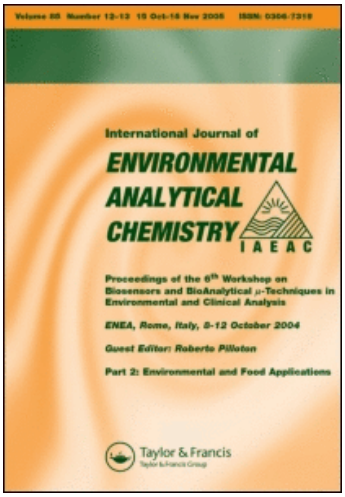

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The Analysis of Dissolved Metals in Natural Waters After Preconcentration on Biosorbents of Immobilized Lichen And Seaweed Biomass in Silica

G. J. Ramelow[a]; L. Liu[a]; C. Himel[a]; D. Fralick[a]; Y. Zhao[a]; C. Tong[a]
[a] Department of Chemistry, P. O. Box 90455, McNeese State University, Lake Charles, LA, U.S.A.

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THE ANALYSIS OF DISSOLVED METALS IN NATURAL WATERS AFTER PRECONCENTRATION ON BIOSORBENTS OF IMMOBILIZED LICHEN AND SEAWEED BIOMASS IN SILICA

G. J. RAMELOW, L. LIU, C. HIMEL, D. FRALICK, Y. ZHAO and C. TONG

Department of Chemistry, P. O. Box 90455, McNeese State University, Lake Charles, LA 70609, U.S.A.

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A new type of sorbent has been developed in which dried lichen and seaweed biomass is entrapped in silica gel. Biomass of the lichens *Bryoria* sp., *Letharia* sp. and the brown seaweed *Sargassum* sp. were immobilized in silica gel. The immobilized biomass was investigated for use as an absorbent for copper, zinc, cadmium, lead, chromium, nickel, iron, cobalt, aluminum, silver, gold, and mercury. Metal solutions were loaded onto columns containing one gram of biosorbent at pH 5.5 and then stripped with 0.05 M sodium or ammonium acetate solution at pH 1.5–2 or 1 M HNO_3. A complexing agent such as 0.1 M thiourea was required to completely strip gold. The use of biosorbent columns for preconcentrating metal ions from natural waters was demonstrated by spiking deionized water with Cu, Pb, Cd, and Zn. Recoveries for all metals were close to 100%. Biosorbent columns were used to concentrate dissolved metals ten-fold from drinking waters before analysis by atomic absorption spectrometry. The performance of biomass-based sorbents compared favorably with a commercial iminodiacetate chelating resin. The biosorbents developed are stable and reusable. They have great potential for concentrating metals from solution prior to chemical analysis and for removing toxic metals from waste streams.

KEY WORDS: analysis, metals, natural waters, preconcentration, biosorbents, immobilized biomass.

INTRODUCTION

The binding of metals by cell-wall constituents of algae, fungi, bacteria and other microorganisms is a very rapid process. The degree of binding in dilute solutions often approaches 100 percent. For many metals binding is pH dependent. The nature of the chemical groups responsible for binding is not well known, but recent experiments have indicated that the carboxyl group is primarily responsible for the binding of the most metals[1,2]. Diamine groups may also be involved. The carboxyl group thus functions as a pH-dependent ion exchanger. Metals may also be sorbed through processes involving chelation and complexation.

Although biomass of microorganisms has great potential for sorbing metal ions from solution, biological material cannot be used directly as sorbents because of the tendency of biomass particles to clump together when wetted. To solve this problem Darnall *et al.*[3] immobilized cells of the green alga *Chlorella vulgaris* in polyacrylamide gel. Although this material was able to bind metals, the gel was not found to have the necessary mechanical strength. These workers later immobilized algal biomass in silica which worked well.

Another potential metal biosorber is lichen biomass. Lichens are organisms composed of a fungus and an alga in a symbiotic relationship. Lichens have been widely used as air pollution monitors because of their ability to strongly bind and accumulate many metals[4]. Lichens have also been found to bind metals in a strongly pH-dependent manner[5]. Non-living lichen biomass was found to bind metals better than living biomass[6]. This feature makes them attractive as potentially useful ion exchangers.

Seaweed algae have also been found to bind metals to a high degree. The brown seaweed *Ascophyllum nodosum* was found to bind cobalt strongly in a pH-dependent manner[7]. *Sargassum* sp. was shown to effectively bind gold[8].

