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Influence of reactive sulfide (AVS) and supplementary food on Ag, Cd and Zn bioaccumulation in the marine polychaete *Neanthes arenaceodentata*

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ABSTRACT: A laboratory bioassay determined the relative contribution of various pathways of Ag, Cd and Zn bioaccumulation in the marine polychaete *Neanthes arenaceodentata* exposed to moderately contaminated sediments. Juvenile worms were exposed for 25 d to experimental sediments containing 5 different reactive sulfide (acid volatile sulfides, AVS) concentrations (1 to 30 µmol g^{-1}), but with constant Ag, Cd, and Zn concentrations of 0.1, 0.1 and 7 µmol g^{-1}, respectively. The sediments were supplemented with contaminated food (TetraMin®) containing 3 levels of Ag-Cd-Zn (uncontaminated, 1× or 5× metal concentrations in the contaminated sediment). The results suggest that bioaccumulation of Ag, Cd and Zn in the worms occurred predominantly from ingestion of contaminated sediments and contaminated supplementary food. AVS or dissolved metals (in porewater and overlying water) had a minor effect on bioaccumulation of the 3 metals in most of the treatments. The contribution to uptake from the dissolved source was most important in the most oxic sediments, with maximum contributions of 8% for Ag, 30% for Cd and 20% for Zn bioaccumulation. Sediment bioassays where uncontaminated supplemental food is added could seriously underestimate metal exposures in an equilibrated system; *N. arenaceodentata* feeding on uncontaminated food would be exposed to 40–60% less metal than if the food source was equilibrated (as occurs in nature). Overall, the results show that pathways of metal exposure are dynamically linked in contaminated sediments and shift as external geochemical characteristics and internal biological attributes vary.

KEY WORDS: Bioaccumulation · Metals · AVS · Dietary uptake

INTRODUCTION

Sediments constitute a concentrated reservoir of trace metals introduced via natural weathering processes and anthropogenic activities in most aquatic environments. Metals in sediments can be concentrated by benthic organisms and may have adverse effects at elevated levels (Long et al. 1995, Hornberger et al. 2000). The geochemical nature of the metal-sediment association and the relative distribution of metals between porewater and sediments could have considerable influence on the eventual fate and bioavailabililty of metals to aquatic organisms (Luoma & Bryan 1982, Di Toro et al. 1990, Lee & Luoma 1998). Transfer of metals from sediments to benthic organisms may also be affected by biological attributes of animals such as feeding behaviors, life habits, reproductive cycles, growth, and size (Cain & Luoma 1990, Arifin & Bendall-Young 1997, Warren et al. 1998, B. G. Lee et al. 2000a)

Studies (e.g., Morse et al. 1987, Di Toro et al. 1990, Ankley et al. 1996) have recognized reactive sulfides (AVS, acid volatile sulfide) in sediments as a major factor controlling porewater metal chemistry. The AVS consists largely of amorphous iron sulfides and is typically extracted with cold weak acid (1 N HCl). Most of

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these studies show that the relative concentration of reactive metal (SEM, simultaneously extracted metals with AVS) and AVS, expressed as [SEM – AVS], determines porewater metal concentrations. An important conclusion of these studies is that metal bioavailability and toxicity to organisms in contaminated sediments are controlled by porewater metal concentrations and therefore are modulated by [SEM – AVS]. This AVS normalization approach has been proposed as a basis for sediment quality criteria for metals (Ankley et al. 1996). In contrast, another body of work has employed microcosm studies or biokinetic models (Luoma et al. 1992, Wang et al. 1996, Munger & Hare 1997, Selck et al. 1998, B. G. Lee et al. 2000a) to demonstrate that uptake from ingestion of contaminated particles is a major route of metal bioaccumulation to various marine invertebrates. The conclusions about dominant uptake routes differ between these 2 bodies of work, but these differences are only beginning to be addressed (Luoma & Fisher 1997, B. G. Lee et al. 2000a). Understanding which pathways affects exposure of aquatic organisms to metals under various environmental conditions and how this occurs is essential for environmental risk assessment of contaminated sediments.

Some chronic toxicity test procedures for contaminated sediments (e.g., animal growth and reproduction test) require use of supplementary food to stimulate the growth and reproduction of test organisms (John et al. 1990, Dillon et al. 1993, Murdoch et al. 1997, Anderson et al. 1998). For example, protocols measuring effects of contaminated sediments on growth and reproduction of the polychaete worm *Neanthes arenaceodentata* supply 6 to 24 mg ind.$^{-1}$ wk^{-1} supplementary food. Rarely, if ever, is supplementary food spiked with contaminants in a bioassay, although in nature food sources are contaminated in a contaminated system. A number of biological complications could arise from feeding test animals uncontaminated food in a bioassay, if diet is an important source of metal exposure. For example, exposure pathways could be altered. Selective feeding behavior has been observed for many benthic animals (Bayne et al. 1993, Arifin & Bendall-Young 1997). Selective feeding of test animals on uncontaminated nutritious supplementary food may discourage ingestion of contaminated sediments and shift the exposure route to contaminants in the dissolved phase. Further, ingestion of nutritious food typically enhances the growth of an organism and may result in dilution of concentrations of contaminants by rapid growth of uncontaminated tissue mass. Yet, effects of supplementary food on exposure of animals to metals in contaminated sediments have not been addressed experimentally.

The primary objective of this study was to evaluate relative uptake routes of Ag, Cd and Zn for the deposit-feeding polychaete *Neanthes arenaceodentata*, from moderately contaminated sediments with different reactive sulfide concentrations. Another goal was to evaluate the effect of contaminated supplementary food on bioaccumulation from contaminated sediments. A mass balance approach was employed to separate the routes of metal bioaccumulation in the worms. The uptake routes considered were natural background sediments, contaminated sediments, contaminated supplementary food and the dissolved phase (overlying water and porewater). *N. arenaceodentata* is an ubiquitous infaunal polychaete found in shallow marine and estuarine benthic habitats around world (Dillon et al. 1993). This worm was chosen since it has a short life cycle, is easy to culture in the laboratory and has been used in many metal toxicity and bioaccumulation experiments (Reish 1985, Mason et al. 1988, Pesch et al. 1995, B. G. Lee et al. 2000a). The 3 metals used in this study are characterized as highly toxic metals and have been studied for evaluation of AVS normalization approaches (e.g., Ankley et al. 1996, DeWitt et al. 1996, Berry et al. 1999).

