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Measurement of Heavy Metal Speciation over Redox Gradients in Natural Water−Sediment Interfaces and Implications for Uptake by Benthic Organisms

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The sediment or fauna incubation experiment (SOFIE) is an experimental research tool that was developed to analyze concentrations and chemical speciation of heavy metals in pore waters of natural, undisturbed sediments or water−sediment interfaces over time, while simultaneously conducting exposure tests with sediment-dwelling organisms. In this way, concentrations of chemical species are directly linked to accumulation by biota. It is shown that discrete gradients of redox-sensitive metals and nutrients occur over very small intervals. These gradients differ from those of free metal ion activities. Speciation affects the uptake of metals by sediment-dwelling organisms, which, in their turn, have a significant effect on metal speciation. With reaction kinetics that differ per metal, uptake of metals by organisms from the water phase may be hindered (e.g., Cu, Zn) or promoted (e.g., Ni, As). Time-varying exposure concentrations of metals were incorporated in uptake and elimination models. Body concentrations of Cd, Cu, Ni, and Zn in the aquatic oligochaete *Limnodrilus* could best be described by the time-varying free ion concentration in the overlying water. Body concentrations of As and Pb were best described by sediment pore water concentrations. It is concluded that SOFIE provides the necessary experimental tool to support, in a mechanistic way, environmental risk assessments of contaminants.

Introduction

Sediments of rivers, river banks, and flood plains are commonly characterized by elevated levels of heavy metals such as Cd, Cu, Cr, Ni, Pb, Zn, and As (*1*). Organisms that occur in these sediments are known to accumulate these metals (*2, 3*), which, in turn, are passed on through the food web. However, the development of well-defined quality criteria for (aquatic) sediments is seriously hindered mainly for the following three reasons: (i) Laboratory exposure tests that are conducted with organisms (bioassays) do not consider the geochemical composition of the sediment in situ and do not link temporal changes in chemical speciation with toxicological effects. (ii) It is usually unclear which exposure and uptake routes contribute to accumulation in organisms and dictate the adverse effects. (iii) Although many researchers focus on the free metal ion concentration as the primary factor controlling biological uptake of metals (*4*), controversy exists about the relative contributions of various metal species to biological uptake.

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In past years, surprisingly little has been achieved to link chemical speciation to bioavailability (e.g., accumulation in organisms) in natural systems. Beside some useful chemical models, very few techniques have become available to actually quantify (i.e., measure) free ion activities, let alone to measure these periodically during exposure of organisms. The concept of the free ion activity model (FIAM) assumes that free aqueous metal ion concentrations, rather than the total or dissolved concentration, largely determine the toxicological or biological effect which is observed in organisms that are exposed to water or sediment containing heavy metals. Only few examples of useful studies exist so far that may confirm the free ion activity model. It was demonstrated for zinc under regimes of varying concentrations of EDTA, a highly effective, organic, metal-complexing agent. Zn free ion activities were reported to determine growth of coastal diatoms (*5*), blue-green alga (*6*), and common alga (*7*). Sunda and Lewis (*8*) proposed a link between Cu activity concentrations and effects to alga in surface waters.

In a critical literature review, Campbell (*4*) 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 underdefined). The few quantitative studies (*8−12*) are however not in agreement, and the applicability of FIAM to natural waters (with DOM) remains to be demonstrated. It is concluded that "future progress would be greatly aided by the development of methods capable of measuring the free ion concentration of metals in the presence of DOM".

Although exceptions have been reported by, for example, Giesy et al. (*9*) and Borgmann and Charlton (*10*), DOM reduces metal toxicity due to decreased metal ion activities. DOM, however, does increase total dissolved fractions. As stated before, most studies do not consider natural DOM or chemical speciation. In cases where DOM and speciation were taken into consideration, other problems arose. In some cases (e.g., refs *9* and *13*), free ion activities are calculated with chemical equilibrium models. This approach, however, excludes a priori kinetic considerations. Others used ion-selective electrodes to quantify metal activities (Meador (*12*), for example, reported relations for $[Cu^{2+}]$ and *Daphnia*). However, the use of ion-selective electrodes in natural waters is often complicated by interference with other elements (e.g., sulfide or other metals), unstable electrode potentials, and relatively long response times, which was demonstrated by Xue and Sunda (*14*). Moreover, ion-specific electrodes may respond in some way to organic matter, as was shown by Daly et al. (*11*), leading to an overestimation of the true free ion activities. Other "in situ" techniques, such as DGT (introduced by Davison and Zhang in 1994 (*15*) and modified by Zhang et al. in 1998 (*16*)) or dialysis or millipeeper methods (used, for example, by Doig and Liber (*17*) and Schults et al. (*18*)), may in some cases be useful to estimate free ion activities, but these methods are destructive to the sample and therefore not capable of monitoring concentration shifts during exposure to organisms. In addition, these techniques do not provide data on relevant macrochemical variables.

Experimenters that expose test organisms to contaminated water or water−sediment systems often assume constant external concentrations. It is highly questionable, however, to do so, since many processes may disrupt equilibrium conditions during the test. In those cases where time varying concentrations are recognized (e.g., ref *19*) and modeled (e.g., refs *20* and *21*), speciation, again, is not accounted for.

