United States
Environmental Protection
Agency

Industrial Environmental Research
Laboratory
Cincinnati OH 45268

Research and Development

EPA-600/S2-82-100 Mar. 1983

EPA

Project Summary

Factors Influencing Metal Accumulation by Algae

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Shallow beds of algae (algal meanders) have proved to be highly effective at removing heavy metals and organometallics from lead-zinc mine and mill wastes. A laboratory-scale research program was initiated to determine conditions under which algae were most effective at concentrating significant quantities of As, Cd, Cu, Hg, Ni, Pb, or An, for the purpose of potential application of the meander technology to new types of wastewater-metal problems.

Studies were performed on 20 species of algae to determine interactions of experimental variables affecting metal adsorption, species and strains of algae, type, form, and concentrations of metal, pH, culture age, micronutrient composition of culture medium, exposure to metal, and light intensity and exposure period. These numerous variables were studied by means of a rapid analytical technique, the Titertek™* supernatant collection system, to determine the adsorption of metal radionuclides.

Metal removal was observed to be fast (three hours), young growing cultures were seen to be most effective, and concentration factors > 1 x 10^4 were observed. The pH had little effect on accumulation of metals except lead. Heavy metals could be stripped from algal mats with 0.01 M EDTA or 0.1 N HNO_3. Ca and Mg were not effective competitors for the binding sites of Hg, Pb, and Cd. Neither Zn nor As was bound significantly at any pH. Experimental and literature data indicate that algae remove certain metals economically from a variety of waters and that the meander system can be used to recover these metals for processing.

This report is submitted in fulfillment of Grant No. R804734 by Syracuse University under the sponsorship of the U. S. Environmental Protection Agency. This report covers the period from September 13, 1976 through January 1, 1979 and work was completed as of June 30, 1979.

This Project Summary was developed by EPA's Industrial Environmental Research Laboratory, Cincinnati, OH, to announce key findings of the research project that is fully documented in a separate report of the same title (see Project Report ordering information at back).

Introduction

Effluents from secondary lead smelters are a major source of heavy metals in the aquatic environment. These effluents are frequently low in pH and contain a wide range of heavy metals. The largest single heavy metal constituent is, of course, lead. Relatively large amounts of zinc, cadmium, arsenic, copper, and other toxic cations may also be found, along with a wide variety of anions often associated with these metals (chloride, sulfate, nitrate and chromate for example). Lead smelter effluents also contain toxic trace organics generated when batteries and other materials are crushed, and the heavy metals are removed from their plastic encasements. The fact that heavy metals are dangerous in the aquatic environment has been well documented in the literature.

To protect the environment, the discharge of heavy metals must be minimized. One approach is to apply the type of algal-meander treatment system cur-

*Mention of trade names or commercial products does not constitute endorsement or recommendation for use by the U.S. Environmental Protection Agency.

rently in use for treating mine and mine-mill wastes at one site in the new lead-belt region in southeast Missouri—the world's largest lead mining region. A detailed laboratory study of the factors influencing heavy metal accumulation by algae is the subject of this investigation and an attempt has been made to evaluate the feasibility of using algal meanders to remove a variety of heavy metals.

Description of the Meander Process

In the Missouri algal-meander system, mine and mill wastes were treated in a standard tailings pond followed by a series of shallow meanders in which the growth of algae is encouraged. These algae grow, utilize the waste millings reagents and trap heavy metals, both particulate and dissolved, on their surface. When the algae mat breaks loose, it is then trapped in a settling pond at the end of the meander system. The pond is equipped with baffled weirs which prevent algal overflow into the receiving stream. Based on total heavy metals removed, the system is more than 99-percent effective. Dissolved heavy metals are also removed to levels well below U.S. Public Health Service drinking water standards. Past work has shown also that lead in its highly soluble acetate form is removed effectively by the algae.

To date, the algal-meander system has been used to treat only one waste, a combined lead-zinc mine and mill waste in the new lead-belt mining region. Since 1971, this region has been the site of a large interdisciplinary investigation, supported by the National Science Foundation, of environmental pollution by lead and other heavy metals. It was confirmed in 1974 that below the AMAX-Homestake Lead Tollers, Inc., mining mills, severe stream degradation and heavy metal buildup was occurring. The meandering system was built to a full operating scale and as a result the stream was returned to essentially pre-mining conditions. The history of this treatment system and the quality of the water in receiving streams has been well documented for lead and zinc. This technology, however, has not been applied to wastes containing other toxic metals. This has been largely due to the lack of information on the system's range of application and the newness of the technology. The ability of algae to concentrate heavy metals from their aqueous environment has been reported by radioecologists examining debris from nuclear tests, chemical geologists attempting to explain low-grade deposits of metal, and ecologists concerned with the self-purification of streams below mining operations. While these observations are clearly valuable, a more detailed knowledge of the interaction between algae and heavy metals could aid in understanding these processes. A systematic study of these interactions has been difficult because a wide range of variables affects metal accumulation by algae. These variables include the length of the exposure period, the type of metal, oxidation states, pH, salinity, and presence of organic pollutants. To understand the factors influencing metal accumulation by algae in the meander system, laboratory studies were initiated with pure cultures of representative species from other laboratories and from the algal culture collection at the University of Texas.