MATERIALS AND METHODS

Experimental design. *Neanthes arenaceodentata* were exposed to sediments containing 5 different AVS concentrations (1 to 30 µmol AVS g^{-1}) with a constant level of Ag-Cd-Zn spiked into the sediment (0.1, 0.1, and 7 µmol g^{-1}, respectively) (Fig. 1). The supplementary food was a commercial aquarium fish food (TetraMin®) and was added either uncontaminated ($F_{0\times}$) or spiked with Ag-Cd-Zn at concentrations 1× ($F_{1\times}$) or 5× ($F_{5\times}$) the metal concentrations of the spiked sediments (Fig. 1). Two groups of worms were exposed to unspiked sediments with 1 or 20 µmol AVS g^{-1} (control-oxic and control-anoxic, respectively) and fed unspiked food. An additional group of worms were incubated in the unspiked sediment (10 µmol AVS g^{-1}) but fed $F_{5\times}$ food (control-$F_{5\times}$).

Sediment and supplementary food preparations. The experimental sediment was obtained from a mud flat near Palo Alto, San Francisco Bay, USA. Mud flat sediment naturally high in AVS (ca 40 µmol g^{-1}) was collected, following removal of the surface oxic layer, screened through 1 mm nylon mesh at the site and transferred to laboratory. No attempt was made to enumerate the organisms present in the sediments that passed 1 mm screen. Mean particle size was 6.3 Φ and mean sand, silt and clay content, analyzed by the pipetting method (Folk 1954), were 0.5, 75, and 24.5%, respectively. The loss on ignition (LOI, at 450°C for 5 h) was 6.8 ± 0.5% (mean ± standard deviation).

The experimental containers and glass-ware used for sediment handling, chemical analysis and sample

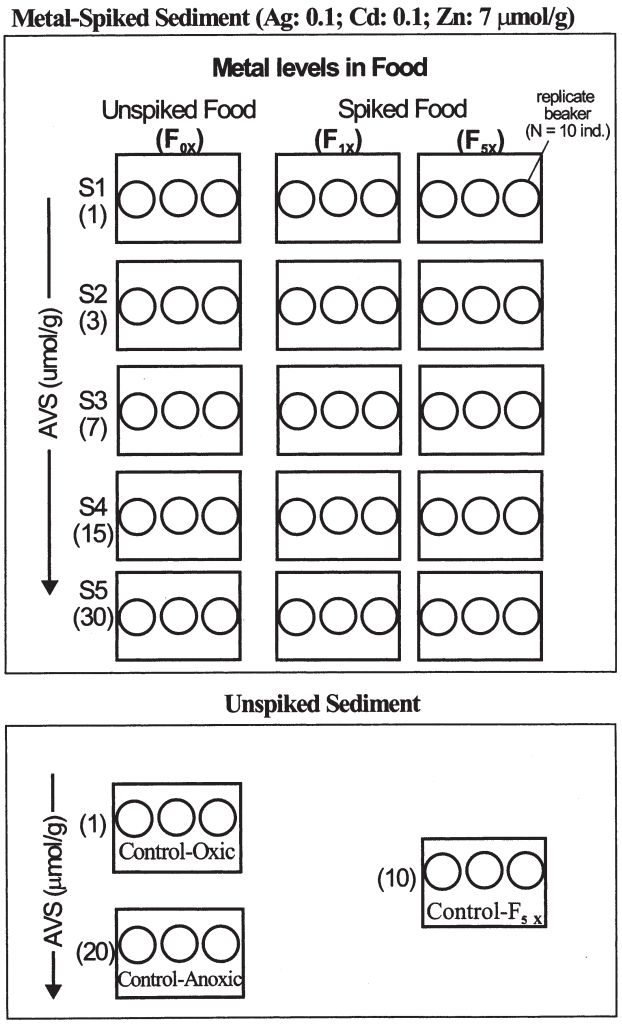

Fig. 1. Schematic diagram of experimental treatments. Metal spiked sediment contained 0.1 µmol Ag g^{-1}, 0.1 µmol Cd g^{-1}, and 7 µmol Zn g^{-1}. None of the 3 control sediments were spiked with metals. Nominal AVS concentrations in experimental sediments increased from 1 (S1) to 30 µmol g^{-1} (S5). The worms were fed either uncontaminated food ($F_{0\times}$), food contaminated with 1×, or 5× sediment metal concentrations ($F_{1\times}$ or $F_{5\times}$)

storage were acid washed, followed by soaking in deoxygenated Milli-Q water for 1 wk. The sediment samples were handled under a glove bag filled with N_2 gas. In the laboratory, a portion of the collected sediment was spiked with metals and manipulated for appropriate AVS levels following a protocol established by Lee et al. (2000b). Briefly, the sediment was mixed with the equal volume of deoxygenated seawater (30 psu) that had been previously spiked with the metal stock solutions prepared with reagent grade $AgNO_3$, $CdCl_2$ and $ZnCl_2$. Following 1 wk of sediment-metal equilibration, half of the metal-spiked sediment was oxidized by bubbling continuously with air for 1 wk and the remaining anoxic metal-spiked sediment was kept in N_2-atmosphere. Aeration of metal-spiked anoxic sediment for 1 week reduced the AVS concentration from ca 40 to ca 1 µmol g^{-1}. The remaining uncontaminated sediments were also manipulated to obtain uncontaminated anoxic and oxic sediments for the control samples (control-anoxic, control-oxic, and control-$F_{5\times}$). The AVS concentration of uncontaminated anoxic sediment declined from ca 40 to ca 20 µmol g^{-1} under N_2 atmosphere, while the metal spiked sediment maintained its initial AVS concentration. A similar phenomenon was observed in other studies (Liber et al. 1996, B. G. Lee et al. 2000b) and was explained by faster oxidation rate of iron sulfides in uncontaminated sediments than other metal sulfides formed in metal-spiked sediments.

The oxidized and anoxic sediments were mixed at appropriate ratios to achieve five nominal AVS levels (1 to 30 µmol g^{-1}). Following the AVS manipulation, 300 ml of sediment slurry was transferred to each of 3 replicate 1 l glass beakers for each treatment (Fig. 1). A total of 800 ml of seawater (30 psu) was added 2 d after sediment transfer and replaced a day prior to animal introduction. The sediments were allowed to consolidate for 9 d prior to animal introduction. The sediment volume was reduced to ca 200 ml during this time. Two replicate subsamples of sediments were collected using syringes at the mid sediment depth at 9 d before ($t = -9$ d) and the beginning of animal incubation ($t = 0$) for geochemical characterization.

Metal contaminated food particles were prepared by suspending ground TetraMin® (<250 µm) into autoclaved deionized water containing appropriate levels of Ag-Cd-Zn (Fig. 1). Deionized water was used to facilitate faster dissolution of metal salts. The metal-food mixture was equilibrated for 1 wk at 2°C and shaken several times daily. Then the metal adsorbed particles were resuspended in seawater (30 psu) for 1 d at 2°C to minimize desorption of metals during feeding processes. The settled particles were freeze-dried, ground finely again (<250 µm) before use, and analyzed for metal concentrations (Table 1).