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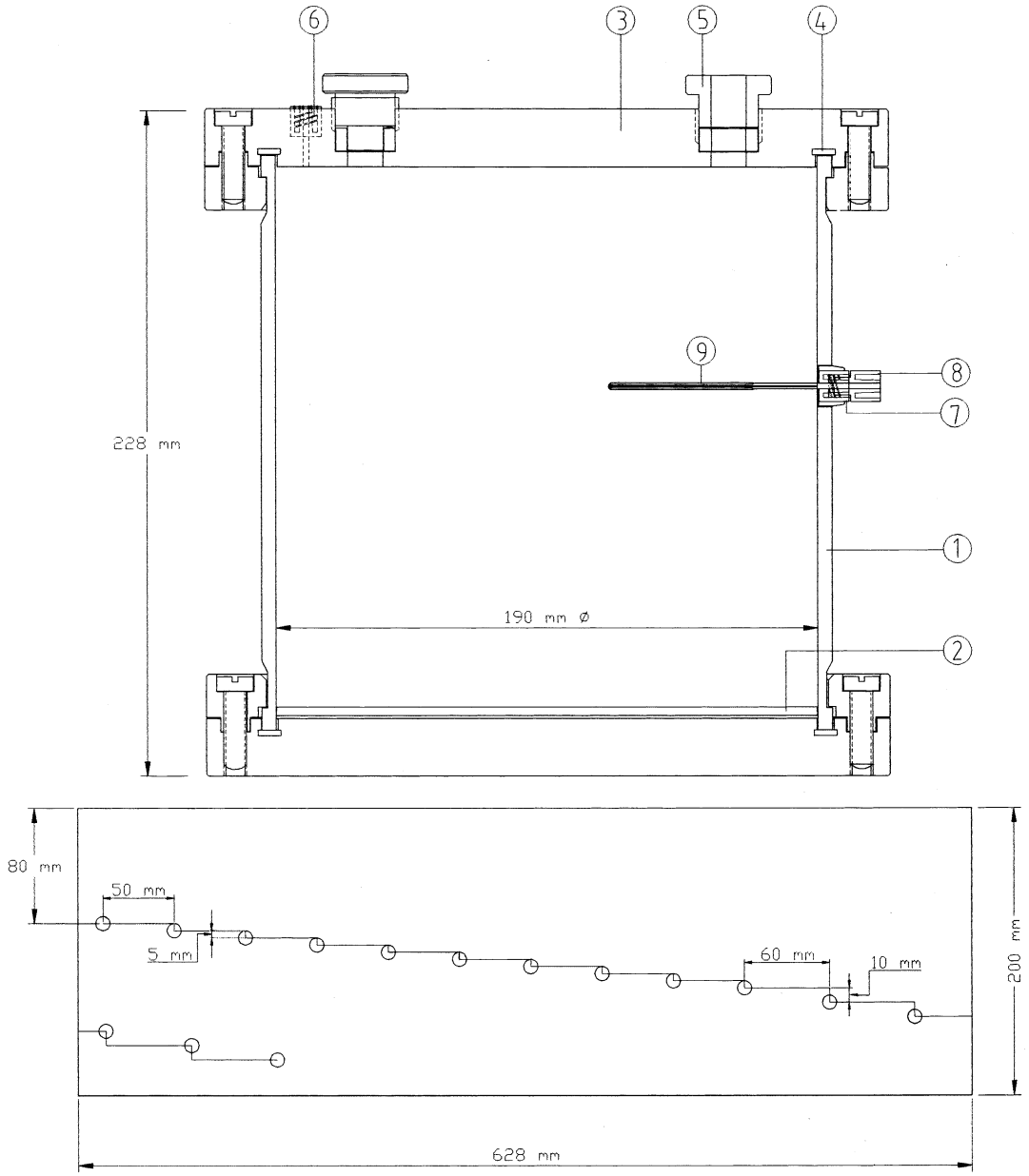

FIGURE 1. SOFIE schematized. Top, vertical cross section; bottom, projection of the cell wall showing the positions of probes.

To tackle the difficulties described above, a novel experimental technique was developed. For the first time, it is possible to quantify very labile metal fractions (among which are free metal ions) in pore water, repeatedly, and in a nondestructive manner to the sample. Bioassays are conducted simultaneously in the same setting. Sediment samples are taken in an undisturbed manner, including the overlying surface water. It is demonstrated that gradients of metal species, among which are free ion activities, can be accurately measured over increments as low as 5 mm. The effect of sediment-dwelling organisms on metal speciation is discussed, accounting for variable exposure concentrations.

Methods

This study was performed with a novel experimental technique (patents 1018200/02077121.8), which is introduced here as sediment or fauna incubation experiment (SOFIE).

This device or "cell" is shown in Figure 1. It consists of a circular coring device (1), 190-mm radius, 200-mm height, which is used as a sampling device to obtain undisturbed sediment. Field samples are taken including the overlying surface water. This guarantees the physical and geochemical integrity of the sample (e.g., bulk density, redox status). After sampling, the core is closed at the bottom edge with a shutter plate (2) and is mounted on a base socket. A top socket (3) is attached, ensuring a gastight fitting with the body core with an internal silicone seal (4). The top plate of this socket has four gastight connectors for electrodes (5) to perform, for example, pH, Eh, EC, or O_2 measurements. These are inserted vertically in the sample. The sample does not leave the body core but is now part of the cell. The cell wall contains 15 gastight connectors (7) for probes (9) (see next paragraph). These connectors are positioned in a circular manner (5.7°) along the core in such a way that the first 10 probes cover a water or sediment layer that is 5 mm lower than the previous one (Figure 1, bottom). The remaining five probes have a vertical distance of 10 mm. These dimensions were calculated for a "worst-case sediment" (i.e., low porosity) and ensure that no interference among probes occurs during pore water sampling.

For experimental scenario purposes, aerobic or anaerobic conditions in the water−sediment system may be manipu-

lated by flushing the atmospheric headspace in the cell with gas (O_2, N_2, CO_2, or mixtures) using valve 6 (Figure 1). Diffusion through the water layer guarantees slow oxidation or reduction kinetics. It is advised that the headspace be flushed with nitrogen gas at least after sampling and during transport to the laboratory.

With the method described above, the top 0.25 m of sediment of the river Rhine, The Netherlands ($X = 194.227$, $Y = 442.577$; "Malburgerhaven") was sampled. The cell was placed in the laboratory and was allowed to acclimatize for seven weeks. A bulk sample was taken from the same location. It was freeze-dried, mixed thoroughly, and analyzed for an array of physical and chemical parameters.

