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Trace Element Transformation During the Development of an Estuarine Algal Bloom

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ABSTRACT: Copper and arsenic underwent large changes in chemical form during the development and senescence of natural phytoplankton blooms in the Patuxent River, a subestuary of Chesapeake Bay in Maryland. Arsenate was rapidly reduced to arsenite and methylated species. At a total arsenic concentration of 20 nmol l^{-1}, arsenate reduction rates ranged from 50 amol $cell^{-1}$ d^{-1} to >230 amol $cell^{-1}$ d^{-1}, with the rate and extent of reduction dependent upon the concentration of arsenic, the dominant phytoplankton present, the season, and the degree of decline in phosphorus concentrations during bloom development. In general, the percentage of organically-associated copper was lowest (20–40% of total copper) during periods of rapid cell growth and highest (60–100% of total copper) during periods of cell decline or periods of dominance by red tide-forming dinoflagellates, a pattern associated with periods of high release of organic compounds during either bloom senescence or dense algal blooms. The end result of biological mediation was to increase the proportion of each element present in a less toxic form, thus affecting the potential toxicity to a natural ecosystem.

Introduction

Algal cells utilize a wide variety of chemical compounds, such as nutrients and vitamins, actively transporting and incorporating the elements necessary for cell growth. However, the transport systems for these compounds often are not completely specific and chemically similar ions often are inadvertently taken up by the cell. When such substances are not required by the cell or are present in excessive concentrations, they may interfere with normal biological processes, producing inhibitory effects. As a result, algae often have the ability to regulate, reject, or sequester some compounds, and can play an important role in the geochemistry, transport, and toxicity of many trace elements. Once competing ions enter the cell, these elements can be incorporated into particulate material or they can undergo considerable restructuring of chemical form. Both processes will result in chemical forms with differing reactivity and altered rates and pathways of transport.

In addition to direct changes following uptake, elements can be affected indirectly by algal metabolism, for example, through complexation by exuded organics. These reactions can yield compounds with dramatically different transport rates and biological reactivity. Because many trace elements are toxic to organisms, such changes in chemical form and reactivity can be of considerable importance to the ecosystem as a whole.

Algal blooms in dynamic systems such as estuaries undergo distinct phases (Margalef 1962; Davis 1982). There is generally an initial phase where a few species exhibit rapid growth and increases in cell density. As environmental conditions change, these species may be replaced in time with other successful species. In later stages, dominant species begin to fail, cell densities decrease, and a variety of different species begin to be more important. There are corresponding changes in the chemical composition of the surrounding water, as well, with pH fluctuating through photosynthesis and respiration, nutrient concentrations falling rapidly, and often concentrations of dissolved organic carbon (DOC) increasing as the bloom senesces. Thus, there are ample reasons why trace element form should be expected to change during algal blooms; yet, there are no current models that one can use to predict possible changes.

We designed a study to investigate how the chemical form and reactivity of model trace elements varied throughout the development of algal blooms in a productive, temperate estuary. The study focused on two elements, arsenic (As) and copper (Cu), as representative, model trace elements with greatly different biogeochemical properties (Table 1). Arsenic is chemically similar to phosphorus in its dominant, inorganic form (arsenate), is readily taken up by phytoplankton, and can undergo reduction and methylation to form a variety of dissolved forms (Andreae 1978; Sanders 1979; Sanders and Windom 1980). Copper can be taken up via a general cation transport system, which also transports zinc and manganese (Sunda

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TABLE 1. Summary of various geochemical properties of copper and arsenic, the likely result of uptake and incorporation by phytoplankton, and the most "important" biogeochemical result.

Property	As	Cu	Reference
Predominant form	Dissolved, as arsenate, anion	Dissolved, as a variety of inorganic, organic complexes	Andreae 1978; Sanders 1980; Eaton and Chamberlin 1982; Coale and Bruland 1988
Other important forms?	Arsenite, methylated arsenicals	Cu^{2+}	Andreae 1978; Sanders 1980; Coale and Bruland 1988; Sunda et al. 1990
Toxic forms	Varies with trophic level	Cu^{2+}	Sunda and Guillard 1976; Nissen and Benson 1982; Blanck et al. 1989
Effects of phytoplankton on trace element behavior:			
1. Dissolved/particulate phase change?	minor	minor	Sanders 1980; Wallace et al. 1983
2. Redox changes?	yes	yes	Sanders 1983, 1985; Moffett and Zika 1987
3. Methylation?	yes, but varies	no	Sanders 1983, 1985
4. Facilitation of photo-reduction?	no	yes	Moffett and Zika 1987
5. Complexation by exuded organics?	no	yes	McKnight and Morel 1979; Newell and Sanders 1986; Coale and Bruland 1988
Important biogeochemical processes	Reduction and methylation increase toxicity	Complexation by DOC reduces toxicity	

et al. 1981); it is also readily complexed by organics exuded from cells, resulting in greatly altered reactivity and biological availability (McKnight and Morel 1979; Fisher and Fabris 1982).

In order to accomplish our goals, we performed three experiments during different seasons in outdoor, large-volume enclosures containing natural phytoplankton communities. The experiments were performed seasonally to allow blooms to grow and decay under differing environmental conditions. In addition, timing of the experiments allowed us to select for dominant algal types. Finally, in an effort to resolve questions regarding the sources of reduced and methylated arsenic in natural systems, we performed a series of unialgal culture studies designed to elucidate the occurrence of these forms.

Materials and Methods

Location

The experiments were performed in the Patuxent River, a subestuary of the Chesapeake Bay, in Benedict, Maryland. River flows within the Patuxent vary widely, with major freshwater inputs in the spring and greatly reduced flows in summer and fall. Average flow is approximately 15 $m^3 s^{-1}$ (Eaton and Chamberlain 1982). This portion of the estuary has an annual salinity range of approximately 5‰ to 15 ‰, a pH range of 7.5 to 8.5, and water temperature extremes of 0°C and 30°C. Thus, this portion of the estuary is quite dynamic and undergoes substantial, seasonal variability. During the course of this study, arsenic concentrations varied between approximately 1 nmol l^{-1} and 10 nmol l^{-1}, while copper concentrations varied between approximately 4 nmol l^{-1} and 25 nmol l^{-1}.

