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Rapid Communication

Cadmium bioaccumulation in Tubificidae from the overlying water source and effects on bioturbation

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Abstract

Cadmium bioaccumulation in tubificid oligochaetes in relation to metal vertical distribution in sediment and bioturbation intensity was studied during a 56-day experiment with a constant contamination source in the overlying water ($20\,\mu g\,L^{-1}$). The indoor microcosms simulate a two-compartment biotope with three experimental treatments based on metal exposure and faunal composition: contaminated water column with or without worms and uncontaminated water column with worms. Cadmium bioaccumulation in worms was studied after 7, 14, 21, 28, and 56 days. Bioturbation was analyzed as a functional parameter representative of organisms' activity and using conservative particulate tracers: luminophores ($\phi = 63-100\,\mu m$ and 100–315 μm) and microspheres ($\phi = 1\,\mu m$). The results show no significant effects of cadmium exposure on bioturbation, despite high bioaccumulation levels in worms ($50\,\mu g\,g^{-1}$ dry wt.), suggesting the existence of detoxification/sequestration processes.

Keywords: Bioaccumulation; Bioturbation; Tubificids; Cadmium; Bioavailability; Bioadvection; Conveyors

1. Introduction

Aquatic ecosystems are the final sink for potentially all toxic metal inputs into the environment, via transfer from natural and/or anthropogenic sources. Measurements of metal distribution in the different abiotic compartments, including water, suspended particulate matter, and sediment, have been widely developed, associated with bioaccumulation analyses in the main trophic levels. Data from these field studies point out that the prediction of ecotoxicological effects requires an understanding of both the physical and biogeochemical pathways controlling metal chemical fate and bioavailability. Among the major processes involved, metal exchanges at the "water column/sediment" interface play a fundamental role along with the storage and release capacities. They are strongly influenced by the physicochemical characteristics of the biotopes but also by biotic factors, such as bioturbation. Bioturbation can be defined as the result of burrowing, feeding, irrigating, respiring, and defecating activities of animal species living at the surface and/or within the sediment superficial layers (Rhoads, 1974). Accurate models enable us to quantify the various benthic bioturbation activities (Robbins et al., 1979; Gerino et al., 1994) and they may provide useful tools to investigate direct and indirect effects of bioturbation on metal accumulation in sediment compartments and toxic effects on benthic communities. To measure the influence of contaminants on benthic organisms, quantification of bioturbation may be a relevant parameter, as this type of activity

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integrates by itself the most important biological functions of the organisms such as respiration, feeding, and defecating.

Within a multidisciplinary research program based on an experimental study of bioturbation effects on cadmium (Cd) transfer between sediment and the water column, with these two compartments acting as initial contamination sources or storage compartments, we selected tubificids as bioturbation models. These oligochaete worms are very widely distributed and frequently dominant in freshwater benthic communities; they have a high level of resistance to unfavorable treatments, especially organic pollution associated with severe hypoxic treatments (Brinkhurst and Cook, 1974). Tubificids are closely associated with superficial sediments: their anterior part burrows into the substrate and the posterior part undulates in the overlying water. Data published in the literature from field or indoor studies show a marked controversy over metal accumulation in tubificids, relating to the predominance of exposure routes from overlying water, sediment pore water, or/and ingested sediment particles (Whitley, 1967; Back, 1990; Sager and Pucsko, 1991; Bervoets et al., 1997; Warren et al., 1998; Bouché et al., 2000) and relationships between exposure treatments and toxic effects (Khangarot, 1991; Klerks and Bartholomew, 1991; Wallace et al., 1998; Deeds and Klerks, 1999; Chapman, 2001; Gillis et al., 2002).

Our experimental approach was based on indoor microcosms, including a two-compartment biotope "water column/natural sediment" and a mixture of three tubificid species as the bioturbation agents. Metal transfers from the water column as the initial contamination source were analyzed using three experimental treatments, where Cd was competing for uptake into sediment and organism phases: {−Cd + Tub}, tubificid worms were added to the sediment and the water column was not contaminated; {+Cd−Tub}, Cd was added to the water and no tubificids were present in the sediment; {+Cd + Tub}, tubificids were added to the sediment and the water column was contaminated with cadmium.

In this article, we focus on Cd bioaccumulation kinetics in worms during a 56-day exposure period. Direct and indirect relationships were determined with Cd vertical distribution in the pore-water and particulate sediment phases. In parallel, Cd impact on bioturbation activity was analyzed using fluorescent particulate tracers (luminophores and microspheres).

2. Material and methods

2.1. Microcosm structure and experimental design

The basic structure, called the "experimental unit" (EU), was based on two compartments, sediment and water column, enclosed in a glass container (Fig. 1). The sediment was sampled from the Garonne River banks, upstream from Bordeaux (Gironde, southwest France). Its main characteristics are reported in Table 1. The background cadmium concentration was $0.75 \pm 0.02\,\mu g\,Cd\,g^{-1}$ dry wt. (dry wt. after 72 h at 60 °C). The sediment was sieved through a 1-mm mesh to remove macrofauna and then frozen at −20 °C for 1 week to kill any organisms. It was introduced into each EU, and then dechlorinated tap water (Table 2) was carefully added to the upper part of the EUs to avoid disturbances at the sediment surface.

