal can be acquired by organisms directly from water and from ingested food. For animals, the contribution of aqueous and dietary sources of metal for the overall body burden can vary among metals and organisms. Metal uptake in animals from one or more dietary sources can also contribute to an animal's overall body burden of a metal. This issue has been less studied than metal accumulation from the dissolved phase, but a considerable amount of data now exists. Much of the work has focused on planktivores (e.g., animals ingesting zooplankton, suspension-feeding bivalves) and much less attention was given to benthic animals. Bryan and Langston (1992) identified several factors that may influence the bioavailability of metals for benthic animals, including chemical speciation, mobilization of a metal into pore water, chemical processes such as methylation, affinity for various ligands, as well as pH, salinity, redox conditions and bioturbation.

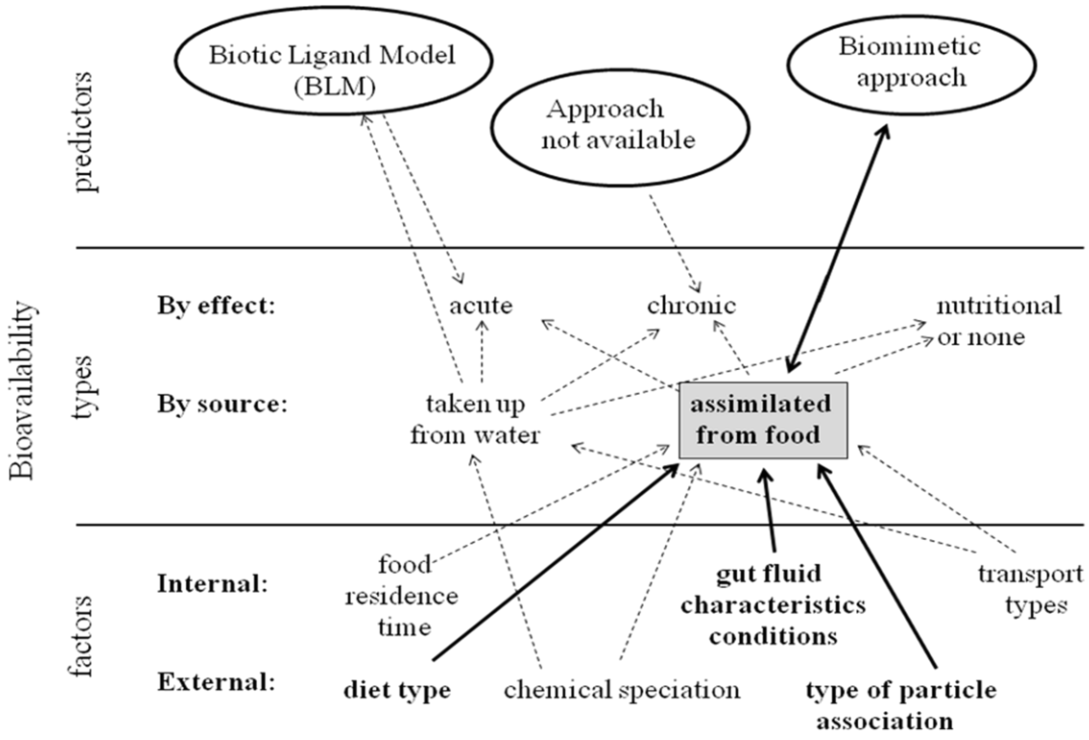


Fig.1. Diagram of bioavailability for feeding (animals) and non-feeding (phytoplankton, bacteria etc.) organisms. Arrows show links between bioavailability type and its factors – for example metal assimilated by an animal can be influenced by food residence time in the gut, diet type (algae, inorganic particles, animal diet *etc*.), metal association with different types of particles etc. Arrowed lines also indicate predictability of bioavailability – for example BLM uses metal concentration and speciation in the water to predict the acute toxicity of the water-borne metal in the organism. Thick arrowed lines indicate the links I focused in this dissertation.

Diet, whether it is prey for carnivores or sediment for deposit-feeders, exemplifies one general factor influencing the bioavailability of metal associated with it (Fig. 1). For example, tissue distribution of the metal in a prey organism (the diet item) can significantly influence its assimilation by the predator such that metals bound to the exoskeleton of a copepod are not assimilated by fish that eat these copepods, whereas the same metal bound to the non-exoskeletal tissues of the copepod do get assimilated (Reinfelder and Fisher 1994). Similarly, it appears that the form of metals bound to marine sediments can vary in their assimilation by deposit-feeding animals (Griscom et al. 2000). For example, the assimilation of some transition metals (e.g., Cd, Zn) that are associated with the organic fraction of marine sediments to deposit-feeding clams differed from the assimilation of metals in the iron oxide fractions (Griscom et al. 2000; Lee et al. 2000b). Sedimentary partitioning of metals and their cellular partitioning in algae used as diet for *N. succinea* polychaetes are investigated in chapters II and III.

A number of studies have explored the influence of metal form on metal bioavailability as a factor leading to bioaccumulation. These studies recognized the importance of metal speciation in sediment and specifically their association with various mineral and organic components (including microorganisms). They have also examined the influences of water salinity and bulk redox conditions of the sediment**.** Thus, factors hypothesized as directly affecting metal bioaccumulation which could limit metal bioavailability in deposit-feeding animals included binding capacity of metal contaminants to sedimentary iron sulfides (AVS), Fe and Mn oxides, and organic matter (Griscom et al. 2000; Rule and Alden 1996a; Rule and Alden 1996b; Zhong and Wang 2006a). An interesting observation was made by Lee et al. (2000b) who determined that AVS-bound metals could be bioavailable for assimilation following ingestion by deposit-feeders. Based on these results, it is difficult to argue that sedimentary AVS concentration alone can be sufficient to predict metal bioavailability for benthic animals. Griscom et al. (2000) found that metal bioaccumulation in the deposit-feeder *Macoma baltica* could vary considerably among metals, with assimilation efficiencies ranging from about 1% for Cr to 42% for Zn. That study showed that metals associated with sulfide and organic fractions of sediments were somewhat bioavailable, but more importantly, that metal assimilability decreased with the time for which the added metal aged within the sediment to which it was added. In another study, bioavailability of Cr to the suspension-feeding bivalve *Potamocorbula amurensis* was evaluated as a function of bacterial abundance as representative of the pool of fresh organic matter. Cr assimilation was far greater in the presence of bacteria than when Cr was bound to nonliving organic matter (e.g., fulvic and humic acids) (Decho and Luoma 1994).

Bryan and Langston (1992) noted that metal bioaccumulation is in part a function of its affinity for specific sites/tissues within the organism and is influenced by competition of other co-occurring metals for these sites. It is also recognized that some elements can mimic other elements based on their physico-chemical properties, and these similar properties may influence their cross-membrane transport. This mimicking can include or lead to displacement of essential elements by non-essential elements (e.g., As for P or Cd for Ca) (Wolfe-Simon et al. 2010; Worms et al. 2006). This area represents an especially challenging and complex problem, because many contaminants can be present in sediment simultaneously and their combined effects are not understood.

One factor affecting the bioavailability of ingested metals is the solubilization of particulate metal in the gut, potentially leading to a greater amount of metal that can cross the gut lining (Weston et al. 2004; Zhong and Wang 2006b). Landrum (1989) considered the bioaccumulation of organic contaminants such as PAHs in a benthic freshwater amphipod *Pontoporeia hoy*i. He recognized the importance of the process of contaminant desorption from particles into the liquid phase (e.g., pore water or gut fluid) in regulating bioavailability. Gagnon and Fisher extended this work in their study of sediment-associated Cd, Co, Ag, and Hg, showing that metal desorption from sediment particles into seawater with its pH adjusted to 5.5 (to simulate the gut pH of a marine mussel) was tightly correlated with assimilation efficiency in mussels feeding on these sediments. This is consistent with work by Mayer, Weston and colleagues, who examined desorption of metals from sediments into gut fluid or bovine serum albumin (BSA) used to simulate gut fluid (Chen and Mayer 1998; Weston et al. 2004; Weston and Maruya 2002). Thus, the connection made between the bioaccessibility of metals present in the diet and the properties of the digestive system of an animal exposed to this diet was explored for both metals and organic contaminants. Chen and Mayer (1999) found that 1 N HCl, which is typically used to extract AVS-bound metals, extracted 35-88% of Cu from sediment, whereas 4-20% of Cu desorbed from that same sediment into the gut fluid of the lugworm *Arenicola marina*. The implication of this work is that acid extraction of marine sediments may not present a realistic assessment of the bioavailable metal for benthic animals. Mayer's approach, which is referred to as biomimetic approach, assumes that the bioavailability of the metals in the diet of an animal is limited by its solubility in the gut fluid of an organism. This approach has since been applied to metals such as Cu, Hg, and methyl mercury, primarily in studies using polychaetes (Zhong and Wang 2006b). The biomimetic approach was to relate the extent to which particle-bound metal would desorb from ingested sediments into gut fluid, potentially leading to its assimilation across the gut lining. However, measurements were generally not made to determine the assimilation efficiency of metal in the worms from the same substrate used in the desorption experiments. Still, Weston and Maruya (2002) demonstrated that bioaccumulation of As, Cu, Hg, Ni, Zn and some organic contaminants bound to sediments ingested by the deposit-feeding polychaete *Arenicola brasiliensis* correlated with the desorption of these metals into *A. brasiliensis* gut fluid. These results suggest desorption from sediments into gut fluid is probably a necessary precursor for a metal's assimilation into the tissues of a

deposit-feeding animal (Weston and Maruya 2002). Mechanisms underlying this observation can only be surmised at present. Thus, a metal bound to certain organic ligands (such as Cu bound to the amino acid histidine) may cross the gut lining of the animal because the gut lining is selected for absorbing the amino acid to meet its nutritional needs. In a sense, this is analogous to the findings of Baines et al. (2005) showing that *Dreissena polymorpha* absorbed specific amino acids from ambient water, where the amino acids were capable of complexing some transition metals. The binding of specific metals to specific organic compounds in the gut fluid may be expected to follow binding strengths of the metal for specific ligands as shown by Lawrence et al. (1999).

METAL SPECIATION IN WATER AND PARTITIONING IN GEOCHEMICAL FRACTIONS IN SEDIMENT (APPLICATION OF THE SEQUENTIAL EXTRACTION METHODOLOGY)

Both sediment and pore water are enriched, relative to the overlying water, in particle reactive chemicals. Sediment is a place of intense decomposition of organic matter, where bigger organic molecules are broken down by sedimentary microorganisms into small organic fractions. The decomposition leads to a change in pH, which can make the surrounding sedimentary environment, i.e. the pore water, more acidic than the overlying water. The same applies to oxygen levels, which are greatly driven by the degradation of organic matter. Therefore, organic-rich sediment deposits are frequently predominantly anoxic, and the oxic surface layer of the sediment is very thin (<1 cm). Both acidity and redox conditions in sediment influence the partitioning and speciation of metals. Other geochemical components of sediments, i.e., specific minerals, type of organic matter, concentration of sulfur or salinity can also be of importance in determining metal partitioning in sediments (Gambrell et al. 1991; Lee et al. 2000a; Lee and Luoma 1998; Riba et al. 2003).

Determining the partitioning of metals to specific sedimentary components, frequently referred to as phases, is not straightforward. Sediment geochemists have devoted much attention to developing methods to differentiate between metal partitioning to various mineral and functional fractions in sediment. Sequential extraction procedures have been widely used for sediments since the 1970's and even longer for soils to operationally determine sediment phases and metals bound to them. The operational character of these extracted phases results from the

impossibility to distinguish the exact reactions and their kinetics during any particular incubation (Du Laing 2010).

The premise of the sequential extraction methodology is that the wet sediment sample is subjected to a number of selective chemical extractions targeting specific geochemical phases. These phases have been referred to in the literature as exchangeable, carbonate, AVS, Fe and Mn oxides or reducible, organic matter – humic and fulvic acids or oxidizable, pyrite, silicates, residual etc. Published methods have aimed at extracting some but not all of these fractions. Also not all of the fractions can be present in all sediments in parallel under specific oxygen conditions if sediment is homogenous. In reality however, sediments include microscopic zones of very different oxygen regime, which allow for reduced and oxidized metal species to coexist on small spatial scales. For example, due to the presence of anoxic micro zones in the bulk sediment that are characterized as oxic, reduced geochemical phases such as AVS and pyrite can be present nearby in oxidized phases such as Fe and Mn oxides. A study by Poulton and Canfield (2005) exemplifies an approach that uses a sequential extraction procedure to identify different iron pools according to Fe reactivity towards sulfides rather than identify more general geochemical pools. Ruttenberg (1992) has best described the concept behind the mechanism of the sequential extraction procedures: "Sequential extraction methods take advantage of the fact that different solid phases show dissimilar reactivity toward different solutions. Sediment is extracted with a series of extractants, each chosen to selectively dissolve a single phase or group of phases of similar chemical characteristics." For example, the operational pool referred to as *exchangeable* represents elements that are weakly bound to the surfaces of particles. The mechanism used to strip these elements off the particle surfaces relies on the application of a $MgCl_2$ solution whose ionic strength is higher than that of pore water. The geochemical pool of *iron and manganese oxides* and metals bound to them can be dissolved when incubated in a reducing solution such as hydroxylamine. However, it is critical to recognize the significance of the kinetics of extractions used in available sequential extraction procedures. The efficiency of dissolution of specific minerals does not only depend on the physico-chemical character of the extractant but also the duration of the extraction. Many details, including the kinetics of different extraction schemes and steps within them, are discussed by Gleyzes et al. (2002). The duration of particular extractions can influence the extent to which metals can be extracted by a given step; for example, in the step targeting the carbonate fraction (second step of extraction), some AVS

(presumably released in the second extraction step) could be released if the sodium acetate extraction at pH=5 is too long (G. Cutter personal communication).

It is important to recognize that there are major limitations to application of sequential extraction procedures (Martin et al. 1987; Nirel and Morel 1990) (Bacon and Davidson 2008; Du Laing 2010; Feyte et al. 2010). It is problematic that chemical solutions used for the selective extraction of particular phases are actually not exactly selective and can unintentionally extract other sedimentary phases. In addition, some of the dissolved phases can be readsorbed to sediment (Feyte et al. 2010). Another problem is the difficulty to interpret the results from different studies, because the different sequential extraction procedures employ different chemical reagents. For example, oxalate, hydroxylamine or citrate can all be used to dissolve iron oxides (Keon et al. 2001; Tessier et al. 1979).

Despite the major limitations, the leaching schemes present a useful proxy in identifying the main operational pools and metals bound to them. In this thesis names of fractions extracted using sequential extraction procedure will referenced in italics to highlight their operational character. Currently, other alternatives to identify specific sedimentary fractions (minerals and organic compounds) are present, e.g., spectroscopic approach such as x-ray absorption near-edge structure (XANES), although more difficult to access. Results from the sequential extractions can be used with caution, for example to study patterns of metal bioavailability in benthic organisms and especially in deposit-feeders such as *N. succinea*. A thorough description of such an application is given in the third chapter.

APPLICATION OF RADIOTRACERS IN BIOACCUMULATION STUDIES

The use of radioisotopes for studying a variety of biological and chemical process has been widely explored and described. Metal bioaccumulation studies have employed radiotracers to follow metal retention in organisms following exposure to radiolabeled water and food (Croteau and Luoma 2005; Wang and Fisher 1999b).

Application of gamma-emitting radioisotopes such as those used in my thesis work i.e., ^{73}As, ^{109}Cd and ^{51}Cr provides an opportunity to follow metal retention in living animals without requiring to sacrifice them. Radioactivity from gamma-emitting radioisotopes can be measured by sodium iodide (NaI) well type of detectors. Measurements are quick, precise and many samples can be processed during the day. Sodium iodide detectors are equipped with variable

size wells and samples of different sizes (e.g., phytoplankton samples, worms, clams or fish etc.) can be measured. In my thesis research, I could follow the retention of ^{73}As, ^{109}Cd and ^{51}Cr for an individual worm, which had the advantage of reducing the biological variation between samples.

Kinetic parameters such as assimilation efficiency (AE) or uptake and release rate constants (k_u and k_e) for a range of different animals have been generated by the application of radiotracer approach. These parameters can be used for metal bioaccumulation forecasting in the field. An excellent synthesis of such studies was published by Luoma and Rainbow (2005). Experiments designed to determine kinetic parameters for As, Cd and Cr in *N. succinea* have been performed in my thesis research and are described in detail in chapters III and IV.

As mentioned above, kinetic parameters continue to be widely employed in predicting metal bioaccumulation for individual metals and animals. Such predictions are performed under the assumption that metal concentrations in animals (C_{ss}) are at steady state.

The biokinetic model was first proposed by Thomann (1981) for zooplankton and fish assuming two uptake routes for metal i.e. from water and from diet and one storage pool of the accumulated metal. Assumption about metal's one storage pool likely simplifies the real bioaccumulation process but due to the lack of empirical data describing different storage pools this is currently the best approach available. In general, the model for animals as derived by Thomann (1981) is described as:

$$\frac{dv''}{dt} = \frac{d(vw)_i}{dt} = \frac{w_i dv_i}{dt} + \frac{v_i dw_i}{dt} = k_{ui} w_i c - K_i v''_i + \alpha_{i,i-1} C_{i,i-1} v_{i-1} w_i ;\ i = 2,3,4$$

where $\frac{dv''}{dt}$ represents the change in average metal concentration in an animal over time, and it is a derivative of the change in metal concentration as a function of average body mass for an individual (w_i). Change in metal concentration in the animal equals the uptake or sorption of metal at concentration *c* from water (k_{ui}), and assimilation ($\alpha_{i,i-1}$ - metal assimilation efficiency) of metal from food (e.g., prey animal in case of carnivores, or phytoplankton for herbivores). Specific consumption rate ($C_{i,i-1}$) is a function of body mass (w_i) and of metal concentration (

v_{i-1}). Loss or excretion of accumulated metal (v'') from animal is described in this equation as K_i. The overall loss term $K_i^{'}$ can be also expressed as:

$$K_i^{'} = K_i + \frac{dw_i}{dt} \div w_i = K_i + G_i$$

considering growth as a dilution factor in the overall metal concentration in the animal (Thomann 1981). Numeral notation *i* indicates the food or aqueous source items from which the animal obtains the metal.

The model describing metal accumulated from two sources: water and food, and stored in one compartment can be derived as shown below.The amount of metal accumulated by the animal from the diet over time is:

$$\frac{dM_{af}}{dt} = i \times AE \times C_f - k_e M_{af}$$

where *i* stands for the food ingestion rate expressed in units of g d^{-1}, and C_f is the metal concentration in the food (g g^{-1}). The amount of metal accumulated by the animal from water over time is:

$$\frac{dM_{aw}}{dt} = k_u \times C_w - k_e M_{aw}$$

where k_u stands for the aqueous uptake rate constant (L g d^{-1}) and C_w is the metal concentration in water. The change of the metal concentration dC_a in the animal over time *dt* is the function of animal growth:

$$\frac{dw}{dt} = g \times M_a$$

In this growth function *g* is the growth rate constant for the storage compartment of metal expressed in the units of g g^{-1} d^{-1}; therefore, the final equation describing metal concentration accumulated by an animal from diet is:

$$\frac{dC_{af}}{dt} = \frac{dM_{Me,f}}{dt} \times \frac{1}{M_a} - \frac{M_{Me,f}}{M_a^2} \times \frac{dM_a}{dt} = (i \times AE \times C_f - k_e \times M_{af}) \times \frac{1}{M_a} - \frac{M_{af}}{M_a^2} \times g \times M_a =$$

$$= \frac{i}{M_a} \times AE \times C_f - k_e \times C_{af} - g \times C_{af};$$

$$\frac{dC_{af}}{dt} = IR \times AE \times C_f - (k_e + g) \times C_{af}$$

where $IR = \frac{i}{M_a}$ is the body mass specific ingestion rate. The equation describing metal concentration accumulated by the animal from water is:

$$\frac{dC_{aw}}{dt} = \frac{dM_{Me,w}}{dt} \times \frac{1}{M_a} - \frac{M_{Me,w}}{M_a^2} \times \frac{dM_a}{dt} = (K_u \times C_w - k_e \times M_{aw}) \times \frac{1}{M_a} - \frac{M_{aw}}{M_a^2} \times g \times M_a =$$

$$= K_u \times C_w - k_e \times C_{aw} - g \times C_{aw};$$

$$\frac{dC_{aw}}{dt} = k_u \times C_w - (k_e + g) \times C_{a,w}$$

At steady state ($dC/dt = 0$) metal accumulated from food and water can be expressed by following equations:

$$C_{af} = \frac{IR \times AE \times C_f}{k_e + g}$$

$$C_{aw} = \frac{k_u \times C_w}{k_e + g}$$

which can be combined as:

$$C_{a,ss} = \frac{IR \times AE \times C_f}{k_e + g} + \frac{k_u \times C_w}{k_e + g} = \frac{IR \times AE \times C_f + k_u \times C_w}{k_e + g}$$

Model describing metal concentration $C_{a,ss}$ stored in two compartments at steady state would be:

$$C_{a,ss} = \frac{M_{af} \times C_{a,f} + M_{aw} \times C_{a,w}}{M_{af} + M_{aw}} = \left(\frac{1}{M_{af} + M_{aw}} \right) \times \left(\frac{i \times AE \times C_f}{k_{ef} + g_f} + \frac{K_u \times C_w}{k_{ew} + g_w} \right)$$

where metal loss is described separately (k_{ew} and k_{ef}) for two storage compartments .

The model developed by Thomann was modified by Wang et al (1996), who has successfully used it to predict metal accumulation in animals in specific field locations. In this model, the concentration of metal obtained by an animal directly from water is a product of an uptake rate constant (k_u), metal aqueous concentration (C_w) divided by a summed loss rate constant (or efflux rate constant; k_{ew}) and the animal's specific growth rate constant (g). In this equation the term describing metal concentration accumulated by the animal from the diet is represented by the product of metal concentration in food (C_f), its assimilation efficiency (AE) and food ingestion rate (IR), which is divided by the sum of metal loss rate constant (k_{ef}) and growth rate constant (g).

The biokinetic model is:

$$C_{ss} = \frac{k_u}{k_{ew} + g} \times C_w + \frac{AE \times IR}{k_{ef} + g} \times C_f$$

if the efflux rate constants following the aqueous and dietary metal uptake are not different, then common efflux rate constant (k_e) could be used this model.

$$C_{ss} = \frac{k_u \times C_w + AE \times IR \times C_f}{k_e + g}$$

This model can be derived as:

$$\frac{dC}{dt} = k_u \times C_w + AE \times IR \times C_f - (k_e + g) \times C$$

For steady state dC/dt = 0 therefore:

$$0 = k_u \times C_w + AE \times IR \times C_f - (k_e + g) \times C$$

$$C_{ss} = \frac{k_u \times C_w + AE \times IR \times C_f}{k_e + g}$$

The relative contributions of metal acquired by animals from diet vs. aqueous phase can be determined from the biokinetic model by rearranging terms. Such an analysis is described for *N. succinea* in chapter IV for all three elements. Past studies have shown that the major source of metal accumulated by animals can come from either diet or water. A comprehensive table synthesizing modeling results from other studies for the dominance of diet or water in the total metal accumulated by animals has been compiled by Wang and Fisher (1999b). As listed in that

table, Ag is the only metal that is acquired by the bivalve *Crassostrea virginica* primarily from water. Arsenic, Cr, Po and Se represent elements that are mainly diet-derived in crustaceans and bivalves. Zinc and Cd can be water or diet derived in bivalves, insects, annelids and crustaceans depending on their diet. Using the biokinetic model, I determined that >98% of predicted As, Cd and Cr body burdens in *N. succinea* are derived from the diet (sediment, pure algal debris or goethite) ingested by the worms (chapter III). This finding motivated me to further investigate possible mechanisms and factors that could influence the dietary metal bioavailability. I hypothesized that some of these factors could be geochemical, e.g., metal partitioning to geochemical fractions (discussed in chapter II and III), while other factors could be related to the chemistry of *N. succinea*'s gut (discussed in chapter V).

OVERVIEW

Presently, knowledge on the dietary bioavailability of sediment-associated As, Cd and Cr in deposit-feeding polychaetes is still limited. The work conducted in my thesis was designed to contribute to a better understanding. I identified the main source (e.g., diet, water) of As, Cd and Cr in *Nereis succinea*, (chapter IV). Secondly, I have determined the extent to which As, Cd and Cr can be assimilated from the ingested diet (chapter III). By using statistical analyses, I have evaluated the influence of As, Cd and Cr associations with various operationally-defined geochemical fractions in sediment on their assimilations in worms (chapter II and III). And finally, I have assessed the impact that the digestive chemistry of the gut fluid can have on As, Cd and Cr assimilation by relating the AEs to the percent of metals released from particles (serving as worm's diet) into the natural and simulated gut fluid (chapter V).

Chapter II: *Determining the phase speciation of sedimentary metals with radioisotopes to support metal bioavailability studies*

ABSTRACT

Solid-phase speciation of ^{73}As(+5), ^{109}Cd and ^{51}Cr(+3) in radiolabeled fresh algal debris and in sediment alone or mixed with algal debris was determined upon their incubation for up to 90 days. This speciation of added radiotracers was determined by conducting a modified sequential extraction procedure. Operationally-defined geochemical fractions were extracted in following seven steps: *exchangeable, carbonate, acid volatile sulfides, iron and manganese oxides, organic I* and *II*, and p*yrite*. Non-extractable *residue* was examined for any remaining radioactivity. Fractionation of ^{73}As, ^{109}Cd and ^{51}Cr was determined to later draw relationships between particular associations (e.g. in *exchangeable*) of radiotracer - their extracted fractions and their bioavailability in deposit-feeding polychaetes. This chapter serves to describe the geochemical aspect of the overall dissertation. Specifically, data sets presented here are used to characterize the geochemical properties of sediment used for further feeding experiments, aimed to determine radiotracer bioavailability from ingested sediments to deposit-feeding worms. Primary results show that fractionation patterns for any of the radioisotopes were similar between sediments collected from three different locations – two in Chesapeake Bay and one in San Francisco Bay, which themselves showed geochemical differences (e.g., organic C content, salinity etc.). As expected fractionation patterns varied between the radioisotopes. Sediment aging with the radioisotopes had no effect on fractionation, except for Cr, which shifted from more easily extractable fractions (*AVS* and *Fe/Mn oxides*) to *pyrite* that was more difficult to extract. Results presented in this chapter were further used in chapter III and IV to help explain the bioavailability of ingested by *Nereis succinea* As, Cd and Cr.

INTRODUCTION

This study was conducted to explore how radioisotopes can be used to gain a better understanding of the geochemical fractionation of arsenic, cadmium and chromium that were initially added in dissolved form to sediments or solid biogenic debris, and how these fractionation patterns change over time under specific conditions. These patterns can then be used to infer metal bioavailability for benthic animals, further complementing radiotracer methods that have been widely used in metal bioaccumulation studies.

Metal contaminants come from a variety of anthropogenic sources, including diverse industrial activities, and can be delivered in dissolved or particulate form to estuaries by rivers, direct discharge, or via atmospheric precipitation (Luoma 1996). Mineral particles, can be composed of clays , which range in size from 4 μm (kaolinite) to 2000 μm (intertidal sand) as summarized by Burdige (2006), iron and manganese oxides, carbonates etc. Organic particles can be of different origin, including allochthonous - terrestrial material and autochthonous - algal debris. Degrading terrestrial and other - non living organic matter can bind with metals in the water column, and algae can passively acquire (through adsorption) or actively incorporate metals into their cells. Once these metals arrive in surface waters, they can be deposited in sediment by being scavenged onto various types of particles that settle out from the water column (Fowler and Knauer 1986; Turner and Millward 2002) and become available to the benthos.

Particle-reactive metals and other types of contaminants that are stored in sediments are in much higher concentrations than metals in the water column, which can be reflected by partition coefficients – Kd indicating several orders of magnitude metal enrichment in particles in relation to overlying water. For example metals that are measured in μmol L^{-1} in the water column can be present in sediments in mmol kg^{-1}. Metals and metalloids in sediments undergo diagenetic processes and cycling, which determine their partitioning into different geochemical pools. Metal(loid)s in these pools have different mobilities. Mobility of particle-bound metals varies due to the properties of metals, due to the properties of these geochemical fractions, and diagenetic conditions (Tessier et al. 1979).

Metal contaminant-enriched sediments are ingested by different deposit-feeding animals (clams, polychaetes, fish etc.) for whom sediment serves as the primary food source. Deposit-

feeding animals can assimilate these ingested metals if the metals are present in a bioavailable form (Wang et al. 1999). Metals that are assimilated and internalized into animal cells can be toxic (Clearwater et al. 2002). Assimilated metals can also be passed through the demersal food chain, if predators consume the metal enriched prey. This trophic transfer poses concern due to harmful effects these contaminants may have for local fauna (Luoma 1996). There is therefore an interest in developing better management strategies for contaminated coastal waters.

Sequential extraction procedures have been used as a tool to approximate the geochemical distribution of metals in operationally-defined (based on the physico-chemical extractability) pools (Huerta-Diaz and Morse 1990; Tessier et al. 1979). These types of extraction procedures intend to selectively target metals in inorganic and organic fractions based on specific reaction kinetics and incubation conditions determined for model fractions. Therefore fractions extracted are, for simplicity, assumed similar to these well characterized model fractions (e.g., specific iron oxide minerals, irons sulfides - AVS, pyrite, etc.) for which the kinetics of solubilization and the reactivity with specific chemical extractants under specific conditions have been determined. Results of such extractions have been used to determine the pools of metals in the bulk sediment that could be potentially bioavailable to animals ingesting these sediments. Bioavailability predictions determined by the sequential extraction procedure could be incorporated into ecological risk assessment protocols.

Sequential extraction methodology has been recognized as problematic due to variable recovery of specific phases or due to non-specificity of chemical extractants that are used in sequential extraction protocols (e.g., specific leaching steps can dissolve more than one targeted geochemical phase) (Martin et al. 1987; Nirel and Morel 1990; Rapin et al. 1986). Despite these problems, sequential extraction remains a widely used approach to study metal distribution in the geochemical fractions that are operationally-defined. Sequential extraction methodology also provides an option to study the geochemical influences on metal bioavailability in deposit-feeding animals (Diks and Allen 1983; Fan et al. 2002; Tessier et al. 1984).

Radiotracer approaches have been used in bioaccumulation studies for many metals and metalloids in aquatic organisms, including deposit-feeding polychaetes . The use of radiotracers allows determinations of metal assimilation efficiencies – a parameter directly related to a

fraction of metal that is bioavailable upon ingestion (Croteau and Luoma 2005; Wang and Fisher 1999b).

In this study I hypothesized that addition of the radiotracers via fresh algal debris, representing metals associated with fresh algal organic matter, to the sediment would show different fractionation patterns of these radiotracers in comparison to sediment labeled directly by metal sorption from solution. Second, I hypothesized that fractionation pattern of radiotracers added directly to otherwise natural sediments would change over time in these wet sediments that were kept sealed in plastic containers. Both of these metal transport scenarios from the water column to seabed will here be mimicked. To address these hypotheses I conducted experiments employing radiotracers of As, Cd and Cr that were introduced to sediment via radiolabeled algal debris and via direct addition of dissolved isotopes. In addition I also conducted sequential extractions of pure radiolabeled algal debris which could represent settling material following algal blooms, particularly in relatively shallow waters where debris could settle to underlying sediments. Radiolabeled wet sediments that were incubated at room temperature in sealed plastic containers for up to 90 days were extracted using sequential extraction procedures. Radiotracers are a useful tool for determining metal fractionation patterns which may control bioavailability of metals to deposit-feeding invertebrates.

MATERIALS AND METHODS

Sediment collection in the field

Sediment samples were collected using a box corer at 3 sites - two sites in Chesapeake Bay: one in Elizabeth River ("ER"; Norfolk, VA; 36°52'32"N, 76°20'09"W), and second in Baltimore Harbor ("BH"; Baltimore, MD 39°12'25"N, 76°31'40"W) both sampled from the RV Fay Slover, and third site located in San Francisco Bay: Mare Island ("MI"; Vallejo, CA; 38°05'15" N, 122°15'15"W) sampled from RV Questuary. Acrylic sub-core tubes (5.7 cm diameter and 30 cm length) were inserted into the sediment in each of the box cores. These tubes were capped and withdrawn from the box core. On board the ship, sediments in these subcores were sectioned at 1 cm intervals from the surface to 10 cm in depth inside a nitrogen-purged glove bag to limit sediment contact with oxygen in the air. A portion of surface (0-1 cm) sediment from each section was used to determine sediment porosity via wet/dry and other chemical analyses described in the following section. A mixture of sediment from the top layer

(~20 cm) remaining in the box core was collected into plastic buckets, transported and stored (4°C, for up to 2 years) for subsequent radiotracer experiments. Sediments used for the experiments were oxidized by leaving them open to air.

