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Effects of pH, Temperature, and Eh on the Uptake of Cadmium by Bacteria and an Artificial Sediment

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Elevated concentrations of heavy metals in the environment are a problem due to their toxicity to plants, animals, fish, and, ultimately, man. These metals may be concentrated in plants or animals and passed throughout the food chain. The role of bacteria in the mercury cycle has been studied extensively, but the influence of bacteria on the movement of other metals such as cadmium is less well understood.

Bacterial activities have been implicated in the mobilization of various metals from sediments to the water column. Bacterial breakdown of detrital organic matter to humic acids which can complex more cadmium resulted in an increase in the metal in the water column (RIFFALDI & LEVI-MINZI 1975). The oxidation of ferrous sulfide by Thiobacillus ferroxidans has been shown to produce acid which in turn may cause the breakdown of CdS, ZnS, and PbS (MALOUF & PRATER 1961). Under acidic conditions Cd and other metals have increased solubility (FORSTNER & WHITMAN 1979).

Studies have been reported which describe the competition between bacteria and sediment for mercury (RAMAMOORTHY et al. 1977) and between algae and sediment for cadmium and copper (LAUBE et al. 1979). This report describes an investigation of cadmium adsorption by a Cd-resistant bacterium and a sediment matrix under various incubation conditions.

MATERIALS AND METHODS

Experimental System

Sorption of cadmium from water by bacteria and sediments was based on the methods outlined by RAMAMOORTHY et al. (1977) and LAUBE et al. (1979) for mercury, cadmium, and copper uptake studies. The system was contained in one liter reaction kettles (Pyrex #6947, Corning Glass Works, Corning, New York) equipped with covers with four openings and consisted of water, bacteria, and sediment components (described separately below) that were aseptically added to the sterile kettles. The system was incubated up to 4 d at pH values of 6, 7, or 8.5, temperatures of 4°, 23°, and 35°C, and at high (air-equilibrated) and low Eh values. The water in each kettle was stirred with a teflon-coated stir

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bar. All water in these experiments used was demineralized and double distilled.

Each experimental parameter was studied by assembling four separate kettles. One kettle contained the water component only, the second had the water and the bacterial component, and the third kettle contained water and the sediment component. The final kettle contained the water, the bacteria, and the sediments with the sediment and bacteria in separate dialysis bags. The standard conditions used for comparison of the test parameters were 23°C, pH 7, and air-equilibrated. Tests involving pH changes were done at 23°C and air-equilibrated, and those experiments involving temperature effects were carried out at pH 7 and air-equilibrated. The study at a lowered redox potential was done at pH 7 and 23°C for 2 d.

The Eh value of the system was initially lowered to 30 mV by purging the water of the sealed kettles with filtered nitrogen gas for 30 min at 8 h intervals. The Eh was measured with platinum combination electrodes (Fisher Scientific Co., Pittsburgh, Pa.) which were sterilized by immersion overnight in undiluted Chlorox (The Chlorox Co., Oakland, Ca.).

Bacterial Component

The Pseudomonas sp. used in these experiments was isolated from sediments of the Ottawa River near Lima, Ohio, identified in our laboratory, and was resistant to 50 μg Cd^{2+} ml^{-1}. The bacterium was grown in Nutrient Broth (Difco Laboratories, Detroit, Mich.) to late log-phase, harvested by centrifugation at 6,000 x g for 20 min, and washed twice with phosphate-buffered saline (0.3 g l^{-1} Na_2HPO_4, 8.5 g l^{-1} NaCl). Phosphate-buffered saline was added to a final level of approximately 10 mg dry cells per ml and 1.5 ml was injected into sterile dialysis tubing tied closed with nylon monofilament line. The remaining cells were washed once with H_2O and dried at 100° ± 5°C to determine the dry weight of cells used.

Sediment Component

Artificial sediments consisted of 1 part of Potting Soil and 3.5 parts of acid washed Kaolin clay (American Standard, Fisher Scientific Co., Fairlawn, New Jersey). Potting soil was pulverized to pass through a 60 mesh screen. The artificial sediments had a cation exchange capacity of 30.4 mEq/100 g and consisted of 12.9% organic matter. Sediments were placed in dialysis bags (0.3 g sediment in 1.5 ml H_2O) and autoclaved suspended in H_2O.

