

# Do Acid Volatile Sulfides (AVS) Influence the Accumulation of Sediment-Bound Metals to Benthic Invertebrates under Natural Field Conditions?

MAARTEN DE JONGE,\* FREJA DREESEN, JOSEFINA DE PAEPE, RONNY BLUST, AND LIEVEN BERVOETS

Department of Biology, Ecophysiology, Biochemistry and Toxicology Group, University of Antwerp, Groenenborgerlaan 171, B-2020 Antwerp, Belgium

Received December 9, 2008. Revised manuscript received March 9, 2009. Accepted April 10, 2009.

The present study evaluates the influence of acid volatile sulfides (AVS) on accumulation of sediment-bound metals in benthic invertebrates under natural field conditions. Natural sediments, pore water, surface water, and two species of widespread benthic invertebrates (*Chironomus gr. thummi* and *Tubifex tubifex*) were collected from 17 historical polluted Flemish lowland rivers and measured for metal concentrations. Different sediment characteristics were determined (AVS, organic matter, clay content) and multiple regression was used to study their relationship with accumulated metals in the invertebrates. Physical and chemical analysis of the field samples indicated low metal concentrations in the water and pore water, but very high metal concentrations in the sediment and the invertebrates, especially for Pb (5.99  $\mu\text{mol/g}$ ). In general, metal accumulation in chironomids and tubificid worms was most strongly correlated with total metal concentrations in the sediment and sediment metal concentrations normalized for organic matter and clay content. Following the results of the linear regression model, AVS did not turn out to be a significant variable in describing variation in metal accumulation. Our study clearly demonstrates that, in addition to the results gained from experiments under lab conditions, benthic invertebrates can accumulate metals from unspiked field sediments even when there's an excess of AVS.

## Introduction

In the aquatic environment, the bioavailability of metals is generally controlled by different water and sediment variables. Sediment characteristics such as organic matter, iron- and manganese oxides, carbonates, and clay content can bind metal ions and therefore reduce their availability to aquatic organisms (1, 2). In anaerobic sediments, sulfate reduction by anoxic bacteria leads to the formation of sulfides, which are called acid volatile sulfides (AVS). AVS is operationally defined as the amount of sulfides volatilized by the addition of 1 N HCl and consists mainly of iron- and manganese sulfides (3, 4). In their reaction with metals, AVS form thermodynamically stable metal sulfide precipitates,

which results in a decreased concentration of free metal ions and therefore reduced metal bioavailability in the sediment pore water (3, 5). The metals that are associated with AVS are called simultaneously extracted metals (SEM). SEM is generally defined as the metal fraction that comprises the sum of molar concentrations of toxicologically important, cationic metals (Cu, Pb, Cd, Zn, and Ni) that have solubility products lower than FeS and MnS and that are extracted together with AVS (6). From this, Di Toro et al. (3) formulated the SEM–AVS model for sediment toxicology at the beginning of the 1990s. This model predicts that, when on a molar basis, AVS concentrations in sediments exceed SEM concentrations (SEM–AVS < 0), all metals will be bound to sulfides and the sediment pore water is considered to be nontoxic. On the contrary, when the sediment contains an excess of SEM (SEM–AVS > 0), metals will be released into the sediment pore water and become potentially toxic to the aquatic life (3–5).

During the past decade, a lot of evidence has been gained regarding the relation between [SEM–AVS] and acute toxicity to both freshwater and marine organisms (3–5, 7–9). Furthermore, the effect of AVS to chronic toxicity of metals has been evaluated (10–13). However, when assessing bioaccumulation, there is significant evidence that some organisms can accumulate metals even when [SEM–AVS] < 0 (14–18). This phenomenon has especially been observed for benthic invertebrates, which live in the sediment and ingest sediment particles as their main feeding source (19–21). Because of this, when entering the gut lumen, sediment-associated metals will be exposed to a chemical environment that strongly differs from naturally environmental conditions (22–26).

Although it is clear that contradictory results have been observed among studies concerning the protective capacity of AVS and the importance of major metal exposure routes (e.g., pore water vs dietary metals) (18), it is absolutely necessary that these results are extrapolated to environmentally realistic conditions. This is because most of the studies regarding the relation between [SEM–AVS] and bioaccumulation used spiked sediments with variable equilibration times, whereas the latter is an important factor in the partitioning and toxicity of metals to aquatic organisms (27). Indeed, metal concentrations in nature are very often introduced gradually over a much longer period, which results in much longer equilibration times and different metal availabilities in nature than is often the case with spiked sediments (15). Therefore, the objective of the present study was to evaluate whether [SEM–AVS] is related to metal bioaccumulation in benthic invertebrates under natural field conditions. This was done by assessing AVS and other important metal binding characteristics in sediments of different lowland rivers in Flanders and relating these characteristics to metal concentrations found in midge larvae (*Chironomidae*, *Diptera*) and tubificid worms (*Tubificidae*, *Oligochaeta*).

## Materials and Methods

**Study Area and Sampling Design.** On the basis of measurements performed by the Flemish Environment Agency (see database available from www.vmm.be, 2008) and results from former studies (2, 20, 21), 17 sample sites from different Flemish lowland rivers were chosen. The sites were selected on containing high metal concentrations in the sediment but low in the water, to minimize possible accumulation of the invertebrates from the surface water. At all sampling sites,

\* Corresponding author phone: +32 3 265 3533; fax: +32 3 265 3497; e-mail: maarten.dejonge@ua.ac.be.

benthic invertebrates, surface water, and natural sediments were collected. All samples were taken in the autumn of 2006.

