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Cadmium Distribution in Sediment and the Lugworm *Arenicola marina* in a Low Concentration Exposure Experiment

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In the central and southern North Sea, and in the Dutch coastal zone, total cadmium (Cd) concentrations in water are 0.02 ± 0.01 µg/L and 0.06 ± 0.02 µg/L, respectively. Cadmium in the estuarine waters of the Dutch Wadden Sea varied from 0.03 ± 0.01 µg/L in the western part to 0.08 ± 0.03 µg/L in the eastern part (van Zeijl et al. 1994). In whole sediment, the Cd background concentration for the Wadden Sea is 0.5 ± 0.01 µg/g dry weight (dw), whereas the reference concentration is 0.08 ± 0.02 µg/g dw (Anon 1994). The concentrations of total-Cd in surface bulk sediments (0-2 cm) of the central North Sea (Oyster Grounds), and of intertidal mud-flats in the western Wadden Sea varied from 0.05 to 0.15 µg/g dw and from 0.13 to 0.46 µg/g dw, respectively (calculated from Kahn et al. 1992). These concentration ranges match the reference Cd concentration for Wadden Sea whole sediment (0.5 ± 0.01 µg/g dw; Anon 1994). Cadmium concentrations in surface sediments of the Dutch coastal zone and estuaries are only slightly elevated compared to the 0.2 µg/g dw, considered as the background concentration in pristine areas, but well below the level of 10 µg/g dw at heavily contaminated sites (Bryan and Langston 1992).

The relationship between cadmium levels in sediments and the biota is uncertain and contradictory. Bryan and Hummerstone (1973) described already 2 decades ago a linear relationship between cadmium concentrations in the polychaete worm *Nereis diversicolor* and in surface sediments. However, in the closely related polychaete *Nereis virens*, cadmium is absorbed from the interstitial water rather than from sediment particles (Ray et al. 1980). Also, more recent data indicate that uptake from sediment fractions either does not occur or the availability of sediment-bound metals to benthic biota is very restricted (Samant et al. 1990; Bryan and Langston 1992). In general, macrobenthic organisms accumulate cadmium from the water by absorption and diffusion across the body surface and by ingestion of food and particulates. Cadmium concentrations in blood, intestine and body-wall of the polychaete worm *Arenicola marina* from the field varied from 5-25 ng/mL, 0.2-3.6 µg/g dw, and 0.5-1.5 µg/g dw, respectively (Everaarts *unpublished data*) .

The present experimental laboratory study reports on the distribution of cadmium in the sediment column, and the uptake in the blood/coelomic fluid, intestine and body-wall of lugworms at low cadmium concentration exposure. The aim of the study was to determine possible interaction between the vertical distribution of sediment-bound cadmium and the bioturbating activity of lugworms.

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MATERIALS AND METHODS

Specimens of the polychaete annelid *Arenicola marina* (L.) were collected from the intertidal mud-flat area of the western Dutch Wadden Sea (Balgzand; Fig. 1), and washed free of adhering sediment and other particles before being used in the experiment. The experiment was carried out applying a continuous flow-through filtered seawater system with tidal movements, in a 15 °C (± 0.5 °C) temperature controlled room, with 12:12 hr lightdark regime. The seawater had a salinity of 28 ‰, a pH of 8.0 (± 0.05) and was oxygen saturated. Six aquaria, each divided into two parts by means of glass partitioning, contained an approx. 18-cm deep layer of homogeneously mixed, wet-sieved (2-mm sieve removing coarse material) sediment from the natural habitat of the lugworm. Ten specimens were placed in each compartment. Cadmium was dosed to the clean seawater as the chloride salt to achieve a nominal concentration of 0.25 µg/L (250 ng/L), which is about 10 times the background concentration of the western Wadden Sea. Two aquaria were used as controls. The experiment lasted 36 days, with intermittent Cd dosing from days 0 to 5 and from days 15 to 25. The animals were not fed during the course of the experiment; no significant wet- or dry-weight loss was measured, and no indications of starvation or decreasing bioturbating activity of the lugworms were observed.

