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Cadmium sorption by bacteria and freshwater sediment

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SUMMARY

Sorption of cadmium by sediment bacteria and freshwater sediment was investigated using diffusion chambers to simulate the water–sediment interface. Diffusion chambers were constructed to provide two compartments separated by a dialysis membrane. Diffusion of cadmium across the membrane was monitored after pure cultures of sediment bacteria or lake sediments were added to the sediment side of a diffusion chamber. Cellular accumulation of cadmium by cadmium-sensitive and cadmium-resistant bacteria removed between 20% and 80% of the dissolved cadmium from the simulated water column and pore water. Cellular accumulation of cadmium was greatest for cadmium-sensitive isolates that were tested. Sediment with an intact microbial community sequestered 80% of the cadmium added to sediment, whereas autoclaved sediment retained 97% of the metal that was added. Addition of glucose to cadmium-amended sediment decreased retention of cadmium by untreated and autoclaved sediments, resulting in elevated concentrations of dissolved cadmium in the simulated water column.

INTRODUCTION

Although cadmium (Cd) has no demonstrable biological function, this heavy metal has received considerable attention due to its toxicity [6] and because it is a proven mutagen, carcinogen, and teratogen [4]. Freshwater sediments can serve as an important sink for Cd that reaches aquatic environments by either atmospheric distribution or point-source contamination [7]. Soluble Cd, similar to other toxic metals, can be removed from the water column or sediment pore water by complexing with organic [11] and inorganic components in sediment [9]. In addition, Cd may accumulate in sediments by absorption onto or transport into bacterial cells. Mackaskie and Dean have shown that Cd can comprise up to 8% of the dry weight of some bacterial cells that were exposed to the metal under laboratory conditions [5]. In a different study, cells of *Pseudomonas* species accumulated more Cd than did artificial sediments exposed to the metal under identical conditions [12].

Cadmium toxicity is usually correlated with concentrations of the dissolved metal. However, after binding to sediment particles, including sediment bacteria, Cd contamination can enter food chains as a consequence of protozoan grazing [8]. When these food chains lead to man, severe health effects have been noted [6]. Furthermore, sediment-bound Cd has the potential to be mobilized and released back into the water column if the physiochemical environment in the sediments or water column is altered [3]. In an attempt to model the partitioning of Cd between the water column and sediments in aquatic environments, and to study the role of sediment bacteria in the sorption of the metal in freshwater sediment, we have constructed practical, inexpensive diffusion chambers to simulate the diffusion of Cd across the water column-sediment interface. This study was designed to assess the role of intact lake sediment and selected Cd-sensitive and Cd-resistant lake sediment bacteria in removing Cd from the water column and sediment pore water. In addition, the effect of nutrient loading on the ability of freshwater sediments to sequester Cd was determined.

MATERIALS AND METHODS

Sample collection and selection of bacterial isolates

Contaminated sediments, averaging 20 ppm Cd, were collected with a Ponar dredge from the Cuyahoga River in Cleveland, OH. Cd-resistant and Cd-sensitive bacteria were obtained from sediments by streaking for isolation on plate count agar (PCA; Difco) amended with 0 or 60 ppm Cd added as $CdCl_2$. Pure cultures of each isolate were classified as Cd-resistant if they were capable of growth on PCA containing 60 ppm Cd and Cd-sensitive if no growth occurred at this metal concentration.

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Sediments for use in diffusion chamber experiments were collected from the Old Women Creek Estuary in Huron, OH. These sediments had a low organic carbon (C) content (1–6% C) and were comparatively Cd-free, containing a maximum of 2 ppm Cd. Before use in chamber studies, sediments were passed through a 100-mesh sieve to remove large sediment particles and debris such as twigs and leaves. Sieved sediments were stirred to produce a uniform mixture before aliquots were transferred to diffusion chambers. Sterile sediments for diffusion chamber experiments were prepared by four consecutive 90-min autoclavings alternated with 1-day incubation at ambient temperature.

Lake Erie water was collected at Edge Water Park in Cleveland, OH. Lake water was stored on ice until filter-sterilized through a 0.45-μm membrane filter. Filter-sterilized water was stored in polyethylene bottles at 4 °C in the dark until used in diffusion chamber experiments.