Experimental Design

We examined the role of seasonally important phytoplankton communities in the uptake, transformation, and release of trace elements. Three experiments were performed in different seasons, so as to focus on different groups of dominant algal species. The first experiment was performed in the late spring, the second in early fall, and the third in mid-winter. The parameters for each experiment are detailed in Table 2. The late spring experiment was timed to highlight a diatom-dominated community; the early fall experiment to investigate a flagellate-dominated algal assemblage; the mid-winter experiment was timed to highlight the dinoflagellates.

The experiments utilized a large-volume outdoor culture system used extensively for the investigation of algal response to changing water chemistry (e.g., Sanders and Cibik 1985, 1988; D'Elia et al. 1986; Sanders et al. 1987, 1989); therefore, only a brief description is appropriate here. The tanks are 500 l in size and are immersed in a flowing water bath held outdoors on the shore of the Patuxent River in Benedict. The relatively large number of tanks allows replication of treatments, allowing an experimental design with reasonable

TABLE 2. General characteristics of experiments.

Parameter	First Experiment	Second Experiment	Third Experiment
Dates	5/10–6/3	9/21–10/21	1/20–2/15
Temperature range, °C	17.5–24.5	15.0–26.5	3.0–9.5
Salinity, ‰	8.8	12.0	8.3
pH range	—	7.69–8.59	7.62–8.21
Arsenic concentration range, nmol l^{-1}	2.9–6.5	2–10	1–5.5
Copper concentration range, nmol l^{-1}	13–25	—	4–12

statistical reality. In these experiments, we operated the tanks in a slightly different fashion from past studies. The tanks were filled with filtered water (1 μm nominal pore size), then inoculated with 5 l (1% of tank volume) of natural phytoplankton assemblage. Tanks are bubbled gently with air to maintain cells in suspension. The Patuxent River (and many other areas of Chesapeake Bay) is quite shallow, and is well mixed and unstratified for most of the year. Thus, this manipulation is a reasonable simulation of natural conditions. These assemblages rapidly grew in density, then leveled off and sometimes declined, just as dominant species do in the course of an algal bloom. This design allowed us to follow trace element chemistry through the development of an algal bloom, before the onset of rapid growth, during the peak growth period, and as the bloom declined.

At the beginning of each experiment, six tanks were spiked with 15 nmol l^{-1} of copper or 13 nmol l^{-1} arsenic (three each). Three tanks were left unspiked. Each experiment continued for approximately 2 wk; periodic samples were taken for an additional 2–3 wk. Each culture tank was sampled daily for in vivo fluorescence, a rapid measure of phytoplankton biomass (Goldman et al. 1973; D'Elia et al. 1986), and three times weekly for phytoplankton cell density and species composition and for the concentration and speciation of the dissolved trace elements. Beginning with the second experiment, pH was measured daily.

Laboratory Cultures

A number of representative algal species in culture were examined for their ability to reduce and methylate arsenate. Clones (Table 3) were maintained either in f/2 medium (Guillard and Ryther 1962) with P levels equivalent to f/10 to ensure P limitation or in a modified Erdschreiber (ERDS) medium (McLachlan 1973) with reduced P content equivalent to f/10. The algal species were spiked with 133 nmol l^{-1} arsenate, and changes in arsenic speciation were followed in the filtrate over the next 48–144 h.

Trace Element Analysis

Samples were taken from each tank, filtered through acid-cleaned GF/F filters held in acid-cleaned, all plastic holders and placed in rigorously-cleaned (Boyle and Huested 1983) CPE bottles. Samples for As analysis were quick-frozen in either liquid nitrogen or an alcohol/dry ice mixture. Samples for total Cu analysis were acidified with 0.2% redistilled HNO_3 (pH < 2). Samples for organic Cu analysis were extracted immediately and stored as methanol extracts until analyzed.

The concentration and chemical form of As in each sample was determined after hydride generation by subsequent detection of specific As hydrides using atomic absorption spectrometry (Braman et al. 1977). This method of analysis permits the determination of the total concentrations of As and also its chemical form. Detection limits in our laboratory are approximately 5 pmol of As for

TABLE 3. Arsenic speciation (nmol l^{-1}) after 48-h growth in clonal cultures spiked with 133 nmol l^{-1} arsenate. As(V) = arsenate. As(III) = arsenite. MMA = methylarsonate. DMA = dimethylarsinate.

Species	Clone	As(V)	As(III)	MMA	DMA
Exuviella baltica	EXUV	102	4.0	ND[a]	14
Isochrysis galbana	TISO	94	10	ND	0.5
Unid. diatom	IRD2	125	7.7	ND	ND
Pavlova lutheri	MONO	100	10	ND	1.1
Thalassiosira pseudonana	3H	103	23	ND	3
Prorocentrum mariae-lebouriae	PROML	32	2.4	ND	98
Skeletonema costatum	SC	96	18	ND	4.0
Chlorella sp.	AS-1	98	6.0	8.7	2.0
Cerataulina pelagica	CPEL	130	5.6	ND	3.1
Dunaliella sp.	DUN	111	24	ND	ND
Katodinium rotundatum	—	88	7.6	ND	36
Katodinium rotundatum	—	75	—[b]	24	13

[a] Less than limit of detection, 0.2 nmol l^{-1}.
[b] No value available.

arsenite and arsenate and 10 pmol for the methyl arsenicals. Maximum sample size is 50 ml, giving minimum detectable concentrations of 0.10 nmol l^{-1} and 0.20 nmol l^{-1}. The standard deviation for aqueous samples based on ten replicate analyses is approximately 10%.

Copper was separated into two operational forms: Cu associated with organics (as defined by retention on a C_{18} column and referred to as C_{18}-extractable Cu) and total dissolved Cu.

Total Cu analysis was performed on 250-ml samples after extraction by Chelex-100 resin columns (Riley and Taylor 1968). Prior to extraction, water samples were digested with $K_2S_2O_8$ to destroy chelating organics (Evans et al. 1977). Calcium and magnesium, which interfere with the analyses, were eluted from the columns with 20%, pH 6, ammonium acetate buffer (Kingston et al. 1978). Samples were then eluted with 10 ml of 2 N redistilled HNO_3 and analyzed by GFAAS.

