THE USE OF ALGAE TO CONTROL HEAVY METALS IN THE ENVIRONMENT

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ABSTRACT

Aqueous effluents from a lead mining and milling operation located in southeastern Missouri, USA, caused a degradation of stream quality despite treatment by a large tailings pond. The receiving stream was choked with algal mats which accumulated unexpectedly large amounts of manganese, lead and zinc. A wastewater treatment system was designed to utilize algae and benthic macrophytes to remove metals from the tailings pond effluent. The system has proved successful and water quality in the receiving stream has been improved to drinking water standards.

Experiments were conducted to understand more fully the phenomenon of heavy metal accumulation by algae. Radionuclides (^{210}Pb, ^{203}Hg, ^{65}Zn, ^{109}Cd) were used in conjunction with commercially available microculture apparatus to screen several species of algae for heavy metal accumulation. It was found that all species of algae studied concentrated mercury, green algae were more efficient accumulators of cadmium than blue-green algae, one alga *(Chlamydomonas)* proved best at removing lead from solution and no alga studied removed zinc.

INTRODUCTION

The discovery of lead near Viburnum, Missouri in 1955 and the subsequent development of the deposits in the world's largest lead mining area provided a unique opportunity to study the effects of the heavy metal mining industry system[1]. Many treatments have been used in this region, including the standard tailings lagoons, complete wastewater recycling and the algal meander system with which this paper is primarily concerned.

The algal meander system was developed in the early 1970's when it was observed that lagoon effluents from lead/zinc mining and milling operations were causing dense matlike growths of algae in receiving streams[2]. These benthic growths collected fine solids and unexpectedly large amounts of heavy metals. Representative values for heavy metal accumulation by stream flora are shown on Table 1. Table 2 gives data on metal concentrations in the stream water. The data for these tables were collected from two streams: Strother Creek which received tailing pond effluent and Upper Bee Fork Creek which received no industrial inputs and served as a control[3].

TABLE 1. *Heavy Metal Concentration (μg/g dry weight) in Stream Flora, Missouri, USA*

Date	Sample	Description	Pb	Zn	Cu	Mn
A. Strother Creek						
8 Oct 1972	B3	*Mougeotia*	9829	4898	299	13,612
8 Oct 1972	P35	*Potamogeton*	4695	2431	181	13,563
8 Oct 1972	P41	*Cladophora & Hydrodictyon*	247	262	26	4,535
15 May 1973	P88	*Myriophyllum*	4858	3686	215	2,921
23 May 1974	P389	*Oscillatoria*	5617	3260	173	—
23 May 1974	P394	*Oscillatoria*	5483	4030	189	—
B. Upper Bee Fork Creek						
23 May 1972	—	*Cymbella*	8	23	—	793
15 May 1973	P93	*Sedge*	0	39	1	196
21 July 1973	P154	*Spirogyra*	1	26	7	2,557

Source: Adapted from reference 3, Appendix T.

Economic considerations precluded water recycling at this site at the time the study was undertaken. Thus the use of algae as a wastewater treatment method--the algal meander system--was suggested by our own observations and reports in the literature that algae may concentrate large quantities of heavy metals. This treatment system, shown schematically in Figure 1, followed the normal lagoon treatment system and employed an extensive

TABLE 2. *Heavy Metal Concentration (mg/l) in Stream Waters, Missouri, USA.*

Element	Mean / Std. Dev. / No. of Samples	Strother Creek Unfiltered	Strother Creek Filtered	Upper Bee Fork Creek Unfiltered	Upper Bee Fork Creek Filtered
Pb	$\bar{x}$	0.077	0.014	0.041	0.015
	σ	0.165	0.015	0.164	0.035
	n	31	21	31	21
Zn	$\bar{x}$	0.041	0.026	0.012	0.014
	σ	0.048	0.021	0.009	0.016
	n	31	21	31	21
Cu	$\bar{x}$	0.012	0.010	0.011	0.010
	σ	0.005	0.002	0.004	
	n	31	21	31	21
Mn	$\bar{x}$	0.131	0.101	0.012	0.011
	σ	0.164	0.177	0.005	−0.003
	n	28	21	28	21

Source: Adapted from reference 3, Appendix Q.