Conclusions

The ability of representative algae to concentrate large quantities of specific heavy metals was demonstrated by adapting the Titertek™ supernatant collection system (Flow Laboratories, Rockville, Maryland) 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. Algal-metal combinations used in batch studies are presented in Table 1.

The removal and concentration of heavy metals from their aqueous environment by algae was first studied in detail in the 1950s and 1960s as part of the problem of the release of nuclear debris from weapons testing, and from reactor cooling water effluent. Scientists took advantage of the released isotopes, as well as newly developed analytical techniques, to study pathways of various elements in the environment. One early result of this work was the observation that certain isotopes were concentrated by biota to a much greater extent than the concentration of that isotope in water. From these observations came the concept of the concentration factor (CF) such that

$$CF = \frac{C}{C^1}$$

where C and C^1 are the concentration of the radionuclide in the aquatic organism and in the aqueous medium respectively. The concept is also applicable to stable isotopes (Polikarpov, 1966).

Calculation of concentration factors for heavy metals revealed significant differences between species of the same genus and between strains of the same species (i.e., *Nostoc*). Some species regularly had concentration factors of 1×10^4. In general, young cultures exhibited very much higher concentration factors than old cultures. Most of the metal removed by algae can be removed by a rinse with 0.01 M ethylenediaminetetraacetic acid (EDTA) or 0.1 N nitric acid (HNO_3). This information suggests that surface adsorption sites are the principal repository for the metallic ions or particles.

The ability of algae to remove metals is generally in this order: mercury > lead > cadmium. Neither zinc nor arsenic was removed significantly at any pH during a three-hour exposure. The presence of chelating agents in the medium (such as EDTA) inhibited the removal of the heavy

Table 1. *Algal-Metal Combinations Used in Batch Studies*

		Cations							
Alga	*Age (days)*	*As(III)*	*As(V)*	*Cd*	*Cu(II)*	*Hg(II)*	*Ni(II)*	*Pb*	*Zn*
Chlamydomonas	*12*	+	+	+	+	+	+	+	+
Chlorella	*12*			+	+		+	+	
Spirogyra	*12*		+	+	+	+	+	+	
Ulothrix	*12*	+	+	+	+		+	+	+
Ulothrix	*20*	+			+		+	+	
Gleotrichia	*12*				+		+	+	
Gleotrichia	*20*	+							
Gleotrichia	*40*	+							
Nostoc *31*	*40*	+	+						
Nostoc *586*	*12*	+	+	+	+	+	+	+	
Nostoc *586*	*20*	+							
Oscillatoria	*12*	+	+	+	+	+	+	+	+
Oscillatoria	*20*	+							

metals. Calcium and magnesium were not effective competitors for the binding sites of mercury, lead, and cadmium. There was remarkably little effect of pH on metal accumulation in the range of pH 5 to 8, when young cells were employed. Lead was the principal exception because it was removed more efficiently at pH 4 to 5 than at other pH values.

The removals of lead and mercury from nutrient solution by algae are found to be rapid phenomena, usually accomplished in three hours or less at room temperature. If the cells were placed in buffer after the initial adsorption of lead, a limited, slow release of lead occurred for a few hours, perhaps while some surface polymer-lead complexes were released from dead or dying cells. Mercury was removed from solution in nearly identical fashion by two strains of *Nostoc* and showed little evidence of being released. In the control wells without algae there were reproducible losses of ^{203}Hg that occurred within the first 24 hours at pH 6. This may be related to reports in the literature claiming volatilization of mercury by algae.

Chlamydomonas (a green flagellated form) proved to be dramatically superior to all other species in its ability to remove lead. Concentration factors for the organism of 1.9×10^4 were noted. This organism showed essentially the same concentration factor for lead in the pH range of 4 to 9. In other experiments, *Ulothrix* and *Chlorella* had concentration factors for cadmium greater than 10^4.

