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The origin of speciation: Trace metal kinetics over natural water/sediment interfaces and the consequences for bioaccumulation

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Chemical speciation of heavy metals dictates the accumulation patterns of benthic species by free ion activities in surface waters.

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ABSTRACT

The speciation of heavy metals was measured over a variety of natural and undisturbed water/sediment interfaces. Simultaneously, two benthic species (oligochaete *Limnodrilus* spp. and the midge *Chironomus riparius*) were exposed to these sediments. Under occurring redox conditions, free ion activities of trace metals Cd, Cu, Ni, Pb, and Zn were measured with a chelating exchange technique, while geochemical conditions (i.e., redox) remained in tact. Free ion activities were compared with total dissolved concentrations in pore waters and surface waters in order to relate speciation to bioaccumulation. *Limnodrilus* spp. and *C. riparius* have accumulation patterns that could be linked to time-dependent exposure concentrations, expressed as chemical speciation, in the surface water and the sediment's pore water. Concentrations of free metal ions in the overlying surface water, rather than in sediment pore water, proved to be the best predictor for uptake. For the first time, measurements are obtained from sediments without disturbing physical–chemical conditions and thus bioavailability, a major restriction of other studies so far.

1. Introduction

The assessment of ecological risks of heavy metals in the aquatic environment is historically linked to generic quality standards, based on total contents in sediment. It is not widely known however that the quality standards for sediments are basically derived from aquatic tests, converting LC5-values via equilibrium partitioning to solid phase. The European Water Framework Directive (WFD) now aims at assessing risks of pollutants in the water phase by allowing chemical or bioavailability to be taken into account.

The general concept of (bio)availability of metals becomes clear when body concentrations of field-collected organisms are compared to those from laboratory exposure tests using disturbed and oxygenated sediments or waters. Concentrations of organic and inorganic compounds differ significantly, both in the close environment as in the organism itself (Vink et al., 2005; Simpson and Batley, 2007). This phenomenon is explained by chemical speciation, which originates from the lability of metal species. The most labile form is the freely dissolved ion, M^{2+}, followed by dissolved inorganic metal–ion pairs (e.g., $M–OH^{+}$, $M–Cl^{+}$, $M–CO_3$) and organic forms (M–DOM).

This concept of lability, however, has in recent years been more often theorized than actually shown by empirical data. This is mainly due to the fact that only few techniques have become available that actually measure free ion activities, let alone that these techniques are able to measure free ions repeatedly during exposure of organisms. The concept of the free ion activity model (FIAM), which is incorporated in biotic ligand models (BLM), assumes that the free aqueous metal ion concentration, rather than the total dissolved or sediment concentration, largely determines the toxicological or biological effect which is observed in organisms that are exposed to water or sediment containing heavy metals. Although there appear to be exceptions, many studies have demonstrated that a large variety of benthic taxa accumulate their metals from the water phase, sometimes even exclusively (e.g., Munger and Hare, 1997; Warren et al., 1998; Hare et al., 2001; Buchwalter and Luoma, 2005; Ciutat et al., 2005). Therefore, this study aims to test the hypothesis whether uptake can be predicted from speciation in the water phase.

In a critical review, Campbell (1995) concluded that studies that are suitable for testing the FIAM concept in the presence of natural dissolved organic matter (DOM) are extremely scarce. There are numerous studies that report on the effects of DOM on metal bioavailability, but virtually all of these are qualitative in nature (speciation is under defined). Campbell (1995) concluded that "future progress would be greatly aided by the

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development of methods capable of measuring the free ion concentration of metals in the presence of DOM''. Although some excellent quantitative studies have been carried out in recent years (e.g., Davison and Zhang, 1994; Campbell, 1995; Buchwalter and Luoma, 2005; Ciutat et al., 2005; Kola and Wilkinson, 2005; Luoma and Rainbow, 2005; DeSchamphelaere and Janssen, 2006; Veltman et al., 2008), the results are not always in agreement.

Recently, a technique was introduced that largely overcomes the difficulties described above. This technique was introduced as Sediment Or Fauna Incubation Experiment, SOFIE in short (Vink, 2002). With this technique it is possible to quantify free metal ions, in pore water, at low-oxygen and reduced conditions, repeatedly, and in a non-destructive manner to the sample. Bioassays are conducted simultaneously in the same setting. Sediment samples are obtained in an undisturbed manner, including the overlying surface water.

This paper deals with the gradients and speciation of metal species, among which free ion activities, over natural water/sediment interfaces. The WFD-hypothesis is tested whether bioaccumulation can be predicted from the aqueous phase, taken into account the site-specific speciation. Six distinctly different river systems were sampled. The effect of metal uptake by sediment-dwelling organisms on metal speciation, and vice versa, is discussed.

2. Materials and methods

2.1. Materials

This study was performed with a novel experimental technique (EU-patent nos. 1018200/02077121.8, October 2001, J. Vink, Rijkswaterstaat), which was introduced as Sediment Or Fauna Incubation Experiment, or SOFIE® (Vink, 2002). In short, this ''cell'' consists of a core, 190 mm radius, 200 mm height, which is used as a sampling device to obtain undisturbed sediment, including the overlying surface water. After sampling, this water/sediment system becomes part of the cell. Pore water probes, constructed from a 0.1 μm-permeable polyethersulfone polymer, perform instantaneous microfiltration and yield sterile samples. This is a large advantage, considering the fact that microorganisms mediate many transformation processes, such as redox processes. Probes are connected to the cell wall at 5 mm layer increments. Preconditioned ion-exchange microcolumns (MIC) of 20 mm length and 1.5 mm radius, are connected to the probes. A MIC contains a metal chelating chelex-type polymer, i.e., styrene divinylbenzene copolymers (RI-718, BioRad, USA), containing paired iminodiacetate ions which act as chelating groups and are extremely selective to metals. The possibility to differentiate trace metal species with this chelating polymer is dictated by the lability of the individual species. Dissociation of metal complexes is prevented by matching dissociation rates of ion pairs and complexes (as reported by, e.g., Van Leeuwen and Town, 2005) with contact time between sample and polymer, as was described earlier in detail (Vink, 2002). Retained metals are liberated from the polymer with an ultrapure HNO_3 extraction procedure. In this set-up, ion exchange between pore water and exchange polymer is practically instantaneous, and is therefore executed at the reigning geochemical status, including redox potential, of the probed sediment layer. Total dissolved (pore)water concentrations, and nutrients, were measured by disconnecting the MIC from the probe.