Bioassay procedures. Laboratory cultured juvenile polychaetes, *Neanthes arenaceodentata* (2 wk, post-emergent; obtained from Dr Reish, California State Univ., Long Beach, USA), were acclimated for 3 d at 20°C in 30 psu seawater. Culturing and testing protocols for lethal and sublethal toxicity tests are reported elsewhere (Reish 1980, Dillon et al. 1993, Anderson et al. 1998). Following acclimation, the worms were divided into groups of 10 individuals and introduced randomly into each experimental sediment. Each treatment had 3 replicate beakers (Fig. 1). During the 25 d incubation of the test animals, overlying seawater was continuously aerated and replaced (ca 60%) every 2 d. Test chambers were illuminated with a light:dark cy-

Table 1. Mean and standard deviation (SD) of Ag, Cd, and Zn concentrations in supplementary food (n = 3). $F_{0\times}$ is unspiked supplementary food; $F_{1\times}$ and $F_{5\times}$ are supplementary foods that are 1× and 5× the metal concentrations in spiked sediments, respectively. –: unknown

Treatment	Ag (μmol g^{-1})			Cd (μmol g^{-1})			Zn (μmol g^{-1})		
	Nominal	Measured		Nominal	Measured		Nominal	Measured	
		Mean	SD		Mean	SD		Mean	SD
$F_{0\times}$	–	0.004	0.001	–	0.003	0.000	–	1.4	0.1
$F_{1\times}$	0.10	0.09	0.02	0.10	0.10	0.00	7	11	2
$F_{5\times}$	0.50	0.43	0.15	0.50	0.31	0.01	35	40	8

cles of 14:10 H L:D. During the incubation period, the worms were fed 10 times (7 mg ind.$^{-1}$ for each time) with the previously prepared TetraMin® 6 h before changing overlying water. The worms were fed every 4 d for initial 12 d of incubation and every 2 d thereafter considering growth of animals. Overlying water quality was monitored 1 to 2 times a week. Temperature, salinity, pH and dissolved oxygen (mean ± SD) were 20 ± 1°C, 30 ± 1 psu, 8.02 ± 0.05 and 6.8 ± 1.1 mg l^{-1}, respectively. Additionally, overlying waters at 5 cm above sediments were sampled 1 d prior to water change and were acidified (pH = ca 2) for later metal analysis.

Following 25 d incubation with animals, subsamples of sediment from the surface oxidized layer (0 to 5 mm) and the deep sediment (5 to 20 mm) were collected from each experimental beaker. The sediment samples from 3 replicate beakers for each treatment were pooled into 1 sample and kept at 4°C refrigerator for later chemical analyses. The remaining sediment in the beakers was sieved with a 1 mm nylon screen to collect worms. There was no significant difference in survival rates (94 ± 7%) of the worms among treatments. Worms were allowed to depurate in 0.22 μm filtered seawater for 24 h. Both visual observation and tissue aluminum concentration suggested that worms depurated most of the ingested particles during this time. Then the worms from each replicate beaker were pooled into 1 composite sample (3 replicates, composed of ca 10 worms treatment^{-1}), freeze-dried, and dry weight was determined. The mean dry weight of worm increased ca 20 times from 0.87 ± 0.15 to 15.8 ± 1.7 mg ind.$^{-1}$ during the 25 d incubation periods.

Analytical procedure. AVS, SEM, overlying water and porewater analyses were done within 1 wk of sample collection. Two replicate sediments from each treatment were analyzed for AVS and SEM at each sampling time (t = –9, 0 and 25) except for surface sediments at t = 25 which had no replication. Most dissolved metals in porewater and overlying water were analyzed without replication. The detailed analytical protocols are described elsewhere (B. G. Lee et al. 2000b). Freeze-dried worm samples were digested in concentrated hot nitric acid following a method described by Brown & Luoma (1995). Procedure blanks and NBS oyster tissue (SRM 1566a) were accompanied at each digestion for quality control and assurance. Mean recoveries for Ag, Cd and Zn of SRM were 90 ± 2, 95 ± 1 and 91 ± 1%, respectively. Metal concentrations in tissue digest, SEM, porewater, and overlying water samples were determined with ICAP-AES and/or GF-AAS. Porewater and overlying water samples were diluted at least 5 times with 0.1 N nitric acid (Ultrex®) to mitigate chloride interference before direct analysis.

Data analysis. AVS and SEM concentrations, and [SEM – AVS] for 3 metals from all treatments were analyzed using multi-way analysis of variance (ANOVA) to test the effect of initial AVS level, incubation time, sediment depth, and metal levels in supplementary food. Statistical significance was set at $\alpha = 0.05$, unless otherwise noted. Limited statistical analyses were done for the dissolved metal data (overlying water and porewater) due to lack of replication and detection limits. Tissue metal concentrations in worms were analyzed by 3-way ANOVA to test the effects of [SEM – AVS], and metal concentrations in food and sediments. When a significant effect was found from ANOVA analysis, Tukey's multiple comparison test was used to determine differences between specific treatments. Dry weight based concentrations were used for all sediment and tissue data. Statistica® was used for all statistical analysis.

When [SEM – AVS] is calculated for a certain metal, all metals with greater affinity for AVS than the metal of concern should be considered as SEM (Ankley et al. 1996, B. G. Lee et al. 2000b). This practice is required to account for binding of other metals with AVS that might bind as strongly or stronger than the metal of concern. In this paper, for example, when [SEM – AVS] is calculated for Cd, the molar sum of extracted Ag, Cu, and Pb concentrations, in addition to concentrations of extracted Cd, represent SEM. Therefore, the [SEM – AVS] values for Cd in specific sediment is calculated by subtracting concentrations of AVS from the sum of Ag, Cu, Pb and Cd concentrations determined for the sediment. The sum of mean Cu and Pb SEM was 0.51 μmol g^{-1}.