The empirical successes of the algal-meander system for removing lead from surface waters can be explained in part by the concentration and sequestering of lead and cadmium on the surface of algal cells. Very little heavy metal was leached from these cells at pH 5 to 9 in the presence of divalent cations and other inorganic constituents of algal growth media.

Results

Algal Growth

Algal growth for 20 species provided a representative selection of algae for the experiments. Some species proved impossible to culture and others exhibited unusual rates of growth. Table 2 reveals that no growth was obtained from any form in soil-water media. The choice of African violet potting soil was probably unfortunate because this soil is high in humus. Success with this medium depends on the selection of a garden soil with a medium humus content.

A few algal forms were characterized by unusual growth rates. *Mougeotia* and *Zygnema* grew only slowly and to a low final biomass. *Nostoc* 31, *Nostoc* W and *Gleotrichia* exhibited a long lag phase. For each of these forms, inoculation was followed by about 15 days of apparent inactivity. After this period, growth to a useful final biomass was rapid. For these forms, then, no young culture data are available.

Table 2. *Sources and Cultivation of Algae*

Algae	Source*	Type	Growth on media† B	D	SW
A. *Baccillariophytes (diatoms)*					
Navicula pelliculosa	*UTEX 668*	*Pennate diatom*	–	+	–
B. *Chlorophytes (green algae)*					
Chlamydomonas *sp.*	*Dr. C. Kuehnert Syracuse University*	*Unicellular, 2 flagella*	+		
Chlorella pyrenoidosa	"	*Unicellular*	+		
Chlorotyllium *sp.*	*Dr. N. Lazaroff SUNY Binghamton*	*Filamentous*	+		
Kirchnerella *sp.*	"	*Colonial, enclosed in gelatinous matrix*	+		
Mougeotia *sp.*	*UTEX 758*	*Filamentous, planktonic*	+		
Scenedesmus obliquus	*UTEX 2016*	*Colonial, 4-8 cells, planktonic*	+		
Spirogyra *sp.*	*Dr. N. Lazaroff*	*Filamentous, planktonic*	+		
Ulothrix fimbrinata	*Dr. N. Lazaroff*	*Filamentous, benthic*	+		
Zygnema	*UTEX 923*	*Filamentous, enclosed by mucilaginous sheath*	+		
C. *Cyanophytes (blue-green algae)*					
Gleotrichia *sp.*	*Dr. Lazaroff*	*Colonial*	+		
Nostoc muscorum *A*	"	"	+		
Nostoc *F*	"	"	+		
Nostoc muscorum *H*	"	"	+		
Nostoc *L*	"	"	+		
Nostoc muscorum *W*	"	"	+		
Nostoc *31*	"	"	+		
Oscillatoria *sp.*	"	"	+		
Nostoc *586*	"	"	+		
Schizothrix calcicola	"	"	+		
D. *Mixed Cultures*					
Cazenovia Lake			+	+	
Onondaga Lake			+	+	

**University of Texas Culture Collection and Identification.*
†B = Bold's medium; D = diatom medium; SW = soil water medium.
+ = success
– = failure

Batch Studies

The results of the batch studies are presented in Figures 1 through 8. The figures show the concentration of metals in the sample as a function of the time the samples were withdrawn from the culture. It should be noted that, for reasons of clarity, not all data points are shown on the figures.

Arsenic III—The data show no obvious trends in the removal of arsenic III. The highest removal (about 35%) was accom-