Water Component

The water component of the system was a phosphate-carbonate buffer system (SHARP et al. 1980) with added salts. The comsition of the buffering systems at different pH values is shown

in Table 1. Components were autoclaved separately and then made up to volume after cooling. The buffer needed for each group of kettles was made up in one flask, cadmium was added (1 μg Cd^{2+} ml^{-1}, nominal), and the system was allowed to equilibrate for one day. One liter of buffer was added to each kettle, bacteria and sediments in separate dialysis bags were added to the proper kettles, and samples were withdrawn after incubation for various intervals of time.

TABLE 1

Composition of water component.

	grams per liter		
	pH 6	pH 7	pH 8.5
KH_2PO_4	3.50	1.30	0.91
Na_2CO_3	0.10	0.34	0.75
$MgSO_4 \cdot 7H_2O$	0.04	0.04	0.04
$CaCl_2 \cdot 2H_2O$	0.03	0.03	0.03
$NaNO_3$	0.025	0.025	0.025

Analysis of Cadmium in Components

Cadmium in water was analyzed by direct aspiration into the flame of an atomic absorption spectrophotometer (AAS) (Perkin-Elmer, Model 403, Norwalk, Conn.). Sediment was centrifuged, dried at 65° ± 5°C, and pulverized. Cadmium in the artificial sediment was analyzed following concentrated HNO_3 digestion for 3 h at 50°C. Bacterial samples were diluted 1:1 with concentrated HNO_3 and digested for 3 h at 50°C. The digests were then analyzed for Cd content by AAS. Cadmium recovery rates were determined based on the total Cd added initially versus the Cd found in the three components after sampling.

RESULTS

When Pseudomonas sp. was placed in dialysis tubing and incubated in a phosphate-carbonate buffer system with 1 μg Cd^{2+} ml^{-1}, the bacteria took up Cd at rates and quantities which varied with the incubation conditions. The standard conditions to which other treatments were compared was pH 7 - 23°C, and air-equilibrated (about 280 to 300 mV). The uptake of Cd by bacteria at pH 7 - 23°C was approximately constant throughout the 4 d incubation (Figure 1A). The rate of Cd uptake at pH 6 - 23°C was initially the fastest of the treatments examined, but pH 7 - 35°C reached the highest final Cd concentration in the water-bacteria system. Cadmium uptake was the lowest at pH 8.5 - 23°C, and uptake at pH 7 - 4°C was less than 7 - 23°C. Lowering the EH value (30 mV initially and 90 mV after 2 d) resulted in a 2 d Cd level midway between that of pH 7 - 23°C and pH 7 - 35°C. The

final level of Cd reached in bacteria of the water-bacteria system was in the range of 1 to 3 μg Cd mg^{-1} of bacterial cells (dry weight) for the more efficient treatments.

Uptake of Cd by the artificial sediment matrix in a water-sediment system was related to the incubation conditions in a manner similar to bacteria (Figure 1B). Incubation at pH 7 - 23°C resulted in a relatively constant rate of Cd uptake as did pH 6 - 23°C, pH 7 - 4°C, and pH 8.5 - 23°C. The initial rate of Cd adsorption at pH 7 - 35°C was the highest of the pH - temperature treatments, and the final level of Cd reached after 4 d was also the highest. Cadmium levels reached after 4 d at pH 6 - 23°C and pH 7 - 23°C were similar. Cd levels in pH 7 - 4°C were higher than pH 8.5 - 23°C, but both were lower than the standard conditions. Lowering the Eh value resulted in a higher 2 d Cd level than any of the other treatments.

Comparison of the water-bacteria-sediment systems (Figure 2) with the water-bacteria and water-sediment systems showed that there was little difference in the final levels of Cd reached in bacteria or sediment or in the rates of adsorption between methods of exposure to Cd. The pH 6 - 23°C and pH 7 - 35°C treatments in the bacterial samples were reversed, and the pH 7 - 23°C and pH 6 - 23°C incubation were reversed in the sediment when bacteria and sediment were incubated together. In both methods of exposure of Pseudomonas sp., the rate of Cd uptake at pH 6 was higher initially, and then the rate slowed between 1 and 4 d, while the pH 7 and pH 8.5 adsorbed Cd at a more constant rate throughout the 4 d incubation periods (Figures 1 and 2).

