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# PERIPHYTON EFFECTS ON ARSENIC TRANSPORT IN WHITEWOOD CREEK, SOUTH DAKOTA

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## ABSTRACT

*Increased periphyton abundance in Whitewood Creek, South Dakota, during the summer months suggests that chemical interactions involving arsenic and phosphorus between biota and the overlying water may significantly affect As transport and distribution in this mining-affected stream. Data used to predict arsenic transport for algae (for example, first-order uptake-rate constants, standing crop and accumulation factors) collected in the creek from upstream of mine discharge through a 57 kilometer affected reach have been determined. Cultures of *Achnanthes minutissima* (Bacillariophyceae) were isolated from four sites along a longitudinal gradient of dissolved arsenic within the study reach and then maintained at ambient dissolved arsenic concentrations. Arsenic uptake-rate constants for these isolates were determined as a function of dissolved arsenate and orthophosphate. All isolates appeared to have some exclusion mechanism by which phosphate was preferentially taken up over arsenate or by which excessive cell-associated arsenic was released. Initial uptake of both arsenate and orthophosphate appear to follow first order kinetics closely. Although uptake-rate constants increased slightly with increased dissolved arsenate concentrations, algae isolated from a site with elevated dissolved arsenic showed a significantly slower arsenic uptake relative to the same species isolated from the least contaminated site upstream. Over a 4-month sampling period during the summer of 1987, periphyton abundance increased downstream, then abruptly decreased at the site farthest downstream. Dissolved arsenic, temperature, physical substrate, and turbidity may explain these trends in periphyton standing crop.*