At every sampling site, pH, temperature, dissolved oxygen, and conductivity of the surface water were measured in situ using a portable WTW multiline F/SET-3 field kit; each time a water sample was collected as well for metal analysis in the lab. The sediment top layer (0–2 cm) was sampled manually in triplicates using a well lockable 50 mL polyethylene tube to measure sediment characteristics and metal concentrations. At the lab, these samples were immediately frozen at  $-20\text{ }^{\circ}\text{C}$ , which is the recommended storing procedure to maintain AVS concentrations within the sampled sediment (29). Benthic invertebrates were collected using a stainless steel Petit Ponar grab sampler (Wildco cat. no. 1728, 235  $\text{cm}^2$ ). The collected invertebrates were depurated by placing them for 24 h in artificial OECD (Organization of Economic Cooperation and Development) water (2 mM  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ , 500  $\mu\text{M}$   $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 771  $\mu\text{M}$   $\text{NaHCO}_3$ , and 77.1  $\mu\text{M}$  KCl). The samples were dried for at least 24 h at  $60\text{ }^{\circ}\text{C}$ . Subsequently, the biological material was digested with concentrated ultrapure nitric acid (96%) in a microwave and stored until analysis (30). To determine the effect of depuration on metal accumulation, the metal contents of 10 invertebrate samples of different locations and both taxonomic groups were compared before and after a 24 h depuration period. All chironomids analyzed for metal content were identified as *Chironomus* gr. *thummi* and tubificids as a complex of different species including *Tubifex tubifex*.

**Water and Sediment Characterization.** Dissolved organic carbon content (DOC) in the water samples was measured using a total organic carbon analyzer (TOC-5000/5050, Shimadzu Corporation, Kyoto, Japan). Water hardness was calculated by expressing Ca and Mg as  $\text{CaCO}_3$  (mg/L). The latter was done using the formula  $2.5 * [\text{Ca}] + 4.1 * [\text{Mg}]$ .

Regarding the sediment samples, a subsample of each sediment was centrifuged (10 000  $\times$  g) to collect pore water (PW) (31). The supernatant was decanted and filtered through a 0.22  $\mu\text{m}$  membrane filter and acidified with concentrated nitric acid ( $\text{HNO}_3$ ). Acid volatile sulfides were extracted from wet sediment using the purge-and-trap method of Allen et al. (6); the extracted amount of sulfides was measured with an ORION 96–16 ion-selective sulfur electrode (Ionplus, Beverly, MA, USA). Afterward the wet/dry ratio of the sediment sample was determined to convert the measured sulfide value to dry weight. The remaining SEM fraction was stored in a fridge at  $4\text{ }^{\circ}\text{C}$  until metal analysis. The organic matter content of the sediment was determined through loss of ignition (LOI). For this purpose, dry sediment was incinerated at  $550\text{ }^{\circ}\text{C}$  for 4 h. Furthermore, particle-size distribution of the sediment was analyzed by laser diffraction (Malvern Mastersizer S., Worcestershire, UK) (32). At last, the total metal content was measured by drying the sediments at  $60\text{ }^{\circ}\text{C}$  for 48 h and adding a mixture of  $\text{HNO}_3$  and HCl (4:1, v/v); subsequently, samples were transferred to Teflon bombs and digested in a microwave oven (ETHOS 900 Microwave Labstation, Milestone, Italy) (33). After digestion, samples were filtered and diluted with ultrapure water (Milli-Q, Bedford, MA, USA) up to 50 mL and stored until metal analysis.

**Metal Analysis.** As, Ca, Cd, Co, Cr, Cu, Mg, Zn, Ni, and Pb concentrations were measured in filtered (0.22  $\mu\text{m}$  membrane) water samples (both surface water and pore water), sediment samples (both total and SEM), and in the digested invertebrate samples using an Inductively Coupled Plasma Mass Spectrometer (ICP-MS, Varian UltraMass 700, Victoria, Australia), with yttrium as internal standard. Analytical accuracy was achieved by the use of blanks and certified reference material (CRM) of the Community Bureau of Reference (European Union, Brussels, Belgium), including standards for trace elements in river sediment (CRM 320)

and mussel tissue (CRM 278). Recoveries were within 10% of the certified values.

**Statistical Analysis.** Prior to analysis, all data were tested for homogeneity of variance by the Folded-F test and for normality by the Shapiro-Wilkinson test. The differences in accumulation between depurated and nondepurated organisms and the differences in accumulation between species were tested using the *t* test. To determine the relation between metal accumulation and sediment variables the Pearson correlation-coefficient was used. Subsequently, linear multiple regression was used to assess the contribution of different environmental variables to the accumulation of metals in both chironomids and tubificids. For this purpose, water variables, such as pH, dissolved oxygen, conductivity, DOC, hardness, and metal concentrations, as well as sediment variables (i.e., organic matter, clay content, AVS, SEM, metal levels in the pore water, and total metal concentrations) were used as independent variables. The significance level is represented as \*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$ . All statistical analyses were performed using the software package SAS (SAS 9.2, SAS Institute Inc., Cary, NC, USA).