In the work described here biomass adsorbers are evaluated as potential ion exchangers for concentrating metals prior to analysis, and also for removing interfering elements. The use of biosorbents for the concentration of some metals in drinking water prior to atomic spectral analysis is described.

EXPERIMENTAL

Preparation of silica gel-immobilized biomass

A known amount (usually 1 g) of dried and powdered biomass which had been passed through a 250-mesh (63-micron) sieve was weighed into a beaker, 25 ml of 6N H_2SO_4 was added and the suspension rapidly stirred. This was followed by the addition of 25 ml of 1:1 sodium silicate solution. After continuous stirring for about 1 hr, the mixture gelled to a firm consistency. This gel was cut into small pieces, washed for about 2 hrs until all excess H_2SO_4 was removed, as evidenced by a yellow color with thymol blue indicator[9], and dried in an oven at 80°C for 48 hrs. After drying the gel was gently ground to a fine powder and separated into various mesh size ranges with standard testing sieves.

Preparation of sorbent columns

The first sorbents were prepared by mixing dried and sieved biomass with silica gel (60–100 mesh) and then pouring the mixture into a small plastic column fitted with a stopcock. Later biomass immobilized in silica gel was used. A 1-g amount of the silica gel-immobilized biomass was weighed into a beaker, a small amount of water was added to make a slurry, then the slurry was poured into a small plastic column fitted with a stopcock and plugged with glass wool. The sorbent was stirred in the column to remove any air bubbles, then a layer of glass wool was placed on top. The columns were soaked overnight in pH 1.5–2

sodium or ammonium acetate solution. Before a sample was run the column was rinsed with two 5-ml portions of pH 5.5 acetate.

Comparison of silica-based biosorbents with a chelating and a complexing resin

Columns containing dried biomass of the lichens *Bryoria* sp. and *Letharia* sp., and the brown seaweed alga *Sargassum* sp. immobilized in silica gel were evaluated by comparison with a commercial chelating resin (Sigma no. C-7901, 50–100 mesh) and a SPADNS-modified Amberlite anion exchange resin[10]. Each column contained 1 g of sorbent. The biomass columns were first cleaned with pH 1.5–2 acetate, as described above, then conditioned with pH 5.5 acetate. The chelating column was cleaned with nitric acid, followed by two 5-ml rinses with pH 5.5 acetate. A 100-ml portion of a solution containing the metal(s) of interest at a concentration of 5 μg/g was passed through each column at a flow rate of 1.7–2.0 ml/min. The sorbed metals were stripped with various stripping agents—pH 1.5 acetate, pH 2 acetate, 1 EDTA at pH 10, 1 M nitric acid, and 0.1 M thiourea. Two 5-ml portions of stripping agent were used in each case.

Analysis of metals in tap water

A 100-ml water sample at pH 5.5 was run through a *Bryoria* 60–115 mesh column at a rate of 1.7–2 ml/min. The sorbed metals were stripped by using two 5-ml portions of pH 2 acetate. The two eluents were combined, thus yielding a concentration factor of 10X. Larger volumes of water can be used when concentrations of dissolved metals are very low. Percent recoveries of each metal were evaluated separately by spiking 100 ml of deionized water with enough metal standard to give an initial concentration of 1; μg/g. The metals were bound as stripped as described previously for tap water samples.

RESULTS AND DISCUSSION

The sorption properties of various types of biomass were first demonstrated by simply mixing various amounts of dried biomass (60–115 mesh) with 60–100 mesh silica gel (Aldrich, no. 23,679–9). The mixture was then placed in small columns to test the sorption properties of the mixtures. These columns demonstrated the feasibility of concentrating metals at a pH of about 5.5, and then stripping the sorbed metals at a pH of 2 or less. Several different ratios of biomass to silica gel were evaluated; a 1:1 mixture of biomass and silica gel was the most efficient.

A mixture of biomass and silica gel is not the most efficient way of utilizing the sorbing properties of microbial biomass. It is difficult to produce a uniform mixture of the same composition. Also, the biomass material has a tendency to clump and form undesirable physical characteristics. Thus, Darnall *et al.*[3] immobilized dried biomass of the alga *Chlorella vulgaris*, first in polyacrylamide gel and later in silica. They demonstrated that biomass immobilized in an inert polymeric material could function well as an ion exchanger

or adsorber. Metals were bound at a relatively high pH and then stripped from the columns by lowering the pH or using a complexing agent.