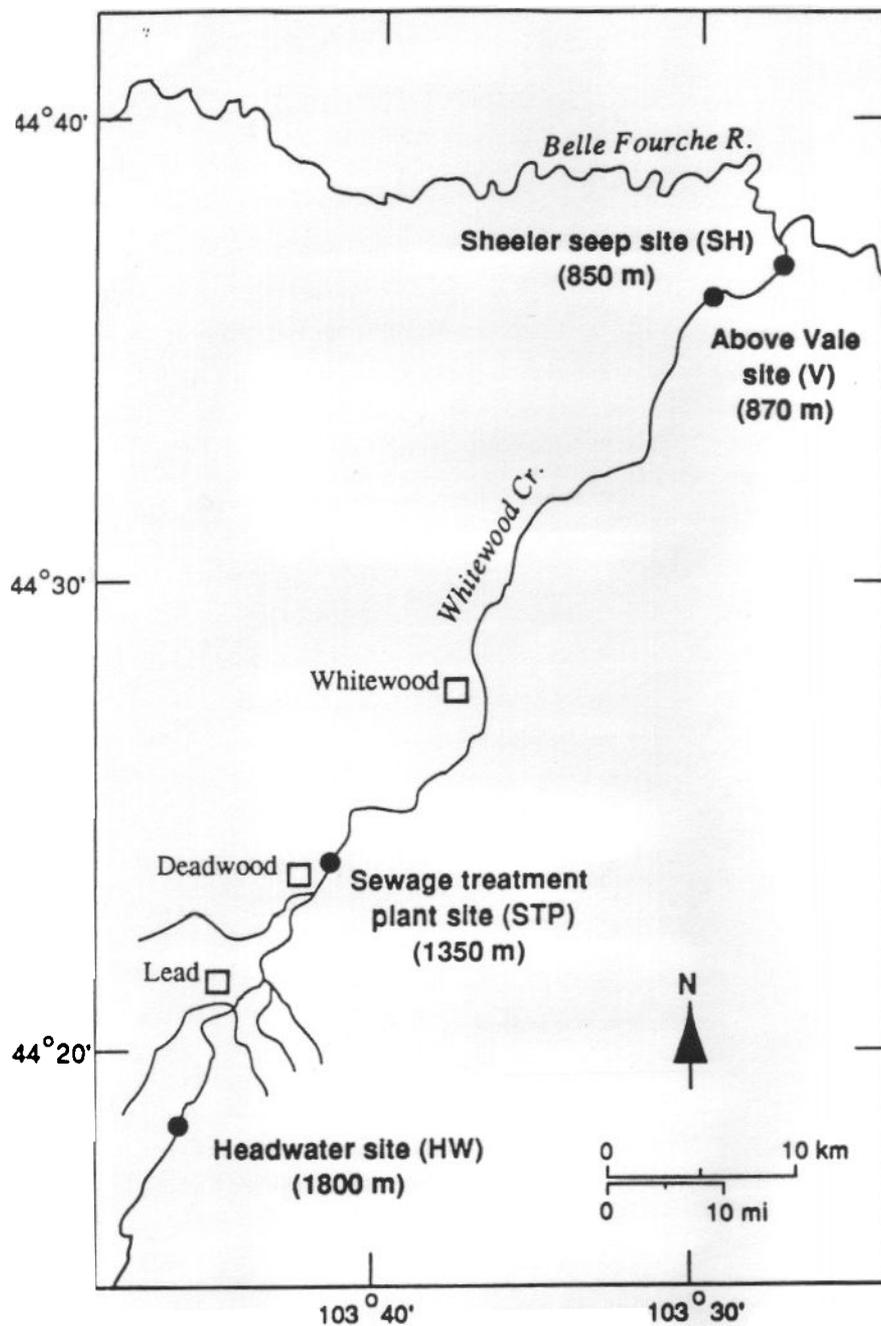
## INTRODUCTION

Biological processes can play an important role in the transport of reactive solutes in streams (Kuwabara and Helliker, 1988). For example,

solute uptake by organisms may retard downstream transport, whereas metabolic reactions may result in a change in chemical speciation of that solute resulting in a change in its surface activity and transport behavior. The effects of these biological processes are difficult to quantify because characteristics of the benthic and planktonic communities may change dramatically temporally and spatially. These changing characteristics may influence, and be affected by, interacting chemical and hydrologic processes. Attempts to model the role of periphyton effects on arsenic (As) transport within an aquatic environment are complicated by a number of factors: (1) arsenate inhibits the growth of certain algal species at submicromolar concentrations, presumably because of interference with phosphate metabolism (Button and others, 1973; Sanders, 1979); (2) the effects of As speciation on uptake rates and periphyton concentration factors is not well known; (3) the manner in which prolonged exposure to elevated As concentrations affects metabolic reaction rates is not well understood; and (4) understanding the effects of physical and chemical factors (for example, light intensity and surface reactions with inorganic particles) is only now being developed. This paper presents results of a field and laboratory study that estimated As transport characteristics for periphyton along a mining-affected reach of Whitewood Creek, S. Dak., under various dissolved arsenate and orthophosphate concentrations. A first-order rate equation was assumed for this periphyton component.

Whitewood Creek (lat. 44°30' N. long. 103°45' E., fig. D-18), which is a perennial, snow-fed stream within the Black Hills of South Dakota, has served since 1876 as the receiving water for effluents from gold mining and municipal activities (for example, sewage treatment and power-generator cooling). Four sites along the creek were

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**Figure D-18.**—Map showing locations of sampling sites for this study (as filled in circles), and neighboring towns (marked by squares.)

selected for this study upstream from the mining activities to approximately 1-km (kilometer) upstream from the confluence of Whitewood Creek and the Belle Fourche River (fig. D-18). Although direct discharge of mine tailings into the creek ended in 1977, residual mine tailings form most of the bank and bottom sediment material over the 57-km study reach. These tailings contribute to a dissolved-As concentration gradient that increases in the downstream direction. Conversely, inputs of phosphorus (P) and other macronutrients from a sewage-treatment facility, at Deadwood, provides a dissolved P gradient that decreases in the downstream direction, except in downstream (arable) areas where irrigation waters from fertilized soils intermittently flow into the stream. Each year after snowmelt, a dense community of attached algae and submerged macrophytes quickly forms. A dense cover remains through the summer months. Coincidentally, peak dissolved As concentrations in the streamwater occur during the summer at downstream sites, possibly because of (1) a periphyton driven, diurnal pH fluctuation that causes release of particle-bound As; and (2) summer inputs of ground water that contains elevated dissolved As concentrations (Fuller and others, 1988, Goddard and others, 1988). The study reach, therefore, represents ideal field conditions for the examination and quantification of potentially important contributions of the periphyton to As mobilization and transport.