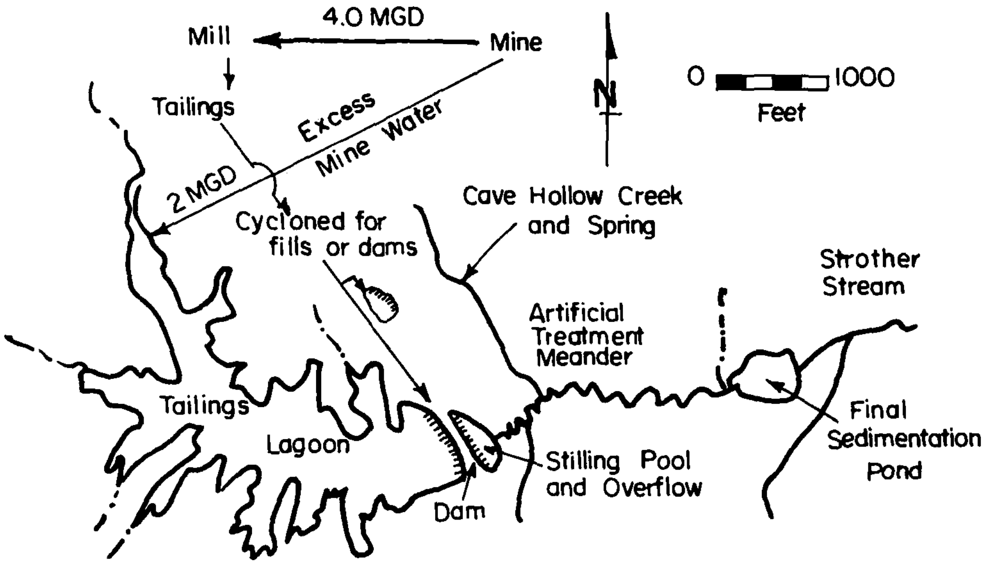

Fig. 1. Algal meander system, showing water flows in million gallons per day (MGD).

series of shallow meanders approximately one meter in depth. These shallow, rapidly flowing meander systems encourage the growth of benthic flora, including algae, which trapped both particulate and dissolved heavy metals. The meanders empty into a final settling pond with baffled weirs to prevent algal overflow into receiving baffled weirs to prevent algal overflow into the receiving stream. This treatment system has been thoroughly studied and reported in the literature[4,5,6]. Work by Ryck and Whitely[7] has shown that the receiving streams which had been severely affected returned to normal conditions within a year after the meander system was built. Additional work by Callier and Jennett[8] showed that the system also was effective in removing the toxic trace organics from the milling reagents. There is considerable evidence that it was these milling reagents which killed the predators that consume algae in the receiving streams and caused the benthic algal mats to form in the first place.

This empirically designed system has been proved to remove more than 99% of the influent heavy metals. It has been effective at returning the receiving stream to its 0original biological and chemical level. Research at Syracuse University for the last two years has concentrated on developing rapid techniques to study the conditions under which algae remove metals efficiently and to evaluate such parameters as metal type, algal type and age, exposure time and pH that modulate the process.

EXPERIMENTAL METHODS

Representative algae were studied for their ability to concentrate heavy metals. The selection was dictated by several factors. First, it was desired to have species of algae from the major taxonomic groups, i.e., cyanophytes, chlorophytes, etc. Second, it was thought necessary to select algae which exhibited colonial, filamentous and unicellular forms. Third, the species selected had to be amenable to laboratory culture in relatively simple culture media. Fourth, the algae had to grow to a respectable biomass in a relatively short period of time.

The species of algae employed included the diatom *Navicula pelliculosa;* the green planktonic algae *Chlamydomonas* sp., *Chlorella pyrenoidosa* and *Scenedesmus obliquus;* the greeen benthic algae *Chlorotyllium* sp.,*Spirogyra* sp.,*Ulothrix fimbrinata* and *Zygnema* sp., and the blue-green algae *Gleotrichia* sp., *Oscillatoria* sp., *Schizothrix calcicola* and *Nostoc* (8 strains). These were maintained on a 16 hour-8hour dark cycle at 20°C. All cultures were grown in 75ml of synthetic media. Diatoms were cultured in Darley and Volcani medium [9] and the other algae in Bold's Bristol medium[10]. Algae were obtained from culture collections at the University of Texas, State University of New York at Binghamton and Syracuse University.

SCREENING METHODS

The ability of these representative algae to concentrate specific heavy metals was determined by adapting the Titertek* supernatant collection system[11] for assaying radionuclides of heavy metals. This technique permitted the simultaneous study of several factors affecting heavy metal accumulation and provided a sufficient number of replicates for statistical analysis.