With these cells, water/sediment interfaces from 6 different river systems in The Netherlands were sampled (Rhine, Waal, IJssel-south, Hollands Diep, Meuse, IJssel-north; Fig. 1). Sites were selected for their degree of contamination, physical properties, and their general representativeness for sediments commonly found in the river system. Water depths ranged from 0.5 to 6 m. At shallow depths, samples were taken manually. At depths over 2 m, the sample was collected with a ship using a 1 × 1 m box corer operated from a crane, from which the sample was cored.

The chemical steady state conditions were determined by probing the water/sediment interface on three occasions. Location MB was probed every 5 mm to obtain maximum detail; the other locations were probed in intervals that coincided with the layering of the sediment, which could be observed through the transparent cell wall. After the introduction of test organisms, chemical speciation was monitored in time and depth.

2.2. Exposure tests with benthic organisms

Species of the oligochaete *Limnodrilus* (Family Tubificidae) and the midge *Chironomus riparius* were chosen for accumulation tests because of their wide abundance in aquatic systems and their importance in the ecological food chain. Both species are sediment-dwellers, and live in close contact with the sediment. Populations of both species were bred in the laboratory in circulated flow chambers under contaminant-free conditions. Oligochaete populations consisted mainly of *L. claparedeanus* and *L. hoffmeisteri* in equal amounts. Individuals with corresponding life-stages (characterized as L2 life stage) were introduced in the cell (surface area is 314 cm^2) in field realistic densities (appr. 1250 oligochaetes and 220 chironomids; acc. to Brils and De Poorter, 1993). For locations MB and

Fig. 1. The Rhine-Meuse tributary, and the six sampling locations.

Table 1
Sediment properties.

River system	Location					
	MB	GA	KV	HD	AS	SW
	Rhine	Waal	IJssel-south	Hollands Diep	Meuse	IJssel-north
Depth (m)	3.5	0.8	1	5.8	0.6	0.5
Dry wt (%)	55	80	73	34	45	43
<63 μm (%)	72.6	4.1	15.7	21.8	54.2	65.5
<16 μm (%)	59.3	3.1	9.7	17.8	23.3	49
<2 μm (%)	35.2	1.8	5.65	10.4	14	29.55
Org. C (%)	2.4	0.2	0.55	1.14	2.25	4.15
Inorg. C (%)	1.1	0.6	1.3	0.87	0.55	0.56
pH (−)	6.9	8.1	7.4	7.6	7.2	7.6
Eh (mV)	−448	−163	−149	23	−147	−171
Al (g/kg)	23.4	5.6	3.5	17.3	9.2	11
As (mg/kg)	nd	5.6	4	6.5	8.5	22
Ca (g/kg)	24.1	10.7	19.6	35.1	23.2	32
Cd (mg/kg)	7.49	0.37	0.14	2.59	1.57	2.35
Co (mg/kg)	15.3	7.8	3.3	7.7	8.6	12
Cr (mg/kg)	102.1	32.4	16.8	45.9	27.7	57.5
Cu (mg/kg)	93.1	11.4	4.9	26.5	25.7	48
Fe (g/kg)	29.3	7.6	8.0	18.2	22.2	24.5
Hg (mg/kg)	1.5	0.2	0.055	0.31	0.205	1.20
Mg (g/kg)	7.79	2	3.06	5.24	3.48	8
Mn (mg/kg)	654	241.7	196.5	430.5	524.5	530
Ni (mg/kg)	35.6	6.6	8.35	19.4	20.8	31.5
Pb (mg/kg)	703.6	28	11.5	39.8	79	91.5
Zn (mg/kg)	1091	133	33	231	320.5	365