The tubificid worms (Annelida, Oligochaeta) consisted of a mixture of three species which are closely related taxonomically and are similar in size: *Tubifex tubifex*, *Limnodrilus hoffmeisteri*, and *Limnodrilus claparedeianus*. They were collected in a natural environment (GREBIL, Arry, France) and were acclimated for 15 days in the laboratory, at 20 °C, in large tanks with the same Garonne River sediment. Batches of 250.0 ± 0.5 mg wet wt. of worms were made, with no

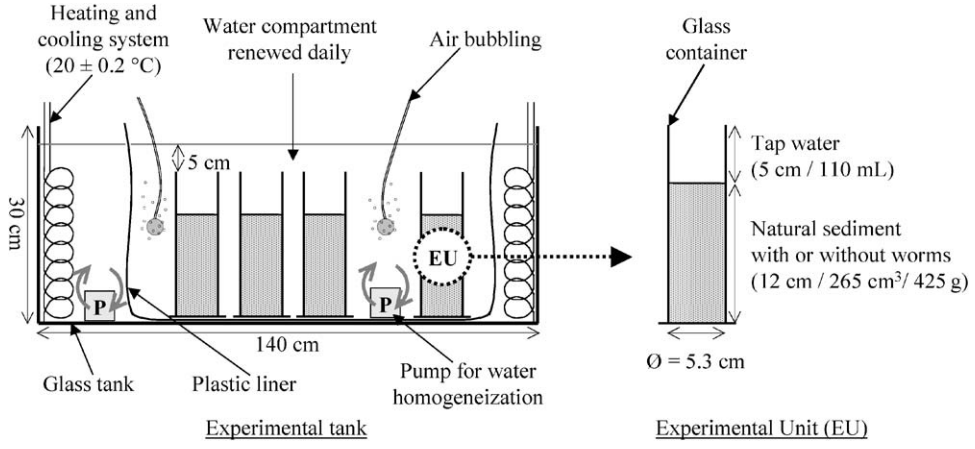

Fig. 1. Experimental units (EUs) and equipment for the regulation of abiotic factors.

Table 1
Sediment particles size distribution, particulate organic carbon (POC, expressed as percentage of dry weight), and porosity[a]

Grain size fraction (%)	
<15 μm	46.4
15–30 μm	27.7
30–63 μm	20.0
63–125 μm	4.4
125–250 μm	1.4
250–500 μm	0.1
>500 μm	0.0
Organic carbon (%)	1.55
Porosity (%)	0.74

[a]The sediment was collected on the banks of the Garonne River, upstream from Bordeaux (southwest France).

Table 2
Main physicochemical characteristics of the tap water used in the experiment

Concentration ($mg\,L^{-1}$)	
Calcium	16.20
Magnesium	8.30
Potassium	3.80
Sodium	39.65
Sulfates	6.55
Chlorures	43.70
Silicates	12.35
Ammonium	0.17
Nitrites	<0.01
Nitrates	<0.25
Orthophosphates	<0.05
Dissolved oxygen	1.10
Total organic carbon	0.50
pH at 20 °C	8.15
Turbidity (FTU)	0.2
Conductivity at 20 °C ($\mu S\,cm^{-1}$)	310

prior sorting with respect to the heterogeneous size distribution of natural communities. Each batch corresponded to 133 ± 5 worms/EU, or about 60,000 worms m^{-2}, which was close to the maximal densities found in the natural environment (McCall and Fisher, 1980). Additional batches were prepared to determine the wet wt./dry wt. ratio (5.6 ± 0.1 dry wt. after 72 h at 60 °C) and the background Cd concentration in organisms ($[Cd] = 1.0 \pm 0.1\,\mu g\,g^{-1}$ dry wt.).

The bioturbation activity of tubificids within the sediment was analyzed using fluorescent particulate tracers: luminophores and microspheres. Luminophores are natural sand particles coated with fluorescent paint. Two different sizes were used: $\phi = 63-100$ and 100–315 μm (Geologisch-paleontologisches Institut and Museum of Kiel University, Germany). Microspheres are fluorescent balls of latex ($\phi = 1\,\mu m$) within a liquid phase (Fluoresbrite YG Microspheres, Polysciences Europe GmbH, Eppelheim, Germany). In each EU, a mixture of 10 g (wet wt.) of Garonne sediment with 0.45 and 0.6 g of the two types of luminophores and 0.3 mL of microsphere suspension was deposited at the sediment surface, in the form of 3-mm-thick frozen mud cakes, 24 h after introduction of the worms.

The EUs corresponding to the three experimental treatments—{−Cd + Tub}, {+Cd−Tub}, {+Cd + Tub}—were distributed into three different experimental tanks (Fig. 1). Time zero for contamination began just after the mud cakes with tracers were deposited in the EUs. The nominal Cd contamination level in the water of the two contaminated tanks ({+Cd + Tub} and {+Cd−Tub} treatments) was fixed at $20\,\mu g\,L^{-1}$, similar to concentrations that can be found in freshwater natural environments downstream from industrial sites (Andrès et al., 1999). To maintain the contamination pressure throughout the experiment, all the water in each tank was renewed daily; a similar procedure was applied to the uncontaminated tank, so that all the EUs underwent identical disturbances.