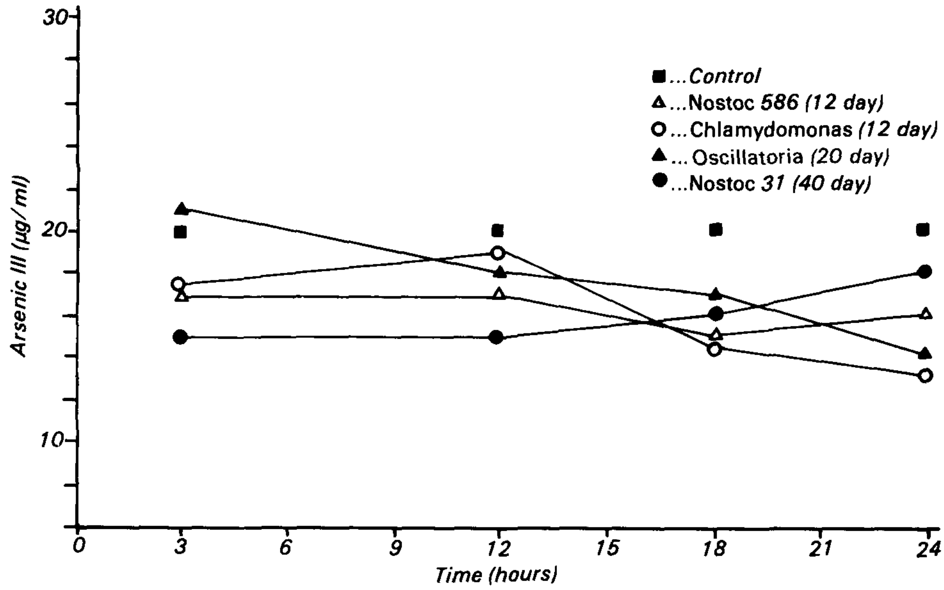

Figure 1. *Uptake of arsenic (III) by various algae.*

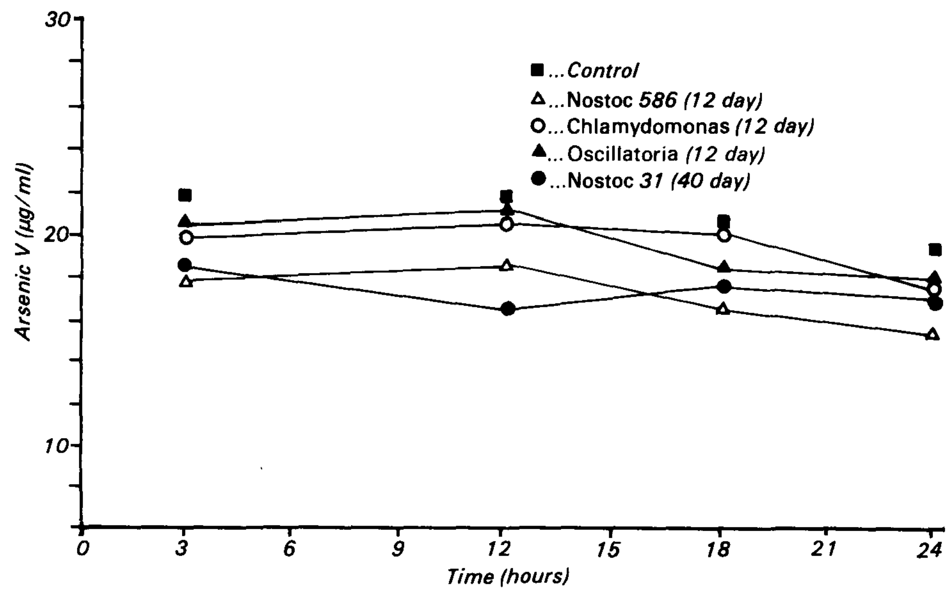

Figure 2. *Uptake of arsenic (V) by various algae.*

plished by *Chlamydomonas*, which also had the lowest biomass (0.12 mg/ml).

Arsenic V—There are no obvious trends in the removal of arsenic V. Most experimental values are too close to the control value to be considered significantly different. The lowest experimental value (16 µg/ml, compared to a control value of 20 mg/ml) was attained by *Nostoc* 586, which had among the highest biomass (0.40 mg/ml).

Cadmium—Cadmium was likewise not dramatically removed by the algae used in this part of the study. The lowest experimental values (about 10 µg/ml) were achieved by *Ulothrix*, which had the highest biomass (0.34 mg/ml). That the acid rinse for both *Ulothrix* and *Nostoc* 586 (with the next lowest experimental value) had high concentrations of cadmium indicates a surface adsorption mechanism for cadmium removal.

Copper—The highest removal of copper (about 30% compared to the controls) was attained by *Gleotrichia*, which had a biomass of about 0.14 mg/ml. This alga, though usually a dark brown mass of filaments, turned greenish on exposure to copper.

Lead—Lead appeared to be removed by all the forms studied in this experiment, with removal (compared to the 18-hour control) of from 15% for *Spirogyra* to about 87% for *Oscillatoria*. The green filamentous *Ulothrix* showed high values of lead removal, and the morphologically similar *Spirogyra* showed values little different from the controls, even though both algae had nearly identical biomasses. The high values of lead in the acid rinses suggest again a surface adsorption.

Mercury—The most dramatic removal of mercury was accomplished by *Nostoc* 586, which had an experimental value of 6 µg/ml as compared to the control value of 34 µg/ml after only three hours. Again the values of mercury in the acid rinses were high.

Nickel—Although all algae seemed to exhibit some removal of nickel (17% to 37% after 24 hours), no trends could be discovered from the data.

Zinc—The algae that seemed to accumulate zinc were *Spirogyra* (with 41% removal after 18 hours) and *Nostoc* 586 (with 37% removal during the same length of time). Again, the acid rinses of these two algae had high values of zinc, suggesting surface adsorption sites.

