

Greedy algae reduce arsenate

*Ferdi L. Hellweger*¹

Earth and Environmental Engineering Department, Columbia University, New York, New York 10027; HydroQual, Mahwah, New Jersey 07430

Kevin J. Farley

Environmental Engineering Department, Manhattan College, Riverdale, New York 10471; HydroQual, Mahwah, New Jersey 07430

Upmanu Lall

Earth and Environmental Engineering Department, Columbia University, New York, New York 10027

*Dominic M. Di Toro*²

Environmental Engineering Department, Manhattan College, Riverdale, New York 10471; HydroQual, Mahwah, New Jersey 07430

Abstract

Algae reduce and methylate arsenate, producing arsenite (As(III)) when the growth rates are high and dimethylarsinic acid (DMA) when the growth rates are low. In lakes, this leads to high As(III) concentrations in the early stages of spring and fall blooms and high DMA concentrations in the summer. We hypothesize that under phosphorus (P)-limited conditions, which usually exist in the summer, algae take up phosphate (PO₄) and, because of similar chemical characteristics, As(V) as well. Inside the cell, As(V) is reduced to As(III), methylated to monomethylarsonic acid (MMA) and DMA, and then excreted. However, under non-P-limited conditions, which exist in the early stages of blooms, algae up-regulate their PO₄ transport system to take up excess P, a phenomenon known as luxury uptake. Since As(V) is taken up by the PO₄ transport system, As(V) uptake also increases at this time. Within the cell, the reduction of As(V) to As(III) is fast, but methylation is slower, causing As(III) to build up in the cell and be excreted, which, in turn, causes an increase in extracellular As(III). This mechanism permits the synergistic (luxury uptake) and antagonistic (competition) effects of PO₄ on As(V) uptake and can therefore explain the seemingly contradictory results found in the literature. A mathematical model is constructed on the basis of existing established algal–nutrient interaction models and is used to simulate As transformation in two laboratory batch experiments. In addition to algal and P responses, the model can reasonably well reproduce the observed As(III) peak during the log growth phase and the more gradual appearance of DMA during the stationary phase.

It has been >20 yr since Sanders and Windom (1980) pointed to a pattern in As transformation by algae. In their words, “Arsenic reduction takes place only when the phytoplankton assemblage is in the log phase of growth, therefore the spring bloom [. . .] should contribute a large majority of the reduced arsenic species, with a smaller contribution from the smaller fall bloom.” After the log phase, As reduction ceases, and the reduced As(III) is oxidized to As(V), often at comparable rates, leading to As(III) peaks in the spring and fall. Subsequently, Howard et al. (1982) observed the occurrence of methylated As, mostly DMA, during the

summer. These trends are evident in the data from Lake Biwa, Japan, where As(III) peaks occur coincidentally with the early stages of spring and fall blooms (when the algae are in the log growth phase), and DMA is highest during the summer (Fig. 1).

Previous work—Seasonal changes in As speciation have been reported for several waterbodies, besides Lake Biwa. In the River Beaulieu, United Kingdom, As(III) concentrations generally peaked in the spring and were followed by a more gradual appearance of methylated species during the summer (Howard et al. 1984). The same pattern was observed in Southampton Water, United Kingdom (Howard and Apte 1989). In Upper Mystic Lake, Massachusetts, spring and fall As(III) peaks were observed. DMA was highest in the summer, with the buildup lagging As(III) in the spring (Aurilio et al. 1994). In Davis Creek Reservoir, California, a weak As(III) peak was present in the fall (no samples were taken in the spring), and DMA was found in the summer (Anderson and Bruland 1991). As(III) was produced in the spring, but it persisted throughout the summer, in Lake Greifen, Switzerland (Kuhn and Sigg 1993). Neither As(III) nor DMA was observed in the epilimnion of Crowley Lake, Cal-

¹ To whom correspondence should be addressed. Present address: HydroQual, 1 Lethbridge Plaza, Mahwah, New Jersey 07430 (ferdi@hydroqual.com).

² Present address: Civil and Environmental Engineering Department, University of Delaware, Newark, Delaware 19716.

Acknowledgments

We thank Hiroshi Hasegawa for providing the data from his experiments. Paul Dombrowski and two anonymous reviewers provided useful comments on the manuscript. This work was funded by the National Institute of Environmental Health Superfund Basic Research Program grant P42ES10344.

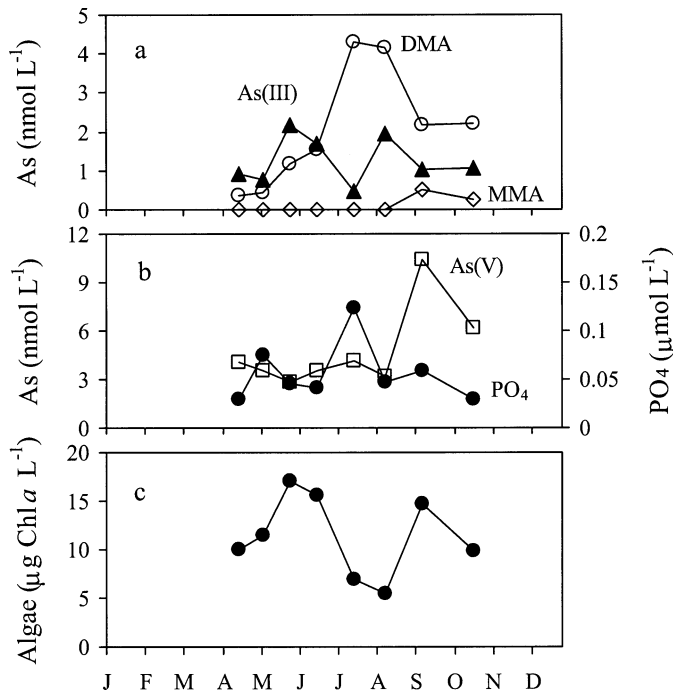


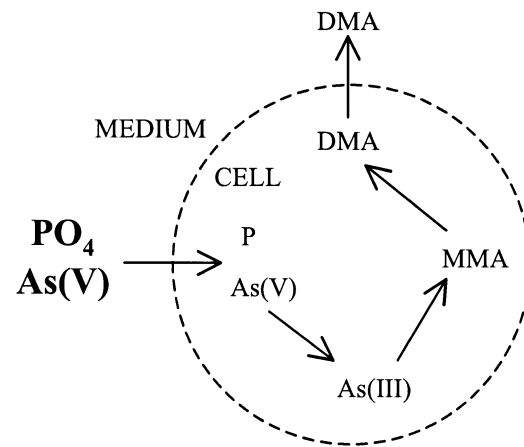
Fig. 1. As speciation in Lake Biwa, Japan. (a) As(III), MMA, and DMA; (b) As(V) and PO_4 ; and (c) algae concentrations as a function of time. Data for Sta. S3, epilimnion, 1993, from Sohrin et al. (1997).

ifornia (Kneebone and Hering 2000). Data were collected only three times during this study, and it is possible that, because of the short duration of the As(III) peaks, they were missed.

Similar behavior has been observed in batch experiments. During the log phase, As(III) peaks, and methylated species, mostly DMA, occur gradually during the course of the experiment. This is evident in laboratory batch experiments (Hasegawa et al. 2001) and in field experiments in Chesapeake Bay (Sanders and Riedel 1993). In a mesocosm experiment in Loch Ewe, Scotland, no As(III) or methylated species were found near the surface (3 m) (Apte et al. 1986). However, the water at the bottom of the bag, where settled algae collected, contained 64% DMA. Therefore, despite some exceptions, this phenomenon—i.e., that As(III) is produced at high growth rates and DMA at lower growth rates—appears to be common to many waterbodies and can be reproduced in batch experiments. To date, no mechanistic explanation has been presented for these observations.

Proposed mechanism—We hypothesize that these seasonal changes in As speciation are linked to the luxury uptake of P by algae as depicted in Fig. 2. Under P-limited conditions, which exist in the summer, algae take up As(V), reduce it to As(III), methylate it to MMA and DMA, and then excrete it. However, under non-P-limited conditions, which exist in the early stages of blooms, algae up-regulate their PO_4 transport system to take up excess P, a phenomenon known as luxury uptake. Large quantities of As(V) are also taken up by the PO_4 transport system at this time. Within the cell, the reduction of As(V) to As(III) is fast, but meth-

(a) P-limited



(b) Not P-limited (luxury uptake)

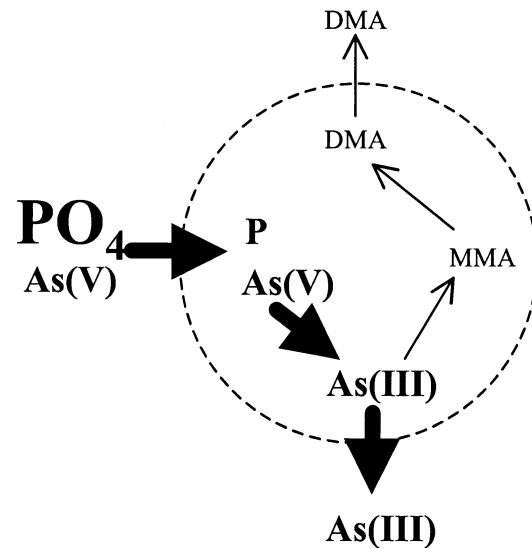


Fig. 2. As transformation by algae. (a) P-limiting conditions and (b) non-P-limiting conditions. Size of text corresponds to species concentrations. Size of arrows corresponds to rates. The general reaction sequence is as proposed by Cullen et al. (1994a).

ylation is slower, causing As(III) to build up in the cell. Intracellular As(III) is then excreted, causing the increase in extracellular As(III).

This paper presents a mathematical model to support this hypothesis. The model builds on existing established algal-nutrient interaction models, which are reviewed in the next section. The extension of the model to arsenic is then presented. Each process in the proposed reaction scheme (Fig. 2), including uptake, excretion, reduction and methylation, and extracellular reactions, is discussed. Finally, we present the application of the model to data from the laboratory batch experiments of Hasegawa et al. (2001).

