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Mobilization and Accumulation of Sediment Bound Heavy Metals by Algae

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Heavy metals (HMs) are known to be insidious toxic pollutants and their presence in the environment, especially aquatic, is a current major concern. It is evident from the numerous studies on HM pollution in aquatic systems (OTTAWA RIVER PROJECT 1977, DEGROOT and ALLERSMA 1975), that HM ions are partitioned among the three major compartments of such ecosystems - specifically among water, sediment and biota.

Detectable levels of HM ions may be found in waters polluted by human activities or by natural geochemical leaching. The amount and form of these ions in the water depends primarily on a number of physico-chemical factors of the compartment itself (GARDINER 1974a, STIFF 1971). The vast majority of the HM ions entering natural waters, however, are sorbed and accumulated by bottom sediments acting as a sink (GARDINER 1974b). Consequently, comparatively small amounts of these ions remain in the water column, e.g. 600 ppm Cu and 45 ppm Cd were detected in Rhine sediments as compared to low ppb values of these same metal ions in the water (DEGROOT and ALLERSMA 1975).

Sorption of HM ions to sediments is dependent on the properties of the sediment as well as of the metal ion. Surface area, organic content and cation exchange properties of the sediment have been shown to be valid indices of HM ion sorption (RAMAMOORTHY and RUST 1976). Furthermore, sediments rich in sulfides and organic nitrogenous compounds are most effective in sequestering Cu and Cd which are classified as soft acids (AHRLAND 1966).

The Ottawa River water used in this study has a HM binding capacity of 21 $\mu M\ M^{2+}/L$ (RAMAMOORTHY and KUSHNER 1975). This value was reaffirmed for the samples used in this study. Analysis showed non-detectable levels of Cu and Cd in the water; sediments contained approximately 50 ppm Cu and non-detectable levels of Cd. Similar results for Ottawa River sediments have been reported (OLIVER and KINRADE 1972).

Since there is a definite distribution of HM ions in natural waters and sediments, it was desirable to follow HM ion flux and HM bioavailability. Accumulation by primary producers, such as algae, could result in the movement of HM ions up the food chain to higher trophic levels.

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Earlier laboratory studies examining the bioavailability and accumulation of HM ions, concentrated mainly on two compartments of water ecosystems (GÄCHTER et al. 1974, STEEMAN-NIELSEN and WIUM-ANDERSON 1970). Recently, a three compartment set-up for examining the role of bacteria in the movement of mercury, was reported (RAMAMOORTHY et al. 1977). In our study, we have used such a multi-compartmental approach to examine the intercompartmental interactions and distributions of Cd and Cu in river water, sediment and algae.

Since there exists a general lack of knowledge as to the uptake and concentration of Cd and Cu in natural and polluted aquatic ecosystems, it was of interest to study the flux of these HM ions in such systems. Cadmium is known to be a hazardous trace substance and increasing concentrations of Cd are entering aquatic ecosystems via effluents from sewage treatment operations, metallurgical plants, plating works etc. (FLEISHER et al. 1974). Analysis of industrial wastes being discharged into natural waters has yielded values as high as 50 ppm Cd (TENNEY and STANLEY 1967). Copper, in contrast to cadmium, is essential in trace amounts. High concentrations of this metal, however, are known to be toxic. Since it is also widely used, industrial and municipal effluents discharge considerable amounts of Cu into natural waters. Analysis of one of the major components of natural ecosystems, sediment, near such areas, has yielded readings as high as 236 ppm (OLIVER and KINRADE 1972).

EXPERIMENTAL

The alga used was grown at 22°C ± 1°C under white light (16 h on, 8 h off), to mid-log phase, Anabaena 7120 in GO medium (STANIER et al. 1971) and Ankistrodesmus braunii (isolated from Ottawa River water) in Chlamydomonas medium (ONAD et al 1967). The cells were then harvested and washed with distilled water. Seven dialysis sacs were prepared into each of which the alga of concern was placed in amounts equivalent to 0.008-0.040 g dry weight of Anabaena 7120 or 0.031-0.094 g dry weight of A. braunii in 6 mL of distilled water.

The sediment (Ottawa River sample) was of a clay consistancy. It was composed primarily of poorly crystallized kaolite and illite and had the following characteristics: 25.5 m^2/g dry wt surface area; 10.1% organic content; 0.35 mm mean grain size; 18.58 mEq/100 g dry wt cation exchange capacity (RAMAMOORTHY and RUST 1976). The sediment was also placed in seven dialysis sacs, each sac containing 0.3g dry weight plus 6 mL of distilled water.