Pore Water Probing. Polyethersulfone (PES; obtained from X-flow Industries, Almelo, The Netherlands) was selected for a semipermeable polymer from which small tubes (1-mm diameter, 50-mm length) were manufactured. PES grade MF0.1 was used, meaning that the micropores that are present in this polymer discriminate colloidal fractions of $>0.1\,\mu$m. This mesh size is impermeable to bacteria, so pore water that passes the polymer is sterile. This type of polymer was applied earlier (*22*) to act as an artificial plant root in soil.

One end of the polymer tube is closed with an inert resin. A 0.5-mm fiberglass needle was mounted inside the tube for robustness. The other end is mounted on an impermeable HDPE tube (1-mm diameter, 20-mm length). Segmented in this way, and mounted in the fittings of the cell's body core, the area adjacent to the wall is avoided during pore water extraction by the probe.

Probes are flushed with nitrogen gas prior to their use to expel oxygen from the polymer's pores. This prevents oxidation processes from occurring during sampling. By applying a small vacuum to the probes, pore water passes the polymer. Adhesive force along the fiberglass core guides the sample to the outlet.

This way of pore water sampling has the following advantages: (i) Instantaneous microfiltration during sampling is near-ideal (contamination is excluded). (ii) Pore water samples maintain their characteristic redox status and therefore their (geo)chemical composition (e.g., free ion activities are maintained). (iii) Probes have a very low dead volume (0.04 mL). (iv) Probes can be used repeatedly.

In the experiment that is described here, pore water was sampled every 2 days for a large number of characteristics. Due to optimization of analytical techniques (see section Chemical Analysis), a sample volume of only 3 mL sufficed to measure all necessary parameters. Steady-state conditions over the water−sediment interface were determined at three sampling times over a 40-day period.

Determination of Free Metal Ions by Ion Exchange. To separate free metal ions from other forms of organic and inorganic metal ligands in solution, use was made of a chelating polymer (Chelex100, Bio-Rad Laboratories). This polymer, which was initially developed for the purification of chemicals and liquids, consists of styrene−divinylbenzene copolymers containing paired iminodiacetate ions, which act as chelating groups in binding positively charged ions. It has a high selectivity for metal ions, in particular the polyvalent ones, and has a high bonding strength. Its selectivity for divalent over monovalent ions is approximately 5000 to 1. Properties of this and other natural and synthetic chelating agents have been discussed by Vasconcelos and Gomes (*23*), among others.

The possibility to differentiate trace metal species with this chelating polymer is dictated by the lability of the individual species. For this purpose, labile fractions were determined over a short time frame (40 s) and were compared with speciation calculations. These were performed with the chemical speciation model CHARON (*24*) which was modified

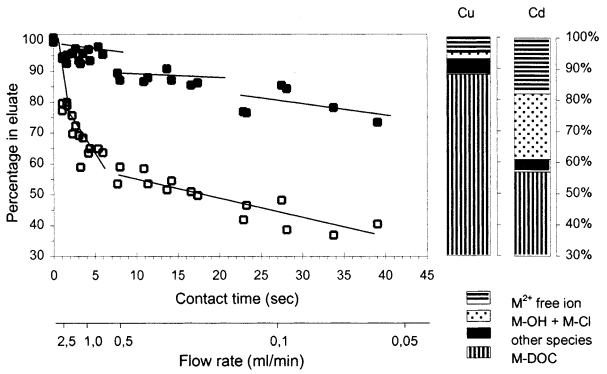

FIGURE 2. Identification of labile metal fractions for Cu (■) and Cd (□) species. Dissociation rates associate with contact time in MICs. The bars are calculated fractions.

with the NICA−Donnan concept for DOC complexation of metals (*25*).

Microsized, preconditioned ion-exchange columns (MIC; 1-mm diameter, 20-mm length) were designed to contain ~600 mg of reactive polymer. MICs were tested extensively on dimensions, preconditioning methods, metal affinities, contact time, and extraction procedures. MICs are connected directly onto the outlet of the probes (7 and 8, Figure 1). In this way, ion exchange is practically instantaneous and is therefore executed at the reigning geochemical status of the probed sediment layer. Reliability of MICs were best when purified with Ultrex-HNO_3 and brought in a saturated ammonium state using NH_4OH solution executed in a batch. This medium retains free metal ions (*26*−*28*) but passes any other fraction (*26*, *29*), provided that dissociation of metal pairs or complexes is prevented. This is exemplified in Figure 2.

It shows that the contact time between the sample and the polymer is closely related with dissociation rates of individual metal species. There is a close match between dissociation coefficients of metal species (related to a defined contact time) and calculated fractions. The most labile fraction was identified as the free ion concentration. From a large number of these tests it was concluded that free metals were isolated from solution by maintaining a contact time between pore water and the exchange polymer of 2 s (flow rate 1.5 mL·min^{-1}). Prolonged contact time results in the dissociation of labile inorganic ion pairs such as M−OH or M−Cl, which may occur within 5−20 s (especially the case for Cd, Pb, and Zn) and may falsely attribute these concentrations to free metal concentrations. Contact times of 25 s or more may give rise to relevant desorption of metals from DOC.

The most labile fraction, further denoted as the free metal ion concentration [M^{2+}], was quantified by stepwise, sequential extraction of the MICs by 6 mL of 2 M Ultrex-HNO_3 and 4 mL of Milli-Q water. Inorganic fractions and DOC-bound fractions were determined from the mass balance with the total dissolved fraction.

