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Microplate Technique for Determining Accumulation of Metals by Algae

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A microplate technique was developed to determine the conditions under which pure cultures of algae removed heavy metals from aqueous solutions. Variables investigated included algal species and strain, culture age (11 and 44 days), metal (mercury, lead, cadmium, and zinc), pH, effects of different buffer solutions, and time of exposure. Plastic, U-bottomed microtiter plates were used in conjunction with heavy metal radionuclides to determine concentration factors for metal-alga combinations. The technique developed was rapid, statistically reliable, and economical of materials and cells. Results (expressed as concentration factors) were in reasonably good agreement with literature values. All species of algae studied removed mercury from solution. Green algae proved better at accumulating cadmium than did blue-green algae. No alga studied removed zinc, perhaps because cells were maintained in the dark during the labeling period. *Chlamydomonas* sp. proved superior in ability to remove lead from solution.

Many species of algae have been reported to accumulate metals from their aqueous environment. This characteristic is expressed quantitatively by a concentration factor (CF), i.e., the ratio of the metal concentration in the algae (micrograms per gram of dry cells) to that in the surrounding water (micrograms per milliliter) (26). This approach makes it possible to compare the metal avidity of different species and strains of algae. However, broad application of this concept has been hindered by the time-consuming nature of the metal assays and the large number of interdependent variables which influence metal removal, e.g., pH, algal species, metal concentration, competing salts, light, culture age, etc. Despite these limitations, several investigators have demonstrated that metal removal by algae is probably largely due to chelation and surface adsorption and constitutes an important response of aquatic flora to metal pollution.

Polikarpov (26) studied a number of freshwater and marine organisms which concentrated radionuclides from nuclear power plant effluents and weapons testing debris and calculated the CF for radionuclides such as ^{106}Ru, ^{137}Cs and ^{90}Sr. The CF for algae were found to be high, often several orders of magnitude. More recently, concern has shifted from radiological contamination to broader problems concerning industrial discharges of metals, notably lead, mercury, cadmium, zinc, copper, arsenic, chromium, and nickel. Trollope and Evans (31) determined CF for samples of algae in waters near abandoned mining areas in Wales and reported values ranging from 10 to 10^5 for copper, iron, nickel, lead, and zinc. Differences were noted in CF within a genus (*Tribonema*), between genera (e.g., *Mougeotia*, *Zygnema*, and *Spirogyra*), and between sample sites located at various distances from zinc smelting wastes. The authors were unable to conclude with certainty whether the differences were related to environmental conditions at the various sites or to biological variation.

Cadmium was removed by pure cultures of the diatom *Skeletonema costatum*, but no uptake occurred until phosphate was depleted from the medium (24). The rate and extent of cadmium uptake by *Nitella* sp. were shown to depend on the amount of calcium and magnesium present in solution with about twice as much cadmium accumulation in *Nitella* sp. cultured in soft water as compared with the same alga cultured in hard water (21). The marine blue-green alga *Synechococcus* sp. has been shown to bind cadmium by producing a metallothionein-like protein (25).

It is quite clear that metal removal is altered when the physiological and nutritional conditions change. The uptake of ^{203}Hg by pure cultures of *Pediastrum boryanum* occurred at a maximum rate during the first 0.5 h after inoculation into fresh media (27). *Chlorella pyrenoidosa* was able to convert mercury which was present in the culture medium as $HgCl_2$ to a more volatile form by a light-mediated reaction

(2-4). Zinc uptake has been found by several investigators to be light dependent (7, 8, 10, 22). Multiple additions of subtoxic does of metals to growing cultures permit levels of accumulation to increase more or less continuously (9).

To date there has been no systematic survey of algae for their ability to accumulate metals. As a first step in such a task we acquired cultures of representative green and blue-green algae and developed a microplate analytical technique which can be used to determine the uptake of metals that have a convenient radionuclide. With this method, it was feasible to survey the effects of several combinations of environmental factors on the removal of metal by algae and to describe the kinetics of metal uptake. The microculture technique described in this paper is statistically reliable and economical of materials, cells, and laboratory space.

MATERIALS AND METHODS

Algal cultures. Cultures of algae were obtained from either the University of Texas (UTEX) culture collection or from local laboratories. Those cultures obtained from UTEX included the diatom *Navicula pelliculosa* (UTEX 668), the chlorophytes *Mougeotia* sp. (UTEX 758), *Scenedesmus obliquus* (UTEX 2016), *Spirogyra* sp. (UTEX 916), *Ulothrix fimbrinata* (UTEX 638) and *Zygnema* sp. (UTEX 923). Species obtained locally included *Clamydomonas* sp., *C. pyrenoidosa*, and the blue-green algae *Gleotrichia* sp., *Oscillatoria* sp., *Schizothrix calcicola* and *Nostoc* spp. (eight strains). All algae were grown in 75 ml of synthetic inorganic medium in 125-ml Erlenmeyer flasks. Flasks were maintained on a gyratory table under a 16-h light–8-h dark regime at a nominal 20°C. Diatoms were grown in Darley and Volcani medium (6), and all other species were grown in Bold Bristol medium (29).