Chemical analyses

Portions of the surface (0-1 cm) sediments were dried at 50°C and later ground with an agate mortar and pestle. Samples were then sieved through a 150 μm nylon mesh screen and stored in polyethylene bottles for further analyses. These samples were used to determine organic carbon, organic nitrogen, and sulfur using a Carlo Erba 1500 Elemental Analyzer (Cutter and Radford-Knoery 1993). To evaluate metal concentrations, sediment samples from the top 1 cm were also digested for 8 hours first by treatment with nitric acid in a boiling water bath. After cooling, concentrated perchloric acid was added to sediment samples to complete the digestion. All metals but aluminum (Al), were recovered with an efficiency of 98 ± 3% (n = 6). A large fraction of Al bound to silicates remained unextracted and only its extraction with hydrofluoric acid (HF) yielded full recovery. The sediment digest solutions were analyzed for metal concentrations using ICP-MS (Finnigan Element 2), with the standard additions method of calibration to assure accuracy.

Sequential extraction procedure

The extraction scheme used in the present study is illustrated in Fig. 1. The objective of the sequential extraction procedure is to selectively dissolve specific geochemical phases. Although these fractions do not represent pure mineral and/or organic phases and likely include other components, we maintained the original names for these fractions to be consistent with previous geochemical studies (Harvey and Luoma 1985; Lee et al. 2000c; Tessier et al. 1979). The seven distinguished fractions were (in order of their recovery): *exchangeable, carbonate, acid-volatile sulfides (AVS), iron and manganese oxides* (*Fe/Mn oxide*), *organic I*, *organic II*, and *pyrite*. Methods were modified from Tessier et al. (1979) for oxic sediments and Huerta-Diaz and Morse (1990) for anoxic sediments. The *AVS* fraction was extracted according to Cutter and Oatts (1987).

We thus employed the following extraction procedure: First, 2 g (wet wt) of sediment was subjected to a 1-h incubation with 8 mL of 1 M $MgCl_2$ to desorb weakly-bound or adsorbed

ions. Chloride ions could also complex Cd present in the $MgCl_2$ solution (Ruttenberg 1992; Stumm and Morgan 1996). After this first step extracting the *exchangeable* fraction, the remaining sediment was incubated for 1 h with 8 mL of sodium acetate (NaOAC) solution buffered at pH 5, targeting the *carbonates*. Some iron sulfides that are typically defined as the AVS and other pH sensitive minerals remaining in the residue of the sediment could be removed at this lower pH. During method testing nearly 20% of AVS incubated with the sodium acetate solution was dissolved. Further, in this incubation anionic metal groups such as arsenate could exchange with the acetate functional group. This extraction step was designed to target carbonates and will be referred to as *carbonates*, despite the recognized possibility that other fractions (e.g., some weak acid soluble and anion exchangeable materials) could be also removed during this extraction. The third extraction step was designed to remove *AVS*. It involved 30 minutes incubation at 21°C with 10 mL of 0.5 M HCl. Some iron oxides may have also been extracted in this step (Schwertmann 1991) and metals in clays could be released as a result of lower pH (Tessier et al. 1979). *Fe/Mn oxides* were the next targeted fraction and the remaining sediment was incubated for 6 h with 15 mL hot (96 ± 3°C) 0.04M hydroxylamine dissolved in 25% (v/v) acetate - a standard solution used in quantitative solubilization of iron and manganese oxides. The extraction of *Fe/Mn oxides* fraction was followed by a sequence of a basic (20 mL of 1 N NaOH, at 80°C) 8-h leach followed by a 6-h hydrolysis in sulfuric acid (10 mL of 5 M H_2SO_4), and it targeted two organic fractions referred herein as fractions *organic I* and *organic II*. According to Huerta-Diaz and Morse (Huerta-Diaz and Morse 1990) the incubation of sediment with H_2SO_4 would decrease the organic carbon content if the previous step (i.e., 1 N NaOH) was insufficient. It is also important to note that these two steps address the extraction of the organic matter, which may represent refractory compounds such as humic and fulvic acids - extractable by NaOH, and other remaining organic compounds - extractable by H_2SO_4. Previous steps of the sequential extraction procedure – for example the hydrochloric acid extraction, could potentially remove some of the labile organic compounds (e.g., proteins) if these labile compounds were present in analyzed sediment. Hence NaOH extraction was assumed to target detrital organic matter as modified from Harvey and Luoma (1985) and H_2SO_4 was used to extract the remaining organic carbon as described by Huerta-Diaz and Morse (Huerta-Diaz and Morse 1990). Importantly, incubation of sediment with H_2SO_4, was assumed not to solubilize pyrite. The last step of the sequential extraction procedure targeted a fraction that presumably

included primarily *pyrite*. This fraction was targeted with 3 mL of cold 11 N HNO_3 by incubating the remaining sediment for 2 h. It is presumed that if any unextracted refractory organics were left over from the NaOH and H_2SO_4 incubations the last step (nitric acid) of the sequential extraction would fully recover the remaining organic matter in the sediment. At the end of each extraction, sediment was separated from the extract by centrifugation at 834 *g* for 15 minutes. The remaining sediment was twice rinsed with 8 mL of deionized water, and the liquid was again separated from the sediment by centrifugation, and added to the previously collected extract for further analyses, described below.

Radiolabeling of the sediment

Addition of dissolved radiotracers

In one series of experiments, the binding of As (arsenate), Cd (cadmium chloride) and Cr (chromic chloride) to different geochemical phases in sediments was determined using gamma-emitting radioisotopes (^{73}As, ^{109}Cd and ^{51}Cr) added directly to the sediments. Briefly, three replicate aliquots (2 g wet weight) of sediment representing a mixture from 0-20 cm from each site were placed in separate containers (50 ml polypropylene conical tubes) and radiolabeled by addition of ^{73}As ($t_{1/2}$ = 80.3 d) or ^{109}Cd ($t_{1/2}$ = 461.4 d) combined with ^{51}Cr ($t_{1/2}$ = 27.7 d). Depending on the activity of the radioisotope stock solution, up to 20 µl was withdrawn from 0.1 M (^{73}As and ^{109}Cd) or 0.5 M HCl (^{51}Cr) stock solutions with a pipette, mixed with dilute NaOH to neutralize the acid, and pipetted into the sediment. In most cases the entire surface of the sediment was covered by the liquid containing the radiotracers. Following isotope additions, sediments were aged for 2, 30 and 90 d (except sediments labeled with ^{51}Cr, which were only aged for 2 and 30 d due to its short half-life) in closed 50 mL Falcon tubes at 21°C. The 2 d time point allowed evaluation of the rapid metal association with the particles while the 30 and 90 day time points provided material to study any further time-dependent changes in fractionation of the added radiotracers. During the incubation some black spots developed in the sediment indicating anoxia. Activities per mass and molar concentrations of added radiotracers are specified in Table 1. Molar concentrations were small and did not increase the overall metal concentrations in sediments by more than 0.01%.

Addition of radiotracer to the sediment via phytoplankton

In another series of experiments, ^{73}As, ^{109}Cd and ^{51}Cr were added to sediments via mixing with radiolabeled diatom cells. Cultures of *Thalassiosira pseudonana* were grown in f/2 medium (Guillard and Ryther 1962) prepared with surface seawater (salinity of 35) collected 8 km offshore of Southampton (Long Island, NY) in the presence of ^{109}Cd and ^{51}Cr or in the presence of ^{73}As (at f/20 levels of phosphate, or 3.6 µM). Algae were grown to stationary phase and were harvested by filtration onto 3.0 µm Nuclepore polycarbonate filters. Filters were then immersed into plastic conical vials filled with 10 ml of seawater so that the diatom biomass could be resuspended. Containers with such concentrated algae were centrifuged at 834 *g* for 5 minutes to separate the liquid from the biomass. Microscopic observations of cells after centrifugation led me to believe the cells stayed intact. Wet algal biomass, whose dry mass equivalent was 22.3 ± 4.5 mg (Fisher and Schwarzenbach 1978) of these radiolabeled algae were mixed with 2 g wet wt of oxidized sediments collected with a box corer. This increased the total C content of the original sediment from 5.0% to 5.4% in BH, from 2.0% to 2.4% in ER and from 1.5% to 1.9% in MI based on an assumption that the C content in a cell of *Thalassiosira pseudonana* is 8 pg (Pechenik and Fisher 1979), and equal to 35% of cell dry wt. Labeled sediment was held at 21 °C for 30 days and development of anoxia was visible at the surface of incubating sediments. Activities per mass and molar concentrations of radiotracers added via algae are specified in Table 1.

To evaluate the influence of the sequential extraction procedures on As, Cd, and Cr extractability from pure phytoplankton, aliquots of the radiolabeled algae (not mixed with sediments) were also extracted directly using the first three extraction steps, without mixing with sediments. By doing so, I evaluated the influence of these extraction steps on the removal of metals that are largely bound to organic compounds in cells, at least for As which is known to primarily associated with arsenosugars (Cullen and Reimer 1989). The biochemical association of Cd and Cr in algal cells is less well-characterized, but an appreciable amount of the Cd is found in the cytoplasm of diatoms (Ettajani et al. 2001; Reinfelder and Fisher 1991).

Measurement of radioactivity

Initial radioactivity of sediment samples and the radioactivity of samples containing the extracted radioisotopes was measured using a well-type NaI(Tl) gamma detector (Canberra).

Radiolabeled sediments and liquids collected at the end of each sequential extraction step were kept in plastic conical Falcon tubes that could hold up to 50 mL of liquid. The final volume of liquid in extracts varied. This required correcting for a variability due to counting geometry changes (i.e. different sample volumes). A function correcting for the counting geometry error was developed based on data generated in a test experiment. Two sets of three conical Falcon tubes (max volume = 50 mL) were inoculated with ^{109}Cd alone (first set), and with a combination of ^{73}As and ^{51}Cr (second set). ^{73}As and ^{51}Cr were combined because their emission energies did not interfere with each other. Initial radioactive counts were recorded for each tube containing no liquid in addition to the µL amount of the radiotracers. Further, volumes of deionized water were added to reach 24, 26, 31 and 36 ml, which were matching the volumes of extracts mixed with rinse water from each of the extractions. Radioactive counts of ^{73}As, ^{109}Cd and ^{51}Cr were recorded for individual tubes for each of the volumes with the instrument counting error of << 1%. Based on the results of the test, the counting geometry correction was applied to each radioactive reading by using the following functions:

$^{73}\mathrm{As}$ $\quad \%\ cpm\ detected = -0.0252\mathrm{vol}^2 + 0.7768\mathrm{vol} + 96.56;\ R^2 = 0.999$

$^{109}\mathrm{Cd}$ $\quad \%\ cpm\ detected = -0.071\mathrm{vol}^2 + 3.4518\mathrm{vol} + 50.446;\ R^2 = 0.991$

$^{51}\mathrm{Cr}$ $\quad \%\ cpm\ detected = -0.0375\mathrm{vol}^2 + 1.3248\mathrm{vol} + 68.975;\ R^2 = 0.972$

Liquids that were extracted during the sequential extraction procedure that were mixed with the rinse solution were counted for periods of time assuring errors <5%, usually < 5 minutes.

Reproducibility of extractions and statistical analyses

Reproducibility of the overall extraction procedure was evaluated with coefficients of variation (CV) for different treatments. CVs for all treatments were found typically below 20% for the dominant phases in Baltimore Harbor and Elizabeth River sediments labeled directly with radioisotopes, and 33% for Mare Island sediments. No systematic differences were noted in reproducibility between metals, sediment phases, or ages in sediments labeled directly. For sediments radiolabeled via addition of algal debris, CVs were 7% for Baltimore Harbor sediments (for dominant phases in all treatments), 13% for Elizabeth River sediments, and 17% for Mare Island sediments. CVs for at all sites were 12% for As, 17% for Cd, and 5% for Cr.

Statistical analyses of replicate (n = 3) samples used PASW 18.0 software. All the data expressed as % metal in a given sedimentary fraction were arcsine transformed to normalize their distributions. One-way ANOVAs were used to detect significant differences in metal partitioning between treatments, including sites, specific sedimentary fractions, and ages.

Assimilation efficiencies

Assimilation efficiency or AE is a parameter directly related to the bioavailability of ingested metal. Assimilation efficiency data of ^{73}As, ^{109}Cd and ^{51}Cr for a deposit-feeding polychaete *Nereis succinea* that were feeding on radiolabeled sediments are from Baumann and Fisher (2011). Briefly, a series of pulse-chase feeding experiments was conducted to estimate AE values for sediments from all three locations. Sediments labeled directly or by addition of radiolabeled algae to sediments and incubated for 2 and 30 days were placed in feeding chambers and worms were given ~ 4 hours to feed. Polychaetes used in these experiments were previously acclimated to laboratory conditions and specific diet type. Feeding on the pulse of the radioactive sediment was followed by measurement of radioactivity for individual worms in a gamma-counter. After radioanalysis, individuals were placed back into their feeding chambers filled with nonradioactive sediment and refreshed seawater. At this stage worms were allowed to clear their guts off of sediment containing unassimilated radioisotope. The amount of radioactivity in worms was monitored every few hours during the first 24 hours and then daily. AEs were calculated based on radioisotope percentages retained in worms plotted against time, as y-intercepts of the trend line describing the slowly turning over radioisotope pool in worm tissues. Details of AE calculations are provided in Wang et al. (1996).

RESULTS

Sediment characterization

The organic carbon content differed considerably between sediments from MI (1.5%) and ER (2%) and BH (5%). The lower atomic C: N ratios in MI sediments (14) than in Chesapeake Bay sediments (ER: 19 and BH: 18), indicating marine vs. terrestrial inputs of organic matter because the C: N ratio of marine algae is 6.6, while the terrestrial organic matter C: N ratios are higher (~15-30; Hedges et al. 1997). The sulfur fraction in sediments from Chesapeake Bay was

slightly higher than in MI sediments (0.5 and 0.6% vs. 0.4%), resulting in higher atomic C: S ratios in BH (10.0) than in MI (3.8) sediments (Table 2).

Metal concentrations in ER sediment were 5-8 fold higher for Fe, ranging between 3.2 and 23.2 mg g^{-1}, and 3-5 fold higher for Mn than in BH and MI sediments ranging between 53.9 and 94.7 µg g^{-1} (Table 2). Concentrations of Ca were 2 orders of magnitude lower in ER sediments (8.6 µg g^{-1}) than in BH and MI (134 and 718 µg g^{-1}, respectively). Both As and Cr in BH were an order of magnitude higher (47.2 and 322.7 µg g^{-1}, respectively) than in ER (6.4 and 33.3 µg g^{-1}, respectively) and MI (1.4 and 47.4 µg g^{-1}, respectively), and Cd showed the least variation among the metals, being lowest in ER and highest in MI (0.5 – 2.4 µg g^{-1}; Table 2). Ratios of metals to Al in sediments were higher than in Earth crust for Fe (1.2 – 987.2 vs. 0.5), Mn (0.02 – 12.0 vs. 0.01), As (only for BH and ER: 0.02 – 0.3 vs. 0.01), and for Cr (0.018 – 1.4 vs. 0.001) indicating anthropogenic contamination (Table 2). In contrast, Cd did not appear to be a contaminant in the sediments I investigated as the ratios of Cd/Al were lower than in Earth crust.

Metals extracted from pure algae

The recovery of the radioisotopes from the triplicate samples of pure (i.e., not mixed with sediment) radiolabeled algal debris that were subjected to the first three steps (1 M $MgCl_2$ targeting the *exchangeable* fraction, NaOAc at pH 5 targeting the *carbonate* fraction, and 0.5 M HCl targeting the *AVS* fraction) of the sequential extraction procedure was less than 100% for ^{73}As and ^{51}Cr. For As, 80 ± 9% (mean ± 1 SD) was extracted, and 80 ± 7% of ^{51}Cr was extracted, whereas all of ^{109}Cd was recovered during these extraction steps (Fig. 2). The percentage of recovered^{109}Cd from algal debris was > than 100% due to measurement uncertainty. Much of the originally added radioisotope - ^{73}As (38 ± 1%), ^{109}Cd (69 ± 1%) and ^{51}Cr (54 ± 0.4%) was extracted with the acidic (pH =5) solution of sodium acetate in the second step of the procedure. The first extraction step, initially designed to strip off the metals loosely bound to sedimentary particles in the so called *exchangeable* fraction, removed less ^{73}As and ^{51}Cr (14 ± 1%; 9 ± 10%, respectively) than in the two remaining steps. This was likely due to osmotic stress that cells could experience due to high ionic strength of used extractants. A quarter of the ^{109}Cd was already extracted from the algal biomass during the first step. After 65% of ^{73}As was extracted in the first two steps, HCl (third step) leached out an additional 28 ± 8% of this isotope, leaving

some of the ^{73}As unextracted. In this third step, only 13 ±1% of ^{109}Cd and 17 ± 2% of ^{51}Cr was extracted from the remaining algal debris.

Radiotracers extracted from sediments mixed with algae

After 30 d incubation of radiolabeled algal debris with sediments, ^{73}As, ^{109}Cd and ^{51}Cr partitioned to different sedimentary fractions (Fig. 3). At the end of the sequential extractions 100% of ^{109}Cd, 98-99% of ^{51}Cr and 82 – 86% of ^{73}As were recovered (Fig. 3). Two main sedimentary fractions accounting for 38 – 58% of ^{73}As were the fourth and fifth in the sequence i.e., Fe/Mn oxides and organic I, which were extracted with a reducing solution of hydroxylamine and solution of sodium hydroxide. Most of the ^{109}Cd was extracted from sediments mixed with algae by the neutral solution of $MgCl_2$ and the acidic solution of NaOAc in the two most reactive fractions i.e., *exchangeable* and *carbonate*, accounting for 41-78% of this radioisotope. Much (13-39%) of the ^{109}Cd was also in the fraction extracted by HCl (step 3), thought to represent the AVS. Similarly to ^{109}Cd, much of the ^{51}Cr (41-66%) was also released during the hydroxylamine extraction. This was the primary pool of ^{51}Cr in all of the sediments. Between 10 and 13% of ^{51}Cr was released during the HCl incubation, and 12-20% of the ^{51}Cr was extracted from sediment by oxidizing it with sulfuric acid.

Time dependent changes in ^{73}As, ^{109}Cd and ^{51}Cr fractionation in sediments labeled directly

Isotopes introduced to sediments via direct injection partitioned to various operationally defined fractions. Arsenic was the only radioisotope not completely recovered during the sequential extraction procedure. For sediments from all three sites the average percentage of ^{73}As in the final *residual* fraction was <17% at 2 days of exposure and increased, although not significantly, up to 25% at 30 and 90 days (Fig. 3). Only 2% or less of the ^{51}Cr remained in the final residue, whereas ^{109}Cd was fully recovered by the sequential extraction procedure for all of the sediment treatments.

For all sediment types exposed to ^{73}As for 2, 30 and 90 days, 30 - 43% was extracted by incubating the sediment with the oxidizing solution of sodium hydroxide (i.e., the organic I fraction). Sulfuric acid extracted (*organic II* fraction) 11-16% of ^{73}As from BH sediments, hot hydroxylamine (*Fe/Mn oxides* fraction) extracted 14-29% and 8-27% of ^{73}As for ER and MI, respectively. In Chesapeake Bay sediments (BH and ER) the concentration of ^{73}As in the AVS fraction decreased from the initial 9 or 29% (BH, ER) to less than 3% at the end of the three

month incubation (Fig. 3). This decrease was the only significant temporal change (one-way ANOVA, $p < 0.05$) for ^{73}As fractionation during the three month incubation experiment.

Overall, in both of the Chesapeake Bay sediments ^{109}Cd was predominantly extracted by $MgCl_2$ (*exchangeable* fraction). There was a significant increase (one-way ANOVA, $p < 0.05$) of ^{109}Cd in the *exchangeable* fraction between 2 and 30 days in ER sediments and between 30 and 90 days for BH sediments, and a parallel significant decrease (one-way ANOVA, $p < 0.05$) by up to 7-fold, in ^{109}Cd fractions extracted as *AVS* and *Fe/Mn oxides*.^{109}Cd from BH and MI sediments exposed for 30 d was extracted in similar proportions in the first four extraction steps (Fig. 2).

Distribution of ^{51}Cr showed the greatest level of agreement among the sediment locations. In all of the sediments at 2 days of exposure, ^{51}Cr was primarily extracted by HCl (40-59%) and hydroxylamine (29-35%) representing fractions identified as *AVS* and *Fe/Mn oxides*. During the 30 d incubation ^{51}Cr, decreased by 56-63% in *AVS* and *Fe/Mn oxides* fractions and significantly increased by 41-56%in pyrite fraction for all sediments (one-way ANOVA, $p < 0.05$).

^{73}As assimilation efficiencies and concentration in exchangeable + carbonate fractions from sediment labeled with algae and via direct addition

In *N. succinea* the assimilation efficiencies of algal ^{73}As incubated with sediments from BH, ER and MI for 30 days were higher than from sediments labeled directly - and incubated for 30 days (Fig. 4). While values for As AEs in algae labeled sediments were available for all sediments (BH, ER and MI), AEs for As labeled directly for 30 days were not available for ER sediment. Arsenic assimilation patterns for sediments labeled by these two methods are in parallel with ^{73}As concentrations as extracted in the pooled operationally defined *exchangeable* and *carbonate* fractions, which are also higher for sediments labeled by algae vs. sediments labeled by direct addition (Fig. 4).

DISCUSSION

Utility of the sequential extraction procedure for algae enriched sediments

Sequential extraction procedures have been designed to extract sedimentary phases from primarily inorganic sediments with small organic matter fractions (Tessier et al. 1979). Traditionally, the refractory organic matter pool represented by the operationally defined *fulvic*

and *humic acids* have been extracted by sodium hydroxide and sulfuric acid but extraction of fresh algal organic matter has not previously been addressed in the sequential extraction procedures.

Our results indicate differences in radioisotope extractability from substrates containing fresher and more labile organic matter derived from phytoplankton cells and older, more diagenetically reworked organic matter that is buried in sediment, where the radioisotopes associated initially with algae were more easily extracted. In algal cells organic arsenic, such as arsenosugars (Cullen and Reimer 1989), was extracted by chemical solutions designed to target inorganic sediment phases. This is not surprising because solutions used in the first three steps of the procedure could extract some arsenosugars and other organic As compounds. There are however specific protocols designed to extract specific compounds such as arsenosugars, which were not part of the sequential extraction procedure used here (Raber et al. 2000). In the case of elements that are taken up by living organisms such as algae and converted into organic molecules, these molecules could be extracted by solutions other than those targeting the humic and fulvic acids used in the sequential extraction procedures. Therefore, one could mistakenly assume that if these metals are extracted by solutions designed for inorganic fractions these metals are inorganic, while in fact this may not be the case. This realization is important when interpreting extraction results of estuarine sediments, particularly when collected following algal blooms. Sediments at these times could be enriched with relatively fresh algal organic matter containing organometals or metals that are weakly and not covalently associated with organic compounds in decomposing cells (Kowalski et al. 2009). Perhaps these metals could be extracted by $MgCl_2$ solutions but this remains to be tested. Metals associated with microflora living in sediment could also serve as a source of "organic – associated" metals, although this fraction is likely very small due to the small microbial biomass relative to the mass of the sediment.

An assumption that adding to the sediment algae-associated radiotracer would result in its increased concentration within the operationally-termed *organic* fractions is supported by results for ^{109}Cd and for ^{51}Cr in sediments from BH and MI, which show an increased concentration of these radiotracers in the algae mixed sediments in comparison to sediments labeled directly. Specifically, ^{109}Cd in sediments labeled directly that were aged for 2 and 30 days was lower in the two *organic* fractions combined than in sediments radiolabeled by algae, and ^{51}Cr was higher

in these *organic* fractions in sediments from BH and MI labeled by algae than in sediments labeled directly and aged for 2 days. This assumption is however not supported by ^{73}As results, although addition to sediment algae associated ^{73}As results in its higher concentration in the most easily extractable fractions i.e., exchangeable and *carbonate* pool in comparison to sediments labeled directly. This finding is important due to previously reported linkage between easily extractible sedimentary metals and their higher assimilation in benthic animals (Baumann and Fisher 2011b; Lee and Luoma 1998).

Application of the sequential extraction procedure for investigating ^{73}As, ^{109}Cd and ^{51}Cr bioavailability in a deposit-feeder *N. succinea*

The natural organic matter from field-collected sediments had 2-3 times higher C: N ratios (14-19) than fresh algae (C: N ~6.6), and 1.4 -1.9 higher than algae degraded by bacteria (C: N ~10) (Ferguson et al. 2003). This suggests therefore that sediment from MI which had the lowest C: N ratio was more impacted by the marine algal biomass. Despite the relatively small amount of added algal biomass in this study, it is possible that labile organic matter, which is also highly nutritious, could increase the metal bioavailability in deposit-feeding animals. The greater content of the fresh algal organic matter vs. more degraded and less nutritious organic matter would likely alter the ingestion rates in *N. succinea*. Further, a study by Lee and Luoma (1998) showed that assimilation efficiency of ingested Cr ranged between 2.0 and 2.5% in the clam *Potamocorbula amurensis* feeding on sediment and between 11.6 – 12.2% for clams feeding on microalgae. Baumann and Fisher (2011b) found that the assimilation efficiency in the polychaete *N. succinea* of ingested As from algal cells was 72%, but only 1-12% when As was bound to unamended sediment. Sequential extraction procedures showed that 80% of ^{73}As was extracted from radiolabeled algae in the first three steps (Fig. 2), similar to its assimilation efficiency from algal cells.

Using approaches relying on chemical extractions of toxic metals to study metal bioavailability does not mimic the physiological processes that take place in the polychaete guts. Instead they serve as a proxy estimating the pool of metal that could be assimilated by polychaetes. Furthermore, ^{73}As AEs are higher in *N. succinea* feeding on 30 d old algae-labeled sediment than for those feeding on 30 d old directly-labeled sediment (Fig. 4). This difference is even more pronounced in fresh sediments (Baumann and Fisher 2011b). The greater

bioavailability of ^{73}As from sediment mixed with labeled algae is consistent with the greater percentage of ^{73}As extracted in the first two steps of the sequential extraction procedure from these mixed sediments (Fig. 4).

Combined metal pools extracted in steps 1(by $MgCl_2$) and 2 (by NaOAc) have indeed been demonstrated to be positively related to bioavailability to *N. succinea* for As, Cd, and Cr (Baumann and Fisher 2011b). Diks and Allen (1983) related Cu fractionation in sediment with its bioavailability to freshwater worms, where Cu bioavailability showed a positive relationship to Cu in the "adsorbed/exchanged" fraction, extracted in the same manner as the exchangeable fraction in the present study. Fan et al. (2002) similarly found that assimilation efficiencies of Cd in mussels and clams increased with Cd fractionation in the exchangeable pool ($MgCl_2$ extracted).

Radiotracers are useful for investigating sedimentary metal bioavailability in deposit-feeding polychaetes. Radiolabeled sediments can be used to experimentally to determine metal assimilation efficiencies, and they can be used to determined operationally-defined radiotracer fractionation by sequential extraction methodology. Future studies are required to evaluate the application of this method for other metal contaminants and other deposit-feeding animals. This approach can ultimately lead to improved modeling of sedimentary metal transfer in the benthic food chains.

TABLES

Table 1. Concentration of radiotracers expressed in units of radioactivity Bq/g and moles in sediment from all three locations at the time of the experiment

		Bq/g (wet wt)			nmol/g (wet wt)		
Label	Equilibration time (d)	^{73}As	^{109}Cd	^{51}Cr	^{73}As	^{109}Cd	^{51}Cr
	2	200	59	71	10	44	49.2
direct	30	209	36	59	10.2	26.6	47.4
	90	104	31		5	22.2	
algae	30	68	47	353	3.3	34.8	232.2

Table 2. Geochemical properties such as elemental concentrations and porosity (φ) in surface (0-1cm) sediment, salinity of the overlying water collected from Baltimore Harbor (BH), Elizabeth River (ER) and Mare Island (MI). Elemental composition of Earth's crust and metal to Al ratios are also provided as a reference for potential contamination.

		Location							
	units	Concentrations				metal/Al ratio			
		BH	ER	MI	Earth Crust	BH	ER	MI	Earth Crust
Fe	mg g^{-1}	4.8	23.2	3.2	36	2.4	987.2	1.2	0.5
Al		1987 [a]	23.5 [a]	2657 [a]	6.9E+04	1.00	1.00	1.00	1.00
Ca		134	8.6	718		0.07	0.4	0.3	
Mn	μg g^{-1}	94.7	281	53.9	525	0.05	12.0	0.02	0.01
As		47.2	6.4	1.4	787	0.02	0.3	0.001	0.01
Cd		1	0.5	2.4	2.0E+05	0.0005	0.02	0.0009	2.9
Cr		322.7	33.3	47.4	71.2	0.16	1.4	0.018	0.001
C		5	2	1.5					
N	wt %	0.3	0.1	0.1					
S		0.5	0.6	0.4					
atomic C:N		18	19	14					
Salinity	ppt	8.5	19.5	23					
Φ		0.8	0.7	0.8					

Concentration of Al is not representative of the total; HF extraction of sediment was not conducted

FIGURES

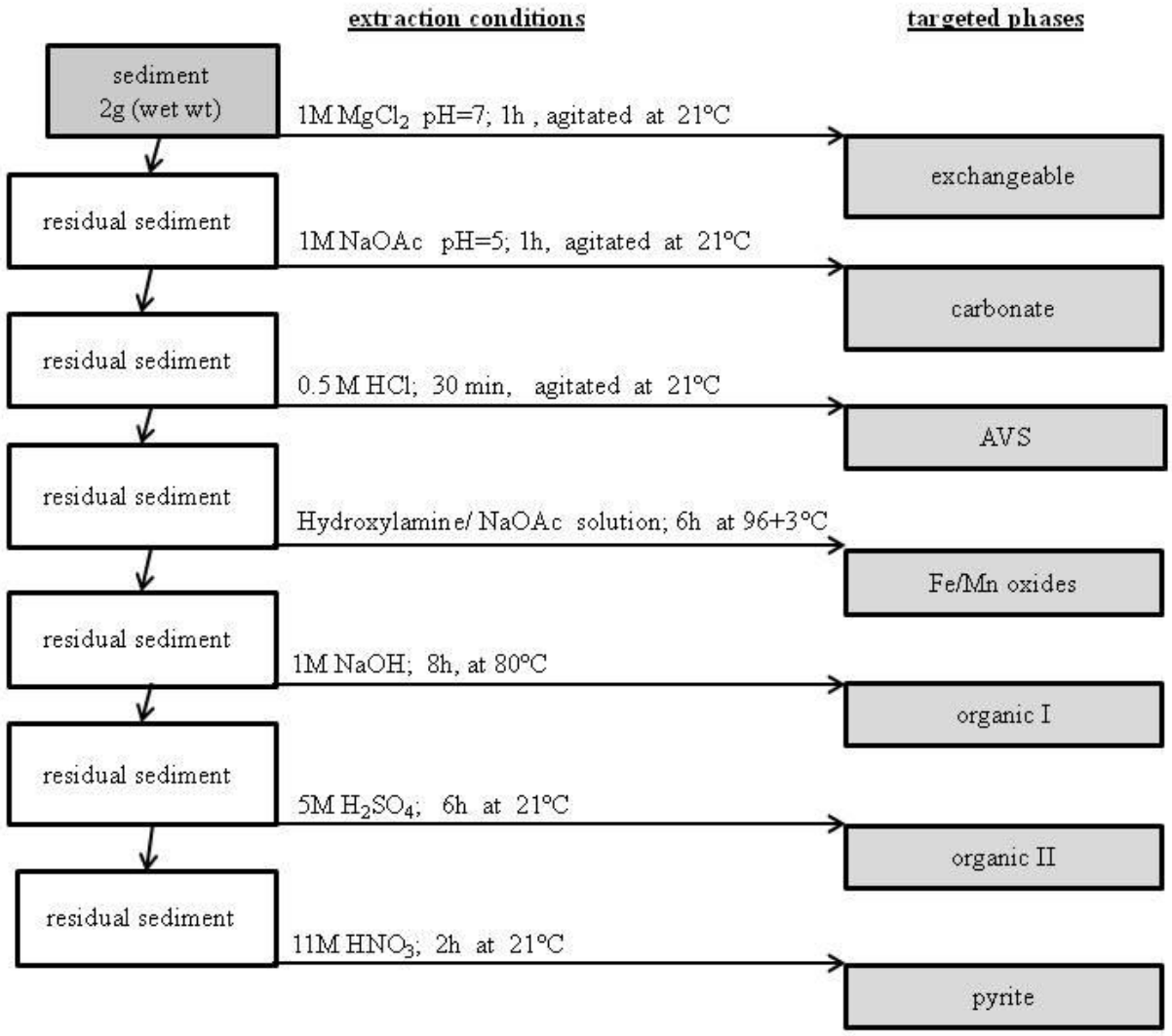


Fig. 1. Scheme showing the steps of the sequential extraction procedure used in this study.