Water, sediment and biota were sampled on days 0, 1, 3, 6, 12, 15, 18, 24, 30 and 36. On each day, only one of the twelve compartments was sacrificed for sampling of sediment and lugworms. Five sediment cores (16 cm, ∅ 1.6 cm) were taken, stored at -20 °C and sliced into two (upper) layers of 1 cm and seven layers of 2 cm. The corresponding slices of the five cores were pooled and the percentage of the fine fraction (grain-size < 63 µm) determined. The 10 specimens of *A. marina* present in this particular compartment were removed and coelomic fluid was collected in a tube after making a longitudinal slit in the anterior-dorsal part of the body-wall; blood was sampled by puncturing the protruding blood vessel. Particulate matter in the blood/coelomic fluid was removed by centrifugation at 2000g for 15 min. The intestine was dissected and separated from the body-wall.

Water samples (500 mL) taken at the aquarium-outflow were acidified with HCl, dissolved cadmium was complexed with a mixture of 1% ammonium-pyrrolidine-dithiocarbonate (APDC) and 1% diethyl-ammonium-diethyl-ditbiocarbonate (DDDC), and extracted into methyl-isobutyl-ketone (MIBK). After phase separation, the metal-complexes in the MIBK were decomposed by addition of concentrated nitric acid. Cadmium was back extracted and 50-fold pre-concentrated into 10 mL double-distilled water, and stored in teflon tubes, (Danielsson et al. 1978). Cadmium concentrations in the sediment were determined after decomposition of about 500 mg measured to the nearest 0.1 mg with a mixture of 1 mL of aqua regia (1 part HNO_3 + 3 parts HCl) and 5 mL HF in a teflon destruction bomb (Loring and Rantala 1977) for 2 hr at 120 °C. Subsequently, the decomposed samples were diluted with 30 mL saturated H_3BO_3 solution and made up to 50 mL with ultra clean water. From the blood/coelomic fluid about 0.4 mL, and from the dried intestine and body-wall of the lugworms about 400 mg, were decomposed with 3 mL of 65% HNO_3 in a teflon bomb for 2 hr at 120 °C (Paus 1972). The decomposed samples were transferred quantitatively to polypropylene tubes and diluted with ultra-clean water to 10 mL. For the analysis of cadmium flameless atomic absorption spectrophotometry (AAS) was applied, using a heated graphite-furnace atomizer (HGA500) coupled to a Perkin Elmer 5000 AAS. To calculate the cadmium concentration, the standard addition method was applied and calibration curves made on standard solutions of cadmium. The AAS measurements were carried out in duplicate on each decomposed sample. All Cd levels were far above the

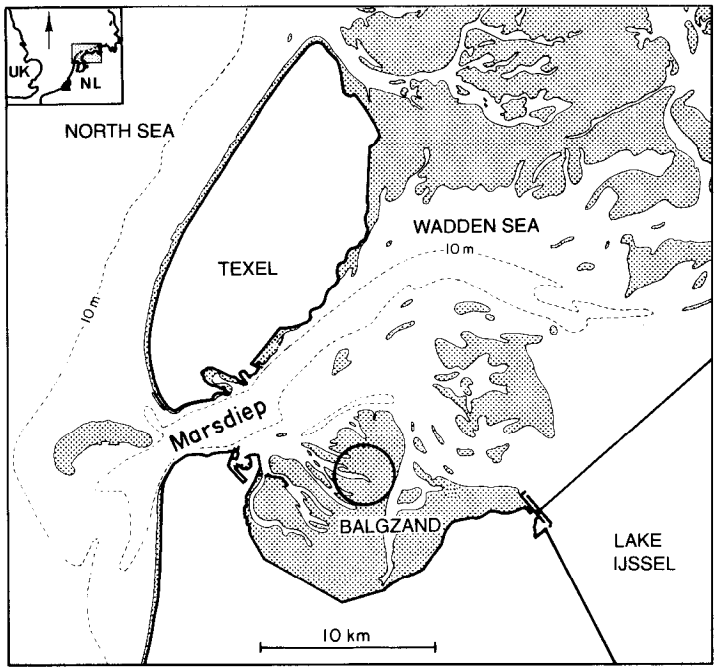

Figure 1. Intertidal mud-flat areas in the western Wadden Sea. Specimens of the polychaete annelid *Arenicola marina* (lugworm) and its habitat sediment were collected from the Balgzand; sampling area indicated by a circle.

detection limits for water (10 ng/L), sediment (0.15 ng/g) and biological material (0.20 ng/g); recovery of the standards varied between 92 and 98 %.

