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Uptake pathway for Ag bioaccumulation in three benthic invertebrates exposed to contaminated sediments

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ABSTRACT: We exposed 3 benthic invertebrates, the clam *Macoma balthica*, the polychaete *Neanthes arenaceodentata* and the amphipod *Leptocheirus plumulosus*, to Ag-contaminated sediments to evaluate the relative importance of various uptake routes (sediments, porewater or overlying water, and supplementary food) for Ag bioaccumulation. Silver bioaccumulation was evaluated at 4 levels of sediment Ag (0.1, 0,3, 1,2 and 3.3 µmol Ag g^{-1}) and 2 levels of acid-volatile sulfide (AVS), <0.5 or ~40 µmol g^{-1}, and compared among food treatments with or without Ag contamination, or with different food rations. *L. plumulosus* were incubated for 35 d in the Ag-contaminated sediments after 3 mo of Ag-sediment equilibration, and *M. balthica* and *N. arenaceodentata* for 19 d after 5 mo equilibration. Ag bioaccumulation in the 3 organisms was significantly correlated with 1 N HCl-extractable Ag concentrations (Ag-SEM: simultaneously extracted Ag with AVS) in sediments. The Ag concentrations in porewater and overlying water were greatest in the sediments with least AVS, consistent with previous studies. Nevertheless, the amphipod and clam exposed to oxic sediments (<0.5 µmol AVS g^{-1}) accumulated amounts of Ag similar to those accumulated by organisms exposed to anoxic sediments (~40 µmol AVS g^{-1}), when Ag-SEM levels were comparable. The dissolved Ag source was important for bioaccumulation in the polychaete *N. arenaceodentata*. Amphipods fed Ag-contaminated food contained ~1.8-fold more tissue Ag concentrations than those fed uncontaminated food. As suggested in kinetic (DYMBAM) modeling studies, ingestion of contaminated sediments and food were the principle routes of Ag bioaccumulation by the benthic invertebrates during chronic exposure, but the relative importance of each uptake route differed among species.

KEY WORDS: Silver · Bioaccumulation · Uptake route · Acid-volatile sulfides · *Macoma balthica* · *Neanthes arenaceodentata* · *Leptocheirus plumulosus*

INTRODUCTION

Silver in aquatic environments has received attention in recent years because it is highly toxic to aquatic organisms even at trace levels, and widely distributed in the vicinity of industrialized areas (Luoma et al. 1995, Purcell & Peters 1998, Andren & Armstrong 1999). Silver is a geologically rare element, but is frequently found at elevated concentrations as a result of anthropogenic activities such as mining and smelting, and also due to the discharge of sewage (Bryan & Langston 1992, Sanudo-Willhelmy & Flegal 1992). In the water column, silver is readily adsorbed onto particles due to its high particle affinity (typical partition coefficient K_d of $10^{4.5}$ to 10^6); thus, sediments constitute a main repository for silver in marine and estuarine environments (Gorsuch & Klaine 1998).

The bioavailability and acute toxicity of water-borne Ag is relatively well documented (Hogstrand et al. 1996, Reinfelder & Chang 1999, Wood et al. 1999,

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Bielmyer et al. 2002, Bury & Hogstrand 2002). For example, models are available to assess site-specific acute Ag toxicity to fishes ('biotic ligand model': Janes & Playle 1995, Paquin et al. 2002), considering the influence of competitive cations for binding sites, Ag^+ complexing anions in water and gill physiology.

In contrast, bioavailability and toxicity are poorly understood for particle-associated Ag, particularly Ag in contaminated sediments. It is established that trace metals in contaminated sediments can be transferred to and adversely affect benthic organisms (Berry et al. 1996, Hornberger et al. 2001). Sediment geochemistry, species-specific biological attributes and experimental conditions have each been shown to affect metal bioavailability to benthic invertebrates, in different experiments (Di Toro et al. 1992, Hare et al. 1994, Lee et al. 2000a, 2001, 2004, Griscom & Fisher 2002), but the relative importance of each is not well known, nor is there agreement as to whether porewater or the sediments themselves are most important in determining metal bioavailability (Luoma et al. 1995, Hirsch 1998b, Berry et al. 1999, Lee et al. 2000a, 2001). The extent and routes of Ag transfer from sedimentary reservoirs into deposit-feeding organisms are especially poorly known.

One model that addresses bioavailability and toxicity of metals in contaminated sediments is equilibrium-partitioning as applied in the acid-volatile sulfide (AVS) normalization approach (Ankley et al. 1996). The model, and supporting studies (Ankley et al. 1996 and references therein), contends that pore waters are the primary source of metal bioavailability and hence toxicity. Metal bioavailability is greatly reduced (usually expressed as an absence of toxicity) when the molar concentration of AVS in sediments exceeds that of the simultaneously extracted metal concentration (SEM) of divalent metals (e.g. Cd, Cu, Ni, Pb and Zn), which can make a very insoluble metal-sulfides compound (Di Toro et al. 1992, Ankley et al. 1996, Berry et al. 1996, 1999). Therefore, the difference between molar concentrations of SEM and AVS ([SEM–AVS]) has been suggested as a tool for predicting metal toxicity in contaminated sediment (Ankley et al. 1996). Silver is mostly monovalent, so each mole of sulfide binds 2 mol of Ag to form Ag_2S. Therefore [Ag-SEM]/2 is used when Ag-SEM concentration is compared with AVS concentration.

A few studies with Ag (Rodgers et al. 1997, Hirsch 1998a, Berry et al. 1999, Call et al. 1999) have shown that acute mortality does not occur when there is AVS in excess of Ag-SEM ([Ag-SEM]/2 – [AVS] < 0). For example, Berry et al. (1999) showed that mortality increased with added silver in sediments from 2 locations, but in order to be toxic, one of the sediments required more silver on a dry weight basis than the other. Acute toxicity of Ag to marine amphipods correlated with the [Ag-SEM]/2 – [AVS] value or with porewater Ag concentration, but not with total Ag concentrations in the sediment. As is often the case with such experiments, exposure periods were relatively short and extreme Ag concentrations were necessary to induce acute toxicity (i.e. conditions were not suitable to evaluate chronic influences, such as dietary bioaccumulation, on bioavailability or chronic toxicity; Berry et al. 1999).