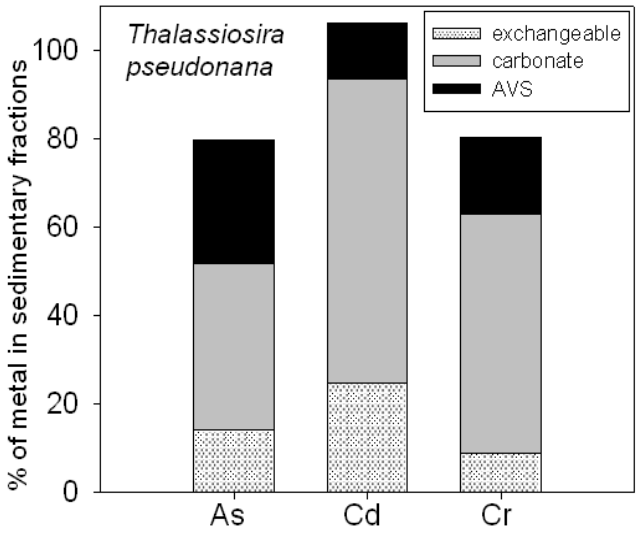


Fig. 2. Per cent of total extracted 73As, 109Cd and 51Cr from pure algal cells in the first three steps (exchangeable, carbonate and AVS phases) of the sequential extraction procedure.

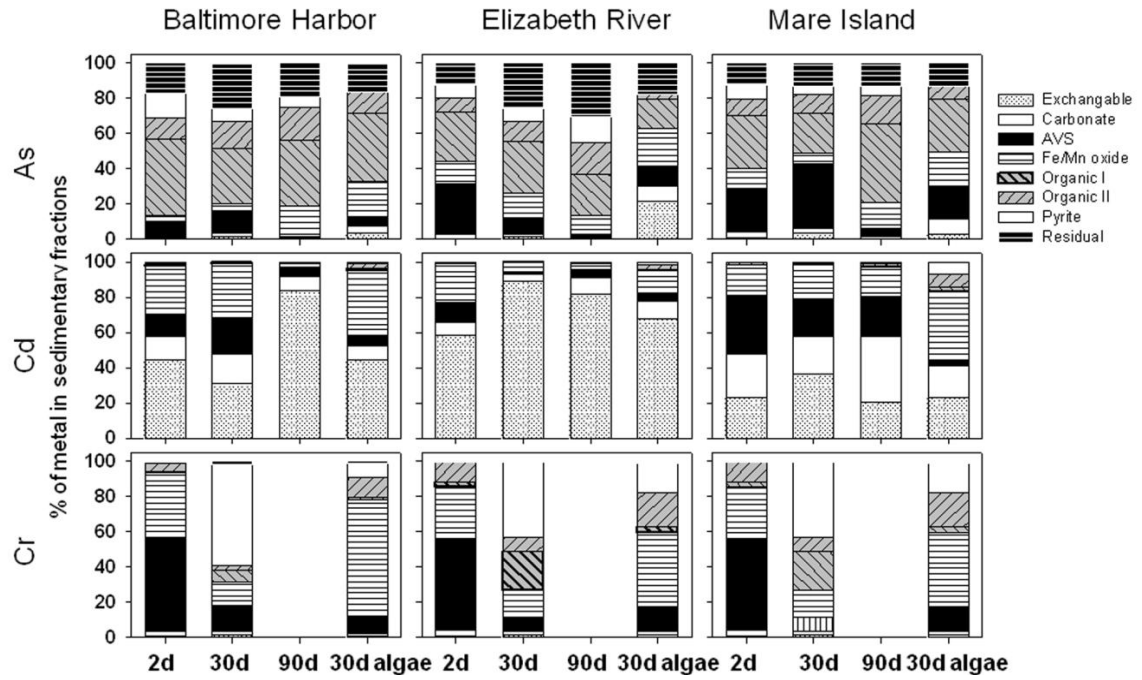


Fig. 3. Phase speciation of ^{73}As, ^{109}Cd and ^{51}Cr expressed as per cent of metal in sequentially extracted geochemical fractions from estuarine sediments that were equilibrated with the radiotracers added directly for 2, 30 and 90 days after and with radiotracers added via previously radiolabeled algae for 30 days

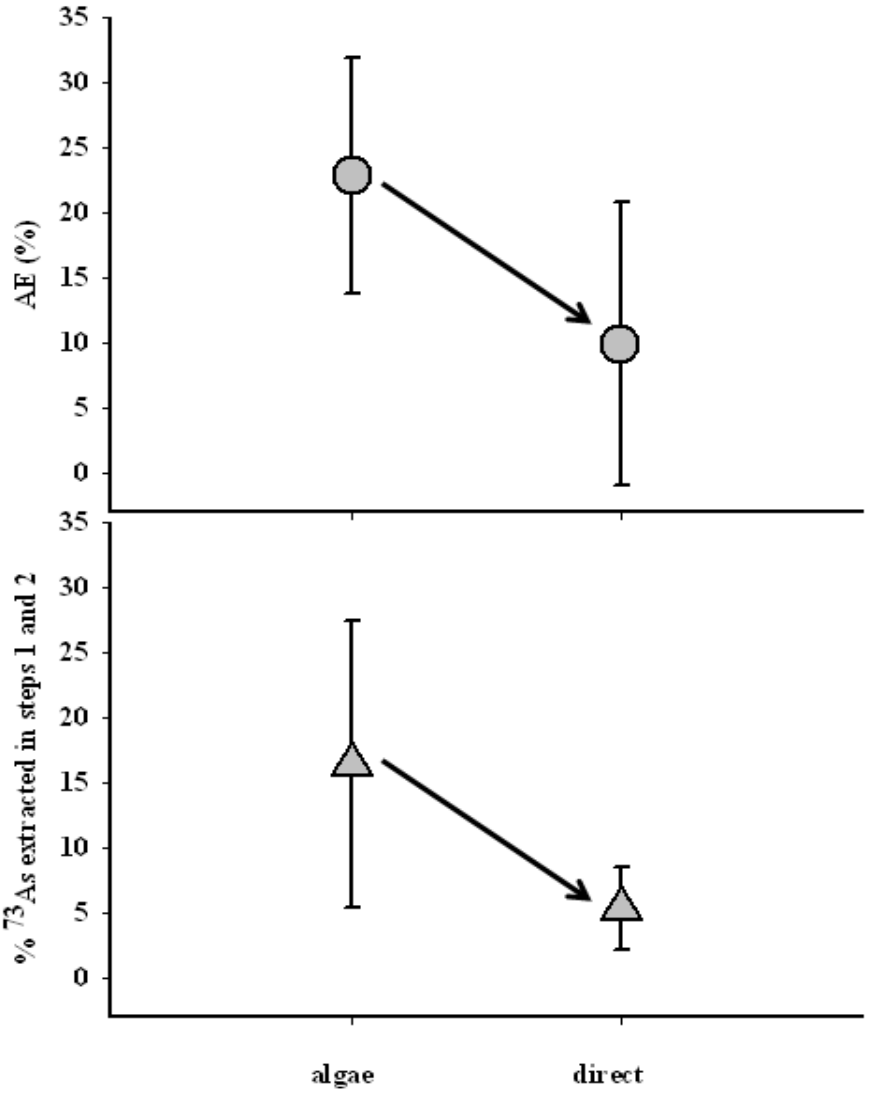


Fig. 4. Mean assimilation efficiencies (± 1 standard deviation; grey circles; top panel) of ^{73}As in *Nereis succinea* fed sediments mixed with radiolabeled algae incubated for 30 days (left) and sediments that were labeled by a direct addition of the radiotracer and aged for 30 days (right). Mean percentage (± 1 standard deviation; grey triangles; bottom panel) of ^{73}As extracted in the first two steps of the sequential extraction procedure (i.e., magnesium chloride and sodium acetate) from sediments mixed with radiolabeled algae that were incubated for 30 days (left) and sediments that were labeled by a direct addition of the radiotracer and aged for 30 days (right). Average values of AEs and extracted in steps 1 and 2 ^{73}As represent all of the sediment types i.e., BH, ER and MI, except AEs for ER sediment (not determined).

Chapter III: *Relating the sediment phase speciation of As, Cd and Cr with their bioavailability for the deposit-feeding polychaete* Nereis succinea

ABSTRACT

We studied the influence of sediment geochemistry on bioavailability of arsenic, cadmium and chromium in deposit-feeding polychaetes. Metal phase speciation in sediments was determined with a sequential extraction scheme, and assimilation efficiencies (AEs) of ingested metals were determined by pulse-chase feeding experiments using γ-emitting isotopes. Worms were fed sediments collected from geochemically diverse estuaries that were labeled by sorbing dissolved radiotracers or mixing with radiolabeled algae. Uptake of sediment-bound metals was compared to that from labeled algae or goethite. Metal AEs showed a positive relationship with the *exchangeable* and *carbonate* sedimentary fractions, while metals in *iron and manganese oxides* and *acid volatile sulfides*, or in *pyrite* and other refractory material were inversely correlated with AEs. Arsenic was most bioavailable from algae (72%), less from sediments mixed with algae (24 -70%) and least from sediments labeled directly (1 – 12%). Arsenic AEs in sediments labeled directly showed a positive correlation with sedimentary Mn and Al and negative correlation with Fe. Cadmium AEs were positively correlated with salinity and negatively correlated with sedimentary organic carbon. AEs of Cr from sediments or algae were < 5%, but 34% from pure goethite. By quantifying the relationship of metal speciation in sediments with their bioavailability for deposit-feeding polychaetes, the present study provides new insight into understanding metal bioaccumulation in benthic invertebrates.

INTRODUCTION

Elevated concentrations of trace metals are presently found associated with sediments on the floors of rivers and their estuaries, often accumulated as historical pulses several centimeters below the surface. Data on total sediment concentrations of specific metals are valuable, but by themselves may be of limited use in evaluating the risks associated with this contamination. Concern over elevated metal concentrations stems primarily from the risks these contaminants can pose for living organisms (including people who might consume contaminated seafood). Because organisms must first accumulate metals before any toxic effect can be manifested, it is necessary to assess the extent to which the metals bound to sediments are accumulated in benthic animals. Contaminated sediments may be a dominant source of metals for benthic animals (Langston and Spence 1995) if the contaminants are in a form that can be accumulated into biological tissue. The non-bioaccessible contaminants irreversibly bound to sediment (Semple et al. 2004) are not assimilated into biota and should have little ecological impact. The extent to which sediments can serve as sources of contaminants for marine organisms and the mechanisms responsible remain under-studied for most sediment types and organisms. Complicating factors include metal partitioning into various sedimentary mineral and organic phases that may affect the uptake and assimilation of the metals by benthic organisms. Metals associate with these phases through surface adsorption, co-precipitation during diagenetic formation or actual incorporation such as covalent bonding in organic matter or precipitation into insoluble metal sulfides.

The chemical speciation of a metal dissolved in water and its solid phase speciation in sediment both can influence its bioavailability (Campbell 1995b; Tessier and Campbell 1988). In this respect, the total concentration of a sedimentary metal could have little relevance to its bioavailability, just as is the case with dissolved metals. The bioavailability of different metals in contaminated sediments is likely to be a function of a metal's characteristics - for example charge, ionic radius, and oxidation state, its phase speciation in the sediment, and the physiological and ecological characteristics of the organism inhabiting the sediment (Griscom and Fisher 2004; Lee et al. 2000b; Luoma and Bryan 1982; Tessier et al. 1993). Consistent with another study that related phase speciation with bioavailability, metals bound to more refractory fractions such as pyrite or part of the sediment matrix itself would be expected to be less

available for animals than metals loosely bound to sediment particle surfaces in *exchangeable* and *carbonate* fractions (Griscom et al. 2000).

Bioavailability of sedimentary metals has been assessed in light of abiotic factors such as ratios of simultaneously extracted metals (SEM) to acid volatile sulfides (AVS) (Ankley et al. 1994; Lee et al. 2000b), metal partition coefficients (Kd) - often related to sediment grain size and organic matter content (Griscom et al. 2000), pore water metal concentrations (Ankley et al. 1994), and geochemical heterogeneity in an animal's immediate microhabitat (Lee et al. 2000a). Biological factors that influence the assimilation efficiency (AE) of ingested metal, a key parameter in kinetic (or biodynamic) bioaccumulation models (Wang et al. 1996), include ingestion rate (Ahrens et al. 2001a; Wang and Fisher 1999a), gut passage time (Griscom et al. 2000), and the gut environment (Chen and Mayer 1998; Chen et al. 2000; Griscom et al. 2002), which determines the amount of metal that can be freed from particles into the gut fluid. For example, gut surfactants help extract organic matter coating mineral particles in the sediment (Ahrens et al. 2001b) and anoxia can help reduce iron oxides, thereby releasing metals bound to them (Griscom et al. 2002).

The assimilation of metals from contaminated sediment in deposit-feeding invertebrates such as polychaetes can effectively transfer the metal from abiotic substrate to living tissue, which can subsequently be transferred to their predators. To provide a better mechanistic understanding of metal transfer into benthic food chains from sediments, the present study involved a series of experiments that used gamma-emitting radioisotopes to evaluate the bioavailability to deposit-feeding polychaetes of sedimentary As, Cd and Cr – all of which often display high concentrations in industrialized estuaries, sometimes reaching concentrations that are toxic to resident organisms (Langston and Spence 1995). These three metals have contrasting biochemical associations in living organisms, contrasting geochemical associations in sediments, and divergent particle-reactivities and residence times in aquatic systems (Fisher and Reinfelder 1995). While each of these metals is an environmental contaminant of concern in its own right, simultaneously studying their behaviors can reveal the behaviors of other metals with similar divergent characteristics. Further, As and Cr exist as oxyanions in solution but also have multiple oxidation states that vary with the oxygen content of the water and sediments. In oxic sediments, As is known to bind with Fe and Mn oxides and with pyrite in reduced sediments (Huerta-Diaz

and Morse 1990). Chromium III and VI both associate with organic matter, although Cr III is far more particle-reactive. In contrast to As and Cr, Cd is a non-redox cation in water that is strongly chloro-complexed (Bruland 1983). Each of these metals shows distinct patterns of mineral/organic association with particulate matter and distinct biological behavior.

Deposit-feeding polychaetes ingest large amount of sediments (Wang et al. 1999), whose nutritional value is typically low in comparison to algae or bacteria but which can vary seasonally with productivity in overlying water. Metals associated with sinking biogenic debris can eventually associate with various geochemical fractions in the sediments. Thus, metals can loosely associate with the surfaces in the *exchangeable* or *carbonate* phases, bind to or precipitate with Fe/Mn oxides, bind to less labile organic matter or iron sulfides including AVS and pyrite in anoxic sediments. Typically only a small fraction of sedimentary metals loosely associates with particles (e.g. exchangeable) and metals that are associated with oxygen or pH sensitive phases can be released into the solution when these conditions shift.

The assimilation efficiencies (AEs) of ingested As, Cd, and Cr from surface sediments collected from two sites in the Chesapeake and one in San Francisco Bay in deposit-feeding polychaetes were compared with their AEs from the same sediments amended with organic matter from algal debris, pure algal detritus, and from goethite - an iron oxide mineral frequently present in sediment. These AEs were regressed against the geochemical fraction patterns of these metals in the sediments. The three study sites were chosen based on differences in their sediment composition (organic carbon content, degree of metal contamination, S content, etc.).

Pulse-chase feeding experiments using radiotracers have been used to determine metal AEs and efflux rates out of animals. These parameters are components of a metal bioaccumulation model that can evaluate the relative importance of dietary and aqueous sources of metals for aquatic animals and enable predictions to be made of steady-state metal concentrations in animal tissues in different geographic locations (Wang et al. 1996). Modeling has shown that many metals are found to be accumulated significantly from diet in diverse invertebrates (Wang and Fisher 1999a). The application of gamma-emitting radioisotopes in bioaccumulation experiments provides the advantage that environmentally realistic metal concentrations can be used and analyses are rapid, accurate and precise. Previous studies have evaluated AEs of metals bound to different types of sediment in diverse marine invertebrates

(Gagnon and Fisher 1997; Griscom and Fisher 2004; Griscom et al. 2002; Griscom et al. 2000) but most earlier studies did not relate AEs with metal fractionation patterns in sediments.

MATERIALS AND METHODS

Choice, collection and handling of the test organism

The present study used the surface deposit-feeding polychaete, *Nereis succinea*, ubiquitous in muddy sediments along the US coastline. This species, whose ecology and physiology are well described, was used previously in experiments that studied contaminant bioaccumulation (Ahrens et al. 2001a). Worms of similar size (~10 cm) were hand collected from a local salt marsh at Flax Pond, from early spring to late fall. Individuals were placed with a small portion of sediment in separate containers to avoid cannibalism, because although *N. succinea* feeds on surface sediment, it is predatory given the opportunity. Animals were transported to the lab, rinsed with Flax Pond water and placed in clean containers with Flax Pond water (salinity 28) and ~0.5 g (wet weight) of Flax Pond sediment. Prior to each feeding experiment ~20 individuals that were regularly producing fecal pellets were selected and further acclimated for 2 to 7 days (depending on water salinity) to the experimental conditions. The final selection of worms (n=5-8 individuals) which were fed radiolabeled sediment was based on the presence of feces in their chambers.

Pulse-chase feeding experiments

Feeding experiments were conducted at 21 °C by placing individual worms in feeding chambers that consisted of two small (φ =5 cm) plastic Petri dishes connected by tygon tubing (length =15 cm; φ = 4 mm). Each chamber was filled with water and ~0.5 g of radiolabeled sediment was placed at the "head" end of the tube; fecal pellets were collected from the other end of the tube (Wang et al. 1999). After worms were placed in the chambers, water was periodically changed or filled to the top and feces were removed.

Study sites

Sediments and water used for feeding experiments were collected from three locations-two sites in Chesapeake Bay and one in San Francisco Bay. They were Baltimore Harbor (BH; 39°12'.25"N, 76°31'40"W) [Baltimore, MD], Elizabeth River (ER; 76°20' 09" W, 36°52' 32" N) [Norfolk, VA] and Mare Island (MI; 38°04'23" N, 122°14'91" W) [Vallejo, CA in San

Francisco Bay]. Sediments from Norfolk were collected in May, BH in June, and MI in October. After collection they were stored at 4°C in plastic containers for up to two years prior to experimentation, where deeper layers of the sediments stored in the bucket were anoxic. Sediment location choices were based on differences in their geochemical properties and extent of metal contamination. For example, BH sediments had the highest content of organic carbon and nitrogen (5 and 0.33%) compared to ER (2 and 0.12%) and MI (1.5 and 0.12%), but lowest salinity (8.5) compared to ER (19.5) and MI (23). The greatest degree of contamination with Cr and As was in BH (Cr: 322.6 and As: 47.19 μg g^{-1} dry wt; ER- Cr: 33.3 and As: 6.37 μg g^{-1} dry wt, and MI: Cr: 47.4 and As: 1.43 μg g^{-1} dry wt), and with Cd in MI (2.39 μg g^{-1} dry wt; BH: 0.96 and ER: 0.46 μg g^{-1} dry wt) (Chapter II).

Chemical analyses

Measurements of organic carbon, organic nitrogen, and sulfur (CNS) were conducted using a Carlo Erba 1500 Elemental Analyzer on sediment samples that were dried (50 °C), ground, and sieved through a 150 μm nylon mesh screen (Cutter and Radford-Knoery 1993). Such prepared sediments were also used for metal concentration analysis. Sediment subsamples were digested in two steps with trace metal-grade concentrated nitric acid and then concentrated perchloric acid in a boiling bath. All of the sediment manipulations were conducted in a clean lab and under a high efficiency particulate air laminar flow bench. Sediment digest solutions were analyzed using ICP-MS (Finnigan Element 2).

Food types and radiolabeling

Feeding experiments considered 4 different foods, each uniformly radiolabeled with gamma-emitting isotopes, either ^{73}As (V) (half-life = 80.3d) or a combination of ^{109}Cd (half-life = 461.4d) and ^{51}Cr (III) (half-life =27.7d): (1) unamended sediment from the upper 10 cm at each site, mixed thoroughly immediately prior to radioisotope additions, (2) sediment to which radiolabeled diatom debris was added and mixed in, (3) pure diatom debris, and (4) pure goethite purchased from Sigma-Aldrich (goethite ~35% Fe; EC No. 2437464). Radioisotopes were added as arsenate for ^{73}As, cadmium chloride dissolved in 0.1 M HCl and chromic chloride dissolved in 0.5 M HCl.

Unamended sediments were radiolabeled by a direct addition via pipette of radioisotope dissolved in dilute HCl; microliter quantities of dilute NaOH were immediately added to the unamended sediment so that the pH was not affected by isotope addition. Amended sediments received radioisotopes by mixing 22.3 ± 4.5 mg (dry wt) of previously radiolabeled diatoms (*Thalassiosira pseudonana*, clone 3H) with ~2 g (wet wt) of sediment. The ratio of dry to wet weight for all the sediments ranged between 0.7 and 0.5 as determined by Baumann et al. (in preparation). Both types of sediments were aged at 21 °C prior to the feeding experiment for 2 or 30 days to assess the influence of sediment aging on metal partitioning and bioavailability over this time period. Given the radioactive half-lives of the isotopes, it was not practical to conduct experiments involving bioavailability of all three isotopes for longer time periods.

Radiolabeled *T. pseudonana* was produced as described previously (Wang et al. 1996). Because the assimilation efficiencies (AEs) of ingested metals in marine invertebrate herbivores from phytoplankton diets reflects the cytoplasmic distribution of metals in the algal cells (Fisher and Reinfelder 1995; Reinfelder and Fisher 1991), the cellular distribution of each metal in aliquots of the diatom cells was determined by differential centrifugation of algal components after cells were broken (Reinfelder and Fisher 1991). Most radioactive diatom cells were harvested for mixing with the sediments to feeding to the worms. Radioactive diatoms were first harvested by filtration on 1 μm polycarbonate membranes, then resuspended in 10 ml of seawater and centrifuged at 840 *g* for 5 minutes; the resulting radioactive pellet containing the cells was mixed thoroughly with the sediments or fed without sediment to the worms. Goethite (2.5 g dry wt) powder was radiolabeled after suspension in 2 ml of seawater. Radioactive goethite upon thorough mixing was briefly centrifuged to remove excess seawater and then goethite pellets were fed to *N. succinea*. For sediments which received direct addition of dissolved radioisotopes a small amount of dilute sodium hydroxide was added to neutralize the acid associated with radioisotope additions (dissolved in dilute HCl). The radioactivity and the added metal concentration in each food, determined using the specific activity of each added radioisotope, are given in Table 1. The radioisotope additions contributed only a small fraction of the measured background concentrations of these metals in surface sediment (<<1%) (Chapter II). Carbon additions in the form of added algal debris were 8% of background organic matter in the BH sediment, 19% in ER sediment, and 27% in MI sediment.

Measurement of radioactivity

Radioactivity of worms was measured following 1-6 h of feeding, depending on the presence of feces in the chamber. Worms were removed from their feeding chambers and rinsed 3 times with filtered seawater and twice with an Ethylenediaminetetraacetic acid (EDTA) solution in seawater [10^{-4} M] to remove adsorbed metal and adhering particles. To assay their radioactivity, individual worms were placed into 50 ml plastic containers, filled with a small amount of water to assure their position on the bottom of the container, and inserted into a well-type NaI(Tl) gamma detector. Counting times were typically 5 min, yielding propagated counting errors that were typically < 5%. Radioactivity of ^{73}As was detected at 53 keV, of ^{109}Cd at 88 keV, and of ^{51}Cr 256 keV. This kind of radioactive counting is non-destructive, so individual worms could be counted repeatedly over different time periods. For all radiolabeled worms, after counting, individuals were returned to their feeding chambers which were filled with new water and nonradioactive sediment that they could ingest to purge their guts of unassimilated radioactive material. After determining the radioactivity of worms following their radioactive feeding, the retention of the radioisotopes post feeding on nonradioactive food over time in the individual worms was determined to quantify the AEs of the ingested metals for each treatment, as described in Wang and Fisher (1999a).

Sequential extraction procedure

Sedimentary metal phase speciation was evaluated using a modified scheme originally described by Tessier et al. (1979) and Huerta-Diaz and Morse (1990). We determined seven sedimentary fractions, nominally identified as: *exchangeable*, *carbonate*, *acid volatile sulfides (AVS)*, *Fe/Mn oxides*, *two organic pools*, and *pyrite*. It is important to note that these fractions were determined chemically and names assigned to each of them are strictly operational; details are given elsewhere (chapter II). Briefly, metals in the *exchangeable* pool were extracted for 1 h by 1M $MgCl_2$, in *carbonate* for 1 h by sodium acetate solution at pH =5, for 0.5 h in *AVS* by 0.5 M HCl. Sediments were incubated for 6 h in a hot reducing solution of hydroxylamine to dissolve the *Fe/Mn oxides* and metals associated with them. Metals in the *organic* fractions were extracted first for 8 h by a hot 1N NaOH solution and later concentrated H_2SO_4 for 6 h. Metals remaining in sediments, thought to associate with *pyrite*, were extracted for 2 h with 11 M HNO_3. After each extraction step the extractant was separated from the sediment by

centrifugation at 834 *g* for 10 min and transferred to a separate container. This extract was then analyzed for the amount of radioactivity due to each of the elements. After the final extraction there was a small "residual" fraction of radioisotopes remaining in sediment.

The geochemical fractionation of As, Cd, and Cr in operationally defined sedimentary fractions (chapter I) was related to AE values for all metals, sediment locations, label types and ages.

Statistical analysis

To determine relationships between assimilation efficiencies and radiotracer percent concentrations in single or combined fractions that are operationally-defined, multiple regression was performed using PASW 18.0 software. Data (e.g., percentages of metal extracted in a particular fraction and assimilation efficiencies) for different types of sediment labels (direct vs. algal), locations (BH, ER, and MI) and radiotracers (^{73}As, ^{109}Cd and ^{51}Cr) were combined and these categories were specified as independent variables in the multiple regression analysis. There were 13 fractions (single and combined) used in this analyses, therefore a Bonferroni correction was applied changing the confidence interval from 95% to 99.6%. All data expressed in percent were normalized by their arcsine transformation.

RESULTS

Assimilation efficiencies

Individual worms feeding on radiolabeled sediments ingested at least 1 mg wet wt of sediment (exact ingestion rates though likely varied for different sediment types and were not estimated), corresponding to 1 - 32 Bq of ^{73}As (or 0.02 - 0.53 fmol of As), 4-169 Bq of ^{109}Cd (or 0.38 – 16.17 fmol of Cd) and 2 - 87 Bq (or 0.01 – 0.50 fmol of Cr). In nearly all cases, there was sufficient radioactivity in each worm to measure the depuration rates of radioisotopes from individual worms over time. Assimilation efficiencies of ingested metals, determined by analyzing the loss patterns of metal during depuration, were determined for all experimental treatments; Figure 1 shows representative results of pulse-chase feeding experiments in which the retention of initially ingested radioactivity in *N. succinea* over time after feeding on radioactive food is given for ^{73}As, ^{109}Cd, and ^{51}Cr. Typically a two-phase loss pattern is observed, a sharp decline within 24 h reflecting loss of unassimilated metal via defecation,

followed by a much slower loss, representing physiological turnover of assimilated metal (Wang and Fisher 1999a). It is evident that *N. succinea* generally retained more As and Cd than Cr, resulting in correspondingly higher AEs for As and Cd (Table 2). Assimilation efficiencies of Cr in most cases were very low (<5%) except when worms were fed pure radiolabeled goethite, yielding AEs of about 34% (Table 2). There were significant differences in AEs for metals that were labeled via direct addition of radioisotopes to the sediment or via addition of radioactive algal debris to the sediment. The AEs of As were much higher for all sites when worms fed on sediments with added algal debris than on unamended sediment (Table 2). These differences were between sediments from BH and MI that were labeled with ^{109}Cd aged for 2 days (one-way ANOVA: $p < 0.01$), and between 2 and 30-day old MI sediments labeled with Cr (one-way ANOVA: $p < 0.05$). Significant differences in metal AEs were noted for As between all sample sites and for Cd and Cr between some sites (one-way ANOVA: $p < 0.01$; Cd: BH vs. ER and BH vs. MI, Cr: BH vs. ER and ER vs. MI). Generally, AEs of As and Cr decreased (one-way ANOVA: $p < 0.01$) from sediment with aging, regardless of whether or not the sediments were amended with diatom debris.

Sub-cellular distribution of metals in *Thalassiosira pseudonana*

The cellular distribution of As, Cd and Cr in the radiolabeled diatoms indicated that 83% of Cr, 55% of As, and 43% of Cd were bound to cell surfaces (Table 3). AEs of As in *N. succinea* that fed on pure algal debris were much higher than when fed goethite (72 ± 3 *vs.* 2.5% ± 0.7; Table 2). The opposite trend was apparent for Cr (2.8% ± 1.6 *vs.* 34 ± 6; Table 2). For Cd, the method of labeling the sediment had no significant effect on AEs (23% ± 20 *vs.* 24 ± 2).

Distribution of ^{73}As, ^{109}Cd and ^{51}Cr in sedimentary fractions

The geochemical fractionation patterns of the metals in the sediments that were fed to the worms are shown in Table 4. Arsenic was primarily in *organic* fractions, with smaller amounts in other fractions among which *oxides* dominated. Cadmium was primarily in the *exchangeable* fraction, but *carbonate, oxide* and, to a lesser extent; *AVS* fractions also contained this metal. In the sediments radiolabeled directly with Cr, this metal was predominantly in the *AVS* and *oxide* phases after 2 d incubation, but, unlike the other metals, the distribution of Cr changed significantly over time so that the *oxide* and *AVS* fractions were largely replaced by *pyrite* after 30 d.

Relationship of AEs with metal fractionation

The relationships of all metal AEs with their geochemical fractionation patterns in all sediments are given in Table 5, which presents regression slopes between AEs of all metals and the per cent of metal in individual or combined geochemical fractions of all sediments. These relationships are also presented for all metals as a function of sediment with or without added algal debris, by each individual metal, or as a function of sediment age (Table 5). Table 5 shows results for two models (1 and 4) of multiple regression. Model 1 does not include the independent variables of label, location or radiotracer, while model 4 does, and therefore model 4 is considered to have more predictive power. Results produced by model 4 represent the more complex analysis whose r^2 values are improved in comparison to model 1.

In model 4, the significant ($p < 0.05$) positive relationships indicated by positive slopes (Beta in Table 5) were between metal AEs and radiotracers in operationally determined *exchangeable* and *carbonate* fractions alone or in combination (listed as *"carbonex"*) as well as *"carbonex"* plus *AVS*; the significant negative ($p < 0.05$, and negative Beta in Table 5) relationships were between AEs and radiotracers in operationally-defined *Fe/Mn oxides*, *organic I* and *II*, *pyrite* and *residual* fractions alone or combined pools of *AVS* and *Fe/Mn oxides* fractions, *organic I* and *II* fractions, and finally *pyrite* and *residual* fractions.

The significant (one-way ANOVA: $p < 0.05$) positive relationship of metal AEs with their fractionation in the *exchangeable + carbonate* sediment fractions (*exchangeable* and *carbonates*) for all metals and sample sites with unamended and amended sediments (both 2 and 30 d) is shown in Fig. 2. The significant (one-way ANOVA: $p < 0.05$) inverse relationship of metal AEs with their fractionation in oxides and AVS fractions in sediment labeled via algal detritus and aged for a month is shown in Fig. 3. Figure 4 summarizes the general pattern of slopes from regressions (model 1 and 4) relating metal AEs and sediment fractionation for all metals, sampling sites, sediment labeling methods, and sediment age.