Plastic microculture plates with 96 U-bottom wells each capable of holding 200 ul of solution were used. The well contained 25 ul droplets of buffer, radionuclide solution (^{109}Cd, ^{210}Pb, ^{203}Hg, ^{65}Zn), maintenance medium and algae or distilled water. The drops were delivered by calibrated micropipettes. After exposure of the algae to the nuclides under appropriate conditions the supernatants were collected in cellulose acetate adsorption cartridges from 48 wells at a time. Glass fiber filter discs on the bottom of the cartridges prevented removal of the metal-labelled algal cells from the wells. The cartridges were dried and transferred to glass scintillation vials. The residual activity was determined with a gamma counter. Figure 2 shows schematically the process, which is described more fully elsewhere[12]. The above technique represents an improvement in speed and of precision over

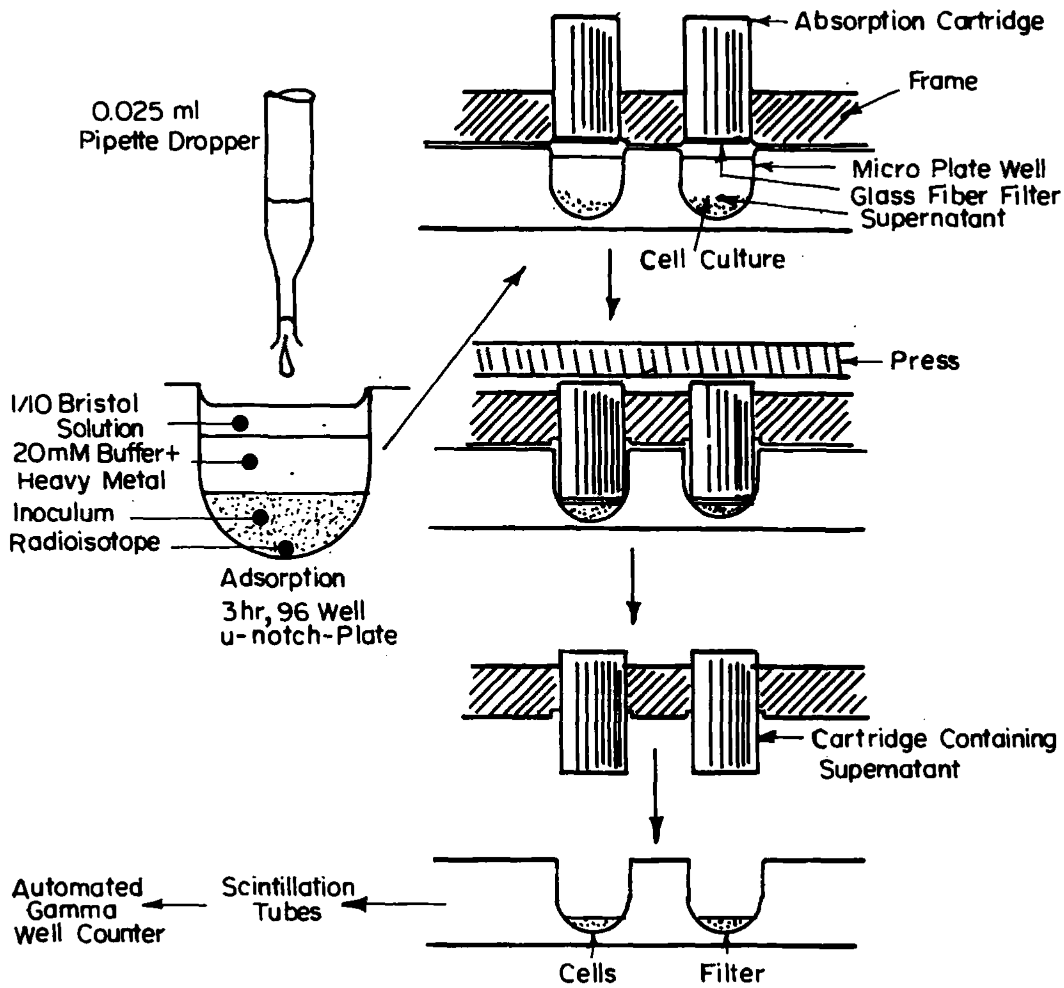

Fig. 2. Details of microtiter technique.

an earlier technique, in which the algae-nuclide complex was separated by centrifugation and the supernatant collected by Takatsy loops[13].