Discussion of Project Results

With the exception of zinc, these results show reasonably good agreement with the literature, with all concentration factors between studies agreeing within a factor of 2 to 3.

Previous studies also examined the kinetics of heavy metal accumulation by algae and in general show that uptake was an extremely rapid phenomenon. The results of the 24-hour experiments in this study clearly confirmed the earlier observations in this regard. The negative results for the cadmium experiments cannot be explained at this time.

One obvious manner in which the present study extends previous work is the calculation of concentration factors for species of algae previously unstudied. These data were obtained under identical conditions and record both intraspecific (in the case of *Nostoc*) and interspecific differences in heavy metal accumulation by algae. New data are provided on the effects of culture age, and initial pH

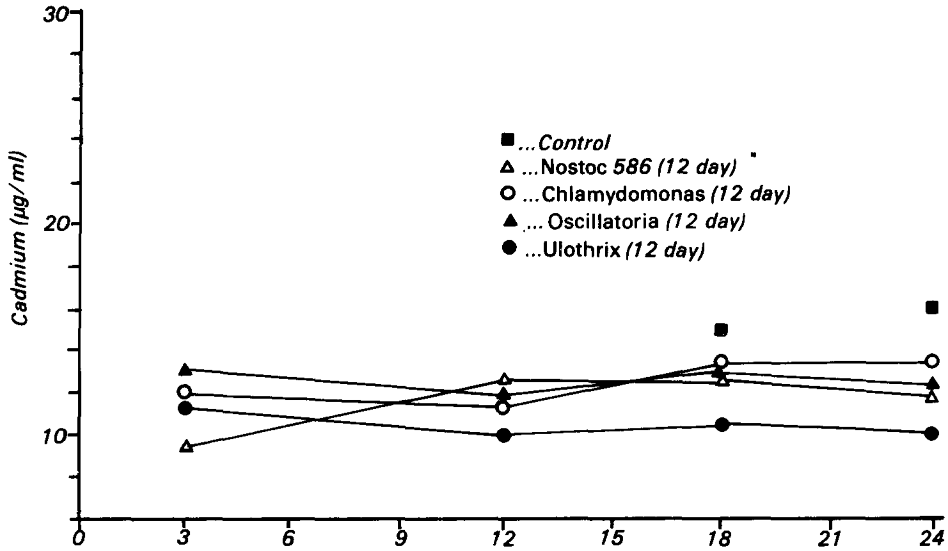

Figure 3. *Uptake of cadmium by various algae.*

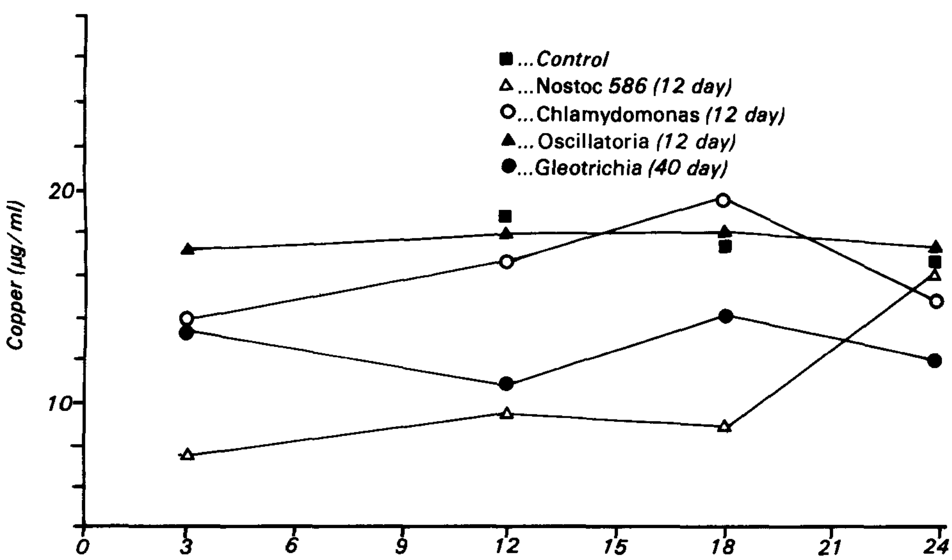

Figure 4. *Uptake of copper by various algae.*

during the labelling period, on the accumulation of heavy metals by algae.

The present study about doubles the number of algae for which concentration factors for cadmium have been determined. This is not only important to waste treatment applications, but also to environmental impact studies. Also noteworthy is the close agreement of concentration factors from the literature with those of this study, with CFs on the order of 10^3 common to both. What is not immediately apparent is the generally lower CFs for the blue-green algae. In fact, three 10-day old 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. Although the total number of species examined is small, the present study suggests that green algae are somewhat more efficient at accumulating cadmium than the blue-green algae.