In the present study the known metal-sorbing properties of lichen[11] and seaweed[12] biomass have been utilized in preparing sorbents of lichen biomass immobilized in silica gel. The immobilized biomass was able to concentrate metals from aqueous samples by binding and stripping steps. For many metals such as copper, lead, cadmium, zinc, chromium, iron, nickel, the process is based on the pH-dependence of binding. For other metals such as gold, silver, and mercury, where pH is of lesser importance, a complexing agent can be used to strip the metals from the biosorbent.

The sorption properties of two lichen varieties were studied by immobilizing dried biomass of each species in silica gel. Two mesh sizes of each lichen were used:32–60 and 60–115 mesh (referred to hereafter as -32 and -60 columns, respectively). One-gram amounts were packed into plastic columns fitted with stopcocks. The percent recoveries of Cu, Zn, Pb, and Cd using columns of the two immobilized lichens in two mesh sizes, as well as a silica gel control column, are given in Table 1. It is clear that both organisms effectively

Table 1 Percent recoveries of copper, lead, cadmium, and zinc using columns of *Bryoria* sp. and *Letharia* sp. immobilized in silica gel[a]

	Percent recovery			
Column	*Cu*	*Pb*	*Cd*	*Zn*
Bryoria-32				
fraction 1 (eluate)	2	3	3	9
fraction 2 (rinse)	2	1	1	5
fraction 3 (strip 1)	15	13	33	35
fraction 4 (strip 2)	61	53	62	66
Bryoria-60				
	0	3	0	1
	0	1	0	1
	66	44	86	92
	36	43	13	17
Letharia-32				
	9	7	12	12
	6	5	3	8
	18	14	23	25
	53	52	58	61
Letharia-60				
	1	4	1	2
	1	4	0	5
	82	7	92	95
	17	21	6	7
Silica gel control	52	48	54	53
(32–60 mesh)	34	30	34	36
	8	10	4	10
	4	6	0	1

[a]Procedure used: 1) Add 5 ml of 5 μg/g mixed-metal solution at pH 5.5 to column and collect eluted solution; 2) Rinse column with 5 ml of pH 5.5 acetate and collect wash; 3) Add 5 ml of pH 2 acetate to strip metals and collect eluate; 4) Add another 5 ml of pH 2 acetate and collect eluate.

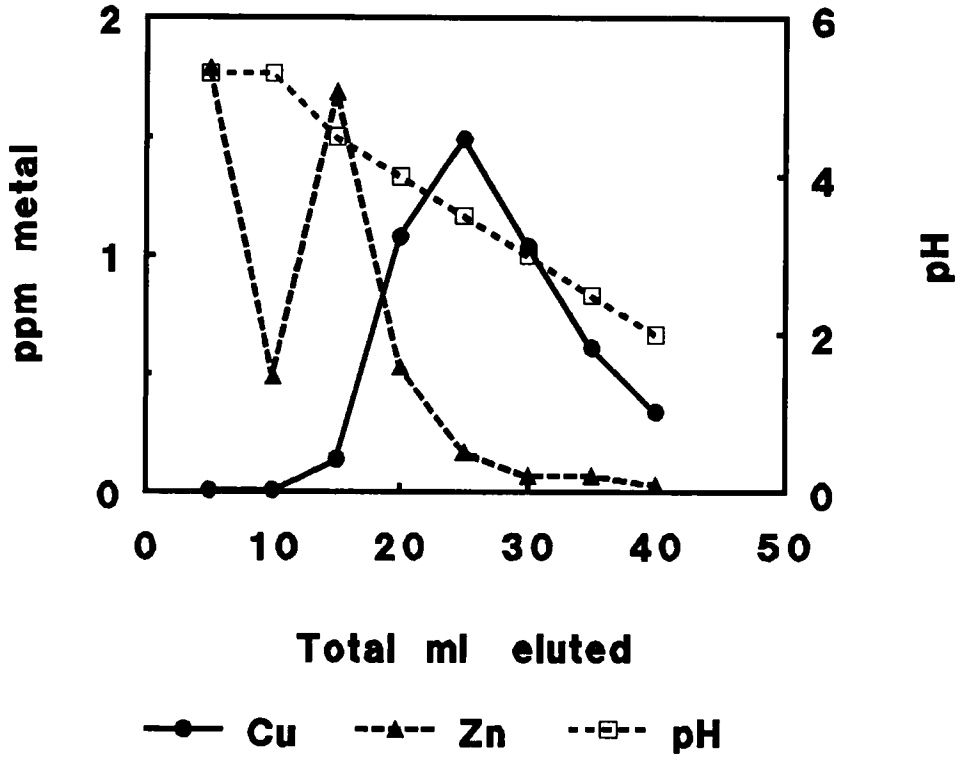

Figure 1 Elution of bound copper and zinc from a Letharia-32 column using a pH gradient.

trap these metals. The smaller mesh-sized sorbent is more effective. It is also clear that two 5-ml portions of stripping agent are needed to completely elute the metals. The control column without biomass did not retain these metals to any significant degree.