Organically-bound Cu was concentrated from 125 ml of sample using reverse-phase liquid chromatography techniques and eluted with 5 ml of MeOH (Mills et al. 1982). While the fraction operationally defined in these studies as C_{18}-extractable does not include all forms of Cu associated or complexed with organics, it does provide a systematic portrayal of a significant fraction of the organically associated Cu and provides a convenient tool for assessing biologically mediated changes in Cu complexation through time. This method has the advantage of being a direct measurement of organic copper (as opposed to a measurement by difference), has low blanks, has good reproducibility, and is rapid and simple to perform. C_{18} cartridges (Sep Paks-Waters) were precleaned with 10 ml 5% redistilled HNO_3, 10 ml deionized water, 10 ml methanol (HPLC grade), and 10 ml deionized water. Subsamples (125 ml) were passed through the prepared cartridges at a flow of <10 ml min^{-1} using a vacuum pressure of approximately 5 psi. The cartridges were rinsed with 10 ml deionized water and the sample was extracted with methanol using an acid-washed glass syringe. All of the apparatus between the sample and the cartridges consisted of acid-washed teflon or polyethylene. Samples were stored in acid-washed polyethylene vials and analyzed by GFAAS. Process blanks were approximately 0.3 nmol l^{-1}.

Results

Experiment One—Late Spring

The development of the bloom in the tanks took 2 wk (Fig. 1 top), with the maximum biomass being attained in approximately 7 d. The initial bloom died off slowly over the next 7 d, remained relatively constant over another 7 d, then increased toward the end of the experiment. The biomass within all tanks were similar, with no differences noted between tanks or treatments.

The initial bloom consisted almost solely of diatoms, dominated by a small centric, *Thalassiosira pseudonana*. Other species, primarily a dinoflagellate, *Katodinium rotundatum*, and a cryptophyte, *Chroomonas* sp., also were present, but in greatly lower densities compared to the dominant diatom species (Fig. 1 middle). Diatoms comprised 90–95% of cell densities during the first 10 d, then quickly fell off to low percentages for the remainder of the experiment (Fig. 1 bottom). They were replaced by more slowly growing species, a dinoflagellate, *Gymnodinium* sp., and a variety of unidentified small flagellates. No differences were noted in either abundances or dominant species between treatments.

Phosphate concentrations (initially 0.6 μmol l^{-1}) declined rapidly as the bloom developed, remaining at approximately 0.1 μmol l^{-1} after the first week. Dissolved organic carbon concentrations increased slowly during the first week, then increased rapidly as the bloom senesced, reaching concentrations of 11–15 mg l^{-1} and remaining elevated until the experiment was completed.

Copper and As exhibited different behaviors during the development of the algal bloom. Total dissolved Cu varied in a systematic pattern, exhibiting an initial increase that may represent regeneration from particles or cells fragmented during the filtration, rapid decrease during the bloom, and a slow increase for the remainder of the experiment (Fig. 2). Copper also underwent a shift between inorganic and organically-associated phases during the bloom. As the biomass of phytoplankton increased within the tanks, the percentage of C_{18}-extractable Cu declined (Fig. 2). After biomass peaked and as the bloom declined, the proportion of C_{18}-extractable Cu doubled to 60%. As biomass leveled off, C_{18}-extractable Cu slowly declined to pre-experiment levels. There was no difference in Cu patterns between tanks containing ambient levels of Cu and tanks spiked with an additional 15 nmol l^{-1}.

Arsenic underwent reduction and methylation during the course of the bloom. As the biomass attained peak levels, arsenite was produced in both ambient and As-spiked tanks, reaching peak concentrations when biomass peaked (Fig. 3). Methylated As species began to appear as the bloom peaked, and continued throughout the course of the experiment. As with Cu, the patterns of As speciation were the same in both ambient and As-spiked tanks. The only methylated compound present in tanks was dimethylarsinate (DMA).

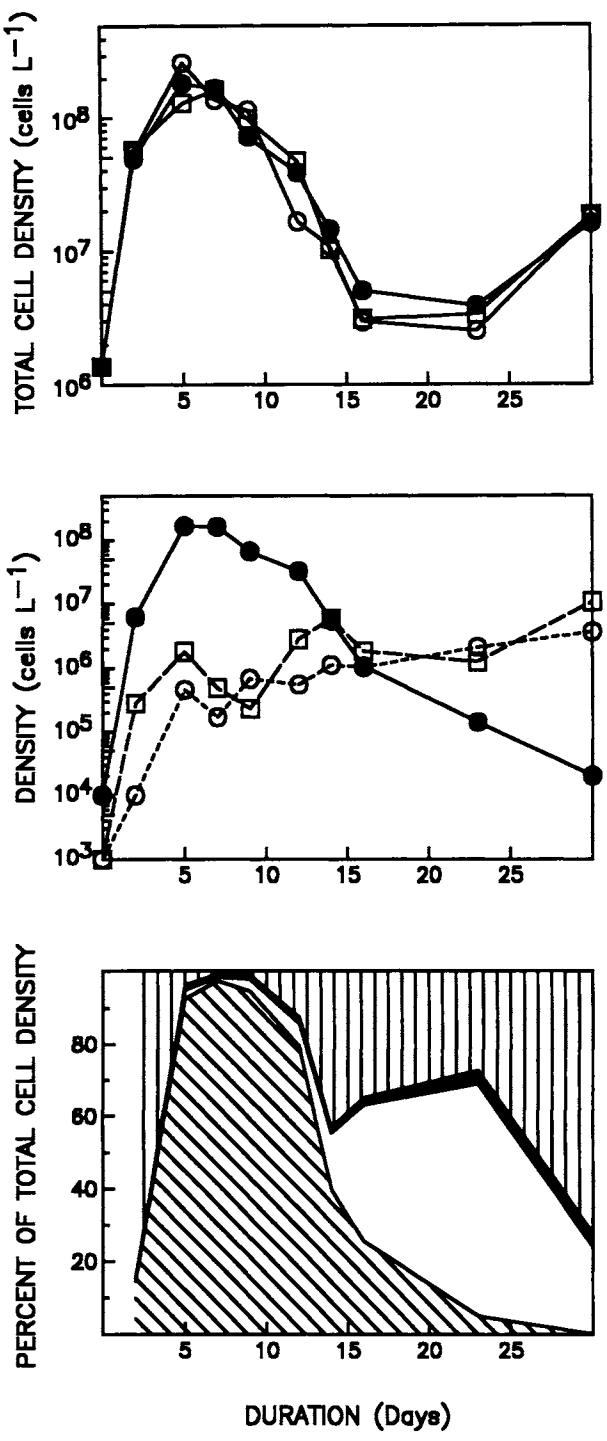

Fig. 1. Changes in phytoplankton density, dominant species, and the importance of taxonomic groups through time, in Experiment One. Top) Total cell density; averages of each group of triplicate tanks. ●——● = contro, ○——○ = Cu-dosed, ■——■ = As-dosed. Middle) Succession of dominant species, control tanks only. ●——● = *Thalassiosira pseudonana*, □- - -□ = small flagellates, ○- - -○ = *Gymnodinium* sp. Bottom) Relative importance of taxonomic groups, control tanks only. ▨ = centric diatoms, □ = dinoflagellates, ■ = cryptophytes, ▥ = other.