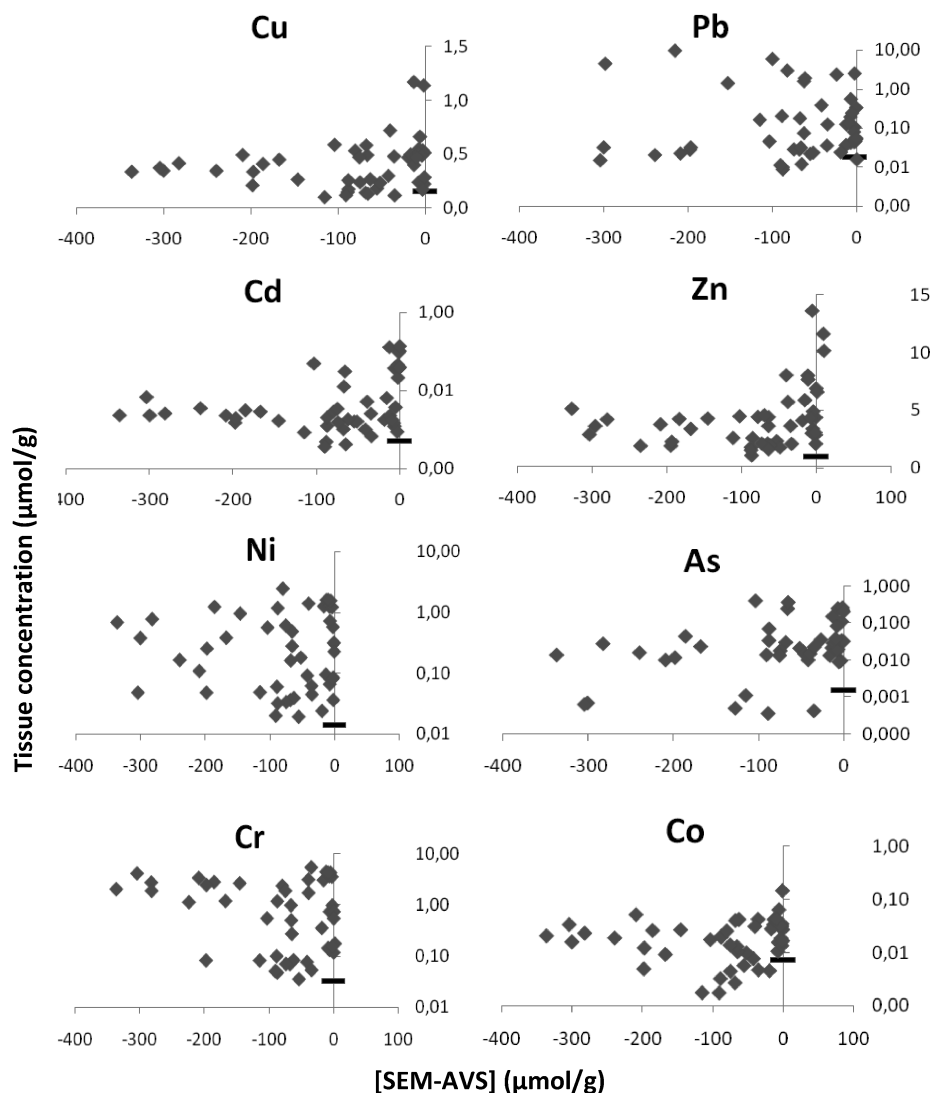
## Results

**Metal Concentrations in Water and Sediment.** Table S1 of the Supporting Information summarizes the results of dissolved metal levels in the water samples of the different sampling sites. Metal concentrations were generally low, ranging from below the detection limit up to  $90.5 \pm 68.7$  nmol/L for Cr,  $156 \pm 87.7$  nmol/L for Co,  $461 \pm 1.08$  nmol/L for Ni,  $74.7 \pm 3.73$  nmol/L for Cu,  $96.5 \pm 80.1$  nmol/L for As,  $25.7 \pm 31.8$  nmol/L for Cd, and  $37.2 \pm 52.2$  nmol/L for Pb. Only for Zn, exceptionally high concentrations ( $31.0 \pm 6.34$   $\mu\text{mol/L}$ ) were measured at site 8.

Total metal concentrations measured in the sediment samples are presented in Table S2 of the Supporting Information. Total sediment concentrations of Cr, Ni, Cu, Zn, As, Cd, and Pb were quite high and very variable among the different sampling sites. For example, Cu concentrations ranged from  $0.235 \pm 0.142$   $\mu\text{mol/g}$  until  $10.2 \pm 16.2$   $\mu\text{mol/g}$ . Exceptional high Pb concentrations were measured at sample site 15 ( $75.0 \pm 41.5$   $\mu\text{mol/g}$ ) and 16 ( $439 \pm 197$   $\mu\text{mol/g}$ ).

**Water and Sediment Characteristics.** General characteristics of the sediment and the water samples are summarized in Table S3 of the Supporting Information. AVS concentrations showed a high variability among the sampling sites, ranging from low ( $0.763 \pm 0.443$   $\mu\text{mol/g}$  dry wt) until very high ( $205 \pm 70$   $\mu\text{mol/g}$  dry wt). At most sites, standard deviations were rather high. The clay content ranged from  $1.12 \pm 0.08\%$  up to  $11.2 \pm 0.7\%$ ; the LOI concentration varied from  $0.651 \pm 0.010\%$  up to  $24.4 \pm 4.0\%$ . Standard deviations for both clay content and LOI concentrations were rather low and showed less variation than was the case for the concentrations of AVS. The pH of the water samples ranged from 6.53 up to 7.86. The level of dissolved oxygen in the water varied from very low (1.40 mg/L) up to near saturation (7.86 mg/L) and electrical conductivity of the water differed from 241  $\mu\text{S/cm}$  until 9860  $\mu\text{S/cm}$ . DOC concentrations varied from  $4.49 \pm 0.06$   $\mu\text{g/l}$  until  $34.3 \pm 0.18$   $\mu\text{g/l}$ ; water hardness ranged from soft water (40.9 mg/L  $\text{CaCO}_3$ ) until very hard water (3864 mg/L  $\text{CaCO}_3$ ). Water temperature varied from  $11.3\text{ }^{\circ}\text{C}$  until  $19.5\text{ }^{\circ}\text{C}$ .

Table S4 of the Supporting Information gives an overview of the measured SEM concentrations (Cu, Pb, Cd, Zn, and Ni are presented), extracted together with the AVS. SEM concentrations were very variable between the sampling sites and were rather high for most of the individual metals; total SEM concentrations ranged from  $0.548 \pm 0.070$   $\mu\text{mol/g}$  until  $67.8 \pm 16.1$   $\mu\text{mol/g}$ . However, SEM concentrations almost nowhere exceeded the AVS concentrations on a molar basis.



**FIGURE 1.** Relation between metal accumulation in chironomid larvae and [SEM-AVS]. The black bars indicate background levels of metals in larvae from unpolluted freshwater sediments (2).

This resulted in negative  $\text{SEM}_{\text{Tot}}-\text{AVS}$  values at almost every sampling site, except for sites 8 and 14.

Metal concentrations in the pore water were rather low for most of the considered metals (results not shown). Generally no significant relations were found between metal concentrations in the sediment pore water and [SEM-AVS] (Figure S1 of the Supporting Information).