To study the elution profiles of metals, 5 mL of a 5-μg/g solution of Cu, Pb, Cd, Zn, and Fe was added to the *Letharia*-32 column at pH 5.5. After rinsing the column twice with pH 5.5 acetate, the sorbed metals were eluted by passing acetate eluent through the column at progressively lower pH's. Starting with pH 4.5, each 5-ml portion was 0.5 pH units lower than the previous solution. This was continued until pH 2.0 was reached, at which point 30 ml of pH 2 acetate was passed through the column. The metal concentration in each 5-ml portion of eluent collected, as well as the pH of each portion was determined. The results for copper and zinc are plotted in Figure 1. It is seen that each metal was stripped from the column at a different pH—about 4.5 for Zn and 3.5 for Cu. Cadmium and lead show very similar elution profiles, but elute at slightly different pH values—4.5 for Cd and 4.0 for Pb. Thus, it would appear to be possible to separate a mixture of copper and lead under the conditions employed. Iron showed no peak elution. A better separation of metals could probably be achieved with a column of smaller mesh size and by a slower pH gradient.

The binding of silver and gold was also investigated in some detail since these elements, particularly Au, are not bound in a pH-dependent manner as the other elements[12]. Thus, a

Table 2 Percent recoveries of silver and gold with sodium acetate and thiourea eluents using columns of *Bryoria* sp. and *Letharia* sp. immobilized in silica gel[a]

	Percent recovery			
	Ag		*Au*	
Column	*acetate*	*thiourea*	*acetate*	*thiourea*
Bryoria-32				
fraction 1 (eluate)	2	8	6	4
fraction 2 (rinse)	8	22	20	4
fraction 3 (strip 1)	46	56	2	38
fraction 4 (strip 2)	42	38	0	46
Bryoria-60				
	0	0	4	2
	2	2	12	2
	70	88	2	60
	10	4	0	16
Letharia-32				
	22	18	30	18
	24	10	23	14
	26	42	2	32
	36	32	2	32
Letharia-60				
	0	2	23	4
	6	0	17	4
	64	92	2	78
	8	6	1	12
Silica gel control	42	42	52	46
(32–60 mesh)	34	40	46	46
	8	11	4	10
	4	8	0	3

[a]Procedure used: 1) Add 5 ml of 5 μg/g metal solution at pH 5.5 to column and collect eluted solution; 2) Rinse column with 5 ml of pH 5.5 0.05 M sodium acetate and collect wash; 3) Add 5 ml of pH 2 acetate or pH 2 thiourea to strip metals and collect eluate; 4) Add another 5 ml of pH 2 acetate or pH 2 thiourea and collect eluate.

complexing agent may be required to completely elute these metals. From the data given in Table 2 it is seen that gold definitely requires a complexing agent to remove bound metal. Thiourea appears to be very effective in stripping gold from the columns. The 60–115 mesh columns both performed well. As for silver, both 60–115 mesh columns worked well. Thiourea does not appear to be necessary to elute silver; pH 2 acetate is equally effective. Gold and silver are not retained appreciably by silica gel alone.

The total sorption capacity of the *Bryoria*-60 biocolumn was evaluated by adding solutions of Cu, Pb, Cd, and Zn in 5-ml increments, collecting the eluates, and analyzing the eluates for each metal. The procedure was terminated when significant amounts of metal began to appear in the eluate, indicating that the maximum capacity of the sorbent had been reached. The resulting "breakthrough curve" is shown in Figure 2. In this figure it is seen that the capacity of biomass is greatest for Pb. Breakthrough of Pb did not occur until about 1000 μg of Pb had been loaded onto the *Bryoria* column, which was three times the capacity of the column for Cu. Very similar results were seen for the *Letharia*-60 column, but it had