Existing algal–nutrient interaction models

Mass balances—When modeling the uptake and transformation of a constituent (e.g., P) by algae, it is convenient to start with the mass balance of the constituent in the medium.

$$\frac{dS}{dt} = -VX + WX \quad (1)$$

and in the algal cells

$$\frac{dQ}{dt} = VX - WX \quad (2)$$

where S (mol L⁻¹) is the concentration of constituent in the medium, Q (mol L⁻¹) is the concentration of constituent in the algae (per unit volume of water), X (mg carbon [C] L⁻¹) is the algal biomass, V (mol g C⁻¹ d⁻¹) is the specific uptake rate, and W (mol g C⁻¹ d⁻¹) is the specific excretion rate. It is convenient to normalize the mass in the algae to the algal biomass, which is called the cell quota ($q = Q/X$). The mass balance for the cell quota is as follows:

$$\frac{dq}{dt} = V - W - \mu q \quad (3)$$

where μ (d⁻¹) is the specific growth rate. The last term in Eq. 3 accounts for the decrease in cell quota due to an increase in biomass. This is also referred to as growth dilution.

Algal growth—Under nutrient-limiting conditions, the specific growth rate of the algae is a function of the cell quota of the limiting nutrient (Droop 1974).

$$\mu = \mu_{\max} \left(1 - \frac{q_0}{q} \right) \quad (4)$$

where μ_{\max} (d⁻¹) is the hypothetical maximum specific growth rate at infinite q , and q_0 (mol g C⁻¹) is the subsistence cell quota at which growth is zero. For growth on multiple nutrients, the rate is controlled by the nutrient in minimal supply (Liebig's law of the minimum). Equation 4 is extended using a threshold operator (Droop 1974).

$$\mu = \mu_{\max} \min \left[\left(1 - \frac{q_{0,A}}{q_A} \right), \left(1 - \frac{q_{0,B}}{q_B} \right), \dots, L_H \right] \quad (5)$$

The subscripts A, B, \dots refer to different nutrients, and the constant L_H is the growth-limitation threshold, which accounts for limitation by a nutrient not explicitly considered (e.g., a hypothetical nutrient H , as proposed by Droop 1975) or some other factor.

Uptake—Uptake of nutrients and other constituents can be active or passive, but in this application, we are only concerned with active uptake. Active transport is conventionally modeled using the Michaelis–Menten enzyme saturation equation.

$$V = V_{\max} \frac{S}{K_M + S} \quad (6)$$

where V_{\max} (mol g C⁻¹ d⁻¹) is the maximum uptake rate, and

K_M (mol L⁻¹) is the half-saturation constant. PO₄ uptake is conventionally modeled using Eq. 6, but it often deviates from Michaelis–Menten kinetics. Most importantly, it varies with the intracellular concentration (cell quota), with increased uptake rates observed at both low (P starved) and high (luxury uptake) cell quotas as discussed below.

Uptake by P-starved cells—It has been observed that algae with low P cell quotas (i.e., P starved) take up PO₄ at a much faster rate than algae with higher cell quotas. This can be modeled by adding a noncompetitive inhibition term to Eq. 6 (Rhee 1973).

$$V = V_{\max} \frac{S}{K_M + S} \left(\frac{1}{1 + i/K_i} \right) \quad (7)$$

where i (mol g C⁻¹) is the inhibitor concentration, and K_i (mol g C⁻¹) is the inhibition constant. Rhee (1973) finds that a number of internal P compounds (e.g., polyphosphates, RNA P, lipid P) can serve as the inhibitor.

Luxury uptake—At the opposite end of the spectrum under non-P-limiting conditions, PO₄ uptake also deviates from Michaelis–Menten kinetics. Algae, when grown under non-P-limited conditions (e.g., N-limited conditions), take up PO₄ at a much higher rate (Droop 1974; Rhee 1974). This can be modeled as a separate uptake system that “switches” on or off depending on the nutrient status of the algae. Rhee (1974) suggests the following luxury uptake model, where the net uptake rate is proportional to the growth rate.

$$V_{\text{net}} = V - W = \mu q_* \quad (8)$$

where V_{net} (mol g C⁻¹ d⁻¹) is the net specific uptake rate, and q_* (mol g C⁻¹) is the steady-state cell quota for non-P-limited conditions.

Luxury uptake is evident in batch culture, as illustrated by the data of Droop (1975) and Hasegawa et al. (2001) presented in Fig. 3 (the model lines will be discussed later in the paper). The data show that P cell quotas are high during the log phase and low during the stationary phase. The reason for this behavior is that during the log phase, cells are not P limited, and as in continuous culture, they up-regulate their PO₄ transport system, causing high P cell quotas. Later in the experiments, the algae are P limited, luxury uptake ceases, and P cell quotas drop accordingly.

Generally, higher P cell quotas during the log phase do not by themselves demonstrate luxury uptake. At first glance, it appears that the Michaelis–Menten uptake model could predict this because the PO₄ concentrations decrease during the course of the experiment. However, quantitatively, the data do not agree with the Michaelis–Menten model (*see* Web appendix 1 at http://www.aslo.org/lo/toc/vol_48/issue_6/2275a1.pdf). To further illustrate luxury uptake, we calculate uptake rates for PO₄, assuming the cell quotas are at steady state, which is a reasonable assumption for timescales greater than a few days (Di Toro 1980), and neglecting excretion (Eq. 3, $V = \mu q$). The uptake rates are plotted against extracellular concentrations in Fig. 4. The As data will be discussed later in the paper. The rates at the highest concentrations (circled points in Fig. 4), corresponding to the log

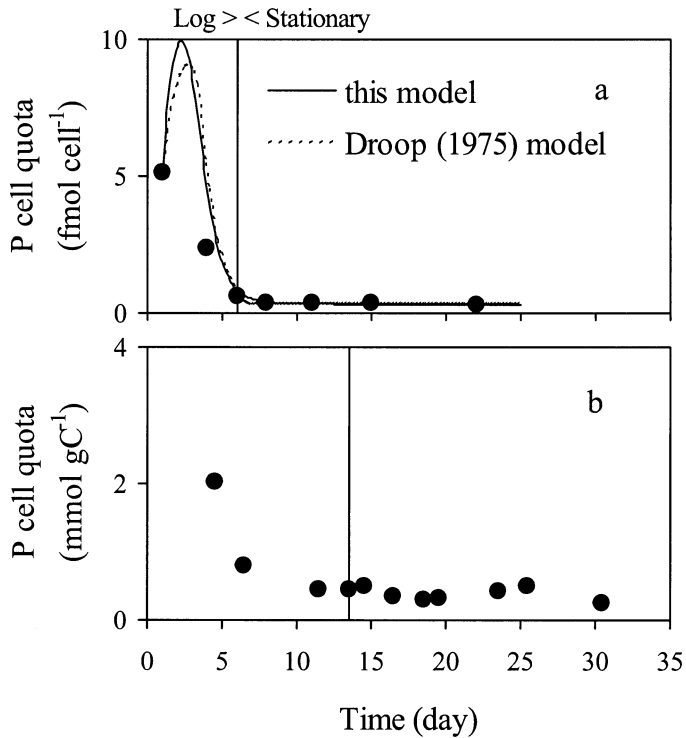


Fig. 3. Luxury uptake of PO_4 in batch culture. P cell quota as a function of time. Note different units between panels. Transition between log and stationary phase is defined to occur at the minimum $d\mu/dt$. (a) Data for *Monochrysis lutheri* from Droop (1975). The lines are the model's prediction (see text). Parameters for "This Model": same as in Droop (1975), except $q_{*,\text{PO}_4} = 50 \text{ fmol cell}^{-1}$, $K_{M^*,\text{PO}_4} = 3.0 \mu\text{mol L}^{-1}$, and $W_{\text{PO}_4} = 0.6 \text{ fmol cell}^{-1} \text{ d}^{-1}$. (b) Data for *Closterium aciculare* from Hasegawa et al. (2001).

growth phase, are very high and cannot be explained by the Michaelis–Menten model (line in Fig. 4).

As demonstrated by Droop (1975), luxury uptake in batch culture can be simulated using a combination of Michaelis–Menten and luxury uptake models, and Droop's model results are presented in Fig. 3a. The key is to dynamically differentiate between P-limited and non-P-limited conditions and use the appropriate uptake model. Whether the algae are P limited or not is determined by the threshold equation for growth limitation (Eq. 5), with P-limited conditions assumed to exist when the P-limitation term $(1 - q_{0,p}/q_p)$ is the lowest of all the terms in the minimum operator.

Excretion—Excretion can be active or passive, but in this application, we are only concerned with passive excretion. For passive transport, the rate is proportional to the concentration gradient across the cell membrane. The excretion rate is defined as follows:

$$W = P_m A_{\text{cell}} (q_E \rho_{\text{cell}} - S) \quad (9)$$

where P_m (cm s^{-1}) is the membrane permeability coefficient, A_{cell} ($\text{m}^2 \text{ g C}^{-1}$) is the specific cell surface area, q_E (mol g C^{-1}) is the cell quota subject to excretion, and ρ_{cell} (g C L cell^{-1}) is the volumetric cell C density, i.e., the concentration of C per unit cell volume. In Eq. 9, the cell quota subject to excretion (q_E) can be different from the total cell quota

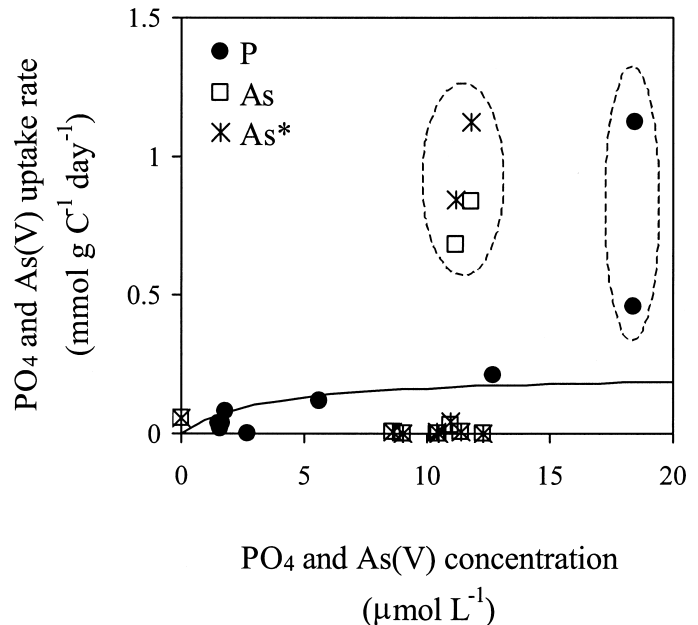


Fig. 4. Luxury uptake of PO_4 and As(V). PO_4 and As(V) uptake rates as a function of medium concentration for algae grown in batch culture. Observed uptake rates are calculated on the basis of steady-state mass balance (Eq. 3, $dq/dt = 0$, $W = 0$, $V = \mu q$). Line corresponds to Michaelis–Menten uptake model (Eq. 6) fit by eye to PO_4 data. Circled data are affected by luxury uptake and are not included in the curve fit. As^* is the hypothetical "no-excretion" As cell quota (see text). Data for *Closterium aciculare* from Hasegawa et al. (2001) (high As(V) experiment).