RESULTS

Sediment geochemistry

A range of AVS concentrations were successfully achieved by the direct oxidation and mixing method (Table 2). AVS concentrations in the deep sediments

Table 2. Mean SEM, AVS and [SEM – AVS] in surface (0 to 5 mm) and deep (5 to 20 mm) sediments determined during equilibration ($t = -9$), at $t = 0$ and 25 d. Sediment samples at $t = -9$ and $t = 0$ were samples only at 1 cm depth. All values for S1–S5 treatments at $t = -9$ and 0 are the means of 2 replicate samples, and values for S1–S5 treatments at $t = 25$ are the means of 3 food metal treatments

Treatment	Measured AVS (μmol g^{-1})				Measured SEM (μmol g^{-1})								[SEM-AVS] (μmol g^{-1})							
					Ag				Cd				Zn				Cd			
					$t = 0$	$t = 25$			$t = 0$	$t = 25$			$t = 0$	$t = 25$			$t = 0$	$t = 25$		
	$t = -9$	$t = 0$	$t = 25$																	
			Surface	Deep		Surface	Deep			Surface	Deep			Surface	Deep			Surface	Deep	
S1	1.3	1.4	3.4	1.5	0.075	0.036	0.064	0.079	0.045	0.079	7.1	6.1	7.0	−1.3	−3.4	−1.5	−0.7	−2.8	−0.9	6.3	3.3	6.1
	(0.2)[a]	(0.5)	(0.3)	(0.2)	(0.006)	(0.003)	(0.005)	(0.005)	(0.003)	(0.000)	(0.5)	(0.4)	(0.0)	(0.5)	(0.3)	(0.2)	(0.5)	(0.3)	(0.2)	(0.0)	(0.6)	(0.2)
S2	3.4	2.2	3.5	3.2	0.065	0.033	0.053	0.078	0.053	0.080	7.2	6.0	7.2	−2.2	−3.5	−3.2	−1.5	−2.9	−2.6	5.6	3.1	4.6
	(0.5)	(0.6)	(0.6)	(0.1)	(0.002)	(0.002)	(0.003)	(0.003)	(0.000)	(0.000)	(0.9)	(0.5)	(0.1)	(0.6)	(0.6)	(0.1)	(0.6)	(0.6)	(0.1)	(0.3)	(0.9)	(0.1)
S3	6.6	5.4	3.6	7.0	0.059	0.033	0.050	0.077	0.068	0.074	7.1	6.5	6.8	−5.3	−3.6	−7.0	−4.7	−3.0	−6.4	2.3	3.5	0.5
	(2.6)	(2.5)	(0.2)	(0.9)	(0.014)	(0.003)	(0.002)	(0.003)	(0.007)	(0.002)	(1.0)	(0.3)	(0.2)	(2.5)	(0.2)	(0.9)	(2.5)	(0.2)	(0.9)	(1.5)	(0.5)	(0.9)
S4	18.1	14.6	4.9	17.9	0.053	0.032	0.044	0.079	0.045	0.074	7.2	5.0	6.9	−14.5	−4.9	−17.8	−13.9	−4.3	−17.2	−6.8	0.7	−10.2
	(1.9)	(2.8)	(0.4)	(0.6)	(0.001)	(0.001)	(0.009)	(0.002)	(0.000)	(0.002)	(0.4)	(0.2)	(0.2)	(2.8)	(0.5)	(0.6)	(2.8)	(0.5)	(0.6)	(2.4)	(0.4)	(0.6)
S5	40.4	33.6	6.3	30.7	0.041	0.035	0.036	0.077	0.047	0.071	7.2	4.5	6.7	−33.6	−6.2	−30.6	−33.0	−5.7	−30.0	−25.9	−1.2	−23.4
	(1.4)	(6.2)	(0.4)	(4.3)	(0.024)	(0.001)	(0.004)	(0.002)	(0.002)	(0.002)	(0.6)	(0.5)	(0.2)	(6.1)	(0.4)	(4.3)	(6.1)	(0.4)	(4.3)	(5.5)	(0.8)	(4.5)
Control–Oxic	nm[b]	0.2	0.5	0.3	0.003	0.003	0.003	0.002	0.002	0.002	1.0	1.1	1.1	−0.2	−0.5	−0.3	0.3	0.0	0.2	1.2	1.1	1.3
		(0.1)	–[c]	(0.0)	(0.001)	–	(0.001)	(0.001)	–	(0.001)	(0.1)	–	(0.0)	(0.1)	–	(0.0)	(0.1)	–	(0.0)	(0.0)	–	(0.0)
Conrol–Anoxic	19.6	14.4	1.9	19.3	0.002	0.002	0.002	0.001	0.002	0.001	0.9	1.0	1.0	−14.4	−1.9	−19.3	−13.9	−1.4	−18.8	−12.9	−0.5	−17.8
	(1.2)	(3.0)	–	(1.5)	(0.000)	–	(0.000)	(0.000)	–	(0.000)	(0.1)	–	(0.0)	(3.0)	–	(1.5)	(3.0)	–	(1.5)	(2.9)	–	(1.4)
Control–F5×	nm	8.6	2.2	11.4	0.002	0.009	0.001	0.004	0.013	0.002	1.0	2.4	1.0	−8.6	−2.2	−11.4	−8.0	−1.7	−10.9	−7.0	−0.6	−9.9
		(0.0)	–	(2.9)	(0.000)	–	(0.000)	(0.002)	–	(0.000)	(0.2)	–	(0.0)	(0.0)	–	(2.9)	(0.0)	–	(2.9)	(0.2)	–	(2.8)

[a]Values in parenthesis for $t = -9$ and $t = 0$ are the differences between 2 replicate values, and those for $t = 25$ are standard deviations of 3 food metal treatments
[b]Not measured
[c]No replication

(0.5 to 2 cm) did not change significantly ($p > 0.05$) during 25 d incubation time. Following 25 d incubation, AVS in the surface decreased in the sediments that were initially anoxic (control-anoxic, control-$F_{5\times}$ and S4-5 treatments), but increased in the oxic sediments (control-oxic and S1-2 treatments) ($p < 0.05$), probably due to a combination of bioturbation by the test organism *Neanthes arenaceodentata*, oxidation by aerated overlying water and decomposition of supplementary foods at the sediment surface (Table 2). Concentrations of AVS were similar in the sediments with 3 different food treatments ($p > 0.05$). Therefore, mean AVS concentrations of these 3 food treatments are reported and used for the [SEM – AVS] calculation (Table 2). The organic matter content (determined as LOI) was changed little ($p > 0.05$) in the deep sediments (6.6 ± 0.4%), but increased significantly ($p < 0.001$) in the surface (8.1 ± 0.5%) from the initial value (6.8 ± 0.6%) probably due to addition of supplementary food.

The SEM concentrations in the surface of the contaminated sediments declined significantly ($p < 0.01$) over 25 d (Table 1). SEM concentrations of Cd and Zn in the deep sediments varied little as AVS concentration increased from S1 to S5 or with incubation time ($p > 0.05$). Extractable Ag decreased significantly with AVS ($p < 0.001$) and incubation time ($p < 0.05$). Addition of supplementary foods containing 3 different levels of metals had insignificant ($p > 0.05$) effects on SEM concentrations in the metal spiked sediments. Therefore, the mean SEM values from these treatments are reported and used for the [SEM – AVS] calculation (Table 1). By design, [SEM – AVS] related inversely to AVS levels. The [SEM – AVS] of Ag and Cd for all the treatments, with the exception of control-oxic treatment, were <0. The [SEM – AVS] of Zn were mostly >0, except for some deep sediments with high AVS (S4 and 5) and unspiked sediments with high AVS (control-anoxic, control-$F_{5\times}$).