DISCUSSION

The positive relationship of metal assimilation efficiencies with *exchangeable + carbonate* sediment fractions for As(V), Cd, and Cr(III) is consistent with reports that suggested that metals bound to these two sediment fractions can be more bioavailable for benthic invertebrates than other fractions (Tessier et al. 1984). Ligands in the gut fluid of deposit-feeders

provide a site for metal binding (Chen et al. 2000) and cations and anions in the gut fluid likely stimulate ionic exchange with metals loosely sorbed to ingested sedimentary particles. Therefore, the *carbonate* fraction extracted by sodium acetate - known to stimulate ionic exchange (i.e., anions could exchange with the acetate functional group) and the *exchangeable* fraction extracted by $MgCl_2$ together could represent the metal pool that is more bioavailable for assimilation in the gut. Generally, oxic sediments have more metal associated with these phases and therefore metals in oxic sediments would be expected to be more bioavailable than metals in anoxic sediment; exceptions have been noted however, such as for Cd, Cr, and Zn in the suspension-feeding mussel *Mytilus edulis* (Griscom et al. 2000). Metals bound to operationally identified phases of *iron and manganese oxides*, *organic phases*, and *pyrite* in sediments showed an inverse relation with assimilation efficiencies in *N. succinea* (Fig. 4), indicating that metals associated with these phases have low bioavailability for *N. succinea*. This polychaete is a surface deposit-feeder and hence feeds primarily on oxic sediment.

In comparing the behavior of metals in sediments from different sample sites, assimilation of As showed a negative relationship with total concentrations of Fe (r^2 =0.97) and Mn (r^2 = 0.99) in sediment but a positive relationship with Al (r^2 =0.99) in sediments from all three study sites (Fig. 5). It is widely accepted that in sediments arsenic shows a strong association with iron and manganese rich phases such as *Fe/Mn oxides*, *AVS* and *pyrite* (Oremland and Stolz 2003). Lower As AEs occurred for sediments with higher Fe and Mn concentrations (Fig. 5), further supporting the observations that *Fe/Mn* and *AVS* (Fig. 4) phases control the bioavailability of ingested As. In sediments, Al can be found in the mineral structure of aluminosilicates. Al's positive correlation to As AEs may be explained by a weak metal binding with the surfaces of aluminosilicates, which can more easily release metal ions into the gut fluid than Fe and Mn oxides (Lin and Puls 2003). The particle reactivity of Cd is inversely related to chloride concentration (Muller 1996) and as salinity increases Cd's retention by ingested particles would be expected to decrease, releasing more Cd into the gut where it could be eventually assimilated, consistent with observations shown in Fig. 5. The organic carbon content in sediments from our study sites showed an inverse relation to Cd AEs (Fig. 5). Degraded organic matter can bind metals and thereby limit their bioavailability. The negative relationship of Cd AEs with its association with extracted organic fractions was also significant

(Table 5), further supporting this relationship. AE values for Cr were very low and variable, and relationships with sediment characteristics are more tentative.

Generally, these findings suggest that metals must be released from ingested particles into the gut fluid before they can be transported across the gut lining and become assimilated into tissues. As such, these findings support the contention of Mayer and colleagues that metal and organic contaminants must be released into gut fluid before they can be assimilated. Most of these earlier studies focused on release from particulates to the dissolved phase in gut fluid and did not assess their AEs, although some studies determined both the solubilization and absorption of some organic contaminants (Ahrens et al. 2001a; Weston and Mayer 1998).

Assimilation efficiencies of As in *N. succinea* were highest when diet consisted of fresh algal debris or sediments amended with algal debris, suggesting that As, which is bound to some labile sugars in algal cells (Andreae and Klumpp 1979), is the bioavailable form and arsenic bound to sedimentary organic matter such as humic or fulvic acids is not assimilable in worms. Further, arsenobetaine, a common form of As in marine invertebrates (Larsen et al. 1993) is far more assimilable (42%) by crustacean predators, *Crangon crangon*, than inorganic As species – arsenate (1.2%; Hunter et al. 1998), consistent with our observations that inorganic As bound to goethite was much less assimilable (2.5%) by *N. succinea* than organic As in algal debris (72 %). Because sugars that contain As in cells are likely labile and degrade over time, the decreased assimilation of As over 30 days in sediment mixed with algal debris was expected as these sugars decompose over time. There are few other studies that determined AEs of As in marine invertebrates. AEs of As in another nereid worm *Nereis diversicolor* reached 62% (Waring and Maher 2005), comparable to our results but much higher than AEs reported for the deposit-feeding polychaete *Arenicola marina* (4-11%; Casado-Martinez et al. 2009).

Studies by Griscom et al. (2000) and in the second chapter of this dissertation illustrate the shift of metals from *exchangeable + carbonate* phases to more refractory phases over time, which might possibly result from microbial degradation of labile organic matter or an increase in *Fe/Mn oxide*-metal associationscompound. Organic C associated with fresh algae is considered labile because it is highly bioavailable for assimilation (e.g. AE = 55-95% in *N. succinea*) in comparison to degraded organic matter in sediments (e.g. AE = 5-18%; Ahrens et al. 2001b). This difference between labile and refractory organic matter coincides with decreased AEs of As

in *N. succinea* (the present study), Cd and Ag in the clam *Macoma balthica* (Griscom et al. 2000), Cd, Cr and Zn in the clam *Ruditapes philippinarum,* and Cd in the mussel *Perna viridis* (Chong and Wang 2000), although Wang et al. (1999) found that AEs of physiologically regulated Co, Se and Zn were unaffected by metal-sediment exposure time. Other studies that examined bioavailability and toxicity of chemicals for worms in soils similarly showed a decrease in bioavailability with increased exposure time (Lanno et al. 2004).

With further sediment aging, ferric iron associated with iron oxides would be expected to be ultimately reduced and dissolve as ferrous iron. Ferrous iron upon reaction with dissolved sulfide species would precipitate as iron sulfides, contributing to the AVS phase. In anoxic sediments AVS can be transformed to pyrite. AVS and pyrite, when present in sediments, can bind metals and organic matter that are dissolved in pore water (Lee et al. 2000a). Metals bound to these phases are more tightly bound and can only be released by strong acids (e.g., HCl and HNO_3), which are harsher than the digestive fluids of marine invertebrates.

Unlike As, the AEs of Cd and Cr did not consistently decline with sediment aging (Table 2) suggesting that bioavailability of these metals may not be tied directly to their association with labile organic C in sediments. This is further supported by the observation that AEs of Cd bound to pure goethite were comparable to those bound to pure algal debris. Presumably, Cd is released equally well into gut fluid from goethite and algal debris. Cd showed a wide range of AEs (from <1% to nearly 69%), whereas others reported a narrower AE range for *N. succinea,* from 29 to 39% (Wang et al. 1999). Bivalves appeared to assimilate similar amounts of Cd in comparison to worms. Filter-feeding mussels assimilated from 9.5 to 44.5% (Chong and Wang 2000; Wang and Fisher 1999a), and clams assimilated 31-51% of Cd (Chong and Wang 2000; Griscom et al. 2002). The estuarine crustacean *Palaemonetes pugio* (Wallace and Lopez 1997) assimilated 57% of Cd from an oligochaete diet. The AE of Cd in mussels from pure mineral phases was lower than from algal cells (Wang et al. 1996).

As seen in many previous studies with diverse invertebrates, Cr displayed lower AEs than the other metals (Wang and Fisher 1999a). Commonly, its AE from ingested food is <10%, particularly for deposit-feeding animals (Lee and Luoma 1998). In San Francisco Bay, the clam *Potamocorbula amurensis* feeding on particles rich in labile organic compounds collected after a spring bloom assimilated <5.5% of Cr, compared with AEs of <1.8% during a non-bloom period

(Lee and Luoma 1998). It is noteworthy that mussels feeding on algae assimilate up to 10% of Cr(VI) but <2% of Cr(III), the difference being explainable by Cr(VI)'s greater ability to penetrate into the cytoplasm of the algal cells (Wang et al. 1997).

Analogous to their fractionation in sediments, metal fractionation in algal cells can significantly influence their assimilation in animals. Previous work has shown that AEs of ingested metals in marine herbivores are strongly correlated with the cytological distribution of the metals in algal cells, with AEs showing a nearly 1:1 relationship with cytoplasmic distribution of the metals in the algal cells that comprised the diet (Reinfelder and Fisher 1991). The low AE of ingested Cr from pure algal debris (2.8%) coincides with its predominant association with diatom cell walls and membranes, in contrast with Cd and As, consistent with earlier findings. The AEs of algal As (69-76%) and Cd (up to 43%) are comparable ($p < 0.05$) to their extraction in the exchangeable fraction (Cd: 43%) alone or in the exchangeable + carbonate pool (As: 65%) within the algae. Nevertheless, although 18% of algal Cr was extracted in the exchangeable pool (Z. Baumann et al. in preparation), its assimilation in *N. succinea* was low (2.8%). Reasons for this discrepancy are not known, but it is possible that trivalent metals can not readily penetrate the gut lining. Cr also displayed low AEs (< 5%) from sediments (Table 2) and its association with exchangeable fractions in these sediments was correspondingly low (Z. Baumann et al. in preparation).

Thus, the bioavailability of As, Cd and Cr to the surface deposit-feeder *N. succinea* is positively related to their exchangeable + carbonate fraction in sediment and negatively related to their fractions in the AVS, Fe/Mn oxides and pyrite and non-extractable phases. We therefore suggest combining the exchangeable and carbonate pools into a "carbonex" pool recognizing that geochemical and physiological processes can positively impact sedimentary metal assimilation in deposit-feeding polychaetes through an ion-exchange process (ion exchange in both $MgCl_2$ and NaOAc extractions; presence of anions and cations in the gut fluid that can facilitate ionic exchange).

The present study did not accurately evaluate ingestion rates in *N. succinea* in these experiments. Ingestion rates can vary in deposit-feeding polychaetes depending on the particle being ingested (e.g., natural sediment vs. goethite). It is recognized that gut retention times can influence the AEs of ingested metals (Wang and Fisher 1999a), and while this may possibly

explain differences noted in AEs between metals bound to goethite and algal-supplemented sediment, both of these radiolabeled food sources were purged with the identical unlabeled sediment. It is therefore unlikely that the gut passage times of the various radioactive foods differed significantly and accounted for AE differences.

Worms assimilate more As when fed pure algae and less when algae are mixed with sediment. AEs of As from directly labeled sediment were lower than As AEs from ingested sediments mixed with algae, and were positively related to total Mn and Al concentrations in sediments but negatively related to sedimentary Fe. AEs of ingested sedimentary Cd increased with salinity and decreased with sedimentary organic carbon. The present study confirms that Cr has generally low bioavailability for deposit-feeding polychaetes. Further appreciation of metal assimilation in deposit-feeders will result from physiological and biochemical studies that also consider the sediment geochemistry.

TABLES

Table 1. Radioactivity added to natural sediments, pure algae and goethite used for the pulse-chase feeding experiment; nd: not determined

location	label	age (days)	kBq g^{-1} (wet wt)			pmoles g^{-1} (wet wt)		
			^{73}As	^{109}Cd	^{51}Cr	^{73}As	^{109}Cd	^{51}Cr
Elizabeth River	direct	2	9	102	15	0.15	9.76	0.09
		30	nd	9	28	nd	0.86	0.16
	algae	2	3	11	6	0.05	1.05	0.03
		30	10	55	31	0.17	5.26	0.18
Baltimore Harbor	direct	2	7	44	17	0.12	4.21	0.10
		30	1	13	2	0.02	1.24	0.01
	algae	2	4	8	6	0.07	0.77	0.03
		30	5	4	2	0.08	0.38	0.01
Mare Island	direct	2	11	169	4	0.18	16.17	0.02
		30	9	10	3	0.15	0.96	0.02
	algae	2	12	72	8	0.20	6.89	0.05
		30	3	62	19	0.05	5.93	0.11
pure algae			3	12	87	0.05	1.15	0.50
goethite			32	13	16	0.53	1.24	0.09

Table 2. Assimilation efficiencies (AE%) of As, Cd and Cr in *N. succinea*, when diet containing those elements consisted of: natural sediments that were radiolabeled by a direct addition of radioisotopes or by mixing the natural sediment with previously radiolabeled algal detritus, pure radiolabeled algal detritus or goethite labeled by a direct addition of radioisotope.

		unamended sediment		sediment mixed with algae	
		2 d	30 d	2 d	30 d
Elizabeth River (NV)		1.2 ± 1.1	nd	69.7 ± 9.7	16.8 ± 4.1
Baltimore Harbor (BH)		7.8 ± 6.2	6.59 ± nd	51.6 ± 11.6	30.1 ± 1.4
Mare Island (MI)	^{73}As	10.2 ± 6.8	12.1 ± 12.5	50.7 ± 9.0	24.0 ± 12.2
fresh algae				72.4 ± 3.3	
goethite		2.48 ± 0.68			
Elizabeth River (NV)		30.8 ± nd	43.6 ± 16.4	9.9 ± 3.5	21.5 ± 6.1
Baltimore Harbor (BH)		1.5 ± 1.29	2.4 ± nd	68.7 ± nd	21.6 ± 14.9
Mare Island (MI)	^{109}Cd	46.1 ± 18.7	58.9 ± 6.6	9.4 ± 4.0	7.6 ± 4.6
fresh algae				22.9 ± 19.7	
goethite		24.2 ± 1.8			
Elizabeth River (NV)		4.5 ± nd	1.0 ± 0.5	0.9 ± 0.2	3.6 ± 1.7
Baltimore Harbor (BH)		4.2 ± 3.3	4.6 ± nd	1.2 ± nd	0.8 ± 1.0
Mare Island (MI)	^{51}Cr	4.0 ± 3.1	0.7 ± 1.3	5.0 ± 2.7	0.3 ± 0.6
fresh algae				2.8 ± 1.6	
goethite		34.2 ± 6.5			

Table 3. Cellular distribution of As, Cd and Cr in *Thalassiosira pseudonana*. 1st pellet - nuclei, plasma membranes, cell walls; 2nd pellet - mitochondria, lysosomes, peroxisomes; 3rd pellet - ER, Golgi bodies, ribosomes, polysomes; supernatant - soluble enzymes, lipid, small molecules.

sub-cellular fraction	As	Cd	Cr
1st pellet	54.6 ± 1.0	43.3 ± 0.5	82.9 ± 5.4
2nd pellet	12.1 ± 2.7	8.7 ± 3.8	10.9 ± 4.1
3rd pellet	30.2 ± 1.3	44.7 ± 3.2	5.1 ± 2.0
supernatant	3.0 ± 1.8	3.2 ± 3.3	1.1 ± 1.4

Table 4. Distribution (%) of As, Cd and Cr in geochemical fractions of sediments collected from Chesapeake Bay (Baltimore Harbor and Elizabeth River) and Mare Island in San Francisco Bay. EX = *exchangeable* fraction, CARB = *carbonate* fraction, AVS = *acid volatile sulfide* fraction, OX = *Fe/Mn oxide* fraction, ORG = *organic* fractions combining humic and fulvic acids, and PYR = *pyrite* fraction). Radioisotopes were added either directly from aqueous solution or via previously radiolabeled algal biomass. Sediments were aged for 2 or 30 days when labeled directly or for 30 days when sediments were radiolabeled via algae. Data indicate means of three replicate sediment batches. Details of sediment extraction are given in Chapter II.

		Baltimore Harbor			Elizabeth River			Mare Island		
	fraction	2 d direct	30 d direct	30 d via algae	2 d direct	30 d direct	30 d via algae	2 d direct	30 d direct	30 d via algae
^{73}As	EX	0	2	4	0	2	21	1	3	3
	CARB	1	1	5	2	1	9	3	3	9
	AVS	9	13	6	29	10	11	25	37	18
	OX	4	4	20	13	14	21	11	6	19
	ORG	55	46	50	36	41	20	40	34	38
	PYR	14	7	0	7	7	0	8	4	0
^{109}Cd	EX	45	32	39	59	90	62	24	37	21
	CARB	14	17	9	8	4	10	24	21	17
	AVS	12	21	7	11	1	5	33	21	5
	OX	29	31	33	22	5	13	17	21	34
	ORG	1	0	7	1	0	6	1	0	13
	PYR	1	0	3	0	0	3	0	0	8
^{51}Cr	EX	1	1	1	1	1	2	1	1	1
	CARB	3	2	1	4	2	3	3	2	2
	AVS	54	14	10	40	7	11	52	8	14
	OX	37	13	66	35	10	55	29	15	41
	ORG	6	10	13	19	22	18	14	30	23
	PYR	0	57	8	0	58	10	0	43	16

Table 5. Results of the Multiple regression on arcsine-transformed assimilation efficiencies (AEs) and % of radioisotope in single or combined fractions (listed in the left column of the table). The confidence interval, which was initially 95% was corrected according to the Bonferroni method due to multiple comparisons and resulted in the updated confidence interval of 99.6%. Model 1 and 4 indicate inclusion of independent variables such that model 1 compares AEs against % of radioisotopes in fractions and model 4 includes the independent variables - label type of sediment, radioisotope and location, leading to updated regression Beta – the slopes of regression, which are significant at $p < 0.05$.

Fraction(s)	Model	R^2	Unstandardized Coefficients		Standardized Coefficients	p-value
			B	Std. Error	Beta	
Exchangeable	1	0.083	.261	.060	.296	.000
	4	0.253	.242	.054	.274	.000
Carbonate	1	0.027	.227	.088	.179	.011
	4	0.232	.362	.097	.285	.000
AVS	1	0.006	-.149	.102	-.102	.147
	4	0.18	.059	.109	.041	.588
Fe/Mn oxides	1	0.127	-.474	.091	-.364	.000
	4	0.157	-.275	.160	-.211	.087
Organic I	1	-0.003	.082	.113	.055	.468
	4	0.229	-.616	.140	-.410	.000
Organic II	1	0.1	-1.258	.277	-.324	.000
	4	0.261	-1.350	.257	-.348	.000
Pyrite	1	0.046	-.388	.126	-.227	.002
	4	0.175	-.314	.120	-.183	.010
Residual	1	0.006	.300	.207	.109	.149
	4	0.212	-1.117	.285	-.405	.000
Carbonex	1	0.12	.228	.043	.353	.000
	4	0.311	.257	.042	.397	.000
Carbonex + AVS	1	0.078	.187	.044	.287	.000
	4	0.3	.228	.039	.350	.000
AVS + Fe/Mn oxides	1	0.1	-.248	.055	-.324	.000
	4	0.162	-.138	.068	-.181	.044
Organic I + II	1	-0.03	-.064	.090	-.053	.482
	4	0.254	-.482	.095	-.401	.000
Pyrite + Residual	1	0.011	-.179	.103	-.130	.084
	4	0.199	-.339	.097	-.246	.001

FIGURES

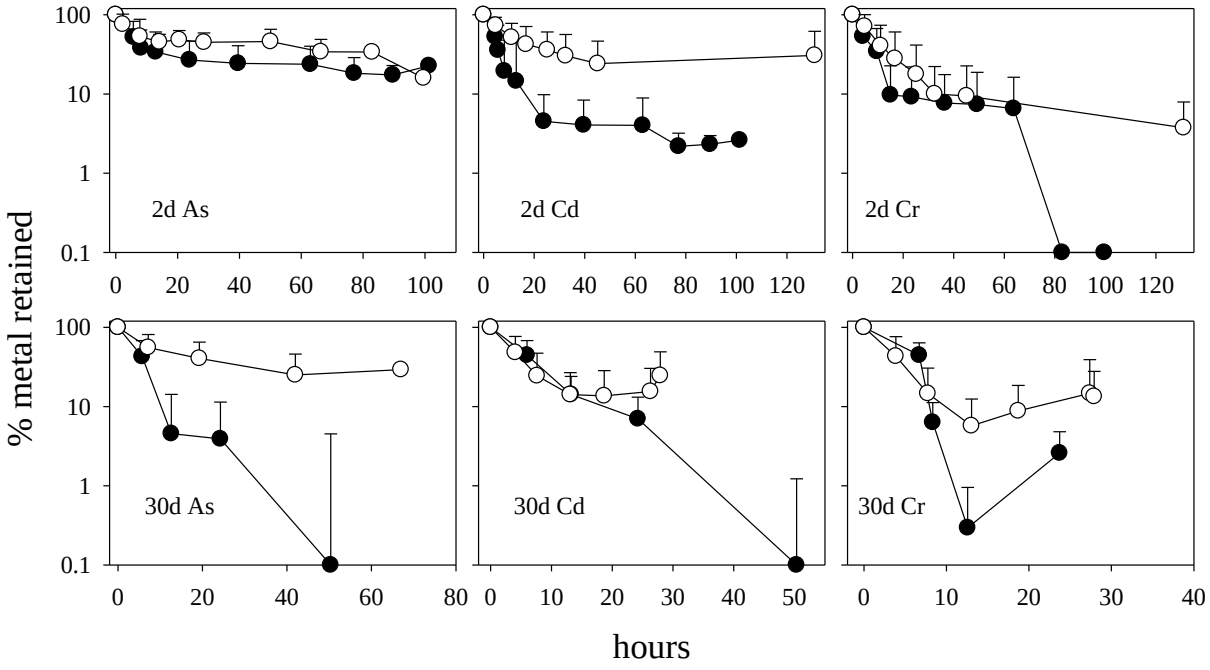


Fig. 1. Metals retained in *N. succinea* after feeding on a pulse of 2 and 30 day old radiolabeled sediment from Baltimore Harbor; data points indicate mean values of % metal retained at time; open circles indicate data for sediment mixed with radiolabeled algal detritus and solid circles indicate data for sediments labeled via direct injection of isotope. The asymmetric error bars indicate one standard deviation.

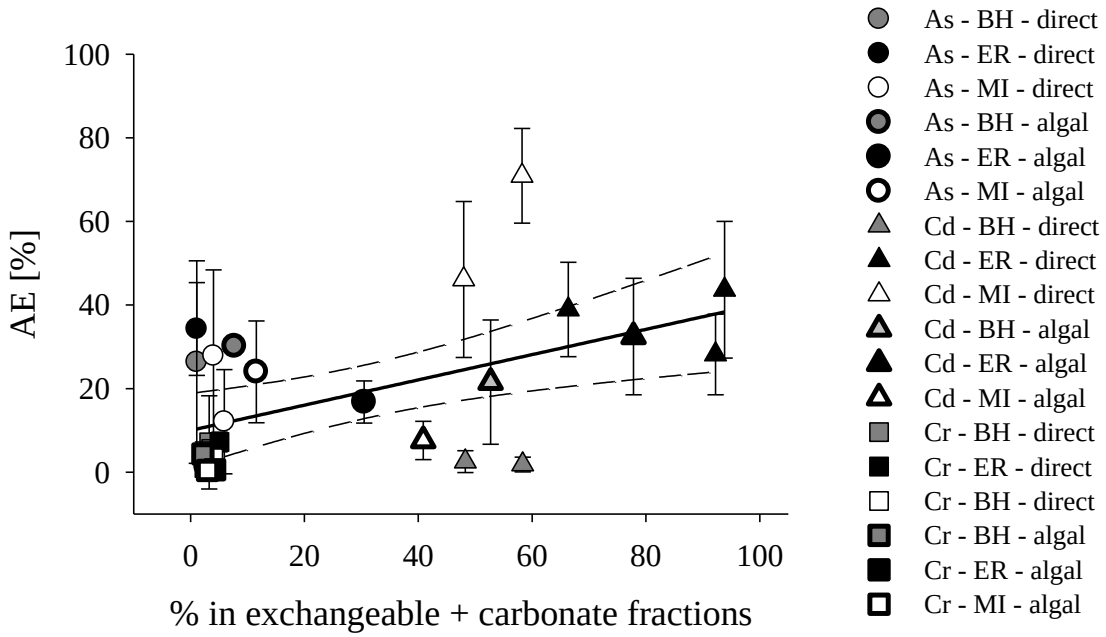


Fig. 2. Significant regression ($p < 0.05$) between AE and concentration of metal (As - circle, Cd - triangle, and Cr - square) in *exchangeable + carbonate* fractions in sediments collected from Baltimore Harbor (BH), Elizabeth River (ER) and Mare Island (MI), which were labeled directly and via algal debris and incubated for 30 days. Dashed line indicates 95% confidence interval around the regression line (solid).

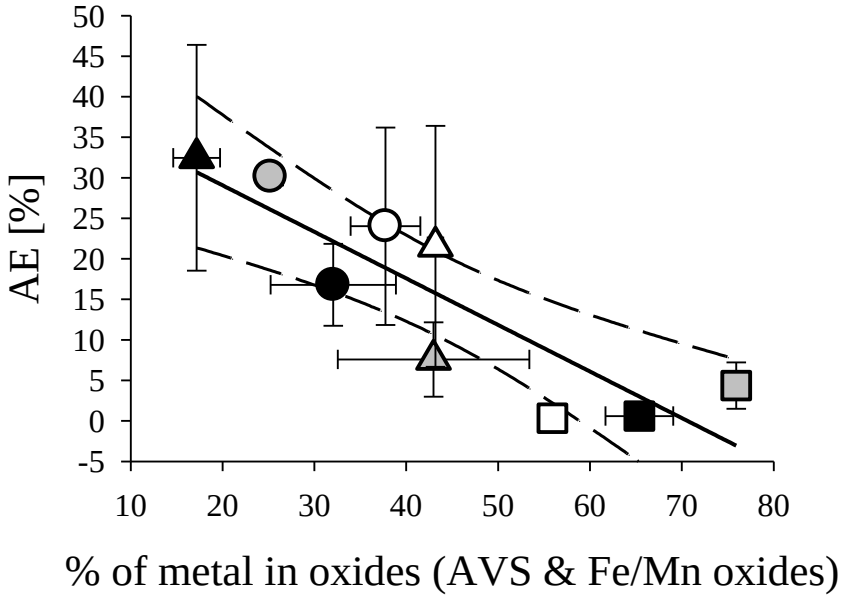


Fig. 3. Significant regression ($p < 0.05$) between AE and concentration of metal (As, Cd, and Cr) in AVS + Fe/Mn oxides fractions in sediments collected from BH (gray), NV (black) and MI (white) and labeled via mixing with radiolabeled algal detritus. Dashed line indicates 95% confidence interval around the regression line (solid).

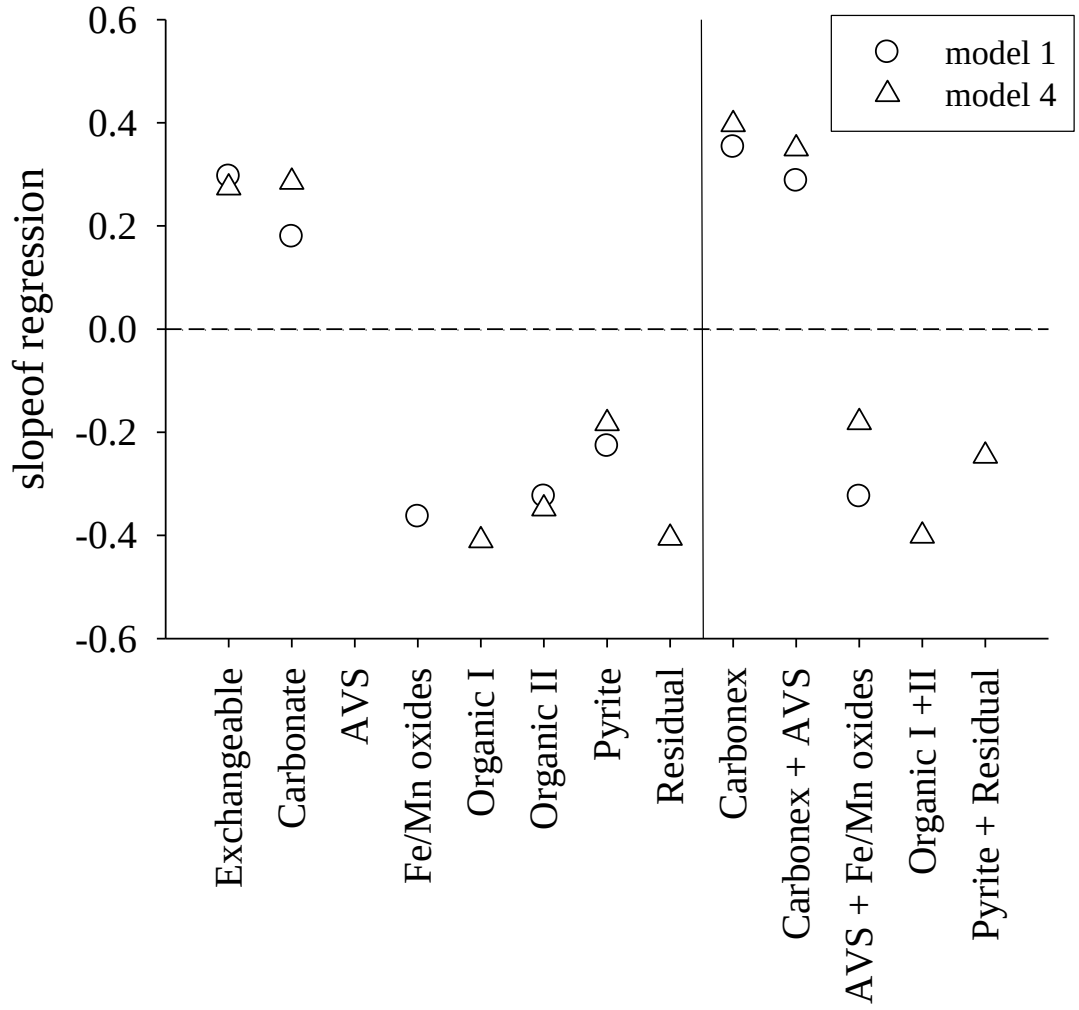


Fig. 4. Slopes for model 1 and 4 of significant ($p < 0.05$) regressions between assimilation efficiencies and % of radioisotope in single and combined fractions. Vertical solid line separates the single (left) and pooled fractions (right).

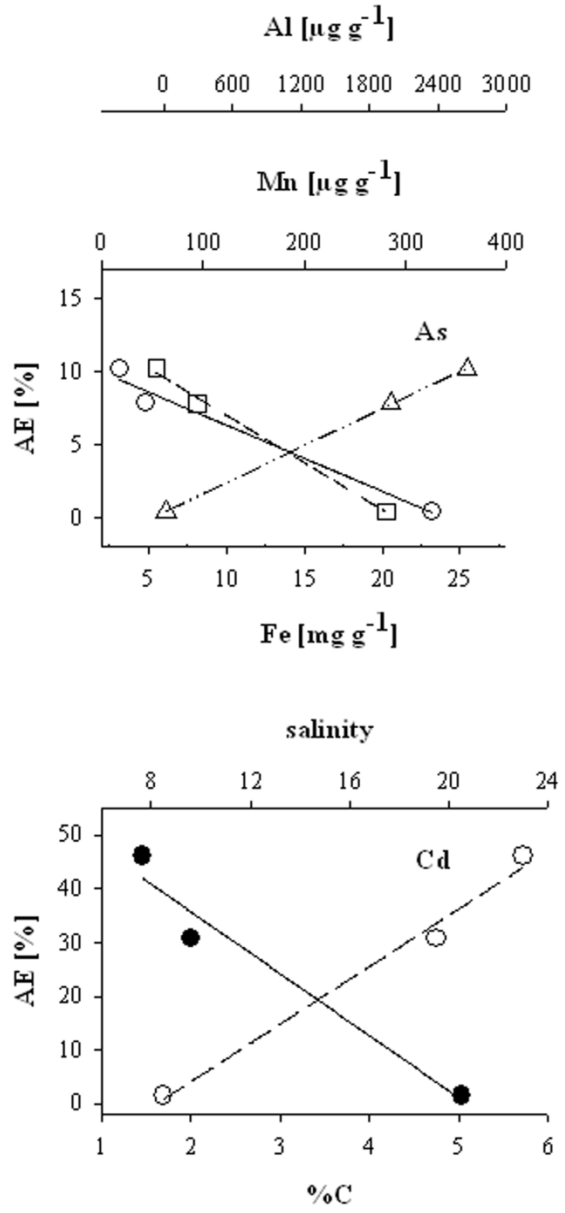


Fig. 5. Significant ($p < 0.05$) relationships between As AEs and the total concentrations of aluminum (triangles), manganese (squares), iron (circles) in sediments, and Cd AEs and % C in sediments (black circles) and salinity of overlying water at sediment collection sites (open circles). AEs of As and Cd are for sediments labeled directly and aged for 2 days; AE errors are ignored for clarity of figure and are given in Table 2. *In situ* metal (Al, Mn and Fe) concentrations are from Chapter II. No other significant relationships were found for metal AEs and total elemental concentration in sediment.