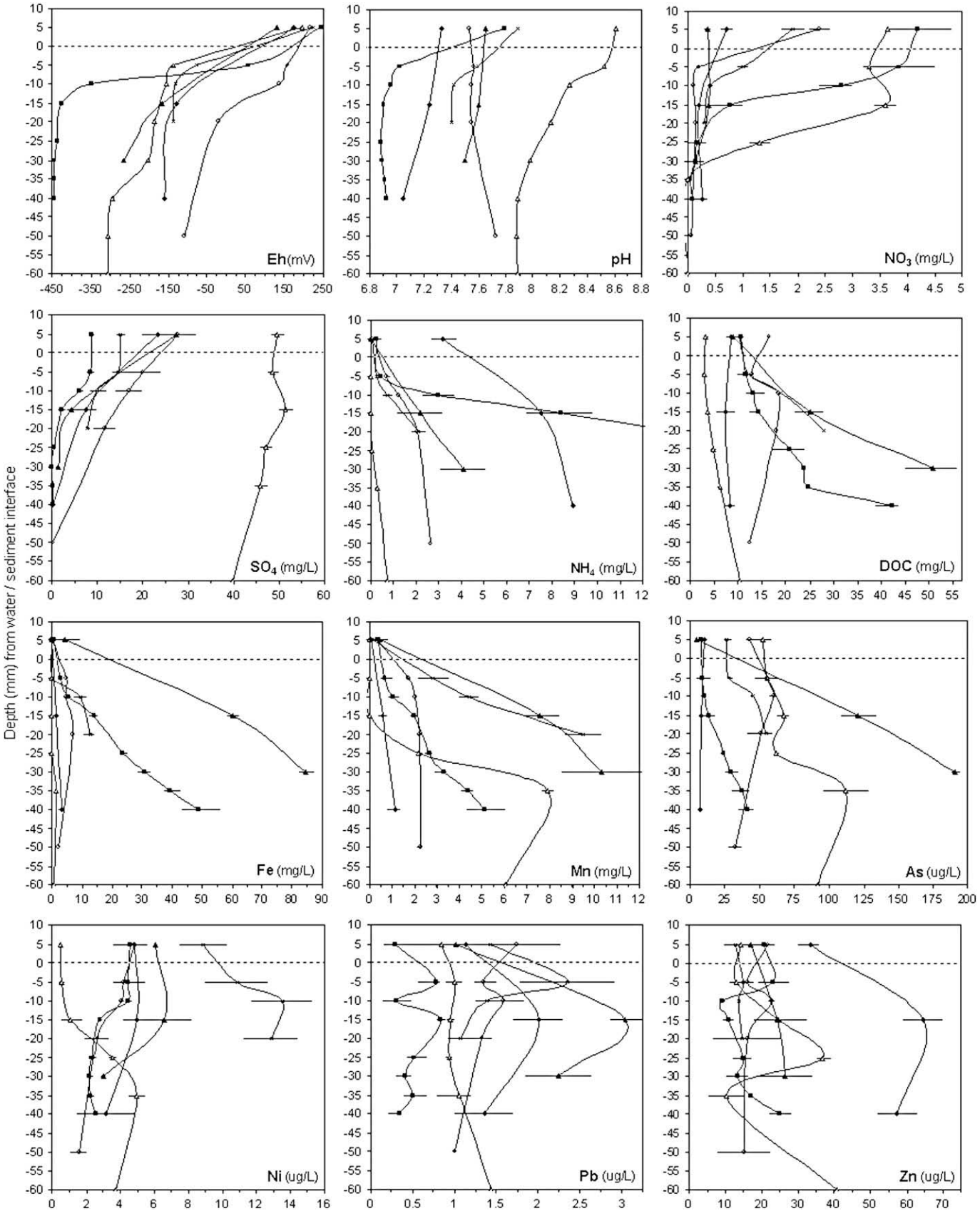

Fig. 2. Aqueous steady state characteristics for the six sites. MB (■); GA (Δ); KV (×); HD (O); AS (◆); SW (▲). Horizontal bars are standard errors ($n = 3$). Cu is shown in Fig. 3.

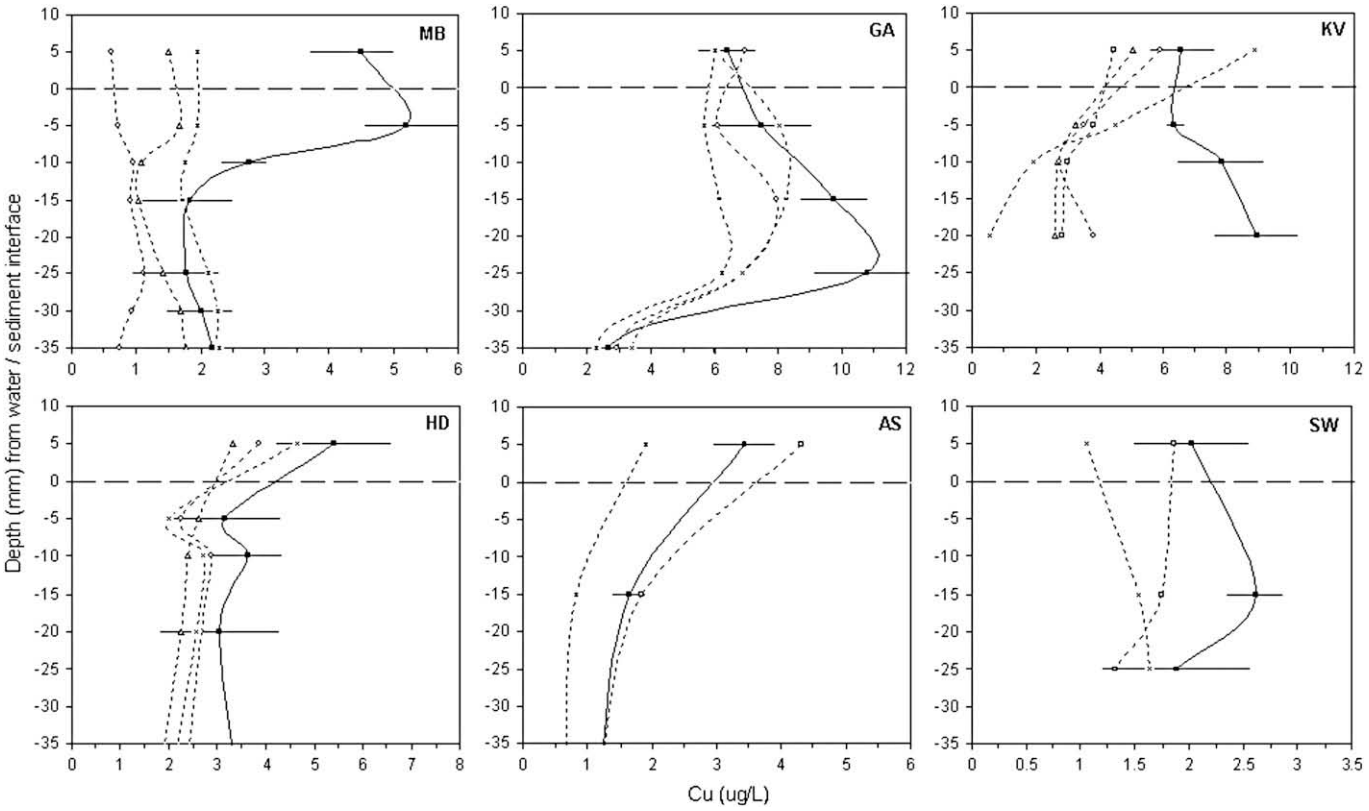

Fig. 3. Dissolved copper concentrations at 6 locations over a 4 cm water/sediment interface at steady state (■), and after the introduction of test organisms: day 2–4 (□); day 5–9 (Δ); day 10–14 (○); day 15–19 (×); >20 days (*). In all cases, Cu concentrations become rate limiting for uptake.

SW, only oligochaetes were used in the exposure tests. Oxygen concentrations in the overlying water were permanently monitored, and if necessary (<5 mg O_2 l^{-1}) the water was aerated.

Body concentrations of both species were measured periodically (7×) by sampling individuals from the populations in the cells in 2–3 days intervals. Prior to analyses, they were allowed to void their gut for 48 h, freeze-dried for 48 h, and dry weight was determined. Digestion was done in 500 μl of 14.9 M HNO_3(Ultrex) at 180 °C. Metal concentrations in the digests were determined with High Resolution Inductively Coupled Plasma Mass Spectrometry (HR-ICP-MS, Thermo-Finnigan 2). Dolt-2, certified by the Community Bureau of Reference, BCR, Brussels, Belgium, was used as biological reference material to validate the trace metal recovery during the digestion.