In contrast, microcosm studies or biokinetic models (Luoma et al. 1992, Wang et al. 1996, Munger & Hare 1997, Lee et al. 2000a, 2001) have demonstrated that dietary uptake can be a major source of metal bioaccumulation by various marine invertebrates, with minor influences from porewater, under conditions conducive to chronic metal exposure. This is important with regard to Ag, because mode of exposure under chronic conditions (dietary versus dissolved route) can result in Ag toxicity to invertebrates at exposures well below those predicted by acute toxicity tests (Hook & Fisher 2001), and can affect partitioning of Ag to different subcellular binding sites within organisms that influence the expression of toxicity (Berthet et al. 1992).

The relative importance of dissolved versus dietary Ag bioavailability in sediment-dwelling animals is not well known. Ag uptake rates from solution are among the fastest for trace elements (Reinfelder et al. 1998). Nevertheless, the modeling approaches used by Wang et al. (1996) and Griscom & Fisher (2002) suggested that food would be an important source for Ag uptake in mussels *Mytilus edulis* and clams *Macoma balthica*, if their assimilation efficiencies of Ag from natural diets were similar to those from phytoplankton. However, it has not been tested how either of the above results would apply to Ag bioavailability from complex sediments under chronic exposure conditions. Ag partitions to sediment particles with one of the highest K_d (partition coefficient) values among trace elements, and assimilation of Ag from sediments by bivalves, is more complex than from phytoplankton. Assimilation efficiencies from Ag-spiked sediments are typically lower than from phytoplankton (Griscom et al. 2000). Silver also forms one of the most insoluble metal sulfides (Ag_2S) in sediments (Berry et al. 1999), although Ag can be taken up from Ag_2S by mussel *Mytilus edulis* and clam *Macoma balthica* with an efficiency of ~15% (Lee et al. 2000a). Finally, Ag bioavailability during chronic exposure is not known for sediment-dwelling invertebrates other than bivalves, although the transfer of metals from sediments to benthic organisms can be affected by attributes that differ widely among species, such as feeding behavior, life habits, reproductive cycles, growth and size (Cain & Luoma 1990, Arifin & Bendell-Young 1997, Warren et al. 1998, Lee et al. 2000a).

Thus, while acute bioassays and modeling studies are effective approaches to forecasting potential influences on Ag bioavailability, neither have unambiguously resolved the biological or geochemical factors that might determine bioavailability during chronic exposure to contaminated sediments. In the present study we have chronically exposed 3 common estuarine species to realistically contaminated estuarine sediments containing contaminated food material and with very different geochemistry. We have directly evaluated the pathways (sediment, food and dissolved sources) by which these invertebrates accumulate Ag and compare the results to the alternative approaches. Thus, in one set of experiments, we compare the influences of biology, geochemistry and experimental conditions on the bioavailability of Ag.

We chose 3 benthic invertebrates with different biological attributes, but representative of coastal environments: the amphipod *Leptocheirus plumulosus*, the clam *Macoma balthica*, and the polychaete *Neanthes arenaceodentata*. These species have been widely used in many ecotoxicological studies using standard test protocols and can be easily obtained (DeWitt et al. 1992, Luoma et al. 1992, Lee et al. 2001).

MATERIALS AND METHODS

We conducted 2 sets of sediment bioaccumulation tests using the same Ag-contaminated sediment source but with different Ag-sediment equilibration times. Expt I employed the estuarine amphipod *Leptocheirus plumulosus* to measure Ag bioaccumulation from sediment that had been equilibrated for 3 mo. In Expt II, the bivalve *Macoma balthica* and the polychaete *Neanthes arenaceodentata* were used with sediment that had been equilibrated for 5 mo. Sediment equilibration time affects metal distribution between porewaters and particulate materials (Lee et al. 2004), and can skew the results of bioaccumulation pathway analysis, but logistical limitations required that the 2 experiments be conducted separately.

Sediment preparation. Experimental sediment that was naturally high in AVS (~40 µmol g^{-1}) was obtained from a mud flat near Palo Alto, San Francisco Bay, USA. The sediment was screened through 1 mm nylon mesh at the site and transferred to the laboratory. Mean particle size was 8.1Ø (phi) and mean sand, silt and clay content, analyzed by the pipetting method, was 0.4, 43.8, and 55.8 %, respectively. Loss on ignition (LOI) was determined at 450°C for 4 h to estimate organic carbon content.

In the laboratory, several batches of sediment slurry were made by mixing 10 l of the collected sediment with 10 l of deoxygenated seawater. Then the slurries were spiked with an adequate amount of 30 mM $AgNO_3$ stock solution (prepared in Milli-Q water) to yield total Ag concentrations of 0.1, 0.3, 1.2, 3.3 µmol Ag g^{-1} dry wt (denoted as M1, M2, M3, M4, respectively). Additionally, the sediment without Ag addition was used as a control (M0). The ranges of spiked Ag concentrations in the present study are comparable to and slightly higher than the Ag concentrations (0.01 to 0.6 µmol Ag g^{-1}) in the sediments of San Francisco Bay, USA (Luoma & Phillips 1988). The Ag-sediment slurry was kept under N_2-atmosphere and mixed mechanically several times a day for 1 wk. Following 1 wk of Ag-sediment equilibration, each sediment treatment was divided into 2 portions and manipulated to achieve either < 0.5 or 40 µmol AVS g^{-1} dry wt (denoted as oxic and anoxic sediment, respectively). The methods for the manipulation of AVS in sediments are described in detail elsewhere (Lee et al. 2000b). These sediment slurries with a range of Ag contamination and 2 AVS levels were allowed to equilibrate for up to 5 mo at 20°C.