The experiment lasted 56 days, with five sampling times: 7, 14, 21, 28, and 56 days. For each treatment and each exposure duration, eight EUs were set up: two replicates for the bioturbation study, three for the measurement of Cd concentrations in worms, and three for sediment analyses.

2.2. Physicochemical measurements, sampling procedure, and cadmium determination

Temperature, turbidity, pH, dissolved oxygen concentration and Cd concentration in the water column were measured daily in each experimental tank, before renewal. Dissolved oxygen profiles were determined in sediment using a voltammetric gold amalgam minielectrode (Anschutz et al., 2000). Unfiltered or filtered (0.2 μm) water samples (10 mL) collected for Cd determination were acidified with 200 μL of nitric acid (65% HNO_3, Merck, Darmstadt, Germany), stored at +4 °C, and analyzed within 3 days.

Worms were collected at the five time exposures by sieving sediment through a 500-μm mesh. External water was removed by placing worms on absorbent paper sheets, weighed (wet wt.), and kept for 72 h at 60 °C to determine the "wet wt./dry wt." ratio, without depuration of the ingested sediment. They were then digested with nitric acid (65% HNO_3) at 100 °C for 3 h under pressure treatments. The digests were diluted with ultrapure water.

Vertical distribution of Cd in sediment pore-water and particulate fractions was analyzed for the three experimental treatments, after the five exposure durations. The water column was carefully removed from the EUs and the sediment was cut into six layers: 0–0.5, 0.5–1, 1–2, 2–3, 3–5, and 5–12 cm. Each layer was weighed (wet wt.) and centrifuged under a nitrogen

atmosphere for 20 min at 5000 rpm (20 °C). The supernatants were collected and filtered through 0.2-μm filters (Membrane SFCA, Nalge Nunc International Corp., New York, USA). The filtrates were acidified with HNO_3 and stored at +4 °C until Cd determination for dissolved metal quantification in sediment pore water was carried out. The pellets were lyophilized and weighed to determine the wet wt./dry wt. ratio for each sediment layer. For particulate metal quantification, samples of 60–80 mg of lyophilized sediment were taken and digested with nitric acid attack (65% HNO_3). The digests were diluted with ultrapure water before Cd determination.

Cd was determined in worms, water, and sediment samples by atomic absorption spectrophotometry using a Varian AA 400 spectrophotometer equipped with a GTA 96 graphite tube atomizer, autosampler, and Zeeman correction. Water samples or sediment digestates (10 μL) were mixed before atomization with 4 μL of a mixture of 50% Pd ($0.2\,g\,L^{-1}$) and 50% $Mg(NO_3)_2$ ($0.5\,g\,L^{-1}$), to avoid interference. The detection limit was $0.1\,\mu g\,L^{-1}$ (3 SD of the reagent blanks). The accuracy of the analytical procedure was monitored by analysis of standard reference materials together with the samples series (PACS-2: marine sediment, $2.11\pm0.15\,\mu g\,Cd\,g^{-1}$; MESS-3: marine sediment, $0.24\pm0.01\,\mu g\,Cd\,g^{-1}$; TORT-2: lobster hepatopancreas, $26.7\pm0.6\,\mu g\,Cd\,g^{-1}$; from NRC-CNRC, Ottawa, Canada). Values were consistently within the certified ranges (data not shown).

To analyze the vertical distribution of luminophores and microspheres in the sediment compartments, the water column was carefully removed with a syringe and the sediment was cut into six layers: 0–0.5, 0.5–1, 1–2, 2–3, 3–5, and 5–12 cm. Each layer was mechanically mixed, and 5 mL was collected and lyophilized. Three 100.0 ± 0.5 mg samples of each lyophilized sediment layer were analyzed using an epifluorescence UV microscope at 360 nm (Olympus Optical Co., BH2-RFC Reflected Light Fluorescence Attachment, Hamburg, Germany). All the luminophores of both sizes were counted at 4× magnification. Luminophore concentrations in the sediment are expressed in grams per cubic centimeter. Microsphere quantification consisted of counting three times the number of microspheres in the ocular grid at 20× magnification, for each of the three samples. All tracer concentrations were standardized for homogenizing graph scales.

2.3. Data treatment

Statistical computations were performed with Statistica 5.1 (Ed. 97, StatSoft, Tulsa, OK, USA), using two-way ANOVA additive models, followed by a post hoc test (least significant difference (LSD) test). The assumption check (normality and homoscedasticity of the error terms), based on residue analysis, was done both graphically and using ad hoc tests (Kolmogorov–Smirnov goodness of fit test and Levene test). When assumptions were not fulfilled, we used a BOX-COX data transformation. Significance of the observed effects was assessed at the $P<0.05$ level.

3. Results

Physicochemical measurements in the water column during the 56-day experiment showed no significant differences between the three experimental treatments with respect to temperature (19.8 ± 0.1 °C), pH (8.4 ± 0.2), and dissolved oxygen concentration ($8.1\pm0.3\,mg\,L^{-1}$). Significant differences were observed in turbidity measured at the end of the renewal cycles: the mean values for all measurements during the 56 days were 10.3 ± 1.3 FTU for the {+Cd+Tub} and {−Cd+Tub} treatments and 0.2 ± 0.1 FTU for the {+Cd−Tub} treatment.