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#### EXPERIMENTAL PROCEDURE

The experimental approach involved determination of As transport characteristics for algae and macrophytes collected at four sampling sites (fig. D-18): (1) a site 7 km upstream from the mining activities (1,800 meters elevation); (2) 15 km downstream from the first site and within the town of Deadwood (0.25 km downstream from treatment plant discharge, 1,350 meters elevation); (3) 39 km farther downstream at a U.S. Geological Survey gaging station (870 meters elevation); and

(4) approximately 1 km upstream from the confluence of Whitewood Creek and the Belle Fourche River (850 meters elevation). These sites are hereafter referred to as the Headwater (HW) site, the Sewage Treatment Plant (STP) site, the Whitewood above Vale (V) site, and the Sheeler Seep (SH) site, respectively (Kuwabara and others, 1988). Abundant ground-water seepage occurs springs along the banks of the creek at the farthest downstream site.

Transport characteristics estimated include standing crop ( $\rho_b$ ) or the accessible periphyton mass per unit streambed area, net sorption rate constant ( $\lambda_b$ ), and accumulation factor (that is, biological partitioning coefficient,  $K_b$ ), and may be used to describe a biological component of a comprehensive transport model describing hydrologic, chemical and biological processes. Assuming a first-order process, the rate of change of dissolved As resulting from periphyton accumulation would take the form:

$$(\rho_{bj}/D_j)R_{bj} = -(\rho_{bj}/D_j)(\partial C_{bj}/\partial t) = -\lambda_{bj}(\rho_{bj}/D_j)(C_{bj} - K_{bj}C_j),$$

where the index  $j$  is a finite interval of the stream reach,  $D$  is the mean interval channel depth,  $R_b$  represents the temporal change in solute concentration associated with the periphyton ( $C_b$ ), and  $C$  is the solute concentration in the stream as a function of time ( $t$ ).

The As uptake by algal cells was studied in laboratory experiments as a function of dissolved arsenate and orthophosphate concentrations in media formulations. Unialgal cultures of the diatom *Achnanthes minutissima* (Kuetzing) Cleve collected along the study reach were used to determine uptake-rate constants. After isolation in liquid media and enriched agar plates, algal stocks were maintained in chemically defined media with added arsenate and orthophosphate concentrations similar to ambient levels at the sampling sites. *A. minutissima* was among the few species isolated from all four sites. *Silicococcus* spp. and *Scenedesmus* spp. also were isolated from all four sites and used in preliminary studies but were not as easily enumerated with the particle counter used because of the frequent formation of doublets and cell chains. Arsenate and phosphate uptake rate constants were determined from  $^{75}\text{As}$

and  $^{32}\text{P}$ -labeled experiments using cells, heat killed as described by Fisher and others (1984). Previous experiments (Kuwabara and others, 1988) indicated that use of heat-killed cells for short-term As and P uptake experiments yielded results comparable to living-cell cultures and avoided the need for biomass corrections. A  $3^2$  full-factorial experimental design ( $n$  (number of treatment replicates) = 3) was used to examine the interactive effects of As and P on uptake rates and accumulation of these solutes using *A. minutissima* isolates from the HW and above V sites. Results presented here consider only results of As uptake in these experiments.  $^{73}\text{As}$  and  $^{32}\text{P}$ -labeled stock solutions were used to achieve 0.0, 0.5, and 1.0  $\mu\text{M}$  (micromolar) initial concentrations of dissolved As and P in the nine chemically defined media formulations. An inoculum of heat-killed cells was then added ( $10^6$  cells per milliliter). After periods of 0.5, 1, 2, 4, 8 and 24 hours aliquots of the cell suspensions were centrifuged at 13,000 r/min (revolutions per minute) for 15 minutes. The supernatant was then removed and counted by liquid scintillation to determine solute removal from solution by the cells.

Periphyton abundance ( $\rho_b$ ) at each of the four sites was estimated monthly from late May to September 1987, using ash-free dry weight and chlorophyll-a measurements ( $n = 9$ ) of measured streambed areas (Franson, 1985). Measurements of channel width ( $n = 3$ ), depth ( $n = 9$ ) and channel velocity ( $n = 9$ ) were made at all sites, as were temperature, specific conductance, dissolved oxygen and pH (table D-6). Arsenic concentrations in dominant periphyton species were determined by hydride generation, atomic absorption spectroscopy (Johns and Luoma, 1988).