Radionuclides. Radionuclides were used to monitor the uptake of metal by algae. These included ^{210}Pb (Amersham-Searle), ^{109}Cd and ^{203}Hg (New England Nuclear Corp.), and ^{65}Zn (University of Missouri Research Reactor). The first three nuclides were obtained as nitrate salts; ^{65}Zn was supplied as elemental zinc and subsequently dissolved in 3 N HCl. The concentration of metal used in each experiment was calculated from data supplied by the nuclide manufacturer; no "cold" carrier metal was used.

Preparation of algae for assay. An amount of 10 to 20 ml of an algal culture was pipetted into a centrifuge tube, washed twice in distilled water, and suspended to one-fourth the original volume with distilled water. The fourfold concentrate was dispensed by means of a 25-μl dropper pipette. Filamentous and colonial forms such as *Spirogyra* sp., *U. fimbrinata*, *Mougeotia* sp., *Zygnema* sp., *Gleotrichia* sp., and *Nostoc* sp. 31 clogged the pipettes; therefore, these cultures were placed in a laboratory Waring blender at high speed for 90 s. It has been shown that such treatment produces filaments of reasonably homogeneous lengths with a minimum of cell damage (B. L. Robinson, Ph.D. Thesis, Syracuse University, 1968).

Preparation of microculture plate. The method employed commercial equipment developed for use in the cell-mediated lympholysis reaction with ^{51}Cr as a label (16). The equipment, known as the Titertek Supernatant Collection System, consists of rigid, disposable, polystyrene microtiter plates with 96 U-bottomed wells, disposable harvesting frames, a transfer fork, and a press (Flow Laboratories, Inc. Rockville, Md.). Each well in a plate was filled with 100 μl of the reagents by means of 25-μl dropper pipettes. The wells received 25 μl of buffer solution first. Buffers used were as follows: for pH 3, 0.05 potassium hydrogen phthalate and 0.022 M HCl; for pH 4, 0.05 M potassium hydrogen phthalate and 0.0001 M HCl; for pH 5, 0.05 M potassium hydrogen phthalate and 0.023 M NaOH; for pH 6, 0.05 M potassium dihydrogen phosphate and 0.006 M NaOH; for pH 7, 0.05 M potassium dihydrogen phosphate and 0.029 M NaOH; for pH 8, 0.05 M potassium dihydrogen phosphate and 0.047 M NaOH; for pH 9, 0.013 M sodium tetraborate (borax) and 0.005 M HCl; and for pH 10, 0.013 M borax and 0.02 M NaOH (28).

Next, all wells received 25 μl of Bristol solution (diluted 1:10 with distilled water) followed by 25 μl of either distilled water or algal cell suspension prepared as described above. This returned the algae to the same concentration as the culture from which they were derived. Finally, 25 μl of radionuclide solution was dispensed to each well. The multiwell plates were then stored in the dark at high relative humidity at 22°C for 3 h to permit uptake of heavy metals.

After this labeling period, a harvesting frame (a plastic holder containing 48 cellulose acetate absorption cartridges, each tipped with a glass fiber filter disk) was placed over the plate in register with 48 wells. A press was used to force the 48 cartridges simultaneously into the wells. A 2-s contact proved sufficient to absorb 80 to 85% of the well contents. The glass fiber filter disks, being slightly larger in diameter than the microplate wells, formed a tight seal with the well wall and were left behind after the frame with the cartridges was withdrawn. This prevented uptake of the algal cells. This procedure is shown schematically in Fig. 1.

The rack of cellulose acetate cartridges with the absorbed contents of the wells was then placed over a rack of 48 transfer tubes. The cartridges were pushed into the tubes with a plastic transfer fork designed for the purpose. The molded plastic transfer tubes were then separated and dropped into 15- by 115-mm glass scintillation tubes. The rack of scintillation tubes (containing the adsorption cartridges within the plastic tubes) was dried for 6 h at 60°C. Drying was found to increase the precision of replicate counts, apparently because drying distributed the radionuclide along the length of the cartridge and thereby standardized the counting geometry.

Experimental procedure. Since the microculture plate technique utilized small volumes of cell culture and reagents, several alga-pH-metal combinations could be studied simultaneously with sufficient controls and replicates to provide data for statistical analysis. Control wells received 25 μl of distilled water rather than algal cell suspension; in all other respects,