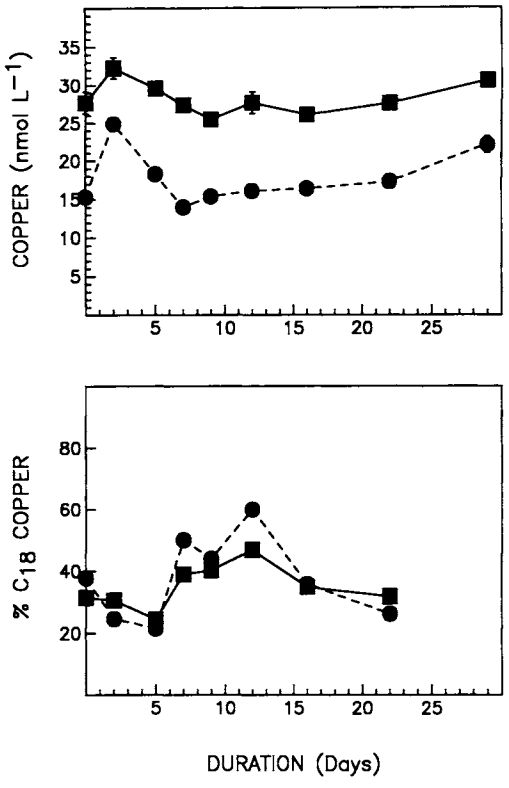

Fig. 2. Concentrations of total dissolved Cu and the % C_{18}-extractable Cu in both control and Cu-dosed tanks; Experiment One. ●——● = controls; ■——■ = Cu-dosed tanks. Bars are ±SE.

Experiment Two—Early Fall

A somewhat different pattern of bloom development was observed in this experiment. Instead of a single biomass peak, followed by decline, biomass increased rapidly over the first 5 d, declined for 3 d, then increased again for 2 d, reaching a second peak on day 10 (Fig. 4 top). From this point,

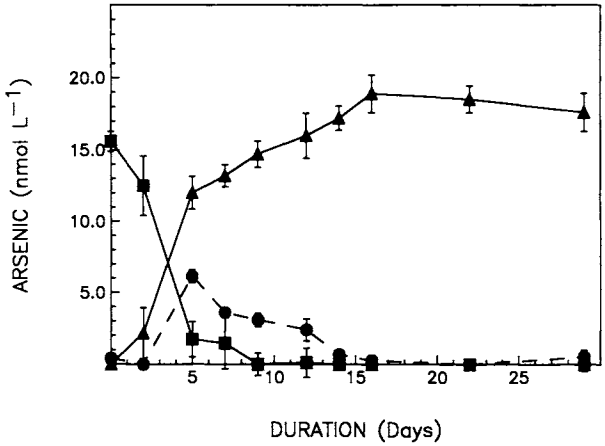

Fig. 3. As concentration and speciation in As-dosed tanks; Experiment One. ●- - -● = arsenite, ■——■ = arsenate, ▲——▲ = DMA. Bars are ±SE.

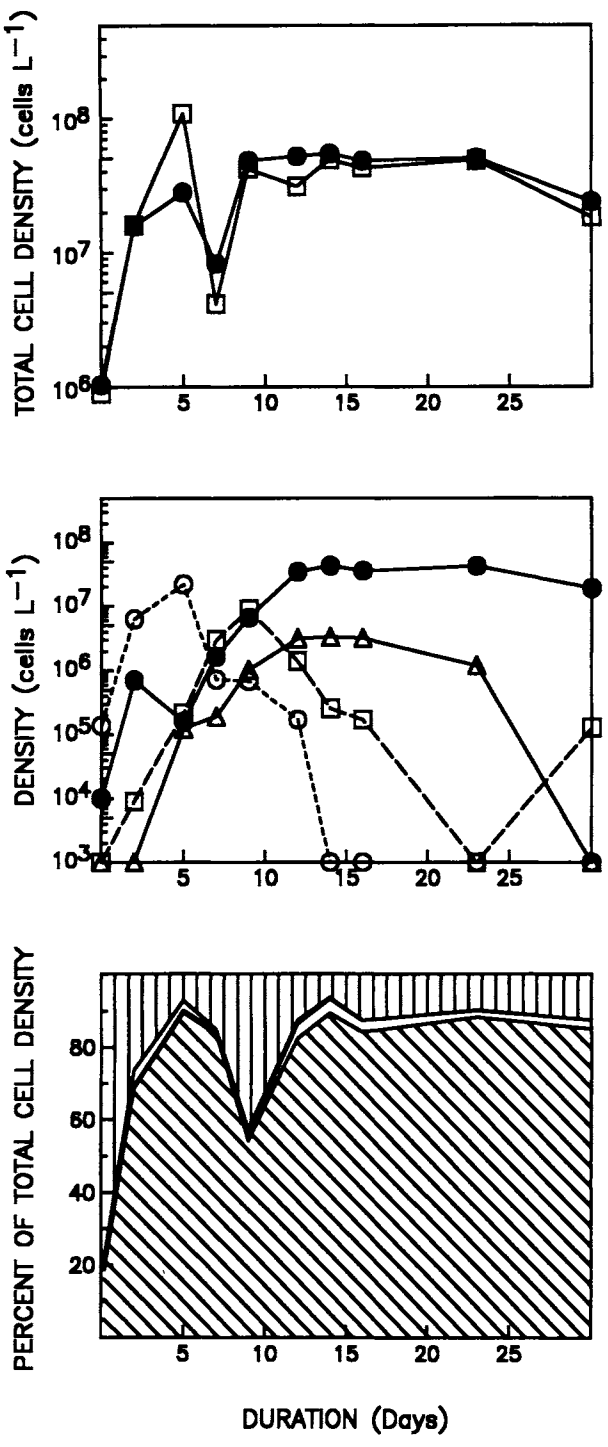

Fig. 4. Changes in phytoplankton density, dominant species, and the importance of taxonomic groups through time, in Experiment Two. Top) Total cell density; averages of each group of triplicate tanks. ●——● = control, □——□ = As-dosed. Middle) Succession of dominant species, control tanks only. ○- - -○ = *Thalassiosira pseudonana*, □- - -□ = *Leptocylindrus danicus*, ●——● = *Chaetoceros* sp., △——△ = *Rhizosolenia fragilissima*. Bottom) Relative importance of taxonomic groups, control tanks only. ▨ = centric diatoms, □ = dinoflagellates, ■ = cryptophytes, ▥ = other.