(q) when a fraction of the constituent is in a form unavailable for diffusion (e.g., structural P vs. dissolved PO_4).

The excretion of PO_4 is a significant process, as illustrated by the radioactive $^{32}\text{PO}_4$ tracer data of Lean and Nalewajko (1976) (Fig. 5a). In these experiments, a small amount of $^{32}\text{PO}_4$ is added to a culture, and the distribution between the algae and medium is followed as time progresses. The algae quickly take up most of the $^{32}\text{PO}_4$, and after about 60 min, equilibrium is established. The data can be used to estimate the excretion of PO_4 , as described by Lean and Nalewajko (1976), who provide first-order excretion rate constants. Since the intracellular PO_4 concentrations are typically several orders of magnitude greater than the external concentrations (e.g., Rhee 1973), S can be assumed negligible in Eq. 9. The first-order rate constants can therefore be equated to $P_m A_{\text{cell}} \rho_{\text{cell}}$. Assuming reasonable values for ρ_{cell} and A_{cell} , the first-order excretion rate constants were converted to permeability coefficients to provide a range of values for this application (see caption of Fig. 5).

To calculate excretion, the intracellular dissolved PO_4 concentration (q_{PO_4}) needs to be estimated. It is reasonable to assume that q_{PO_4} would be correlated to the total P cell quota. In that case, the excretion rate would increase with P cell quota and growth rate. However, this is not supported by the experimental evidence. The experiments of Lean and Nalewajko (1976), conducted with algae at various growth stages, show no relationship between excretion and growth rates. Olsen (1989) estimated decreasing excretion with increasing

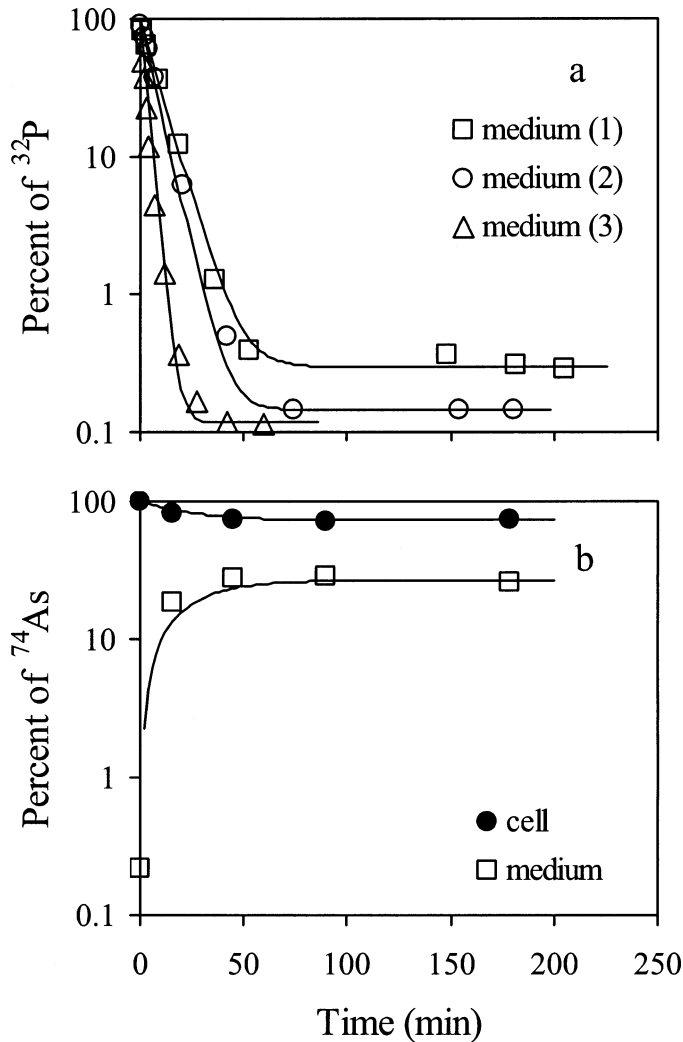


Fig. 5. PO_4 and As(V) excretion. Radioactive $^{32}\text{PO}_4$ and $^{74}\text{As(V)}$ concentrations as a function of time. (a) Data for (1) *Chlorella pyrenoidosa* ($P_m = 0.13\text{--}0.27 \times 10^{-9} \text{ cm s}^{-1}$), (2) *Scenedesmus quadricauda* ($P_m = 0.68\text{--}1.9 \times 10^{-9} \text{ cm s}^{-1}$), and (3) *Anabaena flos-aquae* ($P_m = 6.9\text{--}24 \times 10^{-9} \text{ cm s}^{-1}$) from Lean and Nalewajko (1976). (b) Data for *Chlorella vulgaris* from Maeda et al. (1992a) ($P_m = 33 \times 10^{-9} \text{ cm s}^{-1}$). Assumed $\rho_{\text{cell}} = 40 \text{ g C L}^{-1}$ (see Table 5), and $A_{\text{cell}} = 15 \text{ m}^2 \text{ g C}^{-1}$ for a sphere with a diameter of $10 \mu\text{m}$.

growth rates. The reason for this is that algae store excess P mostly as polyphosphates and less as PO_4 . This was illustrated by Rhee (1973), who found that although the intracellular concentration of PO_4 increased with growth rate, the relationship was “ill defined” compared to that of polyphosphates. For simplicity, we assume a constant PO_4 cell quota, which results in a constant excretion rate.

Extension to arsenic

General model description—The model presented in this paper includes state variables for algal biomass, P, and As species as listed in Table 1. The mass balance equations for each constituent are written on a volume water basis as in Eqs. 1 and 2 (Eqs. 1-1 through 1-11, Table 1). The model

Table 1. Model state variables and mass balance equations.

X = algal biomass concentration (mg C L^{-1}):

$$\frac{dX}{dt} = \mu X \quad (1-1)$$

S_{PO_4} = external PO_4 concentration (mmol P L^{-1}):

$$\frac{dS_{\text{PO}_4}}{dt} = -V_{\text{PO}_4}X + W_{\text{PO}_4}X \quad (1-2)$$

$Q_P = q_P X$ = internal P concentration (mmol P L^{-1}):

$$\frac{dQ_P}{dt} = V_{\text{PO}_4}X - W_{\text{PO}_4}X \quad (1-3)$$

$S_{\text{As(V)}}$ = external As(V) concentration (mmol As L^{-1}):

$$\frac{dS_{\text{As(V)}}}{dt} = -V_{\text{As(V)}}X + W_{\text{As(V)}}X + k_{\text{O,As(III)}}S_{\text{As(III)}} \quad (1-4)$$

$Q_{\text{As(V)}} = q_{\text{As(V)}}X$ = internal As(V) concentration (mmol As L^{-1}):

$$\frac{dQ_{\text{As(V)}}}{dt} = V_{\text{As(V)}}X - W_{\text{As(V)}}X - V_{\text{R,As(V)}}X \quad (1-5)$$

$S_{\text{As(III)}}$ = external As(III) concentration (mmol As L^{-1}):

$$\frac{dS_{\text{As(III)}}}{dt} = W_{\text{As(III)}}X - k_{\text{O,As(III)}}S_{\text{As(III)}} + k_{\text{DM,MMA}}S_{\text{MMA}} \quad (1-6)$$

$Q_{\text{As(III)}} = q_{\text{As(III)}}X$ = internal As(III) concentration (mmol As L^{-1}):

$$\frac{dQ_{\text{As(III)}}}{dt} = -W_{\text{As(III)}}X + V_{\text{R,As(V)}}X - V_{\text{M,As(III)}}X \quad (1-7)$$

S_{MMA} = external MMA concentration (mmol As L^{-1}):

$$\frac{dS_{\text{MMA}}}{dt} = W_{\text{MMA}}X + k_{\text{DM,DMA}}S_{\text{DMA}} - k_{\text{DM,MMA}}S_{\text{MMA}} \quad (1-8)$$

$Q_{\text{MMA}} = q_{\text{MMA}}X$ = internal MMA concentration (mmol As L^{-1}):

$$\frac{dQ_{\text{MMA}}}{dt} = -W_{\text{MMA}}X + V_{\text{M,As(III)}}X - V_{\text{M,MMA}}X \quad (1-9)$$

S_{DMA} = external DMA concentration (mmol As L^{-1}):

$$\frac{dS_{\text{DMA}}}{dt} = W_{\text{DMA}}X - k_{\text{DM,DMA}}S_{\text{DMA}} \quad (1-10)$$

$Q_{\text{DMA}} = q_{\text{DMA}}X$ = internal DMA concentration (mmol As L^{-1}):

$$\frac{dQ_{\text{DMA}}}{dt} = -W_{\text{DMA}}X + V_{\text{M,MMA}}X \quad (1-11)$$

follows the reaction sequence presented in Fig. 2. Kinetic equations (i.e., uptake rate) are presented in Table 2 and are discussed in detail below.

The model contains state variables for As(V), As(III), MMA, and DMA, which are the dominant arsenic species in surface waters. It should be noted that other more complex organic arsenic compounds (i.e., arsenosugars; Lunde 1973; Francesconi and Edmonds 1997) make up a significant fraction of arsenic inside the algal cells. These compounds, however, have not been detected outside the algal cell, and information on their formation/transformation reactions inside the cell is limited. In this application, we are concerned with the uptake, transformation, and excretion of arsenic by algae,

Table 2. Model kinetic equations.