Exposure Tests with Oligochaetes. Species of the oligochaete *Limnodrilus* (phylum Annelida, order Oligochaeta, family Tubificidae) were chosen for accumulation tests because of their wide abundance in aquatic systems and their importance for the ecological food chain. *Limnodrilus* lives in close contact with both the sediment and the overlying surface water. Populations were bred in the laboratory under contaminant-free conditions and consisted mainly of *Limnodrilus claparedeanus* and *Limnodrilus hoffmeisteri*. After steady-state conditions of the sediment were quantified on three occasions with 14-day time intervals, the oligochaetes were introduced in the cell in field-realistic densities (i.e.. 4 organisms/cm^2, which represented ~1250 individuals or 8.3

TABLE 1. Sediment Characteristics

dry weight	55%	Al	23.4 g·kg^{-1}
<63 μm	72.6%	Mn	654 mg·kg^{-1}
<50 μm	71.8%	Cr	102.1 mg·kg^{-1}
<16 μm	59.3%	Ni	35.6 mg·kg^{-1}
<10 μm	52.2%	Cu	93.1 mg·kg^{-1}
<2 μm	35.2%	Zn	1091 mg·kg^{-1}
organic C	2.4%	Cd	7.49 mg·kg^{-1}
inorganic C	1.1%	Pb	703.6 mg·kg^{-1}
Mg	7.79 g·kg^{-1}	Hg	1.5 mg·kg^{-1}
Fe	29.3 g·kg^{-1}	Co	15.3 mg·kg^{-1}
Ca	24.1 g·kg^{-1}		

g of biomass wet weight (1.139 g of dry weight) in the cell). Accumulation in *Limnodrilus* was measured periodically by sampling individuals from the population on seven occasions in 2−3-day intervals. They were allowed to void their guts for 48 h, which, based on dissection, was an adequate period. The organisms were 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 using inductively coupled mass spectrometry (ICPMS). Dolt-2 (certified by the Community Bureau of Reference, BCR, Brussels, Belgium) was used as biological reference material. No systematic correction was applied to the analytical data, since recovery was always between 99 and 114%.

Chemical Analysis. Pore water concentrations of NO_3^-, NO_2^-, SO_4^{2-}, PO_4^{3-}, and Cl^- were measured with segmented ion chromatography (Dionex AS50 chromatography compartment, ED50 electrochemical detector). Limits of detection (LODs) ranged from 0.025 mg·L^{-1} for NO_2^- to 0.05 mg·L^{-1} for PO_4^{3-}. As, Al, Ca, Cd, Cu, Cr, Fe, Mg, Mn, Ni, Pb, and Zn analyses were carried out with ICPMS (Perkin-Elmer Elan 6000; LOD, 0.003 μg·L^{-1} for Cd to 6.0 μg·L^{-1} for Zn). NH_4^+ analyses were performed with a Skalar segmented flow analyzer SAN+ 6250 matrix fotometer (LOD, 0.022 mg·L^{-1}). Dissolved organic carbon was determined with a Shimadzu 5000 TOC analyzer (LOD, 0.45 mg·L^{-1}).

Results and Discussion

Bulk sediment characteristics and composition are presented in Table 1. The sediment is clayey, has a moderate content of organic matter, and is buffered by a fair amount of carbonates such as calcite. Heavy metal contamination levels are high, exceeding quality standards by far. Steady-state partitioning coefficients for most metals are large, indicating strong retention to the soil matrix.

In Figure 3, results are shown for some pore water characteristics. For reasons of survey ability, the steady-state composition is presented as a single solid line with minimum and maximum values (horizontal bars), and only data for 7, 9, and 13 days after the introduction of test organisms (dotted lines) to the system are presented.

Reproducibility of steady-state measurements is good, as illustrated by small variations in analytical values on different days. Thus, the system was at steady state prior to the introduction of the test organisms. Some interesting findings can be drawn from the steady-state situation. First, the steep gradient of the redox potential (Eh) over the first 10 mm, which was expected but is nevertheless remarkable when measured in this way. Denitrification precedes sulfate reduction, and anaerobic conditions were reached within 15 mm from the water−sediment interface. The increasing reduction of ferric(III) oxyhydrides 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 Cu, Ni, Cd, and Zn decrease with depth and coincide with sulfate reduction patterns. These metals most probably associate with sulfides and therefore become insoluble. Heavy metals, however, compete for binding opportunities between the reactive sulfide phase and relatively large amounts of dissolved organic matter at 35−40 mm. This competition was demonstrated quantitatively in a previous kinetic study (*30*) and explains why high dissolved metal concentrations may occur in sulfidic zones.

An illustration of a metal's binding affinity, or selectivity, to specific sediment constituents is shown by lead. Under the initial steady-state conditions, dissolved Pb concentrations decline significantly at −10 mm, only to increase again with depth. This may most probably be explained by the occurrence of a thin but pronounced layer of dark manganese (hydr)oxide, which could be observed through the cell's transparent wall. The stability of manganese (hydr)oxide depends highly on Eh, and the steep gradient of the latter explains the small layer thickness. Findings of Nelson et al. (*31*) suggest that this is biogenic manganese(III/IV) oxide, prepared by enzymatic oxidation of Mn(II) by bacteria, and shows strong Pb adsorption. This layer disappeared within 5 days after introduction of the test organisms.

Immediately after introduction of *Limnodrilus* to the system, equilibrium concentrations shifted. *Limnodrilus* is a sediment dweller. It lives in burrows and irrigates these with oxygen-rich surface water. Consequently, it oxidizes its immediate environment and, therefore, directly affects the chemical reactivity and bioavailability of contaminants that remain in the sediment. The effect of the colonization on chemical and physicochemical pore water characteristics is pronounced. Eh rises significantly, and pH increases by half a unit. Sulfides are oxidized, and phosphates are released. Macronutrients are mobilized but are at the same time taken up by the organisms for metabolism and growth. Next to these alterations, and maybe more importantly, the chemical speciation of heavy metals is affected considerably. This new dynamic state of the system is complex; metals are mobilized with reaction kinetics that differ for each metal. Simultaneously, organisms accumulate and eliminate metals. These measurements illustrate that biological kinetics (uptake) are for most metals faster than chemical kinetics (release from the sediment). Only for arsenic and nickel was a steady flux from sediment to pore water observed. Exposure can therefore only be estimated and modeled when time-related changes in metal concentrations are taken into account. The importance of rate-limiting kinetics, and the need to resolve related questions by actual measurements, was put forward by Hudson (*32*), but there are more criteria that have to be met. From a risk-assessment point of view, the question remains whether uptake of heavy metals by *Limnodrilus* is dictated mainly by the concentration in the overlying water or by concentrations in oxic or reduced pore waters of sediments. Findings of Hare et al. (*3*) point in the first direction. However, the question on the contribution of the metal's free ion activity is still unknown and needs to be resolved.