All of the dialysis bags were then suspended in 5.5 L of Ottawa River water (Eh: +303 - +333 mv; pH: 7.30 - 7.63; pCl: 3.43 - 3.60; conductivity: 52 µmhos - 80 µmhos) and mechanically rotated in a multiple dialyser. The water was agitated using a magnetic stirrer. All experiments were performed at 22°C ± 1°C.

Sediment and algal sacs were taken out at specified time intervals. Atomic absorption spectroscopy was used to analyse the samples for Cd and Cu (detection limit - 10 µg/L Cd, 5 µg/L Cu). In this way, after Cd or Cu (in the form of nitrate salts) was added, either to the water compartment or loaded onto the sediment, the accumulation by the other two compartments could be monitored.

During the experiments, algal cells were examined under the light microscope to detect lysis or major cellular perturbations.

RESULTS AND DISCUSSION

When the HM ion was added to the river water, it was taken up by both the algal compartment and the sediment. In the case of Cd, Anabaena steadily accumulated the metal ion over a 72 hr period reaching an equilibrium (as determined by the ratio of HM ion accumulated by alga to the HM ion accumulated by sediment) in 12-24 h. The ratio is 1.67 for Anabaena and 2.22 for A. braunii (Table 1). On a dry weight basis Anabaena accumulated 31 times and A. braunii 13 times more Cd than the sediment (Fig. 1A).

Similar results were obtained for the copper-fortified water experiments. An equilibrium was reached after 12 hrs. The ratios of accumulation of Cu (alga/sediment) were lower than those for Cd accumulation: Anabaena - 0.65, A. braunii - 1.02 (Table 2). Therefore the two algae were more successful in accumulating Cd than Cu. This is reaffirmed by the dry weight analysis (Fig. 1). It can be concluded from these results, that the algae examined compete very effectively with the sediment in accumulating Cd and Cu.

Anabaena was more efficient in metal ion accumulation than A. braunii. At 12-24 h , in the presence of Cu or Cd in the water, Anabaena is observed to have lysed with the concomittant release of blue pigments, probably phycocyanin, which remained in the dialysis sac. It is likely that lysis released a variety of other Cu and Cd-binding ligands of varying molecular weights. Phosphates, cysteinyl and histidyl side chains of proteins, purines, pteridines and porphyrins are candidates for metal binding in the algal sacs (VALLEE and ULMER 1972). Most of these chelators probably do not diffuse out of the dialysis bags since the enclosures have a molecular weight cut-off of 6,000. Microscopic examination of A. braunii showed that the cells remained intact.

The response of these two algae, to the presence of Cd and Cu, is in agreement with experiments now in progress in our laboratory. The addition of 0.64 ppm or 6.35 ppm of Cu (as nitrate salt) to growing mid-log phase cells of Anabaena result in initial extensive lysis whereas similar additions to A. braunii cells in the same stage of growth show little if any change in growth pattern. Sensitivity of blue-green filamentous algae to heavy metals has been reported (WHITTON 1973).

TABLE 1

The distribution of Cd^{2+} in a multicomponent system after the addition of Cd^{2+} to the water compartment

	Time (hr)	pH	AQUEOUS CONC. (mg) per litre	AQUEOUS CONC. (mg) total	CONC. IN CLAY (µg) per bag	CONC. IN CLAY (µg) total	CONC. IN ALGAE* (µg) per bag	CONC. IN ALGAE* (µg) total	TOTAL RECOVERY OF ADDED Cd in mg	TOTAL RECOVERY OF ADDED Cd as %
I	0	-	1.02	5.61	.40	2.80	0	0 (0) **	5.61	100
	1	7.40	.96	5.27	11.50	69.40	29.10	165.90 (2.40)	5.51	98
	6	7.61	.89	4.89	32.10	172.40	76.20	387.20 (2.20)	5.45	97
	12	7.63	.79	4.32	41.60	210.40	92.30	460.70 (2.19)	4.99	89
	24	7.64	.74	4.03	91.50	360.10	133.00	591.30 (1.60)	4.98	89
	48	7.64	.65	3.53	121.00	419.10	168.00	659.90 (1.57)	4.61	82
	72	7.63	.70	3.79	94.00	392.10	224.00	722.60 (1.84)	4.91	88
II	0	7.12	1.05	5.72	.20	1.40	0	0 (0)	5.72	100
	1	7.03	.96	5.27	7.50	46.40	16.50	107.25 (2.30)	5.42	94
	6	7.12	.88	4.82	32.70	171.20	60.60	349.80 (2.04)	5.34	93
	12	7.13	.73	3.99	56.80	267.60	112.50	527.10 (1.97)	4.78	84
	24	7.15	.73	3.98	74.40	320.40	193.60	770.40 (2.40)	5.07	89
	48	-	.66	3.58	133.00	437.60	284.00	922.80 (2.11)	4.94	86
	72	-	.66	3.57	148.00	452.60	304.00	971.20 (2.15)	4.99	87

