Indian Journal of Marine Sciences
Vol. 10, December 1981, pp. 309-313

Arsenic Geochemistry in a Controlled Marine Ecosystem

HERBERT L WINDOM & JAMES G SANDERS*

Skidaway Institute of Oceanography, Post Office Box 13687, Savannah, Georgia 31406, USA

Received 30 *May* 1981; *revised received* 1 *September* 1981

Arsenic added to controlled enclosures changed in chemical speciation as predicted from laboratory measurements. The reduction of As(V) and production of As(III) and DMA(dimethylarsinic acid) in the As(V) enriched enclosure resulted in As species in proportions very similar to those measured in productive coastal marine systems. Further, the rates of As(V) reduction were remarkably similar to those measured in phytoplankton cultures exposed to As(V). Arsenite in the As(III) enriched enclosure was oxidized at a rate similar to that calculated in the laboratory. Microbial activity and the presence of dissolved organic matter could have contributed to the oxidation. The results support earlier suggestions that biota mediate and control the rate of As(V) reduction in marine systems. It is suggested that the concentration of As(III) is probably controlled by both chemical oxidation and the biota, and is in equilibrium between them. However, the importance of microbial oxidation and dissolved organic matter catalysis should not be ignored and needs further study.

The inorganic chemically stable form of As in oxygenated natural waters is arsenate [As(V)], occurring as As acid species[1,2]. In oxygenated sea water anomalous concentrations of reduced As species such as arsenite [As(III)] are found along with methylarsonic acid and dimethylarsinic acid (DMA)[3-6]. Discovery of the latter forms suggests that As reduction in sea water is mediated by biological processes, and a number of laboratory studies using cultures of marine organisms have demonstrated this[7-10].

The results discussed in this paper are from a study of the geochemical behaviour of As in large experimental marine ecosystem enclosures used in Controlled Ecosystems Pollution Experiments (CEPEX)[11]. This has been an attempt to evaluate the biological mediation of As reduction under more natural conditions than normally found in laboratory experiments.

Methods

Three 1/4-scale enclosures (referred to hereafter as CEEs) containing about 68 m^3 of water were used[11]. They were raised from a depth of 13 m and theoretically should have captured an entire water column intact. In this instance, 75 - 80 % of the column was captured; the remainder was pumped from 17 m.

The enclosures located in Saanich Inlet near Victoria, B.C., Canada, were launched on 24 June 1977. As sufficient to enrich the concentration by 5 μg/litre was added to 2 of the enclosures on 30 June; As(V), as Na_2HAsO_4, to CEE-B, and As(III) as Na AsO_2, to CEE-C. CEE-D was used as a control. The enclosures were sampled over the next 22 days for nitrate, phosphate, silicate, and chlorophyll *a* using the techniques outlined in Strickland and Parsons[12], and were examined visually to determine phytoplankton species composition. Cell counts were converted to algal carbon by calculating cell volumes[13] and using Strathmann[14] regression of volume on carbon. On 3 occasions, *in situ* primary productivity was determined by measuring the uptake of ^{14}C (as HCO_3^-) over a 4 hr period.

Nitrate and silicate were added to the enclosures on day 5 (5 μM each) and on day 12 (10 μM each). Phosphate was added to the control enclosure only.

Water samples were taken 8 times between days 1 and 22 to determine As concentration and speciation using a D.C. arc-induced-plasma emission technique[15]. The detection limit (signal - 2 × background) was approximately 0.5 ng for As(V) and As(III) and 1 ng for the methyl arsenicals. The maximum sample size used was 40 ml, giving minimum detectable concentrations of 0.01 μg As(V) or As(III)/litre and 0.03 μg DMA/litre. The precision for water samples, based on replicate analysis, was approximately 10 %.