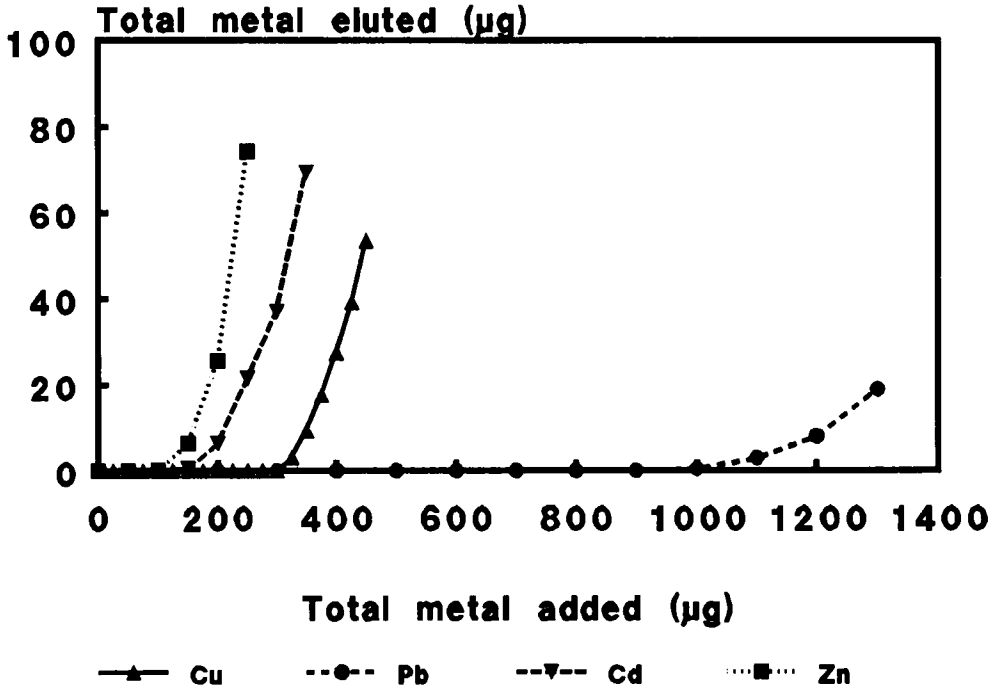

Figure 2 Total adsorptive capacity (breakthrough curves) of a *Bryoria*-60 column for Cu, Pb, Cd, and Zn.

slightly less capacity for Pb. When different mesh sizes were evaluated in separate experiments, the *Bryoria*-60 column showed the highest capacity for Pb.

In the latter phase of the study columns were also prepared with a common brown seaweed alga, *Sargassum* sp. (probably a mixture of S. *natans* and S. *fluitans*), an organism that has been shown to accumulate metals from solution[8,12]. The performances of columns containing 32–60 mesh *Bryoria*, and *Sargassum* were compared with a column containing a commercial chelating resin (60–100 mesh). Another complexing resin, SPADNS, prepared from Amberlite IRA 400 ion exchange resin according to instructions given in the literature[10], was also included in the evaluation.

In Table 3 the sorption properties of the four columns for Cu, Pb, Cd, and Zn are compared. It is apparent that the *Bryoria* columns performed well, both in terms of the trapping of the metals, and the subsequent stripping with pH 2 acetate eluent. The *Sargassum* column was able to trap all the metals studied, but pH 2 acetate was not effective in stripping Cu and Pb from this column. This eluent also could not strip any of the sorbed metals from the chelating resin. From the data in this table it is also apparent that the SPADNS-modified resin essentially did not trap any of the four metals; therefore, this column was not used for any further studies.

Several different eluents were evaluated for stripping Cu, Pb, Cd, Zn, Cr, Fe, Ni, Co, and Al from 60–115 mesh columns of *Bryoria* and *Sargassum* and compared with the commer-

Table 3 Comparison of *Bryoria* and *Sargassum* biocolumns with an iminodiacetate chelating resin and SPADNS-modified Amberlite for binding of copper, lead, cadmium, and zinc[a]

Column material	*Percent recovery* Cu	Pb	Cd	Zn
Bryoria/silica				
fraction 1 (eluate)	0	2	0	0
fraction 2 (rinse)	0	2	4	2
fraction 3 (strip 1)	66	64	88	93
fraction 4 (strip 2)	22	28	8	8
Sargassum/silica				
	0	2	0	0
	0	2	0	0
	0	2	10	10
	14	2	78	80
Chelating resin				
	2	2	2	2
	2	2	2	2
	0	2	1	0
	0	2	1	0
SPADNS/Amberlite				
	37	58	62	62
	20	26	38	38
	14	8	8	8
	12	4	2	0