Diffusion chambers

Diffusion chambers were constructed of plexiglass as illustrated in Fig. 1. Each chamber was assembled with a Spectra/por 4 dialysis membrane (Spectrum Medical Industries) positioned to simulate the sediment–water interface by dividing each chamber into two compartments designated side A and side B. Side A was used to simulate the water column, and side B was used to simulate the sediment or sediment-pore water. The diffusion chambers, with the exception of the dialysis membranes, were sterilized using ethylene oxide and assembled aseptically. Dialysis membranes were prepared by boiling in a 2% sodium bicarbonate solution for 15 min, followed by 15 min of boiling in double distilled water (ddH_2O). Cadmium chloride stock solutions (50 ppm or 100 ppm Cd) and glucose stock solutions (0.25%) were sterilized by autoclaving.

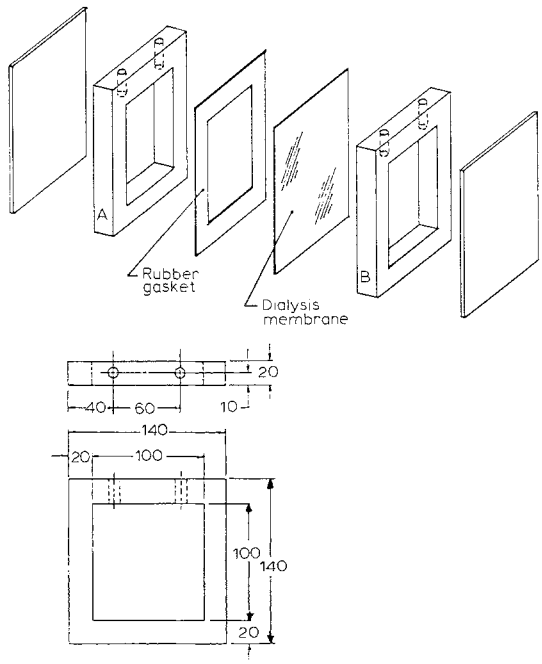

Fig. 1. Dialysis chamber assembly used to model the partitioning of Cd between the water column and sediment (all dimensions shown in mm).

Atomic absorption spectrophotometry

Cadmium concentrations were determined by direct aspiration atomic absorption spectrophotometric analysis (AAS) using a Perkin-Elmer (Norwalk, CT) model 403 atomic absorption spectrophotometer. All samples were analysed using an air-acetylene flame and wavelength of 228.8 nm. Quantification of the metal was achieved by comparison of sample concentrations with a standard curve prepared from commercially available AAS standard solutions (Fisher Scientific).

Cadmium accumulation in lake water by selected sediment bacteria

Pure cultures of three Cd-sensitive isolates (MC102, MC103, MC107) and three Cd-resistant isolates (MC215, MC315, MC312) were tested in the diffusion chambers to determine each isolate's ability to remove soluble Cd from lake water. Cells of each isolate were grown overnight in tryptone broth (Difco). Overnight cultures were concentrated by centrifugation and resuspended in filter-sterilized lake water to a final cell density of 65 Klett units. Side B of the diffusion chamber was filled with 200 ml of cell suspension, while Side A was filled with 200 ml of filter-sterilized lake water. Throughout these experiments, both sides A and B of the diffusion chambers were constantly mixed and aerated by continuously bubbling filter-sterilized air into both sides of the chambers. At time zero, Cd from a 100 ppm $CdCl_2$ stock solution was added to Side A to give a final concentration of 2 ppm Cd. Each diffusion chamber was incubated for approx. 22 h at ambient temperature (22–26 °C). The Cd concentration in both sides of the chamber was determined at approx. 1-h intervals during the first 5 h when the rate of Cd diffusion was greatest. During the remainder of each study, sampling was carried out at the time intervals indicated in the results. Samples from Side A were analysed directly for Cd by AAS. Individual 2-ml samples from Side B were prepared for Cd analysis by centrifuging at 13000 × g for 5 min to separate the cells from the suspending lake water. After centrifugation, the cell-free supernatant was decanted into a clean microfuge tube and analysed for Cd by AAS. Cell pellets from samples taken from Side B were resuspended and digested in 100 μl of

concentrated nitric acid for 1 h at 100 °C. After digestion, the volume of each sample was increased to the original volume (2 ml) with ddH_2O and analysed for Cd by AAS. To determine the viability of cells used in each experiment, serial dilutions of the cell suspensions were prepared in peptone water and plate counts were made using PCA at time 0 and again after 3, 7, and 22 h of incubation. Cell density and pH were both monitored each time samples were removed for Cd analysis. After each experiment, the dry weight of cells in the diffusion chamber was determined for use in calculation of the final level of Cd accumulated by cells of each isolate that was tested.