Chapter IV: *Modeling metal bioaccumulation in deposit-feeding polychaetes from labile sediment fractions and from pore water*

ABSTRACT

Estuarine sediments are often highly enriched in particle-reactive metals and animals feeding on sediments may acquire metals from their diet. Laboratory experiments were performed in which the surface deposit-feeding polychaete species, *Nereis succinea,* was exposed to As(+5), Cd, and Cr(+3) in pore water or in estuarine sediments with and without enrichment with algal debris. These experiments permitted estimation of metal uptake parameters (assimilation efficiency of ingested metal [AE], uptake rate constant of dissolved metal, efflux rate constants following dietary or aqueous metal exposures) used in a kinetic model of metal bioaccumulation. The model showed that >97% of the body burden of these metals is accumulated through ingested sediment. The kinetic model was further modified to consider the geochemical reactivity of the metals in the sediments because metals bound to some mineral or organic fractions were shown to be unavailable to these polychaetes. The modified model substituted the AE term for each metal by the percentage of metal extracted in neutral and weak acid exchangeable fractions (termed "carbonex" fraction) multiplied by the slope of the regression between the metal AE and its fractionation in carbonex. The modified model generated predictions of As, Cd, and Cr body burdens in polychaetes at three different estuarine sites that correlated with sedimentary metal concentrations at these sites (r^2 =0.84 for sediments without organic enrichment, r^2 =0.87 with organic enrichment). Model predictions that relied on total metal concentrations showed weaker relationships (r^2 =0.11–0.50). This study adds to the evidence for the dominance of dietary uptake of metals in aquatic animals and identifies a key sedimentary fraction of metals that can account for bioavailability of sediment-bound metals.

INTRODUCTION

It has been widely recognized that coastal sediments can be greatly enriched in particle-reactive contaminants, including many metals and metalloids associated with industrial activities (Kennish 1997). Considerable attention has been paid toward evaluating the extent to which sediment-bound metals are available for uptake by benthic organisms (Griscom and Fisher 2004; Lee et al. 2000a; Tessier et al. 1984), but uncertainties remain (Luoma and Fisher 1997). In particular, it has been shown that not all metals bound to sediment are in a bioavailable form. Thus, metal bioavailability is thought to be related to the composition of the sediment, diagenetic conditions, association of metals with particular mineral or organic components, and the feeding and digestive properties of the organisms of interest (Baumann and Fisher 2011b; Griscom et al. 2002; Tessier and Campbell 1987; Tessier et al. 1984). Sediments are complex mixtures and metals bound to different sediment components can display very different bioavailability for deposit-feeding animals. Bioavailable components have been shown to include labile organic compounds (e.g., simple sugars or amino acids) (Mayer et al. 1995), and less bioavailable components may include some mineral fractions (Diks and Allen 1983; Geiszinger et al. 2002).

Benthic animals can acquire metals from both water and diet, although for many animals diet has been shown to be the dominant pathway (Casado-Martinez et al. 2009; Croteau and Luoma 2005; Wang et al. 1997). To help quantify the relative importance of each uptake pathway, metal bioaccumulation models have been used for both marine and freshwater organisms (Croteau and Luoma 2005; Luoma and Rainbow 2005; Mathews and Fisher 2009).The so-called biokinetic model or biodynamic model not only delineates the relative importance of each uptake pathway but also can be used to predict metal concentrations in animal tissues on a site-specific basis. Model predictions have generally been shown to closely match independent field measurements, suggesting that this model accounts for the major processes governing metal concentrations in filter-feeding aquatic animals and that the kinetic parameters used in the model are applicable to field situations (Roditi et al. 2000b; Wang et al. 1996). These kinetic parameters are commonly assessed experimentally using radiotracers and protocols that are well suited for determining uptake and release rates of metals in animals (Fisher et al. 1996; Wang and Fisher 1999a).

As described in detail elsewhere (Wang et al. 1996), the model (eq. 1) describes a metal's concentration in an animal at steady state (C_{ss}) as a sum of uptake from the aqueous phase (Eq. 2) and from diet (Eq. 3), where k_u = the uptake rate constant from the aqueous phase, C_w = the concentration of metal in the dissolved phase, AE = the assimilation efficiency of ingested metal from food, IR = the ingestion rate, C_f = the metal concentration in food, $k_{ew\ slow}$ and k_{ef} = the loss rate constants of metal from the animal following uptake from water and food, respectively, and g = the growth rate constant of the animal (generally negligibly small compared to k_e values for most metals). The relative proportion of metal uptake from diet is described in Eq. 4.

$$C_{ss} = \frac{k_u}{k_{ewslow} + g} \times C_w + \frac{AE \times IR}{k_{ef} + g} \times C_f \quad (1)$$

$$C_{ss,w} = \frac{k_u}{k_{ewslow} + g} \times C_w \quad (2)$$

$$C_{ss,f} = \frac{AE \times IR}{k_{ef} + g} \times C_f \quad (3)$$

$$\%\cdot dietary = \frac{C_{ss,f}}{C_{ss}} \times 100\% \quad (4)$$

Currently, there are no published kinetic model evaluations of metal bioaccumulation from contaminated sediments that incorporate metal phase speciation in sediments. This study therefore addresses modeling the bioaccumulation of sediment-bound metals for a deposit-feeding polychaete while considering the geochemical phase speciation of each metal. Three metals of environmental concern were considered: As(V), Cd, and Cr(III), each of which displays distinct chemical and biological behaviors (Greenwood and Earnshaw 1984). In addition, three estuarine sites (two in the Chesapeake, Baltimore Harbor (BH) and Elizabeth River (ER), Norfolk Virginia, and one in San Francisco Bay, Mare Island (MI)) with distinct sediment characteristics were examined to compare the influence of different geochemical properties on metal bioavailability. The characteristics of the sediments at each site are described in detail in Chapter II. Briefly, at the time of collection salinity was lowest at BH (8.5) and higher at the ER (19.5) and MI (23) sites; the dissolved organic carbon concentration in extracted from bulk sediment pore water was lowest at ER (10.2 mg C L^{-1}) and highest at MI and BH (29.5

and 33.0 mg C L^{-1}, respectively). Contents of organic carbon (BH-5.0%, ER-2.0%, and MI-1.5%), nitrogen (BH-0.3%, ER-0.1%, and MI-0.1%) and sulfur (BH-0.5%, ER-0.6%, and MI-0.4%) in the surface layer of sediments also varied among the sites. Iron levels varied from 3.2 mg g^{-1} dry wt in MI to 23.2 mg g^{-1} dry wt in ER sediments. Levels of As, Cd and Cr contamination in sediments also varied among study sites such that Cr and As were most enriched in BH sediments (As – 47.2 µg g^{-1} dry wt; Cr - 322.7 µg g^{-1} dry wt) and Cd was most enriched in MI (2.4 µg g^{-1} dry wt) sediments.

The modeling specifically assessed the influence of geochemical fractionation of sediment-bound metals on their bioavailability for benthic animals and quantified which uptake route contributes most to metal body burdens in polychaetes. This study builds on a recent study that described the assimilation of these metals from these estuarine sites by the polychaete *Nereis succinea* (Baumann and Fisher, 2011). In that study, it was shown that metals in the easily extractable sedimentary phases were also most assimilated in deposit-feeders.

In the present study, I describe experiments which determined metal accumulation in these polychaetes from pore water from each estuarine site and combine the kinetic parameters (k_u, k_{ew}. k_{ef}) from these experiments with AEs, and geochemical fractionation patterns of each metal and site from Baumann and Fisher (2011).

MATERIALS AND METHODS

Experimental organism

Experiments used surface deposit-feeding polychaetes *Nereis succinea*, commonly present in muddy sediments along eastern US coastline. The ecology and physiology of *N. succinea* are well described and this species is regarded as suitable for laboratory experiments determining contaminant bioaccumulation (Ahrens et al. 2001a; Wang et al. 1999). To obtain experimental animals, individuals of similar body length (about 10 cm) were hand collected from a local salt marsh at Flax Pond on Long Island NY, from early spring to late fall and placed with a small portion of sediment in separate containers to avoid cannibalism. Animals can attack, bite but not eat each other if they are densely populating the sediment. After transport to the lab all worms were rinsed with Flax Pond water (salinity 28) and placed in clean containers with Flax Pond water and ~0.5 g (wet wt) of Flax Pond sediment. Prior to each experiment, 20 individuals were selected and further acclimated to the experimental conditions for 2-7 d depending on water

salinity, where longer acclimation periods were needed for greater salinity changes (e.g., Flax Pond to Baltimore Harbor water, from salinity 28 to 8.5). Only worms that regularly produced feces were chosen for feeding experiments. Worms designated for the aqueous metal exposure experiments were further incubated in polycarbonate containers filled with seawater but without sediment so that their guts were emptied prior to exposure to the experimental water containing the metals. This ensured that fecal material would not scavenge the dissolved metals from the water during the metal uptake experiments.

Kinetic parameters

Parameters used in the kinetic model were generated in two series of experiments. Both series used a radiotracer approach that is well-suited for measuring, rapidly and accurately, the assimilation and retention of metals in marine animals while using environmentally realistic metal concentrations (Fisher 1992). I used ^{73}As, ^{109}Cd and ^{51}Cr, with half-lives of 80.3, 461.4, and 27.7 d, respectively. These isotopes were added as arsenate (As +5), cadmium chloride and chromic chloride (Cr +3). Pulse-chase feeding experiments yielded data used to calculate assimilation efficiencies (AE) of ingested metals and efflux rate constants of the metals following dietary uptake (k_{ef})., The uptake rate constants (k_u) of metals from the aqueous phase and efflux rate constants ($k_{ew\ fast}$ and $k_{ew\ slow}$) following uptake from the aqueous phase were calculated from experiments in which *N. succinea* were exposed to radiolabeled pore water from each estuarine site. Efflux rate constants (k_{ef}, $k_{ew\ fast}$ and $k_{ew\ slow}$) were calculated as the slopes of % metal retained during the fast and slow turnover phases (Wang et al. 1999). Aqueous exposure experiments consisted of two parts - uptake and efflux. Uptake rate constants (k_u) equaled the metal concentration accumulated per body mass per day divided by the concentration of metal in solution (Wang et al. 1996). Efflux rate constants from the worms, expressed in units of % d^{-1}, were calculated from regression analysis of % of metal retained in the worms over time.

Eq. 1 was used to determine steady-state total metal concentrations in *N. succinea* based on the experimentally determined kinetic parameters for each metal and each estuarine site. Steady state concentration was also calculated by assuming that the efflux rate constants were equal following the aqueous and dietary metal uptake. For this modeling exercise I used loss rate constants measured following uptake of metal from water.

To determine the metal concentration in the water that would be required for polychaetes for obtaining equal amount of metal from food and from water, the steady state concentration formula:

$$C_{ss} = \frac{k_u}{k_e} \times C_w + \frac{AE \times IR}{k_e} \times C_f \quad (5)$$

as modified such that:

$$C_{ss} = \frac{k_u}{k_e} \times C_w + 0.5 \times C_{ss} \quad (6a)$$

and

$$C_w = \frac{k_e}{k_u} \times 0.5 \times C_{ss} \quad (6\ b)$$

In this calculation I used three assumptions: metal is accumulated at steady state, growth rate is negligible and therefore term g (from eq. 1) is ignored, loss rate constant following aqueous metal uptake equals the loss rate constant following the dietary metal uptake and is indicated by term k_e.

In this study, the metal bioaccumulation model was modified to also consider the influence of different sediment components on metal bioaccumulation in *N. succinea*. The metal fraction extracted in neutral (*exchangeable* extracted with $MgCl_2$) and weak acid extractable (*carbonate* extracted with sodium acetate at pH 5) fractions of sediments (termed "carbonex" fraction when pooled together) has the strongest positive relationship with metal AE in this polychaete (Baumann and Fisher, 2011). Therefore, this modification took into consideration the % of metal in the carbonex sedimentary fraction ($z_{carbonex}$) and the regression slope between the metal AEs and % of metal in a given sedimentary fraction ($b_{carbonex}$). For example, if 25% of total sediment-bound metal is in the carbonex fraction ($z_{carbonex}$) and the regression of percentages of metal in carbonex and AEs has a slope of 0.4 ($b_{carbonex} = 0.4$), then 10% of total metal in sediment is assumed to be assimilated.

$$C_{ss} = \frac{k_u}{k_{ewslow} + g} \times C_w + \frac{z_i \times b_i \times IR}{k_{ef} + g} \times C_f \quad (7)$$

$$C_{ss,f} = \frac{z_i \times b_i \times IR}{k_{ef} + g} \times C_f \quad (8)$$

The metal content in the carbonex pool included the metal associated with the operationally defined exchangeable and carbonate sediment fractions. To determine these fractions, exactly the same types of radiolabeled sediment that were used for the pulse-chase feeding experiments were subjected to sequential chemical extractions, as described in detail elsewhere (Chapter II). Briefly, sediment was first incubated with 1M $MgCl_2$, after which the sediment was centrifuged and the supernatant containing the *exchangeable* portion of the radiotracer was transferred into another container. The remaining sediment was then incubated with sodium acetate solution at pH 5 to extract metals in the *carbonate* and similarly reactive phases. The radioactivity in the extracted exchangeable and carbonate fractions were assessed by gamma-spectrometry.

Feeding experiments

For determinations of polychaete ingestion rates, I assumed that ingestion rate equaled defecation rate. Defecation rates, represented by units of dry mass of defecated sediment per dry mass of worm per time (g g^{-1} d^{-1}), were calculated based on the dry mass of feces that were periodically collected. Fecal pellets were filtered onto tared GFF filters, dried, and weighed. The lengths of the experimental worms were measured with electronic calipers. Individual worms and collected feces were dried over >24 h at 68 °C.

Equation 4 was used to determine the % of polychaete metal accumulated from diet. All kinetic parameters used in the modeling were calculated based on experiments presented here, except AEs and k_{ef} values which are taken from Baumann and Fisher (2011). A brief description of the pulse-chase feeding experiments is given here. Feeding experiments were conducted at 21 °C. Individual worms were placed into the feeding chambers constructed of tygon tubing (length =15 cm; φ = 4 mm) connecting two plastic Petri dishes (φ =5 cm), as described in Wang et al. (1999). The tubing simulated the burrow in which these worms live in nature. Chambers were filled with seawater and about 0.5 g of radiolabeled sediment.

Field collected sediments were radiolabeled by a direct addition of radiotracer dissolved in dilute acid to the sediment or by mixing the sediment with previously radiolabeled algae (*Thalassiosira pseudonan*a) that were separated from water by filtration. Such labeled sediments

were incubated in plastic containers at 21°C for 2 and 30 days prior to the feeding experiment. Exact details of sediment labeling procedure are provided in Baumann and Fisher (2011). Polychaetes spent most of the time inside the tubes. Sediment was placed at the "head" end of the tube; fecal pellets were collected from the other end of the tube. When worms were first presented the radiolabeled sediment, they were given about 4 hours to feed on it and were then removed from their feeding chambers and placed inside the well detector of the gamma counter to determine the radioactivity in their bodies. Immediately after feeding on radiolabeled sediments, the radioactivity of the labeled polychaetes was determined (note this is non-destructive analysis) and the worms were replaced into individual containers and fed unlabeled sediment for up to 5 d. Their radioactivity was periodically determined over time and the retention of each isotope in each individual worm was tracked and k_{ef} values of these isotopes from the worms were determined. Exact procedures describing the feeding experiments are given in Baumann and Fisher (2011).

Pore water experiments

Pore waters used for experiments that determined metal bioaccumulation from the aqueous phase were extracted from sediments collected from three different field sites- Baltimore Harbor (BH; 39°12'.25"N, 76°31'40"W) [Baltimore, MD] in June, Elizabeth River (ER; 36°52' 32" N, 76°20' 09" W) [Norfolk, VA] in May, and Mare Island (MI; 38°04'23" N, 122°15'15" W) [Vallejo, CA in San Francisco Bay] in October. They were transported to the lab and stored at 4°C in plastic buckets. Field locations were chosen based on differences in sediment and water geochemistry and the extent of metal contamination characterized in chapter II. Pore water was separated from the sediment by centrifugation at 7500 g for 10 min. Any particles (including microorganisms) remaining in the extracted pore water were removed by filtration through 0.2 µm polycarbonate membranes. The pore waters were then autoclaved at 121°C and 15 psi for 20 minutes to ensure axenic conditions and the water was cooled prior to injecting the radioisotopes. Water was radiolabeled with γ-emitters either by addition of a single radioisotope ^{73}As or combined ^{109}Cd and ^{51}Cr. The gamma detectors that we used did not allow sufficient resolution to combine all three radioisotopes due to their overlapping energies of emission. The isotopes were taken from stock solutions in dilute HCl, and the acidity from the microliter additions was neutralized by addition of microliter quantities of dilute NaOH. Pore

water labeling resulted in the following concentrations of radioisotopes: for ^{73}As 6.8, 11.8, and 11.5 Bq mL^{-1} were added to ER, BH, and MI pore waters, respectively; for ^{109}Cd 80.0, 55.4, and 59.2 Bq mL^{-1} were added to ER, BH, and MI, respectively; and for ^{51}Cr 0.3, 27.7, and 28.3 Bq mL^{-1} were added to ER, BH, and MI, respectively. The amount of activity in the pore water was checked prior to the uptake experiment and was monitored throughout the experiment to assure that the added radioisotopes remained in solution. These additions corresponded to additions of 0.03, 0.06, and 0.06 fmol mL^{-1} of As for ER, BH, and MI, respectively; 8.0, 5.5, and 5.9 fmol mL^{-1} of Cd for ER, BH, and MI, respectively; and 0.14, 13.15, and 13.44 fmol mL^{-1} of Cr for ER, BH, and MI, respectively. Given the background concentrations of these metals in the pore waters from each site (Table 1), the radioisotope additions resulted in additions of <1% of background concentrations of each of the metals examined.

Metal accumulation experiments using radiolabeled pore waters were conducted at 17 °C by placing individual worms in separate polycarbonate containers filled with 50 mL of radiolabeled water for 2-4 h. Worms were then removed from their containers and rinsed three times with filtered seawater to remove adsorbed sediment grains and twice with a 10^{-4} M solution of ethylenediaminetetraacetic acid (EDTA) in the corresponding seawater (ER, BH or MI) to remove adsorbed radioisotopes from body surfaces. After counting the radioactivity of the worms immediately following exposure to radiolabeled pore water, the same individual worms were placed into plastic containers holding unlabeled pore water and their radioactivity was periodically determined over time. The retention of each isotope in each individual worm was tracked for up to 14 d and the k_{ew} values of these isotopes were determined.

For assaying their radioactivity, individual worms were placed into empty 50 mL plastic counting tubes and inserted into one of the large well gamma detectors. This detector was intercalibrated with an LKB Compugamma well-type NaI(Tl) gamma detector that was used for counting the radioactivity of all samples (water, fecal pellets, sediment) other than worms. Counting times were typically 1 min, yielding propagated counting errors < 5%. Radioactivity of ^{73}As was detected at 53 keV, of ^{109}Cd at 88 keV, and of ^{51}Cr 256 keV.

Field data

As, Cd and Cr in surface sediments, pore waters and various polychaete species collected from the study sites (BH, ER and MI) and pore waters were measured by ICP-MS. Metal

concentrations in surface sediments were determined as described in Chapter II. I used experimentally determined kinetics parameters combined with metal concentrations in sediments and pore waters from field samples to predict metal concentrations in *N. succinea*. These model estimates were compared with metal concentrations in field-collected polychaetes at each of the three estuarine sites.

Statistical analyses

Statistical analyses were performed using PASW 18.0 software. One-way ANOVA combined with ad-hoc Tukey's tests were conducted to detect significant ($p < 0.05$) differences among kinetic parameters such as k_{ef}'s and $k_{ew\ slows}$'s for a specific metal at each location or for a specific location between metals. The number of replicates for each k_{ef} or $k_{ew\ slow}$ typically ranged from 5 to 8. Data expressed as % were arcsine transformed to normalize their distribution prior to ANOVA.

RESULTS

Table 1 shows metal concentrations in surface sediment, pore water and metal partition coefficients – Kd for metals in sediments from the three in estuarine sites. For the purpose of this paper Kd is defined as the ratio of the mass of metal kg^{-1} of bulk sediment to the mass of dissolved metal in a liter of water. Kd values were highest for Cr. The total average concentrations of As in surface sediment were highest in BH and lowest in MI, Cd was highest in MI and lowest in ER, and Cr was highest in BH and lowest in ER. Polychaetes collected at the different sites generally contained measurable concentrations of As, Cd and Cr (Table 2). Mean concentrations of As were similar for worms from BH and ER, although the range was higher in worms from ER. Arsenic concentration in MI worms was below detection. Mean Cd concentrations were similar in worms from ER and MI, however due to insufficient number of polychaetes it was not possible to establish a concentration range for MI. There was no measurable Cd in BH polychaetes. Worms that were collected at all three sites contained detectable Cr, with highest values but fewest samples noted for MI worms (Table 2). Metal concentrations in field-collected worms did not show any relationship with metal partition coefficients (Kd) in the surface sediments.

The fractionations of ^{73}As, ^{109}Cd, and ^{51}Cr in the combined carbonate and exchangeable (carbonex) pools in sediments from each estuarine site that were radiolabeled by direct injection

of radioisotope to the sediments or by addition of radiolabeled algal debris are given in Table 3. Cd was most enriched in the carbonex pool (38-94%), regardless of site or means by which the sediments were labeled, and Cr was least enriched in this pool (2-5%). Aging of the directly labeled sediments from 2 to 30 d had no consistent effect on the association of the metals with the carbonex fraction.

N. succinea exposed to radiolabeled metals in pore water generally showed a linear uptake of metal over time, but Cr uptake from ER pore water and As uptake from MI pore water leveled off after 1 d (Fig. 1). Metal uptake rate constants (k_us, Table 4) were highest for As (ranging from 0.021 L g^{-1} d^{-1} in BH to 0.180 L g^{-1} d^{-1} in MI) and lowest for Cd (ranging from 0.0006 L g^{-1} d^{-1} in BH and MI to 0.005 L g^{-1} d^{-1} in ER); differences among metal k_us were significant (one-way ANOVA, $p < 0.001$). Uptake rate constants of Cd and Cr increased significantly ($p < 0.05$) with salinity (from 8 to 20 ppt) and decreased significantly (one-way ANOVA, $p < 0.05$) for Cr as pore water DOC increased from 10 to 30 mg L^{-1} (Fig. 2). Metals that were accumulated by *Nereis succinea* from water were lost in two phases, most clearly seen for Cr in ER water (Fig. 3). The overall percentage of metal rapidly lost ($k_{ew\ fast}$) was nearly always < 15%, except Cr in ER (74%; Fig. 3, Table 4). The rapid metal removal from worms lasted no longer than 24 h and was followed by a slow turnover - $k_{ew\ slow}$. Given the relatively high variability in the data, no systematic differences for k_{ef}'s were evident among metals, sediment labeling schemes, sites or sediment age (Table 5).

Following feeding on radiolabeled sediment, the average specific ingestion rate (IR) was 0.27 g dry sediment g^{-1} dry worm d^{-1}; ingestion rates were inversely related to worm lengths (Fig. 4). Modeling metal concentrations in polychaetes using whole sediment metal concentrations (C_f) (Eq. 1) used AEs shown in Table 6, an IR of 0.27 g g^{-1} d^{-1}, and $k_{ew\ slow}$ values in Tables 4 and 5; growth rate (g) was assumed to be negligible. The kinetic parameters in the modified metal bioaccumulation model (Eq. 7) also used $z_{carbonex}$ values given in Table 1 and a $b_{carbonex} = 0.353$ (Baumann and Fisher, in 2011). Using this modified model, it was shown that virtually all (≥97%) of the metal in worms was acquired from ingested sediment, for all metals, sediments, and treatments (Table 6); for As in directly labeled ER sediments after 2 d exposure, 85.5% of the As was shown to be acquired from diet (Table 6). However, when using $k_{e\ aq\ slow}$ as the loss rate constant (Eq. 5, 6 a,b), in both the dietary and aqueous metal uptake biokinetic

model terms, some predictions suggested greater importance of the aqueous metal in the total metal body burden. Uptake from the aqueous phase dominated for Cd in nearly all cases but one - ER sediment labeled via algae for 30 days. More than 50% of As was water-derived in worms exposed to water and sediments from ER and MI. This is likely due to high uptake rate constants for As in ER and MI water, which are more saline (19.5 and 23 ppt, respectively).

Listed in Table 7 are the predicted aqueous metal concentrations that would be required to match the dietary metal as a source of total metal body burden i.e., metal concentration in the water that would result in 50% of metal accumulated by the worms from the water and 50% would be acquired from the diet). Calculated ratios of new metal concentration (required for equal metal contribution from water and diet) to the old metal concentration range from 1 to > 1000-fold (Table 7).

Model predictions of metal concentrations in polychaetes using Eq. 7, which considered the geochemical fractionation of the metals in the carbonex phases ($z_{carbonex}$ and $b_{carbonex}$), were compared with independent measurements of metal concentrations in field-collected polychaetes (Fig. 5A, B). Regression analyses for all metals and sediment sites showed significant relationships between the two (linear regression, $r^2 = 0.87$ for directly labeled sediments, $r^2 = 0.84$ for sediments labeled via algal debris). The slope for the directly labeled sediment (0.51, Fig. 5A) suggests that the model underpredicts by a factor of 2 the metal concentration in the polychaetes, whereas the slope for the sediments mixed with radiolabeled algal debris (1.88, Fig. 5B) suggests that the model overpredicts by a factor 2 the metal concentrations in polychaetes relative to field-collected worms. However, when using total metal concentrations in sediments (Eq. 1), model predictions showed much weaker relationships with field measurements (linear regression, $r^2 = 0.50$ for directly labeled sediments, $r^2 = 0.11$ for sediments labeled with algal debris) (Fig. 5C, D). Thus, consideration of the metal fractionation in the sediment enables more accurate modeled predictions of metal body burdens in polychaetes than modeling with total metal concentrations in sediments.

DISCUSSION

Dietary exposure

That model predictions showed metal primarily deriving from a dietary source is consistent with findings for other metals in benthic animals. Diet represents a larger relative source of metals for polychaetes than for copepods and bivalves (Wang and Fisher 1999b). Table 8 summarizes dietary metal contributions for several different invertebrates including the marine mussel *Mytilus edulis,* and clam *Macoma balthica*, freshwater zebra mussel (*Dreissena polymorpha*), amphipod *Leptocheirus plumulosus,* and deposit-feeding polychaetes *Arenicola marina* and *N. succinea* and different elements such as Cd, Cr, As, both elemental and methyl Hg, Zn, Se and Ag . In general, the biokinetic model predicts that Se, Hg and As are dominantly diet-derived, while other elements show some variability for different invertebrates. In a study by Yoo et al. (2004) two invertebrates *Neanthes arenaceodentata* and *Leptocheirus plumulosus* acquired Ag primarily from their diet as did the freshwater bivalve *Corbicula* sp. for Cu (Croteau and Luoma 2005). Croteau and Luoma (2008) proposed evaluating dietary uptake by multiplying the dietary uptake rate constant (k_{uf}) by the metal concentration in the food and dividing by the efflux rate constant, which allows for a more direct comparison with the rate of aqueous metal uptake. Croteau and Luoma (2008) demonstrated that despite a three order of magnitude difference between k_{uf} and $k_{u\ aq}$ values ($k_{uf} < k_{u\ aq}$), dietary metals (Cd, Cu and Ni) accounted for most of the overall metal body burden in animals. Similarly, diet has been shown to dominate the uptake for many metals in pelagic vertebrates in marine and freshwater systems (Mathews and Fisher 2009; Pickhardt et al. 2006).

The finding that acquisition of As, Cd, and Cr is related to their association with labile (easily extracted) sedimentary fractions is consistent with earlier findings for other metals (Tessier et al. 1984). By using the percentage of metal in neutral and weak acid exchangeable pools ($z_{carbonex}$), and $b_{carbonex}$, which is the slope of regression between the AEs and z (Baumann and Fisher, 2011). I show here that this geochemical pool of metal in diverse sediments can be used to explain observed metal concentrations in deposit feeding polychaetes in the field. Indeed, a positive regression was found between Cu in exchangeable and carbonate sediment fractions and Cu concentrations in freshwater tubificid oligochaetes (Diks and Allen 1983), comparable to our findings for As, Cd and Cr (Baumann and Fisher, 2011 or chapter III). The positive

relationship between measured metal concentrations in field-collected polychaetes and model predictions based on the carbonex associations of these metals (Fig. 5) provides further evidence that metals that are weakly bound to sedimentary particles are more bioavailable. This is presumably due to the fact that weakly-bound metals are more readily released from ingested sediment particles into the gut of the deposit-feeder (Chen and Mayer 1999; Mayer et al. 1996) and therefore more likely to cross the gut lining and be assimilated (see also chapter III and V). The underprediction of polychaete metal from sediment labeled directly may be attributed to the fact that the sediments had no fresh organic detritus associated with it, and thus less metal bound to labile organic matter than would occur in natural field conditions. The overprediction of metal body burden acquired by polychaete from sediment labeled by mixing with algal detritus may be attributable to greater bioavailability of metals associated with depositing algal debris, which is not present at all times throughout the year and is higher during post-bloom periods.

The mean ingestion rate measured here for *N. succinea* (0.27 g $g^{-1}d^{-1}$) was identical to that described for similarly sized *N. succinea* at the same experimental temperature reported by Cammen (1980a) and an order of magnitude lower than that cited by Wang et al. (1999). I am unaware of published reports of As, Cd, and Cr concentrations in polychaetes in the Chesapeake to compare with our measurements, but Cd concentrations in resident oysters and mussels have been described (Sinex and Wright 1988; Wright et al. 1985). In San Francisco Bay (but not at Mare Island), As concentrations in several species of polychaetes were 110-130 µg g^{-1}dry wt (Meador et al. 2004) in comparison to polychaetes collected in Mare Island (0 µg g^{-1}dry wt; present study). There is more information available regarding metal concentrations in animals representing other taxonomic groups living in San Francisco Bay (e.g. bivalves, birds). For example the suspension-feeding *Potamocurbula amurensis* collected in San Francisco Bay and Lower Suisun Bay had ~4 µg g^{-1}dry wt of Cd and ~6 µg g^{-1}dry wt of Cr (Brown and Luoma 1995), another suspension-feeder *Corbicula* sp. collected all throughout the bay system had As levels in a range of 5.4 to 11.5 µg g^{-1}dry wt, and deposit-feeding *Macoma balthica* contained 9 µg g^{-1}dry wt of As (Johns and Luoma 1990). Among the waterfowl monitored in San Francisco Bay, the American coot (*Fulica americana*), collected near Mare Island, had an average 3.7 µg g^{-1}dry wt of As, 105 µg g^{-1}dry wt of Cr, and no measurable Cd in its ingested food. Its liver was also enriched with As and Cr (5.1 µg g^{-1}dry wt of As, 3.3 µg g^{-1}dry wt of Cr, 1.3 µg g^{-1}dry wt of Cd) (Hui 1998).

Aqueous exposure

Cd aqueous uptake rate constants from our study were 0.06 to 0.5 times those reported for previously published results for *N. succinea* (Wang et al. 1999). Cadmium k_us calculated from data reported by Ng et al. (2008) for the polychaete *Perinereis aibuhitensis* were approximately 0.003 L^{-1} g^{-1} dry wt d^{-1} (assuming dry wt = 20% wet wt) when worms were exposed to 4.12 μg Cd L^{-1}. The k_u measured for *N. succinea* in the present study was approximately 0.005 L^{-1} g^{-1} dry wt d^{-1} in ER pore water, which had a comparable Cd exposure concentration (5.12 μg L^{-1}) and significantly lower ($p<0.05$) k_us (0.0006 L^{-1} g^{-1} dry wt d^{-1}) when worms were exposed to lower Cd concentrations in BH and MI pore waters (0.16 and 0.52 μg Cd L^{-1}, respectively). Yan and Wang (2002) reported a Cd k_u of 0.0018 L^{-1} g^{-1} dry wt d^{-1} for the polychaete *Sipunculus nudus* and a Cr k_u of 0.019 L^{-1} g^{-1} dry wt d^{-1}. This Cr k_u is within the range of k_us reported here for *N. succinea* (Table 5). I am unaware of any k_us reported for As in marine polychaetes.

The positive relationship between As k_u and salinity is consistent with recent findings for As accumulation in the killifish *Fundulus heteroclitus* (Dutton and Fisher in press). Although Cd is a chloro-complexed metal, the lowest salinity water investigated here still has sufficiently high chloride to complex all the Cd in the water; thus the lack of a salinity response over the salinity range 8 to 23 is not surprising. Only the k_u for Cr was inversely related to the DOC concentration in pore water for all sites, suggesting that organic complexation of this metal may reduce its bioavailability for *N. succinea*.

The efflux rate constants from *N. succinea* following aqueous and dietary exposures did not significantly differ for any of the metals (one-way ANOVA; $p>0.05$), similar to findings of Norwood et al. (2006) who evaluated loss of As and Cr in *Hyalella azteca*, and suggesting that the uptake route has no appreciable influence on the biological turnover rates of assimilated metal from the polychaete tissues.