Mean Cd and Zn concentrations in porewater and overlying water increased with [SEM – AVS], suggesting that AVS influenced dissolved metal concentra-

tions (Fig. 2). Dissolved Ag was not detectable by GF-AAS (<0.01 µM) in all the treatments. The concentrations of Cd and Zn in porewater increased sharply near [SEM – AVS] = 0, while those in overlying water gradually increased with [SEM – AVS] (Fig. 2). At the beginning of incubation (t = 0), porewater Cd ranged from 0.034 ± 0.021 to <0.001 µM and porewater Zn from 7.5 ± 3.6 to 0.3 ± 0.2 µM, from most oxic (S1) to anoxic (S5) sediments (n = 3 for all). At the completion of incubation, porewater Cd in all treatments except S1 (0.003 ± 0.004 µM) were <0.001 µM and mean porewater Zn decreased to concentrations that were 14 to 67% of the initial values. Metal levels in the supplementary food had an insignificant effect ($p > 0.05$) on porewater Cd and Zn concentrations. Similarly, the mean overlying water metals, measured 6 times, decreased from S1 (0.036 ± 0.005 µM Cd and 5.0 ± 0.2 µM Zn) to S5 (0.012 ± 0.000 Cd and 0.60 ± 0.20 µM Zn). Overlying water Cd and Zn had significant ($p < 0.01$) temporal variations but without consistent trends. The treatments with the most contaminated food ($F_{5\times}$) had 0.006 ± 0.004 µM and 0.69 ± 0.80 µM more overlying water Cd and Zn, respectively, than those with uncontaminated food. These results suggest that some Cd and Zn adsorbed to food particles were released during feeding processes.

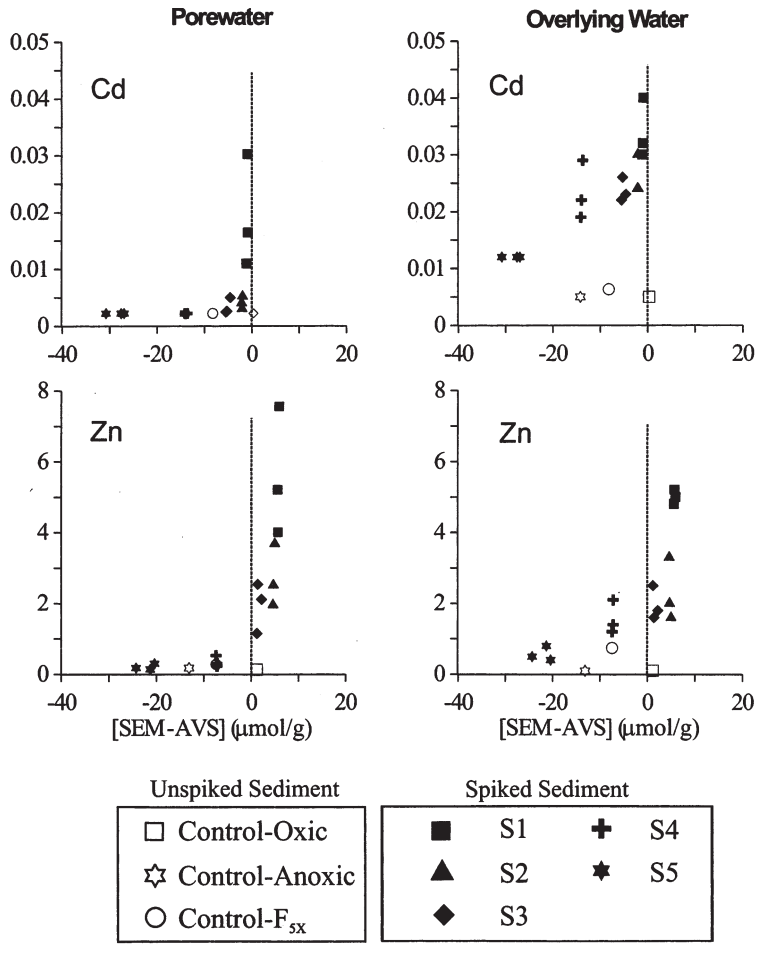

Fig. 2. *Neanthes arenaceodentata*. Time-averaged dissolved Cd and Zn concentrations in porewater (t = 0 and t = 25) and overlying water (t = 0, 4, 9, 17, 21 and 25) with relation to [SEM – AVS] determined for unspiked (open symbols) or metal-spiked (filled symbols) sediments. The 3 replicate symbols represent treatments with 3 different metal levels in foods. Nominal AVS concentration increased from S1 (1 µmol g^{-1}) to S5 (30 µmol g^{-1}). Depth and time averaged [SEM – AVS] values were used here. Dotted vertical line: [SEM – AVS] = 0

Metal bioaccumulation

Worms exposed to metal-spiked sediment ($F_{0\times}$) accumulated more than 3× the Ag and Cd found in the control worms (control-oxic and control-anoxic), although [SEM – AVS] was <0 (Figs. 3 & 4). Worms fed contaminated supplementary food ($F_{1\times}$ or $F_{5\times}$) accumulated significantly more ($p < 0.001$) Ag and Cd than worms fed uncontaminated food ($F_{0\times}$). For example, the worms fed supplementary food spiked with 5× sediment metal concentrations ($F_{5\times}$) had 3.4 × Ag and 2.3 × Cd than worms fed uncontaminated food ($F_{0\times}$). The mass of metal added as supplementary food at $F_{5\times}$ treatment was only about 3% of the total mass in contaminated sediments. The worms fed 5× metal contaminated food, but in unspiked sediment, had ca 10× Ag and Cd than the control worms (Figs. 3 & 4). Tissue Ag and Cd in the worms treated with the various food metal levels was not ($p > 0.05$) influenced by [SEM – AVS], except for tissue Ag in the $F_{5\times}$ which decreased with [SEM – AVS].

In all treatments (and controls), worms contained ca 3 µmol Zn g^{-1} (Figs. 3 & 4). Tissue Zn concentrations were not different ($p > 0.05$) from the controls in any treatment except the low AVS treatment (S1). There, [SEM – AVS] was considerably higher than other treatments and tissue Zn was ca 30% greater than in control worms (Figs. 3 & 4). Yet the differences of Zn concentrations in porewater between S1 and S5, and in foods between uncontaminated and most contaminated, were >20 times. Regulation of Zn in the various animals, especially in polychaetes, has long been reported (Bryan & Langston 1992). In the present experiments, *Neanthes arenaceodentata* seemed to regulate Zn.