RESULTS AND DISCUSSION

The courses of cadmium concentrations in the several compartments of the experimental microcosm, being the waterphase, the sediment and the different body compartments of the lugworms are shown in Figure 2. Within 6 hr after the start of the exposure, the actual concentration of total Cd in the water approximated the nominal concentration of 250 ng/L (Fig. 2a). This 10-fold increase in water Cd level was reflected almost simultaneously in the surface sediment Cd concentration (Fig. 2b), whereas in deeper sediment layers a significant delay in the increase of the Cd concentration was measured (Fig. 2c). Also, the Cd concentration increased within the first 12 hr of exposure in the different body compartments of the lugworms. The blood/koelomic fluid Cd level (Fig. 2d) showed almost a linear increase from 10 to 48 ng/mL during the first 6-d exposure period, whereas in the intestine (Fig. 2e) Cd showed an initial strong increase. In the body wall, Cd increased from 0.35 to 0.65 µg/g dry weight immediately after exposure (Fig. 2f), and remained at the same level during the experiment. A gradual release of Cd from all compartments was established after the first 6-d exposure period, and total Cd levels in the water reached the reference (control) level after 15 days. The Cd concentration in the blood/coelomic fluid, the intestine and body-wall of the lugworms did not reach the reference levels before starting the second exposure period. The same pattern of increasing Cd concentration in all compartments of the microcosm occurred during the second exposure period, except in the