In summary, the present study shows that diet is the dominant source of sediment-bound metals for deposit-feeding polychaetes, regardless of the metal or sediment type. Further it is important to recognize that bulk sediment is a mixture of minerals and organic compounds to which metals may bind, and that metal bioavailability varies among geochemical fractions. For all metals and estuarine sites considered, modeling metal bioaccumulation in polychaetes most

accurately quantifies metal body burdens when considering geochemical fractionation of the metals in the sediments.

TABLES

Table 1. Total concentrations of As, Cd and Cr in surface sediments (SS; 0-1 cm), pore water (PW; 0-1 cm), and partition coefficients (Kd) of As, Cd and Cr in these sediments.

Location		unit	As	Cd	Cr
Mare Island	SS	mM	0.019	0.021	0.911
	PW	µmolal	0.021	0.005	0.003
	Kd	L kg^{-1}	9.12×10^{2}	4.61×10^{3}	3.08×10^{5}
Baltimore Harbor	SS	mM	0.630	0.009	6.205
	PW	µmolal	0.026	0.001	0.007
	Kd	L kg^{-1}	2.41×10^{4}	5.94×10^{3}	9.52×10^{5}
Elizabeth River	SS	mM	0.085	0.004	0.640
	PW	µmolal	0.380	0.046	0.016
	Kd	L kg^{-1}	2.24×10^{2}	0.90×10^{2}	4.01×10^{4}

Table 2. Ranges and means (in parentheses) of metal concentrations in field-collected polychaetes. BH n = 4; ER n = 5; MI n = 1 and therefore no range provided for metals in MI. < det indicates below detection limit.

	metal concentrations [µg g^{-1} dry wt]		
	BH	ER	MI
As	6.16 - 21.631 (13.45)	0.70 – 21.10 (13.14)	< det
Cd	< det	0.20 – 3.40 (1.55)	- (0.98)
Cr	2.09 – 31.78 (12.30)	1.2 – 5.8 (3.04)	- (49.34)

Table 3. Percent of ^{73}As, ^{109}Cd and ^{51}Cr in carbonex fraction (exchangeable + carbonate) of sediments from 3 estuarine sites labeled directly with radioisotopes or via mixing with previously radiolabeled algae and aged for 2 (just directly labeled sediment) or 30 days (both)(Baumann and Fisher, 2011).

location	label	days	% of radioisotope in carbonex pool ($z_{carbonex}$)		
			^{73}As	^{109}Cd	^{51}Cr
Baltimore Harbor	algae	30	9	48	2
Elizabeth River			30	72	5
Mare Island			12	38	3
Baltimore Harbor	direct	2	1	59	4
Elizabeth River			2	67	5
Mare Island			4	48	4
Baltimore Harbor		30	3	49	3
Elizabeth River			3	94	3
Mare Island			6	58	3

Table 4. Kinetic parameters for uptake (k_u) from pore water and efflux from worms ($k_{ew\ slow}$ and $k_{ew\ fast}$) following metal uptake by *N. succinea*; terms in parentheses represent the % of metal in the slowly exchanging metal pool; values represent means ± 1 SD for n = 5 - 7 individuals; in places where error is not specified individual worms were pooled together to obtain a sufficiently high radioactivity count and hence inter-individual differences were not observed. A fast loss phase was not evident for ^{73}As and ^{109}Cd in ER.

	Baltimore Harbor (BH)	Mare Island (MI)	Elizabeth River (ER)
k_u [L g^{-1} d^{-1}]			
^{73}As	0.021 ± 0.019	0.180 ± 0.166	0.122 ± 0.067
^{109}Cd	0.0006 ± 0.0002	0.0006 ± 0.0003	0.005 ± 0.0027
^{51}Cr	0.0048 ± 0.0045	0.008 ± 0.0043	0.076 ± 0.068
$k_{ew\ slow}$ [% d^{-1}]			
^{73}As	3.4 ± 2.0 (95)	0.2 ± 0.9 (95)	1.6 (100)
^{109}Cd	2.0 ± 0.3 (86)	1.5 ± 0.5 (92)	1.5 ± 1.0 (100)
^{51}Cr	2.3 ± 2.1 (94)	1.7 ± 1.7 (95)	0.8 (26)
$k_{ew\ fast}$ [% d^{-1}]			
^{73}As	16.9 ± 5.6	5.6 ± 4.7	-
^{109}Cd	11.7 ± 11.7	10.1 ± 4.7	-
^{51}Cr	5.4 ± 3.6	6.4 ± 5.5	415.1 ± 179.6

Table 5. Efflux rate constants [k_{ef}; % d^{-1}; mean ± 1 SD, n = 5 - 8] of ^{73}As, ^{109}Cd, and ^{51}Cr in *N. succinea* during depuration after pulse feeding on sediment radiolabeled by direct addition of radioisotopes or by mixing with previously radiolabeled algal detritus. Also shown are k_{ef}s following feeding on pure radiolabeled algal detritus or goethite. nd: not determined

diet		efflux rate k_{ef} [% d^{-1}]			
		directly labeled		labeled algae	
		2 days	30 days	2 days	30 days
Elizabeth River (ER)	^{73}As	nd	nd	7.6 ± 5.3	1.2 ± 0.3
Baltimore Harbor (BH)	^{73}As	3.5 ± 2.5	1.4	nd	nd
Mare Island (MI)	^{73}As	6.3 ± 5.9	nd	3.4 ± 1.2	1.6 ± 0.8
fresh algal detritus	^{73}As			4.29 ± 1.58	
pure goethite	^{73}As	1.0 ± 0.6			
Elizabeth River (ER)	^{109}Cd	5.4 ± 4.3	2.5 ± 1.8	0.3 ± 0.2	5.9 ± 4.4
Baltimore Harbor (BH)	^{109}Cd	0.3 ± 0.3	0.3	5.4 ± 5.5	10.6 ± 9.6
Mare Island (MI)	^{109}Cd	1.8 ± 1.3	6.4 ± 1.5	0.9 ± 1.0	0.6 ± 0.4
fresh algal detritus	^{109}Cd			2.1 ± 2.0	
pure goethite	^{109}Cd	2.5 ± 1.5			
Elizabeth River (ER)	^{51}Cr	2.4 ± 2.7	0.1 ± 0.1	0.05 ± 0.01	0.12 ± 0.17
Baltimore Harbor (BH)	^{51}Cr	1.7 ± 2.0	1.4	nd	1.89 ± 2.01
Mare Island (MI)	^{51}Cr	1.0 ± 0.6	nd	1.7 ± 0.7	0.22 ± 0.26
fresh algal detritus	^{51}Cr			0.6 ± 0.3	
pure goethite	^{51}Cr	3.1 ± 1.7			

Table 6a. Model predictions (Eq. 4) of dry wt based body burden of metals in *N. succinea* following feeding on sediment with or without algal detritus and aged for up to 30 d; AEs from Baumann and Fisher (2011). Percentages showing AEs and dietary source rounded to closest 0.1%.

	location	metal	age	AE	from food	from water	C_{ss}	dietary
			days	%	$\mu g\ g^{-1}$	$ng\ g^{-1}$	$\mu g\ g^{-1}$	%
algal detritus	BH	As	2	51.6	112.1	0.123	112.10	100.0
			30	30.1	65.54	0.123	65.54	100.0
		Cd	2	68.7	0.64	0.000	0.64	100.0
			30	21.6	0.10	0.000	0.10	100.0
		Cr	2	1.2	29.85	0.007	29.85	100.0
			30	0.8	85.95	0.007	85.95	100.0
	ER	As	2	69.7	15.32	173.1	15.50	98.9
			30	16.8	6.59	173.1	6.77	97.4
		Cd	2	9.9	2.00	0.948	2.00	100.0
			30	21.5	0.22	0.171	0.22	99.9
		Cr	2	0.9	154.9	0.371	154.92	100.0
			30	3.6	59.58	0.371	59.58	100.0
	MI	As	2	50.7	5.71	1.764	5.71	100.0
			30	24	5.74	1.764	5.74	100.0
		Cd	2	9.4	10.23	0.002	10.23	100.0
			30	7.6	12.32	0.002	12.32	100.0
		Cr	2	5	61.62	0.015	61.62	100.0
			30	0.3	32.44	0.015	32.44	100.0
directly injected radiotracer	BH	As	2	7.8	23.07	0.123	23.07	100.0
			30	6.6	34.65	0.123	34.65	100.0
		Cd	2	1.5	0.25	0.0005	0.25	100.0
			30	2.4	0.40	0.0005	0.40	100.0
		Cr	2	4.2	113.0	0.007	113.00	100.0
			30	4.6	149.6	0.007	149.61	100.0
	ER	As	2	1.2	1.02	173.1	1.19	85.5
		Cd	2	30.8	0.35	0.171	0.35	100.0
			30	43.6	1.06	0.171	1.06	100.0
		Cr	2	4.5	15.89	0.371	15.89	100.0
			30	1	86.82	0.371	86.82	100.0
	MI	As	2	10.2	0.62	1.764	0.62	99.7
			30	12.1	0.73	1.764	0.73	99.8
		Cd	2	46.1	24.98	0.002	24.98	100.0
			30	58.9	8.97	0.002	8.97	100.0
		Cr	2	4	88.59	0.015	88.59	100.0
			30	0.7	14.69	0.015	14.69	100.0

Table 6b. Model predictions (Eq. 5) of dry wt based body burden of metals in *N. succinea* following feeding on sediment with or without algal detritus and aged for up to 30 d; AEs from Baumann and Fisher (2011). Loss rate constants following the aqueous and dietary uptake were assumed equal and and $k_{eaq\ slow}$ was used for this model prediction. Percentages showing AEs and dietary source rounded to closest 0.1%.

	location	metal	age	AE	from food	from water	C_{ss}	dietary
			days	%	µg g^{-1}	ng g^{-1}	µg g^{-1}	%
algal detritus	BH	As	2	51.6	193.4	1.2	194.6	99.4
			30	30.1	112.8	1.2	114.0	98.9
		Cd	2	68.7	8.9	0.005	8.9	99.9
			30	21.6	2.8	0.005	2.8	99.8
		Cr	2	1.2	45.5	0.07	45.5	99.8
			30	0.8	30.3	0.07	30.4	99.8
	ER	As	2	69.7	74.9	216.9	291.8	25.7
			30	16.8	18.1	216.9	234.9	7.7
		Cd	2	9.9	0.8	1.7	2.5	32.4
			30	21.5	1.8	1.7	3.5	51.1
		Cr	2	0.9	10.1	7.9	18.0	56.2
			30	3.6	40.5	7.9	48.3	83.7
	MI	As	2	50.7	97.9	141.3	239.2	40.9
			30	24	46.3	141.3	187.6	24.7
		Cd	2	9.4	4.0	0.021	4.1	99.5
			30	7.6	3.3	0.021	3.3	99.4
		Cr	2	5	37.6	0.071	37.7	99.8
			30	0.3	2.3	0.071	2.3	97.0
directly injected radiotracer	BH	As	2	7.8	29.2	1.211	30.4	96.0
			30	6.6	24.7	1.211	25.9	95.3
		Cd	2	1.5	0.2	0.0048	0.2	97.6
			30	2.4	0.3	0.0048	0.3	98.5
		Cr	2	4.2	159.1	0.071	159.2	100.0
			30	4.6	174.2	0.071	174.3	100.0
	ER	As	2	1.2	1.3	216.9	218.1	0.6
		Cd	2	30.8	2.6	1.7	4.3	59.9
			30	43.6	3.6	1.7	5.3	67.9
		Cr	2	4.5	50.6	7.9	58.5	86.5
			30	1	11.2	7.9	19.1	58.8
	MI	As	2	10.2	19.7	141.3	161.0	12.2
			30	12.1	23.4	141.3	164.7	14.2
		Cd	2	46.1	19.8	0.021	19.9	99.9
			30	58.9	25.3	0.021	25.4	99.9
		Cr	2	4	30.1	0.071	30.2	99.8
			30	0.7	5.3	0.071	5.3	98.7

Table 7. Metal concentrations (C_f) measured in sediments from BH, ER and MI and metal concentrations in the water (C_w^*) that were calculated (Eq. 6b) to match the dietary contribution of the total metal body burden. Ratio of C_w* vs. C_w represents the ratio between the measured metal concentration (C_w) and metal concentration that would lead to metal accumulation equal to metal accumulation from ingested sediment.

				C_{w*} [µg mL^{-1}]		C_f [µg g^{-1}]		Cw* vs. Cw	
label	location	metal	age days	algal detritus	directly added radiotracer	algal detritus	directly added radiotracer	algal detritus	directly added radiotracer
algal detritus	BH	As	2	0.00196	0.0020	47.19	47.19	80	12.6
			30	0.00196	0.0020	47.19	47.19	47	10.7
		Cd	2	0.00016	0.0002	0.96	0.96	928	21
			30	0.00016	0.0002	0.96	0.96	292	33
		Cr	2	0.00034	0.0003	322.65	322.65	321	1121
			30	0.00034	0.0003	322.65	322.65	214	1228
	ER	As	2	0.0284	0.0284	6.37	6.37	0.7	0.5
			30	0.0284		6.37		0.5	
		Cd	2	0.0051	0.0051	0.46	0.46	0.7	1.2
			30	0.0051	0.0051	0.46	0.46	1.0	1.6
		Cr	2	0.0008	0.0008	33.30	33.30	1.1	3.7
			30	0.0008	0.0008	33.30	33.30	3.1	1.2
	MI	As	2	0.0016	0.0016	1.43	1.43	0.8	0.6
			30	0.0016	0.0016	1.43	1.43	0.7	0.6
		Cd	2	0.00052	0.0005	2.39	2.39	98	477
			30	0.00052	0.0005	2.39	2.39	79	610
		Cr	2	0.00015	0.0002	47.38	47.38	267	214
			30	0.00015	0.0002	47.38	47.38	16	38

Table 8. Percent contribution of diet derived metal in the total body burden as predicted by biokinetic models from various referenced studies.

Species	AE [%]							Reference
	Cr	Se	Cd	Ag	Zn	Hg	As	
Mytilus edulis	62-87*	95-98	24-49					Wang and Fisher 1999b
		>98		41-46	48-67			Wang et al. 1996
Macoma balthica			33-82[a]	49-93[a]				Griscom et al. 2000
			90[b]	>98[b]				
Dreissena polymorpha	9-45		75-91	63-90		57-82		Roditi et al. 2000b
Leptocheirus plumulosus			>90			>90[c]	>90	Williams et al. 2010
Arenicola marina			>90	>70	>90			Casado-Martinez et al. 2009
Nereis succinea						~60[c] and 40-80[d]		Wang et al. 1998

*Cr (+3), [a]- deposit-feeding, [b]- filter-feeding, [c]- elemental Hg, [d]- methyl-Hg

FIGURES

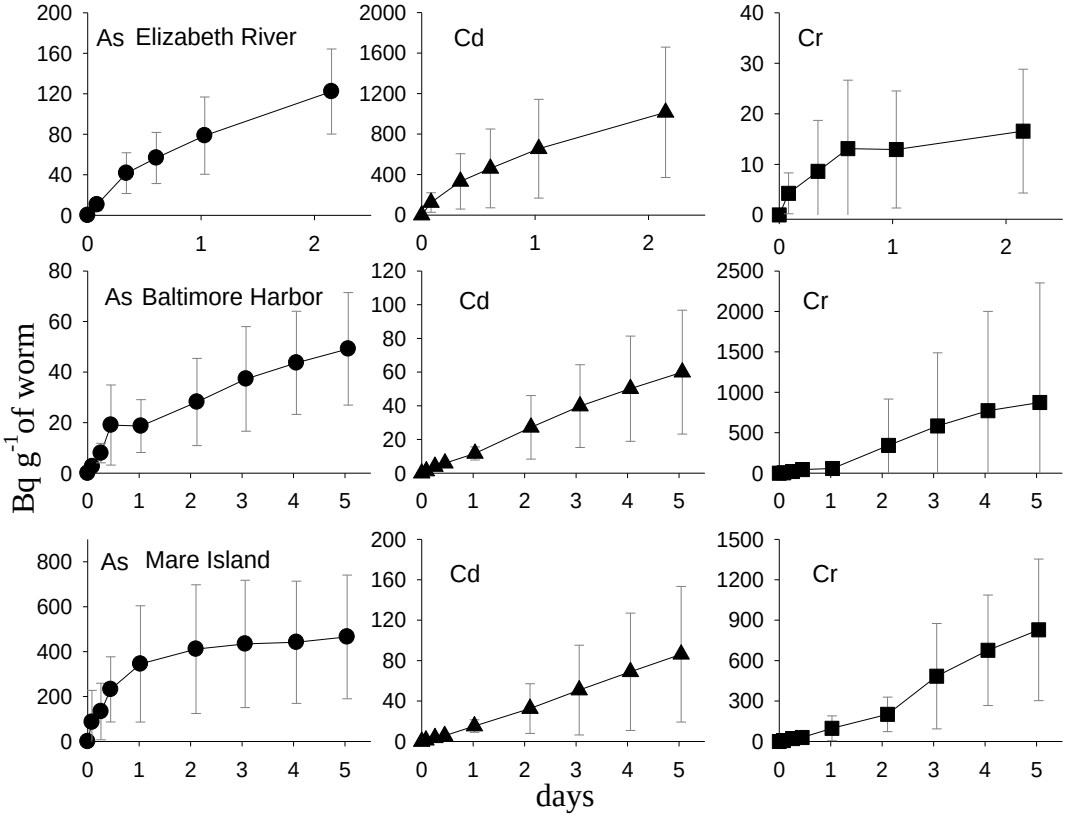


Fig. 1. Accumulation (Bq g^{-1}) of ^{73}As, ^{109}Cd, and ^{51}Cr from pore water by *N. succinea* over time. Data points denote means (n = 5 – 7) ± 1 SD.

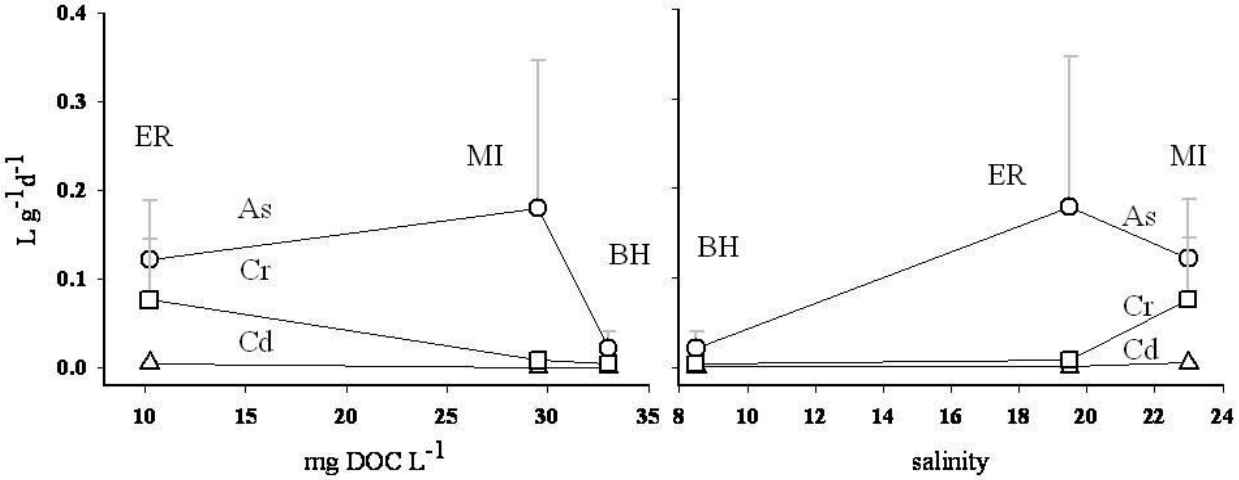


Fig. 2. Relationship between metal uptake rate constants (k_u, L g^{-1} d^{-1}) in *Nereis succinea* exposed to radiolabeled pore water and the dissolved organic carbon concentration in the pore water or the salinity of the pore water. Pore waters were extracted from field collected sediments from Baltimore Harbor (BH), Elizabeth River (ER), and Mare Island (MI). Data points denote means (n = 5 – 7) + 1 SD.

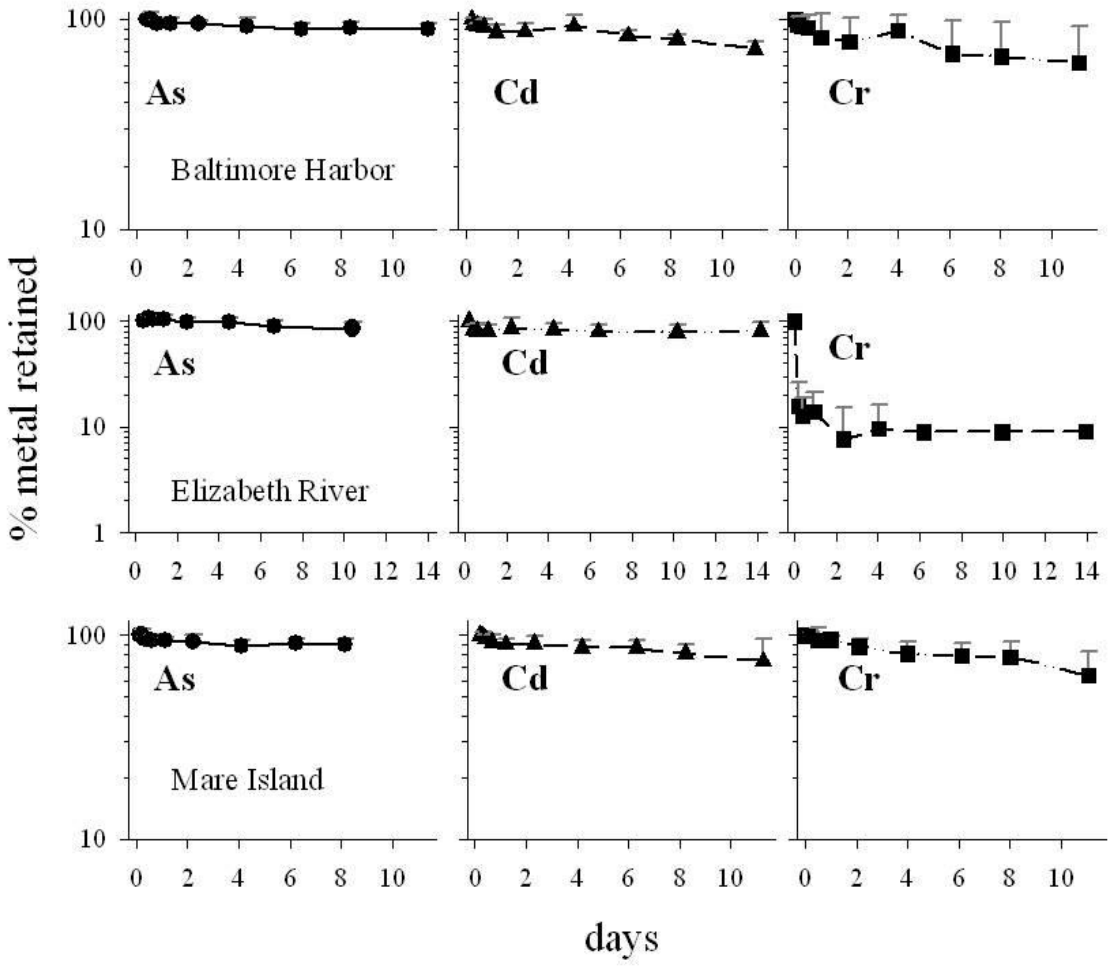


Fig. 3. Percent metal retained by *N. succinea* during depuration after uptake from pore water and 10^{-4} M seawater (from BH, ER, MI) solution of EDTA rinse. Data points denote means (n = 5 – 7) + 1 SD.

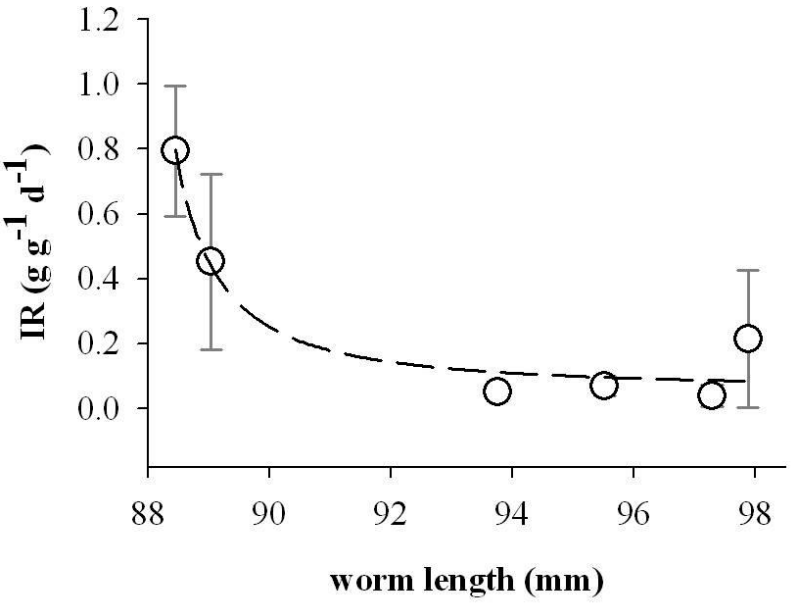


Fig. 4. Specific ingestion rates (g dry sediment g^{-1} dry wt worm d^{-1}) of *N. succinea* as a function of worm length. Mean value is 0.27 g $g^{-1}d^{-1}$; data points denote means of fecal matter collected over three time points (n = 3) ±1 SD. When error bars not visible SDs were very small.

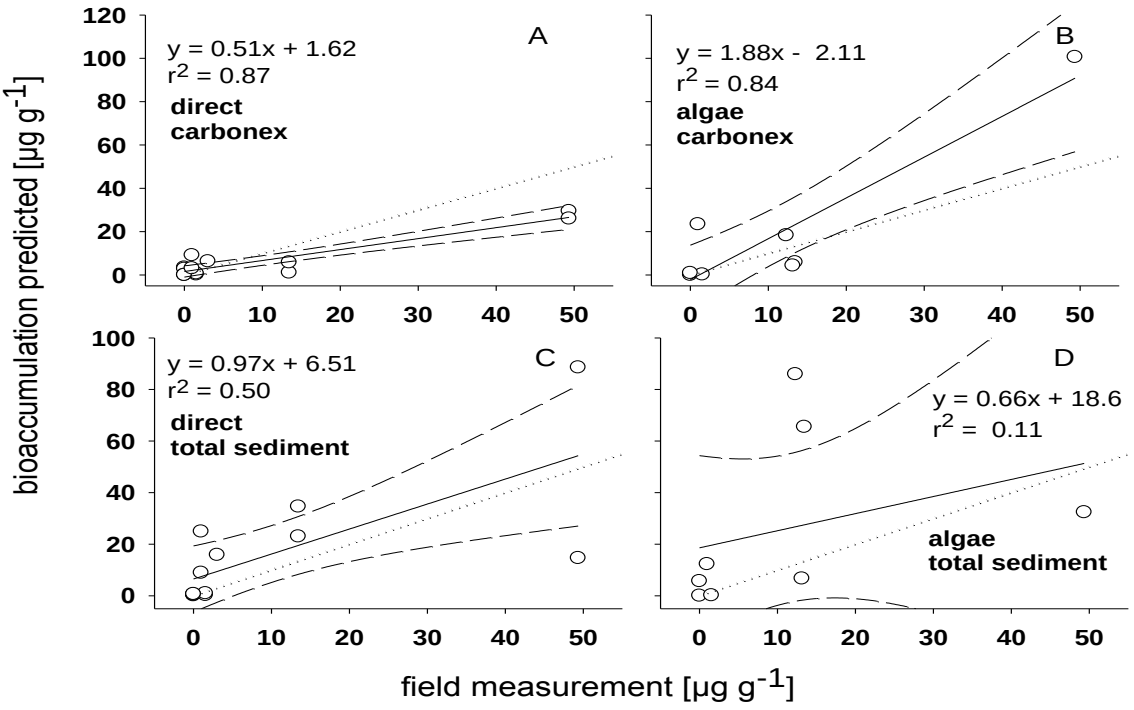


Fig. 5. Regressions (solid line) between field observed metal concentrations in polychaetes and model-predicted metal concentrations in worms for As, Cd, and Cr. Regressions for model predictions using carbonex pool of sedimentary metals (A, B) and model predictions using total metal concentration in sediments (C, D) from ER, BH and MI. Panels A and C show the model predictions for directly labeled sediment and B and D show model predictions using sediments mixed with radiolabeled algae. Dashed lines indicate 95% confidence intervals of the regression line; 1:1 lines are dotted.

Chapter V: *Factors influencing the assimilation of arsenic in a deposit-feeding polychaete*

ABSTRACT

I investigated mechanisms leading to assimilation of particle-bound Arsenic (As) ingested by the deposit-feeding polychaete *Nereis succinea* using a radiotracer approach. As release from different particle types into gut fluid and bovine serum albumin (BSA), a gut fluid mimic, was measured. In addition, gut fluid proteins were analyzed by separating proteins via 2D gel electrophoresis, and protein peptide sequences were determined by mass spectrometry. Major ions in the gut fluid were measured by ion chromatography and metals by mass spectrometry. Percentages of particulate As release were related to As assimilation efficiencies (AEs) in polychaetes feeding on different particle types. AEs of As were highest from radiolabeled pure algae (72%) and radiolabeled algae added to sediment (51%), lower from radiolabeled sediment (10%), and lowest from a radiolabeled iron oxide mineral, goethite (2%). It appears that As release from particles is a necessary but not sufficient requirement of As assimilation. For example, 15% of As was released from goethite into the gut fluid but only 2% was assimilated by *N. succinea*. Our results suggest that the likelihood of As assimilation is higher when it is bound to an organic compound of nutritional value in the ingested particles.

INTRODUCTION

Given the importance of sediments serving as a source of metal contaminants for benthic invertebrates, I investigated processes that may influence metal assimilation in a deposit-feeding polychaete, *Nereis succinea*. Polychaetes can serve as conduits of metals from contaminated sediments to benthic predators such as bottom-feeding fish, which in turn can be consumed as seafood. I have recently shown that metals are acquired principally from ingested sediment in *N. succinea* (Baumann and Fisher 2011a). Previous work concluded that a metal's solubilization from ingested sediment into gut fluid is necessary for that metal to be assimilated across the gut lining (Chen and Mayer 1998). Building on this earlier work, I conducted a series of experiments with sediment-bound arsenic to relate the release of As from ingested particles in polychaete guts to its assimilation in polychaete tissues. Baumann and Fisher (2011b) showed that sedimentary As can be assimilated with efficiencies of ~72% from ingested organic particles in *N. succinea*. In the present study, I consider the form of the As in the ingested particle and the chemical composition of *N. succinea* gut fluid in influencing the assimilation of this metalloid. Arsenic is a nonessential element that can be enriched in estuarine sediments resulting from natural sources as well as coal combustion and industrial activities (Cullen and Reimer 1989). It has been shown to accumulate in high concentrations in estuarine sediments and in resident benthic fauna (Chen et al. 2000; Cullen and Reimer 1989; Fattorini et al. 2005; Fattorini et al. 2006) and has been implicated in risk assessments as a priority pollutant in many coastal sediments (Jones et al. 1999).

It can be argued that metals bound to ingested particles, including sediment particles for deposit-feeding invertebrates, must first be released from that particle into the gut fluid of an animal before it can cross the gut lining and become incorporated into tissues (Chen and Mayer 1998). Release of metals from sediment particles may be dependent on the characteristics and composition of the sediments and on the chemical composition of the gut fluid. Further, the metal must be released in a form that is able to cross the membranes of the gut lining. A biomimetic approach, in which a solution of bovine serum albumin (BSA) is used here to mimic natural gut fluid for a given animal, is one tool that has been used to better understand the mechanisms governing dietary assimilation of ingested metal. With this approach, the relationship between the metal released from sediments into solution and its assimilation efficiency (AE) in *N. succinea* was evaluated by comparing solutions of BSA, chloride and BSA,

unamended deionized water, and natural *N. succinea* gut fluid as extractants of As from natural sediments and goethite.

A biomimetic approach was proposed by Mayer et al. (1996) and applied by others in studying metal and organic contaminant release from sediments for deposit-feeding invertebrates (Voparil and Mayer 2004; Weston and Maruya 2002). The biomimetic approach provided a measure of metal release from ingested sediment particles into a deposit-feeder's gut, inferring that this was the metal pool that could be available for uptake across the gut lining. It was developed based on an observed relationship between release of sediment-bound metals and organic contaminants into the gut fluid of a deposit-feeder *Arenicola marina* and its release into a solution of a commercially available protein - BSA. BSA was shown to influence the solubilization of some sedimentary metals similar to the gut fluid of a deposit-feeding polychaete *A. marina* (Mayer et al. 1996). Experiments involving sediment incubations with gut fluid and BSA led to the conclusion that proteins, amino acids, and other abundant organic ligands in gut fluid can be effective in binding metals and perhaps transporting them across the gut lining, consistent with an animal's "objective" of assimilating nutritious organic compounds (Mayer et al. 1997). A biomimetic approach that relies on BSA is attractive because it is very difficult to obtain sufficient quantities of gut fluid from polychaetes.