Time-Varying Exposure Concentrations. It is shown that dissolved metal concentrations change with time and so do specific metal species such as M^{2+}. These time-varying changes may follow first-order reaction kinetics:

$$dC/dt = kC$$

so the external concentration, or exposure concentration, at a given time $C(t)$ is 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 the rate term. In analogy, the free ion activity concentration

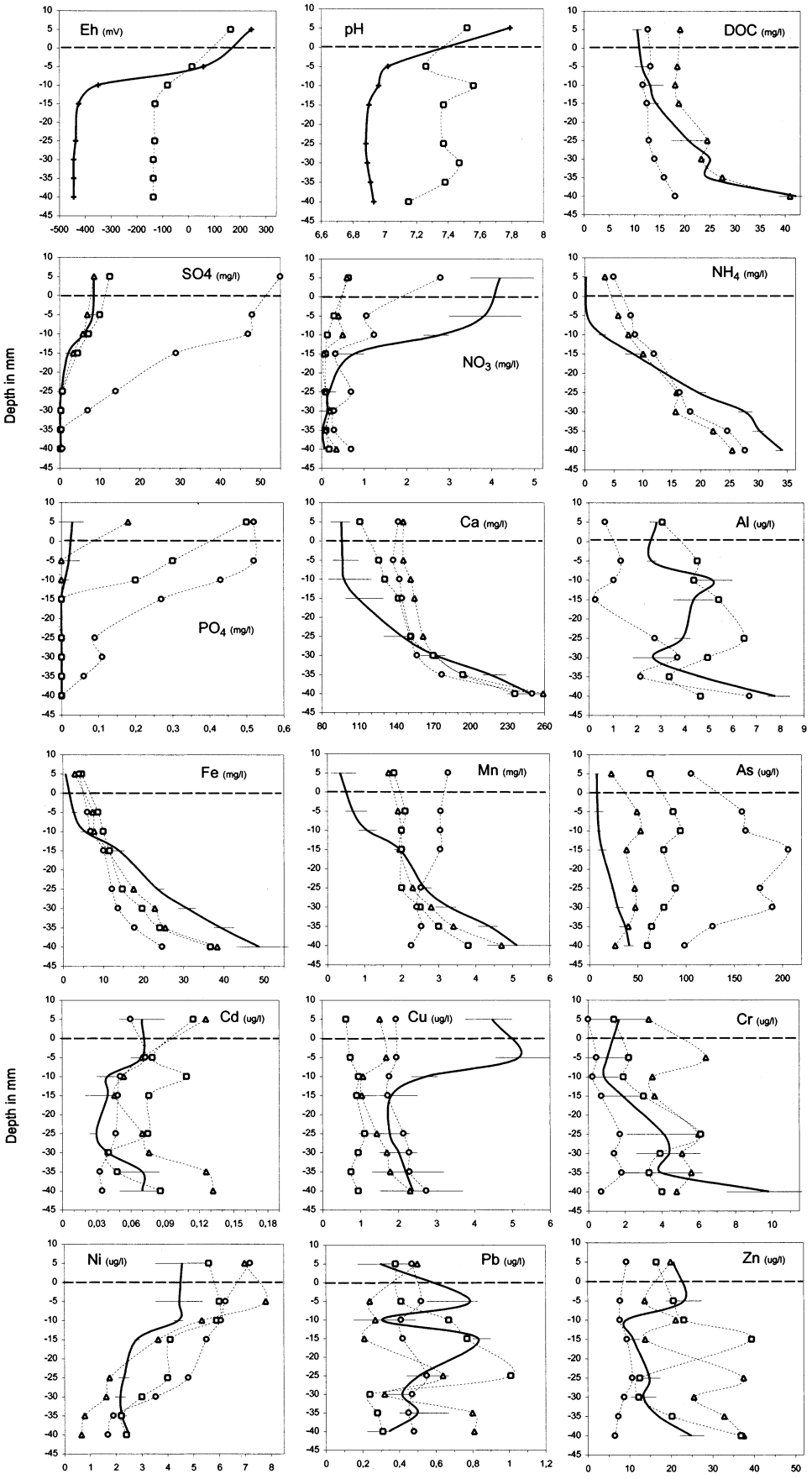

FIGURE 3. Time−depth characteristics over the water−sediment interface at steady-state conditions (solid line) and 7 (△), 9 (□), and 13 (○) days after introduction of *Limnodrilus* to the system.