The statistical significance between residual activity in controls and in experimental wells was determined using Dunnett's *t* criteria[14]. This permitted several experimental mean activities, one for each alga, to be compared with a common control mean activity, all at a given pH. When significant differences in activity were found, concentration factors were calculated using the control and experimental means, isotope mass data and also dry weights obtained from subsamples of the algal cultures.

TABLE 3. *Concentration Factors for Cadmium (Cd)*[a]

Organism	Type	Culture Age[b]	pH[c]	Concentration Factor[d]
Navicula pelliculosa	Diatom	Young	6–8	$3.6 - 4.9 \times 10^3$
		Old	5, 6, 8	70–320
Chlamydomonas	Green, flagellated	Young	6	5.5×10^3
Chlorella	Green, colonial	Young	5–9	$1.7 - 3.2 \times 10^4$
Scenedesmus obliquus	Green, colonial	Young	6–8	$2.7 - 3.6 \times 10^3$
Ulothrix fimibrinata	Green, filamentous	Young	5, 6	$1.1 - 1.4 \times 10^4$
Zygnema	Green, filamentous	Young	5	8.6×10^3
Gleotrichia	Blue-green, filamentous	Old	4–6	70–160
Nostoc 586	Blue-green, colonial	Young	5, 6, 8	$1.1 - 1.5 \times 10^3$
		Old	5, 6	50–90
Oscillatoria	Blue-green, filamentous	Young	6, 7	$1.2 - 1.3 \times 10^3$
Nostoc H	Blue-green, colonial	Old	4–6	40–130

Notes:

(a) Cadmium as the nitrate; concentrations = 0.37 μg/ml for young culture experiments; 0.32 μg/ml for old culture experiments.

(b) Young = 11 day; Old = 44 day.

(c) pH's for which significant removals were found.

(d) $\text{Concentration Factor} = \dfrac{\text{concentration of metal in algae}}{\text{concentration of metal in medium}}$