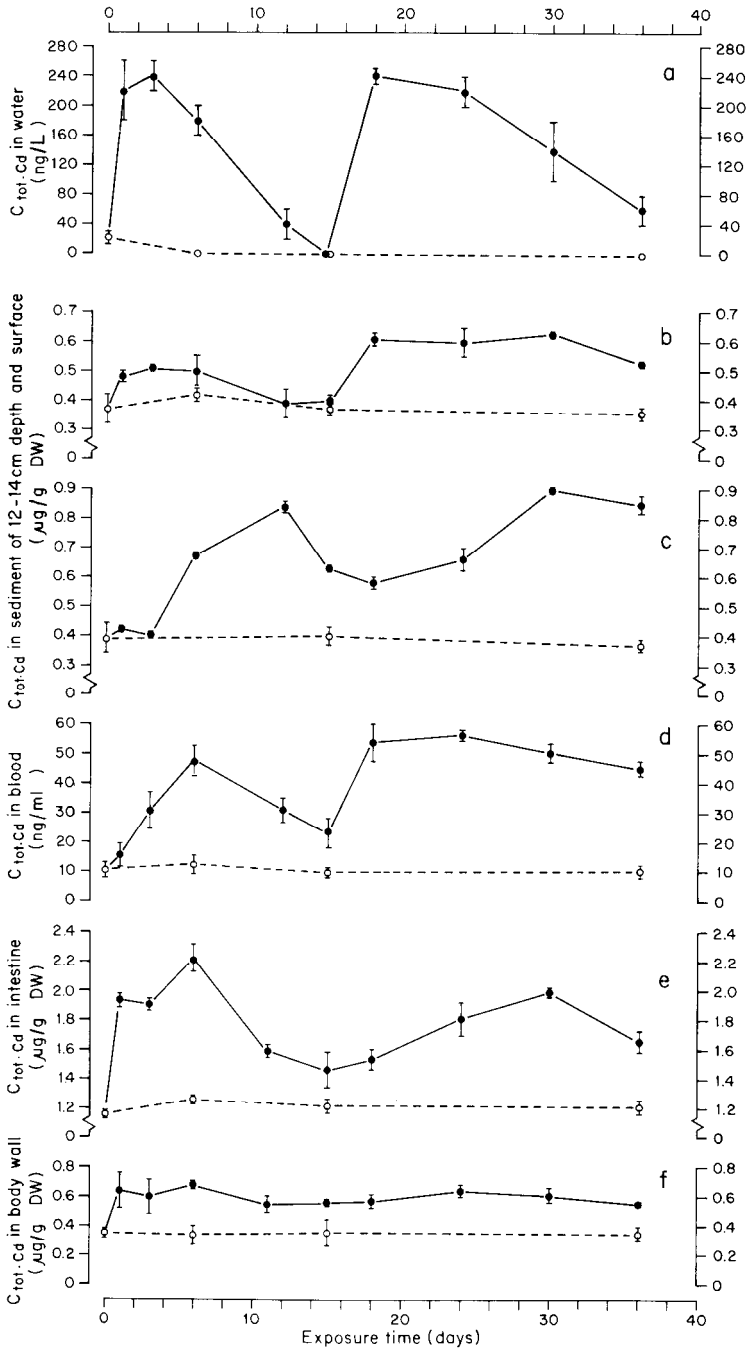

Figure 2. The cadmium concentration (from top to bottom) in [a] the waterphase (ng/L), in the whole sediment of [b] the surface layer (0-1 cm) and [c] a deeper layer (12-14 cm) (µg/g dry weight [dw]), and in [d] the blood/coelomic fluid (ng/mL), [e] the intestine and [f] the body-wall (µg/g dw) of the lugworms (*Arenicola marina*) intermittently exposed to the chloride salt of Cd, dosed at a nominal concentration of 0.25 µg/L (250 ng/L), on days 0 to 5, and 15 to 25. Dotted lines reflected the cadmium concentration in the controls.

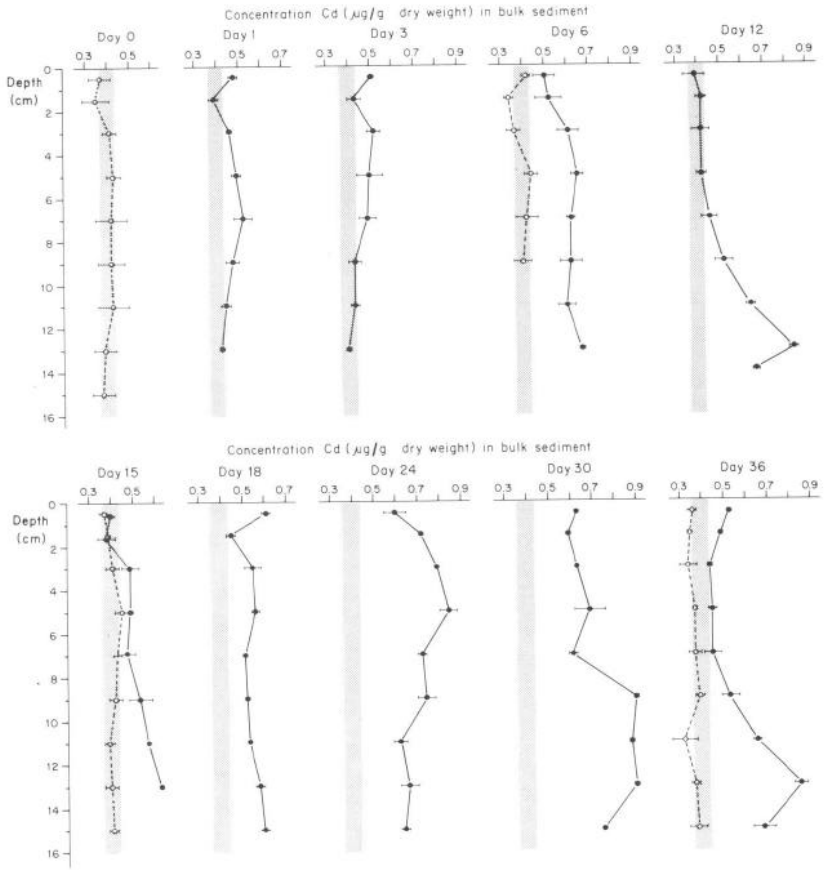

Figure 3. The distribution of cadmium in the sediment column after intermittent exposure to the chloride salt of Cd, dosed at a nominal concentration of 0.25 µg/L (250 ng/L). The hatched vertical bar represents the average Cd concentration (0.4 µg/g dry weight) and the standard deviation in the different sediment layers of the control aquaria, which was measured at days 0, 6, 15 and 36.

body-wall where no significant increase of Cd was measured. The delay of Cd increase in deeper sediment layers was obvious again. After stopping exposure at day 25, release of Cd from the blood, intestine and body-wall did not occur at the same rate or not at all to any extent, respectively. Even though the Cd concentration in the overlying waterphase decreased dramatically (almost to the reference level) during the 12 days after the second Cd exposure was stopped, the Cd concentration in the surface sediment remained the same (approx. 0.6 µg/g dry weight, which is a factor of 2 higher than the reference value of the control sediment), whereas Cd in the deeper sediment layers showed a delayed increase until 6 days after stopping exposure.