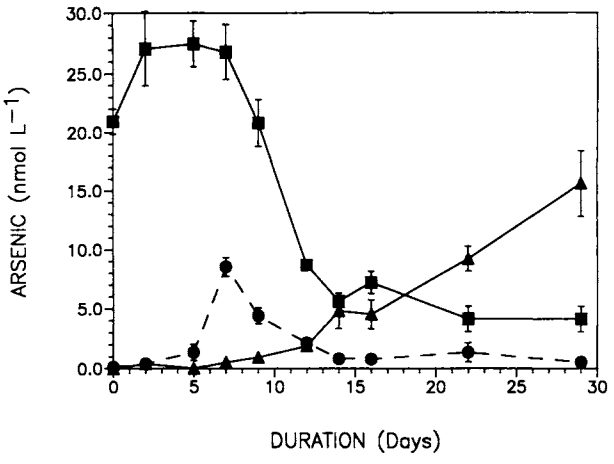

Fig. 5. As concentration and speciation in As-dosed tanks; Experiment Two. ●- - -● = arsenite, ■——■ = arsenate, ▲——▲ = DMA. Bars are ±SE.

biomass gradually decreased over the remaining 21 d of the experiment. As before, the biomass levels were similar in all treatments, with the exception of the As-dosed tanks. In this treatment, biomass levels greatly exceeded (approximately double) other treatments during the first bloom peak, then did not differ for the remainder of the experiment.

The first bloom peak was composed almost solely of a small (<10 μm), centric diatom, probably a species of *Thalassiosira*. This species rapidly attained densities of 20–40 (100 in As-spiked tanks) million cells l^{-1} by day 5, then as quickly disappeared. It was replaced by a succession of diatom species, initially by *Leptocylindrus danicus*, then by *Chaetoceros* sp. (Fig. 4 middle). While some flagellated species were present, none attained significant densities. Once the bloom developed, centric diatoms comprised 65–85% of total density (Fig. 4 bottom).

Phosphate concentrations declined over the first 7 d of the experiment, from an initial concentration of 2.4 μmol l^{-1} to approximately 0.1 μmol l^{-1}. Dissolved organic carbon concentrations rose steadily during the development of the bloom, peaked at 16 mg l^{-1} on day 12, then remained elevated at 10–12 mg l^{-1} for the duration of the experiment.

As in the first experiment, arsenic underwent reduction and methylation, but rates were not as rapid. Total As concentrations slowly declined throughout the experiment (Fig. 5). Very little arsenate reduction occurred until day 7, then arsenate concentrations fell rapidly, and were replaced initially by arsenite and then by DMA. The beginning of arsenate reduction was coincident with minimum phosphate concentrations.

Copper concentrations during this experiment varied erratically; exhibiting an unexplained, rapid increase in the middle of the experiment. The erratic behavior is indicative of widespread contamination; thus, these data are not presented here.

Experiment Three—Mid-winter

Phytoplankton biomass exhibited a quite different pattern during the mid-winter experiment. Initial cell densities were quite high because the dominant species, *Katodinium rotundatum,* a small, unarmored dinoflagellate, passed easily through the filter. Cell densities rose steadily from this initial level, doubling by the end of the first week, remained steady through day 13, then fell dramatically to a minimum at day 21. From this point, cell densities rose to the end of the experiment (Fig. 6 top). Overall, however, cell densities were approximately 10 times lower than those measured in the experiment in late spring.

Species composition initially was dominated by *Katodinium.* After day 15, *Katodinium* disappeared from the assemblage and was replaced by a variety of flagellates (a mixture of chrysophytes, prasinophytes, and unidentified species). None of these species attained significant densities individually; however, as a group they dominated (Fig. 6 middle). At the end of the experiment, the increase in cell density was due to diatom growth, especially *Chaetoceros* sp. Thus the bloom was composed of three different algal groups, dinoflagellates, naked flagellates, and diatoms, each succeeding the other through time (Fig. 6 bottom).

Phosphate concentrations were low, less than 0.12 μmol l^{-1}, for the first half of the experiment. Following the decline of *Katodinium* on day 13, phosphate concentrations rose somewhat, then declined as *Chaetoceros* sp. bloomed. Inorganic nitrogen concentrations were high throughout (10–15 μmol l^{-1}). Concentrations of DOC were highest (6–7 mg l^{-1}) during the *Katodinium* bloom, then declined.

The behavior of Cu was somewhat different in this experiment than in the earlier experiments. Dissolved Cu concentrations were much lower in both ambient and Cu-dosed tanks during this experiment, relative to concentrations observed in earlier experiments. Copper concentrations rose initially, then dropped in all tanks after 1 wk. After 2 wk (when the dinoflagellate bloom in the tanks began to subside), dissolved Cu rose slightly, where it remained for the duration of the experiment (Fig. 7). From the beginning of the experiment, virtually all of the Cu was C_{18}-extractable, a pattern which remained until the *Katodinium* bloom began to subside on day 13. The degree of organic as-