The amount of cadmium taken up by bacteria averaged about 10 times higher than Cd adsorbed by the sediment when based on dry weight. The difference was most pronounced during the initial day of Cd exposure and then lessened after 4 d in the presence of Cd. Cadmium recovery rates are shown in Table 2.

TABLE 2

Total recovery of Cd as a percentage of the added Cd after four days of incubation.

Parameter	System			
pH - °C	Water	Water Bacteria	Water Sediment	Water Bacteria Sediment
7 - 23	99.1	98.8	98.0	98.3
6 - 23	98.0	99.1	97.6	98.0
8.5 - 23	89.3	90.8	95.9	92.4
7 - 35	96.2	93.4	97.7	94.0
7 - 4	92.6	94.9	95.5	94.2

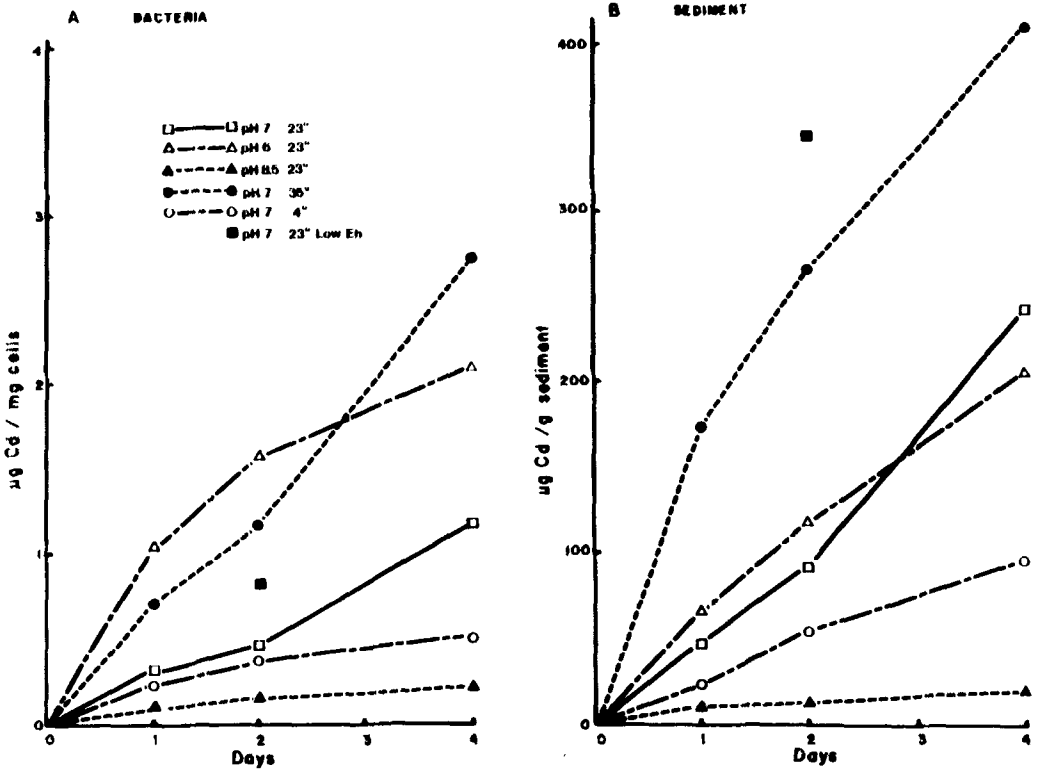

Figure 1. Uptake of Cd^{2+} by bacteria (A) and sediment (B) when incubated separately.