* Algae I = Anabaena 7120, II = Ankistrodesmus braunii
Cd^{2+} added to water compartment as $Cd(NO_3)^2$: 15 mg/5.5 l = 1 ppm Cd^{2+}

** Cd^{2+} accumulated by alga/Cd^{2+} accumulated by sediment

TABLE 2

The distribution of Cu^{2+} in a multicomponent system after the addition of Cu^{2+} to the water compartment

	Time (hr)	pH	AQUEOUS CONC. (mg) per litre	AQUEOUS CONC. (mg) total	CONC. IN CLAY (µg) per bag	CONC. IN CLAY (µg) total	CONC. IN ALGAE* (µg) per bag	CONC. IN ALGAE* (µg) total	TOTAL RECOVERY OF ADDED Cu in mg	TOTAL RECOVERY OF ADDED Cu as %
I	0	7.01	1.02	5.61	16.25	97.50	1.25	7.1 (0.07)**	5.71	100
	1	6.98	.92	5.05	30.75	170.00	8.75	43.43 (0.26)	5.26	92
	6	7.40	.85	4.66	66.25	312.00	45.00	163.45 (0.52)	5.14	90
	12	6.96	.82	4.48	80.00	353.25	63.75	232.22 (0.66)	5.07	89
	24	6.60	.77	4.23	101.25	395.75	69.76	256.16 (0.65)	4.88	85
	48	6.40	.70	3.79	153.00	447.50	110.25	298.75 (0.67)	4.54	80
II	0	7.07	1.04	5.72	19.20	134.40	1.20	8.28 (0.06)	5.86	100
	1	7.08	.83	4.56	30.00	199.20	13.20	80.00 (0.40)	4.84	83
	6	7.06	.83	4.55	63.00	364.20	50.70	287.17 (0.79)	5.20	89
	12	6.82	.83	4.54	77.28	421.32	78.28	408.74 (0.97)	5.37	92
	24	7.03	.73	4.00	99.96	489.36	118.44	553.05 (1.09)	5.02	86
	48	6.80	.73	4.00	123.00	535.44	140.40	532.79 (0.99)	5.07	87
	72	6.20	.63	3.35	140.40	552.84	156.60	558.82 (1.01)	4.46	76

* Algae I = *Anabaena* 7120, II = *Ankistrodesmus braunii*
Cu^{2+} added to water compartment as $Cu(NO_3)_2$: 21 mg/5.5 l ≈ 1 ppm Cu^{2+}

** Cu^{2+} accumulated by alga/Cu^{2+} accumulated by sediment

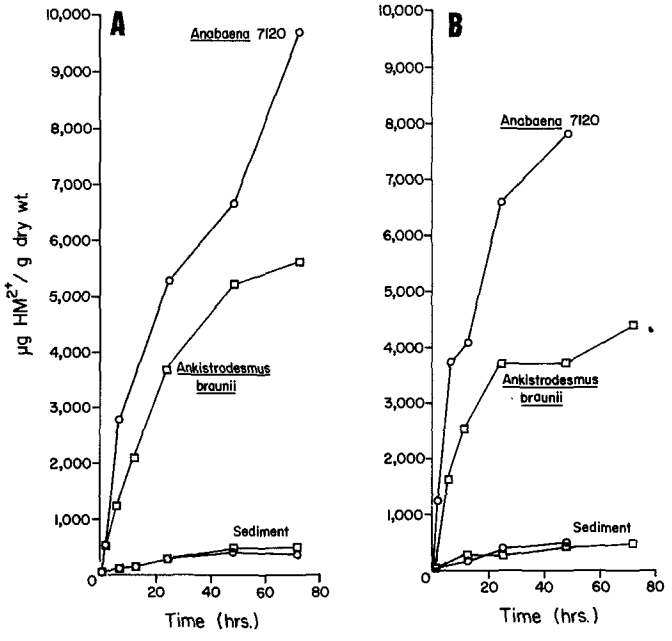

Fig. 1 Accumulation by algae and sediments of Cadmium (1A) and Copper (1B) added to river water.