Algal growth

$$\mu = \mu_{\max} \min \left[\left(1 - \frac{q_{0,P}}{q_P} \right), L_H \right] \quad (2-1)$$

Uptake

$$V_{\text{PO}_4} = \begin{cases} V_{\max, \text{PO}_4} \frac{S_{\text{PO}_4}}{K_{\text{M,PO}_4} (1 + S_{\text{As(V)}/K_{\text{M,As(V)}}) + S_{\text{PO}_4}} & \text{if P-limited*} \\ \mu q_{*, \text{PO}_4} \frac{S_{\text{PO}_4}}{K_{\text{M}^*, \text{PO}_4} (1 + S_{\text{As(V)}/K_{\text{M}^*, \text{As(V)}}) + S_{\text{PO}_4}} & \text{if not P-limited*} \end{cases} \quad (2-2)$$

$$V_{\text{As(V)}} = \begin{cases} V_{\max, \text{As(V)}} \frac{S_{\text{As(V)}}}{K_{\text{M,As(V)}} (1 + S_{\text{PO}_4}/K_{\text{M,PO}_4}) + S_{\text{As(V)}}} & \text{if P-limited*} \\ \mu q_{*, \text{As(V)}} \frac{S_{\text{As(V)}}}{K_{\text{M}^*, \text{As(V)}} (1 + S_{\text{PO}_4}/K_{\text{M}^*, \text{PO}_4}) + S_{\text{As(V)}}} & \text{if not P-limited*} \end{cases} \quad (2-3)$$

Excretion

$$W_{\text{PO}_4} = P_{\text{m,PO}_4} A_{\text{cell}} (q_{\text{PO}_4} \rho_{\text{cell}} - S_{\text{PO}_4}) \quad (2-4)$$

$$W_{\text{As(V)}} = P_{\text{m,As(V)}} A_{\text{cell}} (q_{\text{As(V)}} \rho_{\text{cell}} - S_{\text{As(V)}}) \quad (2-5)$$

$$W_{\text{As(III)}} = P_{\text{m,As(III)}} A_{\text{cell}} (q_{\text{As(III)}} \rho_{\text{cell}} - S_{\text{As(III)}}) \quad (2-6)$$

$$W_{\text{MMA}} = P_{\text{m,MMA}} A_{\text{cell}} (q_{\text{MMA}} \rho_{\text{cell}} - S_{\text{MMA}}) \quad (2-7)$$

$$W_{\text{DMA}} = P_{\text{m,DMA}} A_{\text{cell}} (q_{\text{DMA}} \rho_{\text{cell}} - S_{\text{DMA}}) \quad (2-8)$$

Reduction and methylation

$$V_{\text{R,As(V)}} = k_{\text{R,As(V)}} q_{\text{As(V)}} \quad (2-9)$$

$$V_{\text{M,As(III)}} = \begin{cases} k_{\text{M,As(III)}} q_{\text{As(III)}} & \text{if P-limited*} \\ k_{\text{M}^*, \text{As(III)}} q_{\text{As(III)}} & \text{if not P-limited*} \end{cases} \quad (2-10)$$

$$V_{\text{M,MMA}} = k_{\text{M,MMA}} q_{\text{MMA}} \quad (2-11)$$

* P-limited conditions exist when $(1 - q_{0,P}/q_P) < L_H$ in Eq. 2-1.

and we therefore limit the model state variables to the species directly involved in the general reduction and methylation sequence ($\text{As(V)} \rightarrow \text{As(III)} \rightarrow \text{MMA} \rightarrow \text{DMA}$). Because of this simplification, the ability of the model to predict the full arsenic speciation in algal cells is limited.

Uptake—The first step in the overall transformation reaction is the uptake of As(V) by algae. Killed or metabolically inhibited algae do not take up significant amounts of As(V) (e.g., Andreae and Klumpp 1979; Klumpp 1980), which shows that As(V) is taken up by an active transport system. As(V) is toxic, and there is no known metabolic function for it, making it unlikely that the algae have an active transport system specifically for As(V). It is reasonable to assume that, because of the chemical similarities of As(V) and PO_4 , the PO_4 uptake system cannot differentiate between As(V) and PO_4 and that As(V) is taken up by the PO_4 transport system. The Michaelis–Menten uptake model (Eq. 6) is a reasonable first approximation for simulating PO_4 uptake, and As(V) uptake has been observed to exhibit saturation kinetics (e.g., Klumpp 1980). However, the deviations from Michaelis–Menten kinetics observed for PO_4 (i.e., luxury uptake, as discussed above) are important for As(V) uptake as well. Strong evidence exists for the luxury uptake of arsenic, as shown below.

Luxury uptake—A key element to our hypothesis is that As(V) uptake is also affected by luxury uptake. Unfortunately, rigorous continuous culture experiments, such as

those of Droop (1974) and Rhee (1974), have not been performed with As(V), but data from batch experiments are available to support our contention. For example, P and As cell quotas from the experiments of Hasegawa et al. (2001) and Yamaoka et al. (1988) are presented in Fig. 6 and show that As cell quotas follow the same pattern as P cell quotas affected by luxury uptake during log growth (Fig. 3). This is evident in the As cell quotas for the experiments using high As(V) concentrations (Fig. 6a,c). The experiment with lower As(V) concentrations (Fig. 6b), however, does not appear to follow that pattern. That is because As(V) is transformed and excreted by the algae, as is evident by the presence of As(III), MMA, and DMA in the medium (these data are presented Figs. 9, 10). The As cell quotas are therefore not representative of the total amount of As(V) taken up by the cells. To better illustrate the effect of luxury uptake, a “hypothetical no-excretion As cell quota” (As^*) is computed by converting the concentration of As(III), MMA, and DMA in the medium to an equivalent cell quota and adding that to the observed cell quota, since those species must have been internal As prior to transformation and excretion. The As^* cell quota has a pattern similar to that of the P cell quota (Fig. 6a,b). No As speciation was performed by Yamaoka et al. (1988); hence, As^* cannot be computed for Fig. 6c.

As with PO_4 uptake, higher As cell quotas during the log phase are qualitatively consistent with the Michaelis–Menten model, but a more quantitative analysis demonstrates that this model cannot explain As(V) uptake (see Web appendix

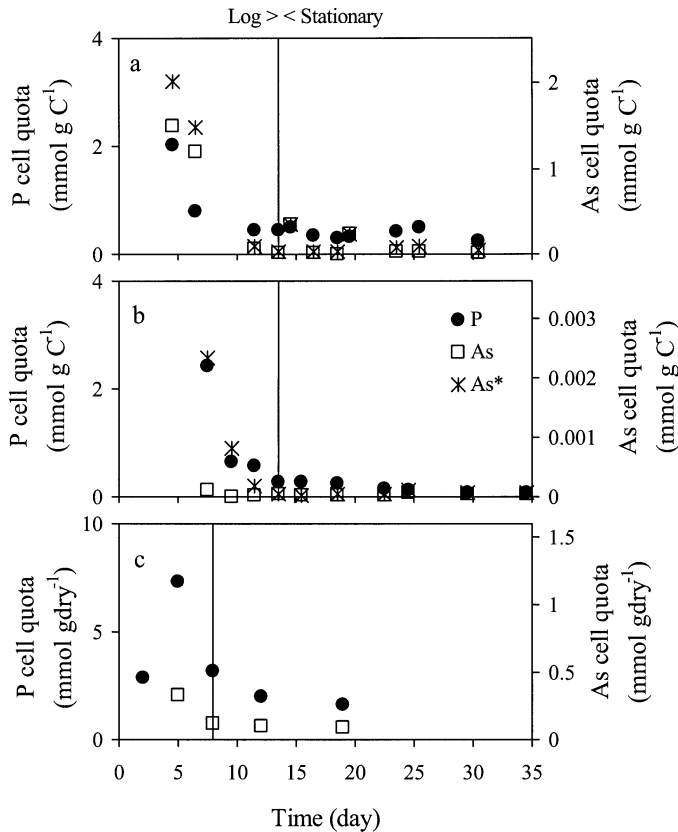


Fig. 6. Luxury uptake As(V) in batch culture. P and As cell quotas as a function of time. Note different units between panels. Transition between log and stationary phase is defined to occur at the minimum $d\mu/dt$. The y-axis values for P and As cell quotas are scaled on the basis of the initial PO_4 :As(V) ratio in the medium. As* is the hypothetical “no-excretion” As cell quota (see text). (a, b) Data for *Closterium aciculare* from Hasegawa et al. (2001). (c) Data for *Dunaliella* sp. from Yamaoka et al. (1988). Initial medium concentrations ($\mu\text{mol L}^{-1}$): (a) $\text{PO}_4 = 19$, As(V) = 12; (b) $\text{PO}_4 = 11$, As(V) = 0.010; and (c) $\text{PO}_4 = 33$, As(V) = 5.4.

1). As was performed for PO_4 , As(V) uptake rates were calculated and plotted against the extracellular concentrations in Fig. 4. Again, the rates at the highest concentrations (circled in Fig. 4), corresponding to the log growth phase, are very high and do not conform to the Michaelis–Menten model.

The luxury uptake model used in this application for both arsenic and P is based on Rhee’s (1974) model (Eq. 8) with two modifications. First, since our model explicitly accounts for excretion, Rhee’s model is used for gross uptake rather than net uptake. Second, a Michaelis–Menten term is added to the model. This is done even though the uptake rate is not observed to vary with extracellular PO_4 concentration. The reasons for including the Michaelis–Menten term are that it provides a more mechanistic description of uptake via an active uptake system and that it will provide a basis for describing competitive uptake as discussed below. The luxury uptake model used is as follows:

$$V = \mu q_* \frac{S}{K_{M^*} + S} \quad (10)$$

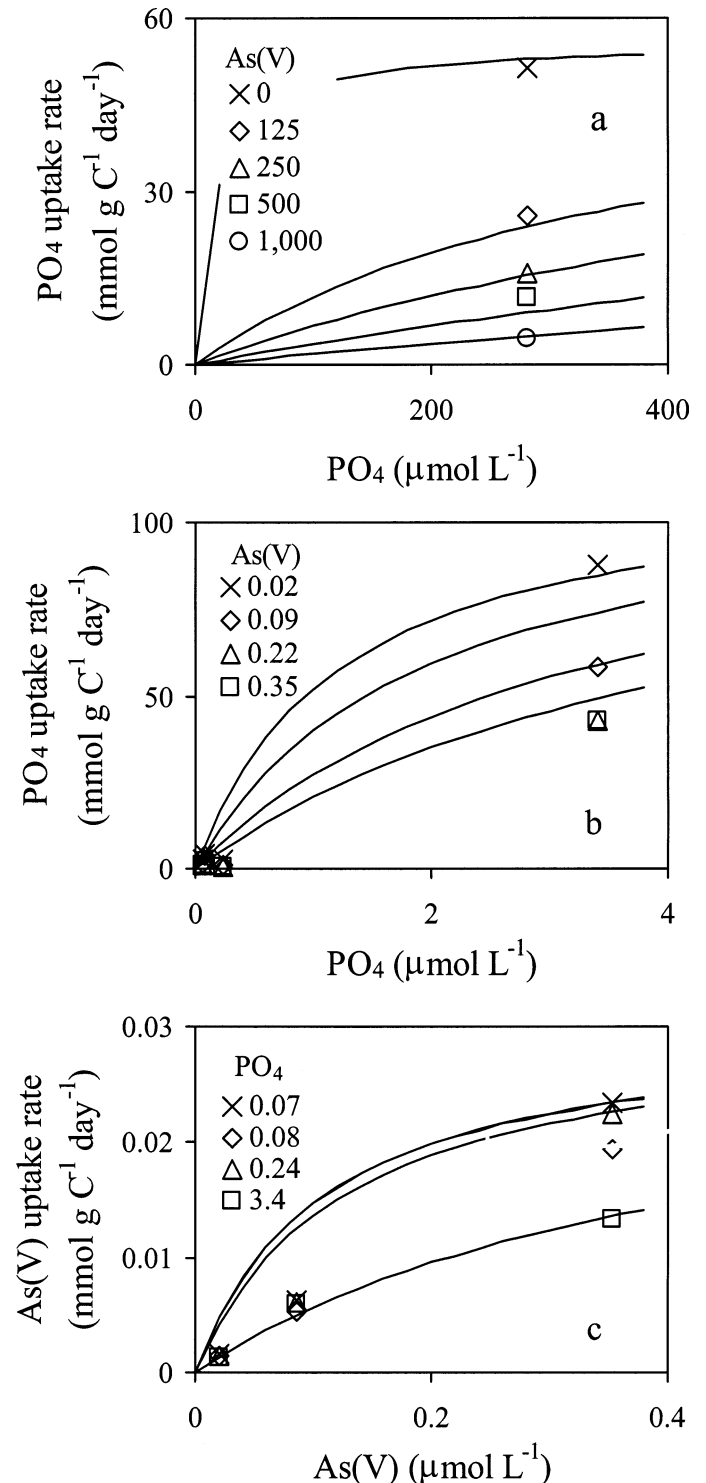


Fig. 7. PO_4 and As(V) uptake competition. PO_4 and As(V) uptake rates as a function of substrate concentrations for various inhibitor concentrations. Lines correspond to Eq. 12 with parameters listed in Table 3. (a) Data for *Euglena gracilis* from Blum (1966). (b, c) Data for *Skeletonema costatum* from Sanders and Windom (1980).

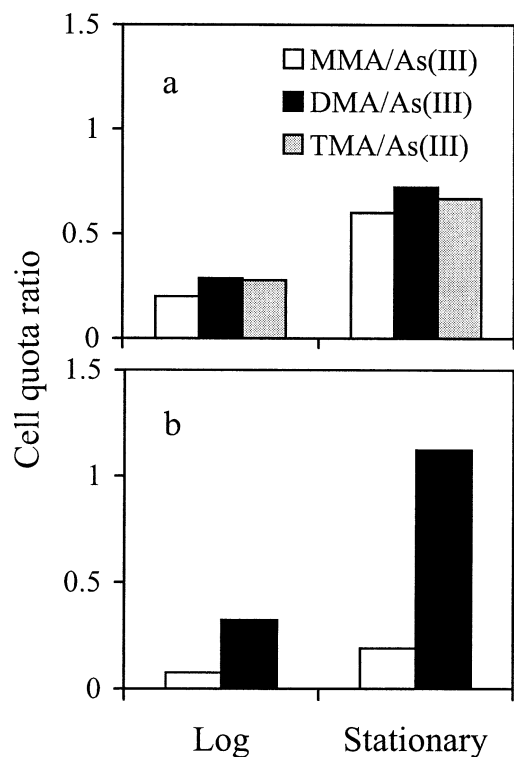


Fig. 8. Intracellular As speciation. Ratio of methylarsenic to As(III) cell quotas during log and stationary growth phases. (a) Data for *Chlorella vulgaris* from Suhendrayatna et al. (1999). TMA, trimethylarsenic compounds. (b) Data for *C. vulgaris* from Goessler et al. (1997).

where q_* (mol g C⁻¹) is the steady-state cell quota, and K_{M^*} (mol L⁻¹) is the half-saturation constant for nonlimiting conditions. Following the modeling strategy of Droop (1975), the uptake model (“regular” or luxury uptake) used depends on whether the algae are P limited.

$$V = \begin{cases} V_{\max} \frac{S}{K_M + S} & \text{if P-limited} \\ \mu q_* \frac{S}{K_{M^*} + S} & \text{if not P-limited} \end{cases} \quad (11)$$

P-limited conditions are assumed to exist when the P-limitation term in the growth-limitation equation (Eq. 5) is the minimum. This model can produce a time course of P cell quotas similar to that in the model by Droop (1975), as illustrated by the solid line in Fig. 3a.

Uptake competition/preference—If As(V) is taken up by the PO₄ transport system, then As(V) and PO₄ should compete for uptake sites on the cell membrane, causing mutual inhibition of transport. This is indeed observed, as illustrated by the data of Blum (1966) (Fig. 7a) and Sanders and Windom (1980) (Fig. 7b,c). As shown, the uptake rate of PO₄ decreases with increasing As(V) concentration and vice versa. It should be noted that although mutual inhibition is generally observed, some exceptions have been reported (e.g., Budd and Craig 1981). Competitive uptake can be modeled by extending the Michaelis–Menten model.

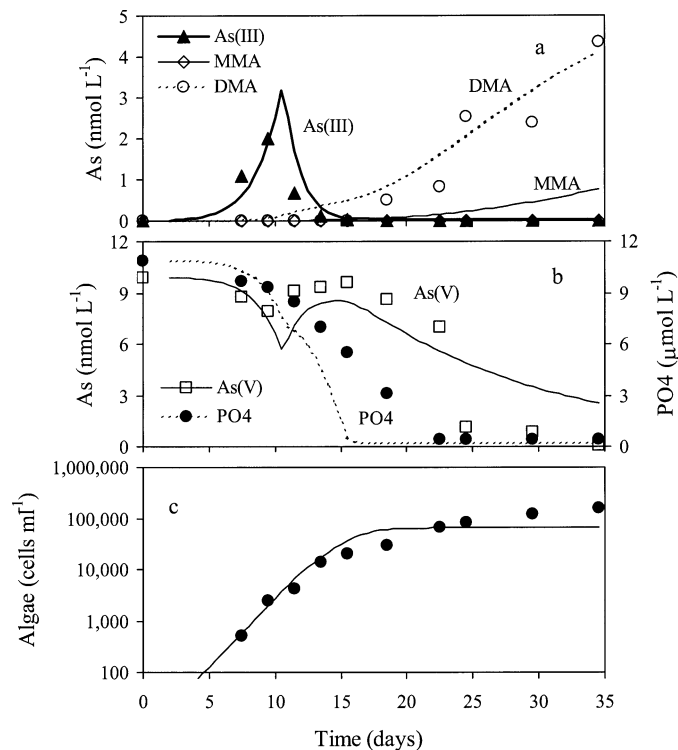


Fig. 9. As transformation by algae at ambient As concentration. (a) As(III), MMA, and DMA; (b) As(V) and PO₄; and (c) algae concentrations as a function of time. Data for *Closterium aciculare* from Hasegawa et al. (2001). The lines are the model’s prediction (see text).

$$V = V_{\max} \frac{S}{K_M(1 + I/K_I) + S} \quad (12)$$

where I (mol L⁻¹) is the inhibitor concentration, and K_I (mol L⁻¹) is the inhibition constant. For As(V) transport, the inhibitor is the PO₄ concentration, and the inhibition constant is the PO₄ half-saturation constant and vice versa. Table 3 lists the parameter values used to fit the data of Blum (1966) (Fig. 7a) and Sanders and Windom (1980) (Fig. 7a,b) as well as the parameter values presented by Takahashi et al. (2001). Parameters for the model application to the Hasegawa et al. (2001) data (discussed subsequently) are also included.

The complete uptake model for PO₄ and As(V) is presented in Table 2 (Eqs. 2-2 and 2-3). A separate equation is used for P-limited and non-P-limited conditions. P-limited conditions are assumed to exist when the P-limitation term (1 - $q_{0,P}/q_P$) in Eq. 2-1 is lower than L_H . Mutual inhibition of PO₄ and As(V) uptake is included for both conditions.

Antagonistic and synergistic effect of PO₄ on As(V) uptake—The uptake model proposed in this paper provides useful insights into the effect of PO₄ on As(V) uptake. As(V) uptake is competitively inhibited by PO₄, which means PO₄ has an antagonistic effect on As(V) uptake. However, increasing PO₄ concentrations can change the algae from P-limited to non-P-limited status and induce luxury uptake, causing a large increase in As(V) uptake. This represents a synergistic effect of PO₄ on As(V) uptake, which is not ex-

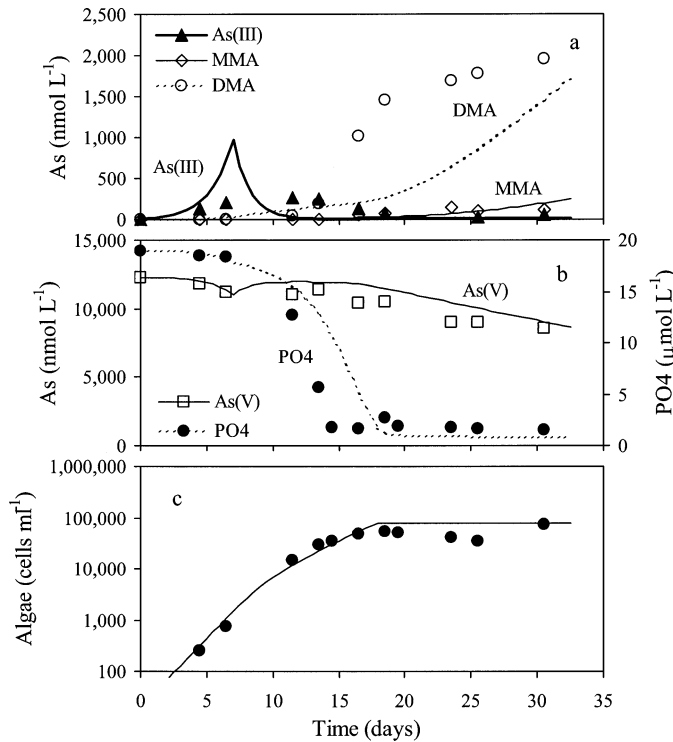


Fig. 10. As transformation by algae at high As concentration. (a) As(III), MMA, and DMA; (b) As(V) and PO₄; and (c) algae concentrations as a function of time. Data for *Closterium aciculare* from Hasegawa et al. (2001). The lines are the model's prediction (see text).

pected and opposite to the obvious competitive interaction. In fact, this mechanism can settle the controversy in the literature regarding the effect of PO₄ concentration on As(V) uptake and toxicity. Increasing PO₄ concentration caused a decrease in As(V) uptake in the experiments by Sanders and Windom (1980) but an increase in As(V) uptake in the experiments by Andreae and Klumpp (1979). In a similar manner, increasing PO₄ concentration caused a decrease in As(V) toxicity in the experiments by Sanders (1979a) and an in-

crease in As(V) toxicity in the experiments by Fuhua et al. (1994). This model can account for both an antagonistic (competition) and a synergistic (luxury uptake) effect of PO₄ concentration on As(V) uptake.