Prior to each bioaccumulation test, 200 ml of wet sediment from each treatment was added to 1 l glass beakers and re-equilibrated with the overlying seawater (700 ml) for 5 d. On the 5th day of re-equilibration, the overlying seawater was replaced with clean seawater, and test organisms were added.

Test organisms. The sediment-dwelling infaunal amphipod *Leptocheirus plumulosus* was obtained from laboratory culture. This species has both filter-feeding and deposit-feeding habits (DeWitt et al. 1992). Amphipods were maintained in 15 psu seawater at 20°C, and fed with ground commercial fish food, TetraMin®, and the diatom *Phaeodactylum tricornutum.* The facultative deposit-feeding clam *Macoma balthica* was collected from the Palo Alto mudflat, San Francisco Bay, USA, 2 d prior to the bioaccumulation test and kept in the laboratory in 20 psu seawater at 20°C. The deposit-feeding polychaete *Neanthes arenaceodentata* was obtained 3 d prior to the bioaccumulation test from a laboratory culture and maintained in 30 psu seawater at 20°C. The clams were supplied with *P. tricornutum* and the polychaetes with TetraMin® during the acclimation period. The salinity of the experimental seawater was adjusted by diluting 0.2 µm-filtered seawater (35 psu) with Milli-Q water considering each test speces' optimal salinity range.

Bioassay procedure. The amphipod bioaccumulation experiment was designed to evaluate how Ag bioaccumulation is influenced by (1) spiked Ag levels (AM0 to AM4) in the anoxic sediment, (2) AVS levels in the sediments (AM4 versus OM4), and (3) Ag contamination levels in the supplementary diet (contaminated versus uncontaminated) (Table 1). Additionally, 4 treatments in anoxic sediments (AM0, AM2, AM3,

Table 1. Experimental design. Each treatment comprised 3 replicate containers for biological analysis and 1 for chemical analysis. UF, SF, $UF_{1/5}$: uncontaminated food, Ag-spiked food, and uncontaminated food with one-fifth of regular ration, respectively; AVS: acid-volatile sulfide (A = anoxic, O = oxic); AM, OM: anoxic and oxic metal treatments, respectively; M1–M4: spiked-Ag levels; –: no treatment

	Expt I (amphipod) *Leptocheirus plumulosus* 35 d, 15 psu, 20°C					Expt II (clam, polychaete) *Macoma balthica* 19 d, 20 psu, 20°C			*Neanthes arenaceodentata* 19 d, 30 psu, 20°C	
	TetraMin® (UF)		TetraMin® (SF)		TetraMin® ($UF_{1/5}$)	Algae (UF)		Algae (SF)	TetraMin®-sediment mixture	
AVS (µmol g^{-1}):	40 (A)	<0.5 (O)	40 (A)	<0.5 (O)	40 (A)	40 (A)	<0.5 (O)	40 (A)	40 (A)	<0.5 (O)
Sediment [Ag] (µmol g^{-1})										
M0 (Control)	AM0	–	–	–	AM0	AM0	–	–	AM0	–
M1 (0.1)	AM1	–	AM1	–	–	AM1	OM1	AM1	AM1	OM1
M2 (0.3)	AM2	–	AM2	–	AM2	AM2	–	–	AM2	–
M3 (1.2)	AM3	–	AM3	–	AM3	AM3	OM3	AM3	AM3	OM3
M4 (3.3)	AM4	OM4	AM4	OM4	AM4	AM4	OM4	–	AM4	OM4

AM4) were provided with one-fifth the regular uncontaminated food ration to evaluate the influence of food ration on Ag bioaccumulation. There were 15 treatments and each had 4 replicates (Table 1); 1 replicate from each treatment was used for geochemical analysis and 3 for amphipod exposure.

To initiate the bioassay, 20 juvenile amphipods that were retained on a 250 µm mesh after passing through a 450 µm mesh were transferred to each replicate beaker containing the appropriate Ag-contaminated sediment and overlying water. Amphipods were exposed to experimental sediments for 35 d at 15 psu and 20°C with a photoperiod of 16 h light:8 h dark. During exposure, the overlying water was continuously aerated, and half of the water was replaced with new seawater every other day, and supplementary diets were provided 1 d before water renewal. The food ration for each feeding was 1.5 mg ind.$^{-1}$ for the first 2 wk, and 3 mg ind.$^{-1}$ for the remaining period, in accordance with the amphipod's growth (US EPA 2001). The mean overlying water temperature, salinity, pH and dissolved oxygen concentration, monitored 3 times a week, were 20.4 ± 0.17°C, 15.2 ± 0.15 psu, 7.9 ± 0.11 and 7.3 ± 0.28 mg l^{-1}, respectively.

Similarly, clam and polychaete bioaccumulation studies were conducted separately and designed to evaluate how Ag bioaccumulation is influenced by (1) spiked Ag levels in the anoxic (AM0–AM4) and oxic (OM1, OM3, OM4) sediments, and (2) AVS levels (OM1, OM3, OM4 versus the respective treatment in the anoxic sediment at the same Ag level) in sediments (Table 1). Additionally, the influence of contaminated versus uncontaminated algal diets on Ag bioaccumulation by *Macoma balthica* was determined in the 2 anoxic sediments (AM1 and AM3). Clams were provided with 2.6 mg ind.$^{-1}$ d^{-1} of algae and worms with 1 mg ind.$^{-1}$ d^{-1} of TetraMin®. The bioaccumulation experiment for clams comprised 10 treatments and that for polychaetes 8 treatments. Each treatment had 4 replicates; 1 replicate from each treatment was used for geochemical analysis and 3 for animal incubation.

We placed 6 clams of similar size (24 ± 2 mm shell length; 79 ± 21 mg dry wt) or 6 individual polychaetes of similar size (4.0 ± 0.5 mg dry wt) on the surface sediment in each replicate beaker. Individuals that did not burrow into the sediment within 1 h were replaced with new individuals. The clams and polychaetes were exposed for 19 d in 20 psu (clam) or 30 psu (worm) seawater at 20°C. All the experimental conditions and water quality data were similar to those described earlier for the amphipod experiments, except for salinities (20.5 ± 0.5 psu for the clams and 30.4 ± 0.7 psu for the worms) and the supplementary food ration.