Partitioning of cadmium between water column and sediment

The potential for sediments to bind and retain Cd was tested using the diffusion chambers under the conditions listed in Table 1. For each condition tested, 2 g of sieved Old Women Creek sediment was suspended in 200 ml of ddH_2O or filter-sterilized lake water and added to the sediment side (B) of a diffusion chamber. 50 μg of Cd (1 ml of 50-ppm stock solution) were added daily to side B of each chamber during the 16 days each chamber was monitored. Samples (2 ml) for Cd analysis were taken from the simulated water column (side A) 24 h after Cd additions made on day 2, 4, 6, 8, 10, 12, 14, and 16. Each sample was acidified by the addition of 0.5 ml of concentrated nitric acid and analysed by AAS. Throughout the diffusion experiment, the sediments in Side B were mixed by a magnetic stir bar. Total colony forming units in Side B were enumerated at the beginning and the end of each experiment using PCA to prepare spread plates from serial dilutions.

RESULTS

Cadmium accumulation by selected bacteria

The capacity for Cd-sensitive and Cd-resistant sediment bacteria to remove Cd from lake water was determined using the diffusion chambers. In cell-free control chambers, during 22 h of incubation, Cd diffused from the simulated water column (side A) to the simulated pore water (side B) and established an equilibrium concentration of 1 ppm throughout the diffusion chamber (Fig. 2). When Cd-sensitive cells were added to the sediment side of diffusion chambers, the cellular accumulation of Cd reduced the equilibrium concentration of dissolved metal to 0.31, 0.50 and 0.48 ppm Cd for MC102, MC103, and MC107 cells, respectively (Fig. 3). The measurable reduction in dissolved Cd was accounted for by the cell-associated Cd that was measured in cell pellets obtained by centrifugation of samples from side B. After dissolved Cd had diffused to equilibrium, cell-associated Cd reached 61.0, 53.9, and 54.0 nmol Cd/mg cells (dry weight) for Cd-sensitive isolates, MC102, MC103, and MC107, respectively (Fig. 3). In contrast, cellular accumulation of Cd by the Cd-resistant isolates during the same incubation period reached a maximum of 34.0 nmol Cd/mg cells (dry weight) for cells of isolate MC215. Total Cd accumulated by MC315 and MC312 cells reached 18.6 and 8.5 nmol Cd/mg cell (dry weight), respectively (Fig. 4). The average Cd accumulation measured in cells of the three Cd-sensitive strains (56.3 nmol Cd/mg cell dry weight) was significantly greater than the average Cd accumulated by the Cd-resistant cells (20.4 nmol Cd/mg cell dry weight) that were tested (Table 2).

TABLE 1

Set-up of diffision chambers used to study partitioning of Cd by lake sediment

	Additions to simulated sediment and water column	
	Side B (sediment/pore water)	Side A (water column)
Chamber 1	200 ml sterile glucose solution (0.25%), Cadmium chloride solution (50 ppm Cd) (control)	200 ml sterile ddH_2O
Chamber 2	2 g autoclaved sediment, 200 ml sterile ddH_2O, Cadmium chloride solution (50 ppm Cd)	200 ml sterile ddH_2O
Chamber 3	2 g autoclaved sediment, 200 ml sterile glucose solution (0.25%), Cadmium chloride solution (50 ppm Cd)	200 ml sterile ddH_2O
Chamber 4	2 g natural sediment, 200 ml filtered lake H_2O, Cadmium chloride solution (50 ppm Cd)	200 ml filtered lake H_2O
Chamber 5	2 g natural sediment, 200 ml filtered lake water, 0.5 g glucose, Cadmium chloride solution (50 ppm Cd)	200 ml filtered lake H_2O

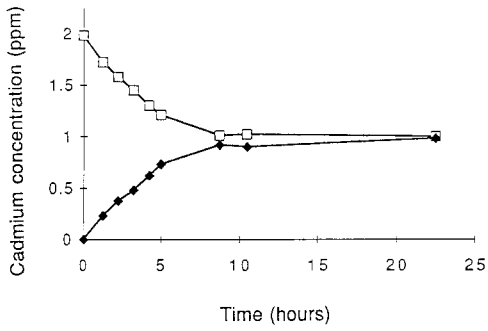

Fig. 2. Diffusion of Cd from the simulated water column (□) to the simulated sediment (◆) in the absence of sediment or cells.