Average Cd concentrations determined from the whole set of data measured in the unfiltered water samples collected at the beginning and the end of each renewal cycle, during the 56 days of the experiment, were 13.9 and $18.3\,\mu g\,L^{-1}$ for the two contaminated treatments {+Cd+Tub} and {+Cd−Tub}, respectively. Mean dissolved Cd concentrations represented 86% of total Cd concentrations for the {+Cd+Tub} treatment and 98.4% for the {+Cd−Tub} treatment. When no Cd was added to the EUs ({−Cd+Tub} treatment), all measurements in the water samples were below the detection limit ($0.1\,\mu g\,L^{-1}$).

For the {+Cd+Tub} treatment, the temporal changes in vertical Cd distribution in the dissolved pore-water (<0.2 μm) and particulate fractions of the six sediment layers are shown in Fig. 2A, after 7, 21, and 56 days. A marked increase in Cd accumulation was observed in the different layers up to 3–5 cm, with a strong correlation with exposure duration. After 56 days, Cd concentrations in the first layer (0–0.5 cm) reached $3.1\pm0.4\,\mu g\,L^{-1}$ for dissolved Cd and $20.9\pm0.6\,\mu g\,g^{-1}$ dry wt. for particulate Cd. In the contaminated units without worms ({+Cd−Tub} treatment), a significant increase in Cd concentrations was observed only in the sediment top layer (Fig. 2B); maximal values were $5.5\pm0.7\,\mu g\,L^{-1}$ in the dissolved pore-water fraction and $39.8\pm13.4\,\mu g\,g^{-1}$ dry wt. in the particulate fraction at the end of the experiment. Note that in the absence of Cd contamination ({−Cd+Tub} treatment), Cd concentrations in the pore water were systematically below the detection limit ($0.1\,\mu g\,L^{-1}$); corresponding measurements in the particulate fraction did not differ significantly from the background level ($[Cd]=0.75\pm0.12\,\mu g\,g^{-1}$ dry wt.). The amount of Cd transferred from the water to the sediment was

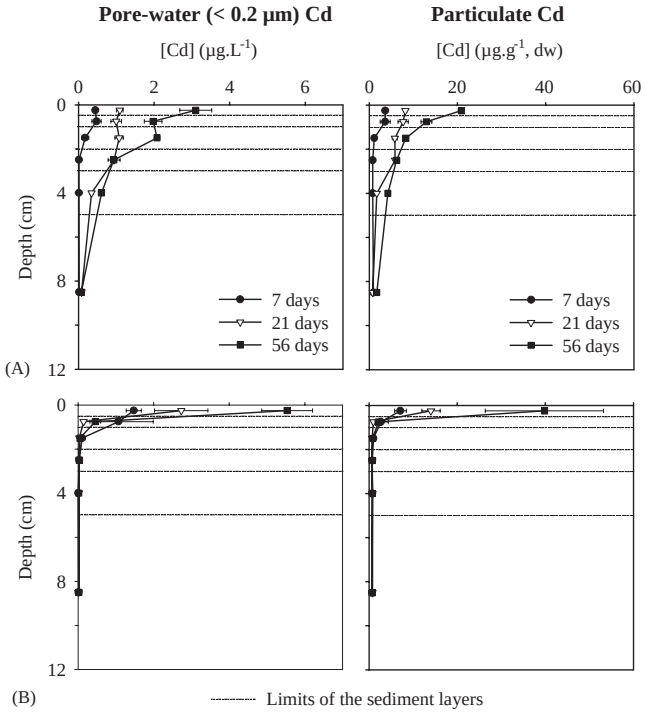

Fig. 2. Vertical profiles of cadmium concentrations measured in the pore-water (<0.2 µm) and particulate fractions of the sediment, after 7, 21, and 56 days: (A) {+Cd+Tub}, water column contaminated with cadmium and tubificid worms added to the sediment; and (B) {+Cd−Tub}, cadmium in the water column and no worms added to the sediment. Means±SD ($n=3$).

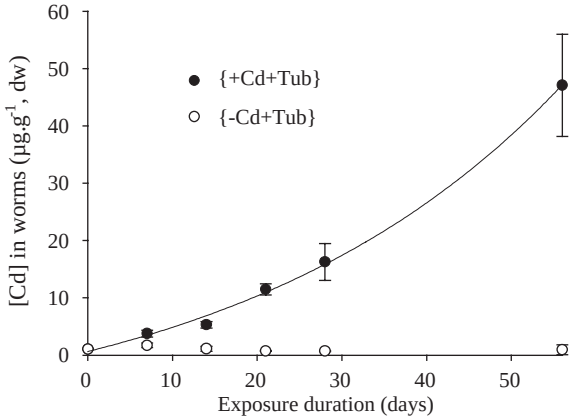

Fig. 3. Evolution of cadmium concentrations in worms during the 56-day exposure to the two experimental treatments: {−Cd+Tub}, no cadmium in the water column and tubificid worms added to the sediment; {+Cd+Tub}, water column contaminated with cadmium and tubificid worms added to the sediment. Means±SD ($n=3$). Analysis of data shows significant differences between the two experimental treatments for the five exposure durations ($P<0.05$). Regression model for {+Cd+Tub} treatment: [Cd] = −13.74 + 14.38 × exp(0.026 × time), $R^2=0.998$, $P=0.0001$.

determined from the average Cd concentrations measured in the different sediment layers, after correction for background Cd. After 56 days, the total net increase in Cd in the sediment was significantly different between the worm-free and worm-present units, at 318±103 and 671±21 µg, respectively.