As(V) excretion—Maeda et al. (1992a) performed excretion experiments using radioactive ⁷⁴As(V) (Fig. 5b). Cells were grown in a medium containing ⁷⁴As(V) and then transferred to a medium without ⁷⁴As(V). ⁷⁴As is excreted rapidly, reaching an equilibrium in about 60 min, which is qualitatively similar to the ³²PO₄ experiments of Lean and Nalewajko (1976) (Fig. 5a). An analysis similar to that of Lean and Nalewajko (1976) was used to estimate the permeability coefficient ($P_{m,As(V)} = 33 \times 10^{-9} \text{ cm s}^{-1}$) is outside the range of values for PO₄ $P_{m,PO_4} = 0.13\text{--}24 \times 10^{-9} \text{ cm s}^{-1}$, see caption of Fig. 5). The reason for this is unclear. The algae were allowed to accumulate the ⁷⁴As(V) for 1 week, and it is possible that a significant amount of the ⁷⁴As(V) was reduced and methylated in the cell. Once excreted, those forms are unavailable for uptake, which could distort the equilibrium, leading to the higher ⁷⁴As concentrations in the medium. Unfortunately, speciation data are not available to verify this. Given this uncertainty, the large range of permeability coefficients for PO₄, and the lack of further evidence for higher permeability of As(V), we believe that the differences in the permeability coefficients are not significant. We therefore consider the As(V) and PO₄ permeability coefficients equal in this application.

To calculate excretion using Eq. 9, it is necessary to define the internal concentration. For PO₄, the intracellular dissolved concentration was assumed constant because P is stored using various P compounds (i.e., polyphosphates), which are not explicitly included in the model. For As, the intracellular reactions are explicitly included, and excretion is therefore based on the total concentration ($q_E = q_{As(V)}$ in Eq. 9). Equation 2-5 in Table 2 constitutes the As(V) excretion model.

As(III) excretion—The field and laboratory data indicate that significant amounts of As(III) are excreted only during

Table 3. As(V) and PO₄ uptake parameters.

Species	$V_{max,As(V)}^*$	V_{max,PO_4}^*	$K_{M,As(V)}^\dagger$	K_{M,PO_4}^\dagger	Comment
<i>Euglena gracilis</i>	5.9	56	5.5	16	Blum (1996)‡
<i>Synechococcus</i> sp.	3.9	0.13	140	1.8	Takahashi et al. (2001)§
<i>Skeletonema costatum</i>	0.031	110	0.1	1.0	Sanders and Windom (1980)
<i>Closterium aciculare</i>	0.004	0.15	3.0	0.90	Model application¶
<i>C. aciculare</i>	4.5	1.5	10	10	Model application¶#

* mmol g C⁻¹ d⁻¹.

† μmol L⁻¹.

‡ Calculated on the basis of data presented in reference. V_{max} converted from cell number-based units, using $1.1 \times 10^{10} \text{ cell g C}^{-1}$, on the basis of assumed cylindrical cell shape with diameter = 10 μm and length = 30 μm and ρ_{cell} from Table 5. $K_{M,As(V)}$ value presented by Blum (1966) is 56 μmol L⁻¹, which is inconsistent with other parts of the paper and thus assumed to be an error. See Fig. 7a.

§ Converted from dry weight-based units using 40% C.

|| Calculated on the basis of data presented in reference. Converted from cell number-based units, using $3.2 \times 10^{10} \text{ cell g C}^{-1}$, on the basis of assumed cylindrical cell shape with diameter = 10 μm and length = 10 μm and ρ_{cell} from Table 5. See Fig. 7b,c.

¶ Approximated for comparison as $V_{max} = \mu_{max} q_{*} \cdot q_{*P} = 2.0 \times 10^{-3} \text{ mol g C}^{-1}$ ($q_{*P} = 0.7 \times 10^{-3} - 3.3 \times 10^{-3}$, various species, Sommer [1998]; $q_{*P} = 7 \times q_{0,P}$, net uptake, *Scenedesmus* sp., Rhee [1973, 1974]). $q_{*,As(V)} = 6.0 \times 10^{-3} \text{ mol g C}^{-1}$.

For non-P-limiting conditions.

the log growth phase. As reviewed above (Fig. 6), total As cell quotas are higher during the log phase, and As(III) cell quotas are also higher (Goessler et al. 1997), suggesting that excretion is by passive diffusion. However, As(III) excretion has been studied in detail for bacteria and fungi, and for these organisms, it is an active transport process (Silver and Phung 1996). Nevertheless we use Eq. 9—the generic passive diffusion model—in the model for simplicity but recognize that the processes could actually be active and more complicated. Equation 2-6 in Table 2 constitutes the As(III) excretion model.

MMA and DMA excretion—The transport of MMA and DMA across biological membranes is by passive diffusion (Cullen et al. 1990). Using liposomes (artificial cells), Cullen and Nelson (1992) measured permeability coefficients for MMA and DMA of 1.4×10^{-13} and 4.5×10^{-11} cm s⁻¹, respectively. These are much lower than those of PO₄ ($0.13\text{--}24 \times 10^{-9}$ cm s⁻¹) and As(V) (33×10^{-9} cm s⁻¹). The reason for the difference could indicate a difference in the diffusion mechanism (i.e., simple diffusion across the lipid bilayer vs. ion channel, Stein 1986) for the methylated species. On the other hand, differences in the permeability of DMA and MMA are in part related to differences in speciation. MMA and DMA are acids, and only the neutral, fully protonated species are expected to be subject to significant diffusion (i.e., $q_E = \alpha_0 q$, in Eq. 9, where α_0 is a neutral fraction). At ambient pH (7.4), 6% of DMA is neutral, whereas 0.1% of MMA is neutral. In addition, Cullen et al (1994b) found that the permeability of the neutral DMA species is higher than that of the neutral MMA species. This is presumably due to structural differences (i.e., OH vs. CH₃ group). The lower permeability of MMA undoubtedly contributes to the low MMA concentrations in water, as suggested by Cullen et al. (1994a). However, speciation data such as those presented in Fig. 8, which are discussed in more detail below, consistently show lower intracellular MMA concentrations compared to DMA. The lower intracellular MMA concentration also contributes to the lower MMA concentrations in the medium. Equations 2-7 and 2-8 in Table 2 constitute the MMA and DMA excretion models, respectively.

Reduction and methylation—Once inside the cell, As(V) is reduced to As(III). This reaction has been studied in detail for bacteria and fungi, and the enzymes responsible for the reduction have been identified (Silver and Phung 1996). The methylation of As(III) in algae has been investigated (e.g., Maeda et al. 1992b), but the experimental data are not sufficient to support the development of a kinetic model for the reactions.

Mechanistically, the most attractive model may be one in which the methylation capacity is limited and exceeded during periods of luxury uptake, thus causing an increase in intracellular As(III), which is excreted to the medium. This is consistent with the intracellular As speciation presented in Fig. 8, where data show lower ratios of methylated As to As(III) during the log growth phase. This indicates that the methylation reaction could be saturated during the log phase. However, the data of Hasegawa et al. (2001) (Figs. 9, 10, discussed in more detail below) show that the same general

pattern of As transformation is observed at As concentrations varying by three orders of magnitude. A methylation reaction that saturates at the lower As concentrations could not produce the large amounts of DMA observed in the high As experiment.

In the absence of more detailed kinetic information, we use the simplest possible model that is consistent with the data. This consists of a set of first-order rate expressions for As(V) reduction and the two methylation reactions. The rate constant for As(V) reduction is constant (Eq. 2-9, Table 2). The rate constants for As(III) methylation are different for P-limited and non-P-limited conditions to allow a higher methylation rate during the stationary growth phase, as discussed above (Eq. 2-10, Table 2). The rate constant for MMA methylation is constant (Eq. 2-11, Table 2).

Extracellular reactions—Once excreted into the oxic medium, As(III), MMA, and DMA are converted back to the thermodynamically stable As(V). We assume the reaction sequence is DMA → MMA → As(III) → As(V), with first-order kinetics and rate constants $k_{DM,DMA}$, $k_{DM,MMA}$, and $k_{O,As(III)}$, respectively. Rate constants from the literature as well as those used in the model application (discussed subsequently) are summarized in Table 4. The abiotic rates are generally low, and in natural waters, the conversion of DMA and As(III) is often microbially mediated. Abiotic As(III) oxidation appears to be a function of the light intensity (Johnson and Pilson 1975, Table 4).

Model application

The model is applied to the data of Hasegawa et al. (2001), who grew algae isolated from Lake Biwa in axenic batch cultures under various As(V) and PO₄ concentrations. Controlled experiments like these are ideal for a first application of the model, because they eliminate any other possible effects on As speciation. In particular, bacteria that can transform As(V) (e.g., Johnson 1972) are not present. Three experiments were conducted. In the first experiment, As(V) concentrations were comparable to ambient concentrations in Lake Biwa. We will refer to this as the low As experiment. In the second experiment, the high As experiment, As(V) concentrations were three orders of magnitude higher and close to PO₄ concentrations. This is a very large change in arsenic concentration and, therefore, is a stringent test of the model. The model is applied to those two experiments. The third experiment, which was conducted under possibly N-limited conditions, was not considered because N is not included in this version of the model.

General experimental results—The data are presented together with the model results in Figs. 9, 10. The experiments begin with high concentrations of PO₄, and as a result, the algae are not P limited and are growing fast. Since the algae are not P limited, the luxury uptake system is active, causing the algae to take up large quantities of PO₄ and As(V). The resulting reduction in PO₄ and As(V) medium concentrations is remarkable considering the low biomass concentrations. Inside the cell, As(V) is reduced to As(III) at rates too fast for the methylation reaction to keep up. The As(III) cell

Table 4. Extracellular reaction rate constants.