2.3. Analytical procedures

Aqueous concentrations of NO_3^-, NO_2^-, SO_4^{2-}, PO_4^{3-}, and Cl^- were measured with an ion chromatograph using a High Capacity analytical column AS9-HC (Dionex), based on conductivity detection with chemical suppression (limit of

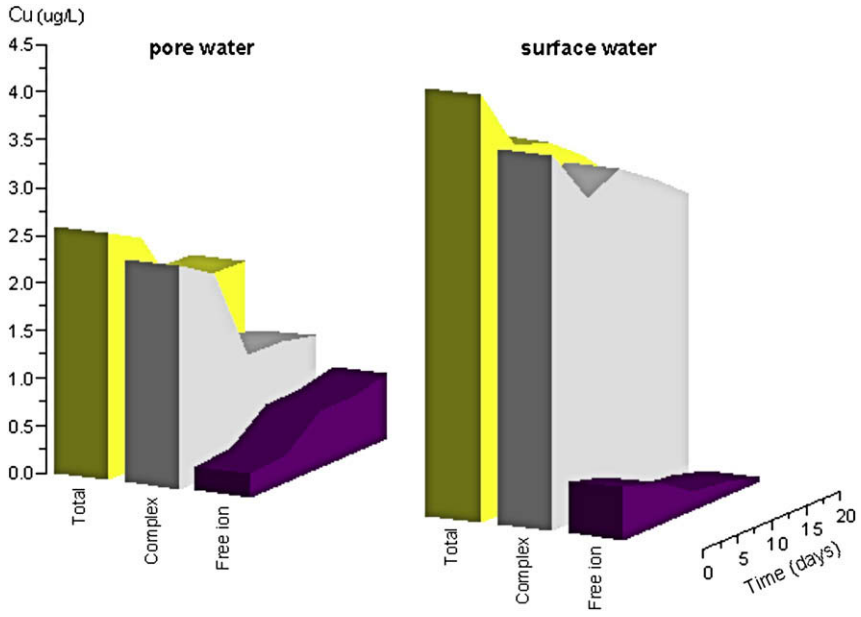

Fig. 4. Example of time-dependent speciation shifts (Cu) in overlying surface water and pore water (20 mm below water/sediment interface) during an exposure test. As a result of oxidation, the free ion activity in pore water increases, and is exhausted from the overlying water due to uptake by biota.

detection (lod) min/max: 0.025 mg l^{-1} for NO_2^- to 0.05 mg l^{-1} for PO_4^{2-}). All anions were thus measured simultaneously under similar analytical conditions.

As^{75}, Al^{27}, Ca^{43}, Cd^{111}, Co^{59}, Cu^{63}, Cr^{52}, Fe^{56}, Mg^{24}, Mn^{55}, Ni^{60}, $Pb^{206,207,208}$, and Zn^{66} analyses were carried out with High Resolution ICP-MS (lod min/max: 0.003 μg l^{-1} for Cd to 6.0 μg l^{-1} for Zn). All lead isotopes were measured and subsequently averaged to compensate for variations from geological origin. NH_4^+ analyses were done with a Skalar Segmented Flow analyzer SAN+ 6250 matrix photometer (lod: 0.022 mg l^{-1}). Dissolved organic carbon was determined with a Shimadzu 5000 TOC analyzer (lod: 0.45 mg l^{-1}). All measurements in this study were checked with 6-point calibration curves from standard reference stock solutions.

3. Results and discussion

Table 1 shows the solid phase characteristics of the six sites. The undisturbed (steady state) concentrations in pore water and overlying surface water are shown in Fig. 2.

Typically, steep redox gradients occur over the water/sediment interface, more or less pronounced for the various sites. Redox-sensitive compounds follow these gradients: denitrification precedes sulfate reduction, and anaerobic conditions were reached in most cases within 20 mm from the water/sediment interface. The reduction of ferric(III)oxyhydroxides to Fe(II), and manganese(V)oxides to Mn(II) results in increasing concentrations in depth of aqueous Fe^{2+} and Mn^{2+}. Pore water concentrations of Cd, Cu, Ni, Pb, and Zn in most cases tend to decrease with depth and coincide with sulfate reduction patterns. These metals associate with sulfides and therefore become insoluble. Heavy metals however compete for binding opportunities between the reactive sulfide phase and the relatively large amounts of dissolved organic matter (see Fig. 2: DOC), which keeps them in a dissolved state.

3.1. Time-varying exposure concentrations

After introduction of the test organisms to the water/sediment systems, steady state concentrations started to shift. Oligochaetes and chironomids are sediment-dwellers; they live in burrows, which they irrigate actively (chironomids) or passively (oligochaetes), with surface water. Consequently, their immediate environment is oxidized; sulfate concentrations increase due to sulfide oxidation (data not shown), and a lighter colouring of the occupied layers was observed. This oxidation directly affects the chemical reactivity of adsorbed and dissolved metals, as will be demonstrated later.

Because of the sheer magnitude of acquired data (i.e., time series, locations, depths, elements, and organisms), only graphic examples for copper are shown. In the Water Framework Directive, copper is marked as a priority pollutant. All trends, however, are summarized in Table 4, which is discussed later.

Fig. 3 shows concentration shifts for copper for all locations. In Fig. 4, an example (site MB) is shown of how aqueous concentrations of copper species change over a 4 cm interface during the exposure test.

Time-varying changes of dissolved metal concentrations follow first-order reaction kinetics, e.g., $dC/dt = kC$. The actual exposure concentration at a given time $C(t)$ is then written as

$$C(t) = C_i\, e^{k_0 t} \quad (1)$$

in which C_i is the initial concentration in (pore)water, and k_0 is rate term that describes the increase or decrease rate of the initial steady state concentration. In analogy, the free ion concentration at a given time, $M^{2+}(t)$, is written as

$$\left[M^{2+}\right](t) = \left[M_i^{2+}\right] e^{k_{0,act} t} \quad (2)$$

With the classical Boltzmann equation, free concentrations can be converted to activities.