TABLE 2. Free Metal Ion Activities as Percentage of Total Dissolved at Equilibrium and 7 and 21 Days after the Introduction of Test Organisms

		free metal ion activity (% of total dissolved)		
metal	distance from water−sediment interface (mm)	steady state	7 days after introduction of *Limnodrilus*	21 days after introduction of *Limnodrilus*
Cd	+5	29	48	<1
	−5	<1	1	40
	−10	<1	26	12
	−15	<1	<1	29
	−25	<1	nd[a]	17
	−30	<1	<1	20
	−35	<1	13	<1
	−40	nd	<1	<1
Cu	+5	4	13	1
	−5	8	5	<1
	−10	4	10	<1
	−15	<1	1	<1
	−25	<1	nd	<1
	−30	<1	<1	<1
	−35	<1	<1	<1
	−40	nd	<1	<1
Ni	+5	29	26	18
	−5	1	23	5
	−10	<1	8	1
	−15	<1	19	<1
	−25	<1	nd	<1
	−30	<1	<1	<1
	−35	<1	<1	<1
	−40	nd	<1	<1
Pb	+5	1	34	1
	−5	1	1	1
	−10	<1	15	1
	−15	<1	1	<1
	−25	1	nd	<1
	−30	5	1	<1
	−35	<1	2	<1
	−40	nd	1	<1
Zn	+5	38	44	32
	−5	55	35	28
	−10	1	47	1
	−15	36	1	1
	−25	34	nd	5
	−30	4	1	1
	−35	5	34	1
	−40	nd	1	1

[a] nd, not determined due to malfunction of MIC.

at a given time, $M^{2+}(t)$, may be written as

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

where, if the FIA assumption for uptake is valid, $k_{0,act}$ must have a value different from k_0 which accompanies total dissolved concentrations.

Equation 2 was quantified with the measurements performed by MICs. Because of the large amount only part of these data is presented in Table 2. These results show that relatively large fractions of free ion activities occur in the overlying oxic surface water. These free metal fractions may increase after some days when organisms are introduced. Uptake of metals by the test organisms results in declining fractions in time, as will be demonstrated later. It should be clear, however, that large fractions of free ion activities are not necessarily equivalent to high concentrations. For example, the increase of Cu^{2+} fractions from 4 (steady state) to 13% (after 7 days) represents declining Cu^{2+} concentrations from 0.5 to 0.17 $\mu g \cdot L^{-1}$, respectively. Another example is the reported Cu^{2+} fractions of Sunda and Lewis (*8*) in the lower range of 0.4−3.5%, measured potentiometrically in surface waters. These fractions, however, represent similar Cu^{2+} concentrations as those measured in this study.

TABLE 3. Internal Body Concentrations at $t = 0$ Days (Q_0) and $t = 11$ Days (Q_{max}), Linear Uptake Constant (a), and Correlation Coefficient (r^2)

element[a]	$Q_{0,org}$ ($mg \cdot kg^{-1}$ dw)	$Q_{max,org}$ ($mg \cdot kg^{-1}$dw)	a ($mg \cdot kg^{-1} \cdot d^{-1}$)	r^2 ($n = 5$)
Cd	0.02	2.15	0.164	0.88
As	9.42	21.16	0.566	0.83
Ni	0.59	12.04	0.999	0.97
Cr	0.44	33.22	2.540	0.82
Cu	20.30	57.50	2.800	0.90
Pb	4.43	135.60	11.96	0.99
Mn	12.50	279.80	21.39	0.84
Zn	249.5	671.10	37.42	0.76
Fe	1374	9943	686.7	0.86
Ca	4914	13997	696.9	0.90

[a] Elements are ranked with increasing uptake rate.

Uptake and Elimination by *Limnodrilus* spp. Metal uptake and excretion rates by *Limnodrilus* were approximated by a time/concentration dynamic, two-compartment model. It is assumed that body concentrations vary in time and depend on uptake from the water phase at a certain rate. At the same time, uptake is accompanied by elimination, which is kinetically directed by the organism itself. Hence, one may write

$$dQ/dt = k_1C(t) - k_2Q(t)$$

where Q is the internal body concentration ($\mu g \cdot g^{-1}$), k_1 and k_2 are uptake and elimination rate constants, respectively (day^{-1}), and t is time. This yields, for $Q(0) = 0$

$$Q(t) = \frac{k_1C_0}{k_2 - k_0}(e^{-k_0t} - e^{-k_2t}) \quad (3)$$

where C and k_0 are derived from eqs 1 and 2.

In analogy for the free ion activity,

$$Q(t) = \frac{k_1[M^{2+}]}{k_2 - k_{0,act}}(e^{-k_{0,act}t} - e^{-k_2t}) \quad (4)$$

With the introduction of k_0, time-dependent concentrations determine the overall exposure of organisms and, thus, $Q(t)$. Note that elimination may partly be attributed to growth of the organisms during the exposure period. This separate loss term is not considered in the model.

To determine the consequence of *not* considering time-dependent concentrations, a one-compartment model was included for comparison. With this model, a constant uptake rate, a, is assumed, as opposed to concentration-dependent uptake. Body concentrations are then described as

$$dQ/dt = a - kQ$$

which yields

$$Q(t) = C_0e^{-kt} + (a/k(1 - e^{-kt}) \quad (5)$$

Initial body concentrations of *Limnodrilus* prior to exposure and maximum body concentrations 11 days after exposure are summarized in Table 3. This time frame showed remarkable linearity for uptake ($dQ/dt = a$) for a range of elements ($r^2 = 0.76$−0.99). Maximum body concentrations increase in the order Cd < Ni ∼ Cr < As < Cu < Pb < Mn < Zn < Fe < Ca. Linear regression coefficients a (d^{-1}) increase in the order Cd < As < Ni < Cr ∼ Cu < Pb < Mn < Zn <