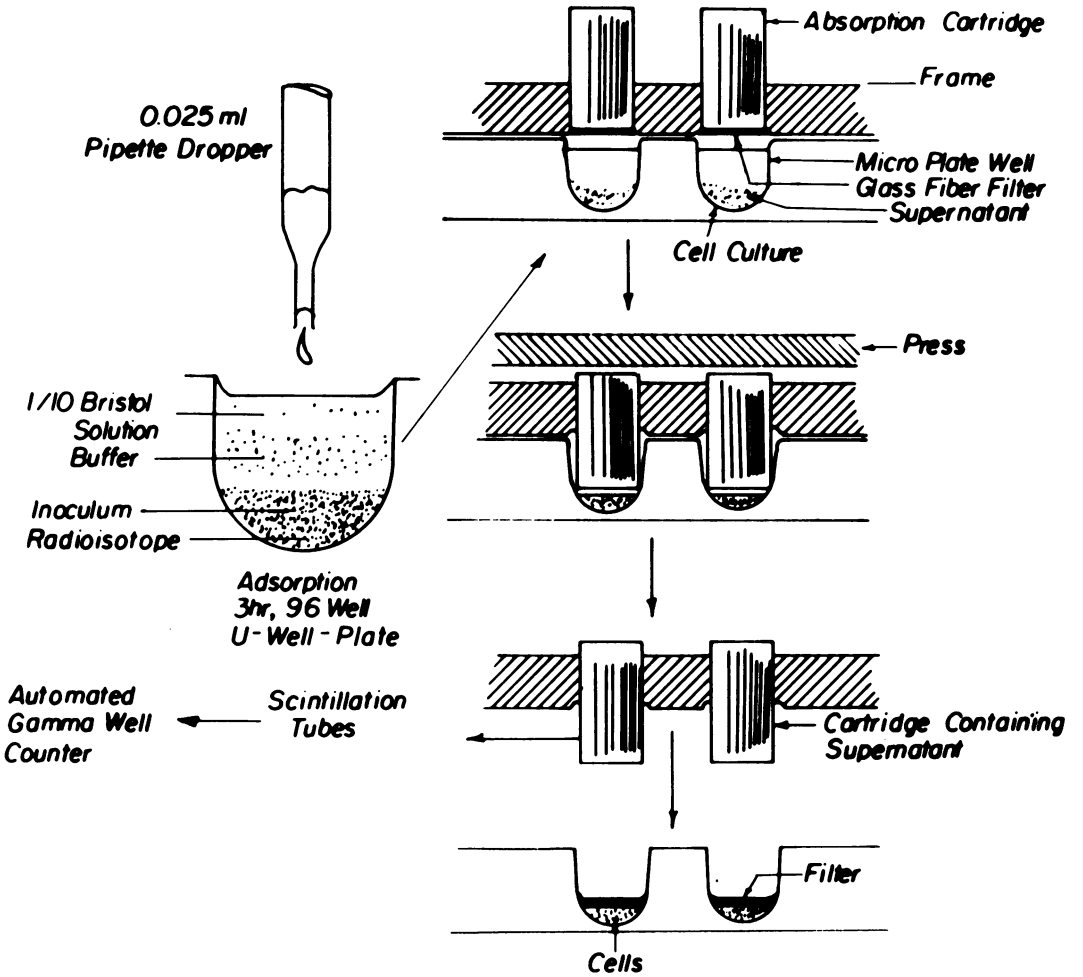

FIG. 1. *Schematic representation of experimental procedure used in assaying the concentration of radionuclides by algae.*

the wells were similar, as described above. The control plate for a specific radionuclide had one row of 12 wells at each pH interval from 3 to 10. These 12 wells were maintained at a designated pH with the buffers and provided the basis of comparison for experimental wells at the same pH.

Two algae were tested on each experimental plate and supplied with a single radionuclide (Fig. 2). Experimental plates had 6 replicate wells for each algae-pH combination for comparison against the 12 replicate wells at the same pH on the control plate. Rows corresponding to pH 3 on the experimental plate received 25 μl of distilled water rather than algal cell suspension; these were utilized as an interplate control.

Statistical methods. Data obtained from the gamma counter (counts per minute of radionuclide remaining in the supernatant) were used with algal dry weight (determined by drying a sample of washed cells to constant weight at 103°C) to calculate the following information: (i) removal efficiency = $[(\bar{X}$ control $- \bar{X}$ experimental$)/(\bar{X}$ control$)] \times 100$; (ii) accumulation coefficient (AC) = (micrograms of metal removed)/(grams of algae dry weight); (iii) CF = (AC)/(micrograms of metal per milliliter of medium).

Significant differences between mean control and mean experimental counts were determined by use of Dunnett's t criterion. This utilizes an estimate of the pooled variance and one tabulated t-value to determine significant differences between each of several experimental means (in this case, mean counts from the six experimental wells for an alga-metal-pH combination) and a common control mean (i.e., mean count from the 12 wells on the control plate) (30). It is important to note that experimental means were compared with control means at the same pH. There were then, in effect, eight assays being performed simultaneously, corresponding to each row of the plates.