The penetration depth of oxygen in the sediment remained similar in the presence or absence of worms: the concentration of O_2 decreased with depth, from values close to saturation just above the sediment–water interface to zero between 3 and 5 mm below this interface. Deeper dissolved O_2 due to bioirrigation was not observed.

Results relating to Cd bioaccumulation in tubificid worms are illustrated in Fig. 3. In the uncontaminated EUs ({−Cd+Tub} treatment), average Cd concentrations did not significantly differ from the background level measured at time zero: 1.0±0.1 µg Cd g^{-1} dry wt. When Cd was added to the water column ({+Cd+Tub} treatment), bioaccumulation in worms increased exponentially during the 56-day experiment, with a maximal value of 47.1±8.9 µg Cd g^{-1} dry wt. which is about 50 times higher than the background level. Data treatment based on a two-way ANOVA followed by an LSD test showed a significant difference between the Cd concentrations in worms from the two experimental treatments (with Cd in the water column and without) from 7 days.

Luminophore and microsphere vertical profiles from uncontaminated and contaminated units where tubificids were added ({−Cd+Tub} and {+Cd+Tub} treatments) are shown in Figs. 4A and B (two replicates/treatment), after 7, 14, and 21 days. Luminophore profiles show an increase in the depth location of the maximal concentration. This tracer subduction gives evidence of sediment bioadvective processes induced by conveyor feeding organisms with sediment ingestion at depth and fecal pellet accumulation at the sediment surface. As early as 7 days after the tracers were added, no luminophores were detected in the surface layer (0–0.5 cm); after 21 days, more than 80% of the tracer loads initially deposited at the sediment surface were present in the 3–5-cm layer. Microspheres undergo similar bioadvection but approximately 25% of the tracer load reappears in the surface layer after 7 days. In units without bioturbation ({+Cd−Tub}), all particulate tracers were found in the top sediment layer (0–0.5 cm) at the end of the experiment.

Tracer profiles were simulated using the bioadvection–biodiffusion model (Officier and Lynch, 1982; Gerino et al., 1994). This model was applied under non-steady-state treatments after a pulse input of tracers at the sediment surface at the beginning of the experiment:

$$\frac{\delta C}{\delta t} = D_b \frac{\delta^2 C}{\delta z^2} - V \frac{\delta C}{\delta z},$$

where t is the time, z the depth, and C the tracer concentration. This model allows the estimation of suitable values for the two parameters V (the bioadvective rate that quantifies the burial velocity of tracers)

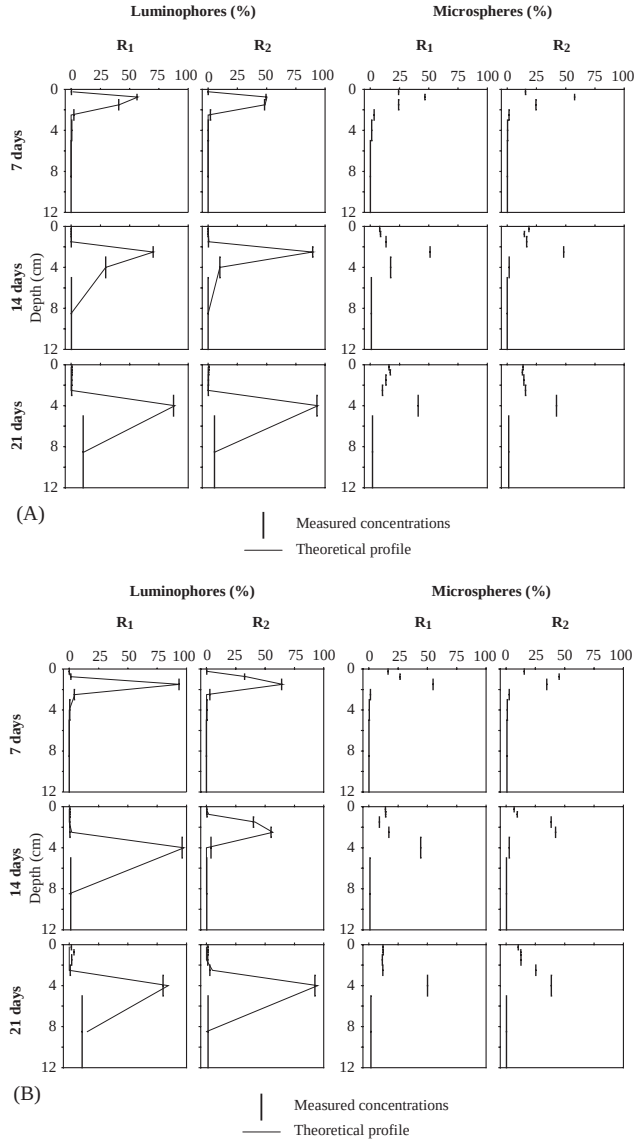

Fig. 4. Vertical profiles of particulate fluorescent tracers in the sediment—luminophores (63–315 μm) and microspheres (1 μm)—after 7, 14, and 21 days, for the two experimental treatments: (A) {+Cd+Tub}, water column contaminated with cadmium and tubificid worms added to the sediment; (B) {−Cd+Tub}, no cadmium in the water column and tubificid worms added to the sediment. *R*, replicate.

and D_b (the biodiffusive rate that quantifies omnidirectional dispersion).