At the end of the experiment, test organisms were collected and depurated for 2 to 3 d in clean seawater. Then, the tissues were freeze-dried and digested in hot concentrated HNO_3 (Brown & Luoma 1995). The samples for geochemical analysis were taken twice, at the beginning and end of the experiment, from the beaker used for chemical analysis. Porewater samples were taken by centrifuging (3000 × *g* for 20 min) ~30 ml of sediment under nitrogen atmosphere (Bufflap & Allen 1995). The supernatant was filtered using a 0.45 µm syringe filter, acidified to pH ~2, and stored in a refrigerator until analysis. The overlying water was treated in the same way as the porewater, but without centrifugation. An aliquot of sediment (0.2 g dry wt) per treatment was also digested with concentrated HNO_3 (4 ml) for near-total extraction (Brown & Luoma 1995).

Supplementary food. The supplementary TetraMin® diet for *Leptocheirus plumulosus* was prepared by suspending finely ground TetraMin® in 500 ml of 15 psu

seawater that contained appropriate amounts of $AgNO_3$ stock solution to yield silver concentrations of 0.3, 1.0, 3.6, 10 µmol g^{-1} TetraMin®. Following 1 wk of equilibration at 4°C, the TetraMin® settled on the bottom of a flask was freeze-dried, ground and sieved through 250 µm mesh before use as food. Uncontaminated food was prepared by following the same procedure as that used for contaminated food, but without spiking Ag in the media. The Ag concentrations in the diets corresponded to ~3× the sediment Ag concentrations in the AM1 to AM4 treatments fed to the amphipods in the respective treatments.

Diatoms *Phaeodactylum tricornutum* were also used as supplementary food. Diatoms were grown in seawater with 9 or 160 nM Ag for 1 wk, which yielded Ag concentrations similar to those of the sediment (0.04 ± 0.02 µmol g^{-1} dry wt algae for AM1 and 0.6 ± 0.3 µmol g^{-1} for AM3 [N = 6]). During the 19 d exposure, clams in AM1 and AM3 were fed 20 ml of Ag-enriched algae (= 2.6 mg dry wt ind.$^{-1}$) per feeding occasion. The former algal diet was provided in the AM1 treatment and the latter in the AM3 treatment. Algal diets for uncontaminated food treatments were cultured without Ag spiking in the culture media.

For *Neanthes arenaceodentata*, 30 mg of ground TetraMin® was mixed with 2 g of Ag-contaminated sediment from each treatment and equilibrated for 4 d at 4°C. Since only a single food type was added to each sediment treatment in the *N. arenaceodentata* test chambers, the effect of food treatment on Ag bioaccumulation could not be evaluated.

Chemical and data analysis. The experimental containers and glassware used for chemical analysis and sample storage were acid-washed, followed by soaking in deoxygenated Milli-Q water. Sediment AVS was analyzed by the cold-acid (1N HCl) purge-and-trap technique described by Boothman & Helmstetter (1992). The concentrations of Ag in the porewater and overlying water were analyzed with a GFAAS (graphite furnace atomic absorption spectrometer) after 10× dilution with Milli-Q water. Silver concentrations in the sediment and tissue extracts were determined by flame-AAS with appropriate dilution. Procedural blanks and NBS (National Bureau of Standards, USA) oyster tissue (standard reference material 1566a) were used for quality control and assurance. Mean recovery for Ag was 96.4 ± 1.0% (N = 6), and the method detection limit (MDL) for Ag in porewater was 7.4 nM.

The Ag-SEM and AVS values used for comparison with tissue Ag data were the geometric mean of values determined from sediments sampled at time $t = 0$ (initial) and $t = f$ (final). Unpaired *t*-tests were conducted to compare tissue Ag concentrations between different treatments (oxic versus anoxic, contaminated versus uncontaminated food, and food rations). The statistical significance was set at $\alpha = 0.05$, unless otherwise noted.

RESULTS

Sediment geochemistry

The AVS concentrations in Expt I decreased by 38 to 57% during 35 d incubation and those in Expt II decreased by 66 to 88% during 19 d incubation (Table 2). The decrease in AVS was probably due to the combination of bioirrigation/bioturbation by the test organisms and oxidation of sediments by constant aeration of the overlying water. The greater decrease in AVS with the clams and polychaetes was probably due to their larger sizes and greater ventilating activities compared to that of amphipods in Expt I. The organic matter content (as LOI) was constant among all sediment treatments (9.5 ± 0.6%). In both Expts I and II, Ag extracted by weak acid (1N HCl) with AVS (Ag-SEM) in the sediments generally changed little over the 35 d (Expt I) or 19 d (Expt II) exposure periods. The exception was at the highest sediment Ag treatments (AM4, OM4) where Ag-SEM decreased by 15 to 52% during the incubation period. These changes in SEM over time probably reflected the transition of Ag to more recalcitrant sediment phases (Lee et al. 2004). This is supported by 2 lines of evidence. First, Ag concentrations extracted by near-total digestion with concentrated HNO_3 were similar between the samples collected at the beginning and end of each sediment incubation. Second, the Ag-SEMs determined at the end of Expt I was similar to those determined at the beginning of Expt II (Table 2). Expt II was initiated at the completion of Expt I with the same sediment source, that had been aging continuously.

The molar [Ag-SEM]/2 – [AVS] values for all the anoxic sediments (AM0 to AM4) were negative; i.e. the AVS concentrations in these treatments were greater than Ag-SEM (Table 2). The [Ag-SEM]/2 – [AVS] values in the oxic sediments (OM1, OM3, OM4) were close to or greater than 0. Porewater and overlying water Ag concentrations in most treatments were below the detection limits (7.4 nM). Detectable amounts of dissolved Ag were present in high-Ag sediments (AM4 and OM4) in Expt I (Table 3). In those treatments, the porewater and overlying water-Ag concentrations were higher in oxic sediment (OM4) than in anoxic sediment (AM4), and also higher at the beginning than at the end of incubation. Ag concentrations in the overlying water were generally higher in sediment treatments provided with contaminated diets than those treated with uncontaminated diets, possibly due to desorption from contaminated TetraMin®.