## RESULTS

Temporal changes in periphyton abundance differed among sites. As observed in the 1986 samples (Kuwabara and others, 1988), periphyton mass per unit streambed area generally increased with distance downstream from the HW site to the V site and then decreased abruptly at the most downstream (SH) site (table D-6). At the HW site, which was dominated throughout the summer

by a submerged macrophyte (*Ranunculus longirostris*), biomass slightly increased from an average of 37  $\text{g}/\text{m}^2$  (grams per square meter) in late May to 60  $\text{g}/\text{m}^2$  in September (table D-6). Monthly differences were, however, not significant at the 95-percent confidence level (table D-6). The large confidence intervals relative to average  $\rho_b$  values, even with nine replicates per site, is indicative of a patchy periphyton distribution. At the STP site, a community dominated by *Ranunculus* during June and July gradually changed to a dense growth of the pondweed *Zannichellia palustris* and the chlorophytes *Cladophora spp* and *Ulothrix spp*. during August and September. Similar to the HW site, monthly standing crop values were not statistically different (table D-6). Because of high-flow conditions, periphyton growth was not established at the V site during the first sampling period in June. However, by July, a thick (approximately 20 cm (centimeters)) mat of *Ranunculus* and epiphytic diatoms blanketed more than half of the stream channel. As at the STP site, August and September samples showed a shift toward *Zannichellia* and filamentous chlorophyte species at the V site. Once the periphyton was established, standing crop was clearly most abundant at the V site, where estimated biomass was an order of magnitude higher than at other sites. Elevated stream discharge in late May also inhibited the establishment of a periphyton community at the SH site, but *Zannichellia* was observed in abundance by July under much lower flow conditions (table D-6). Epiphytic diatoms and filamentous chlorophytes dominated periphyton communities in August and September samples. Standing crop was consistently lower at the SH site than the other three upstream sites.

Autotrophic indices (AI, mass ratios of periphyton dry weight to chlorophyll-a) ranged from 80 at the SH site in July to 310 at the V site in September (table D-6). Tabled values represent the mean of three replicates with 95-percent confidence intervals consistently less than 10 percent of the mean. This index has been used as an indicator of environmental stress, whereby increasing values indicate increasing abundance of senescent or stressed cells or an increasing

proportion of heterotrophic growth (Weber, 1973). All four sites in late May and July displayed AI values typical of unstressed periphyton communities (approximately  $100 \pm 50$ ), whereas elevated values were measured at the V and SH sites in August and at the HW, V, and SH sites in September.

The arsenic concentrations in algal tissues consistently increased between the late May and August sampling dates at all four sites (table D-7). Concentrations observed in August and September samples were similar or showed a decrease in September. There was also a discernible increase in tissue As concentrations with distance downstream. However, the range of

Table D-6.—Field data collected monthly during the summer of 1987 from four sites along Whitewood Creek, South Dakota: Headwater (HW), Sewage Treatment Plant (STP), Above Vale (V), and Sheeler Seep (SH) sites

5/27	930	HW	420	8.2	8.3	2.6±0.1	0.12±0.05	0.82±0.02	0.3	37±15	120
5/27	1430	STP	545	15.2	8.1	7.7±.5	.38±.09	.94±.12	2.8	34±17	110
5/28	1300	V	890	18.4	8.2	21.6±1.6	.35±.12	.30±.11	2.3	-	-
5/28	1550	SH	980	20.0	8.2	3.9±.4	.38±.15	.77±.20	1.1	-	-
7/7	1255	HW	450	16.0	8.3	2.4±.3	.10±.02	.58±.10	.1	52±19	110
7/8	1040	STP	760	17.0	8.4	7.3±.5	.24±.04	.74±.11	1.3	71±51	120
7/9	1300	V	1180	21.5	8.6	17.9±.7	.05±.02	.39±.14	.4	469±65	90
7/10	1610	SH	1310	28.0	8.6	3.4±.5	.24±.11	.86±.16	.7	28±5	80
8/19	940	HW	429	9.0	8.7	2.5±.3	.15±.03	.51±.16	.2	51±27	140
8/19	1300	STP	899	17.6	8.3	7.1±.3	.23±.06	.65±.19	1.1	54±19	100
8/26	1400	V	1108	17.0	8.0	18.0±.5	.21±.18	.13±.03	.5	280±30	180
8/26	1015	SH	1245	14.5	8.1	3.6±.3	.27±.04	.72±.20	.7	29±14	160
9/16	1315	HW	563	11.1	8.3	1.7±.2	.10±.02	.25±0.05	.1	60±34	170
9/16	1730	STP	930	16.8	7.9	6.5±.7	.19±.04	.53±0.07	.7	82±60	120
9/28	1745	V	1167	18.3	8.1	17.8±.8	.33±.06	.07±0.01	.4	237±25	310
9/28	1435	SH	1222	19.5	8.3	3.7±.4	.24±.05	.46±0.09	.4	7±4	280