[a]Five mL of 5-μg/g mixed-metal standard loaded onto columns at pH 5.5; bound metals stripped with pH 2 acetate.

cial chelating resin. The stripping agents used were 1M HNO_3 (pH = 1.4), pH 1.5 acetate, and 1% EDTA at pH 9. The results obtained are presented in Table 4. Essentially all three eluents were effective in stripping Cu, Pb, Cd and Zn from the three columns. It does appear that 1 M HNO_3 has a slight edge in efficiency. It is also apparent that all four metals are stripped from the *Bryoria* column more efficiently than from the other columns, as evidenced by the larger amount of metal in the first strip compared to the second strip.

Four different eluents were evaluated for stripping bound gold, silver, and mercury from *Bryoria* and *Sargassum* biocolumns and the chelating column. The stripping agents used were 1 M HNO_3, 1% EDTA at pH 9, pH 1.5 acetate, and 0.1 M thiourea at pH 2. From the results shown in Table 5, it is clear that gold is completely eluted only by thiourea. EDTA is an ineffective eluent for gold and silver, but elutes mercury well. Acetate solution at pH 1.5 is effective for eluting silver from all three columns. None of the three metals were completely eluted by 1 M HNO_3.

To test the long-term stability of the biocolumns, a *Sargassum*-60 column was subjected to successive binding and stripping cycles by adding 5 ml of a 5-μg/g solution of Cu and Zn at pH 5.5, and then stripping the metals with pH 1.5 acetate. The procedure was carried out ten times. The concentrations of Cu and Zn in two combined 5-ml portions of acetate eluate are given in Table 6. The percent relative standard deviation of both Cu and Zn was found to be 6.4%, indicating acceptable precision. There was no observable deterioration of column performance with repetitive usage. Also, a *Bryoria* column which had been stored

Table 4 Evaluation of three different eluents for stripping bound copper, lead, cadmium, zinc, chromium, iron, nickel, cobalt, and aluminum from *Bryoria*-60 and *Sargassum*-60 biocolumns and a chelating resin

		Percent recovery								
Eluent	*Column*	*Cu*	*Pb*	*Cd*	*Zn*	*Cr*	*Fe*	*Ni*	*Co*	*Al*
1 M HNO_3 (pH 1.4)	*Bryoria*									
	fraction 1	0	2	0	0	13	7	46	46	0
	fraction 2	2	2	2	2	2	32	18	44	1
	fraction 3	80	80	88	90	6	36	8	8	82
	fraction 4	20	18	8	8	4	18	2	2	18
	fraction 5	2	2	2	2	2	5	0	0	3
	fraction 6	2	0	0	2	2	3	0	0	2
	fraction 7	2	0	0	2	0	2	0	0	1
	Sargassum									
		0	2	2	0	0	2	2	0	0
		0	2	2	0	2	10	34	16	1
		64	64	70	72	40	46	48	64	57
		32	32	26	26	12	22	6	8	28
		4	4	2	4	3	8	0	0	4
		2	2	0	2	3	3	0	0	2
		4	2	0	0	2	2	0	0	1
	Chelating resin									
		0	4	0	2	2	0	0	0	3
		2	4	4	2	2	0	2	0	1
		58	56	62	60	52	64	66	66	81
		36	34	30	34	12	22	24	24	12
		4	4	4	4	2	4	2	2	1
		4	4	0	4	2	2	0	0	0
		0	0	0	0	0	2	0	0	0
1% EDTA (pH 9)	*Bryoria*									
		0	0	0	0	32	0	32	30	1
		2	0	4	4	42	0	40	44	1
		58	70	76	79	2	12	6	6	6
		22	18	10	10	2	22	2	4	5
		6	12	2	2	0	21	0	2	3
		2	8	2	2	0	20	0	6	0
		2	8	2	2	0	8	0	0	1
	Sargassum									
		0	0	0	0	0	0	0	0	1
		2	0	0	0	0	0	6	2	1
		56	76	72	72	0	12	64	54	13
		28	28	22	22	0	20	8	18	11
		6	10	4	3	0	20	2	6	5
		4	8	2	2	0	12	0	0	4
		2	6	2	2	0	8	0	0	3
	Chelating resin									
		2	0	4	3	2	0	0	2	7
		2	0	2	2	2	0	0	2	4
		59	78	76	56	0	34	40	4	6
		22	22	14	14	30	13	10	10	28
		6	8	2	6	18	6	6	0	32
		2	8	2	4	7	0	2	0	23
		2	4	2	2	2	0	0	0	8