Rate constant (day ⁻¹)	Conditions	Comment
DMA ($k_{DM,DMA}$)		
0	Acidified or sterile filtered seawater	Andreae (1979)
0.1	Abiotic	Sanders (1979b)*
0.02	Axenic algal culture	Model application
MMA ($k_{DM,MMA}$)		
0.02	Axenic algal cultures	Model application
As(III) ($k_{0,As(III)}$)		
0	Deionized water, 0.68 mol L ⁻¹ NaCl	Johnson and Pilson (1975)
0.01–0.03	Seawater, † dark and diffuse light	Johnson and Pilson (1975) ‡
~0.1	Seawater, † sunlight	Johnson and Pilson (1975) ‡
0.01–0.02	Filtered and sterilized seawater	Sanders (1978)
0.03	O ₂ -saturated distilled deionized water	Cherry et al. (1979)
0.05–0.1	Distilled, artificial, and natural seawater	Scudlark and Johnson (1982) ‡
~0.2	Sterilized, nutrient-enriched seawater	Matsuto et al. (1984)
0.04–0.06	Abiotic oxidation in ambient estuary water	Peterson and Carpenter (1983)
~0.0018	O ₂ -saturated water	Eary and Schramke (1990) §
0.8	Axenic algal culture	Model application

* Based on analysis of data presented in reference.

† Natural and sterile-filtered seawater at various pH values, salinities, and temperatures (using $T < 35^{\circ}\text{C}$).

‡ Abiotic rates, *see reference*.

§ pH = 7, based on initial rate.

quota increases, and As(III) is excreted, causing a rapid increase in As(III) concentration in the medium.

On about day 10, the P cell quota decreases to the point where the P-limitation term drops below the growth-limitation threshold (L_H , Eq. 2-1), and the growth rate starts to decrease. Since the algae are now P limited, luxury uptake ceases, and less PO₄ and As(V) are taken up. At this time, As(V) enters the cell at slow enough rates for the methylation reaction (which is also faster now) to keep up and prevent As(III) from building up inside the cell. As a result, less As(III) is excreted. Without continuous rapid production of As(III) by the algae, the As(III) concentration in the medium drops rapidly due to the ongoing abiotic oxidation. This is accompanied by a corresponding increase in As(V) concentrations.

Inside the cell, As(III) is methylated to MMA and DMA, which are excreted, causing an increase in DMA and, to a lesser extent, MMA concentrations in the medium. Compared to the As(III) production in the log phase, the cell-specific rate of DMA production is slow, due to the lower As(V) uptake rate. However, the resulting increase in the DMA medium concentration is significant because of the higher biomass concentrations. On about day 20, the PO₄ concentration reaches a minimum, and the algae enter the stationary growth phase. At that time, the rate of As(V) uptake increases again because of lessened competition by PO₄.

High versus low As experiments—An As(III) peak occurs during the log growth phase in both experiments. Relative to the initial As(V) concentration, the As(III) peak is higher in the low As experiment. This occurs because the initial As(V) concentration is well above the half-saturation constant ($K_{M^*,As(V)} = 10 \mu\text{mol L}^{-1}$) in the high As experiment. As a result, the As(V) uptake system is saturated and is

operating at its maximum rate. The As(V) uptake rate is no longer a function of the As(V) concentration. The relative uptake of As(V) and the subsequent reduction and excretion of As(III) are therefore slower.

Both experiments illustrate uptake competition for both PO₄ and As(V). For PO₄, inhibition by As(V) affects the PO₄ concentration during the stationary phase. At that time, uptake is balanced by excretion ($V = W$). At higher As(V) concentrations, more PO₄ is required to balance excretion because of competition. The stationary-phase PO₄ concentration is therefore higher in the high As experiment. For As(V), inhibition is not evident in this manner because As(V) continues to be transformed during the stationary phase. The effect of PO₄ inhibition on As(V) transport is evident in the decrease of As(V) concentration. Both experiments show that As(V) is taken up more rapidly once PO₄ is depleted.

Model evaluation—In both experiments, As(III) peaks during the log growth phase, and DMA appears more gradually and later during the experiment. In general, the model reproduces the major features of the data and provides support for the proposed mechanism outlined in Fig. 2. Following is a brief discussion of the parameter values and a more detailed evaluation of the model.

Parameter values are listed in Tables 3–5, and a detailed discussion of how each parameter value was chosen is presented in Web appendix 2 at http://www.aslo.org/lo/toc/vol-48/issue_6/2275a2.pdf. In general, most parameters are in reasonable agreement with literature values, where values are available. However, the As(III) oxidation rate constant that is used in the model is outside the range of values in the literature. The data show the rapid removal of As(III) after the log growth phase, corresponding to $k_{0,As(III)} = 0.8$

Table 5. Model parameters and values used in application.

Name	Symbol	Units	Value	Comment
Cell properties				
Cell carbon density	ρ_{cell}	g C m^{-3}	4.0×10^4	10% dry, 40% C
Specific cell surface area	A_{cell}	$\text{m}^2 \text{g C}^{-1}$	13	Simplified geometry*
Biomass unit conversion factor	—	cell g C^{-1}	1.0×10^9	Simplified geometry*†
Algal growth				
Maximum specific growth rate	μ_{max}	d^{-1}	0.75	$0.69 - 3.6\ddagger$
Phosphorus subsistence quota	$q_{0,P}$	mol g C^{-1}	1.7×10^{-4}	$2.5 \times 10^{-5} - 6.7 \times 10^{-4}\S$
Growth-limitation threshold	L_H	—	0.82	0.76
Excretion				
PO ₄ membrane permeability coefficient	P_{m,PO_4}	cm s^{-1}	1.8×10^{-9}	$1.3 \times 10^{-10} - 2.4 \times 10^{-8}\P$
As(V) membrane permeability coefficient	$P_{m,As(V)}$	cm s^{-1}	1.8×10^{-9}	$3.3 \times 10^{-8}\#$
As(III) membrane permeability coefficient	$P_{m,As(III)}$	cm s^{-1}	1.8×10^{-7}	—
MMA membrane permeability coefficient	$P_{m,MMA}$	cm s^{-1}	6.9×10^{-13}	$1.4 \times 10^{-13}**$
DMA membrane permeability coefficient	$P_{m,DMA}$	cm s^{-1}	2.2×10^{-10}	$4.5 \times 10^{-11}**$
PO ₄ cell quota	q_{PO_4}	mol g C^{-1}	3.2×10^{-5}	$\sim 10\% q_0, \dagger\dagger 3.2 \times 10^{-5} \ddagger\dagger$
Reduction and methylation				
As(V) reduction rate constant	$k_{R,As(V)}$	d^{-1}	1.0×10^3	—
As(III) methylation rate constant (P limited)	$k_{M,As(III)}$	d^{-1}	1.0×10^3	—
As(III) methylation rate constant (not P limited)	$k_{M^*,As(III)}$	d^{-1}	1.0×10^1	—
MMA methylation rate constant	$K_{M,MMA}$	d^{-1}	1.0×10^3	—

* Assumed cylindrical cell shape with diameter = 8 μm and length = 500 μm .

† To convert algal biomass from mg C L^{-1} to cells ml^{-1} in Figs. 9, 10.

‡ Various species, Di Toro (1980).

§ Various species, Sommer (1998).

|| *Monochrysis lutheri*, Droop (1975).

¶ Various species, Lean and Nalewajko (1976), see Fig. 5a.

Chlorella vulgaris, Maeda et al. (1992a), see Fig. 5b.

** Liposomes, Cullen and Nelson (1992).

†† *Scenedesmus* sp., $q_{PO_4} = 0.06-0.21 \text{ fmol cell}^{-1}$, $q_0 = 1.6 \text{ fmol cell}^{-1}$, Rhee (1973).

‡‡ *Scenedesmus quadricauda*, “exchangeable” PO₄, Jansson (1993).

d^{-1} . This is significantly higher than any of the abiotic rates reported in the literature. The cultures were maintained under axenic conditions, which means oxidation is not mediated by bacteria. Johnson and Pilon (1975) found that the rate increases by up to about an order of magnitude under sunlight, compared to dark and diffuse light (Table 4). It is possible the relatively strong illumination of the culture contributes to the high rate observed in the experiments.

Model results for the production of As(III) in the high As experiment are higher than the experimental observations. Several reasons could be considered to explain this overprediction. For example, it is possible that As(V) reduction or As(III) excretion (if active) also saturate during the high As experiment. Since the model does not have a saturation mechanism for those processes, the reaction rate that is appropriate for the low As experiment may be too fast for the high As experiment.

The data show an abrupt decrease in As(V) concentration in the low As experiment on about day 25. This decrease in As(V) is not accompanied by an increase in As(III), MMA, or DMA, which means the arsenic is not excreted. It is unclear what the mechanisms responsible for this behavior are, and the model does not reproduce this feature in the data.

DMA appears after the end of the log growth phase in both experiments. The modeled DMA concentration during the low As experiment matches the data very well. For the

high As experiment, however, the data show a more rapid production of DMA. This may be related to the model producing too much As(III) too early—perhaps because of a saturation mechanism that is not modeled as suggested above—thereby depleting the cellular storage of As that would later be methylated and excreted.

Refinements could be made to the model to better the agreement in the high As experiment. However, this would come at the expense of increasing the complexity of the model. It also must be kept in mind that the As concentrations in the high As experiment are three orders of magnitude higher than the ambient concentrations in Lake Biwa. Therefore, any model refinements to better the fit for the high As experiment would be of little relevance in explaining As transformations under natural conditions, which is the focus of this paper.

Summary and outlook—A model for arsenic transformation by algae was constructed and applied to data from two laboratory batch experiments. The model can reproduce the observed arsenic transformation reasonably well, which supports our hypothesis that As transformation by algae is linked to the P luxury uptake as outlined in Fig. 2. In the early stages of spring and fall blooms, algae up-regulate their PO₄ transport system (luxury uptake), which causes them to take up large quantities of As(V). Inside the cell, the reduc-

tion of As(V) to As(III) is fast, but the methylation is slower, causing As(III) to build up in the cell and be excreted, leading to a peak in As(III) concentration. During the summer, the "regular" PO₄ uptake system operates, which takes up less As(V) than the luxury uptake system, and it operates at rates slow enough for the methylation reaction to keep up. The internal As(III) is methylated to MMA and DMA, which are excreted, leading to higher DMA concentrations in the summer. Research is currently under way to show that this algal model, when integrated into a full-lake model, can explain the field observations (e.g., Lake Biwa, Japan).