concentrations observed during the growth period at the upstream (HW) site was much greater than that observed at the downstream sites (table D-7). Arsenic in submerged macrophyte tissue was slightly higher than found in the macroalgae although general concentrations trends at each site were very similar between plant groups (table D-7).

Short-term uptake-kinetics experiments using *A. minutissima* indicate that a first-order rate equation closely describes the observed algal uptake of arsenate. Fit of the kinetic data for both HW and V isolates at the nine treatment combinations to a first-order model (Dixon, 1985) yielded consistently high coefficients of determination

(table D-8). Uptake rate constants were significantly increased by elevating dissolved arsenate concentrations. A comparison of rate constants for HW and V isolates shows significantly lower constants for the apparently As-tolerant V isolate. The accumulation of As by both isolates was inhibited by increasing orthophosphate concentrations. The V isolate had slightly lower  $K_b$  values than did the HW isolate. Increasing dissolved arsenate concentrations in the media did not result in a corresponding increase in biologically sorbed As (note in table D-8 that the  $K_b$  values significantly decreased at higher dissolved As concentrations).

Table D-7.—Total arsenic concentrations (microgram solute per gram dry tissue) with 95-percent confidence intervals (4 replicates) in dominant periphyton species collected from four sites along Whitewood Creek, South Dakota

[The headwater and most upstream site (HW), sewage treatment plant site (STP), above Vale site (V) and Sheeler seep site (and most downstream sampling site for this study, SH). Symbol "-" indicates that the periphyton community had not yet been established at that site]

Sampling site	5/27-28/87	Date (month/day/year)		
		7/7-10/87	8/19-26/87	9/16-28/87
HW - Macroalgae <sup>1</sup>	1,465 ± 38	1,633 ± 33	1,884 ± 43	1,931 ± 50
- Macrophyte <sup>2</sup>	1,552 ± 16	1,866 ± 9	2,180 ± 52	1,991 ± 83
STP - Macroalgae	1,709 ± 22	1,819 ± 31	1,903 ± 18	1,933 ± 50
- Macrophyte	1,757 ± 14	1,971 ± 8	2,030 ± 49	2,099 ± 112
V - Macroalgae	-	2,051 ± 45	2,151 ± 15	2,117 ± 94
- Macrophyte	-	2,390 ± 8	2,479 ± 39	2,184 ± 95
SH - Macroalgae	-	2,358 ± 91	2,418 ± 24	2,317 ± 189
- Macrophyte	-	2,480 ± 16	2,572 ± 42	2,352 ± 23

<sup>1</sup>Samples dominated by filamentous chlorophytes and epiphytic diatoms. Specific changes in community structure are described in the text.

<sup>2</sup>Early summer samples typically dominated by the submerged macrophyte, *Ranunculus lanuginosus* and associated algal epiphytes, whereas later samples commonly dominated by the pondweed, *Zannichellia palustris*. Specific changes in dominant macrophyte species are discussed in the text.

Table D-8.--Results from experiments examining arsenate uptake by *Achnanthes minutissima* isolates from the Headwater site (HW, low (less than 0.1 micromolar) dissolved arsenic exposure) and the above Vale site (V, elevated (approximately 1 micromolar) dissolved arsenic exposure)

[Uptake rate constants ( $\lambda_b$ , in reciprocal hours) and accumulation factors ( $K_b$ , ratio of micromoles solute per gram algae to micromoles solute per gram media). Confidence intervals (95 percent) reflect the multivariate regression from a  $3^2$  full factorial design ( $n = 3$ ) sampled at elapsed times of 0.5, 1.0, 2.0, 4.0, 8.0 and 24.0 hours after inoculation. The coefficients of determination ( $r^2$ ) are given for a fit of the kinetic data to a first order model (Dixon, 1985). Details of the experimental design are provided in the text]