Controls were maintained in two ways. The interplate control data from each experimental plate were compared with the control plate data. This comparison revealed whether any plate had been handled differ-

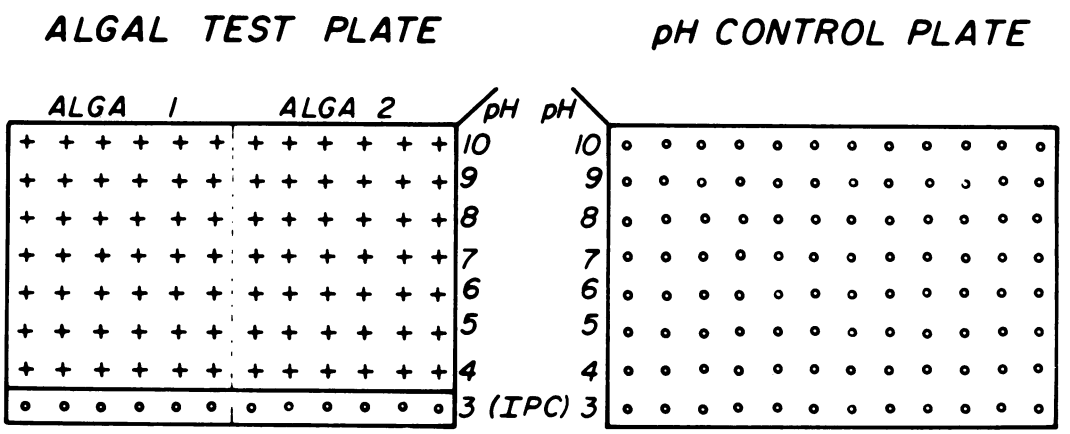

FIG. 2. *Experimental protocol for comparing the removal of heavy metals by algae, maintained 3 hours at different pH. Symbols:* (+) *wells with algal inoculum;* (○) *wells with no algal inoculum. IPC, Interplate control.*

ently than the others, since, as noted above, all interplate control wells were identical. If the interplate control data for any experimental plate were found to be significantly different than the control plate data, the data on that plate were rejected (Fig. 2).

The second control procedure was used on half of an experimental plate. All six wells at each pH received distilled water rather than algal cell suspension. The means (six replicates) for each pH 3 to 10 were compared to the corresponding control plate mean. In this manner, any unusual trends across the range of pH could be detected, such as would be caused by adsorption of the metal on the well surfaces at high pH.

RESULTS

The first series of experiments studied the effects of algal species, pH, and culture age on metal uptake by algae. The algal cells were exposed to a radionuclide for 3 h in the dark at 22°C. In this manner, light-dependent metal removal was minimized. Data (expressed as CF) generated from 11-day-old cultures are displayed in Table 1, and the same information from 44-day-old cultures is illustrated in Table 2. Representative data used to generate Tables 1 and 2 are presented in Tables 3 and 4. The following example uses data from *N. pelliculosa* (Table 4). Removal efficiency is calculated by equation i: $(7{,}570 - 5{,}490)/7{,}570 \times 100 = 22\%$. The accumulation coefficient utilizes dry weight data and the fraction of metal removal by the algae (equation ii): $(0.22 \times 0.317)/0.95 \times 1{,}000 = 72\ \mu g/g$. Finally, the CF uses the initial metal concentration (equation iii): $72/0.317 = 230$.

The data analysis in Tables 3 and 4 were repeated for every pH-metal-culture age combination. The *F*-value in Table 3 indicated whether any treatment mean was significantly different than the control mean; the *t*-values calculated in Table 4 identified specific treatment means different (at the 95% confidence level) from the control mean. The coefficients of variation around a given mean were generally in the range of 0.05 to 0.09 (Table 4).

Differences in CF were observed between species as well as between strains of the same organism (*Nostoc* spp.) and between the same algae at different culture ages (Tables 1 and 2). In general, young cultures exhibited very much higher CF than old cultures. For example, 11-day *Chlamydomonas* sp. cultures showed a CF of 18,600 for lead at pH 6, whereas a 44-day culture of the same organism at the same pH revealed a CF for lead of 360 at pH 6. This trend was consistent for all algae studied at both culture ages.

It is apparent that a given alga can accumulate different metals to different degrees. For example, the pure cultures of *Chlamydomonas* sp. showed CF of 18,600, 9,060, and 5,480 for lead, mercury, and cadmium, respectively, at pH 6. The cells used to test the accumulation of each of the metals were derived from the same culture; therefore, differences in accumulation can only be due to different responses by the alga to the three metals.

In these experiments algae appeared to accumulate metals in the order Hg > Cd > Pb > Zn (expressed as CF). An important exception is *Chlamydomonas* sp. Algae were not found to accumulate appreciable amounts of zinc at any pH, a point which will be discussed later.

Some CF listed in Table 1 are high. For example, 11-day cultures of *N. muscorum* A at pH 6 to 10 had CF approaching 10^5 for mercury. This was due to both fairly high removal efficiencies (33% at pH 8) and a very low biomass (0.005 mg/ml).