The distribution of Cd throughout the sediment column during the experiment is shown in Figure 3. During the first period of exposure (days 0 to 5) a gradual Cd increase was found in the sediment of depths up to 14 cm, which was first evident in the upper sediment layers (*cf:* measurements at day 1 and 3). After finishing the first exposure period and thus during the decrease of water Cd concentration, the Cd concentration in the upper layers

Table 1. The correlation coefficients (R) and p-values at 95% two-sided confidence interval, for the relationship (linear regression) of the cadmium concentration ($ng.mL^{-1}$) in the blood/coelomic fluid of lugworms *(Arenicola marina)* and in sediment layers from different depth (cm). ns = not significant.

	0-1 cm	1-2 cm	2-4 cm	4-6 cm	6-8 cm	8-10 cm	10-12 cm	12-14 cm	14-16 cm
R	0.806	0.803	0.732	0.733	0.684	0.663	0.598	0.577	0.744
p <	0.01	0.01	0.02	0.02	0.05	0.05	ns	ns	0.02

decreased, whereas in the deeper sediment layers Cd initially continued to increase (day 12). Towards the end of the depuration period Cd compared to the reference levels of the control sediments in the upper layers (to approx. 8 cm), but were still enhanced in the deeper layers (8 to 16 cm). During the second cycle of exposure, starting after sampling at day 15 until day 25, about the same dynamics of Cd concentrations were observed as compared to the first exposure period. The only difference observed was in the Cd concentration level, which was slightly higher during the second exposure period. Again, after stopping exposure at day 25, three phenomena were obvious: *(1)* delayed, Cd increase in the deeper layers (*cf:* day 30) *(2)* the depuration of Cd in the upper and middle sediment layers (2 to 8 cm), except the surface layer which showed enhanced Cd levels, and *(3)* the accumulation of Cd into the deeper layers (12 to 16 cm; *cf.* day 36) at the end of the experiment.

The Cd concentration in any of the body compartments of the lugworms did not statistically correlate (linear regression, 95% two-sided confidence interval, $p>0.05$) with Cd in the waterphase, due to the delayed Cd uptake as compared to the almost instantaneous Cd increase in the waterphase after the start of the experiment (*cf.* Fig. 2). However, at the 90% two-sided confidence interval ($p<0.1$), water-Cd correlated with Cd in the intestine and body-wall, indicating most likely a dependency of both water and sediment Cd level. A statistically significant correlation (linear regression) between Cd in the overlying waterphase and any particular sediment layer was found only for the relation water-surface sediment Cd ($R=0.714$, $N=10$, $p<0.02$). Considering Cd uptake in lugworms, significant correlations (linear regression) were established between the Cd concentration in the blood/coelomic fluid and various sediment layers (Table 1). A highly significant correlation existed only between Cd in the bottom sediment layer (which is the bottom of the J-shaped living tube of the lugworms) and Cd in the intestine ($R=0.920$, $N=6$, $p<0.005$) and in the body-wall ($R=0.883$, $N=6$, $p<0.005$). The strong relationship of Cd in the intestine with bottom sediment and the weak relationship with water and surface sediment might indicate uptake from sediment and interstitial water. The initial increase in Cd in the body-wall, reaching a plateau after the very first days of exposure, most likely reflects adsorption and diffusion processes. These data strongly demonstrated the relationship of sediment-biotic compartments. However, the data did not yet bear clear evidence of Cd uptake exclusively via the sediment. Also, the fact that Cd in overlying water correlated significantly with only the surface sediment (diffusion), did not exclude some evidence for Cd uptake in the biota via the waterphase. The data rather indicated the phenomenon of delayed vertical distribution of Cd in the sediment column due to water circulation through the J-shaped living tube of the lugworms in addition to the sediment reworking activity. The Cd concentrations in blood/coelomic fluid, intestine and body-wall

Table 2. The gram size distribution in the various layers of the sediment column, as represented by the percentage of the fine fraction (grain-size < 63 pm). Before start of the experiment samples of all twelve compartments of the aquaria were taken (N=12); after the experiment N depended on the day of sampling and varied from 3 to 12.