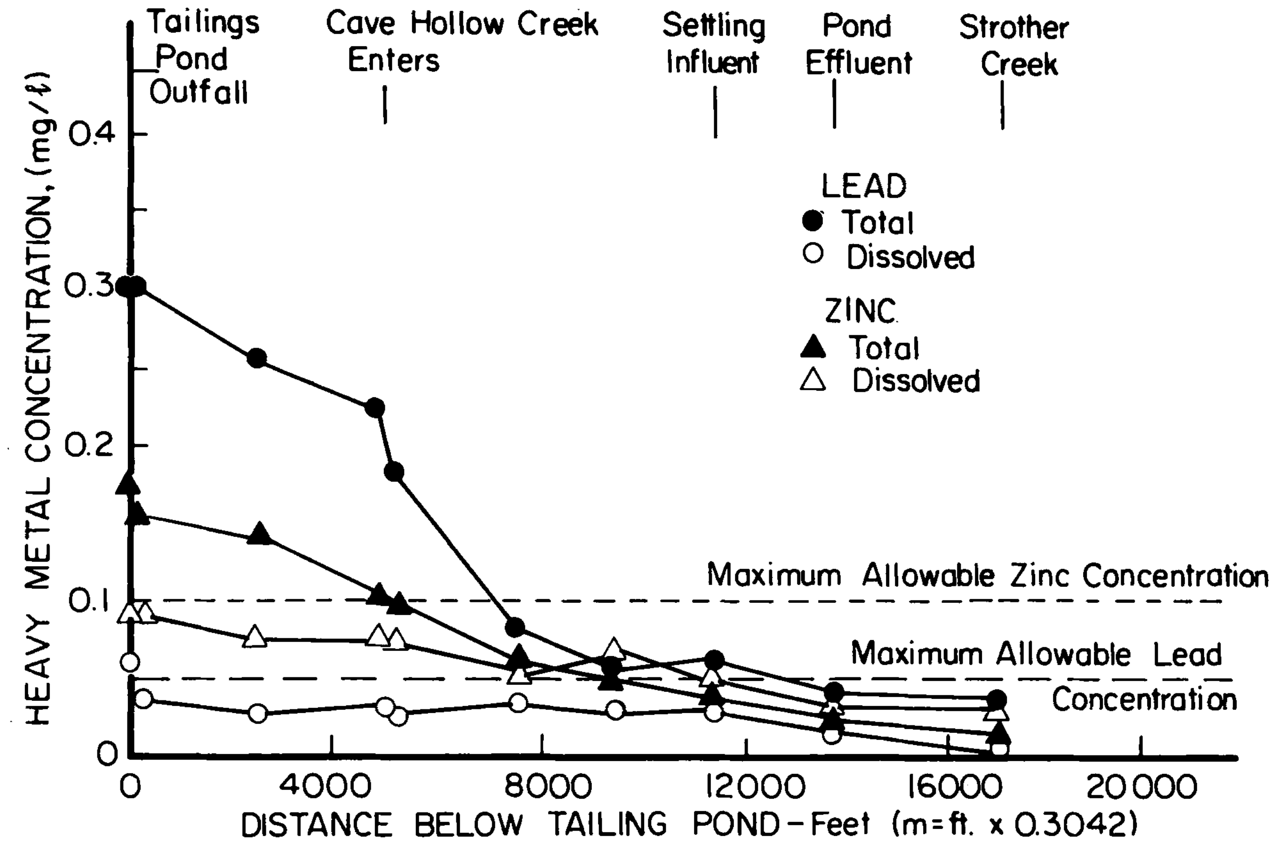

Fig. 3. Heavy metal removal in meander system.

RESULTS AND DISCUSSION

The results of these studies have been summarized in Tables 3, 4 and 5, using the concentration factor (CF) as the means by which an alga's ability to accumulate heavy metals was evaluated.

Several comments about the data in these tables should be made. Although an extensive review of the literature[12] found numerous reports that zinc is accumulated by a wide variety of algae, no zinc was amassed to any significant degree in this study. However, Findenegg[15] indicated that zinc uptake by a species of *Chlorella* was light dependent. Since all cultures in this study were kept in the dark, it was likely that this prevented zinc uptake.

Except for zinc, the results show reasonably good agreemnt with concentration factors reported in the literature. Concentration factors cannot be considered fixed numbers, though, and must be considered dependent upon the environmental conditions of an experiment.

Studies of the kinetics of heavy metal accumulation by algae indicate that it is a rapid phenomenon and adsorption was essentially complete at the end of three hours.

The information presented in Table 3 is one of the few studies of algae for which concentration factors for cadmium have been reported. Concentration factors on the order of 10^3 were observed. What is not immediately apparent is the generally lower CF for the blue-green algae. In fact, three young cultures of blue-green algae, *Nostoc muscorum A, Nostoc H* and *Schizothrix calcicola* did not remove cadmium significantly at any pH, while only one green algae, *Mougeotia* showed such negative results. The green algae appear to be much more efficient at accumulating cadmium than the blue-greens.

Concentration factors for lead (Table 4) have been determined; values in the order of 10^3 to 10^4 were observed for all algae studied. All blue-green algae screened were successful at removing lead, at least at one pH, but the diatom *Navicula pelliculosa* and the green *Chlorella* and *Scenedesmus obliquus* were not successful accumulators of lead at any pH.

Every organism screened in this study removed mercury (Table 5) at at least one pH with concentration factors in the order of 10^3 and 10^4.

This work has tended to confirm and extend suggestions made by other investigators. Hessler[15] has hypothesized that the flagella of *Platymonas subcordiformis* provide the sites for a significant fraction of the lead adsorbed by this alga. While this hypothesis was not specifically tested in this study, the superiority of *Chlamydomonas* in removing lead as compared to the nonflagellated algae, argues for the importance of flagella as a site for lead accumulation

Kerfoot and Jacobs[16] have noted that cadmium was accumulated to a greater extent by a green platymonad than by a mixture of diatoms. We found that green algae were generally more efficient accumulators of cadmium than either the diatom (*Navicula pelliculosa)* or blue-green algae.

Perhaps the most important contribution of this work is the demonstrated feasibility of using microtiter methods to study the large number of variables which influence heavy metals removal by algae. It makes it possible to estimate the multiple interactions between these environmental factors.