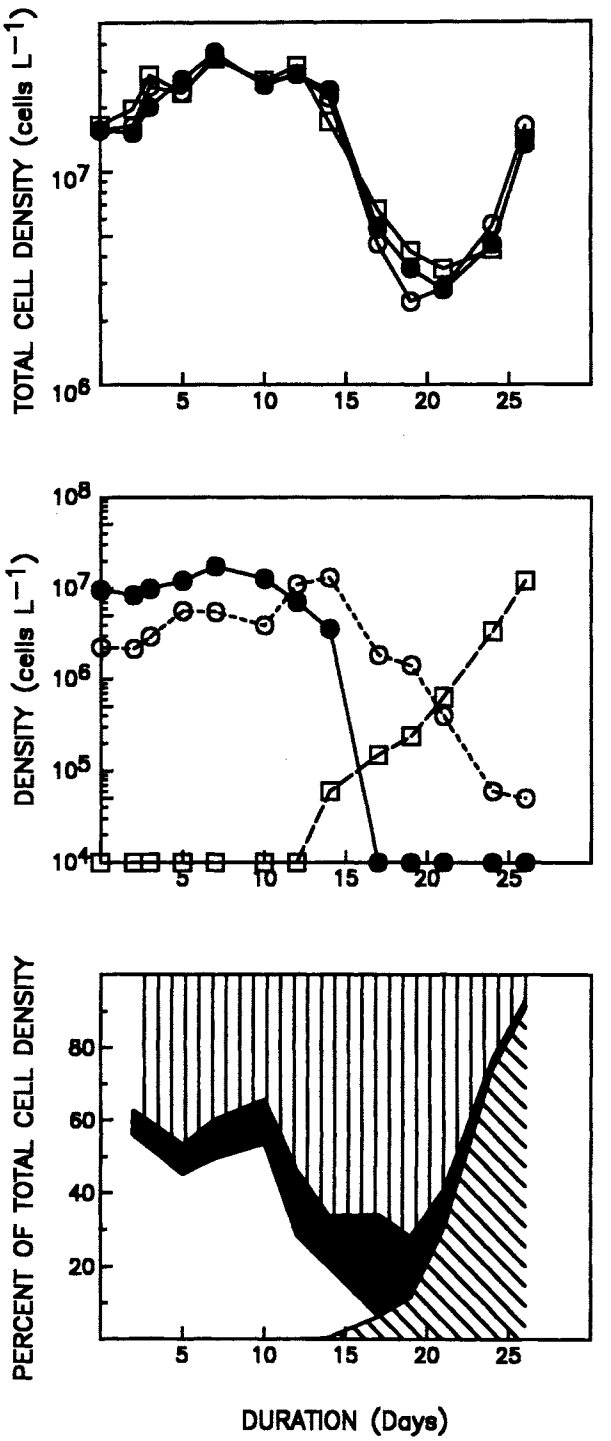

Fig. 6. Changes in phytoplankton density, dominant species, and the importance of taxonomic groups through time, in Experiment Three. Top) Total cell density; averages of each group of triplicate tanks. ●——● = control, ○——○ = Cu-dosed, □——□ = As-dosed. Middle) Succession of dominant species, control tanks only. ●——● = *Katodinium rotundatum,* ○– – –○ = small flagellates, □– – –□ = *Chaetoceros* sp. Bottom) Relative importance of taxonomic groups, control tanks only. □ = dinoflagellates, ▨ = centric diatoms, ■ = cryptophytes, ▩ = other.

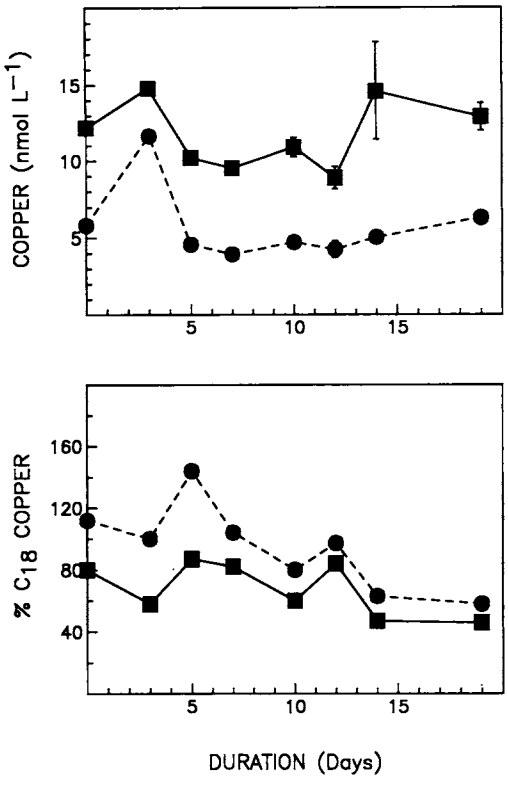

Fig. 7. Concentrations of total dissolved Cu and the % C_{18}-extractable Cu in both control and Cu-dosed tanks; Experiment Three. ●——● = controls, ■——■ = Cu-dosed tanks. Bars are ±SE.

sociation then dropped to approximately 60% for the remainder of the experiment (Fig. 7).

Changes in As speciation in this experiment were considerably different because of initial conditions. Because of the very dense *Katodinium* bloom in the Patuxent River before and during the experiment, most of the As within the water was in the form of DMA. Only traces of inorganic As, as arsenite, were present. Within the As-dosed tanks, As speciation underwent significant changes. The arsenate spike was quickly reduced to arsenite; by the third day essentially all inorganic As was in this form (Fig. 8). After the first week, DMA dominated.

Laboratory Cultures

A survey of 11 species demonstrated that considerable interspecific variation occurs in the ability to reduce and methylate arsenate (Table 3). All species examined were able to reduce arsenate to arsenite and most produced DMA within the 48 h incubation. Only two species, however, *Chlorella* sp. and *Katodinium rotundatum*, produced measurable quantities of methylarsonate (MMA) (Table 3). As a consequence, and because the presence of

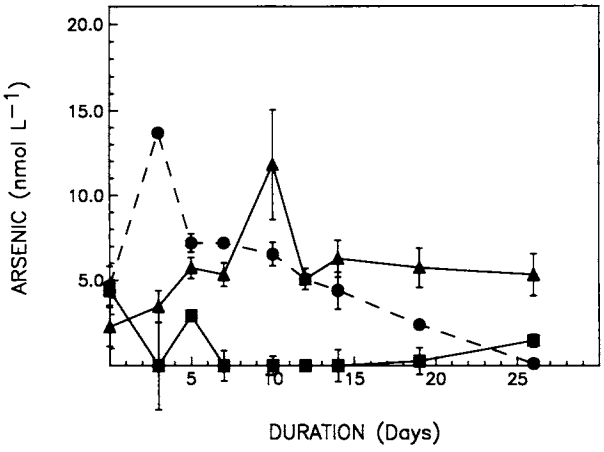

Fig. 8. As concentration and speciation in As-dosed tanks; Experiment Three. ●– – –● = arsenite, ■——■ = arsenate, ▲——▲ = DMA. Bars are ±SE.

winter blooms of *K. rotundatum* in the Patuxent River are associated with the almost total conversion of As to organic forms, this species was subjected to further study.