Table 2
Mean free ion activity, as % of total dissolved after 0.1 μm microfiltration, at steady state for 6 sites. Values in brackets denote the variation between sites.

	Surface water	Pore water
Cd^{2+}	22.4 (±9.8)	33.5 (±16.7)
Zn^{2+}	22.1 (±14.8)	25.8 (±16.1)
Ni^{2+}	14.2 (±6.5)	20.7 (±14.5)
Cu^{2+}	12.5 (±7.3)	15.4 (±12.9)
Pb^{2+}	12.1 (±11.4)	11.3 (±5.9)

In general, the contribution of the free ion concentration to the total dissolved concentration is relatively large for Cd and Zn, and relatively small for Cu and Pb (Table 2). Note that the liquid phase is microfiltrated over the 0.1 μm-permeable polymer of the probe. The fractions that are shown may therefore be somewhat larger than found in larger-sized filtrates (e.g., 0.45 μm). Variations shown in Table 2 are differences between locations, which in some cases are quite large. Weng et al. (2001) also reported for 15 different soils' large variations of free Cu^{2+}, ranging from 3% to 73% of total dissolved.

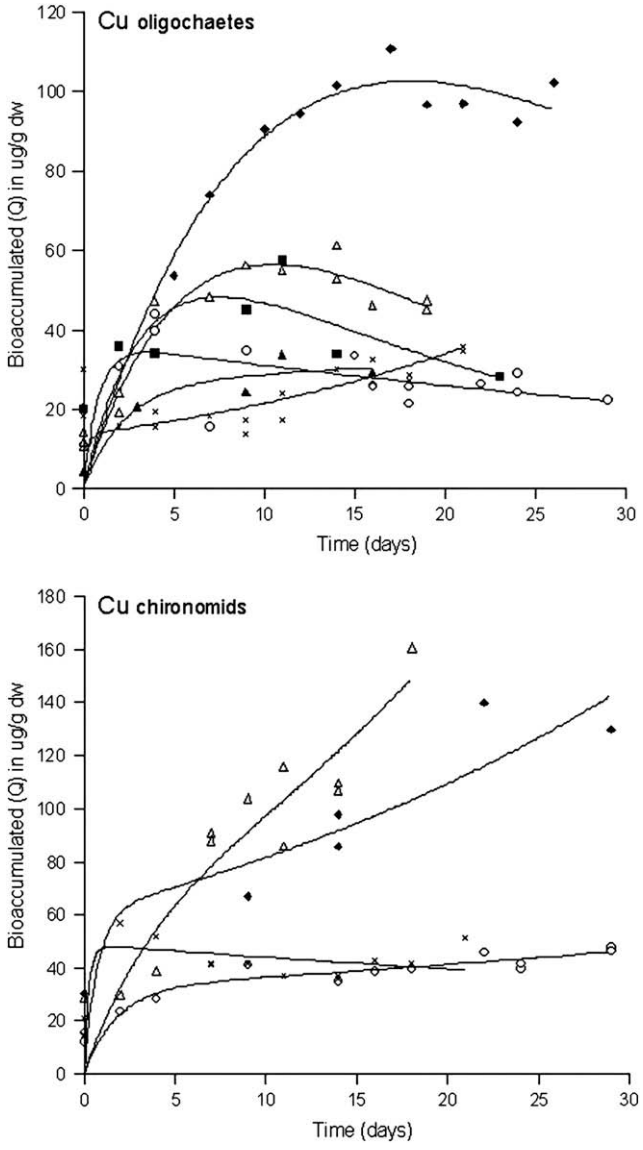

Fig. 5. Cu bioaccumulation patterns in oligochaetes and chironomids for the sampled sites. The solid line is the model prediction based on Eq. (4). MB (■); GA (Δ); KV (×); HD (O); AS (◆); SW (▲).

Table 3
Variance in accumulation patterns of oligochaetes and chironomids between locations. D indicates that accumulation patterns between two locations are significantly different.

Cd	MB	GA	KV	HD	AS	SW	**Cu**	MB	GA	KV	HD	AS	SW
MB		D	ns	ns	ns	ns	MB		ns	D	ns	D	ns
GA			D	D	D	D	GA			D	ns	D	D
KV		D		ns	ns	ns	KV		D		ns	D	ns
HD		D	D		ns	ns	HD		D	ns		D	ns
AS		D	D	ns		ns	AS		ns	ns	ns		D
SW							SW						
Ni	MB	GA	KV	HD	AS	SW	**Pb**	MB	GA	KV	HD	AS	SW
MB		ns	ns	ns	D	ns	MB		D	D	D	D	D
GA			ns	ns	ns	ns	GA			D	ns	ns	ns
KV		ns		ns	D	ns	KV		D		D	D	ns
HD		ns	D		D	ns	HD		ns	D		ns	ns
AS		ns	ns	ns		D	AS		ns	ns	ns		ns
SW							SW						
Zn	MB	GA	KV	HD	AS	SW	**As**	MB	GA	KV	HD	AS	SW
MB		D	D	ns	ns	D	MB		D	D	D	D	ns
GA			D	D	ns	D	GA			ns	D	D	ns
KV		ns		D	ns	D	KV		D		D	ns	ns
HD		D	D		D	D	HD		D	ns		ns	ns
AS		ns	ns	ns		D	AS						ns
SW							SW						
Cr	MB	GA	KV	HD	AS	SW							
MB		ns	ns	ns	D	ns							
GA			ns	ns	D	ns							
KV		ns		ns	ns	ns							
HD		ns	D		D	ns							
AS		ns	ns	ns		D							
SW													

Underlined characters = oligochaetes. Roman characters = chironomids.
D = significantly different.
ns = not significantly different.

The rates at which these concentration shifts occurred significantly affected the shapes of bioaccumulation patterns, which are shown hereafter.

3.2. Uptake and elimination by Limnodrilus and Chironomus

The correlation between total sediment concentration (Table 1) and bioconcentrated metals was very poor (Cd: $r^2 = 0.05$; Cu: 0.001; Ni: 0.05; Pb: 0.09; Zn: 0.48) except for arsenic: $r^2 = 0.91$. This again shows that sediment concentrations are no robust indicators for uptake and risks of metals in natural systems.