Sediment layer (depth in cm)	% fine fraction before start of the experiment	% fine fraction at the time of sampling
0-1	11.5 ± 2.4	9.1 ± 1.5
1-2	10.6 ± 2.5	8.4 ± 1.7
2-4	12.6 ± 2.7	10.2 ± 4.0
4-6	13.3 ± 6.2	11.4 ± 3.3
6-8	14.7 ± 3.9	12.2 ± 4.4
8-10	14.6 ± 5.2	12.0 ± 4.4
10-12	11.8 ± 3.6	12.4 ± 3.3
12-14	12.0 ± 1.9	12.6 ± 3.5
14-16	11.1 ± 4.8	13.8 ± 4.8

may then reflect Cd levels in the oxygen-rich water which moves through the J-shaped living tube. Benthic species, such as the bivalve mollusc *Scrobicularia plana, the* amphipod crustacean *Corophium volutator,* and the lugworm *Arenicola marina,* could mobilise the transuranic elements americium (^{241}Am) and plutonium (^{238}Pu) within the biodisturbed sediment layers (Miramand et al. 1982). Transfer occurred both via interstitial water and directly from sediment to species after ingestion.

During the course of the experiment the texture of the sediment, reflected in the percentage of the fine fraction (grain size < 63 µm), changed considerably throughout the column (Table 2). Before the start of the experiment the similar percentage of the fine fraction in all layers throughout the column reflected the homogeneity of the sediment. After the experiment, taking into account the different sampling times, a clear gradient in the fine fraction was observed. Evidently, the presence of lugworms had a great impact on the redistribution of fine material throughout the sediment column. Probably, bioturbating activity of the lugworms also influenced the sediment's physical/chemical composition, *e.g.*. affecting adsorption and desorption processes of dissolved metals (*cf.* Samant et al. 1990). The more so because of both the intermittent activity of the worms themselves and the tidal movements of the overlying water. Tidal rhythms in mixing surface sediment, thereby bringing particulate matter into the water column, were found to facilitate the uptake and accumulation of cadmium from both water and sediment by the crustacean *Mictyris longicarpus* (soldier crab) (Ahsanullah and Ying 1993). The redistribution of the fine sediment also implicated enhanced Cd concentration in the surface and deeper sediment layers, since the highest Cd concentrations were found in the fraction with a relative large surface area, which is the finest gram size fraction (*cf.* Everaarts and Fischer 1992). This observation compared with results described by Everaarts and Boere (1989) in a similar experiment which applied a 2000 times higher concentration in the waterphase (50 µg/L).

In summary, the phenomena in reference to the Cd distribution in the sediment column and the uptake and depuration in the lugworms indicated a dynamic interaction between the animals and their habitat. Evaluation of the present data strongly supported the following mechanisms of Cd distribution through the sediment column and uptake in the biotic compartments. *(1)* Diffusion of Cd from the overlying water to the surface layer of the

sediment occurred, and in deeper layers exchange to the sediment by adsorption of Cd from the oxygen-rich water pumped through the J-shaped dwelling-tube of the lugworms. *(2)* In case of bioactive reworking of the sediment by burrowing activity of the lugworms, Cd was mobilized from the sediment into the waterphase. *(3)* Apart from these processes, a net increase in sediment Cd was observed towards the end of each exposure period both in the surface and deepest layers of the sediment column. *(4)* Lugworms revealed very poor Cd regulation, since Cd interacted directly with the extracellular blood pigment and coelomic fluid, causing a linear uptake and depuration of Cd. *(5)* Uptake and depuration of Cd in the intestine was related to sediment (interstitial water) and oxygen-rich water pumped through the J-shaped living tube of *A. marina. (6)* Low Cd exposure caused rapid uptake in the body-wall, and accumulation of a factor 2 compared to reference Cd values.

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