Both Cd-sensitive and Cd-resistant isolates reduced the equilibrium concentration of soluble Cd that remained in the simulated water column and pore water after 22 h of incubation. The average equilibrium concentration of

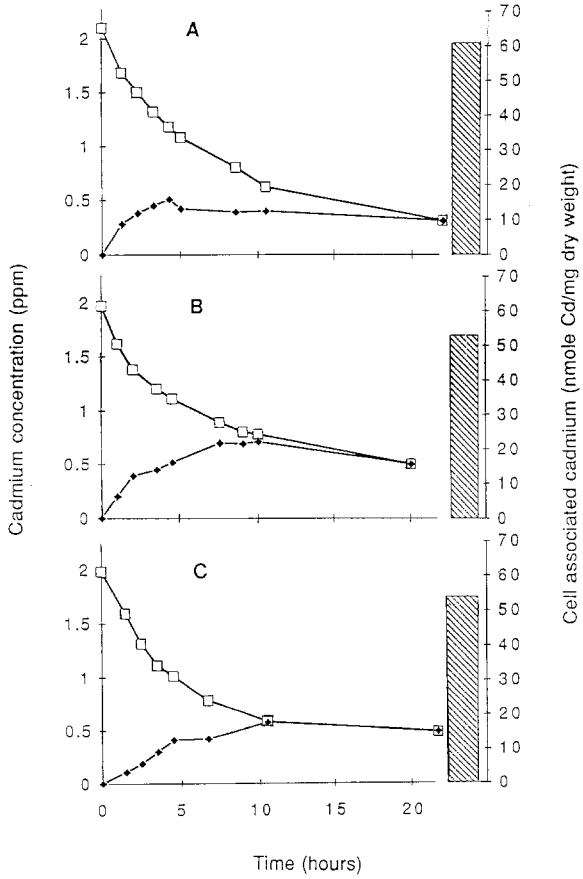

Fig. 3. Diffusion of Cd from the simulated water column (□) to the simulated sediment (◆) and cellular accumulation of Cd (▨) by cells of three Cd-sensitive lake sediment bacteria: **A**, MC102; **B**, MC103, **C**, MC107.

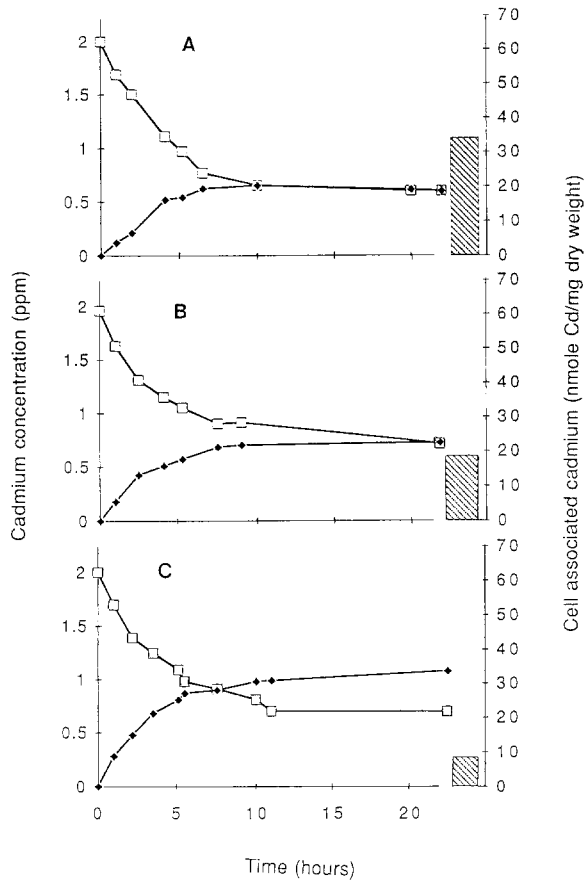

Fig. 4. Diffusion of Cd from the simulated water column (□) to the simulated sediment (◆) and cellular accumulation of Cd (▨) by cells of three Cd-resistant lake sediment bacteria: **A**, MC215; **B**, MC315; **C**, MC312.