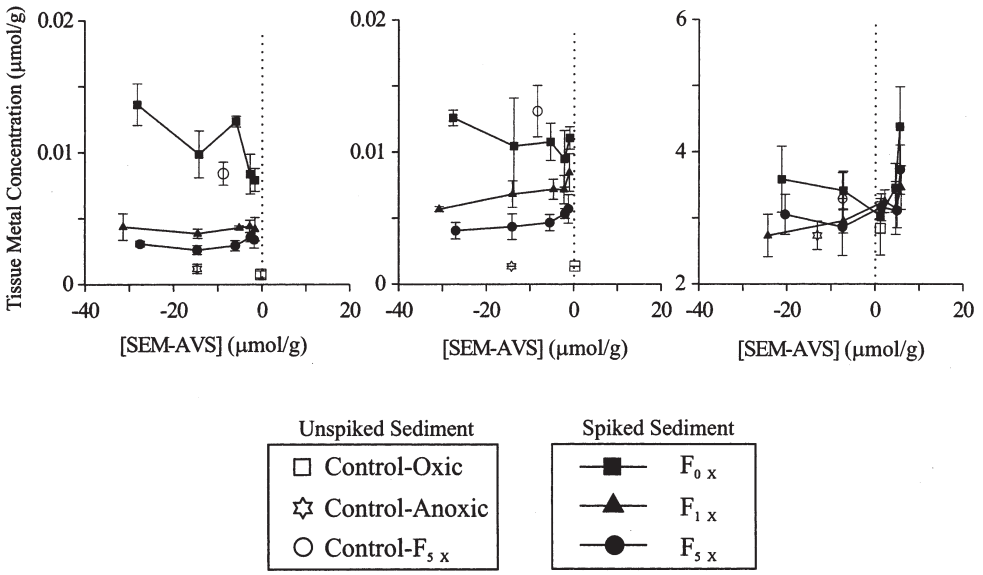

Fig. 3. *Neanthes arenaceodentata*. Mean and standard deviations (error bar) of tissue Ag, Cd and Zn concentrations in *N. arenaceodentata* (n = 3) from unspiked (open symbols) or metal-spiked (filled symbols) sediments with relation to [SEM – AVS]. Depth averaged [SEM – AVS] values were used here. The worms were fed either uncontaminated food ($F_{0\times}$), food contaminated with 1× or 5× sediment metal concentrations ($F_{1\times}$ or $F_{5\times}$). The dotted vertical line represents [SEM – AVS] = 0

Mass balance approach

The experimental design allowed determination of the relative contribution of metals from different sources to bioaccumulation in the worms, using a mass balance approach, as follows,

$$[M]_{Tissue} = [M]_{Back} + [M]_{Sed} + [M]_{Diss} + [M]_{Food} \quad (1)$$

where $[M]_{Tissue}$ is tissue metal concentrations in *Neanthes arenaceodentata* following 25 d exposure, $[M]_{Back}$ is tissue metal obtained from natural background sediments and uncontaminated food, $[M]_{Sed}$ is from ingestion of contaminated sediments, $[M]_{Diss}$ is from dissolved source (overlying water and porewater), and $[M]_{Food}$ is from ingestion of contaminated supplementary food ($F_{1\times}$ or $F_{5\times}$). The mean tissue metals in the

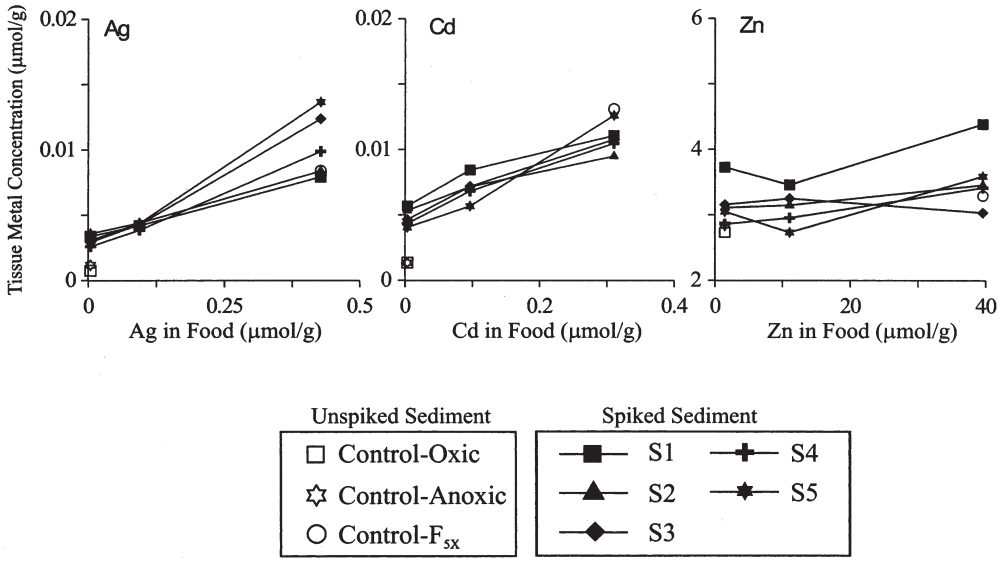

Fig. 4. *Neanthes arenaceodentata*. Mean tissue Ag, Cd and Zn concentrations in *N. arenaceodentata* from unspiked (open symbols) or metal-spiked (filled symbols) sediments with relation to metal concentrations in supplementary food. Nominal AVS concentration increased from S1 (1 µmol g^{-1}) to S5 (30 µmol g^{-1})

control worms (control-anoxic and control-oxic) were considered to be background ($[M]_{Back}$), because these worms were exposed to uncontaminated sediment and food. $[M]_{Sed}$ was calculated by subtracting $[M]_{Back}$ from the tissue metals in the worms fed uncontaminated food (F_{0x}) in spiked anoxic sediment (S5) where dissolved metal concentrations (porewater and overlying water) were minimal. It was assumed that bioavailability of metals from sediment particles having different AVS concentrations were not different (see 'Discussion'). The difference between tissue metals in S1 and S5 treatments for a given food metal level was assumed to result from the difference in dissolved metal concentrations between 2 treatments. Time-averaged dissolved Cd and Zn concentrations in porewater and overlying water in S5 were significantly lower than those in S1, or close to detection limits (Fig. 2). Therefore, contributions of dissolved metals in S5 to bioaccumulation were assumed to be none. Therefore, the maximum contribution of $[M]_{Diss}$ was calculated by subtracting the mean tissue metal concentrations in the most anoxic sediment (S5) from those in the most oxic sediment (S1). $[M]_{Food}$ was calculated by the difference of the mean tissue metal concentration in the unspiked food treatment (F_{0X}) and in the worms exposed to spiked food treatment (F_{1X} or F_{5X}). Efflux or growth dilution of tissue metals was assumed to be similar for all the treatments and therefore was not considered in this mass balance approach.