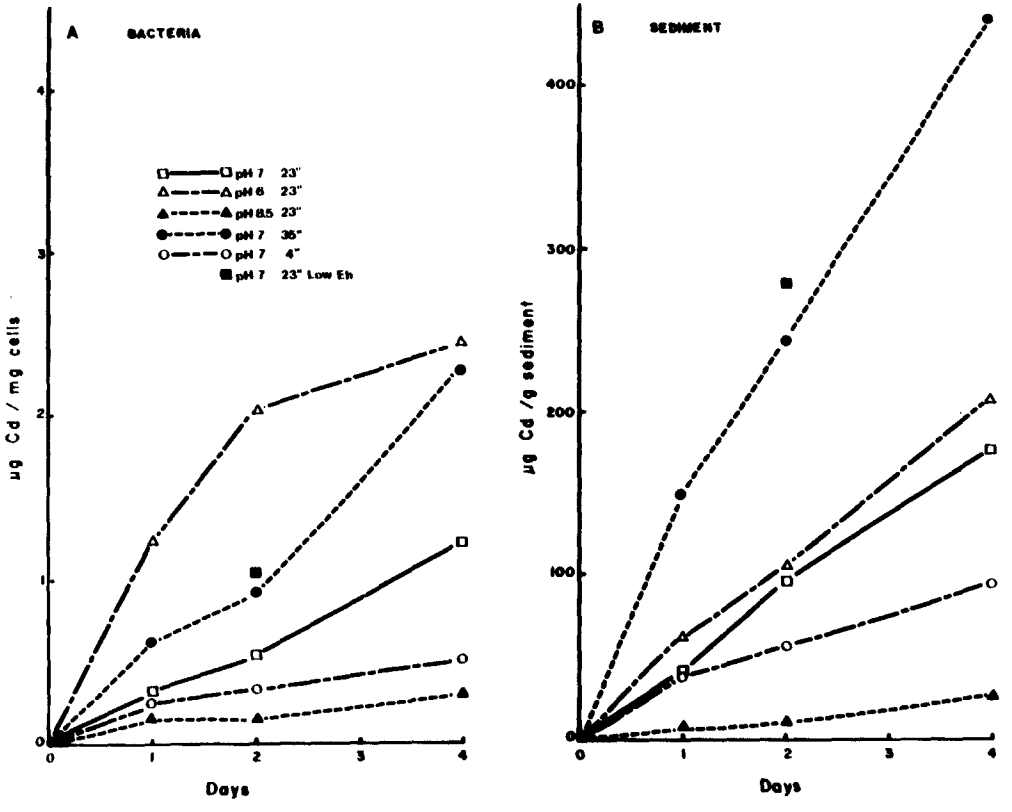

Figure 2. Uptake of Cd^{2+} by bacteria (A) and sediment (B) when incubated in the same kettle.

DISCUSSION

The experiments examined conditions which influenced the rate of Cd uptake from water by sediments and a Cd-resistant bacterial isolate. The results suggested that those conditions under which Cd is most mobile induced the highest uptake of Cd by both bacteria and sediments. The highest rates of uptake were observed at a temperature of 35°C and pH values of 6. At alkaline pH values Cd may form insoluble complexes with the carbonate present in the buffering system. These complexes are not taken up efficiently by either bacteria or sediments. Lowering the temperature of incubation also lowered the amount of Cd adsorbed, probably due to the decreased rate of both biological and chemical processes at lower temperatures. Similarly, the increased rate of uptake at higher temperatures is probably related to the increased rate of chemical and biological reactions at elevated temperatures.

The uptake of Cd at lowered Eh values was examined, and it was found that these conditions accellerated Cd uptake by both bacteria and sediment, with sediment being affected more than bacteria. One plausible explanation of this may be that phosphate and carbonate were replaced by sulfide as the controlling anionic species under more reduced conditions (LU & CHEN 1977). In the sediments there was a higher concentration of available sulfide groups than in the water. When Cd entered the sediment it precipitated as CdS and remained within the dialysis tubing. The level of Cd in the sediment would continue to increase as Cd continued to enter by diffusion without CdS being removed. This explanation assumes that an equilibrium is set up in the air-equilibrated treatments.

The total Cd adsorbed by the Pseudomonas sp. was approximately 10 times higher than the amount adsorbed by the artificial sediment. However, the kinetics of Cd adsorption were similar for both the bacterium and sediment. Pseudomonas stutzeri has been reported to have a cation exchange capacity (CEC) of about 340 mEq/100 g (ZWARM 1973), and the artificial sediment used in these experiments had a CEC of 30.4 mEq/100 g. The higher CEC of bacteria may have accounted for the higher level of Cd adsorbed by the Pseudomonas sp. These experiments indicated that the uptake of Cd by a Cd-resistant bacterium was influenced by environmental factors in the same manner as sediment.

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