Table 2. Near-total [Ag], [Ag-SEM], [AVS], and [Ag-SEM]/2 – [AVS] in sediments (µmol g^{-1} sediment; mean ± SD) at initiation ($t = 0$) and end ($t = f$) of Expts I and II. SEM: simultaneously extracted metal concentrations; Near-total Ag: [Ag] extracted with near-total digestion by concentrated HNO_3 for 1 wk (means of $t = 0$ and $t = f$ values). Ag-SEM values are mean chemical data from treatments with different Ag levels in supplementary food; see Table 1 for experimental design and treatment designations

Treatment	Near-total Ag	Ag-SEM		AVS		[Ag-SEM]/2 – [AVS]	
		$t = 0$	$t = f$	$t = 0$	$t = f$	$t = 0$	$t = f$
Expt I (amphipod)							
AM0	0.01 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	32.9 ± 1.7	14.0 ± 5.3	–32.9	–14.0
AM1	0.09 ± 0.01	0.03 ± 0.01	0.04 ± 0.00	26.8 ± 1.4	15.3 ± 1.9	–26.8	–15.3
AM2	0.32 ± 0.04	0.12 ± 0.02	0.11 ± 0.03	22.6 ± 0.1	14.0 ± 2.3	–22.5	–14.0
AM3	1.26 ± 0.02	0.68 ± 0.02	0.66 ± 0.12	26.6 ± 0.9	17.5 ± 2.6	–26.2	–17.1
AM4	3.34 ± 0.20	2.10 ± 0.26	1.59 ± 0.07	26.6 ± 0.4	15.0 ± 2.6	–25.6	–14.3
OM4	3.24 ± 0.31	2.49 ± 0.16	1.75 ± 0.00	0.13 ± 0.04	0.07 ± 0.05	1.1	0.8
Expt II (clam and polychaete)							
AM0	0.01 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	39.6 ± 3.4	5.5 ± 1.1	–39.6	–5.5
AM1	0.09 ± 0.01	0.03 ± 0.00	0.03 ± 0.00	40.4 ± 2.3	13.7 ± 1.7	–40.3	–13.7
AM2	0.29 ± 0.01	0.11 ± 0.01	0.11 ± 0.02	33.2 ± 2.5	10.8 ± 0.7	–33.2	–10.8
AM3	1.17 ± 0.05	0.52 ± 0.03	0.61 ± 0.11	31.2 ± 0.3	4.5 ± 1.6	–30.9	–4.2
AM4	3.21 ± 0.13	1.48 ± 0.14	1.26 ± 0.42	27.7 ± 1.4	6.3 ± 1.0	–26.9	–5.7
OM1	0.09 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	2.9 ± 0.1	1.0 ± 0.2	–2.8	–1.0
OM3	1.17 ± 0.01	0.79 ± 0.05	0.81 ± 0.06	0.2 ± 0.0	0.1 ± 0.0	0.2	0.4
OM4	3.04 ± 0.21	1.59 ± 0.27	1.04 ± 0.15	0.03 ± 0.00	0.03 ± 0.00	0.8	0.5

Table 3. Mean Ag concentrations (nM) in porewater or overlying water in Expt I (N = 2). Ag concentrations of porewater or overlying water in all other treatments of Expts I and II were below detection limit (7.4 nM). na: not available; other abbreviations as in Table 1

Treatment	Porewater				Overlying water					
	UF		SF		UF			SF		
	$t = 0$	$t = f$	$t = 0$	$t = f$	$t = 0$	$t = 5$	$t = f$	$t = 0$	$t = 5$	$t = f$
AM4	17.1	9.3	na	11.5	7.1	9.6	<7.4	na	27.3	14.9
OM4	67.0	18.4	na	18.9	30.3	7.4	<7.4	36.6	15.8	12.6

Silver bioaccumulation

Tissue Ag concentration in both amphipods and clams increased proportionally with the weak-acid extractable Ag in the sediments (Ag-SEM), regardless of oxidation state (i.e. AVS levels) of the sediment (Table 4). A similar relationship was observed in the tissue of the polychaetes, but organisms exposed to oxic sediments accumulated more Ag than those exposed to anoxic treatments when Ag-SEM levels were comparable (Table 4).

Differences in AVS concentrations (oxic versus anoxic) did not significantly influence Ag bioaccumulation in either *Leptocheirus plumulosus* or *Macoma balthica* (Fig. 1). In contrast, *Neanthes arenaceodentata* exposed to the oxic sediments accumulated 2 to 3× more Ag than those exposed to the anoxic treatments at the same Ag concentration (Fig. 1). All 3 organisms accumulated significantly more Ag than the control organisms even when the Ag-SEM was much less than AVS ([Ag-SEM]/2 – [AVS] << 0) (Fig. 2). Tissue concentrations of Ag in *N. arenaceodentata* increased when [Ag-SEM]/2 – [AVS] > 0; but concentrations in *L. plumulosus* and in *M. balthica* did not increase beyond that expected from the relationship with Ag concentrations in the sediments.

Amphipods provided with Ag-contaminated diets accumulated 1.5 to 1.8× more Ag than those provided with uncontaminated diets. An effect of contaminated diets on Ag bioaccumulation was observed in all test treatments (Fig. 3A). A desorption experiment showed that <5% of Ag desorbed from the food with the highest spiked Ag concentration, desorption from the lower spiked Ag concentration was not detected. Tissue Ag in *Macoma balthica* fed with contaminated algae was not significantly ($p > 0.05$) different from that in individuals fed uncontaminated algae (Fig. 3B).