Concentration factors for lead are generally on the order of 10^3 to 10^4 for all algae studied. All the blue-green algae screened were successful at removing lead at least at one pH, while the diatom *Navicula pelliculosa* and the green *Chlorella* and *Scenedesmus obliquus* were successful at accumulating lead at any pH. *Chlamydomonas* proved to be the most efficient at removing lead from solution.

Mercury was again demonstrated to be concentrated avidly by most species of algae. Every organism screened in this study removed mercury at least at one pH, with concentration factors on the order of 10^3 and 10^4. Algae may be one of the most effective means for removing this element. Algae can reduce mercuric ion to elemental mercury which is subsequently volatilized to the atmosphere. The question might arise whether the same phenomenon occurred in these experiments, giving artifically high concentration factors. Although the possibility cannot be denied categorically, the short time scale of these experiments (three hours) and the quiescent conditions under which the plates were held would argue against loss of very much mercury to the atmosphere. This is especially true since most other studies employed aerated cultures and a time scale of several days. It has also been reported that the reducing factor(s) were extracellular. Since the algal cells in the present study were washed and resuspended in distilled water before introduction to the assay plates, the concentration of reducing factor(s) would be diminished, at least initially. However, the phenomenon of mercury reduction and volatilization by algae clearly deserves further study.

Another contribution of this work was the systematic study of the effect of pH on heavy metal accumulation. The studies cited from the literature were generally field observations, during which the pH of the surroundings varied naturally, or were laboratory studies where the culture media characteristically determined the pH of metal uptake, and therefore, uptake across a wide variety of pH conditions could not be determined. In general, those algae which are most proficient at removing metals will remove them over a wide pH range. For example, *Chlamydomonas* removes lead across a pH range from 4 to 9 at about the same efficiency (0.69-0.80) while the less effective alga *Mougeotia* removes lead only at pH 9 and