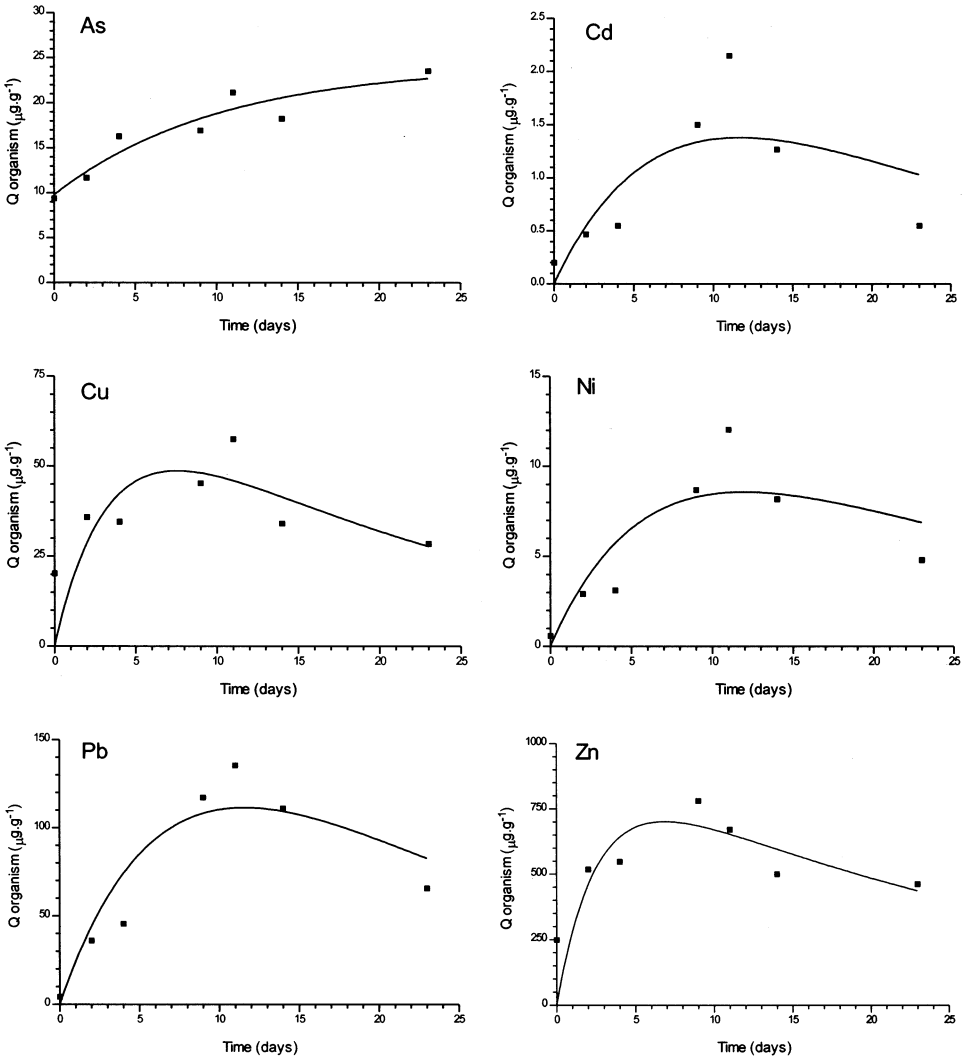

FIGURE 4. Internal body concentrations of *Limnodrilus* with time. The solid line is the accumulation−elimination model prediction from eq 3 (Pb), eq 4 (Cd, Cu, Ni, Zn), and eq 5 (As).

Fe ~ Ca. This simplification evidently disregards the relative contributions of uptake and elimination that occur simultaneously. Mass calculations show that *Limnodrilus* consumes considerably more metals than the amount that is initially available in the dissolved phase at the beginning of the test; this amount is 18 times the total dissolved mass in overlying water and pore water for Cd, 4.4 for Cu, 1,3 for Ni, 122 for Pb, and 10 for Zn. Hence, considerable amounts have to be delivered from various solid phases to the aqueous phase. This delivery is partly facilitated by chemical oxidation of metal sulfides due to burrowing activity and partly by desorption from surfaces and dissociation of precipitated ligands. The amount of bioconcentrated As is only 0.3 times the initial available amount. Concentrations are therefore not kinetically inhibiting uptake.

Data and model results for As, Cd, Cu, Ni, Pb, and Zn are shown in Figure 4. Except for arsenic, body concentrations of all metals decreased after 11 days. Depending on the element, different equations were used to describe uptake and elimination. This is discussed in the next section.

Performance; Relation between Speciation and Body Concentrations. To analyze the performance of the uptake and elimination models with varying exposure concentrations, the overall data set was divided into six subsets: two system compartments (overlying surface water and sediment pore water) each with three chemical "species" (total dissolved metal concentration, free ion activities, and DOC + inorganic fraction). Performance of the uptake−elimination models (eqs 3−5) were tested with each subset. Results of 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 of each model parameter was used rather than the correlation coefficient of the model with the measurements. The standard error is a measurement 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

$$sy \cdot x = \sqrt{\sum (Y - Y_p)^2 / n}$$

By minimizing the sum of squares, values for k_1 and k_2 and corresponding standard errors were derived.

Table 4 shows the results of the reconstruction of k_0 values with accompanying metal concentrations. Three out of six subsets gave satisfactory results when used as input for the uptake−elimination models. The best results were obtained with the underlined values. Negative k_0 values indicate a decrease in concentrations; positive values indicate an increase. Since uptake dominates elimination in the first 13 days by far, only decreasing k_0 values may explain uptake of

TABLE 4. Metal Concentrations and Corresponding Dissipation/Supply Rate Constants k_0[a]

	total dissolved in overlying water			free ion activity in overlying water			total dissolved in pore water of sediment		
metal	$C_{i,T}$ ($\mu g \cdot L^{-1}$)	$k_{0,T}$ (d^{-1})	r^2	$M^{2+}{}_i$ ($\mu g \cdot L^{-1}$)	$k_{0,act}$ (d^{-1})	r^2	$C_{i,T}$ ($\mu g \cdot L^{-1}$)	$k_{0,pw}$ (d^{-1})	r^2
Cu	4.48	−0.180	0.99	0.45	−0.380	0.85	2.51	−0.095	0.88
Cd	0.078	+0.037	0.63	0.025	−0.277	0.47	0.052	+0.033	0.47
Ni	4.57	+0.020	0.27	1.02	−0.042	0.87	2.69	+0.039	0.96
Pb	0.298	+0.024	0.27	0.003	+0.047	0.10	0.833	−0.015	0.10
Zn	20.6	−0.020	0.83	7.83	−0.034	0.82	10.96	+0.114	0.84

[a] Underlined values are combinations that yielded significant relationships with uptake (k_1) and elimination rates (k_2) by *Limnodrilus* spp.