#### Metal Accumulation in Chironomids and Tubificids.

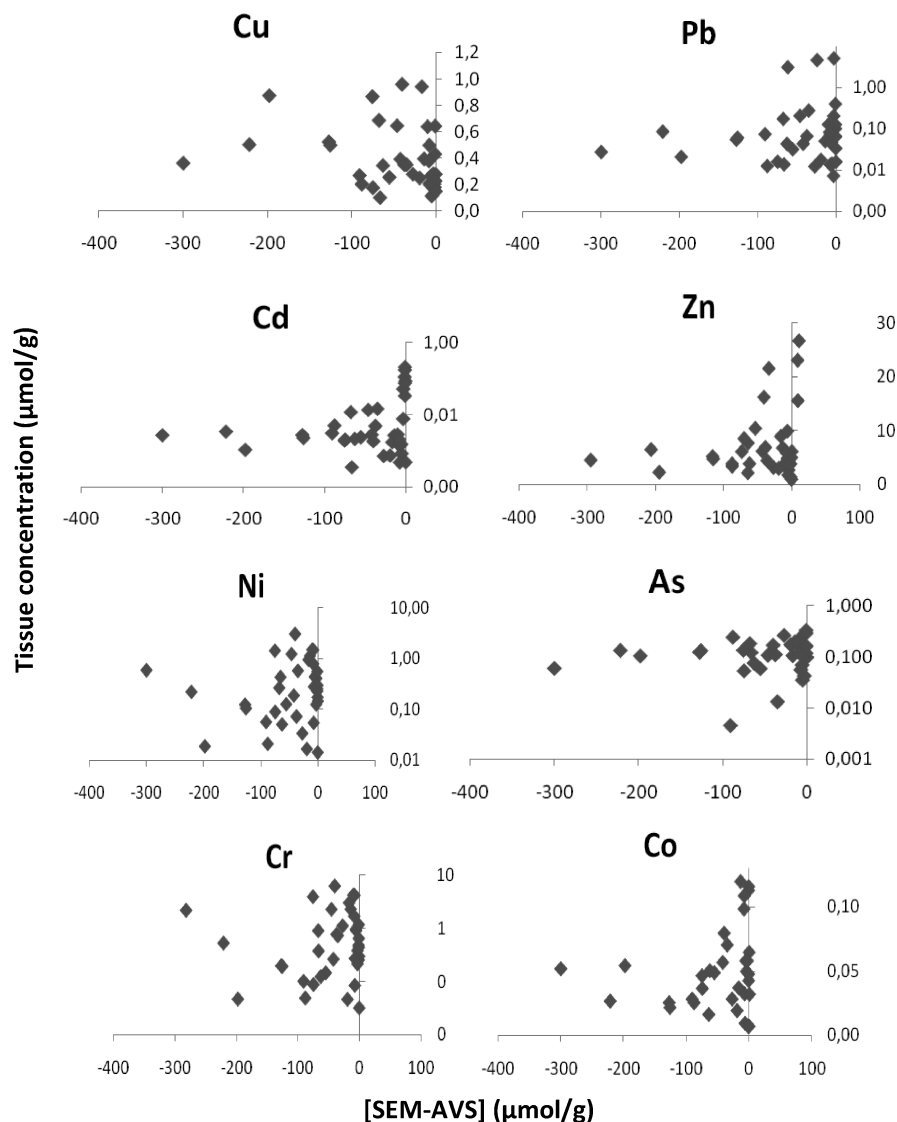
Figure S1 of the Supporting Information summarizes the effect of depuration on the accumulation of Cd in both chironomid larvae and tubificid worms. Cd concentrations measured in chironomid larvae were significantly lower ( $p = 0.0284$ ) when the organisms were depurated previously. Regarding the tubificid worms, there were no significant differences in Cd concentrations between depurated and nondepurated worms. The same results were observed for other measured metals (data not shown).

Figure S3 of the Supporting Information presents the accumulation of all metals measured in depurated chironomid larvae. The larvae were found at every sampling site, except for sites 5 and 7. Metal accumulation in chironomid larvae is generally high but very variable between the sampling sites. A very high accumulation of Pb was found in chironomids from site 15 ( $2.00 \pm 0.48 \mu\text{mol/g}$ ) and 16 ( $5.90 \pm 2.93 \mu\text{mol/g}$ ), also Cu ( $0.942 \pm 0.375 \mu\text{mol/g}$ ), Cd ( $0.106 \pm 0.009 \mu\text{mol/g}$ ), Zn ( $11.6 \pm 5.05 \mu\text{mol/g}$ ), Ni ( $1.46 \pm$

$0.20 \mu\text{mol/g}$ ), As ( $0.270 \pm 0.123 \mu\text{mol/g}$ ), and Cr ( $4.22 \pm 1.02 \mu\text{mol/g}$ ) accumulated in high concentrations in larvae of certain sampling sites.

The accumulation of metals in depurated tubificid worms is presented in Figure S4 of the Supporting Information. No worms were found at sites 2, 4, 10, 11, and 16. Metal accumulation in tubificids generally followed the same pattern as in the chironomids. Tubificid worms significantly accumulated more Zn ( $p = 0.0013$ ), As ( $p < 0.0001$ ), and Co ( $p < 0.0001$ ) than chironomids.