Reduction of food ration to one-fifth of the regular ration caused a significant decrease in amphipod body weight after 35 d exposure; the amphipods fed one-fifth of the regular ration weighed ~30% less than those fed the regular ration (data not shown). These

Table 4. *Leptocheirus plumulosus*, *Macoma balthica* and *Neanthes arenaceodentata*. Tissue Ag concentrations (µmol g^{-1}; mean ± SD) in animals exposed to control and to Ag-spiked sediment and food. na: not available; other abbreviations as in Table 1

Exposure	*Leptocheirus plumulosus*	*Macoma balthica*	*Neanthes arenaceodentata*
Uncontaminated food			
AM0	0.006 ± 0.001	0.04 ± 0.01	0.02 ± 0.00
AM1	0.019 ± 0.001	0.07 ± 0.02	0.05 ± 0.01
AM2	0.037 ± 0.003	0.09 ± 0.02	0.09 ± 0.01
AM3	0.055 ± 0.005	0.22 ± 0.09	0.19 ± 0.05
AM4	0.090 ± 0.004	0.47 ± 0.04	0.61 ± 0.10
OM1		0.07 ± 0.01	0.09 ± 0.01
OM3		0.21 ± 0.06	0.42 ± 0.01
OM4	0.091 ± 0.020	0.42 ± 0.12	2.13 ± 0.16
Ag-spiked food			
AM1	0.029 ± 0.008	0.06 ± 0.01	na
AM2	0.066 ± 0.016		
AM3	0.100 ± 0.009	0.24 ± 0.03	na
AM4	0.144 ± 0.064		
OM4	0.134 ± 0.052		
Uncontaminated food (one-fifth ration)			
AM0	0.004 ± 0.002		
AM2	0.047 ± 0.000		
AM3	0.056 ± 0.001		
AM4	0.158 ± 0.009		

differences in food ration and growth rates had little effect on Ag bioaccumulation, except for the treatment with the highest Ag levels in sediment and diet (AM4). In this treatment, tissue Ag concentration was significantly higher when the amphipods received one-fifth of the food ration (Fig. 4).

DISCUSSION

Benthic animals exposed to metal-contaminated sediments can accumulate metals by ingesting contaminated sediments and other suspended particles, and by exposure to dissolved metals in the overlying water or porewater (Luoma et al. 1992, Wang et al. 1996, Munger & Hare 1997, Lee et al. 2000a, 2001). However, the relative importance of these routes of exposure can be influenced by biology, sediment geochemistry and/or experimental conditions. In the present experiments, biology was the overriding factor of importance. The polychaete appeared to accumulate more Ag from the dissolved source than from the diet, in accordance with the predictions of equilibrium partitioning, but neither the amphipod nor the bivalve responded in the same way. AVS concentrations in the sediments did not affect the bioavailability of Ag to the amphipods and bivalves. Similarly, the amphipod responded to the presence of

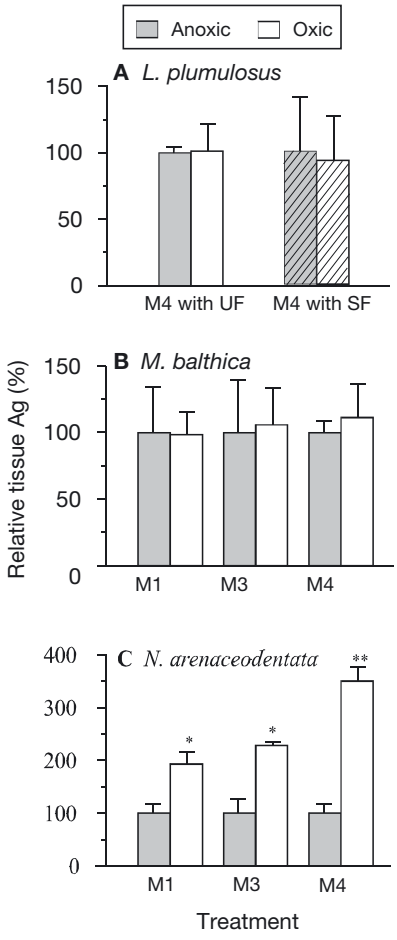

Fig. 1. *Leptocheirus plumulosus*, *Macoma balthica* and *Neanthes arenaceodentata*. Comparison of mean (±SD) tissue Ag concentrations in organisms exposed to oxic and anoxic Ag-spiked sediments. Tissue Ag in oxic sediments was normalized to those in anoxic sediments. M4 with UF, M4 with SF: uncontaminated and metal-contaminated food treatments, respectively. Asterisks represent significant difference in tissue Ag concentration between 2 sediment treatments (* $p < 0.05$, ** $p < 0.01$). Error bars indicate standard deviations

supplementary contaminated food with greater Ag bioaccumulation than from sediment alone, but supplementary food had no influence on Ag uptake by the bivalve. Experimental conditions were important, but their importance differed among species. Similarly, geochemistry (AVS concentrations) was critical for the polychaete, but made little difference in chronic exposure of the amphipod and the bivalve. Finally, the outcome of chronic exposure to Ag contaminated sediment was consistent with results predicted by biokinetic models, but was not consistent with predictions of influences on bioavailability from acute toxicity tests.

These findings, that dietary uptake via ingestion of contaminated sediments and food particles was impor-