The model equation was solved with initial conditions $C(z, t=0)=1$ for $z \in [0; 0.3]$; $C(z, t=0)=0$ for $z>0.3$ cm; and boundary conditions $C(z \to +\infty, t)=0$ and $VC_{(z,t)} - D_b(\delta C_{(z,t)}/\delta z)=0$ at $z=0$. The analytical solution was provided by Officier and Lynch (1982):

$$C_{(z,t)} = \frac{1}{\sqrt{\pi D_b t}} \exp\left[-\frac{(z-Vt)^2}{4D_b t}\right] - \frac{V}{2D_b} \exp\left[\frac{Vz}{D_b}\right] \operatorname{erfc}\left[\frac{z+Vt}{\sqrt{4D_b t}}\right].$$

Estimation of bioadvection and biodiffusion coefficients in our experiment was performed by fitting the theoretical concentrations to the measured luminophore concentrations in the different sediment layers, with a least-squares procedure. Two replicates and mean values of the two coefficients are summarized in Table 3 for the {+Cd+Tub} and {−Cd+Tub} treatments. As the present model is not suitable for simulating reingestion when tracers reach the ingestion zone by advection processes, data obtained with microsphere distributions were not used for bioturbation coefficient determination. After 21 days, it is assumed that the luminophores could have reached the lower limit of the ingestion zone, so estimation of advection rate with a longer experimental duration may be underestimated. The mean bioadvective rate, V, varies between 74 and 87 cm year^{-1} for the uncontaminated treatment, and between 55 and 105 cm year^{-1} when Cd was added to the water column. The corresponding mean biodiffusive rates, D_b, vary between 3.0 and 3.1 cm^2 year^{-1} for the {−Cd+Tub} treatment and between 2.5 and 3.1 cm^2 year^{-1} for the {+Cd+Tub} treatment. Data treatment based on a two-way ANOVA revealed no significant effect of exposure treatment (+Cd or −Cd) or of exposure duration (7, 14, and 21 days) on the two bioturbation coefficients. Note that for the biodiffusive rate, the probability calculated for the effect of exposure treatment equals 0.05, significance limit with a threshold of 5%.

4. Discussion

This experimental approach to cadmium transfer from the water column to the sediment compartment under the effects of bioturbation via tubificid worms shows marked and significant metal accumulation within the inhabited sediment zone. This accumulation occurs in both the particulate and pore-water fractions. The Cd loads in the sediment result from Cd fluxes through the sediment–water interface, driven by diffusion processes alone in the worm-free treatment and superimposition of bioturbation and diffusion processes in the case of worm-present units. Tubificids introduce Cd to deeper horizons: the metal scavenging was twice as efficient in the bioturbated sediment because particles and Cd adsorption sites were constantly renewed at the sediment–water interface (Ciutat, 2003). This result is in agreement with previous works on other metals, such as zinc (Soster et al., 1992). Other bioturbators, such as meiofauna, also have significant effects on Cd partitioning in muddy sediments, with larger amounts of Cd in the pore waters in the presence of benthic harpacticoid copepods or foraminiferans (Green and Chandler, 1994).

Cd accumulation in tubificids during the 56-day exposure reveals high metal concentrations, with

Table 3
Bioadvective rates (V) and biodiffusive rates (D_b) estimated by fitting the luminophores profiles after 7, 14, and 21 days for the two experimental treatments: {+Cd+Tub}, Cd in the water column and worms in the sediment and {−Cd+Tub}, no Cd in the water column and worms in the sediment[a]

Treatment	Time (days)	V (cm year^{-1})			D_b (cm^2 year^{-1})		
		R_1	R_2	Mean	R_1	R_2	Mean
{+Cd+Tub}	7	54	56	55	2.5	2.4	2.5
	14	81	76	78	2.7	2.7	2.7
	21	105	104	105	3.1	3.1	3.1
{−Cd+Tub}	7	87	62	74	2.7	3.3	3.0
	14	98	53	75	2.8	3.3	3.0
	21	106	68	87	3.2	3.1	3.1

[a] R, replicates.

maximal values close to 50 μg Cd g^{-1} dry wt. at the end of the experiment. As worms were not depurated before the metal was measured, part of the Cd accumulated at the whole-organism level may derive from metal present in the sediment within the digestive tract. After 56 days, the maximal Cd concentration measured in the sediment from the {+Cd+Tub} treatment was 20.9 μg g^{-1} dry wt, in the superficial layer (0–0.5 cm). The average metal concentration up to 5 cm, estimated from the Cd concentrations measured in the different sediment layers at the end of the experiment and taking into account differences in thickness and hence in weight, is about 8 μg g^{-1} dry wt. So, in both cases, accumulation levels in the sediment are evidently less than the Cd concentrations measured in the organisms at the end of the experiment. A large proportion of metal is therefore bioccumulated in the worms, with this bioaccumulation including adsorption sites at the ''organism/surrounding environment'' interfaces and sequestration within the tissue and cell compartments. We must stress that no significant amount of bioaccumulation was observed in the worms collected after 56 days in the control EUs ({−Cd+Tub} treatment).