Figure 1 shows the relation between Cu, Pb, Cd, Zn, Ni, As, Cr, and Co accumulation and [SEM-AVS] in chironomid larvae. At several sampling sites a high amount of metals was accumulated, even when  $[\text{SEM-AVS}] \ll 0$ . This high accumulation was most remarkable for Ni, Pb, As, and Cr. The same pattern was found with the tubificid worms, as shown in Figure 2. Table S5 of the Supporting Information presents the Pearson correlations between metal concentrations in organisms, sediment fractions, and water. In general, metal accumulation in chironomids and tubificids was most strongly correlated with total metal concentrations in the sediment and sediment metal concentrations normalized for LOI and clay content. Only one significant correlation was found between metal accumulation and [SEM-AVS] or [AVS]. The latter was the case for Cd accumulation in chironomids. Furthermore, no single significant correlation



**FIGURE 2.** Relation between metal accumulation in tubificid worms and [SEM–AVS].

**TABLE 1.** Linear Regression Models for the Metal Accumulation in Chironomid Larvae and Tubificid Worms<sup>a</sup>

variable	[SEM]	[AVS]	[total Me] <sub>Sed</sub>	[LOI]	[clay]	model <i>r</i> <sup>2</sup>
Chi Pb	0.08915***					0.8127
Tub Pb	0.07714**		0.06174***	−0.00528*		0.9965
Chi Cd			0.38156***	−0.00135*		0.4879
Tub Zn				0.42350*		0.5609
Chi Ni			1.20564***	−0.03081*		0.4539
Tub Ni			0.81500*			0.1525
Chi As	0.69384*					0.4065
Chi Co				−0.00147*		0.1134
Tub Co			0.25979*			0.1680

<sup>a</sup> Parameter estimates of the significant variables are presented. \*: *p* < 0.05; \*\*: *p* < 0.01; \*\*\*: *p* < 0.001. Chi: *Chironomus* gr. thummi., Tub: *Tubifex* sp. Only metals with significant variables are presented.

was found between metal accumulation and metal concentrations in the pore water. On the other hand, metal concentrations in the surface water correlated significantly with some accumulated metals such as Cd and Zn. However, when removing the two sample points with elevated Cd and Zn levels in the surface water, the above-mentioned correlations proved to be not significant any more. Important metal-binding water characteristics such as pH, DOC, and hardness showed no significant correlation with accumulated metals as well.

**Linear Regression Models.** Table 1 summarizes the linear regression models for the accumulation of metals in both chironomid larvae and tubificid worms. Significant relations were found between accumulated and environmental levels for Pb, Cd, Zn, Ni, As, and Co, with between 11.3% and 99.6% of the variation being described. The later was found for Pb and is due to 3 sample points containing extraordinary high Pb concentrations in the sediment. Only the sediment variables SEM, total sediment metal, and LOI turned out to be significant factors explaining part of the variation in metal

accumulation. In none of the cases AVS turned out to be a significant variable in describing variation in metal accumulation. Moreover, no contribution was found of the metal concentrations in pore water and surface water.

## Discussion

In the present study, metal concentrations in the water were generally low and, except for Zn and Cd at certain sites, did not exceed current Flemish or international water quality standards (34–37). On the other hand, at most sampling sites, metal concentrations in the sediment were very high, compared to Flemish background concentrations (38), existing sediment quality standards (35, 36, 39), and to recent literature (40). AVS concentrations measured in this study were high compared to the results of other field based studies (41, 42) but are in accordance with the results of De Cooman et al. (43) and Vangheluwe et al. (44) who also studied Flemish locations. The latter measured AVS concentrations ranging up to 690  $\mu\text{mol/g}$ . Moreover, AVS concentrations were very variable among the different sampling sites and even between the subsamples within sites. This strong variability is not remarkable because AVS can be subject to a large variability both in space and in time (41, 42).

The amount of metals accumulated in chironomids was high compared to the levels of metal accumulation found by Bervoets et al. (2) in larvae from unpolluted freshwater sediments. It is striking that high amounts of Cu, Pb, Cd, Zn, Ni, As, and Cr were measured in both larvae and worms, even when the amount of AVS largely exceeded the amount of SEM. Many studies have already observed bioaccumulation in benthic invertebrates, even when  $[\text{SEM}-\text{AVS}] < 0$  (10, 11, 14–18, 45, 46). Ankley (5) ascribed this phenomenon partly to the fact that, in some studies regarding bioaccumulation, the organisms were not purged before metal analysis (11, 45). Indeed, the fraction of sediment-bound metals in the gut lumen is not absorbed by the organism and this could lead to an overestimation of the actual metal accumulation (47). However, in the present study, all organisms were depurated prior to the metal analysis. Our comparison of both depurated and non-depurated organisms clearly demonstrated that chironomid larvae significantly contained less metals when they were depurated. Moreover, other studies also observed metal bioaccumulation by  $[\text{SEM}-\text{AVS}] < 0$  while organisms were purged (15, 16, 46, 48). The higher accumulation in Zn, As, and Co found by tubificid worms, compared to midge larvae, can probably be related to differences in the accumulation pattern, nature, and degree of storage detoxification of the accumulated metals and contrast in feeding behavior and food preferences. It is known that *Tubifex tubifex* selectively feeds on smaller silt-clay and organic-rich sediment particles (49), which both have an elevated capacity for binding metals (1, 2).

In the current study, only one significant relation was found between metal accumulation and  $[\text{SEM}-\text{AVS}]$  or  $[\text{AVS}]$ . This relation was found for Cd accumulation in chironomid larvae. Despite the fact that some correlations were found between accumulated metals and levels in surface water, which were due to elevated Zn and Cd concentrations in the water at certain sample points, metal accumulation generally was most strongly correlated with total (and presumably correlated bioavailable) metal concentrations in the sediment. Therefore, we can assume that, for the organisms in the present study, the most dominant route of metal uptake occurred through exposure via sediment-bound metals. Besides, the importance of the ingestion of sediment as a major route of metal accumulation has been demonstrated for both chironomid larvae and tubificid worms (20, 21, 48, 50). Following the

results of the linear regression model, no significant contributions of  $[\text{AVS}]$  to metal accumulation were found as well. On the other hand, strong significant relations were found between metal accumulation and metal levels in the sediment as well as metal levels normalized for LOI and clay content and this for both chironomids and tubificids. The total level of metals in the sediment and the LOI also appeared to be the most important environmental variable, significantly explaining a part of the variation in metal accumulation. The sediment's pore water was not related to metal accumulation nor was it significant in the linear regression model.