Table 4 Continued

Eluent	*Column*	*Percent recovery*								
		Cu	*Pb*	*Cd*	*Zn*	*Cr*	*Fe*	*Ni*	*Co*	*Al*
0.05 M acetate (pH 1.5)										
	Bryoria									
		2	2	2	2	28	0	28	7	1
		2	2	2	4	58	4	50	50	1
		76	76	84	86	8	36	10	10	55
		14	12	6	12	4	20	2	6	26
		4	2	2	2	4	8	2	0	7
		2	4	0	2	2	2	0	0	3
		0	2	0	2	0	0	0	0	2
	Sargassum									
		0	0	0	2	2	2	0	0	1
		0	0	0	0	2	2	20	8	1
		58	54	70	70	62	30	58	66	48
		28	28	20	22	12	16	6	8	24
		6	4	2	4	2	6	2	0	5
		2	2	0	2	0	4	0	0	2
		0	1	0	2	0	2	0	0	0
	Chelating resin									
		20	18	40	42	2	0	2	2	17
		4	6	14	14	2	0	2	0	5
		42	48	42	40	62	34	64	62	10
		26	24	14	12	24	20	24	26	31
		6	4	2	2	2	2	2	0	20
		2	2	0	0	0	2	2	0	6
		2	2	0	0	0	2	0	0	3

[a]The procedures used were the same as previously described. All metal standards were 5 μg/g except for Al which was 20μg/g.

in the dry form for several months performed like a new column when rewetted and used.

The *Bryoria* 60–115 mesh column was selected to study the average recovery of Cu, Pb, Cd, and Zn from spiked water samples. Distilled water samples spiked to give a final concentration of 1 μg/g of each metal at a pH of 5.5 were passed through the column. Bound metals were stripped from the column with two 5-mL portions of pH 2 acetate. The percent recoveries are given in Table 7. Recoveries ranged from 99.2 for Cu to 93.1 for Pb.

After the performance of the *Bryoria* column was evaluated, the use of the column for the analysis of drinking water samples was demonstrated by passing 100 ml of various water samples, including tap water collected before and after flushing of pipes, through the column at pH 5.5. The bound metals were subsequently eluted with two 5-ml portions of a pH 2 acetate solution. A concentration factor of 10X was thus achieved. The results for several water samples are shown in Table 8. Copper and Zn were seen in all samples. Lead was seen in two samples drawn from taps early in the morning. The effects of flushing taps prior to use is clearly seen in lower values of Cu, Pb, and Zn. Cadmium was not detected in any samples.

Table 5 Evaluation of four stripping agents for elution of gold, silver, and mercury from *Bryoria*-60 and *Sargassum*-60 biocolumns and a chelating resin

Eluent	*Column*	*Percent recovery*		
		Au	*Ag*	*Hg*
1 M HNO_3	*Bryoria*			
(pH 1.4)	fraction 1	3	0	0
	fraction 2	13	0	0
	fraction 3	6	62	38
	fraction 4	4	4	23
	fraction 5	2	0	8
	fraction 6	0	0	4
	fraction 7	0	0	2
	Sargassum			
		0	0	0
		2	0	0
		6	36	7
		5	38	3
		2	6	2
		0	0	2
		0	0	2
	Chelating resin			
		4	0	3
		2	0	1
		27	54	0
		14	18	6
		2	3	3
		0	0	1
		0	0	1
1% EDTA				
(pH 9)				
	Bryoria			
		2	0	1
		9	0	0
		4	2	63
		2	2	26
		2	2	5
		2	2	4
		2	2	5
	Sargassum			
		2	0	1
		2	0	1
		1	0	14
		0	1	7
		2	2	1
		2	2	1
		2	2	1
	Chelating resin			
		4	2	1
		2	2	1
		2	18	88
		4	14	22
		2	2	2
		2	2	2
		2	0	3