Our research evaluated the use of BSA as a substitute for natural gut fluid of *N. succinea* to study the release of As and its eventual assimilation in this polychaete. To study the extent of sedimentary As solubilization in gut fluid and in a BSA solution, I used a radiotracer approach that is well-suited for assessing rates of As transfer from particulate to dissolved phases and subsequent assimilation in polychaete tissues. Sediment from an industrial part of San Francisco Bay, Mare Island, was labeled by direct addition of ^{73}As or by mixing the sediments with ^{73}As-labeled algae. I also used ^{73}As-labeled goethite to represent the significant pool of As that is typically associated with iron oxides in sediments (Neff 1997). Radiolabeled sediments and goethite were incubated in a number of possible extractant solutions, including solutions containing BSA, as well as in natural *N. succinea* gut fluid to measure the release of particle-bound ^{73}As over a period representative of the gut transit time of ingested particles in this polychaete. The chemical composition of the gut fluid was determined and compared to that of BSA. As noted above, some previous studies considered the release of metals from ingested

particles into natural or simulated gut fluid, while others assessed the assimilation of ingested metals bound to sediments. However, studies that have combined these approaches to provide a mechanistic explanation of measured assimilation of metals are generally lacking. Here I consider observations of arsenic release from sediments and its assimilation in a polychaete and identify relevant chemical properties in the gut that may help explain these observations.

MATERIALS AND METHODS

Experimental species

A surface deposit-feeding polychaete *Nereis succinea*, which is found ubiquitously in muddy sediments along the US coastline, was chosen as the experimental species. *N. succinea* has been used in the past for laboratory studies due to its well known ecology and physiology (Ahrens et al. 2001a). This species has also been examined in a series of metal bioaccumulation studies involving contaminated estuarine sediments (Baumann and Fisher 2011b; Wang et al. 1999). Polychaetes from a local salt marsh at Flax Pond on Long Island NY were hand-collected in summer, placed in individual containers with a small portion of sediment, and transported to the laboratory. Individual worms were rinsed off and placed in individual containers filled with Flax Pond seawater (salinity ~28) to eliminate any undigested sediment remaining in their guts prior to the experiment.

To obtain the gut fluid samples for pH, protein and amino acid analyses, worms were anaesthetized by immersion in 10% $MgCl_2$ after gut clearance was complete. Individual worms were blotted and placed on a clean glass slide. The body wall was cut open along the body length, and coelomic fluid was removed by a gentle application of a cotton swab. After the coelomic fluid was removed, the gut fluid was pulled towards the foregut by a gentle upward rolling of a glass rod. This resulted in formation of a "bulb" in the frontal region of the gut. The bulb was poked with a sharp needle and ~5-50 microliters (depending on the individual worm) of gut fluid was withdrawn by an automatic pipette and immediately transferred into an Eppendorf tube set on ice. Gut fluid from individual worms was pooled together into one tube, later filtered with 0.2 µm PVDF (polyvinylidene fluoride) protein non-binding membranes, and stored at -20°C for further procedures.

Gut fluid analyses

Bacteria

Three samples of 1 µm Nuclepore filtered gut fluid were fixed with 2% borate-buffered formalin, filtered via 0.2 µm black Nuclepore filter and stained with a fluorescent dye DAPI (4'6-diamidino-2-phenylidole), according to Sherr et al. (2001). DAPI stain was excited by ultraviolet light when bacterial cells were observed and bacterial cells were counted using epifluorescence on an inverted microscope (Leica DM IRB).

Total concentration of gut fluid proteins and amino acids

Filtered (0.2 µm PVDF) gut fluid of *Nereis succinea* was analyzed for dissolved amino acids. Three 20 µl gut fluid samples were subjected to 6N HCl for 22-24 h at 110°C under N_2 to hydrolyze the proteins. These samples were analyzed for concentrations of individual amino acids by HPLC (Lindroth and Mopper 1979; Mayer et al. 1995). I was not able to measure the concentration of cysteine due to the lack of adequate standard. The concentration of total proteins in extracted gut fluid and in solutions of bovine serum albumin (for the protein degradation experiment) was measured (Bradford 1976) by determining the absorbance of dissolved protein bound to Coomasie Blue stain measured at 595 nm with a Micro Plate Reader (Perkin Elmer, Wallac Victor2 1420 Multilabel Counter).

Analysis of proteins in extracted gut fluid required a prior protein precipitation with 100% Trichloroacetic acid (TCA) with the final TCA v/v concentration of 10%. A solution that included the precipitated proteins was centrifuged at 16,000 *g* for 15 min. The supernatant thought to include only the small peptides containing <7-14 amino acids in their chain (Mayer et al. 1995) was removed, and the pellet was resuspended in a solution of protease inhibitor cocktail (Enzo Life Sciences, KI-103) to prevent enzymatic protein degradation. Protein degradation in the gut fluid sample was observed on gels (sodium dodecyl sulfate polyacrylamide gel electrophoresis or SDS-PAGE) generated in the preliminary stage of this research. Mostly low molecular weight proteins from the gut fluid samples were initially detected on silver stained SDS-PAGE gels. This suggested that the gut fluid proteins had undergone degradation between the gut fluid extraction times and the time of its analyses. Gut fluid **s**amples were stored at 4°C before running the two dimensional electrophoresis (2-DE).

Qualitative protein analysis: Sample preparation, two-dimensional electrophoresis(2-DE),mass spectrometry and data analysis

Salts and other contaminants where removed from the worm gut extract solution using the Bio-Rad ReadyPrep™ 2-D Cleanup Kit (cat# 163-2130). The resultant precipitated protein was dissolved in first dimension rehydration buffer (ST_{50}) consisting of 7 M urea, 2 M thiourea, 4% 3-[(3-cholamidopropyl) dimethylammonio]-1-propanesulfonate (CHAPS), 0.2% Bio-Rad Biolytes (isoelectric point or pI range = 3-10) and 50 mM dithiothreitol (DTT) and the protein concentration determined using a modification of the Peterson (1977) method that incorporates additional 6% TCA washes to remove DTT, which interferes with the assay.

Two-dimensional electrophoresis (2-DE) was conducted essentially as described in the Bio-Rad ReadyStrip immobilized pH gradient (IPG) strip instruction manual (Bio-Rad catalogue number 163-2099). Briefly, IPG strips (11 cm, pI range 3-10, Bio-Rad catalogue number 163-2014) were passively equilibrated under mineral oil for 18 h at 23 ºC with 47 µg of solubilized protein in 200 µl ST_{50}. The IPG strips were subsequently washed by dipping into deionized H_2O and blotted with filter paper upon which they were transferred to a Protean IEF (Bio-Rad) focusing tray and laid gel side down across wet paper wick covered electrodes and covered with mineral oil. Further, they were focused in a Bio-Rad Protean IEF Cell at 20 ºC, rapid ramp 0-250 V for 1 h; slow ramp 250V-8000 V for 1 h; hold 8000 V to a total of 35000 Vh and subsequently removed from the focusing tray, blotted with moist filter paper and placed gel side up in channels of a clean rehydration/equilibration tray, covered and stored at –80 ºC.

The thawed strips were equilibrated at 23 °C for 10 min in equilibration buffer (EB) composed of 6 M urea, 50 mM Tris-HCl, 2% sodium dodecyl sulfide - SDS, 20% glycerol with 2% (w/v) DTT at pH = 8.8. The equilibration buffer was decanted off and the strip was equilibrated (10 min, 23 °C) in EB with 2.5% (w/v) iodoacetamide. The strips were transferred to IPG wells of pre-cast Criterion 8-16% polyacrylamide gels (Bio-Rad catalogue number 345-0105) and overlaid with agarose. The gels were resolved under constant 200 V for 55 min. The slab gels were stained with 100 ml of SYPRO® Ruby Stain according to the Bio-Rad instruction manual and imaged with a Bio-Rad VersaDoc™ 3000 Imaging System.

Gel spots were cut out, destained, reduced, alkylated and digested with trypsin (Promega Gold, Mass Spectrometry Grade) essentially as described by Shevchenko et al. (1996) with

minor modifications. The resulting concentrated peptide extract was diluted into a solution of 2% acetonitrile (ACN), 0.1% formic acid (FA) (buffer A) for analysis. For solution digest, 10 µl of purified protein was diluted in 40 µl of 50 mM ammonium bicarbonate. The proteins were reduced with 2 mM DTT and alkylated with 4 mM iodoacetamide for 30 min each. Trypsin (0.25 µg) was added to the samples and the digests were incubated for overnight at 37 °C. Protease reactions were stopped with 100% formic acid (final concentration 5%). Ten µl of the peptide mixture was analyzed by automated microcaplillary liquid chromatography-tandem mass spectrometry. Fused-silica capillaries (100 µm inner diameter - i.d.) were pulled using a P-2000 CO_2 laser puller (Sutter Instruments, Novato, CA) to a 5 µm i.d. tip and packed with 10 cm of 5 µm Magic C18 material (Agilent, Santa Clara, CA) using a pressure bomb. Ten µl of the resulting 20 µl concentrate was pressure-loaded onto a 10 cm 100 µm i.d. fused-silica capillary packed with 3 µm C18 reverse phase (RP) particles (Magic, Michrome), which had been pulled to a 5 µm i.d. tip using a P-2000 CO_2 laser puller (Sutter Instruments). This column was then installed in-line with an Eksigent Nano 2D High Performance Liquid Chromatography (HPLC) pump running at 300 nL/min.

Peptides were loaded with an autosampler directly onto the column and were eluted from the column by applying a 30 min gradient from 5% buffer B to 40% buffer B (98% acetonitrile, 0.1 % formic acid). The gradient was switched from 40 % to 80 % buffer B over 5 min and held constant for 3 min. Finally, the gradient was changed from 80 % buffer B to 100 % buffer A over 0.1 min, and then held constant at 100% buffer A for 15 more minutes. The application of a 1.8 kV distal voltage electrosprayed the eluting peptides directly into an LTQ Orbitrap XL ion trap mass spectrometer equipped with a nano - liquid chromatography electrospray ionization source (Thermo Finnigan). Full mass spectra (MS) were recorded on the peptides over a 400 to 2000 *m*/*z* range at 15,000 resolution, followed by one tandem mass (MS/MS) event triplet on the two most intense ions, each triplet containing of an HCD (higher energy collision dissociation) scan, a CID (collision induced dissociation) scan in the Fourier Transform (FT) at 7,500 resolution and a CID scan in the ion trap of the same ion. Charge state dependent screening was turned on, and peptides with a charge state of +2 or higher were analyzed. Mass spectrometer scan functions and HPLC solvent gradients were controlled by the Xcalibur data system (Thermo Finnigan, San Jose, CA). MS/MS spectra were extracted from the RAW with ReAdW.exe (http://sourceforge.net/projects/sashimi).

The resulting mzXML file contains all the data for all MS/MS spectra and can be read by the subsequent analysis software. The MS/MS data was searched with Inspect (Tanner et al. 2005) against a database containing peptide sequences for annelids (3932 protein sequences downloaded on December 1, 2009) plus common contaminants and *E. coli* proteins (2777 protein sequences total) with modifications: +16 on methionine, +57 on cysteine. Only peptides with at least a p-value of 0.01 were analyzed further. In addition, MS/MS data were also analyzed by de novo analysis using PepNovo (Frank and Pevzner 2005). Only hits with a score of >8 were considered for further manual analysis. Possible peptide sequences were used for fasts searches against an NCBI database (downloaded 10/25/2009) (Mackey et al., 2002).

Chemical composition of gut fluid

Worms used for gut fluid extraction and later for ionic composition and trace metal analysis were not anesthetized by 10% $MgCl_2$ recognizing that ionic and metal concentrations in gut fluid could be altered by physiological changes that occur in worm bodies upon exposure to $MgCl_2$. Gut fluid was analyzed for anions (Cl^-, SO_4^{2-}), cations (Mg^{2+}, Ca^{2+}, K^+, Na^+), trace metals (Cr, Ni, Cu, Zn, As, Se, Rb, Cd, Ba and Hg) and pH. The anionic composition was assessed using an AS4A-SC column with 5 mM sodium tetraborate decahydrate as eluent and the cationic composition using AS4A column with 1.7/1.8 sodium carbonate/sodium bicarbonate as eluent at the flow rate of 1 ml min^{-1}. A sample of gut fluid for the metal analyses was acidified and stored in a trace metal-clean plastic container. Metal analyses were performed using inductively coupled plasma mass spectroscopy (ICP-MS) on three replicates gut fluid aliquots from the larger pooled gut fluid volume. The pH of these gut fluid samples was measured with an optical pH sensor using seawater as a buffer Zhu et al. (2006) .

Degradation of BSA when mixed with natural sediment

I conducted an experiment to test if the proteins at a concentration similar to those in gut fluid are rapidly degraded when in contact with sediment. Possible protein degradation was monitored for 24 hours. BSA fraction V at a starting concentration of 10% was diluted to a final concentration of 3.8 mg mL^{-1}, as determine by the Bradford assay described above. Three replicates of 15 mL of BSA solution were mixed with ~1 g (wet weight) of sediments from three different locations: Baltimore Harbor, Elizabeth River from Chesapeake Bay and Mare Island

from San Francisco Bay. The control consisted of BSA solution without added sediment. Falcon tubes with all treatments were incubated at 21°C on a shaker table to assure mixing of the solution with sediment throughout the incubation. Three liquid samples – 100 µl each, were withdrawn for protein concentration analysis after 1, 2, 4, 6, 8 and 24 hours of incubation and injected into a 96 well plate, previously filled with 200 µl per well of Bradford reagent for protein staining. Well plates were inserted into a Micro Plate Reader (Perkin Elmer, Wallac Victor 2 1420 Multilabel Counter) and absorbance was measured at 595 nm. Protein concentrations were calculated based on a standard curve prepared for a series of BSA concentrations (0, 125, 500 and 1500 mg/ml). A new standard curve for BSA concentration was prepared at every sampling time.

Application of radiotracer approach

Radiolabeling of sediments and goethite

Sediment collected from Mare Island was uniformly radiolabeled by two methods with the gamma-emitting radioisotope ^{73}As ($t_{1/2}$ = 80.3d) as arsenate. One labeling method involved direct addition of 7.7 kBq/g (dry wt) of ^{73}As dissolved in 0.1 M HCl to ~2 g wet wt of sediment; the acid from the stock solution of radioisotope was first neutralized by addition of microliter quantities of dilute NaOH prior to radiolabeling so that the pH of sediment was not changed. Portions of sediment (18.3 ± 9.7 mg dry wt) were then transferred into individual tubes for later incubation. The second labeling method involved addition of (32 Bq/g dry wt) previously radiolabeled diatom biomass to the sediments. To produce the radiolabeled diatoms, a culture of the centric diatom *Thalassiosira pseudonana* (clone 3H) was grown in f/2 medium, except phosphate which was at the level of f/20 (or 3.6 µM) (Guillard and Ryther 1962) prepared with surface seawater (salinity of 35) collected 8 km offshore of Southampton (Long Island, NY). This water was inoculated with nutrients and 185 kBq of ^{73}As /L of. Algae were grown to stationary phase and were harvested by filtration onto a 3.0 µm Nuclepore filter. Approximately 22 mg (dry wt) of algal biomass was removed from the filter and mixed into 2 g (wet wt) of Mare Island sediment. Small sediment portions (3.9 ± 1.7 mg dry wt) were transferred from this mixture into individual tubes. Radiolabeled goethite was prepared by suspending dry goethite (from Sigma-Aldrich, goethite ~35% Fe; EC No. 2437464)) in seawater (salinity 35) to which ^{73}As was added to produce goethite containing 10.8 kBq ^{73}As/g (dry wt).

Incubation of radiolabeled sediments

Three different solutions were used as sediment extractants. One of the solutions was gut fluid, one was 3.8 mg bovine serum albumin (BSA) per liter of deionized water, and one was 3.8 mg bovine serum albumin (BSA) suspended in 1 liter of 20 g/L sodium chloride solution; a control consisted of deionized water. In our approach to mimic anoxic conditions in a deposit-feeder's gut, I purged the deionized water (used to prepare the chloride and BSA solutions) with N_2 for 1 h to remove dissolved oxygen. The concentration of BSA was similar to the protein concentration measured in the gut fluid of *Nereis succinea*. Gut fluid used for the experiment was filtered with a 0.2 µm PVDF filter to remove particles such as small sediment grains or microbial cells but not dissolved proteins.

Radiolabeled sediments and goethite were incubated with the solutions for up to 4 h on a shaker table (at 200 rpm) placed inside a glove bag that was purged with N_2. A minicentrifuge and all the tools needed for the liquid collection at the end of incubation times were also placed inside the glove bag so that no oxygen would affect the incubated samples. A volume of 100 µL of each of deionized water, BSA dissolved in deioinized water, BSA dissolved in chloride solution, and gut fluid was collected after 20, 60, and 240 min of incubation. Three replicates (200 µL) for each solution were taken. Because of difficulty in obtaining sufficient gut fluid from *N. succinea,*it was feasible to use three replicates of 200 µL of gut fluid only at the end of the 4 h incubation with sediment labeled directly, sediment mixed with radiolabeled algae, and radiolabeled goethite. Sampling times were chosen to observe the rate of ^{73}As release from the sediment into each solution that could occur in the gut of *N. succinea*.

Based on our observation of *N. succinea* in the laboratory under these conditions, I estimated the retention time of sediment in the gut to average 4 h. At each sampling time, three replicates containing substrate + solution were centrifuged at 734 *g* for 5 min to separate liquid from solids. One hundred µL of the liquid was pipetted out of each sample and transferred into separate tubes for determination of radioactivity.

Measuring radioactivity

To assay their radioactivity, samples were inserted into plastic counting tubes and analyzed using a Pharmacia-Wallac LKB gamma spectrometer equipped with a well-type NaI

(Tl) detector. Radioactivity of ^{73}As was detected at 53 keV and counted for 5 min per sample, yielding propagated counting errors typically < 5%.

Statistical analyses

To determine significant differences between treatments in the incubation experiments, one-way ANOVAs were performed using PASW 18.0 software.

RESULTS

Bacteria, proteins and amino acids in gut fluid

Protein analysis using two-dimensional electrophoresis isolated several proteins in the extracted gut fluid (Fig. 1). Eight protein spots were further analyzed by LC/MS/MS to deduce the sequences from these proteins. Due to the limited protein sequences of *N. succinea* in publicly available databases, *de novo* sequencing of peptide fragmentation patters was attempted. Possible peptides for each protein spot were further analyzed by matching the sequences to the NCBI database by using fasts (Mackey et al., 2002). For two spots (spot 8 and spot 9) I was able to align the possible peptide sequences to protein sequences (Fig. 1). Both aligned proteins, alkaline serine protease from *Alteromonas* for spot 8 and fibrinolytic protease P-III-1 from *Eisenia fetida* (common brandling worm) for spot 9 are proteases, which are normal constituents in gut fluid. However, based on the peptide sequences I was not able to determine if the analyzed proteins are from *N. succinea* or gut bacteria. This can only be done with further experiments. Bacterial cell counts revealed that the gut fluid of *N. succinea* contains on average 1.15×10^8 cells mL^{-1}, therefore, future studies addressing gut fluid proteins originating from bacteria would be worthwile.

The amino acid composition of the gut fluid of *N. succinea* is given in Table 1. Isoleucine was the most concentrated amino acid in the gut fluid (0.39 mg L^{-1} or 15.9% of total measured amino acids), followed by alanine (14.2%) and glutamic acid (9.8%); γ-aminobutyric acid was least concentrated (0.001 mg L^{-1}, or 0.04% of total amino acids). The total concentration of measured amino acids was 20.6 mmol L^{-1} or 2.46 mg L^{-1} (Table 1).

Ions, metals and the pH

Measurement of gut fluid pH using the optical pH sensor showed that the gut fluid is neutral (pH =7.15 ± 0.08; Fig. 2). Ion chromatography analyses of *N. succinea*'s gut fluid

showed that its ionic composition was not similar to seawater except for chloride (471.8 ± 0.7 vs. 546 mmol/kg) (Table 2). Concentrations of other ions (Ca^{2+}, Na^{+}, Mg^{2+}, SO_4^{2-}) were 4.5 - 5.7-fold higher (10-fold for K^{+}) in gut fluid than in seawater (Table 2; Bruland and Lohan 2004). Metal concentrations measured for total body and gut fluid alone indicated that only Cr had similar concentrations in whole worms and gut fluid. Other elements such as Ni, Ba, As, Hg and Se were up to 7.7 times more concentrated in the whole worms, and Cu, Rb, Cd and Zn were much higher (up to 66.2-fold) in the whole worms compared to gut fluid (Table 3).

Sediment incubation experiments

There was no evidence that protein in the BSA degraded notably to peptides consisting of less than 5-10 amino acid units during 24 h incubation with sediments from all 3 estuarine sites (Fig. 3).

Mare Island sediments, labeled by direct injection of ^{73}As or by addition of radiolabeled algal debris, and radiolabeled goethite, released different amounts of ^{73}As into the different extractants over 4 h period (Fig. 4). At the 4 h time point, the fraction of As released into solution was significantly different between gut fluid and control for sediment labeled directly (one-way ANOVA, $p = 0.05$), and between gut fluid and all other solutions for goethite (one-way ANOVA, $p \leq 0.02$). No significant difference (one-way ANOVA, $p > 0.05$) was found between either of BSA solutions and the control solution. The percentage of As released into the gut fluid from sediment labeled by mixing with radiolabeled algal debris was highest (33.7 ± 11.7%), lower for directly labeled sediment (17.4 ± 5.7%) and for goethite (14.7 ± 3.0%). Mean AEs for As in *N. succinea* from ingested sediments (Baumann and Fisher, 2011) were higher than the release of As into any solution except for natural gut fluid.

Percentages of As assimilated by *N. succinea* and that released from goethite into water (control), BSA, or chloride + BSA solutions were not significantly different from one another, however the amount of As released into gut fluid was 18.4, 18.4 and 15.5-fold higher than the other three extractions, respectively (Fig. 4). AEs for As from sediment mixed with algae were highest (50.7 ± 9.0%), lower for sediment labeled directly (10.2 ± 6.8%) and lowest from goethite (2.4 ± 0.7%). The AE of As in *N. succinea* feeding on sediment mixed with radiolabeled algae was significantly different from As released from this substrate into water, BSA in distilled water, and BSA + chloride solutions (one-way ANOVA, $p < 0.0001$) but not different from the

percentage released into gut fluid (one-way ANOVA , $p > 0.05$). Sediments mixed with radiolabeled algae released 5.2, 2.1 and 4.2 times more ^{73}As into gut fluid than into control, BSA solution in distilled water, or BSA chloride solution, respectively.

DISCUSSION

This research aimed to identify key factors affecting As assimilation in the gut of the deposit-feeding polychaete *N. succinea*. First, it was evident that As assimilation can vary depending on the composition of the diet with which As is associated. Assimilation efficiencies of ingested As are much lower from diets in which As is attached to goethite and to particles within the largely inorganic sediment than from sediment enriched with fresh algal organic matter with which As is associated (Fig. 5; Baumann and Fisher 2011b). Second, As release from particles into gut fluid varies among particle types in parallel with AEs. Comparison of AEs and the percentages of As released from unamended sediment and from goethite shows that AEs are either similar to or lower than its release into gut fluid, suggesting that some of the As released from goethite and unamended sediment into gut fluid is not assimilable. This is especially evident for goethite, where As AE was < 3% compared to 15% of As released into the gut fluid. The assimilability of As clearly depends on its association with the particle types that are ingested, such that As bound to inorganic particles (e.g., goethite or minerals in unamended sediment) is much less assimilable by *N. succinea* than As bound to fresh algal organic matter (e.g. arsenosugars in algal cells mixed with sediment) (Andreae and Klumpp 1979; Edmonds et al. 1997). This is further supported by findings that As AEs from pure algal cells (not mixed with sediments) reach 72% for *N. succinea* (Baumann and Fisher 2011b). It would appear that As bound to assimilable organic compounds that are released into gut fluid can be efficiently assimilated. Thus, dissolution of As from ingested particles into gut fluid is insufficient on its own to explain assimilation. This conclusion is consistent with the idea that metals must be released into gut fluid before they can be assimilated into animal tissues. It is also consistent with the idea that animals eat to acquire nutritious organic compounds; if metals are bound to those compounds they are transported across gut linings into the tissues of the animal and become assimilated.

Mayer et al. (1997) argued that gut matrices of deposit-feeding animals containing surfactants and organic ligands are “designed” to solubilize essential nutrients, including

compounds to which inorganic and organic contaminants may be bound. This solubilization can lead to elevated contaminant concentrations in the gut fluid of deposit-feeders (Chen et al. 2000). Consistent with this concept, Roditi et al. (2000) reported that metals bound to dissolved organic compounds were assimilated by the zebra mussel, *Dreissena polymorpha*, than the metals that were not complexed by organic matter. Previous studies also showed that metals like Cu and Zn that are biologically incorporated into fish prey lead to higher body burdens and higher toxicity in these fish than metals in inorganic forms (Clearwater et al. 2002).

The higher fraction of ^{73}As released into *N. succinea* gut fluid than in BSA solutions suggests that not just any (e.g., BSA) dissolved proteins can explain As release from particles. Factors that may influence the release of metals from ingested particles into a soluble form within the gut include enzyme activity, surfactancy of gut fluid, and possibly anoxic or reducing conditions within the gut. The latter, for example, may lead to release of As from Mn and Fe oxides.

In Mare Island sediment 40% of the As was extracted within the operationally defined fractions such as the *organic matter*, 25% in *acid volatile sulfides - AVS*, and 11% in *Fe/Mn oxides* (Baumann and Fisher 2011b). Though these fractions are operational we might expect that among other potentially extracted minerals and compounds As was extracted with refractory organic matter – in the *organic* extractions, and iron oxides that were likely present in the *AVS* and *Fe/Mn oxides* fractions. It is plausible that solubilization from this sediment into the gut fluid would occur for As by complexation with organic matter in the gut fluid, which is supported by Redman et al. (2002) according to whom As – natural organic matter interaction aids As release from particles in nature. For oxide-bound As (such as in goethite) As is mobilized by their partial dissolution in a reducing environment. The reducing environment of *N. succinea*'s gut fluid is likely due to organic matter decomposition (Griscom et al. 2002; Mayer et al. 1997; Plante and Jumars 1992). A study conducted with deposit-feeding clams (*Macoma baltica*) showed that ~25% of ingested Cd was released from oxic estuarine sediments into *M. baltica*'s gut fluid in parallel with release of Fe(II) during passage of ingested sediment in the anoxic gut (Griscom et al. 2002); the acidity (pH 5.5) of the clam gut was shown not to impact this Cd release.

A study (Chen and Mayer 1998) examining the mechanisms of Cu release from contaminated sediment into the holothuroid *Parastichopus californicus* gut fluid found that gut

fluid proteins (as well as proteins in a BSA solution) influenced dissolution of sediment-bound Cu by complexing with this metal. Another study showed that the presence of the enzyme proteinase K influenced the dissolution of Cu, Al, Fe, Pb and Zn (but not As and Mn) from sediment *in vitro* (Turner 2006). Fan and Wang (2003) studied the effects of gut fluid (in this case, of a sipunculan polychaete) as a Cd extractant relative to seawater at pH 8 and pH 5, and seawater plus surfactant (1% sodium dodecyl sulfonate), finding that natural gut fluid was more effective in mobilizing sediment-bound Cd. In contrast to Cd, all solutions were inefficient in extracting sedimentary Cr (Fan and Wang 2003). The minimal dissolution of sediment-bound Cr into gut fluid is comparable to this metal's low assimilation efficiency (usually < 5%) in *N. succinea* and other benthic invertebrates (Lee and Luoma 1998; Wang et al. 1997).

In some cases, seawater has been used as a control to compare the metal extraction capability with gut fluid (Fan and Wang 2003; Lawrence et al. 1999). However, the ionic composition of gut fluid can differ from that of ambient seawater, as shown for *N. succinea* in Table 2. For example, concentration of K^+ is an order of magnitude higher, and Ca^{2+}, Mg^{2+}, Na^+ and SO_4^{2-} are 4.5 and 5.7-fold higher in the gut fluid than in seawater. Moreover, there is an imbalance of charges measured in gut fluid, with cation concentrations being much higher than those of anions. It is therefore plausible that the negative charge of some organic compounds in the gut fluid – e.g., acidic polysaccharides, could balance these inorganic cations if present in high enough concentration (100's of mM; L. Mayer personal communication). The concentration of acidic polysaccharides in the gut fluid of *N. succinea* remains unknown. Concentrations of Ca and Mg in the gut fluid of a sipunculan polychaete were 13 and 7 times higher, respectively, than in *N. succinea*, and 56 and 37 times higher than concentrations in seawater (Fan and Wang 2003). Compositional differences in gut fluid among species may be due to differences in ionic regulation, as described for sulfate and sodium in the marine snail *Aplysia californica* (Gerencser and Levin 2000). Unlike the variability in ionic composition of gut fluids among marine invertebrates, the pH of deposit-feeder gut fluids tends to reflect the pH of the ingested sediment and pore water (Ahrens et al. 2001a; Lopez 2005; Plante and Jumars 1992). Most aquatic invertebrates have a gut fluid pH of 6-8, although the guts of bivalves tend to be more acidic (pH = 5-6) (Lopez 2005). Small pH variations in deposit-feeding polychaetes have been observed due to body length, with greater acidity of gut fluid in juvenile worms (Ahrens et al. 2001a).

Total metal body burdens and concentrations in gut fluid measured in sipunculans, *A. marina,* and *N. succinea* differed by up to three orders of magnitude (Chen et al. 2000; Fan and Wang 2003). The most striking differences are among the gut fluid to tissue concentration ratios in *A. marina* and *N. succinea*. For example, in the study by Chen et al. (2000), *A. marina* gut fluid was much more enriched with metals in relation to its tissues, whereas the opposite was evident for *N. succinea,* where tissues had slightly higher metal concentrations (present study). Reasons for differences between these two polychaetes are not immediately apparent. *A. marina* generally ingests sandy sediments whereas *N. succinea*, ingests finer sediment grains (personal observation; Longbottom 1970). Because sandy sediments are generally less reactive for metals than fine-grained sediment, metals could be expected to desorb more readily from sandy sediments and be found in the gut fluid of *A. marina*. Furthermore *A. marina* individuals are larger than *N. succinea* individuals, and the ingestion rate of deposit-feeding polychaetes is a function of their body volume (Penry and Jumars 1990). Hence the absolute rate of sediment ingestion should be higher in *A. marina.* This higher ingestion rate translates into larger volume of sediment being subjected to gut conditions. It is also noteworthy that sand ingesting deposit-feeders have higher ingestion rates than those feeding on more fine particles (G. Lopez, personal communication). Therefore, in worms ingesting more sediment, potentially greater amount of metal can be released from particles into the gut fluid. This scenario is plausible if the kinetics of metal release from the particles into the gut fluid are faster than the gut transit time. Also, the surface-to-volume relationship in *A. marina* is lower than in *N. succinea.* Therefore absorption of released metal from gut fluid across the gut lining into the tissues of *N. succinea* may therefore is expected to be greater than for *A. marina*.

The abundance of proteinaceous compounds in the gut fluid sampled from a variety of deposit-feeding polychaetes (but not *N. succinea*) has been described by Chen et al. (2000). Proteinaceous compounds have been suggested to complex metals in the gut fluid once they are released from ingested sediment particles. BSA, substituting for natural gut fluid proteins, suggested the importance of proteins for binding a variety of metals and organic contaminants deposit-feeding polychaete guts (Chen and Mayer 1998; Kalman and Turner 2007; Voparil and Mayer 2004). BSA has been shown to have a large capacity to dissolve substantial amounts of sediment-bound metals, but as shown in the present study, BSA can dissolve only a small fraction of sedimentary As, much less than the amount of As released into the gut fluid of *N.*

succinea. Concentrations of proteins in the gut fluid of *A. marina* are an order of magnitude higher than protein concentrations measured in the gut fluid of *N. succinea* (Voparil and Mayer 2004). The concentrations of BSA used in incubation experiments described here and elsewhere were adjusted to be comparable to the total protein concentrations in gut fluids of *N. succinea* and *A. marina* (present study; Chen and Mayer 1998; Voparil and Mayer 2004). Inter-experimental differences in metal solubilization capacity in BSA solutions may be attributable in part to differences in protein concentrations used in each experiment and also due to differences in BSA affinities towards different metals and metalloids.