Our results are in accordance with the findings of Lee et al. (15–17), who also noticed significant accumulation of Cd, Ni, and Zn in marine clams and polychaetes when  $[\text{SEM}-\text{AVS}] < 0$ . In these studies, bioaccumulation also increased linearly with the sediment metal concentrations, independent of AVS, or pore-water metal concentrations. The results of a study by Otero et al. (46), which is quite similar to the present study, indicated that the marine polychaete *Nereis diversicolor* accumulates Ni and Cu from sediments with  $[\text{SEM}/\text{AVS}] < 1$  as well. Griscom et al. (25, 26) found out that both the deposit-feeding clam *Macoma balthica* and the suspension-feeding mussel *Mytilus edulis* assimilated Cd, Co, Cr, Se, and Zn from anoxic sediments. In contrast with our results, Lee and Lee (7) found a significant relation between  $[\text{AVS}]$  and bioaccumulation of Cd and Zn, however not for Ni. The authors suggested that the exposure route differed between Cd/Zn (via water) and Ni (via sediment).

Lee et al. (15–17) ascribed their findings, that metal bioaccumulation from contaminated sediments was better related to extractable, sediment-associated metals rather than to  $[\text{AVS}]$  or pore-water metals, to the fact that benthic organisms ingest sediment-bound metals directly via dietary uptake, regardless of AVS. In addition, surface sediments are subjected to repeated passes along the digestive systems of detritus-eating organisms. The geochemical properties within animal guts can differ substantially from sedimentary environments with regard to pH, redox conditions, DOC, digestive enzymes, and surfactants (22–26). Mayer et al. (22, 23) extracted significantly more metals by using digestive fluids of marine invertebrates than would be predicted from water–solid partitioning with clean seawater. In a radiotracer experiment, Lee et al. (17) directly demonstrated that *Macoma balthica* and *Mytilus edulis* could assimilate sulfide-associated Ag and Cd. Griscom et al. (26) showed that gut fluids from mussels and clams released AVS-bound metals. The results of these previous studies clearly prove that gut biochemistry can influence the stability of mineral phases, trace metal partitioning, and affect metal bioavailability to aquatic organisms as well (22–26).

If metal accumulation from ingested sediment contributes to the total body residues, then studies that use only pore water as a basis for estimating bioavailability (i.e., the  $[\text{SEM}-\text{AVS}]$  model) will underestimate the bioaccumulation and possible related effects of metals. Metals taken up from the diet can represent a significant proportion of total metal intake and therefore contribute to subsequent metal toxicity effects. Although a body of literature exists on the importance of dietary exposure to metal uptake from sediments, clear relations between toxicological effects and food-borne metals are limited.

Because the present study only assessed bioaccumulation in benthic invertebrates, it is not obvious that the same assumptions can be made for other invertebrates, which do not reside most of the time in the sediment. Probably a clear difference has to be made between the uptake route of metals (diet-borne vs water-borne metal)

to the organisms when assessing bioaccumulation and its potential toxicity. Therefore, it is very important that, in future research regarding bioaccumulation, multiple species with different feeding strategies are studied and that ecological processes are taken into account as well. Finally, it is essential that more knowledge is gained concerning the relation between metal accumulation and its toxic effects with an emphasis on the route of exposure.

### Acknowledgments

We would like to thank Dr. Valentine Mubiana and Marcel Selens for the metal analysis. Furthermore, we like to express special thanks to Lien Van Gool for the English revision.

### Supporting Information Available

Results regarding metal concentrations in surface water, sediment, and SEM; sediment characteristics; r-values; the relation between total metal concentration in PW and [SEM-AVS]; and the effect of depuration and accumulated metal concentrations. This material is available free of charge via the Internet at <http://pubs.acs.org>.

### Literature Cited

- (1) Luoma, S. N. Bioavailability of trace metals to aquatic organisms. A review. *Sci. Total Environ.* **1983**, *28*, 1–22.
- (2) Bervoets, L.; Blust, R.; de Wit, M.; Verheyen, R. Relationships between river sediment characteristics and trace metal concentrations in Tubificid worms and Chironomid larvae. *Environ. Pollut.* **1997**, *95*, 345–356.
- (3) Di Toro, D. M.; Mahony, J. D.; Hansen, D. J.; Scott, K. J.; Hicks, M. B.; Mays, S. M.; Redmond, M. S. Toxicity of cadmium in sediments: The role of acid volatile sulfides. *Environ. Toxicol. Chem.* **1990**, *9*, 1487–1502.
- (4) Di Toro, D. M.; Mahony, J. D.; Hansen, D. J.; Scott, K. J.; Carlson, A.; Ankley, G. T. Acid volatile sulphide predicts the acute toxicity of cadmium and nickel in sediments. *Environ. Sci. Technol.* **1992**, *26*, 96–101.
- (5) Ankley, G. T. Evaluation of metal/acid-volatile sulfide relationships in the prediction of metal bioaccumulation by benthic macroinvertebrates. *Environ. Toxicol. Chem.* **1996**, *5*, 2138–2146.
- (6) Allen, H. E.; Gongmin, F.; Deng, B. Analysis of acid volatile sulfides (AVS) and simultaneously extracted metals (SEM) for estimation of potential toxicity in aquatic sediments. *Environ. Toxicol. Chem.* **1993**, *12*, 1441–1453.
- (7) Lee, J.-S.; Lee, J.-H. Influence of acid volatile sulfides and simultaneously extracted metals on the bioavailability and toxicity of a mixture of sediment-associated Cd, Ni, and Zn to polychaetes *Neanthes arenaceodentata*. *Sci. Total Environ.* **2005**, *338*, 229–241.
- (8) Ankley, G. T.; Phipps, G. L.; Leonard, E. N.; Benoit, D. A.; Mattson, V. R.; Kosian, P. A.; Cotter, A. M.; Dierkes, J. R.; Hansen, D. J.; Mahony, J. D. Acid volatile sulfide as a factor mediating cadmium and nickel bioavailability in contaminated sediments. *Environ. Toxicol. Chem.* **1991**, *10*, 1299–1307.
- (9) Hansen, D. J.; Berry, W. J.; Mahony, J. D.; Boothman, W. S.; Di Toro, D. M.; Robson, D. L.; Ankley, G. T.; Ma, D.; Yan, Q.; Pesch, C. E. Predicting the toxicity of metal-contaminated field sediments using interstitial concentration of metals and acid-volatile sulfide normalizations. *Environ. Toxicol. Chem.* **1996**, *15*, 2080–2094.
- (10) Hare, L.; Carignan, R.; Huerta-Diaz, M. A field study of metal toxicity and accumulation by benthic invertebrates — implications for the acid-volatile sulphide (AVS) model. *Limnol. Oceanogr.* **1994**, *39*, 1653–1668.
- (11) Ingersoll, C. G.; Brumbaugh, W. G.; Dwyer, F. J.; Kemble, N. E. Bioaccumulation of metals by *Hyalella azteca* exposed to contaminated sediments from the upper Clark-fork River, Montana. *Environ. Toxicol. Chem.* **1994**, *13*, 2013–2020.
- (12) De Witt, T. H.; Swart, R. C.; Hansen, D. J.; Berry, W. J.; McGovern, D. Interstitial metal and acid volatile sulfide predict the bioavailability of cadmium during a full life-cycle sediment toxicity test using the estuarine amphipod *Leptocheirus plumulosus*. *Environ. Toxicol. Chem.* **1996**, *15*, 2095–2101.
- (13) Vandegehuchte, M. B.; Roman, Y. E.; Nguyen, L. T. H.; Janssen, C. R.; De Schampelaere, K. A. C. Toxicological availability of nickel to the benthic oligochaete *Lumbriculus variegatus*. *Environ. Int.* **2007**, *33*, 736–742.