Katodinium rotundatum rapidly reduced and methylated arsenate in culture. Arsenite was produced within the first few hours, peaking at concentrations of 10.9 nmol l^{-1} after 96 h, then remained steady (Fig. 9). The arsenite production rate averaged 96 amol $cell^{-1}$ over the first 24 h. The production of DMA was also rapid; concentrations rose steadily, implying a constant production rate, attaining maximum concentration of 50–80 nmol l^{-1}, depending upon the culture medium (Fig. 9). DMA production rates averaged 192 amol $cell^{-1}$ d^{-1} over the 96 h sampling period. No appreciable MMA was formed during these incubations.

Discussion

In all experiments, dominant phytoplankton species underwent a progression through time. In both the late spring experiment and the early fall experiment, initial dominants were rapidly growing centric diatoms, whose individual growth rates greatly exceeded the overall growth of the assemblage (2.8 vs 1.4 div d^{-1} in late spring, 1.5 vs 1.0 div d^{-1} in early fall). The mid-winter experiment, conducted during a period of dinoflagellate blooms, initially was dominated by a dinoflagellate. In all experiments, however, early dominants were replaced by more slowly growing species, generally diatoms or a mixture of flagellates, as might be predicted (Margalef 1962; Davis 1982). In each experiment, we were able to produce periods of rapid cell increase, periods of stable densities, and periods of decline.

The dynamics of phytoplankton blooms obvi-

ously can greatly affect the chemical speciation of reactive trace elements. In this study, the two elements underwent large changes in chemical form as natural phytoplankton blooms developed and senesced and as dominant phytoplankton species flourished and disappeared. Copper underwent changes in its degree of organic association, with highest levels of C_{18}-extractable Cu present during bloom senescence or when *Katodinium rotundatum* dominated the assemblage. During periods of rapid cell growth (early during the bloom development), when DOC levels from cellular excretion should be at their lowest (Sharp 1977), C_{18}-extractable Cu is also at minimum levels. During the bloom's decline, when DOC levels are higher (Hellebust 1965; Holmes et al. 1967; Morris and Foster 1971; Sharp 1977), we observed a higher degree of association. Highest levels were concurrent with dense blooms of *Katodinium*, a species that forms dense blooms within the Chesapeake system. High concentrations of DOC have been documented to be associated with dense algal blooms (Brockmann and Dahl 1990); thus it is reasonable to expect a higher degree of organically-associated Cu. It is interesting to note that, although Cu concentrations in the third experiment were much lower than in earlier experiments, the quantity of Cu that was found in our C_{18}-extractable fraction was similar. Although the incorporation of Cu into particulate phases was not measured during the experiments, such a process could account for the lowered dissolved Cu concentrations during the *Katodinium* bloom. Higher incorporation rates of biologically available Cu would lead to lowered dissolved Cu concentrations and similar concentrations of organically-associated (and presumably, unavailable) Cu.

Our results are essentially similar to the recent work of Wangersky and associates (Wangersky et al. 1989; Zhou et al. 1989; Zhou and Wangersky 1989) using both laboratory and larger scale diatom cultures. In their experiments, ligands capable of binding Cu were produced during the log stage of growth; however, much higher quantities were released as the diatom blooms began to senesce and cells disintegrated. Therefore, while our observation that Cu associated with organics declined during periods of rapid cell growth is not consistent with their results, our overall conclusions of the importance of ligand release during bloom senescence are the same. As mentioned earlier, our measurement of organically-associated Cu is an operational one; the C_{18} method captures only a portion of the organically-associated Cu. Nevertheless, it is an efficient means of examining temporal changes in important chelating organic groups. Relatively little is known about the nature

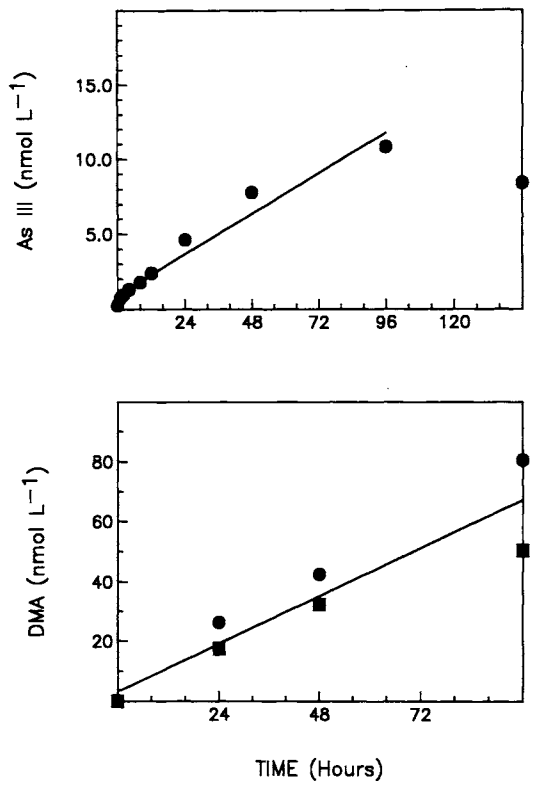

Fig. 9. The production of arsenite (AsIII) and DMA through time by *Katodinium rotundatum* in culture. ● = cells grown in ERDS, ■ = cells grown in f/10.

of metal complexing organics in natural waters. However, very recent work seems to be converging on agreement that there are two classes of ligands, a low concentration-high affinity (pK_{cond} 10–12.5) class and a more abundant, low affinity (pK_{cond} 8–10) class. In the Northeast Pacific, Coale and Bruland (1988) found that the low-affinity ligand was evenly distributed vertically, while the high-affinity ligand was present only in surface waters. This indicates that the low-affinity ligand is stable over the mixing time of the oceans, while the high-affinity ligand is not. In our experiments we see increases and decreases of a ligand in the course of days to weeks, which demonstrates both production of the ligand by the phytoplankton community, and the lability of the compound. Thus, we infer that the C_{18} method is capturing at least a portion of the high-affinity ligand and is effectively modeling the changes associated with algal growth and decline. While the conditions present during these experiments are somewhat artificial, it is reasonable to expect that at least the qualitative, if not the quantitative, aspects of ligand release from cells and subsequent ion binding are consistent with natural systems.