dissolved Cd remaining in the simulated water column in the presence of Cd-resistant isolates (0.67 ppm Cd) was significantly greater than the average equilibrium concentration of Cd (0.43 ppm Cd) observed for the Cd-sensitive isolates (Table 2). The dissolved Cd that remained in chambers equilibrated with cells of the three Cd-sensitive isolates and Cd-resistant cells of strain MC215 or MC315 was partitioned equally between the simulated water column and pore water (Figs. 3 and 4). In contrast, the dissolved Cd remaining in diffusion chambers equilibrated with Cd-resistant MC312 cells was not evenly distributed between the simulated water column and pore water. After 22 h of incubation with MC312 cells, 0.70 ppm Cd remained in the simulated water column, while 1.08 ppm Cd, that could not be removed by centrifugation, remained in the simulated pore water (Fig. 4C).

The pH of each chamber changed only slightly during incubation, with all chambers remaining between pH 6.96 and pH 7.13 throughout the equilibration period. The cell

TABLE 2

Average cell-associated and dissolved Cd after equilibration with Cd-sensitive and Cd-resistant cells

	Cell-associated Cd[a]		Dissolved Cd in water column[b]	
	Cd-sensitive	Cd-resistant	Cd-sensitive	Cd-resistant
Average[c]	56.3	20.4	0.43	0.67
S.D.	4.1	12.8	0.10	0.06
C.V.	7.2	63.0	24.30	9.10

[a] nmol Cd/mg cell dry weight.
[b] ppm Cd.
[c] $n = 3$.

densities of suspensions used in the diffusion chambers also remained nearly constant with all suspensions tested remaining between 62 and 68 Klett units during the equilibration period. The number of viable cells in test suspensions varied by isolate and ranged between 3.8×10^7 cfu/ml and 1.6×10^8 cfu/ml. The total cfu/ml for each cell suspension did not change significantly during incubation in the diffusion chamber (data not shown).

Partitioning of cadmium between water column and sediments

The amount of Cd that diffused into the simulated water column from the sediment pore water as a function of total Cd added to the sediment is shown in Fig. 5. In each instance, Cd was added to the simulated sediment (side B) and the diffusion of Cd into the simulated water column (side A) was monitored. From the sediment side of the sediment-free control chamber, Cd diffused into the water column side of the chamber to produce a uniform concentration throughout the chamber within 24 h after each addition of Cd (Fig. 5). Untreated sediments and autoclaved sediments actively sorbed Cd and prevented the diffusion of more than 80% of the Cd that was released to the simulated water column of control chambers. The smallest amount of metal released to the water column occurred with autoclaved sediments which retained more than 97% of the added Cd (Fig. 5). Untreated sediments retained 80% of the added Cd. The addition of glucose to both autoclaved and untreated sediments decreased the retention of Cd by the sediments. The decrease in Cd retention by glucose amended sediments corresponded with an increase in bacterial cell numbers during the incubation period. Total counts in untreated sediments increased from 1.4×10^7 to 9.5×10^8 cfu/g sediment (wet weight), whereas total counts for autoclaved sediments increased from < 1 to 1×10^3 cfu/g sediment (wet weight) by the end of the 16-day incubation.

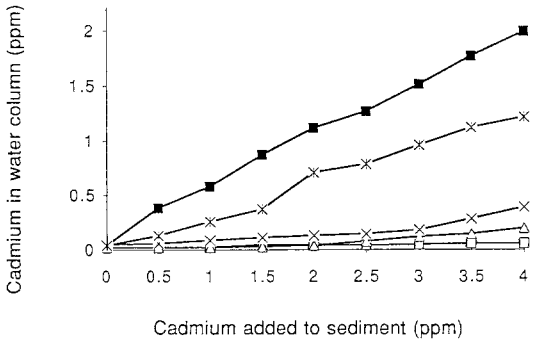

Fig. 5. Diffusion of Cd from the sediment side of diffusion chamber to simulated water column during cumulative additions of Cd: (■) no sediment, control (×) untreated sediment; (∗) sediment with glucose; (□) autoclaved sediment; (△) autoclaved sediment with glucose.