Under our experimental treatments, the tubificids can accumulate Cd via the direct exposure route, from the metal present in the water column and/or in the pore water and, at the same time, via ingested sediment (trophic route). The posterior part of these burrowing organisms is in more or less permanent contact with the water column, mainly for breathing purposes. Indeed, respiration is based on absorption by diffusion of the oxygen present in the water through the epiderm covering the abdomen, with transfers facilitated by the undulations of the hind part of the body. The procedure we adopted for contamination of the EUs during the 56-day exposure period, based on renewal of the water column on a daily basis, enabled us to maintain a Cd concentration between 20 and 13.9 μg L^{-1}, 86% of the metal present being in the dissolved form (<0.2 μm). Under these conditions, the posterior part of the worms, in direct contact with the water column, represents a significant exposure route, given the relatively high Cd concentration in the water in a bioavailable form. Several studies have shown that metals, especially cadmium, can accumulate in oligochaete worms via epidermal uptake: results from Back (1990) showed that tubificids exposed to contaminated water accumulate Cd in the tegument of the posterior part of the body; Bouché et al. (2000) also revealed a high level of Cd accumulation in the epiderm of *T. tubifex* worms maintained in contaminated water. However, in these experimental studies, organisms were maintained in water only, in treatments that were very different from their normal lifestyle. It can be postulated that a metal transfer occurs through this barrier, thus giving access to the coelomic compartment, followed by accumulation in the internal compartments of the worms.

The tubificids can also accumulate Cd from the metal present in the pore water via the part of the body buried in the sediment. Cd vertical profiles in the dissolved phase of the pore water suggest a significant increase in metal concentrations over time, as far as the 3–5-cm layer, but the concentrations measured in the sediment superficial layer for the different sampling periods (0.5 μg L^{-1} after 7 days to 3 μg L^{-1} after 56 days) are much lower than those that are maintained in the water column (17 μg L^{-1} on average during the 56 days). The galleries that are burrowed out by the worms are not irrigated by water currents from the overlying water column (McCall and Fisher, 1980), as is observed with burrowing mayfly nymphs, for example (Matisoff, 1995). Therefore, exposure conditions are very closely dependent on the depth in the sediment compartment. The mucous layer around polychaete or oligochaete external cuticules is known as an efficient ligand site for several metals present as cationic species in the dissolved water phase (Whitley, 1967; Fleming and Richards, 1981; Dhainaut-Courtois et al., 1988; Back, 1990; Bouché et al., 2000).

Vertical profiles of Cd associated with sediment particles exhibit the same changes with time as vertical profiles of Cd in pore water (Figs. 2A and B). The sediment ingested by the worms in the surface layers, to a depth of about 5 cm, is therefore a potential source of contamination via the trophic route. Cd concentrations in worms are very much higher than those measured in the sediment particulate phase, ranging from 20.9 $\mu g\,g^{-1}$ dry wt. in the layer 0–0.5 cm to 4.2 $\mu g\,g^{-1}$ dry wt. in the layer 3–5 cm at 56 days. Bervoets et al. (1997) analyzed Cd accumulation in tubificids collected in the field from contaminated sites: their results showed that metal levels in worms were correlated mainly with the easily reducible cadmium concentration in sediment (Cd associated with Mn oxides), without significant correlations with Cd concentrations in pore-water samples. Sager and Pucsko (1991), on the contrary, found no significant relationships between Cd accumulated in tubificids and Cd concentrations from either pore water or total sediment. Warren et al. (1998) showed in an in situ experiment that tubificids exposed to Cd in both sediment and overlying water accumulated most of the metal from the sediment. These results from the literature are in agreement with experimental studies on other benthic species, such as oligochaetes and insect larvae (Hare, 1992; Hare et al., 2001) and marine annelids (Luoma, 1983).

The changes in the vertical distribution of tracers in the EUs with bioturbation reveal information on the location and depth range of the ingestion zone. Tubificid batches added to the EUs at the beginning of our experiment consisted of three species with organisms of different ages and sizes, which could feed at various depths. Microspheres occur at the sediment surface after 7 days when all luminophores were subducted by bioadvection (Figs. 4A and B). This fact is interpreted as ingestion of microspheres in the tracer accumulation zone over the same time period and egestion at the sediment surface via the fecal pellets. Luminophore particles are coarse enough not to be ingested by the majority of tubificids used in this experiment (Juget, 1978); microspheres were small enough ($\phi = 1\,\mu m$) to be ingested with sediment particles by all worm species and sizes, even the smallest (Juget, 1978; Rodriguez et al., 2001). The occurrence of microspheres at the sediment surface when luminophore maximum depth reached 3 cm indicates that the upper limit of the ingestion zone is in the top 3 cm of sediment. These results are in agreement with those published by Robbins et al. (1979): when a thin layer of clay particles labeled with ^{137}Cs entered the feeding zone of the tubificid populations, the radiotracer reappeared at the sediment surface; with increasing time, the ^{137}Cs activity tended toward a uniform distribution in sediment over the upper 6 cm and decreased exponentially below to undetectable levels under 9 cm. The heterogeneity in the sizes of tubificids used for this experiment could explain the progressive homogenization of microsphere vertical distribution between the sediment surface and the maximal ingestion zone. Under our experimental treatments, worms are able to ingest almost the totality of particles of the EUs (from the Garonne River sediment), which consists of 94% of particles smaller than 63 μm (Table 1), apart from the coarse luminophores. Because advection velocity is controlled by the sediment ingestion rate, the constant vertical velocities over time reveal homogeneity of the feeding rates during these periods. With longer experimental period, the tracer input should stop its vertical migration once it reaches the bottom of the ingestion zone. As no slowdown of bioadvection velocity was noted, it is concluded that luminophores did not reach the maximum depth of the ingestion zone after 21 days. At this time, the position of luminophores in the layer 3–5 cm indicates that the lower limit of the ingestion zone should be deeper than 5 cm.