Treatments (Micromolar solute added)			Transport characteristics	
As	P	$\lambda_b$	$K_b$	$r^2$
<b>HW isolates</b>				
0.5	0.0	0.73 ± 0.08	1,790 ± 70	0.96
.5	.5	.56 ± .08	1,340 ± 90	.92
.5	1.0	.94 ± .13	990 ± 50	.92
1.0	.0	1.08 ± .11	990 ± 20	.96
1.0	.5	1.37 ± .22	800 ± 30	.90
1.0	1.0	1.77 ± .17	500 ± 30	.84
<b>V isolate</b>				
.5	.0	.39 ± .02	1,750 ± 30	.99
.5	.5	.48 ± .06	1,240 ± 30	.98
.5	1.0	.57 ± .07	960 ± 30	.99
1.0	.0	.74 ± .08	870 ± 30	.98
1.0	.5	.81 ± .11	630 ± 30	.94
1.0	1.0	1.09 ± .19	500 ± 20	.97

## DISCUSSION

Inhibition of As uptake with increasing dissolved orthophosphate concentrations that was observed for the freshwater diatom *A. minutissima* is an interesting, but not surprising observation. Preferential uptake of P over As previously has been reported for chlorophytes (Kuwabara and others, 1988) and for marine diatoms (Morris and McCartney, 1984). In contrast, Button and others (1973) found that the yeast *Rhodotorula rubra* sorbed P and As indiscriminately. Although the exclusion mechanism

for the test algae reported on here has not been identified, uptake results from the HW site isolate suggest that the mechanism is not acquired by prolonged As exposure.

Differences between algal isolates in As uptake and accumulation suggest that *A. minutissima* from the V site exhibited As tolerance in two ways. First, this isolate accumulated slightly less As than did controls (the HW isolate). Secondly, the V isolate consistently demonstrated slower As sorption kinetics than did the HW isolate. Both observations represent distinctive advantages for

cells exposed to elevated and fluctuating dissolved As concentrations.

Increasing algal abundance with distance downstream may be attributed to inputs of macronutrients from the water-treatment-plant effluent, ground-water inputs, and irrigation runoff from fertilized land. In addition, temperature effects on algal growth rates may contribute to the downstream increase in algal abundance (table D-6). At the SH site, decreased abundance may be caused by scarcity of firm substrate needed to establish a dense algal community. Visibly high turbidity also may hinder photosynthetic activity at this site. Although periphyton growth was patchy, especially at the SH site, replicate ash-free, dry-weight measurements indicated similar biomass concentrations within the patches.

Elevated AI values, particularly at the V and SH sites in August and September 1987, suggest an adverse change in the condition of the periphyton community. Senescence of periphytic tissue was visibly evident in these samples and probably represents typical growth progression within these mats. It is interesting to note that the elevated AI observed in August 1986, at the STP site (Kuwabara and others, 1988) was not observed in 1987, probably because of a shift in community structure of periphyton at the STP site in 1987 to include new growth of *Z. palustris* and various filamentous chlorophytes.

It is clear from these studies that algal isolates from Whitewood Creek have the ability to take up orthophosphate over arsenate preferentially. Given the As transport characteristics reported here for *A. minutissima*, the rapid growth of periphyton biomass between late May and July at the V and SH sites represents both a rapid and significant accumulation of As (table D-7) within this single biological component. Furthermore, *A. minutissima*, which was exposed to elevated concentrations of dissolved As, seems to be able to slow down the kinetics of cellular As uptake. In view of the above observations, the limitations of these results are equally clear. The trends in the transport characteristics presented above pose a number of additional complex questions related to the modeling of periphyton effects on stream transport of As: (1) What causes large annual

variations in community structure of the type seen between 1986 and 1987 samples? (2) How do solute sorption processes differ between the algae and submerged macrophytes seen in abundance in 1987? (3) How does the state of the periphyton affect As uptake and accumulation (that is, what other physical and chemical perturbations that account for changes in observed autotrophic indices, in addition to elevated orthophosphate concentrations, affect As uptake)? (4) How long does it take for periphyton species to adapt to ambient As concentrations by way of changing uptake characteristics?

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