TABLE 1. *CF for heavy metals[a] by 11-day-old algae, 3 h, 22°C in the dark*

Element	Alga	CF at pH:						
		4	5	6	7	8	9	10
Cadmium	*N. pelliculosa*	NS[b]	NS	3,640	4,370	4,900	NS	NS
	Chlamydomonas sp.	NS	NS	5,480	NS	NS	NS	NS
	C. pyrenoidosa	NS	18,200	16,700	32,000	19,800	NS	17,100
	Mougeotia sp.	NS	NS	NS	NS	NS	NS	NS
	S. obliquus	NS	NS	2,700	3,600	3,100	NS	NS
	U. fimbrinata	NS	14,100	11,400	NS	NS	NS	NS
	Zygnema sp.	NS	8,600	NS	NS	NS	NS	NS
	Nostoc sp. 586	NS	1,260	1,140	NS	1,470	NS	NS
	N. muscorum A	NS	NS	NS	NS	NS	NS	NS
	Oscillatoria sp.	NS	NS	1,160	1,310	NS	NS	NS
	S. calcicola	NS	NS	NS	NS	NS	NS	NS
Lead	*N. pelliculosa*	NS	NS	NS	NS	NS	NS	NS
	Chlamydomonas sp.	19,500	17,900	18,600	16,700	17,400	20,100	NS
	C. pyrenoidosa	NS	NS	NS	NS	NS	NS	NS
	Mougeotia sp.	NS	NS	NS	NS	NS	8,180	NS
	S. obliquus	NS	NS	NS	NS	NS	NS	NS
	U. fimbrinata	NS	NS	NS	NS	NS	19,900	NS
	Zygnema sp.	NS	NS	NS	NS	NS	NS	NS
	Nostoc sp. 586	NS	NS	1,560	1,920	NS	2,400	NS
	N. muscorum A	NS	NS	NS	NS	NS	43,800	NS
	Oscillatoria sp.	NS	NS	NS	NS	NS	1,520	NS
	S. calcicola	NS	NS	NS	1,410	NS	1,320	1,690
Mercury	*N. pelliculosa*	4,700	4,290	6,780	3,460	4,040	5,420	4,130
	Chlamydomonas sp.	15,810	10,700	9,060	NS	NS	10,900	NS
	C. pyrenoidosa	35,100	24,000	44,100	46,700	47,300	44,800	31,700
	Mougeotia sp.	NS	NS	12,800	11,300	NS	10,900	11,600
	S. obliquus	NS	3,540	6,540	5,040	5,190	6,340	5,160
	U. fimbrinata	25,700	NS	18,220	NS	NS	21,100	24,130
	Zygnema sp.	10,200	NS	17,100	9,200	12,000	11,800	11,300
	Nostoc sp. 586	5,120	4,680	4,130	1,950	3,290	4,080	3,270
	N. muscorum A	NS	NS	68,000	43,500	66,900	87,500	66,900
	Oscillatoria sp.	3,380	3,420	3,370	NS	2,260	2,860	NS
	S. calcicola	2,590	2,760	2,390	2,150	3,040	3,070	2,800

[a] Initial heavy metal concentrations: Cd, 0.37 μg/ml (3.3 μM); Pb, 0.25 μg/ml (1.2 μM); Hg, 0.041 μg/ml (0.20 μM).

[b] NS, Not significant at the 95% confidence level.

The influence of pH on metal accumulation by algae is apparently species specific (Tables 1 and 2). *Chlamydomonas* sp. removed lead efficiently at pH 4 to 9, whereas *U. fimbrinata* accumulated lead to a significant degree only at pH 9. *Nostoc* sp. 586, in both young and old cultures, had a CF for mercury three to four times the CF of either cadmium or lead. Mercury was removed from the solution by the blue-green algae at pH 4 to 10, whereas cadmium and lead were removed to a significant degree only at some intermediate pH values (Tables 1 and 2). Differences such as these probably reflect differences in the composition of the cell coverings of the various algae employed in this study.

On the basis of the above experiments, some combinations of alga-pH-metal were selected for further study. *Chlamydomonas* sp.-pH 6-lead and *Nostoc* sp. H- and *Nostoc* sp. 586-pH 6-mercury were among the combinations selected to study the course of metal uptake over a period of 24 h. In this experiment, several identical plates were made up as described above. Supernatants were collected from different plates and assayed after 0, 0.5, 1, 3, 6, 9, 12, and 24 h. Lead and mercury were removed rapidly from nutrient solution by the algae employed (Fig. 3 and 4). The initial adsorption of lead (Fig. 3) was commonly followed by a limited, slow release for a few hours, perhaps as some surface-lead complexes were released from the algal surface. Two *Nostoc* sp. cultures (Fig. 4) removed mercury from solution in nearly identical fashion. There was little evidence of mercury release over the 24-h course of the experiment.