TABLE 5. Model Parameters k_1 and k_2 with Standard Errors[a]

species	chemical descriptor two-compartment	$k_{1,act/T}$ (d^{-1})	sy·x	$k_{2,act/T}$ (d^{-1})	sy·x
Cu^{2+}	free ion activity	45.96	0.01	0.034	0.02
Cd^{2+}	free ion activity	20.16	0.01	0.032	0.04
Ni^{2+}	free ion activity	1.77	2.19	0.146	0.08
Pb_T	sediment pore water	27.20	0.01	0.206	0.12
Zn^{2+}	free ion activity	30.57	0.01	0.390	0.17

species	chemical descriptor one-compartment	k	sy·x	a	sy·x
As_T	sediment pore water	0.098	0.062	2.376	1.145

[a] C_i and k_0 are derived from Table 4.

the metal species. Combinations of 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 remaining variables k_1 and k_2.

Only those combinations that yielded standard errors of k_1 and k_2 that were of the same magnitude as their own values were considered significant. This was the case for the following combinations: total dissolved concentrations and overlying water; free ion activity concentrations and overlying water; total dissolved concentrations and sediment pore water.

Table 5 gives the results for the lowest standard errors that were achieved and therefore provided the best chemical descriptors for body concentrations. Hence, model lines in Figure 4 originate from different equations.

Except for arsenic, the one-compartment model failed in all cases to achieve a model fit to the measurements. Since the numerical iterations resulted in stack overflow errors (the model did not converge), no statistical information could be derived. Obviously, uptake from solution by the organisms was not constant, and kinetic processes do indeed matter. The suitability of the one-compartment model to describe arsenic uptake may be explained by the fact that external concentrations were never limiting during exposure. Hence, the rate of uptake is not concentration dependent.

From a statistical point of view, very reliable model simulations were obtained for all elements. It is shown that Cd, Cu, Ni, Pb, and Zn uptake and elimination could best be described by the two-compartment model. Uptake of Cd, Cu, Ni, and Zn by *Limnodrilus* is related to the free ion activity concentrations that are present in the overlying surface water (eq 4). Standard errors are low, especially for $k_{1,act}$. The fact that the overlying water plays such a significant role in metal uptake is actually quite easy to explain. Benthic organisms

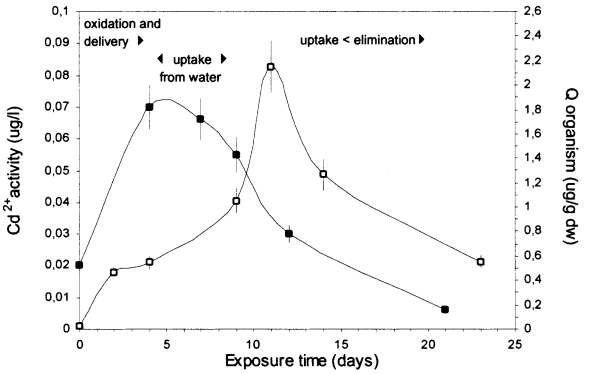

FIGURE 5. Kinetic interaction between Cd^{2+} activity concentration (solid marker, left axis) and body concentration in oligochaetes (open marker, right axis).

are often considered to be 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. As was demonstrated before, free ion activities in overlying water exceeded those in pore water and hence provide a large pool of available metal ions.

Figure 5 exemplifies the relation between time-varying concentrations of Cd^{2+} activities in overlying water and time-varying uptake of Cd by *Limnodrilus*. This simultaneous presentation of C_{act} and Q is unique; it shows that temporal variations in chemical composition occur and that interactions between chemical availability and biological uptake take place. As stated before, the introduction of organisms to the water−sediment system results in a release of metals, mainly due to dissolution of iron/manganese oxy(hydr)oxides and oxidation of sulfide precipitates. Therefore, $[Cd^{2+}]$ increases. As shown in Figure 5, release to and simultaneous uptake from the water phase are, in terms of concentrations, antagonistic processes: body concentrations rise and free ion activities decline.

In contrast to Cd, Cu, Ni, and Zn, uptake of Pb and As could best be described by total dissolved concentrations in pore water. For As, this is explained by the fact that dissolved arsenic does not yield significant free ion concentrations, being a oxyanion; the predominant species are arsenate or arsenite, and these are negatively charged. This does, however, still not explain the lack of correlation with concentrations in overlying water. For Pb, the outcome is yet unexplained. It is occasionally suggested that uptake and elimination of Pb obeys mechanisms different from those in the case of the other heavy metals (i.e., an interaction between the free metal ion and a channel or carrier transport system in the external or internal epithelium), but this is yet to be demonstrated.

These presented findings provide a significant contribution to our understanding of chemical speciation of metals

in water−sediment systems and subsequent metal uptake by organisms. It is demonstrated that the presence of benthic organisms in a water−sediment system has a significant effect on chemical speciation and, thus, has to be considered when bioassays and chemical tests are performed. Body concentrations of *Limnodrilus* are regulated by temporal variations in metal concentrations and chemical speciation. Metal concentrations in the overlying water contribute in particular to overall uptake by tube dwelling oligochaetes, with possible exceptions for As and Pb.

Nevertheless, these findings require a further note. Speciation of heavy metals in water−sediment systems is the effect of many geochemical characteristics and environmental conditions. 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. Still, it may in some cases be insufficient to predict the biological response, since one must also consider potential interactive effects of hydrogen ions (*4*, *13*) or from other macrochemicals, as was suggested by DeSchamphelaere and Janssen (*33*). In any case, it is concluded that an integrated cell such as SOFIE provides the necessary experimental tool to support, in a mechanistic way, environmental risk assessments of contaminants. This knowledge may in time be incorporated in the derivation of better founded quality criteria for sediments.

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