DISCUSSION

The diffusion chambers used in this study permitted the rapid diffusion of dissolved Cd between the simulated water column and sediment while preventing the mechanical mixing of solutions in the two sides of the chamber. For this reason, the chambers described in this work provide a suitable system for modeling the partitioning of Cd between sediments and the water column based on three variables: solubility of Cd; Cd exchange capacity of sediments; and Cd concentration gradient between the water column and sediment-pore water.

When pure cultures of Cd-sensitive sediment bacteria were used, most of the Cd added to the simulated water column was sequestered by cellular accumulation of the metal while the soluble fraction of the metal diffused to a uniform concentration throughout the chamber. Similar results were obtained with two of the Cd-resistant isolates that were tested. However, for one isolate, there was a measurable increase in the concentration of non-cell-associated Cd in the sediment side of the diffusion cham-

ber compared with the Cd concentration in the simulated water column. The unequal distribution of Cd between the water column and pore water was most likely the result of chelation of Cd by an extracellular product released by cells of isolate MC312. Examples of metal-binding microbial polymers have been described [1]. Our results suggest that metal-binding cellular polymers may effectively prevent the release of Cd into the water column even though the metal is not bound to sediment particles or bacterial cells.

Those isolates that demonstrated the greatest sensitivity to Cd accumulated the highest levels of the metal from lake water, whereas the Cd-resistant isolates accumulated comparatively low levels of the metal. These results are consistent with studies in this lab that have demonstrated differential Cd accumulation by Cd-sensitive and Cd-resistant bacteria under laboratory conditions [2]. Results of diffusion chamber studies suggested that Cd-resistance in sediment isolates resulted from reduced cellular accumulation of Cd during exposure to elevated levels of the metal. The correlation between Cd-resistance and cellular accumulation of Cd observed in this study suggests that in the absence of differential binding to abiotic sediment components, sediments containing high numbers of Cd-resistant cells would bind less metal than sediments containing comparatively high numbers of Cd-sensitive cells.

In autoclaved sediment, having less than one viable cell per g sediment, 97% of the added Cd was bound to the sediment particles and prevented from diffusing into the water column. Therefore, even in the absence of viable cells, lake sediment can potentially bind and thereby remove dissolved cadmium from the water column. Based on results obtained with control chambers, it was not expected that sorption of cadmium by autoclaved sediments would be influenced by the addition of glucose. In our experiment, however, the addition of glucose slightly lowered the total Cd bound by the autoclaved sediment as evidenced by increased Cd released into the water column. As noted in the results, glucose-amended, autoclaved sediments were found to contain a small community of bacteria at the end of the study even though no viable cells were recovered from the sediments immediately after autoclaving. The increase in Cd released from glucose-amended, autoclaved sediments and the recovery of viable cells from these sediments at the end of the incubation period is consistent with results obtained with non-sterile glucose amended sediments, which released the greatest quantity of Cd into the water column. Differences in Cd retention by autoclaved sediments with and without glucose was most likely caused by the metabolic activities of contaminating bacteria that survived the sterilization procedure.

Although pure cultures of sediment bacteria accumulated Cd and decreased the equilibrium concentration of soluble Cd in the simulated water column and sediment pore water, sediments containing the greatest number of viable cells released the greatest quantities of Cd into the water column. These results illustrate the complexity of interactions that can influence the sequestering or mobilization of Cd by sediments. It is possible that when glucose was added to sediment in the diffusion chambers, metabolic products of glucose metabolism were released into the sediments. Such metabolites may have decreased the affinity of sediments for the metal and caused Cd that had been bound to cells and sediment particles to be released. However, additional studies will be required to determine the mechanism by which Cd was released from sediments into the water column as bacterial numbers increased. These studies suggest that in an undisturbed, nutrient-limited freshwater environment, dissolved Cd that enters the water column will most likely bind to and be retained in the sediment. Moreover, our results suggest that if microorganisms in Cd-contaminated sediments are provided with appropriate nutrients, some of the toxic metal sequestered in the sediment may be released into the water column as dissolved Cd.

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