Arsenate was readily reduced by all phytoplankton communities examined, but rates of reduction

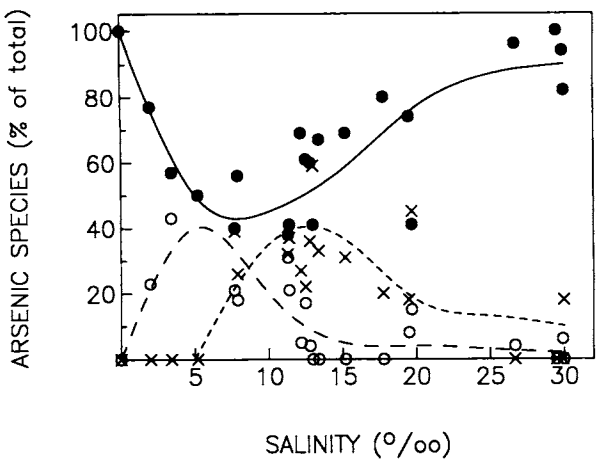

Fig. 10. Spatial progression in As speciation within Chesapeake Bay. Data from Sanders (1985). ● = arsenate, O = arsenite, x = MMA and DMA.

varied. Maximum arsenate reduction rates in unspiked assemblages ranged from 12–25 amol cell^{-1} d^{-1} in late spring and early fall experiments (no estimate of arsenate reduction is possible from the mid-winter experiment, as no arsenate was present). Rates in tanks spiked with 13 nmol l^{-1} arsenate ranged from a low of 50 amol cell^{-1} d^{-1} in late spring to >230 amol cell^{-1} d^{-1} in mid winter. Periods of arsenate reduction were associated with periods of rapid increase in cell densities and rapid declines in phosphate concentrations in the late spring and early fall experiments; in the mid-winter experiment, added arsenate was immediately reduced by the existing *Katodinium*-dominated assemblage. While temperature and nutrient [particularly phosphate (Blum 1966; Sanders and Windom 1980)] conditions play a role in controlling rates, obviously phytoplankton species composition and density also is important. The highest rates of reduction occurred during the *Katodinium*-dominated, mid-winter experiment, when temperatures were lowest and phosphate concentrations were very low: 0.1 μmol l^{-1}. In the early fall experiment, at high temperatures and moderately high phosphate concentrations, arsenate reduction did not occur during the first 7 d of the experiment, a time of rapid growth and dominance by a small, centric diatom, probably a species of *Thalassiosira*. After this initial bloom, phosphate concentrations were depleted further, this diatom rapidly declined in numbers, and a second bloom of several different species occurred. During this second bloom, arsenate was rapidly reduced.

The maximal arsenate reduction rates measured during these experiments are very similar to rates measured earlier [25–50 amol cell^{-1} d^{-1} in unenriched cultures and natural populations (Sanders and Windom 1980; Sanders 1983) and 190–400 amol cell^{-1} d^{-1} in As-spiked systems (Sanders 1983)] and in the *Katodinium rotundatum* culture experiments reported here.

Phytoplankton species composition, in addition to controlling rates of reduction, also likely affects the ultimate form of arsenic produced. In these experiments, only arsenite and DMA were produced; however, in culture and in natural systems often MMA is present during some periods of the year. The annual cycle of arsenic speciation in the Patuxent River has periods of significant DMA concentrations that coincide with both early summer blooms of centric diatoms and the mid-winter *Katodinium* bloom. Arsenite, a relatively unstable chemical form, is present in significant quantities only in late spring. Although MMA was not produced in our experiments, it is present in natural systems in mid-summer in the Patuxent River and the Chesapeake Bay (Sanders 1985), where it appears to be associated with dinoflagellate-, diatom-, and cryptophyte-dominated phytoplankton communities.

The MMA measured in the natural system could also be a breakdown product of DMA; it appears slowly after the DMA peak and persists for some time. This is consistent with the arsenic cycle postulated by Blanck et al. (1989). Further research is necessary to determine whether DMA degradation or biological production of MMA is the major pathway.

In addition to the temporal changes in arsenic speciation apparent both seasonally in the Patuxent River as phytoplankton dominance shifts and through the development of a discrete bloom as evidenced in our experiments, changes in arsenic speciation can also occur along a spatial scale. An estuary, in many ways, can be considered an algal reactor, with nutrients coming in with the freshwater inflow and successive phytoplankton blooms occurring downstream, in both space and time. An example of such a pattern is shown in Fig. 10, where data taken from a Chesapeake Bay transect during summer (Sanders 1985) have been loosely fit with generalized curves, indicating rapid reduction of arsenate, production of an intermediate species, arsenite, followed by production of the more stable methyl species, which persist through space and time, eventually being "diluted" as the flow from the estuary enters the less productive coastal ocean.

In the case of both As and Cu, the end result of biological processes was to transform both elements into less toxic forms. However, one consequence of the propensity of As and Cu to change forms after introduction to the estuary is a greater degree of uncertainty in the possible consequences

of an environmental release of these elements. Transformation of arsenate to arsenite or DMA, while an apparent benefit to the phytoplankton, may be more detrimental to fauna, which are more sensitive to the reduced and methylated forms (Nissen and Benson 1982). Arsenate and arsenite are also readily sorbed to sediment and suspended solids, so that transformation to the less particle-reactive DMA might result in faster flushing from the estuary and less retention in the estuary. Similarly, complexation of Cu would likely reduce its tendency to sorb to particulates. Both elements therefore illustrate the fallacy in attempting to predict potential toxicity to a natural system based upon loading information without adequate consideration of the biogeochemical changes that are likely to occur within an ecosystem.

The uncertainty in the behavior of each element can be reduced, however. Given seasonal, nutritional, and productivity aspects of a coastal ecosystem, coupled with our emerging understanding of geochemical and biological controls over chemical speciation, we can begin to construct general models of likely behavior of toxic trace elements. Such models, in our opinion, can be used for prediction of system impact.

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