References

- ANDERSON, L. C. D., AND K. W. BRULAND. 1991. Biogeochemistry of arsenic in natural waters: The importance of methylated species. *Environ. Sci. Technol.* **25**: 420–427.
- ANDREA, M. O. 1979. Arsenic speciation in seawater and interstitial waters: The influence of biological–chemical interactions on the chemistry of a trace element. *Limnol. Oceanogr.* **24**: 440–452.
- , AND D. KLUMPP. 1979. Biosynthesis and release of organoarsenic compounds by marine algae. *Environ. Sci. Technol.* **13**: 738–741.
- APTE, S. C., A. G. HOWARD, R. J. MORRIS, AND M. J. MCCARTNEY. 1986. Arsenic, antimony and selenium speciation during a spring phytoplankton bloom in a closed experimental ecosystem. *Mar. Chem.* **20**: 119–130.
- AURILIO, A. C., R. P. MASON, AND H. F. HEMOND. 1994. Speciation and fate of arsenic in three lakes of the Aberjona Watershed. *Environ. Sci. Technol.* **28**: 577–585.
- BLUM, J. J. 1966. Phosphate uptake by phosphate-starved *Euglenia*. *J. Gen. Physiol.* **49**: 1125–1137.
- BUDD, K., AND S. R. CRAIG. 1981. Resistance to arsenate toxicity in the blue-green alga *Synechococcus leopoliensis*. *Can. J. Bot.* **59**: 1518–1521.
- CHERRY, J. A., A. U. SHAIKH, D. E. TALLMAN, AND R. V. NICHOLSON. 1979. Arsenic species as an indicator of redox conditions in groundwater. *J. Hydrol.* **43**: 373–392.
- CULLEN, W. R., L. G. HARRISON, H. LI, AND G. HEWITT. 1994a. Bioaccumulation and excretion of arsenic compounds by a marine unicellular alga, *Polyphysa peniculus*. *Appl. Organomet. Chem.* **8**: 313–324.
- , F. G. HERRING, AND J. C. NELSON. 1994b. Employing permeability coefficients to understand the biomobility and bioaccumulation of compounds sensitive to the environment. *Bull. Environ. Contam. Toxicol.* **52**: 171–175.
- , B. C. MCBRIDE, AND A. W. PICKETT. 1990. The uptake of arsenicals by *Candida humicola*. *Appl. Organomet. Chem.* **4**: 119–125.
- , AND J. NELSON. 1992. The use of liposomes in predicting the biological mobility of arsenic compounds. *Appl. Organomet. Chem.* **6**: 179–183.
- DI TORO, D. M. 1980. Applicability of cellular equilibrium and Monod theory to phytoplankton growth kinetics. *Ecol. Model.* **8**: 201–218.
- DROOP, M. R. 1974. The nutrient status of algal cells in continuous culture. *J. Mar. Biol. Assoc. UK* **54**: 825–855.
- . 1975. The nutrient status of algal cells in batch culture. *J. Mar. Biol. Assoc. UK* **55**: 541–555.
- EARY, L. E., AND J. A. SCHRAMKE. 1990. Rates of inorganic oxidation reactions involving dissolved oxygen, p. 379–396. *In* D. C. Melchior and R. L. Bassett [eds.], *Chemical modeling of aqueous systems II*. American Chemical Society.
- FRANCESCONI, K. A., AND J. S. EDMONDS. 1997. Arsenic and marine organisms. *Adv. Inorg. Chem.* **44**: 147–189.
- FUHUA, C., C. WEIQI, AND D. SHUGUI. 1994. Toxicities of four arsenic species to *Scenedesmus obliquus* and influence of phosphate on inorganic arsenic toxicities. *Toxicol. Environ. Chem.* **41**: 1–7.
- GOESSLER, W., J. LINTSCHINGER, J. SZAKOVA, P. MADER, J. KOPECKY, J. DOUCHA, AND K. J. IRGOLIC. 1997. *Chlorella* sp. and arsenic compounds: An attempt to prepare an algal reference material for arsenic compounds. *Appl. Organomet. Chem.* **11**: 57–66.
- HASEGAWA, H., Y. SOHRIN, K. SEKI, M. SATO, K. NORISUYE, K. NAITO, AND M. MATSUI. 2001. Biosynthesis and release of methylarsenic compounds during the growth of freshwater algae. *Chemosphere* **43**: 265–272.
- HOWARD, A. G., AND S. C. APTE. 1989. Seasonal control of arsenic speciation in an estuarine ecosystem. *Appl. Organomet. Chem.* **3**: 499–507.
- , M. H. ARBAB-ZAVAR, AND S. APTE. 1982. Seasonal variability of biological arsenic methylation in the River Beaulieu. *Mar. Chem.* **11**: 493–498.
- , ———, AND ———. 1984. The behaviour of dissolved arsenic in the estuary of the River Beaulieu. *Estuarine Coastal Shelf Sci.* **19**: 493–504.
- JANSSON, M. 1993. Uptake, exchange, and excretion of orthophosphate in phosphate-starved *Scenedesmus quadricauda* and *Pseudomonas* K7. *Limnol. Oceanogr.* **38**: 1162–1178.
- JOHNSON, D. L. 1972. Bacterial reduction of arsenate in sea water. *Nature* **240**: 44–45.
- , AND M. E. Q. PILSON. 1975. The oxidation of arsenite in seawater. *Environ. Lett.* **8**: 157–171.
- KLUMPP, D. W. 1980. Characteristics of arsenic accumulation by the seaweeds *Fucus spiralis* and *Ascophyllum nodosum*. *Mar. Biol.* **58**: 257–264.
- KNEEBONE, P. E., AND J. G. HERING. 2000. Behavior of arsenic and other redox-sensitive elements in Crowley Lake, CA: A reservoir in the Los Angeles Aqueduct System. *Environ. Sci. Technol.* **34**: 4307–4312.
- KUHN, A., AND L. SIGG. 1993. Arsenic cycling in eutrophic Lake Greifen, Switzerland: Influence of seasonal redox processes. *Limnol. Oceanogr.* **38**: 1052–1059.
- LEAN, D. R. S., AND C. NALEWAJKO. 1976. Phosphate exchange and organic phosphorus excretion by freshwater algae. *J. Fish. Res. Board Can.* **33**: 1312–1323.
- LUNDE, G. 1973. The synthesis of fat and water soluble arseno organic compounds in marine and limnetic algae. *Acta Chem. Scand.* **27**: 1586–1594.
- MAEDA, S., K. KUSADOME, H. ARIMA, A. OHKI, AND K. NAKA. 1992a. Uptake and excretion of total inorganic arsenic by the freshwater alga *Chlorella vulgaris*. *Appl. Organomet. Chem.* **6**: 399–405.
- , ———, ———, AND ———. 1992b. Biomethylation of arsenic and its excretion by the alga *Chlorella vulgaris*. *Appl. Organomet. Chem.* **6**: 407–413.
- MATSUTO, S., H. KASUGA, H. OKUMOTO, AND A. TAKAHASHI. 1984. Accumulation of arsenic in blue-green alga, *Phormidium* sp. *Comp. Biochem. Physiol. C* **78**: 377–382.
- OLSEN, Y. 1989. Evaluation of competitive ability of *Staurastrum luetkemullerii* (Chlorophyceae) and *Microcystis aeruginosa* (Cyanophyceae) under P limitation. *J. Phycol.* **25**: 486–499.
- PETERSON, M. L., AND R. CARPENTER. 1983. Biogeochemical processes affecting total arsenic and arsenic species distributions in an intermittently anoxic fjord. *Mar. Chem.* **12**: 295–321.
- RHEE, G.-Y. 1973. A continuous culture study of phosphate uptake, growth rate and polyphosphate in *Scenedesmus* sp. *J. Phycol.* **9**: 495–506.

- . 1974. Phosphate uptake under nitrate limitation by *Scenedesmus* sp. and its ecological implications. *J. Phycol.* **10**: 470–475.
- SANDERS, J. G. 1978. Interactions between arsenic species and marine algae, Ph.D. dissertation. Univ. of North Carolina-Chapel Hill.
- . 1979a. Effects of arsenic speciation and phosphate concentration on arsenic inhibition of *Skeletonema costatum* (Bacillariophyceae). *J. Phycol.* **15**: 424–428.
- . 1979b. Microbial role in the demethylation and oxidation of methylated arsenicals in seawater. *Chemosphere* **3**: 135–137.
- , AND G. F. RIEDEL. 1993. Trace element transformation during the development of an estuarine algal bloom. *Estuaries* **16**: 521–532.
- , AND H. L. WINDOM. 1980. The uptake and reduction of arsenic species by marine algae. *Estuarine Coastal Mar. Sci.* **10**: 555–567.
- SCUDLARK, J. R., AND D. L. JOHNSON. 1982. Biological oxidation of arsenite in seawater. *Estuarine Coastal Shelf Sci.* **14**: 693–706.
- SILVER, S., AND L. T. PHUNG. 1996. Bacterial heavy metal resistance: New surprises. *Annu. Rev. Microbiol.* **50**: 753–789.
- SOHRIN, Y., M. MATSUI, M. KAWASHIMA, M. HOJO, AND H. HASEGAWA. 1997. Arsenic biogeochemistry affected by eutrophication in Lake Biwa, Japan. *Environ. Sci. Technol.* **31**: 2712–2720.
- SOMMER, U. 1998. *Biologische Meereskunde*. Springer.
- STEIN, W. D. 1986. *Transport and diffusion across cell membranes*. Academic Press.
- SUHENDRAYATNA, A. OHKI, T. KUROIWA, AND S. MAEDA. 1999. Arsenic compounds in the freshwater green microalga *Chlorella vulgaris* after exposure to arsenite. *Appl. Organomet. Chem.* **13**: 127–133.
- TAKAHASHI, A., H. KAWAKAMI, K. IWAKIRI, AND S. MATSUTO. 2001. Some characteristics of arsenate transport in a marine cyanobacterium, *Synechococcus* sp. *Appl. Organomet. Chem.* **15**: 291–298.
- YAMAOKA, Y., O. TAKIMURA, AND H. FUSE. 1988. Environmental factors relating to arsenic accumulation by *Dunaliella* sp. *Appl. Organomet. Chem.* **2**: 359–364.

Received: 21 April 2003

Amended: 7 July 2003

Accepted: 7 July 2003