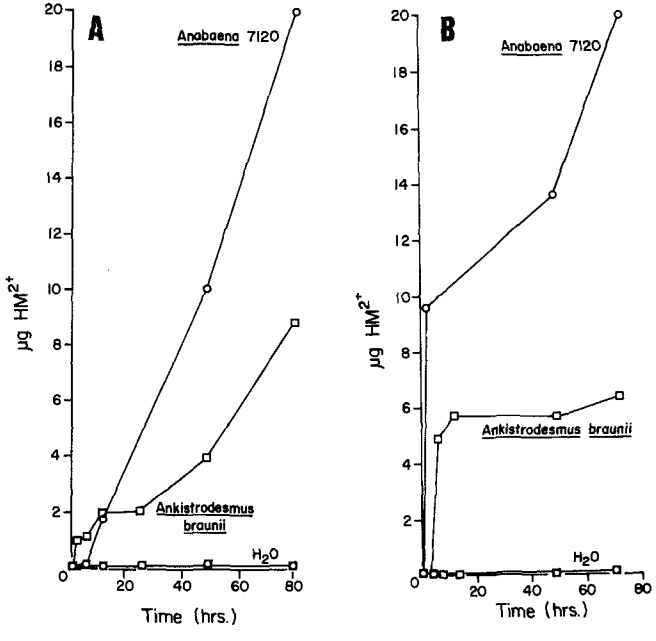

Fig. 2 Accumulation by algae and river water of Cadmium (2A) and Copper (2B) added to sediment.

From experiments with HM ion fortified water, it can be concluded that A. braunii contains substantially more binding sites for Cu and Cd than the clay sediment, on a weight by weight basis. The alga sorbs more Cd than Cu. A. braunii, being a fairly HM tolerant species, would probably be found in waters polluted with HM ions. Separate experiments (LAUBE and KUSHNER, unpublished) confirmed that substantial amounts of Cd and Cu are bound to A. braunii cells. Aquatic organisms feeding on these algae would ingest HMs which could lead to bioaccumulation. Anabaena, on the other hand, would enhance the level of HM in the water compartment of a natural ecosystem via HM chelation.

Control experiments revealed insignificant accumulation of HMs. Loss of Cu and Cd by sorption to the walls of the container and dialysis tubing could explain why less than 100% recovery was observed in these experiments (Table 1 and 2).

When the source compartment of the metal ion was the sediment, algae accumulated considerable amounts of HM ions. Since the volume of water was 5,000 times as great as the volume of the other compartments, it is noteworthy that the water showed negligible amounts of Cd and Cu. The algal accumulation, however, could only have been possible if the water was acting as the medium of metal ion transport.

The sediment, loaded with either Cd or Cu ions, was carefully rinsed a number of times with distilled water to remove non-sorbed metal ions. The water from these washing contained virtually no HM ions.

The sediment contained no detectable natural Cd and was loaded with high levels of Cd (100 ppm) to increase the sensitivity in the analysis of the sediment and especially of the water column. Although the sediment had a natural level of Cu (50 ppm), the sediment was deliberately loaded with additional amounts (1 ppm). The copper ions found in the sediment could have been irreversibly bound i.e. covalently bound and/or sorbed to crystalline lattices, sulfides and/or organic compounds (JONASSON 1970). Both Anabaena and A. braunii, accumulated some of the natural copper and presumably the added Cu. The former alga accumulated 20 ppm, the latter 7 ppm, all on a dry weight basis, over a 72 h period (Fig. 2B). For a period of 72 h, Cd accumulation by the two algae was similar to Cu accumulation- 9 ppm for A. braunii and 20 ppm for Anabaena (Fig. 2A). During this period, the sediment did not show progressive decreases in its HM content.

The present study shows that Cd and Cu present in sediments, can be accumulated in substantial amounts by algae in separate compartments. These results suggest that in natural systems algae may play a very important role in mobilizing sediment-bound HM ions.

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