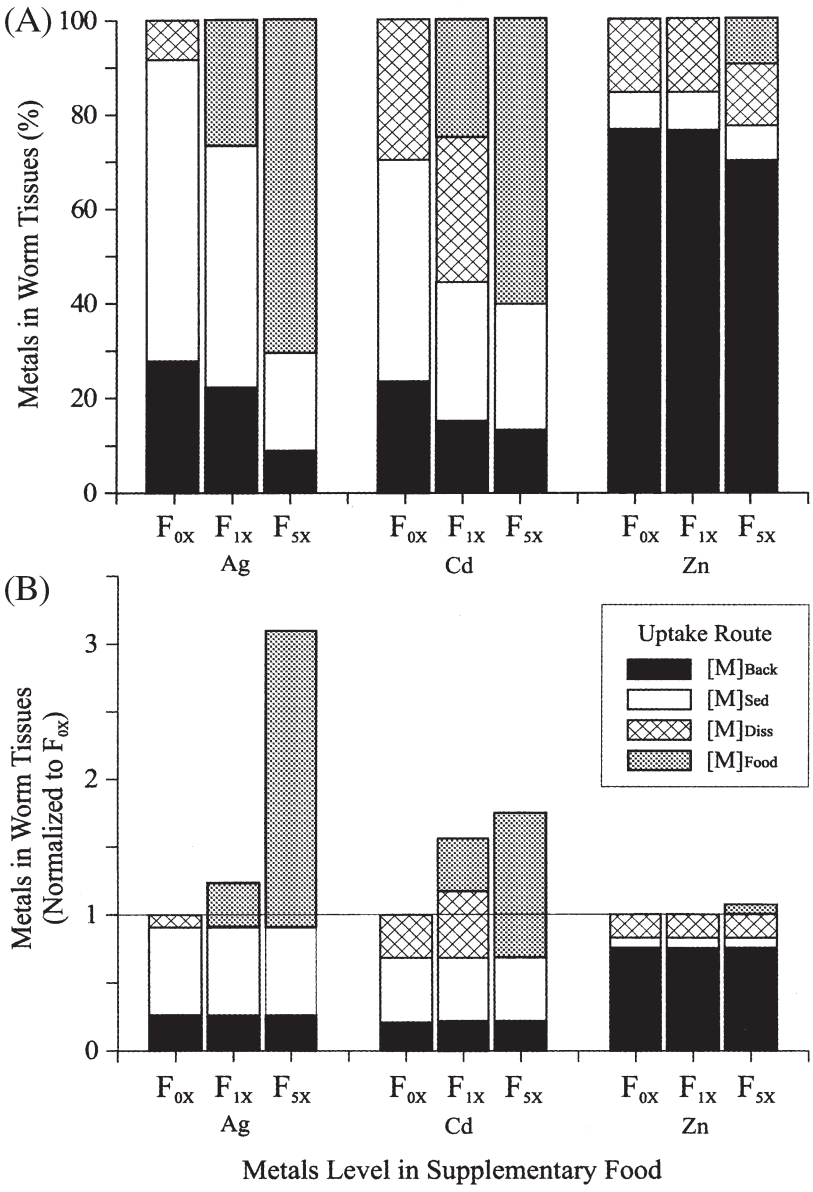

Fig. 5. *Neanthes arenaceodentata.* Estimated tissue metal concentrations in *N. arenaceodentata* from most oxidized sediments (S1) derived from the mass balance equation: $[M]_{Tissue} = [M]_{Back} + [M]_{Sed} + [M]_{Diss} + [M]_{Food}$, where $[M]_{Tissue}$ is tissue metal concentrations in the worm, $[M]_{Back}$ is tissue metal obtained from natural background sediments and uncontaminated food, $[M]_{Sed}$ is from ingestion of contaminated sediments, $[M]_{Diss}$ is from dissolved source (overlying water and porewater), and $[M]_{Food}$ is from ingestion of contaminated supplementary food (F_{1x} or F_{5x}). Tissue metal concentrations are represented as (A) % contribution from 4 different sources, or (B) normalized to tissue metal concentrations in the F_{0x} treatment

The mass balance results showed that the ingestion of contaminated sediment and food was responsible for 50 to 90% of Ag and Cd bioaccumulation in the worms (Fig. 5). The contribution of the dissolved source became negligible and contaminated particles became progressively more important when animals were fed supplementary contaminated food. Metals from the dissolved phase contributed only up to ca 8% of Ag and ca 30% of Cd bioaccumulation from the most oxidized sediments (S1) (as shown in Fig. 5). When animals were exposed to more anoxic sediments than S1, the contribution from the dissolved source was less than shown in Fig. 5 (data not shown). It was questionable whether any bioaccumulation of Zn occurred except in the most oxic sediment. There, the contribution of dissolved source ranged from 13 to 15% of tissue Zn.

DISCUSSION

This study examined the relative contribution of various uptake pathways for Ag, Cd, and Zn bioaccumulation in a deposit-feeding polychaete from moderately contaminated sediments. The overall results suggest that ingestion of contaminated sediments and supplementary food were responsible for most of metal bioaccumulation in the test worm. AVS clearly influenced partitioning of metals in both porewater and overlying water as shown by many previous studies (Hare et al. 1994, Berry et al. 1996, B. G. Lee et al. 2000b). However, neither AVS nor dissolved metal had a great influence on metal bioaccumulation. The contribution of dissolved metals decreased when the more anoxic sediments were considered. These findings are consistent with other studies, emphasizing that benthic organisms bioaccumulate metals in contaminated sediments from ingestion of various food particles, and biological attributes of animals modify exposure conditions (Wang et al. 1996, Lee & Luoma 1998, Selck et al. 1998, B. G. Lee et al. 2000a,b).