The influence of the buffer system used to

TABLE 2. *CF for heavy metals[a] by 44-day-old algae, 3 h, 22°C in the dark*

Element	Alga	CF at pH:						
		4	5	6	7	8	9	10
Cadmium	*N. pelliculosa*	NS[b]	70	230	NS	320	NS	NS
	S. obliquus	40	NS	110	NS	NS	NS	NS
	Gleotrichia sp.	90	70	160	NS	NS	NS	NS
	Nostoc sp. 586	NS	50	90	NS	NS	NS	NS
	N. muscorum A	60	50	NS	NS	NS	NS	NS
	Nostoc sp. H	60	40	130	NS	NS	NS	NS
Lead	*N. pelliculosa*	NS	NS	NS	NS	NS	NS	NS
	Clamydonomonas sp.	350	300	360	300	300	340	NS
	Gleotrichia sp.	140	140	150	NS	NS	60	NS
	Nostoc sp. 586	60	NS	90	90	60	120	NS
	N. muscorum A	NS	NS	NS	NS	NS	NS	NS
	Nostoc sp. H	180	140	230	190	160	220	NS
	Nostoc sp. C	50	NS	NS	NS	NS	60	NS
	Nostoc sp. W	120	130	140	130	NS	150	NS
	Oscillatoria sp.	260	70	190	90	110	190	NS
	S. calcicola	60	NS	90	80	60	70	NS
Mercury	*S. obliquus*	270	320	320	280	260	250	350
	Gleotrichia sp.	440	390	270	140	180	NS	NS
	Nostoc sp. 586	280	360	320	260	250	NS	140
	Nostoc sp. H	350	410	330	240	220	NS	150
	S. calcicola	360	370	330	280	290	330	290

[a] Initial heavy metal concentrations: Cd, 0.32 μg/ml (2.8 μM); Pb, 0.38 μg/ml (1.8 μM); Hg, 0.024 μg/ml (0.12 μM).

[b] NS, Not significant at the 95% confidence level.

TABLE 3. *Analysis of variance for cadmium-44-day algae-pH 6*

Source	df	SS[a]	MS[a]	F[b]
Treatment	8	390	48.7	6.83
Error	51	363	7.13	

[a] Multiply given SS (sum of squares) and MS (mean squares) by 10^5 for actual values; data are counts per minute remaining in the supernatant.

[b] Indicates tht at least one treatment mean was significantly different than the rest ($P < 0.05$). F = ratio of treatment MS to error MS.

TABLE 4. *Data[a] for cadmium-44-day algae-pH 6 used to generate CF*

Alga	Dry wt (mg/ml)	n	$\bar{X}$	σ_x	Calculated t-value[b]
Control		12	7,570	500	
N. pelliculosa	0.95	6	5,940	510	3.86
S. obliquus	1.88	6	6,070	150	3.56
Gleotrichia sp.	1.80	6	5,450	640	5.04
Nostoc sp. 586	2.03	6	6,150	330	3.37
N. muscorum A	1.88	6	6,850	1,980	1.72
Nostoc sp. H	1.76	6	5,900	350	3.97
Control A		6	7,830	1,320	−0.67
Control B		6	7,160	380	0.98

[a] Data are counts per minute remaining in supernatant.

[b] Values exceeding a tabulated t-value of 2.87 were significant at 95% confidence level.

control pH was studied by using lead and 12-day cultures of *Chlamydomonas* sp. With the exception of the citrate buffer, the choice of buffer had little effect on lead accumulation by *Chlamydomonas* sp., at least at pH 6 (Table 5).

Studies on the uptake of mercury by algae have been complicated by losses due to volatilization. The microplate technique was modified to determine the degree of mercury loss in these short-term, quiescent assays. *Chlamydomonas* sp. and *Chlorella* sp. (21-day cultures) were utilized. Four plastic plates were prepared with a pH 7 buffer, $^{203}Hg(NO_3)_2$ (0.5 μg/ml), Bristol maintenance medium, and either washed algal suspension or distilled water. Two rows (12 wells) of control wells (no algae), wells containing *Chlamydomonas* sp. and wells containing *Chlorella* sp. were prepared on each plate. One row of each (control, *Chlamydomonas* sp., *Chlorella* sp.) was then "capped" with two drops (0.05 ml) of sterile mineral oil. Of the four plates, two were placed in the dark and two were placed under laboratory illumination. One light and one dark plate were harvested after 3 h of exposure, the remaining two were harvested after 6 h. This factorial design was 2 (light, dark) × 2 (capped, uncapped) × 2 (3 h, 6 h) × 2 (*Chlamydomonas* sp., *Chlorella* sp.) × 12 (number of replicates). Response was measured by calculating concentration factors, as before. Capped wells contain-