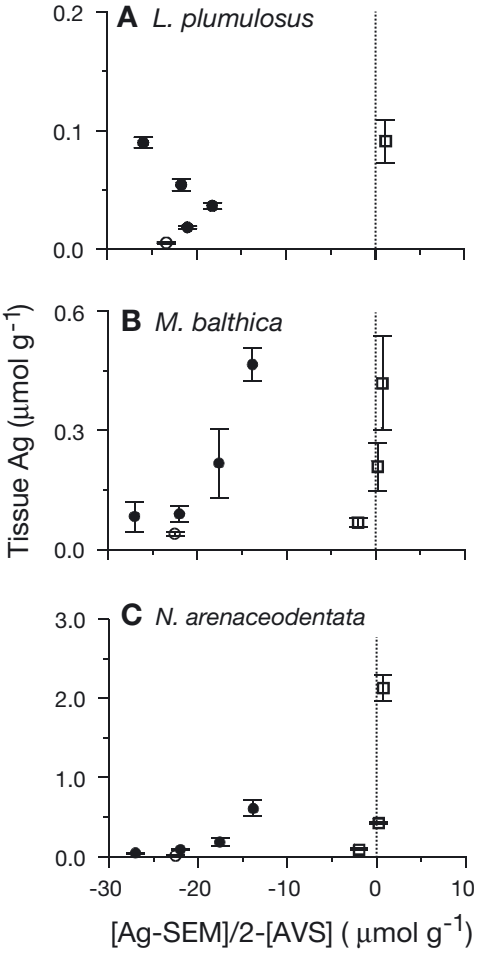

Fig. 2. *Leptocheirus plumulosus*, *Macoma balthica* and *Neanthes arenaceodentata*. Relationship between mean (±SD) tissue Ag concentration and [Ag-SEM]/2 - [AVS]. Vertical dotted line marks zero [Ag-SEM]/2 – [AVS] (abbreviations as in Tables 1 & 2). (●) anoxic-sediment treatments; (□) oxic-sediment treatments; (○) uncontaminated control sediment

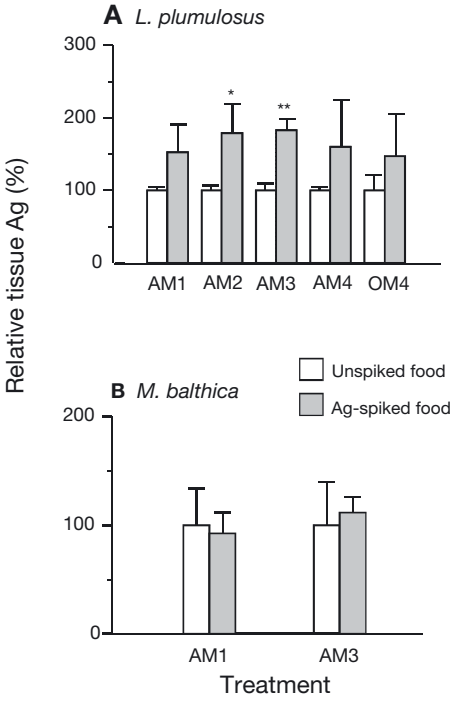

Fig. 3. *Leptocheirus plumulosus* and *Macoma balthica*. Comparison of tissue Ag concentrations in organisms exposed to uncontaminated and Ag-contaminated food. Tissue Ag in contaminated-food treatments was normalized to tissue Ag in uncontaminated-food treatments. AM, OM: anoxic and oxic sediments, respectively; asterisks and error bars as in Fig. 1

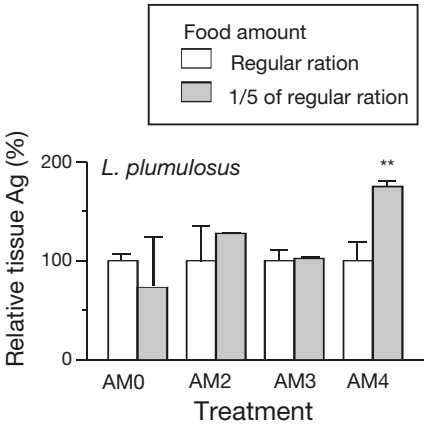

Fig. 4. *Leptocheirus plumulosus*. Comparison of tissue Ag concentrations in amphipods provided with different amounts of uncontaminated supplementary food. Tissue Ag concentration in treatment with one-fifth of regular ration was normalized to that in treatment with normal ration. Asterisks and error bars as in Fig. 1

tant for Ag bioaccumulation, are consistent with those of other studies (Munger & Hare 1997, Lee et al. 2000a, 2001) demonstrating the dominance of the dietary uptake pathway in metal bioaccumulation by sediment-dwelling organisms in moderately contaminated sediments.

Mechanistically, dietary uptake of Ag by these organisms can be explained by the ingestion of Ag associated with sediments and food particles and the subsequent assimilation of Ag via gut epithelia into tissue. Numerous studies (Fisher et al. 1995, Wang & Fisher 1998, Griscom et al. 2000, Lee et al. 2000a, Schlekat et al. 2000) have shown that marine invertebrates can assimilate Ag from various types of food particles, although its assimilation efficiency was generally lower than other elements. In particular, Lee et al. (2000a) demonstrated that *Macoma balthica* and *Mytilus edulis* were able to assimilate Ag by ingestion of both anoxic and oxic particles with similar efficiencies. Later Griscom et al. (2000) reported that Ag assimilation efficiency by *M. balthica* was 20 ± 5.5%

from oxic sediments and 11 ± 1.7% from anoxic sediments. The overriding implication is that a portion of the Ag in anoxic sediments is available when ingested by these bivalves, even though silver sulfides in anoxic sediments are the most insoluble forms of metal sulfides (Bell & Kramer 1999).

Alternatively, a similar geochemical microhabitat created by active burrowing and irrigation of the test organisms may explain the comparable Ag bioaccumulation observed in sediments with high and low AVS treatments. Most benthic animals keep their burrows oxygenated for respiration and to avoid sulfide and ammonia toxicity (Aller 1980, Miron & Kristensen 1993). Often these oxygenated burrows display geochemical gradients and solute fluxes similar to those in the surface sediment (Aller & Yingst 1985, Wang et al. 2001). The clam *Macoma balthica* has a facultative suspension-feeding mode, and the amphipod *Leptocheirus plumulosus* employs both suspension- and deposit-feeding. Therefore, it is plausible that the test animals in both oxic and anoxic sediments created, and were thus exposed to, a geochemical microenvironment in their burrows that was similar to that of the overlying water, resulting in similar tissue Ag concentrations.