The marked exponential trend for Cd bioaccumulation in the tubificids as a function of exposure period (Fig. 3) can be associated with the increase in metal concentrations in the sediment layers, both in the pore water and in the particulate phase (Fig. 2A). Mean Cd concentrations in the whole sediment compartment were calculated for both pore water and particulate Cd (sum of particulate or pore-water Cd burdens in each of the six sediment layers/total wt. of sediment or pore water) and for each of the five exposure periods studied. Both particulate Cd concentrations and pore-water Cd concentrations are in very close correlation with the corresponding concentrations in the organisms ($R^2 = 0.95$ for the particulate fraction and 0.87 for the dissolved pore-water fraction), whereas Cd concentrations in the water column remained practically constant during the entire exposure period. In these treatments, we can hypothesize that a significant amount of Cd bioaccumulation in the organisms derives from the top 5-cm layer of the sediment, which is Cd-enriched as a result of bioturbation. Note that when no worms are present ({+Cd−Tub} treatment), only the first few millimeters at the sediment surface are enriched in metal after 56 days of exposure.

Cd bioaccumulation in tubificids leads to an average concentration of $47.1 \pm 8.9\,\mu g\,Cd\,g^{-1}$ dry wt. after 56 days, which is about 50 times higher than the background level. Such concentrations may have toxic effects on worms. Some biological parameters, based on various biological functions, have been used to reveal and to estimate impacts on tubificids: for example the retraction of the posterior end in the sediment in response to metallic contaminants (Leynen et al., 1999). In our study, we selected bioturbation as a functional parameter to investigate the toxic effects of Cd. All tracer movements recorded in presence of

worms within the EUs ({−Cd + Tub} and {+Cd + Tub} treatments) were directly linked to worm's activity, as tracer distribution in fauna-free units ({+Cd−Tub} treatment) indicates no perturbation. Statistical comparison (two-way ANOVA) between bioadvection (V) and biodiffusion (D_b) rates indicates no significant differences between the contaminated and uncontaminated treatments nor between the three exposure durations (7, 14, and 21 days). Neither were differences observed between the microsphere profiles for the two treatments {−Cd + Tub} and {+Cd + Tub}. The non-significant effects of Cd exposure on bioturbation parameters also provide evidence that worm activities, like feeding and defecating, are not affected by the exposure treatments used for this experiment or by the metal accumulation levels observed in the organisms. It should, however, be stressed that the P value of the effect of Cd on the D_b coefficient was equal to the α risk (0.05). So Cd could have a significant effect on sediment homogenization by worm activity if the exposure treatments via the contaminated water column were a little higher and/or after a longer exposure period. Such effects would be in agreement with reduced sediment-reworking rates observed for several marine polychaetes (*Arenicola marina*, *Arenicola cristata*) in polluted sites (Lee and Swartz, 1980).

This absence of any significant effects of metal exposure on the bioturbation activity of tubificids in our experimental treatments, despite the bioaccumulation levels observed, may be linked to the existence of detoxification processes in worms. Several authors have described resistance to high metal accumulation levels for other oligochaete species, based on efficient detoxification mechanisms, such as intracellular compartmentalization, involving lysosomes, spherocrystals, and/or metal-containing granules (Brown, 1982; Dhainaut-Courtois et al., 1991); metal inactivation by binding to metalloproteins (Dallinger, 1994) or metallothionein-like proteins (Wallace et al., 1998; Gillis et al., 2002); sequestration in sulfur-rich granules located mainly in chloragocytes, body wall, and gut wall cells (Klerks and Bartholomew, 1991).

5. Conclusion

This experimental approach to Cd transfer from the water column into the benthic compartment and impact on tubificid activity shows that bioturbation was not significantly affected despite high levels of Cd accumulation in worms, suggesting that the levels and/or rates of Cd exposure were insufficient to elicit a significant decrease in function or the existence of detoxification/sequestration mechanisms. However, if the worms are protected against the toxic effects of the metal, they represent an important potential contamination source to their predators (insect larvae, fish, etc.). On the other hand, tubificids significantly increase Cd accumulation in the sediment compartment; they also markedly increase the thickness of the Cd-enriched layers within the biologically mixed zone. The present demonstration of intensive worm activity under Cd contamination pressure indicates that bioturbation is able to play a significant role within the biogeochemical cycle of the metal in contaminated aquatic systems.

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