TABLES

Table 1. Amino acid gut fluid composition determined by HPLC; values represent the mean ± one standard deviation (n = 3 samples; gut fluid pooled from 16 individuals).

Amino Acid (AA)	gut fluid (GF)			BSA[1]		
	mean ± SD [mmol L^{-1}]	mean ± SD [mg L^{-1}]	% of total AA in gut fluid	[g g^{-1}]	% of total AA in BSA	BSA : GF
Alanine	3.891 ± 0.231	0.35 ± 0.02	14.2	0.082	6.6	0.5
Arginine	1.244 ± 0.017	0.22 ± 0.00	8.9	0.069	5.6	0.6
Aspartic Acid	1.466 ± 0.385	0.20 ± 0.05	8.1	0.102	8.2	1.0
γ-Aminobutyric Acid	0.010 ± 0.017	0.001 ± 0.002	0.04	-	-	-
Glutamic Acid	1.641 ± 0.199	0.24 ± 0.03	9.8	0.024	2.0	0.2
Glycine	2.984 ± 0.239	0.22 ± 0.02	8.9	0.029	2.3	0.3
Histidine	0.714 ± 0.151	0.11 ± 0.02	4.5	0.135	10.9	2.4
Isoleucine	2.955 ± 0.649	0.39 ± 0.09	15.9	0.0356	2.9	0.2
Leucine	1.161 ± 0.055	0.15 ± 0.01	6.1	0.179	14.4	2.4
Lysine	0.758 ± 0.538	0.11 ± 0.08	4.5	0.195	15.7	3.5
Methionine	0.119 ± 0.206	0.02 ± 0.03	0.8	0.000	0.0	0.0
Phenylalanine	0.488 ± 0.042	0.08 ± 0.01	3.3	0.103	8.3	2.6
Serine	1.281 ± 0.018	0.13 ± 0.00	5.3	0.052	4.2	0.8
Threonine	0.941 ± 0.102	0.11 ± 0.01	4.5	0.067	5.4	1.2
Tyrosine	0.257 ± 0.022	0.05 ± 0.00	2.0	0.089	7.2	3.5
Valine	0.693 ± 0.011	0.08 ± 0.00	3.3	0.078	3.3	1.0
Σ AA	*20.6* mmol L^{-1}	*2.46* mg L^{-1}		1.24[2]		

1-Bovine Serum Albumin (BSA) data is derived from (Shi et al. 2006); 2-Total mass of amino acids is >1.0 g due to recovery efficiency error

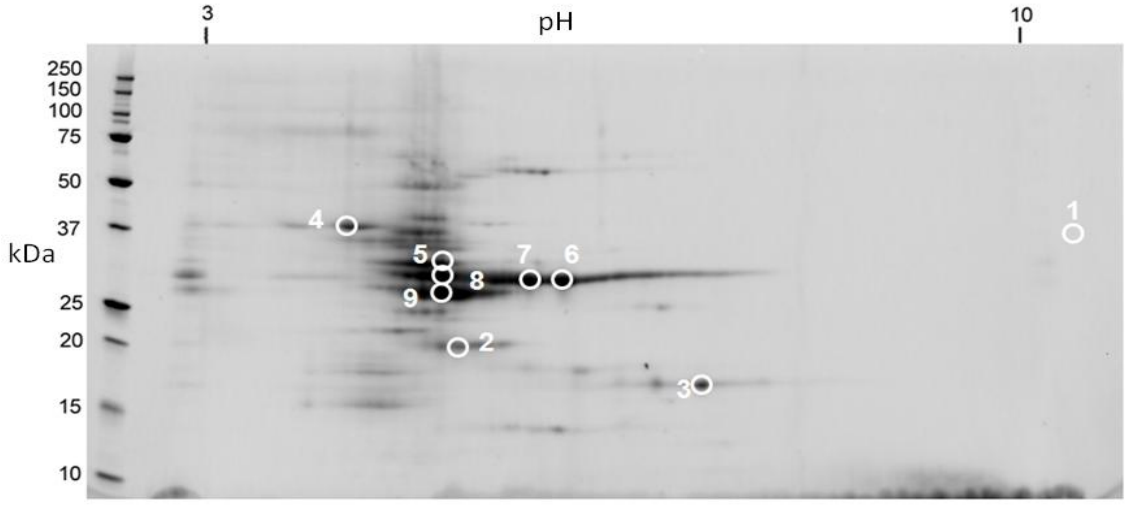
Table 2. Ionic composition of *N. succinea*'s gut fluid (present study), open ocean seawater (Bruland and Lohan 2004) and their ratio; error indicates one standard deviation (n =3 replicates of gut fluid).

ions	mmol kg^{-1}		gut fluid to seawater ratio
	gut fluid	Seawater	
Cl^-	471.8 ± 0.7	546	0.9
SO_4^{2-}	159.0 ± 1.0	28	5.7
K^+	102.8 ± 0.9	10.2	10.1
Na^+	2271.8 ± 3.5	468	4.9
Ca^{2+}	45.9 ± 0.7	10.3	4.5
Mg^{2+}	266.4 ± 3.9	53	5.0

Table 3. Concentrations (dry wt) of metals in whole worms (µg g^{-1}) and in gut fluid (µg g^{-1}) pooled from several 16 individuals of *Nereis succinea*.

metal	whole worm	gut fluid
Cr	1.72 ± 0.92	1.54
Ni	2.87 ± 1.00	1.62
Cu	75.3 ± 12.5	2.60
Zn	394 ± 47	5.95
As	11.4 ± 0.64	3.55
Se	2.01 ± 0.19	0.26
Rb	3.18 ± 0.17	0.10
Cd	1.56 ± 0.79	0.03
Ba	0.44 ± 0.27	0.19
Hg	0.06 ± 0.01	0.01

FIGURES



Possible sequences for separated proteins:
SPOT 1 (blank): AGALCNN, AGEHSSG, AGEYLAMK, ATGGDLNAALER, ATVSLPR, DGLNAALER, ETVVLGHVDSEER, FASEMSR, GSAGENR, HLQLALR, KALTLFY, LAQGVQLVDGFTK, LASYLDK, LATVSLPR, LDGLFKT, LGSEEGR, QALDVFY, QLGQALR, QLGQALR, SGGSLNR, TLLDLDNTR, TSGLELEG, VVTVSLPR, YTVSLPR; SPOT 2: AGGEEVFVGR, DQAFGLK; SPOT 3: VGGDGAVYEGR, TLTLVTR, YSFDDLPK; SPOT 4: LEGEESR, YYLTGNAR, YSFDDLPK; SPOT 5: STLWGLSR, YLHDTSLR; SPOT 6: ENAGEDPGLAR (**dermicidin – sample contamination**), YLHDTSLR, GSLGGGFSSSK, SYDTTDGAGVR, SYDTTDGAGVR; SPOT 7: TTHQDFGGR, YLHDTSLR (**serine protease II**); SPOT 8: R(-17)AATLVAVK, GSQDTGSR, GTVGGGEFGLAK, LELSELNR, LGT(-18)SDTATSTK, LKEWYEK, LTHDDFGGR, RLSFSLGGSR, STLWGLSR, TSDTATSTK, VLGVGSALLNSLR, VYVLDTGVR, YLHDTSLR (**serine protease II**); SPOT 9: **ASGYAGVYAR**, LSLSTSGGS, **TDACQGDSGGPLVVK**, VYLHDTSLR, YEDLAQK (**fibrinolytic protease**).

Fig. 1. Molecular mass distribution of gut fluid proteins on two dimensional SDS-PAGE (gel electrophoresis); mark spots of gel that were sampled, digested and analyzed by HPLC. Each spot yielded several amino acid sequences (specified below the photo).

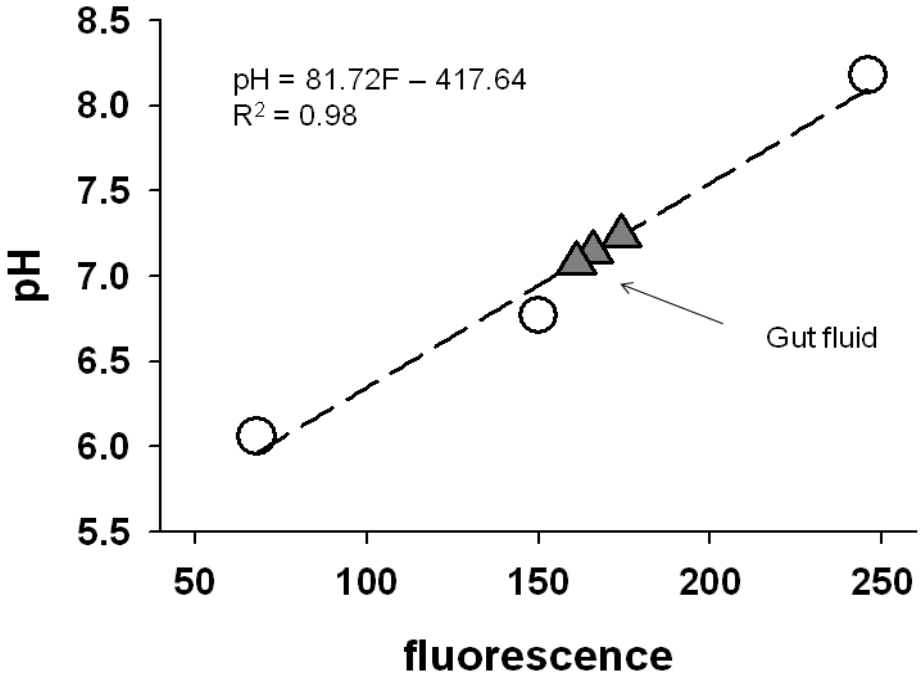


Fig. 2. *Nereis succinea* gut fluid pH (triangles) determined by an optode using seawater as buffer (Zhu et al. 2006); dotted line is the standard curve for samples with known pH (circles); in the pH equation F stands for fluorescence ratio.

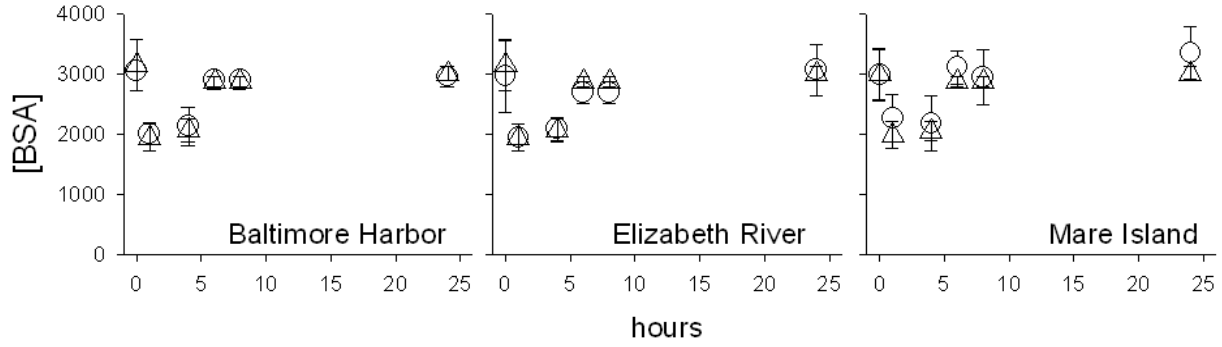


Fig. 3. Bovine serum albumin concentration during its incubation with natural sediments; circles indicate BSA concentration in solution mixed with sediment and triangles indicate BSA concentration in control; there were no significant (one-way ANOVA, $p<0.05$) differences between the treatments and control at any time point.

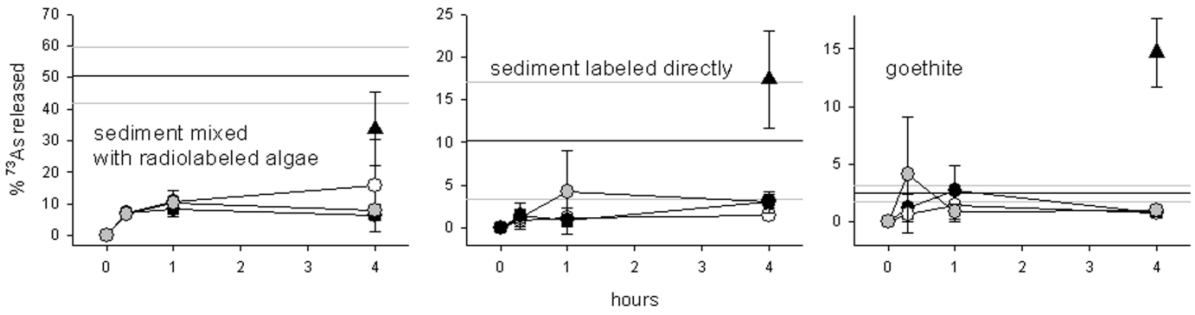


Fig. 4. Release of ^{73}As from sediments that were radiolabeled directly and mixed with radiolabeled algal debris or from radiolabeled goethite in control treatment (distilled water; black circle), BSA solution in distilled water (open circle), BSA solution in 20 g L^{-1} chloride solution (grey circle) or gut fluid extracted from *Nereis succinea* (black triangle); and ^{73}As assimilation efficiency from Baumann and Fisher (2011; mean indicated by a black horizontal solid line ± 1 standard deviation indicated by grey solid horizontal lines above and below the mean).

Chapter VI: *Conclusions*

My thesis addressed the bioavailability of the metalloid arsenic (V) and the metals cadmium and chromium (III) to surface deposit-feeding polychaetes *Nereis succinea*. The core of this work relied on experimental approaches that have been developed in the Fisher laboratory at Stony Brook University and in the Mayer laboratory at the University of Maine. My work focused on understanding the factors influencing bioavailability of metals from dietary sources, which are the dominant metal source for many aquatic animals, including deposit-feeders such as *N. succinea*. Conclusions drawn from my results are summarized in Table 1.

Research on metal bioavailability and bioaccumulation has been largely motivated by concerns about metal toxicity to aquatic animals and to humans who may be exposed to elevated concentrations of toxic metals through seafood consumption. Coastal sediments are known to be especially contaminated with toxic metals, and may serve as enriched sources of these metals for benthic animals, including deposit-feeding polychaetes.

Previously, much attention has been given to research on metal toxicity in animals exposed to metals dissolved in water and less so to metals in their diet. Work of Clearwater et al. (2002) has shown that metals accumulated by animals via diet can be toxic. Although acute and chronic toxicity are important to consider for the health of the species, metal bioaccumulation and its transfer to other animals higher in the food chain is of significance, because it might impair the health of these animals in higher trophic positions and also people who eat seafood. The number of studies addressing metal bioaccumulation from diet is increasing but the mechanisms of this bioaccumulation are not well known.

I have identified two general factors that could influence the bioavailability of ingested As, Cd and Cr in polychaetes and potentially other deposit-feeding invertebrates. The first is the geochemical partitioning of these elements to different types of sedimentary phases (e.g., organic matter or Fe/Mn oxides), while the second concerns the chemistry of the polychaete gut (e.g., ionic composition, pH, oxygen level, enzymatic activities or protein concentration). *N. succinea* was chosen as a model organism, because based on others previous research experiences it was

relatively easy to manipulate in the lab and easy to collect in the local salt marsh. Sediments were collected from three different sites to evaluate the impacts of contrasting sedimentary geochemistries on the bioavailability of ingested As, Cd and Cr in *N. succinea*. I addressed several objectives to gain a more complete understanding of mechanisms and factors influencing metal bioavailability. These objectives were to:

- evaluate the fraction of As, Cd and Cr acquired in worms from water and from diet;
- determine the operationally-defined geochemical distribution of radioisotopes - ^{73}As, ^{109}Cd and ^{51}Cr that were added to experimental sediments;
- compare the extent to which As, Cd and Cr are assimilated *in vivo* from different diets;
- study the influence of the diet type on assimilation of As, Cd and Cr;
- evaluate the *in vitro* efficiency of sedimentary As extraction using natural gut fluid and commercially available protein – bovine serum albumin;
- relate the percent of As extracted by the gut fluid with As AEs in *N. succinea*.

These objectives were addressed by a suite of experiments using an overall application of gamma-emitting radiotracers. Uptake of aqueous metals in *N. succinea* was determined by exposing worms to radiolabeled pore water and measuring the amount of radioactivity in worms during the exposure. AEs of ingested metals from food were calculated based on results from pulse-chase feeding experiments that used radiolabeled diets (e.g., sediments, fresh algal biomass or goethite). The relative contribution of metals from dietary and water sources to the total metal concentration in worms was calculated using a biokinetic model and is described in chapter IV. Once it was obvious that diet – sediment was the main source of metal in the worms, I focused on determining the operationally-defined geochemical distribution of radiotracers in radiolabeled sediments using a sequential extraction scheme. Another set of experiments used natural gut fluid from *N. succinea* and BSA solution as a biomimetic of the gut fluid to assess the extractability of As from sediments.

The suspected mechanism of dietary metal bioavailability of ingested metals demands two major conditions to be fulfilled. First, particle-bound metals must be released into the gut fluid of the animal, and second, these metals released into the gut fluid need to be present in a

form that is biologically available for transport across the gut lining. Metal release from particles was hypothesized to be reflective of the metals' geochemical partitioning in sediment or its partitioning to particular compartments in algal cells when the diet included algae. It is known that different mineral phases and organic materials could have different affinities for a given element based on their thermodynamic preferences. It is also known that due to the thermodynamics, metals bind with sedimentary fractions based on the availability of preferred binding sites in these fractions. For example if sediment is contaminated with As and if this sediment is primarily made of pyrite, organic matter and clay, then the binding sites for As are unlimited at either of these fractions. One could anticipate that in sediment As would likely be found in association with pyrite, because it has a greater affinity for pyrite than for organic matter. If the sediment however contained mostly organic matter and clays and only a small fraction of pyrite so that the binding sites in pyrite would be limited, then arsenate would be expected to associate with the pyrite phase, and the remainder of it would bind to organic matter. The strength of metal binding to a specific sedimentary particle type would result in different amounts of metal released into solution. This release would depend on the presence of other organic and inorganic compounds, including diverse ligands in the gut fluid. For oxygen sensitive phases and metals (e.g., Fe and Mn oxides and metals associated with them), metal release would also be dependent on the difference between the oxygen conditions in the ingested sediment and the gut. Metals bound to pH sensitive phases, such as carbonates and some weakly crystallized Fe oxides, could be released if the pH of the gut fluid was low enough.

For metals in ingested algal cells, depending on their cellular compartmentalization (e.g., cell wall vs. cytoplasm), solubilization of metal could be influenced by the presence of gut fluid ligands, ionic strength of the solution or the presence of surfactants. If the ionic strength of the gut fluid is greater than the ionic strength of the sediment pore water (when diet consisted of sediment mixed with algae), the metals associated with the external site of the algal cell wall could be released into solution and eventually bind to another ligand. Greater ionic strength of the gut fluid could also lead to rupturing of the cell membranes leading to spilling of the cytoplasm and other cellular organelles and hence to spilling of the metals that were contained inside the cells. Similar to the ionic strength, surfactancy could also lead to breakage of the cell membranes. Surfactants are known as membrane phospholipid solubilizers leading to the disruption of the cell membrane integrity and finally to release of the cell contents. This property

of surfactants – especially sodium dodecyl sulfide or SDS is widely used in biochemical research.

Previous studies have shown that metals associated with the cytoplasmic fraction of algal cells tend to be assimilated but metals that bind to cell surfaces are not (Reinfelder and Fisher 1991). It has also been suggested that metals that are biologically transformed into organic compounds in fish prey are more assimilable by fish (Clearwater et al. 2002). In chapter III of this thesis I also described the cellular partitioning of As, Cd and Cr in cells of the diatom *T. pseudonana*, and related partitioning of these metals to assimilation in worms feeding on pure algal debris. One of the conclusions in that chapter is that As, which is predominantly associated with the inside of the cells and presumably with organic compounds such as arsenosugars, is highly assimilable (72%) in *N. succinea*, whereas Cr is mostly bound to cell surfaces and is not likely assimilated by the worms (2.8%). My findings for the surface deposit-feeding polychaetes are consistent with past studies for other animals (Reinfelder and Fisher 1991).

It is critical to recognize that metals bind to diverse organic compounds. Organic compounds in living and decomposing cells are different from other organic compounds (e.g., humic and fulvic acids) that have been diagenetically modified. During the degradation and reworking of the organic matter some of it is not readily degradable and can be preserved in sediments. While fresh and more readily degradable organic matter is easier to extract and classify into specific compounds (e.g., proteins, sugars and lipids), the reworked humic and fulvic acids (extracted with NaOH and H_2SO_4) represent operationally-defined compounds. These can vary with respect to hydrophobicity or aromacity. Chromium represents the type of metal that has a high chemical affinity for dissolved organic matter (Fukushima et al. 1995). Other metals that are dissolved in the water are first taken up by organisms and are then biologically transformed into organometallic compounds inside the cells. An example is As, which in algal cells can be transformed into its methylated congeners and arsenosugars. To examine how algae-associated metals are extracted by the sequential extraction procedure, I performed an experiment using pure radiolabeled algae (chapter II). This revealed that metals associated with fresh algal organic matter are indeed almost entirely extractble prior to reaching the step designed to extract the organic fractions. I observed that algal As is significantly more

assimilable than algal Cr, potentially because the two metals bind to different organic compounds in cells.

The main rationale for determining radiotracer partitioning patterns among the operationally-defined fractions was to relate them subsequently to assimilation efficiencies in polychaetes feeding on radiolabeled sediment (chapter II). Instead of using published *in situ* values of the fractionation of As, Cr and Cd in marine sediments, I evaluated *in vitro* fractionation patterns of these metals using combined radiotracer and sequential extraction approaches. This work demonstrated that in sediments labeled directly ^{73}As was primarily associated with the two organic fractions, whereas ^{109}Cd was mostly associated with the exchangeable and in some cases with Fe/Mn oxide fractions. ^{51}Cr was initially (2 days) associated with the Fe/Mn oxide and AVS fraction, while in aged sediments (30 days) it was mostly found in the pyrite fraction. Interestingly, ^{73}As was never completely recoverable from labeled sediments; up to 25% remained as residue. Recoveries of ^{109}Cd and ^{51}Cr, on the other hand, were either complete (^{109}Cd =100%) or nearly complete (^{51}Cr = 98%). Overall, radiotracer distributions in sediments mixed with radiolabeled algae were comparable to those in sediments labeled directly. However, an important exception was that ^{73}As occurred in significantly higher concentrations in the combined operationally-defined *exchangeable* and *carbonate* pool in sediments mixed with algae than in directly labeled sediments. Radiotracers that were associated with pure algal biomass were extracted during the first three steps. Given that these extractants are originally designed to remove inorganic fractions (i.e., *exchangeable*, *carbonate* and *AVS*), it is inconsistent with the organic nature of algae associated metals. Operationally-defined fractions may therefore be of limited use, particularly when interpreting metal association patterns in sediments from shallow, productive waters, or when following phytoplankton bloom events.

Operationally-defined fractionation patterns of radiotracers in labeled sediments, were combined with estimates of AEs derived from pulse-chase feeding experiments. These data allowed assessing the bioavailability of sediment-associated radioisotopes in *N. succinea*. AEs were linearly regressed on the percentage of a given radiotracer in single or combined fractions, yielding statistically significant positive as well as negative relationships for some fractions. For example, metal AEs were negatively related to their concentrations in the pool combining *Fe/Mn oxides* and *AVS* (for all studied elements and for both labeling methods i.e., direct vs. via algae).

This relationship suggested that radiotracers associated with these sedimentary pools are less likely to be assimilated by worms. In contrast, radiotracers extracted in the exchangeable and carbonate pool (referred to as *carbonex*, chapter VI) showed a positive significant relationship with AEs in *N. succinea*, suggesting that radiotracers, which were weakly bound to sediments in these fractions are more assimilable by *N. succinea*. Being one of the central findings of this research, this relationship has implications for predicting the risks associated with metal bioaccumulation in deposit-feeding invertebrates.

Because metals associate with Fe oxides, I examined more closely the bioavailability of oxide-associated As, Cd and Cr in *N. succinea*. Given the previously noted inverse relationship between AEs and metal concentration in Fe/Mn oxides and AVS (chapter III), I conducted feeding experiments using a pure Fe oxide mineral, goethite. AEs differed substantially between As, Cd and Cr (chapter III). While average AEs for As were low (2.4%), AEs for Cd and Cr were surprisingly high (24 and 34%, respectively), suggesting differences between assimilation mechanism in *N. succinea* for these elements. I hypothesized that in order to be assimilable, metals first have to be released from ingested particles into the gut fluid of the worm. Once released from particles, metals also need to be in a form that can be transported across the gut lining (Chen and Mayer 1998). Chen and Mayer (1998) studied metal release from contaminated sediments by incubating them with natural gut fluid from deposit-feeding polychaetes and with a solution of bovine serum albumin (BSA). Adopting this approach, I used *N. succinea* gut fluid and BSA solutions to evaluate the release of ^{73}As from radiolabled sediments (directly or via algae) and goethite. In all cases, gut fluid was a more effective extractant of ^{73}As than BSA. Results from the incubation experiments showed that proteins, when dissolved in water or in sodium chloride, are insufficiently strong ligands to strip As from sediment particles. Furthermore, for goethite substantially more ^{73}As was released into pure gut fluid (15%) than measured as AE in *N. succinea* (2%), possibly because of the reducing gut environment (Griscom et al. 2002). This finding suggests that the release of ^{73}As from ingested particles is only one step governing the bioavailability this metal. The release of algal As from sediment into the gut fluid may be aided by gut surfactants and enzymes, which could destroy cellular membranes and spill the cell contents. To confirm this mechanism more research is needed. Algal As was found to be highly bioavailable, as reflected by the high measured AEs in worms feeding on algae labeled sediments (51-70%) and on pure algae (72%).

In summary, this work contributed toward a better understanding of metal bioaccumulation in benthic organisms. First, metals are mainly accumulated in worms from food and only ~2% are accumulated from water. Second, metals are more assimilable from the ”carbonex” pool, or from algae containing organically-bound As in the form of arsenosugars. The present experiments also showed that metal association with algal cell membranes is limiting their assimilation by worms. For example Cr, which predominantly associates with algal cell walls (~ 83%), shows very low AEs in *N. succinea* (~3%). Metals associated with operationally-defined fractions of AVS and Fe/Mn oxides generally represent the pool of non bioavailable metals, even though Cd and Cr AEs from goethite were unexpectedly high (>20%). Finally, a critical first step for metal bioavailability appears to be its release from particles into the gut fluid.

There are several limitations to the work I have conducted during this PhD. Perhaps the greatest limitation is the use of only one polychaete species to assess the bioavailability of As, Cd and Cr. Greater number of species could benefit this type of study by comparing two or more gut conditions. Some of these differences are discussed in chapter V. Secondly, in laboratory based studies animals may not behave as they would in the natural setting. Their feeding patterns and other physiological processes could be altered in the laboratory due to stress. However it is difficult or even impossible to quantitatively account for the differences caused by the stress. The sequential extraction of metals comes with a range of limitations that have been extensively discussed in peer reviewed literature (Martin et al. 1987; Nirel and Morel 1990). Nirel and Morel (1990) discouraged any further research that would rely on sequential extraction methodology due to a concern about the lack of leach selectivity and specificity for particular geochemical fractions. This issue can complicate interpretation of results from such extractions, therefore when interpreting results one should be sure to consider the operational character of extracted fractions. More accurate methods to determine specific mineral-metal associations could be used. Electron microscopy, x-ray absorption, and near edge structure spectroscopy could be more accurate, but the access to instrumentation required for these analyses may be a problem.

More studies that relate metal association with operationally-defined geochemical phases with their bioavailability should be undertaken. Due to stepwise mechanism of metal bioavailability i.e., particulate metal release into the gut fluid → metal uptake across the gut

lining, studies should continue focusing on understanding the mechanisms, conditions and kinetics of metals bound to specific particle types release into the gut fluid metals. Such studies have not been conducted for deposit-feeding polychaetes. Experiments evaluating the extent to which metals are released into the gut fluid should be complemented with an approach determining the rates of metal uptake by the gut lining as well as the chemical forms of released into the gut fluid metals. Such chemical analyses should be conducted in various regions of the gut because the biochemical and physico-chemical conditions change along the gut and can influence the chemical speciation of metals (Lopez 2005; Mayer et al. 1997; Plante and Jumars 1992).

It is further recommended that future studies evaluate the dependence of metal bioaccumulation on animal size. Metal assimilation is known to be related to food ingestion rate and gut residence time, which are functions of body size (Penry and Jumars 1990). It is also known that gut fluid pH in *N. succinea* is size dependent (Ahrens et al. 2001a), which may influence the bioavailability of ingested metals, especially those which are pH sensitive or which associate with pH sensitive mineral particles. Metal bioaccumulation could also be impacted by worm density in the sediment and the benthic community structure. Further studies should therefore include these factors as they may alter the sediment geochemistry and metal biological availability (Aller 1978).

While present studies for assessing metal bioavailability use live animals, there could be other possible approaches. Such approaches could use artificial membranes mimicking the gut walls of deposit-feeding invertebrates. To study toxic metal concentrations in the water, diffusive gradients in thin films (DGT) have been developed and tested by Zhang and Davison (1995), based on aqueous uptake models (e.g., Free Ionic Activity Model, FIAM and Biotic Ligand Model, BLM) through gill membranes. To study metal bioavailability in animal guts, suitable membranes would have to be immersed in the fluid mimicking chemical properties of a given animal's gut. Artificial membranes may alleviate the need for animal use on large scale bioaccumulation testing in the future.

Finally, more quantitative approaches are needed that rely on a mechanistic understanding of metal uptake in order to better predict metal bioaccumulation in benthic invertebrate communities. Future models should take into consideration uncertainties and

complexities due to biological, physiological and ecological differences for given group of organisms.

	Conclusions	Chapter			
		II	III	IV	V
1.	In most cases *Nereis succinea* accumulated As, Cd and Cr primarily from ingested diet			✓	
2.	Biokinetic model prediction for total metal body burdens and field measured metal concentrations in deposit-feeding polychaetes are positively related to the metal concentration in carbonex pool		✓	✓	
3.	Bioavailability of sedimentary metals is also positively related to their fraction extracted as exchangeable, carbonate, exchangeable + carbonate + AVS; and negatively related to metals extracted as Fe/Mn oxides, organic I, pyrite, AVS + Fe/Mn oxides, pyrite + residual		✓		
4.	Percentage of metals associated with carbonex in sediments decreases with time of the metal exposure (or sediment aging) to the sediment	✓			
5.	Radiotracers of As, Cd and Cr added can be extracted in different steps of the sequential extraction procedure - e.g., As mainly as organic I and II, Cd mainly as exchangeable, and Cr as AVS and pyrite	✓	✓		
6.	Radiotracers associated with algal cells can be extracted with chemical solutions designed to extract inorganic sediment fraction → implication in extractions conducted on surface sediments enriched with fresh decaying algal debris, benthic algae etc.	✓			
7.	^{73}As is not completely extractible from sediments during the sequential extraction procedure and might be associated with most refractory sediment fractions	✓			
8.	Overall, ^{73}As, ^{109}Cd and ^{51}Cr partition to similar sedimentary operationally-defined pools regardless of sediment geochemistry; the most consistent partitioning patters are for Cr	✓			
9.	For algae exposed to As, Cd and Cr, Cr is primarily associated with cell membranes, while As and Cd are partly associated with cell membranes and partly with cytoplasm		✓		
10.	High (72%) AEs of As in *N. succinea* feeding on pure algae might be related to As association of organic compounds inside the algal cell		✓		✓
11.	Bioavailability of As associated with algal mixed with sediment decreases with the time during which sediment is exposed to the decomposing algae		✓		
12.	Bioavailability of As is higher when As is introduced to sediment via algae rather than via sorption to the particles	✓	✓		
13.	Bioavailability of As is related to its release from particles into the gut fluid, which is not sufficient for this released As to be assimilated				✓
14.	Bioavailability of As associated with sediment particles for short periods of time is greater when sediments are less enriched with Fe and Mn, and more enriched with extractible (in sequential extraction procedure) Al		✓		
15.	Aqueous uptake of As in *N. succinea* is faster in less saline water and Cr uptake is slower in water containing higher concentration of DOC			✓	
16.	Ingestion rate of sediment in *N. succinea* is lower for bigger worms			✓	
17.	Ionic composition of gut fluid must be regulated due to observed differences between ionic composition of gut fluid and seawater (except for chloride)				✓

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