We determined porewater and overlying water Ag concentrations only at the beginning and end of incubation. The detection limit of dissolved Ag (7.4 nM) in the present study was relatively high compared to that in other geochemical studies, but comparable to that in most ecotoxicological studies (e.g. 9.4 nM in Berry et al. 1999) using similar analytical procedures. It is possible that the clam *Macoma balthica* and the amphipod *Leptocheirus plumulosus* accumulated Ag from the dissolved source, but we believe that this dissolved Ag source made but a minor contribution to Ag bioaccumulation in these organisms. Numerous previous studies (e.g. Berry et al. 1996, 1999, Lee et al. 2000b) have demonstrated that the dissolved metal concentrations in both overlying water and porewater are controlled by AVS or [SEM-AVS] values. If dissolved Ag in either the porewater or overlying water was a significant source for bioaccumulation in these organisms, then those organisms exposed to oxic sediments should have accumulated more Ag than organisms in anoxic sediments, due to a greater dissolved Ag concentration in the former. The fact that both *M. balthica* and *L. plumulosus* exposed to either oxic or anoxic sediments had similar tissue Ag concentrations suggests that dissolved Ag was not the principal source for Ag bioaccumulation. In contrast, the greater concentration of tissue Ag in *Neanthes arenaceodentata* exposed to oxic sediments than in those exposed to anoxic sediments was probably due to uptake from comparatively higher dissolved Ag in oxic sediments.

The reasons why porewater Ag had a significant effect only in the polychaetes are not evident. In a study involving exposure of 5 marine benthic invertebrates to Cd-contaminated sediments, Lee et al. (2000a) reported that *Neanthes arenaceodentata* accumulated significant amounts of Cd from dissolved sources while the other 4 benthic invertebrates (the bivalves *Macoma balthica* and *Potamocorbula amurensis*, and the polychaetes *Heteromastus filiformis* and *Spiophanes missionensis*) accumulated Cd largely from ingested particles. Similarly, Wang et al. (1999) reported that the polychaete *Nereis* (*Neanthes*) *succinea* assimilated dissolved Ag at the highest rate among the 5 elements (Ag, Cd, Co, Se and Zn) tested. They further estimated with a biokinetic model that 5 to 35% of Ag in the polychaetes were contributed by the dissolved phase, while most (>98%) of the other 4 elements were from the dietary pathway. Probably, the greater bioaccumulation of dissolved Ag by the worm could be achieved by uptake via its large body-surface epithelia and well developed parapodia along its elongated body, which function in respiration-gas exchange, and ion-regulation. Alternatively, the greater Ag bioaccumulation in the oxic sediments could be explained if the *Neanthes arenaceodentata* assimilated Ag with greater efficiency from oxic sediments than from anoxic sediments, as shown for *Nereis succinea* (Wang et al. 1999).

The elevated Ag bioaccumulation in experiments involving contaminated supplementary diets for amphipods further emphasizes the importance of the dietary pathway. The weight or Ag content of the supplementary food provided to the amphipods comprised only a minute fraction (<0.2%) of the total sediments in the test beakers. The results imply that the amphipods selectively fed on the nutritious supplementary diets and accumulated Ag from these diets (3× higher Ag concentration for each sediment treatment). Other studies (Maloney 1996, Schaanning et al. 1996, Lee & Luoma 1998, Lee et al. 2001) have also shown that some marine invertebrates accumulate considerably more metals by ingestion of organic-rich food particles than by ingestion of organic-poor particles. However, the tissue Ag concentration in *Macoma balthica* was not affected by Ag contamination levels of the algae, probably due to the lower Ag concentration in the algae (half the total Ag concentration in contaminated sediments).

Unfortunately, the 3 species were exposed not only for different durations (19 versus 35 d) but also to Ag-contaminated sediment equilibrated for different periods (3 versus 5 mo). These different experimental protocols for different animal species prevented us from evaluating relative bioaccumulation rates (e.g. the bioaccumulation factor) among the 3 test species.

However, we believe that the different protocols had but a minor influence on the evaluation of the major uptake routes of each test species and the conclusions of this study. We have demonstrated in earlier studies (Lee et al. 2000b, 2004) that porewater metal concentrations decrease most rapidly within 1 mo of equilibration of the metal-spiked sediments, and that partitioning of metals between porewater and sediment change little thereafter. If the different Ag-sediment equilibration times had influenced the partitioning of Ag between the porewater and sediment, and subsequently the Ag bioaccumulation, then the amphipods should have been more exposed to the dissolved source than the clams and polychaetes. However, the bioaccumulation results show that dissolved Ag had only a minor influence on Ag bioaccumulation in the amphipods. Rather, the dissolved Ag source was important only for Ag bioaccumulation in the polychaetes, which were exposed to sediment equilibrated for 5 mo. Further, the dissolved Ag source made but a minor contribution to Ag bioaccumulation in clams exposed to the same sediments as the polychaetes, emphasizing again the importance of the biological processes of each invertebrate species in determining the Ag bioaccumulation pathway.

Overall, the results of this study differ from those of many acute-toxicity tests that have used the AVS normalization approach (Ankley et al. 1996, Berry et al. 1999). Those studies led the authors to conclude that geochemistry is the dominant control on bioavailability and to imply that bioavailability of trace metals (including Ag) from sediments would be negligible when [SEM-AVS] < 0 for all species. In contrast, other studies have also shown that significant bioaccumulation of metals could occur in various benthos exposed to sediments even when [SEM-AVS] < 0. (Hare et al. 1994, Ingersoll et al. 1994, Ankley 1996, Lee et al. 2000c, 2001). Several reasons for such differences have been explained previously (e.g benthic invertebrates ingesting AVS-rich pure-phase particles assimilate metals including Ag; Wang et al. 1999, Griscom et al. 2000, Lee et al. 2000a, 2004). The results of this study emphasize that investigation of the biological mechanisms of uptake is necessary to clarify such dichotomies.

Recently, a growing number of studies have demonstrated that Ag bioaccumulation by some aquatic invertebrates via the food chain or dietary pathway can cause chronic effects, such as reduced fecundity or other reproductive activity (Hook & Fisher 2001, Hornberger et al. 2001, Bielmyer et al. 2002). Therefore, ecologically relevant risk-assessment and management of Ag-contaminated sediments should consider all the relevant uptake pathways, diverse resident animals with various biological attributes, and a range of toxicological effects expressed in different levels of biological organization ranging from cellular level through individuals to communities.

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