Our results are contradictory to earlier studies (Kemp & Swartz 1988, Di Toro et al. 1990, Berry et al. 1996) that concluded AVS or porewater have a major role in controlling metal bioavailability to aquatic organisms in contaminated sediment. The discrepancy could be attributable partly to the differences in experimental protocols between the studies. The earlier studies tested acute toxicity of organisms (typically, a 10 d mortality test) exposed to sediments spiked with extremely high metal concentrations. These experimental protocols accentuated exposure of organisms to the dissolved phase. Use of high metal concentration was necessary in these experiments because porewater metal concentration was controlled by spiking metals to the sediment containing a high, constant AVS concentration. In some of the studies, precipitates of metal salts formed on the surface sediments (e.g., Di Toro et al. 1990, Berry et al. 1996). Use of high metal concentrations can shift the partitioning of metals from particles to porewater, lowering the partitioning coefficient (K_d; Luoma & Fisher 1997, J. S. Lee et al. 2000). K_d values determined in the present study were generally $>10^4$ for Ag and Cd, and $>2 \times 10^3$ for Zn, much greater than values observed in some of the previous studies (see also J. S. Lee et al. 2000).

Metal spiking protocols affect the partition coefficient in several ways. Adsorption isotherms (e.g., Langmuir isotherm) can be non-linear at high adsorbate (metal) concentrations (Bourg 1987, Hassan et al. 1996). For example, Hassan et al. (1996) reported in adsorption of 5 metals to various sediments for 24 h that partitioning coefficients decreased with increased metal concentrations even when concentrations in the metal mixture were relatively low (15 to 700 µg l^{-1}). In addition, a short metal-sediment equilibration time is typically employed in many studies, which can enhance partitioning of metals to porewater relative to sediment particles (J. S. Lee et al. 2000). Partitioning coefficients typically decrease as the metal-sediment equilibration time increases (Di Toro et al. 1996, Sibley et al. 1996, Leonard et al. 1999). For example, Sibley et al. (1996) reported that porewater Zn concentrations decreased up to 3 orders of magnitude at the end of 56 d equilibration, while [SEM – AVS] values varied only $<2\times$. This decrease in porewater Zn concentrations was most pronounced in the sediments with higher Zn concentrations.

The combination of high metal concentrations and short metal-sediment equilibration inevitably accentuates the exposure of animals to metals in porewater, so a significant relationship might be expected between metal bioavailability or toxicity and [SEM – AVS] which largely controls porewater metal chemistry. These extreme conditions could also preclude or attenuate the role of dietary uptake of metals.

Manipulation of SEM to AVS by varying AVS concentrations allowed use of environmentally realistic metal concentrations in the present study (see also J. S. Lee et al. 2000). As found elsewhere, porewater metals were largely controlled by AVS and the pattern of the metal-AVS relationship was similar regardless of the sediment manipulation methods (J. S. Lee et al. 2000). Use of low metal concentrations would shift the partitioning of metals to the particulate phase. Consequently, uptake from ingestion of contaminated particles becomes more important. Other bioaccumulation studies (Selck et al. 1998, Wang et al. 1999) have also reported that marine polychaetes accumulate metals predominately from ingestion of particles when environmentally realistic metal concentrations were used. Studies (Ankley 1996 and references therein) evaluating AVS protocols for bioaccumulation have frequently reported that metal bioaccumulation increased linearly with spiked metal concentrations independent of [SEM – AVS]. In natural conditions, metal contaminants are introduced gradually and equilibrated with sediments over long periods. Dietary uptake of contaminated particles either from sediments or the water column is likely to play an important role in these circumstances.

Worms accumulated more metal overall, and more metal from ingested food particles, when they were exposed to supplementary, contaminated food particles. The magnitude and routes of the animals' exposure to metals were dynamically shifted as the external exposure conditions were altered. The increase in tissue metal concentrations with small additions of supplementary, contaminated food also suggested that the animals selectively fed on the supplementary

food. This was further evidenced by observation of *Neanthes arenaceodentata* emerging from its burrow and feeding on supplementary food particles on the sediment surface, as seen in other studies (Pesch et al. 1987). Maloney (1996), Schaanning et al. (1996) and Lee & Luoma (1998) demonstrated that various benthic invertebrates accumulate metals considerably more from ingestion of organic-rich food particles than organic-poor sediments. For example, Lee & Luoma (1998) reported that assimilation efficiency of Cd, Cr and Zn in the ingested particles by 2 bivalves *Macoma balthica* and *Potamocorbula amurensis* increased as the proportion of algal biomass increased. Similarly, Lutofo et al. (2000) reported that *N. arenaceodentata* bioaccumulated greater amounts of DDT when they were exposed to contaminated TetraMin® than when they were exposed to contaminated sediments only.

Bioassay protocols that use uncontaminated supplementary food, as does the growth test for *Neanthes arenaceodentata*, could underestimate the exposure to contaminants that animals might experience under natural conditions. In the present study, animals exposed to uncontaminated food bioaccumulated 40 to 60% less Ag and Cd than did animals exposed to food contaminated to a similar level as sediments ($F_{1\times}$). Use of nutritious supplementary food thus introduced additional complexity to bioassays. The TetraMin® used as supplementary food in the present study was enriched with organic material (LOI = 90% at 450°C for 5 h) and resulted in rapid growth of the test worms (12.3% dry wt d^{-1}). The rapid growth of test animals probably also diluted tissue metal concentrations and could have reduced their potential toxic effects (see also Lotufo et al. 2000). Additional tissue metal burden (content) data would be needed to evaluate total metal bioaccumulation when different growth regimes (or food quantitiy and quality) are considered.

The contribution of dissolved metals to tissue bioaccumulation was estimated with the assumption that the difference in uptake between the most oxic sediment (S1) and anoxic sediment (S5) was due to differences in dissolved metal concentrations. However, other factors such as differences in metal bioavailability from ingested sediments and changes in burrowing behavior of the worms in response to sulfide concentration changes, could also have contributed the difference in tissue metal concentrations. For example, Wang et al. (1999) reported that the deposit feeding polychaete *Nereis* (*Neanthes*) *succinea* assimilated Ag, Cd and Zn associated with oxic sediments up to 3× more efficiently than those from anoxic sediments. If this occurred in our experiments, the actual contribution from dissolved metals could have been less than the estimations.

CONCLUSION

The marine polychaete *Neanthes arenaceodentata* accumulated Ag and Cd predominately from ingestion of contaminated sediments. Addition of contaminated, supplementary food increased bioaccumulation. Dissolved metals in porewater and overlying water or AVS had a minor influence on metal bioaccumulation in the worm. Thus the magnitude and pathways of metal uptake in the worm were dynamically changed, following changes in external exposure conditions and various biological responses to such changes. A proposed sediment quality criterion for metals based on the AVS normalization approach could have limited application to situations where introduced metals have been equilibrated with sediments and where natural foods, over long periods and exposure pathways other than the dissolved source, dominate. Understanding of feeding ecology, the geochemical and biological factors affecting metal bioavailability, and evaluation of relevant uptake pathways for bioaccumulation, are prerequisites for the development of environmentally relevant sediment quality criteria for metals.

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