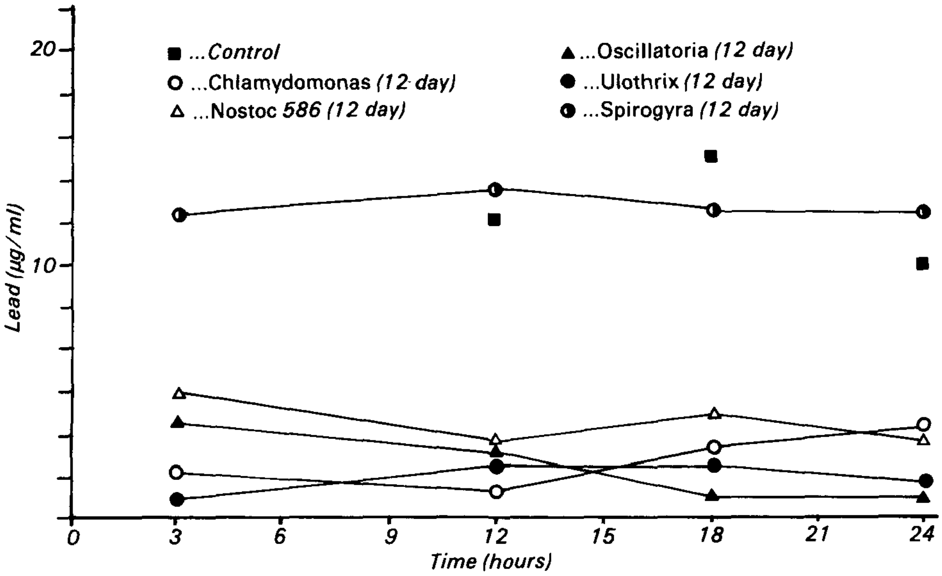

Figure 5. *Uptake of lead by various algae.*

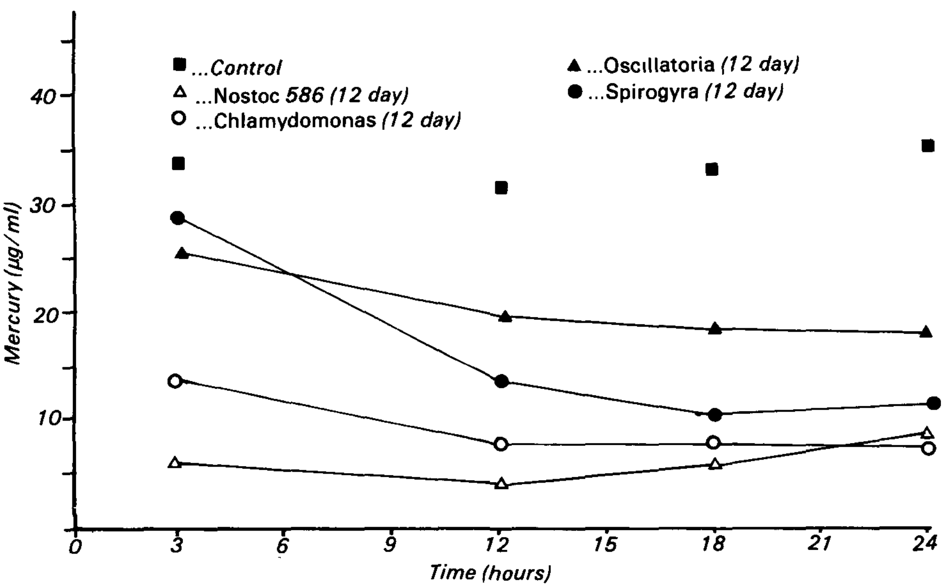

Figure 6. *Uptake of mercury by various algae.*

then only at an efficiency of 0.29. Clearly *Chlamydomonas* is more competent at removing lead from solution.

Other observations made during this study tend to confirm and extend suggestions made by earlier workers. It has been hypothesized that the flagella of *Platymonas subcordiformis* provided the sites for a significant fraction of the lead taken up by this algae. While this hypothesis was not specifically tested in the laboratory, the dramatic superiority shown by *Chlamydomonas* in removing lead, as compared to the other algae evaluated combined with the fact that *Chlamydomonas* was the only flagellated form used, argues for the importance of the flagella as sites of lead accumulation.

Perhaps one of the most important contributions of this work, however, was the adaptation and development of micrc titer equipment, especially the Titertek[T] supernatant collection apparatus, to th study of the problem of algal uptake c heavy metals. The technique and proce dures developed during the course of thi study have already provided usable dat on a relatively limited number of variables Future work will no doubt utilize th rapidity and sensitivity of the technique t accumulate more data covering a wide range of variables.

Recommendations

The remarkable ability of selected alga to concentrate heavy metals on their ce surfaces suggests that the use of inter sive algal culture may be a very practic way to reclaim wastewater and/or re cover useful quantities of metals. Severa directions for future research and develop ment are clearly indicated before thi potential can be realized:

1. The glycocalyx—i.e., the mucopoly saccharide and protein capsule—surrounding all algae must be isolate from metal-avid species and furthe studied for its biochemical structure exchange capacities, and biphasi physical properties. With these dat in hand, it is practical to search fo organisms with glycocalyces havin desirable properties for the adsorp tion of metals.
2. If algal beds are to be maintained on continuing basis, it will be necessar to follow the reproductive capacity o the organism(s) in the presence o excessive levels of metal. In fact, i may be necessary to devise schedule for metal adsorption that are followe by regeneration at low metal levels.

   The present meander technolog depends entirely on the presence o naturally occurring wild algae, prob ably growing as a mixed flora. Sinc some of the species may have con centration factors in excess of 1 x 10 under certain conditions, and other may not concentrate metal at all, i appears profitable to encourage th former by selective algal farming Simple enrichment culture tech niques are feasible for the meande manager.
3. A screening operation should b devised with the objectives of findin natural or induced mutants that a have accumulation coefficients on or more orders or magnitude greate than wild types, b) exhibit greate selectivity for a particular metal io than the wild types, and c) tend t