Table 5 Continued

Eluent	*Column*	*Percent recovery* *Au*	*Ag*	*Hg*
0.05 M acetate (pH 1.5)				
	Bryoria			
		12	0	1
		16	0	0
		5	96	14
		3	26	17
		0	4	11
		0	2	8
		0	2	8
	Sargassum			
		2	0	0
		2	0	1
		6	62	1
		6	54	1
		2	20	1
		2	9	0
		2	4	0
	Chelating resin			
		9	0	0
		4	2	0
		22	72	2
		29	28	1
		8	4	4
		4	2	4
		2	2	0
0.1 M Thiourea (pH 2)				
	Bryoria			
		8	0	0
		6	0	0
		64	74	14
		31	12	8
		12	4	2
		6	2	2
		4	2	3
	Sargassum			
		5	0	1
		3	0	0
		15	12	1
		41	54	3
		34	24	5
		21	6	5
		15	2	5
	Chelating resin			
		5	0	0
		1	0	0
		1	2	0
		4	10	0
		13	38	1
		34	28	6
		30	8	5

Table 6 Long-term stability of biocolumns[a]

Metal	*Concentration (μg/g) in eluent after each successive binding/stripping cycle*	
Cu	5.3	
	4.7	
	4.8	
	4.8	
	5.2	
	5.4	
	5.0	
	4.7	x = 4.90
	4.7	SD = 0.31
	4.5	RSD = 6.4%
Zn	5.6	
	5.1	
	5.2	
	5.5	
	5.9	
	6.0	
	5.3	
	5.1	x = 5.39
	5.1	SD = 0.35
	5.1	RSD = 6.4%

[a]A *Sargassum*-60 column was used.

Table 7 Average recovery of copper, lead, cadmium, and zinc from *Bryoria*-60 column[a]

Element	*Percent Recovery*
Cu	99.2
Pb	93.1
Cd	96.1
Zn	98.5

[a]Deionized water (100 ml) spiked to give 1 μg/g concentration of each metal; pH adjusted to 5.5; flow rate through column: 1.7–2.0 ml/min; stripped with two 5-ml portions of 0.05 M acetate at pH 2.

Table 8 Concentrations of copper, lead, cadmium and zinc in water samples after preconcentration on *Bryoria*-60 column[a]

Water sample	*Cu*	*Pb*	*Cd*	*Zn*
		μg/g *metal*		
McNeese drinking fountain (first draw)	0.22	0.06	<0.001	0.41
McNeese drinking fountain (after 5 min)	0.06	<0.01	<0.001	0.25
Lake Charles city tap (first draw)	0.03	0.01	<0.001	0.15
Lake Charles city tap (after 5 min)	0.01	<0.01	<0.001	0.20
Sulphur city tap (first draw)	0.31	<0.01	<0.001	0.25
Sulphur city tap (after flush)	0.04	<0.01	<0.001	0.02
Deionized water blank	0.00	<0.01	<0.001	0.01

[a]100 ml of tap water adjusted to pH 5.5; flow rate: 1.7–2.0 ml/min; stripped with two 5-ml portions of 0.05 M acetate at pH 2.

References

1. V. Majidi, D. A. Kaude, Jr., and J. A. Holcombe, *Env. Sci. Technol.*, **24**, 1309–1312 (1990).
2. J. L. Gardea-Torresdey, M. K. Becker-Hapak, J. M. Hosea, and D. W. Darnall, *Env. Sci. Technol.*, **24**, 1372–1378 (1990).
3. D. Darnall, B. Greene, M. T. Henzi, J. M. Hosea, R. A. McPherson, J. Sneddon and M. D. Alexander, *Environ. Sci. Technol.*, **20**, 206–208 (1986).
4. D. H. S. Richardson and E. Nieboer, *Endeavor*, **5**, 127–133 (1981).
5. S. J. Wainwright and P. J. Beckett, *New Phytol.*, **75**, 91–98 (1975).
6. D. H. S. Richardson, S. Kiang, V. Ahmadjian and E. Nieboer, in: *Lichen Physiology and Cell Biology* (D. H. Brown, ed., Plenum Publishing Co., 1985) pp. 227–246.
7. N. Kuyucak and B. Volesky, *Biotechnol. Bioeng.* **33** 809–814 (1989).
8. N. Kuyucak and B. Volesky, *Biorecovery* **1** 189–204 (1989).
9. H. F. Walton, *Inorganic Preparations, A Lab Manual* (Prentice-Hall, 1948) pp. 114–115.
10. M. L. Marina, V. Gonzalez, and A. R. Rodriguez, *Bull. Soc. Chim. France*, **11–12** 339–345 (1984).
11. G. J. Ramelow, Z. Yumo, L. Liu, *Microbios*, **66** 95–105 (1991).
12. G. J. Ramelow, D. Fralick, and Y. Zhao, *Microbios*, (1992), in press.