- (14) Pesch, C. E.; Hansen, D. J.; Boothman, W. S. The role of acid-volatile sulfide and interstitial water concentrations in determining bioavailability of cadmium and nickel from contaminated sediments to marine polychaete *Neanthes Arenaceodentata*. *Environ. Toxicol. Chem.* **1995**, *14*, 129–141.
- (15) Lee, B.-G.; Griscom, S. B.; Lee, J.-S.; Choi, H.-J.; Koh, C.-H.; Luoma, S. N.; Fisher, N. S. Influences of dietary uptake and reactive sulfides on metal bioavailability from aquatic sediments. *Science* **2000a**, *287*, 282–284.
- (16) Lee, B.-G.; Lee, J.-S.; Luoma, S. N.; Choi, H.-J.; Koh, C.-H. Influence of acid volatile sulfide and metal concentrations on metal bioavailability to marine invertebrates in contaminated sediments. *Environ. Sci. Technol.* **2000b**, *34*, 4511–4516.
- (17) Lee, J.-S.; Lee, B.-G.; Yoo, H.; Koh, C.-H.; Luoma, S. N. Influence of reactive sulfide (AVS) and supplementary food on Ag, Cd and Zn bioaccumulation in the marine polychaete *Neanthes arenaceodentata*. *Mar. Ecol.: Prog. Ser.* **2001**, *216*, 129–140.
- (18) Yoo, H.; Lee, J.-S.; Lee, B.-G.; Lee, I. T.; Schlekot, C. E.; Koh, C.-H.; Luoma, S. N. Uptake pathway for Ag bioaccumulation in three benthic invertebrates exposed to contaminated sediments. *Mar. Ecol.: Prog. Ser.* **2004**, *270*, 141–152.
- (19) Luoma, S. N.; Johns, C.; Fisher, N. S.; Steinberg, N. A.; Oremland, R. S.; Reinfelder, J. R. Determination of selenium bioavailability to a benthic bivalve from particulate and solute pathways. *Environ. Sci. Technol.* **1992**, *26*, 485–491.
- (20) Bervoets, L.; De Bruyn, L.; Van Ginneken, L.; Blust, R. Accumulation of Cs-137 by larvae of the midge *Chironomus riparius* from sediment: Effect of potassium. *Environ. Toxicol. Chem.* **2003**, *22*, 1589–1596.
- (21) Bervoets, L.; Meregalli, G.; De Cooman, W.; Goddeeris, B.; Blust, R. Caged midge larvae for the assessment of metal bioaccumulation from sediments *in situ*. *Environ. Toxicol. Chem.* **2004**, *23*, 443–454.
- (22) Mayer, L. M.; Chen, Z.; Findlay, R. H.; Fang, J.; Sampson, S.; Self, R. F. L.; Jumars, P. A.; Quetel, C.; Donard, O. F. X. Bioavailability of sedimentary contaminants subject to deposit-feeder digestion. *Environ. Toxicol. Chem.* **1996**, *30*, 2641–2645.
- (23) Mayer, L. M.; Schick, L. L.; Self, R. F. L.; Jumars, P. A.; Findlay, R. H.; Chen, Z.; Sampson, S. Digestive environments of benthic macroinvertebrate guts: Enzymes, surfactants and dissolved organic matter. *J. Mar. Res.* **1997**, *55*, 785–812.
- (24) Chen, Z.; Mayer, L. M. Sedimentary metal bioavailability determined by the digestive constraints of marine deposit feeders: Gut retention time and dissolved amino acids. *Mar. Ecol.: Prog. Ser.* **1999**, *176*, 139–151.
- (25) Griscom, S. B.; Fisher, N. S.; Luoma, S. N. Geochemical influences on assimilation of sediment-bound metals in clams and mussels. *Environ. Sci. Technol.* **2000**, *34*, 91–99.
- (26) Griscom, S. B.; Fisher, N. S.; Aller, R. C.; Lee, B.-G. Effects of gut chemistry in marine bivalves on the assimilation of metals from ingested sediment particles. *J. Mar. Res.* **2002**, *60*, 101–120.
- (27) Lee, J.-S.; Lee, B.-G.; Luoma, S. N.; Yoo, H. Importance of equilibrium time in the partitioning and toxicity of zinc in spiked sediment bioassays. *Environ. Toxicol. Chem.* **2004**, *23*, 65–71.
- (28) www.vmm.be. Flemish Environmental Association: Water monitoring data bank, Belgium, 2008.
- (29) de Lange, H. J.; van Griethuysen, C.; Koelmans, A. A. Sampling method, storage and pretreatment of sediment affect AVS concentrations with consequences for bioassay responses. *Environ. Pollut.* **2008**, *151*, 243–251.
- (30) Blust, R.; Van der Linden, A.; Verheyen, E.; Declercq, W. Evaluation of microwave heating digestion and graphite furnace atomic absorption spectrometry with continuum source background correction for the determination of Fe, Cu and Cd in brine shrimp. *J. Anal. At. Spectrom.* **1988**, *3*, 387–393.
- (31) Ankley, G. T.; Schubauer-Berigan, M. K. Comparison of techniques for the isolation of sediment pore-water for toxicity testing. *Arch. Environ. Contam. Toxicol.* **1994**, *27*, 507–512.
- (32) Queralt, I.; Barreiros, M. A.; Carvalho, M. L.; Costa, M. M. Application of different techniques to assess sediment quality and point source pollution in low-level contaminated estuarine recent sediments (Lisboa coast, Portugal). *Sci. Total Environ.* **1999**, *241*, 39–51.
- (33) Tessier, A.; Campbell, P. G. C.; Auclair, J. C.; Bisson, M. Relationships between the partitioning of trace metals in sediments and their accumulation in the tissues of the freshwater mollusc *Elliptio complanata* in a mining area. *Can. J. Fish. Aquat. Sci.* **1984**, *41*, 1463–1472.
- (34) Flemish Government. Besluit van de Vlaamse Regering van 1 juni 1995 houdende vaststelling van het Vlaamse reglement