In a previous study (Duester et al., 2008), we performed detailed speciation analyses of arsenic over the natural redox gradient. Arsenic is a transitional reactive element that can occur in many forms, both organic (x-methylated) and inorganic. Based on that study, we can now assume that the trivalent and pentavalent oxidation states dominate the dissolved concentrations to which biota are exposed, and that bioaccumulation is related to these species (Caussy, 2003).

Metal uptake and excretion rates were approximated by a time/concentration-dynamic, two-compartment model presented earlier by Vink (2002). In short, this model assumes uptake and excretion to occur simultaneously, and a concentration-dependency from the surrounding environment: $dQ/dt = k_1C(t) - k_2Q(t)$, with Q being the internal body concentration (μg g^{-1} dw), k_1 and k_2 are uptake and elimination rate constants, respectively (k_1 in l kg^{-1} day^{-1}, k_2 in day^{-1}), and t is time, yielding:

$$Q(t) = \frac{k_1 C_0}{k_2 - k_0}\left(e^{-k_0 t} - e^{-k_2 t}\right) \quad (3)$$

where C and k_0 are derived from Eqs. (1) and (2). Free ion activity (C-act) is formulated in analogy. With the introduction of k_0, time-dependent concentrations determine the overall exposure of organisms, and thus $Q(t)$.

The consequence of *not* considering time-dependent concentrations was investigated by using a one-compartment model. This model assumes a constant uptake rate (a). Body concentrations are then described as: $dQ/dt = a - kQ$, which yields:

$$Q(t) = C_0\ e^{-k\cdot t} + \frac{a}{k}\left(1 - e^{-k\cdot t}\right) \quad (4)$$

Recoveries of the certified biological standard Dolt-2 showed acceptable ranges (91–112%), which gave confidence in the reliability of the measured body concentrations of both species. Fig. 5 shows the bioaccumulated amounts of copper by oligochaetes and chironomids for the six sites (all data in Table 5). Typically, metals are taken up at a certain rate (k_1), leading to increased body concentrations. After some time, elimination (k_2) exceeds uptake, and concentrations reach steady state. In general, bioaccumulated amounts are ranked according to Ca > Fe > Mg > Zn > Mn > Cu>Pb > Cr > As ~ Cd ~ Ni. Of both species, chironomids tend to accumulate larger amounts than oligochaetes (normalized to dry weight).

To test whether these sediment-dwellers actually show different patterns on different locations (i.e., is there location-specificity?), the accumulation data of oligochaetes and chironomids were statistically compared (2 tailed F-test, $p < 0.05$). Results are summarized in Table 3 and show that locations do indeed matter. Both test species show the lowest variety in uptake for chromium and nickel. On the other hand, the combinations cadmium-chironomids, and zinc-oligochaetes show a high discriminating potential. The fact that accumulation patterns on various locations are indeed different – also on locations with comparable dissolved concentrations – indicates that there is a difference in the actual bioavailability of metals. This is an

Table 4
Chemical speciation in water/sediment compartments. C-tot = total dissolved concentration (μg/l); C-act = free ion (M^{2+}) activity; k_0 = first-order rate in which concentrations (C-tot and C-act, resp.) change during the exposure test; % act = percentage of free ion concentration of total dissolved in the 0.1 μm fraction.

Metal, site	Total content, mg/kg	Speciation in surface water					Speciation in pore water				
		C-tot	k_0	C-act	k_0	% act	C-tot	k_0	C-act	k_0	% act
Cadmium											
MB	7.49	0.08	0.037	0.025	−0.277	32.1	0.05	0.033	0.01	0.157	19.6
GA	0.37	0.20	−0.003	0.050	0.360	25.0	0.30	0.038	0.07	0.010	23.3
KV	0.14	0.23	0.005	0.060	−0.005	26.7	0.09	0.001	0.03	−0.001	34.8
HD	2.59	0.05	0.002	0.014	0.0003	30.0	0.03	0.001	0.01	0.001	28.8
AS	1.57	0.39	−0.004	0.053	0.0003	13.6	0.12	0.005	0.06	−0.001	53.4
SW	2.35	0.04	−0.001	0.003	0.0001	7.3	0.04	0.001	0.02	−0.001	51.2
Copper											
MB	93.1	4.48	−0.180	0.45	−0.380	10.0	2.51	−0.095	0.33	0.012	13.1
GA	11.4	6.00	0.094	0.70	0.088	11.7	5.38	0.095	2.00	0.096	37.2
KV	4.9	6.56	−0.043	0.57	−0.036	8.7	10.17	−0.043	0.79	−0.043	7.8
HD	26.5	5.41	−0.100	1.23	−0.041	22.7	3.07	−0.070	0.12	−0.012	3.8
AS	25.7	2.74	−0.040	0.07	−0.003	2.6	1.90	−0.066	0.12	−0.006	6.3
SW	48.0	2.55	−0.082	0.49	−0.027	19.2	2.87	−0.063	0.70	−0.041	24.3
Nickel											
MB	35.6	4.57	0.020	1.02	−0.042	22.3	2.69	0.039	0.50	−0.602	18.6
GA	6.60	1.00	0.081	0.05	0.024	5.0	3.00	0.200	0.05	0.021	1.7
KV	8.35	10.27	−0.066	1.16	−0.012	11.3	16.29	−0.086	5.86	−0.086	36.0
HD	19.4	4.82	0.016	0.73	−0.031	15.1	2.65	0.053	0.73	−0.012	27.4
AS	31.5	5.44	0.094	0.58	0.009	10.7	5.70	−0.048	1.97	−0.028	34.6
SW		6.17	0.027	1.27	0.055	20.6	5.16	−0.031	0.30	0.018	5.7
Lead											
MB	703.6	0.30	0.024	0.00	0.047	1.0	0.83	−0.015	0.14	0.080	16.8
GA	28.0	2.00	−0.040	0.10	−0.001	5.0	1.00	−0.028	0.05	0.016	5.0
KV	11.5	1.93	0.100	0.64	−0.007	33.2	1.39	−0.007	0.25	−0.005	17.8
HD	39.8	1.74	−0.080	0.20	−0.009	11.8	1.33	−0.073	0.20	−0.019	14.7
AS	79.0	2.60	−0.081	0.17	0.000	6.5	2.51	−0.004	0.11	0.014	4.3
SW	91.5	3.70	0.135	0.56	−0.030	15.2	4.68	−0.155	0.44	−0.062	9.4
Zinc											
MB	1091	20.60	−0.020	7.83	−0.034	38.0	10.96	0.114	2.78	0.050	25.4
GA	133.0	14.17	−0.144	3.01	−1.117	21.2	37.00	−0.262	19.00	−0.261	51.4
KV	33.0	11.27	−0.006	4.47	−0.006	39.7	13.28	−0.006	4.00	−0.006	30.1
HD	231.0	21.70	−0.125	4.12	−0.043	19.0	15.96	0.048	4.51	−0.157	28.3
AS	320.5	33.40	0.091	4.80	0.018	14.4	64.70	−1.526	11.30	−0.396	17.5
SW	365	23.96	0.539	0.14	−0.017	0.6	18.81	0.216	0.43	0.377	2.3
Arsenic											
MB	nd	8.61	4.865				23.95	7.262			
GA	5.6	44.27	0.949				71.57	0.518			
KV	4.0	23.95	1.633				46.0	−0.072			
HD	6.5	42.44	−0.319				50.76	−0.378			
AS	8.5	12.70	−0.323				5.90	0.599			
SW	22.0	4.60	0.215				107.8	0.589			