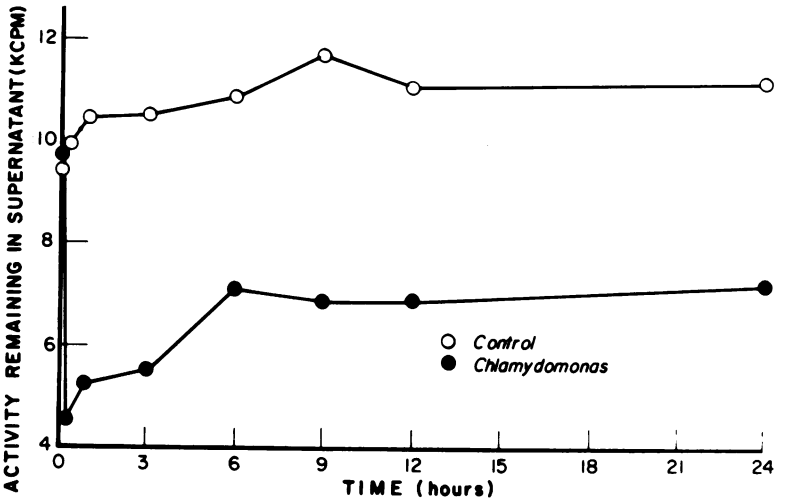

FIG. 3. *Removal of ^{210}Pb by Chlamydomonas sp. at pH 6.*

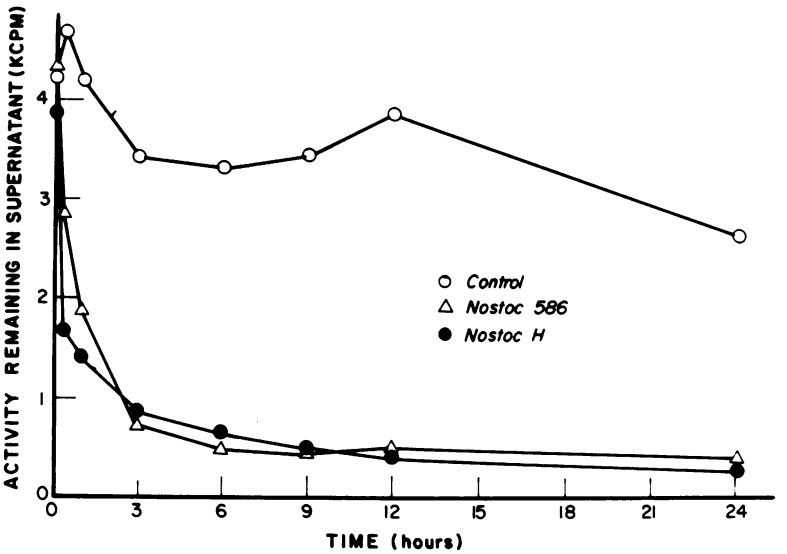

FIG. 4. *Removal of ^{203}Hg by Nostoc sp. 586 and Nostoc sp. H at pH 6.*

TABLE 5. *Effects of buffers (pH 6) on the uptake of lead[a] by 12-day Chlamydomonas sp.*

Buffer	CF	
	3 h	6 h
Borax-KH_2PO_4	1,920	2,040
Sodium citrate-citric acid	270	240
Succinic acid-NaOH	1,980	2,050
Citric acid-Na_2HPO_4	1,710	2,050
Phthalate-NaOH	1,840	1,960
Tris[b]-maleic anhydride-NaOH	2,020	2,210

[a] Initial ^{210}Pb concentration, 0.06 μg/ml (0.3 μM).
[b] Tris, Tris(hydroxymethyl)aminomethane.

ing algae were compared with the capped control wells; open experimental wells were compared with open control wells for purposes of CF calculations.

A summary of the data is provided in Table 6. Analysis of the data by multiple regression techniques revealed that the only significant effect ($P < 0.01$) was due to species; i.e., *Chlorella* sp. versus *Chlamydomonas* sp. Under the conditions of the experiment, none of the factors (light-dark, 3h-6h, or capped-uncapped) proved significant in explaining the response. Furthermore, higher-order interactions (i.e., light ×

TABLE 6. *Removal of mercury[a] by algae[b] as influenced by light, aeration, and exposure time*

Alga	Exposure (h)	CF[c]			
		Light		Dark	
		Capped[d]	Uncapped	Capped[d]	Uncapped
Chlorella sp.	3	2,340 ± 120	2,600 ± 80	2,380 ± 180	2,570 ± 70
	6	2,210 ± 110	2,410 ± 110	2,340 ± 320	2,480 ± 90
Chlamydomonas sp.	3	NS[e]	NS	NS	300 ± 260
	6	NS	NS	NS	300 ± 280

[a] Initial ^{203}Hg concentration, 0.5 μg/ml (2.5 μM).
[b] Culture age, 21 days. Dry weights: *Chlorella* sp., 0.34 mg/ml; *Chlamydomonas* sp., 0.52 mg/ml.
[c] Mean of 12 wells ± 1 standard deviation.
[d] Microplate wells capped with 0.05 ml of sterile mineral oil.
[e] NS, CF not significantly different from zero.

capped) were not found to be significant. In all cases, the CF for *Chlamydomonas* sp. were low, again indicating insignificant removals at this pH (Table 1). The CF for the 21-day culture of *Chlorella* sp. were lower than the 11-day CF for this alga.

DISCUSSION

We have used algae in our work, but there is no reason to believe that the technique would not be useful in similar studies with bacteria, fungi, protozoa, and other microbes. Furthermore, other γ-emitting nuclides, such as ^{54}Mn, ^{75}Sc, ^{133}Ba, and ^{137}Cs, could be used without modification of the technique to determine uptake rates, levels of accumulation, and other data concerning these elements and various microorganisms. Modification involving solubilization of the absorption cartridge and incorporation of the resulting liquid into scintillation fluid would make the technique usable for β-emitting nuclides such as ^{36}Cl, ^{45}Ca, ^{63}Ni, and ^{106}Ru. Experiments in our laboratory have shown the feasibility of this modification in experiments involving the uptake of ^{14}C-labeled polychlorinated biphenyls by algae (L. E. Jennett, M.S. thesis, Syracuse University, 1980). The technique presented here represents an increase in speed and precision over our previous work (18).