- betreffende de milieuvergunning (Vlarem), zoals gewijzigd bij besluit van 17 juli 2000. Belgisch Staatsblad; 2000. 5 augustus, (in Dutch).
- (35) US Environmental Protection Agency: National recommended water quality criteria-correction; Washington, DC, US, 1999. EPA 822/Z-99-001.
- (36) Canadian Council of Ministers of the Environment (CCME). Canadian environmental quality guidelines, Winnipeg, MB, Canada, 1999.
- (37) Nixon, S.; Zabel, T. Proposed environmental quality standards for priority substances - Current compliance and potential benefits, 2005.
- (38) De Cooman, W.; Florus, M.; Devroede, M. P. *Characterisation of Sediments of Flemish Watercourses*; Ministry of the Flemish Government: Brussels, Belgium, 1998.
- (39) MacDonald, D. D.; Ingersoll, C. G.; Berger, T. A. Development and evaluation of consensus-based sediment quality guidelines for freshwater ecosystems. *Arch. Environ. Contam. Toxicol.* **2000**, *39*, 20–31.
- (40) Bervoets, L.; Voets, J.; Covaci, A.; Chu, S. G.; Qadah, D.; Smolders, R.; Schepens, P.; Blust, R. Use of transplanted zebra mussels (*Dreissena polymorpha*) to assess the bioavailability of micro-contaminants in Flemish surface waters. *Environ. Sci. Technol.* **2005**, *39*, 1492–1505.
- (41) van Griethuysen, C.; de Lange, H. J.; van den Heuvel, M.; de Bies, S. C.; Gillissen, F.; Koelmans, A. A. Temporal dynamics of AVS and SEM in sediment of shallow freshwater floodplain lakes. *Appl. Geochem.* **2006**, *21*, 632–642.
- (42) Burton, G. A.; Green, A.; Baudo, R.; Forbes, V.; Nguyen, L. T. H.; Janssen, C. R.; Kukkonen, J.; Leppanen, M.; Maltby, L.; Soares, A.; Kapo, K.; Smith, P.; Dunning, J. Characterizing sediment acid volatile sulphide concentrations in European streams. *Environ. Toxicol. Chem.* **2007**, *1*, 1–12.
- (43) De Cooman, W.; Seuntjes, P.; Bervoets, L. Methodologische studie naar de inventarisatie, de ecologische effecten en de sanering van de bodems van de Vlaamse waterlopen + Bijlagen. Ministerie van de Vlaamse Gemeenschap AMINAL in samenwerking met Administratie waterinfrastructuur en zeezeven, Brussels, Belgium, 1995.
- (44) Vangheluwe, M. L.; Heijerick, D.; Van Sprang, P. Probabilistic assessment of zinc bioavailability in sediments. Final report February 2003. Commissioned by the International Lead and Zinc Research Organization, 2003.
- (45) Carlson, A. R.; Phipps, G. I.; Mattson, V. R. The role of acid-volatile sulfide in determining cadmium bioavailability and toxicity in freshwater sediments. *Environ. Toxicol. Chem.* **1991**, *10*, 1309–1319.
- (46) Otero, X. L.; Sanchez, J. M.; Macias, F. Bioaccumulation of heavy metals in thionic fluvisols by a marine polychaete: The role of metal sulfides. *J. Environ. Qual.* **2000**, *29*, 1133–1141.
- (47) Hare, L.; Campbell, P. G. C.; Tessier, A.; Belzile, N. Gut sediments in a burrowing mayfly (Ephemeroptera, *Hexagenia limbata*): Their contribution to animal trace element burdens, their removal, and the efficacy of a correction for their presence. *Can. J. Fish Aquat. Sci.* **1989**, *46*, 451–456.
- (48) Warren, L. A.; Tessier, A.; Hare, L. Modelling cadmium accumulation by benthic invertebrates in situ: The relative contributions of sediment and overlying water reservoirs to organism cadmium concentrations. *Limnol. Oceanogr.* **1998**, *43*, 1442–1454.
- (49) Rodriguez, P.; Martinez-Madrid, M.; Arrate, J. A.; Navarro, E. Selective feeding by the aquatic oligochaete *Tubifex tubifex* (Tubificidae, Clitellata). *Hydrobiologia* **2001**, *463*, 133–140.
- (50) Steen Redeker, E.; Bervoets, L.; Blust, R. The importance of water and sediment for the uptake of cadmium and zinc by the aquatic oligochaete *Tubifex tubifex*. *Environ. Sci. Technol.* **2004**, *38*, 6193–6200.

ES8034945