important conclusion; it shows that a relation between site-specific chemical speciation and bioaccumulation exists.

3.3. Relation between time-dynamic speciation and body concentrations

To analyze the performance of the uptake and elimination models with varying exposure concentrations, and speciation, the overall data set was divided into 4 sub-sets: two "environment compartments" (overlying surface water and sediment pore water), each with two chemical "species" (total dissolved metal concentration and free ion activities). Performances of the uptake-elimination models (Eqs. (3) and (4)) were tested with each sub-set. Measurements and corresponding model parameters C_i and k_0 were subsequently subjected to statistical analyses. As a descriptor for the goodness of prediction for the uptake-elimination model, the standard error (sy.x) of each model parameter was determined (SPSS 6 software, Sybex). The standard error is a measure of the variability of a data point Y around the predicted value Y_p. It represents information about the goodness of fit in the same manner as the standard deviation does about the spread around the mean. The standard error is written as

$$\mathrm{sy.x} = \sqrt{\frac{\sum (Y - Y_p)^2}{n}} \tag{5}$$

By minimizing the sum of squares, values for k_1 and k_2 and corresponding standard errors were determined. The data set (for this purpose considering only Cu, Cd, Ni, Pb, Zn, and As) was of a considerable size; it consisted over 1800 chemical and 608 biological measurements.

Combinations of C_t and Q_t from the various sub-sets were considered significant when standard errors of the individual parameters were of the same magnitude or smaller than the values of k_1 and k_2 itself. This does not mean that other relationships between compartments, chemical species and accumulation do not exist; this statistical criterion is necessary to identify the best model predictor, and therefore the most significant source of metals to biota.

The outcome of the modelling exercise and the statistical comparisons are summarized in Tables 4 and 5. Chemical speciation, and concentration shifts in the two compartments, is shown in Table 4. The variation of speciation between sites is large. Also, the effect that organisms exercise on the local speciation is significant:

Table 5
Uptake from water/sediment compartments. k_0, k_1, k_2 are rate constants from Eqs. (1)–(4); Q_{max} = maximum concentration in the organism (μg/g dw); r^2 = correlation coefficient. Values in italic are derived with the one-compartment uptake model (Eq. (5)). Only those combinations are shown where the statistical criterion was met (sy.x < k_1, k_2), and gave the best predictions; empty spaces are therefore less significant combinations.

Metal, site	Oligochaetes											Chironomids									
	Q_{max}	Uptake from surface water				Uptake from pore water				k_2	r^2	Q_{max}	Uptake from surface water				Uptake from pore water				k_2	r^2
		k_1-C	sy.x	k_1-act	sy.x	k_1-C	sy.x	k_1-act	sy.x				k_1-C	sy.x	k_1-act	sy.x	k_1-C	sy.x	k_1-act	sy.x		
Cadmium																						
MB	2.2			20.6	0.01					0.03	0.47	nd										
GA	10.0			18.5	6.65					0.03	0.94	29.0	8.2	0.01							−0.03	0.95
KV	0.7			0.31	0.11					0.05	0.58	11.0	401	4.7							8.37	0.13
HD	2.0			48.1	0.01					0.20	0.71	6.3	208	26.3							1.34	0.37
AS	0.6			53.5	0.05					8.18	0.99	7.4	2.4	1.2							0.13	0.88
SW	0.9			10.3	3.15					3.47	0.85	nd										
Copper																						
MB	57.5			46.0	0.01					0.03	0.85	nd										
GA	55.0			20.0	5.34					0.09	0.84	140	3.3	2.3							−0.05	0.82
KV	35.0	37.3	0.01							16.8	0.41	51	2.9	0.9							0.18	0.31
HD	39.7	6.1	1.6							0.76	0.71	48	3.4	1.8							0.55	0.71
AS	103.0	21.3	17.7							1.24	0.55	152	6.7	4.8							0.33	0.78
SW	32.0			31.1	24.9					0.68	0.88	nd										
Nickel																						
MB	12.0			1.77	2.19					0.15	0.87	nd										
GA	5.1			7.34	2.44					0.01	0.75	14.7			14.9	4.7					−0.03	0.81
KV	11.0			0.44	0.36					−0.01	0.54	13.0			11.0	5.7					4.31	0.94
HD	7.6			6.41	2.56					0.42	0.51	17.3			8.6	3.1					0.57	0.94
AS	3.9			0.47	0.24					0.07	0.52	21.0	9.3	0.01							4.36	0.90
SW	9.0			1.28	0.29					0.09	0.91	nd										
Lead																						
MB	135.6					27.2	0.01			0.21	0.10	nd										
GA	35.0					10.6	5.88			0.54	0.71	51.0							105	18.7	0.05	0.92
KV	21.0	0.53	0.18							−0.06	0.66	13.5	0.75	0.18							0.09	0.94
HD	26.4			74.8	18.75					0.44	0.68	32.7			38.7	7.3					0.29	0.96
AS	26.1							18.5	3.61	0.05	0.81	97.0							139	8.0	0.12	0.99
SW	20.3	1.21	0.29							0.05	0.86	nd										
Zinc																						
MB	781			30.6	0.01					0.39	0.82	nd										
GA	480	*0.26*	*0.15*								0.72	1100	*0.13*	*0.07*								0.87
KV	395	*0.19*	*0.12*								0.56	710	*8.7*	*1.4*								0.70
HD	730	*29.3*	*0.01*								0.67	512	*5.1*	*7.8*								0.35
AS	530	*0.17*	*0.03*								0.95	765			17.8	4.8					0.06	0.95
SW	429	*7.27*	*2.52*								0.72	nd										
Arsenic																						
MB	23.0	0.10	0.06								0.90	nd										
GA	7.4	*0.05*	*0.12*								0.54	7.5	*0.06*	*0.05*								0.87
KV	10.0	*−0.09*	*0.05*								0.78	3.3	*0.67*	*0.15*								0.96
HD	10.1	*42.4*	*0.01*								0.12	4.0	0.73	0.55							12.4	0.91
AS	14.6	*0.01*	*0.01*								0.96	nd										
SW	23.1	*−0.25*	*0.02*								0.99	nd										