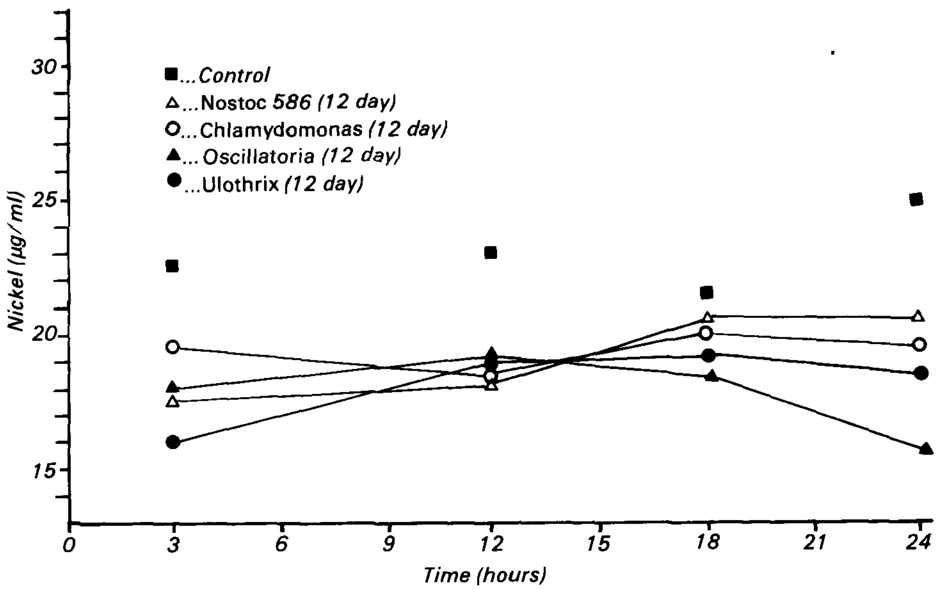

Figure 7. *Uptake of nickel by various algae.*

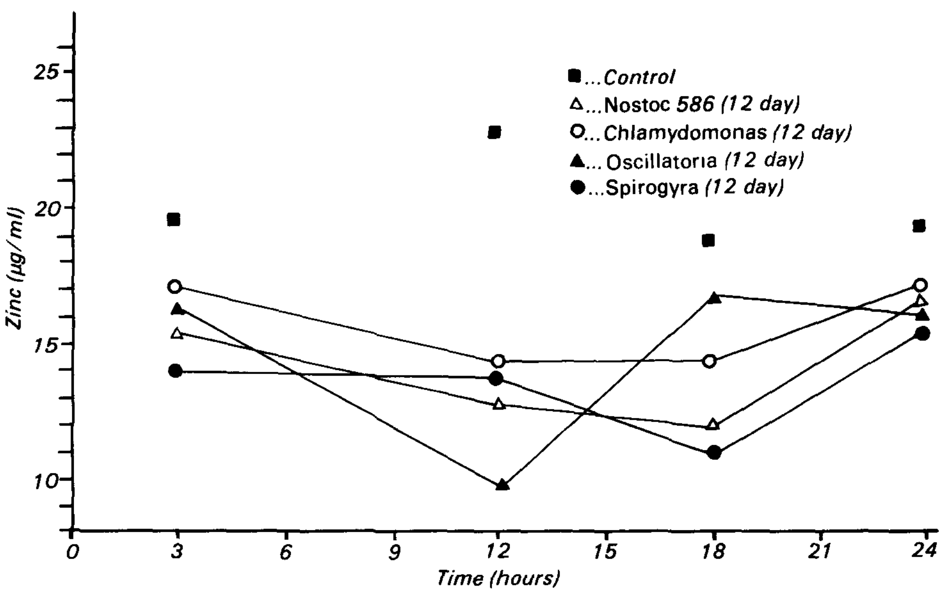

Figure 8. *Uptake of zinc by various algae.*

remain in their benthic form rather than in their free-floating form. The blue-green algae may be the better choice for this work because they are usually more metal-avid than green algae, and because they are haploid. The latter characteristic makes it easier to induce mutations and to select for variants than in diploid organisms.

4. Almost no data are to be found concerning the maintenance of beds of benthic algae on a continuous basis. Experiments are needed to learn the long-term consequences of heavy metal accumulation in such beds, to monitor detention and release of metals during chronic exposures, and to study the detachment of benthic cells coated with heavy metals.
5. Methods must be devised for systematically discharging metals, or for otherwise recovering them, from meander beds. Simultaneously, it must be determined whether or not it is feasible to recycle the algal cells as inoculum or feedstock after they have been stripped of metal by chemical washing.
6. Investigation of practical questions regarding the suitability of metal-stripped secondary sewage effluent for agricultural purposes must be examined. Conversely, the efficacy of sewage as a dependable nutrient source for algae has not been established. Certainly, the presence of oil, cyanates, solvents, etc., could be expected to interfere with the productive synthesis of the glycocalyx adsorptive surface.
7. The effects of light, temperature, and salt concentration have not been evaluated on a continuously operating meander. Quantitative data have not been collected on the optimum output of glycocalyx because the factors which stimulate it are essentially unknown.
8. Future work should determine whether or not an appropriate substitute can be found for the algal cells in the meander (aggregates or other surfaces coated with semi-purified glycocalyx, for example). This search might provide a renewable and/or strippable surface that would eliminate some of the vagaries associated with the maintenance of open algal cultures.

Reference

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☆U. S. GOVERNMENT PRINTING OFFICE: 1983/659-095/1907

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The complete report, entitled "Factors Influencing Metal Accumulation by Algae," (Order No. PB 83-149 377; Cost: $14.50, subject to change) will be available only from:
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