The remarkable differences in CF between young and old cultures of algae was due to at least two factors. First, old cultures had a considerably higher cell concentration than did young cultures. It has been found that heavy metal uptake by yeast cells was largely dependent on cell concentration; at a given equilibrium concentration of mercury, for example, yeast cells adsorbed more mercury at low cell concentration than at higher cell concentration. It was postulated that electrostatic interaction among cells in suspension was the main factor for the cell concentration dependency of metal adsorption, with a larger quantity of cation being adsorbed on the negative charged cell surfaces when the distance between cells was great (17).

The second factor responsible for higher CF in young rather than old cultures concerns change in the cell surface itself. Although the literature on this point is confusing, the fact that Formalin-killed cells of *Chaetoceros costatum* accumulated about two times as much ^{203}Hg as did living cells (14) indicates the importance of the state of the cell surface in short-term metal uptake studies. Another study (1) found that radionuclides (^{60}Co and ^{137}Cs) were taken up rapidly by *Anabaena variabiles* in the logarithmic growth phase and more slowly by the same organism in both the linear and the stationary growth phases, although nearly the same concentration of nuclide in the organism was attained in each case. These experiments were performed over a period of 200 h, whereas our assays measured accumulation over only 3 h. It is therefore possible that the lower CF in old cultures in our work represent slower rates of accumulation. Each of the four metals studied will be discussed in turn.

Cadmium. The CF found in this study are reasonably close to values reported in the literature for cadmium. For example, Polikarpov (26) reported CF for *Cladophora fracta, C. glomerata,* and *Mougeotia* sp. in the range of 2.8×10^3 to 1.7×10^4.

In general, the green algae employed in this study were more efficient accumulators of cadmium than were the blue-green algae (Tables 1 and 2). Three cultures of blue-green algae, *N. muscorum* A, *Nostoc* sp. H, and *S. calcicola* did not remove cadmium significantly at any pH, whereas only one green alga, *Mougeotia* sp., showed such negative results. Why the green algae appear more efficient in this regard cannot be answered from the data at hand.

Lead. As with cadmium the CF observed in

this study for young cultures are reasonably close to literature values for lead. Trollope and Evans (31) found CF for *Mougeotia* sp., *Zygenema* sp., *Ulothrix* sp., and *Oscillatoria* sp. of 5.6×10^3 to 2.5×10^4, with the blue-green alga at the low end of the range.

In our study *Chlamydomonas* sp. proved superior to all other algae studied in its ability to concentrate lead, with 11-day culture CF of 2×10^4 at pH 4 to 9. No other alga studied had as large a CF over as wide a pH range. Since *Chlamydomonas* sp. was the only flagellated form studied, these data provide indirect evidence for the importance of flagella as sites of lead accumulation. This was suggested by Hessler (15), who studied the effects of lead on normal and flagella-free mutants of *Platymonas subcordiformis*.

Mercury. Polikarpov (26) reported CF for mercury of 6×10^3 for *C. fracta* and *C. glomerata*. Others (8, 11) have reported a CF of 1.6×10^2 to 1.2×10^3 (wet weight basis) for *Chlorella* sp. and 5×10^4 (wet weight basis) for the diatom *Synedra ulna*. These values are comparable to the range of values found in this study.

The results of this study indicate that mercury uptake is a general phenomenon with little apparent dependence on pH. No single organism proved superior in its ability to accumulate mercury, although *C. pyrenoidosa* (11-day cultures) exhibited the highest CF (5×10^4) for the widest pH range (pH 6 to 9).

The results of the light-dark, capped-uncapped experiment (Table 6) deserve comment, particularly in view of reports in the literature detailing light-dependent volatilization of mercury (2–4). It is important to note that the literature studies generally involved mixed, aerated cultures and monitored mercury removal over a longer period of time than the 3 or 6 h reported here. It is possible that the quiescent experimental conditions we employed minimized the transport of mercury vapor from the wells. Our CF with respect to mercury should therefore be viewed as initial values which would change with time as volatilization occurs.

Zinc. Several workers have reported CF for zinc on the order of 10^3 to 10^4 (5, 13, 26). This study did not find zinc amassed to any significant degree, probably due to the dark conditions employed during the study. Reports (10, 22) indicate that the uptake of zinc is a light-dependent phenomenon.

The demonstrated ability of algae to concentrate heavy metals has both practical and theoretical importance. A wastewater treatment system designed to use benthic algae to treat mining and milling wastes has been reported (12, 19, 20). On the theoretical side, algal blooms in natural waters may well alter the distribution of heavy metals in the water column and thereby change, on a short-term basis, patterns of bioaccumulation of these metals through food chains. Morris (23) observed changes in distribution of particulate and dissolved zinc, copper and manganese as a result of a *Phaeocystis* sp. bloom in the Menai Straits. The phenomenon of heavy metal accumulation by algae has an impact on many environmental problems.

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