negative k_0 values indicate a decrease in concentrations, positive values indicate an increase. These values were used as input for the biotic uptake models (Eqs. (3) and (4)). These results are shown in Table 5. For survey ability and presentation purposes, only those combinations where the statistical criterion was met (sy.x < k_1, k_2) are shown. Consequently, the other combinations are of less significance.

Combinations of the measured C_i and k_0 values were used as initial constants in the iteration procedure. The advantage of this approach is that variables that have a relative large certainty or reliability do not participate in the iteration process as such. They do not need to be included, since $C(t)$, M^{2+}, and k_0 were actually measured in time. This is a large advantage, since the optimization procedure is now focused on the accurate estimation of the only remaining variables k_1 and k_2, i.e., the uptake and elimination rate.

The most important conclusion from Table 5 is that both benthic species obtain their metals primarily from the overlying surface water, and not from pore water of the sediment. Total concentrations in the sediment (mg/kg) are not indicative or discriminating, and do not reflect the magnitude of total body concentrations. Both test species accumulated the largest amounts of cadmium at site GA (chironomids even faced lethal concentrations), although sediment contents are second-lowest. In all cases, and therefore conclusive, the most important source for cadmium and nickel for oligochaetes is the free ion activity in the overlying surface water. In 100% of the cases, the measured C-act and k_0 combination for free ions in the surface water compartment yielded the best descriptors for accumulation patterns based on the two-compartment model. For copper, this seems valid in half of the cases. In some occasions, free ion concentrations dropped to a point where the contribution as a relevant source becomes (temporarily) negligible (see also Fig. 4). Potential sources of free Cu ions, such as humic complexes, mostly have slow dissociation kinetics (Sunda and Hanson, 1987; Daly et al., 1990; Meador, 1991) and cannot make up for Cu-uptake rates by biota. Chemical dissociation is therefore rate limiting.

Uptake from the sediment pore water was observed only occasionally, and solely, for lead. The source of Pb is more variable, which is – for that matter – in agreement with the fact that the physiological mechanisms of Pb-uptake are yet unclear. It is argued that uptake and elimination of Pb obey different mechanisms than in the case of the other heavy metals (i.e., an interaction between the free metal ion and a channel and/or carrier transport system in the external and/or internal epithelium), but this is yet to be demonstrated.

For zinc and arsenic, the performance of the one-compartment model was in 80% of the cases better than the model that used two compartments. External concentrations (i.e., outside the organism) obviously never became rate limiting for uptake. In river sediments, Zn is mostly present in fair amounts, and the fraction of labile species, among which free ions, is mostly the largest of all metals. Most organisms regulate their Zn body concentrations very efficiently, and steady state is often quickly reached. Arsenic does not yield significant free ion concentrations, being an oxy-anion; the predominant species are arsenate or arsenite, and these are negatively charged.

It is shown that uptake rates are no universal constants. A fair variation of k_1-values over the various locations exists. Kinetic processes therefore do indeed matter. The fact that the overlying water plays such a significant role in metal uptake is actually not very surprising. Sediment-dwellers are in close contact with sediment pore water, but species that live in burrows are actually in closer contact with overlying water because (i) the burrow inhibits direct contact between the organism and pore water, and (ii) organisms exchange the water in their burrows with overlying water, either actively by physical motion or passively through diffusion (Ciutat et al., 2005; Simpson and Batley, 2007).

These findings provide a significant contribution to the understanding of chemical speciation of metals in water/sediment systems and subsequent metal uptake by organisms. In this study, the hypothesis that the water phase can be used as predictor for uptake, and possibly as a biomarker, is confirmed for most metals provided that speciation is taken into account. It is demonstrated that benthic organisms exert a significant effect on chemical speciation (Figs. 3 and 4; Table 4). This has to be considered when performing bioassays and chemical tests. Body concentrations are regulated by temporal variations in metal concentrations, including chemical speciation. The measurements show that biological kinetics (uptake) are for most metals faster than chemical kinetics (dissociation of less labile or complexed metals). Exposure can therefore only be estimated and modelled when time-related changes in metal concentrations are taken into account. The construction of generic models that are capable of transferring chemical information to a biological response has undoubtedly a high priority. Knowledge of the free metal ion activity is a significant step forward, and dynamic metal speciation analysis is emerging as a powerful basis for development of predictions of bioavailability and reliable risk assessment strategies (Allen et al., 1980; Chapman and Wang, 2001; Lofts et al., 2004; Van Leeuwen et al., 2005; Van Straalen et al., 2005). Still, it may in cases be insufficient to predict the biological response, since one must also consider potential interactive effects of hydrogen ions (Brown and Markich, 2000; Campbell, 1995; Francois et al., 2007) or from other macrochemicals (DeSchamphelaere and Janssen, 2002; Buchwalter and Luoma, 2005). This knowledge may be incorporated in biotic ligand models (BLM) (e.g., Paquin et al., 2002; Niyogi and Wood, 2004) in order to derive better-founded quality criteria for aquatic systems.

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