The success of the algal-meander treatment system in removing heavy metals from surface waters (as shown in

TABLE 4. *Concentration Factors for Lead (Pb)*[a]

Organism	Type	Culture Age[b]	pH[c]	Concentration Factor[d]
Chlamydomonas	Green, flagellated	Young	4–9	$1.7-2.0\times10^4$
		Old	4–9	$3.0-3.6\times10^2$
Mougeotia	Green, flagellated	Young	9	8.2×10^3
Ulothrix fimbrinata	Green, filamentous	Young	9	2.0×10^4
Nostoc 586	Blue-green, colonial	Young	6, 7.	$1.6-2.4\times10^3$
		Old	4, 6–9	$1.4-1.5\times10^2$
Nostoc H	Blue-green, colonial	Young	6, 9	$1.5-1.6\times10^3$
		Old	4–9	$1.4-2.2\times10^2$
Nostoc L	Blue-green, colonial	Old	4, 9	50–60
Nostoc W	Blue-green, colonial	Old	4–7, 9	$1.2-1.5\times10^2$
Oscillatoria	Blue-green, filamentous	Young	9	1.5×10^3
		Old	4–9	90–260
Schizothrix calciola	Blue-green, colonial	Young	7, 9, 10	$1.3-1.7\times10^3$
		Old	6–9	60–90

Notes:
[a] Lead as the nitrate, concentration = 0.03 μg/ml for young culture experiments; 0.04 μg/ml for old culture experiments.
[b] Young = 11 day; Old = 44 day.
[c] pH's for which significant removals were found.
[d] Concentration factor $= \dfrac{\text{concentration of metal in algae}}{\text{concentration of metal in medium}}$

Fig. 3) can be explained in part by the concentration of these elements on the surface of algal cells. The accumulation of lead by algae in the meander system and in the laboratory experiments was of the same order of magnitude. The concentration of lead in the meander system influent was six times higher than the 0.05 mg/l maximum contaminant level for drinking water, but it fell below the drinking water standard at the outfall below the final sedimental basin. This dramatic improvement in water quality was achieved with a minimal capital expenditure involved in constructing the meanders and no recurring operational costs.

The algal-meander system is not a panacea. Many industrial operations discharging heavy metals are located in sites that preclude using it as a wastewater treatment system. Where feasible, however, this technique does offer the attractive possibility of reclaiming certain types of valuable trace metals as well as requiring little or no energy for water pretreatment

Further research is needed to investigate more thoroughly the mechanisms by which algae accomplish this removal and to select for algal mutants which have increased avidity for metals, exhibit greater selectivity for specific metals and persist as the anchored or benthic form.

ACKNOWLEDGEMENTS

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TABLE 5. *Concentration Factors for Mercury (Hg)*[a]

Organism	Type	Culture Age[b]	pH[c]	Concentration Factor[d]
Navicula pelliculosa	Diatom	Young	4–10	$3.5-6.8\times10^3$
Chlamydomonas	Green, flagellated	Young	4–6, 9	$9.1\times10^3-1.6\times10^4$
Chlorella	Green, unicellular	Young	4–10	$2.4-4.7\times10^4$
Mougeotia	Green, filamentous	Young	6, 7, 9, 10	$1.1-1.3\times10^4$
Scenedesmus obliquus	Green, colonial	Young	5–10	$3.5-6.5\times10^3$
		Old	4–10	$2.5-3.5\times10^2$
Ulothrix fimbrinata	Green, filamentous	Young	4, 6, 9, 10	$1.8-2.6\times10^4$
Zygnema	Green, filamentous	Young	4, 6–10	$9.2\times10^3-1.7\times10^4$
Gleotrichia	Blue-green, colonial	Old	4–8	$1.4-4.4\times10^2$
Nostoc 586	Blue-green, colonial	Young	4–10	$1.9-5.1\times10^3$
		Old	4–8	$1.4-3.6\times10^2$
Nostoc muscorum A	Blue-green, colonial	Young	6–10	$4.4-8.8\times10^4$
Nostoc H	Blue-green, colonial	Young	4–10	$1.6-3.3\times10^3$
		Old	4–8	$1.5-4.1\times10^2$
Oscillatoria	Blue-green, filamentous	Young	4–6, 8, 9	$2.3-3.4\times10^3$
Schizothrix calcicola	Blue-green, colonial	Young	4–10	$2.2-3.7\times10^3$
		Old	4–10	$2.9-3.7\times10^2$

Notes:
[a] Mercury as the nitrate, concentration = 0.04 μg/ml for young cultures experiments; 0.02 μg/ml for old culture experiments.
[b] Young = 11 days; Old = 44 days.
[c] pH's for which significant removals were found.
[d] Concentration factor $= \dfrac{\text{concentration of metal in algae}}{\text{concentration of metal in medium}}$

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