Sedimented material collected from the bottom of the enclosures was filtered through a 0.4 μm membrane filter. The filtrate was analyzed as above for As species and the sediment was dried at 60°C in an oven, and digested in acid. The digestions were performed in loosely capped teflon vials of 20 ml capacity on a hotplate at 90° C (slightly higher than the boiling point of HNO_3). The vials were placed under a teflon hood, which was flooded with N_2 gas to minimize contamination. The acid used for digestion was 5 ml of concentrated Baker Ultrex HNO_3. The acid was evaporated nearly to dryness and, if necessary, more

*Present Address: Benedict Estuarine Research Laboratory, Academy of Natural Sciences of Philadelphia, Benedict, MD 20612, USA.

was added to complete the digestion. The residue remaining was dissolved in 5 ml of 10% Ultrex HNO_3 and analyzed with no further modification. Occasionally, oxidation of As(III) to As(V) has occurred during digestion[10], thus As concentrations in sediments are reported as inorganic fraction [As(III)+ As(V)] and an organic fraction (DMA and other methyl arsenicals).

To help determine the role of phytoplankton and the microbial assemblage in As speciation changes, 500 ml glass bottles containing portions of the enclosed water were suspended in the enclosures and incubated for 4, 6 and 18 days. Sufficient antibiotics (streptomycin, penicillin, gentamycin, and kanamycin) were added to the bottles to eliminate microbial activity. Other bottles were wrapped with black tape and inoculated with antibiotics to exclude both phytoplankton and microbial activity. On day 18, the *in vivo* fluorescence of chlorophyll in each bottle and in the enclosures was determined to provide a relative measure of phytoplankton activity. At the same time, 15 ml duplicates of each water sample were inoculated with 0.93 μCi of ^{14}C labeled glucose, incubated for 1 hr at 11°C, filtered, then the filters were counted in a Beckman liquid scintillation counter. This procedure was used to provide a relative measure of microbial activity.

Results

Changes in nutrient concentration—Nutrients within the 2 experimental enclosures were similar during the course of the experiment (Fig. 1), with NO_3 ranging between 0.1 and 4.5 μM, SiO_2 between 0.5 and 19 μM, and PO_4 between 0.15 and 1.65 μM. Generally, nutrient concentrations peaked just after additions (days 5 and 12) and then declined. Phosphate was not intentionally added to either enclosure, however, increases after each nutrient addition suggests addition. Due to these additions, the PO_4 concentrations remained above 0.5 μM after day 5 (Fig. 1).

Productivity and phytoplankton composition—Phytoplankton carbon and chl *a* declined rapidly between days 1 and 8, corresponding to decline in the diatom component of the phytoplankton. By day 12, both chl *a* and phytoplankton carbon increased greatly then remained constant as the enclosures experienced a bloom of dinoflagellates and other flagellates (Figs 2 and 3).

Primary productivity measurements yielded rates of carbon uptake of 1.6, 11.7, and 12.3 ($\bar{x}=8.5$) μg C/litre/hr in CEE-B and 1.5, 20.4 and 7.2 ($\bar{x}=9.7$) μg C/litre/hr in CEE-C. The low rate was measured on day 5 when phytoplankton carbon and chl *a* concentrations were quite low (Fig. 2).

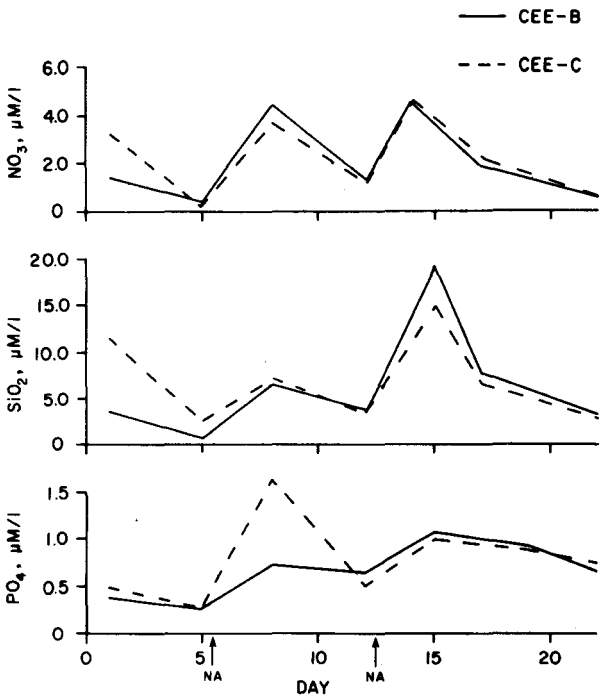

Fig. 1—Nutrient concentration in enclosures [Arrows indicate nutrient additions]

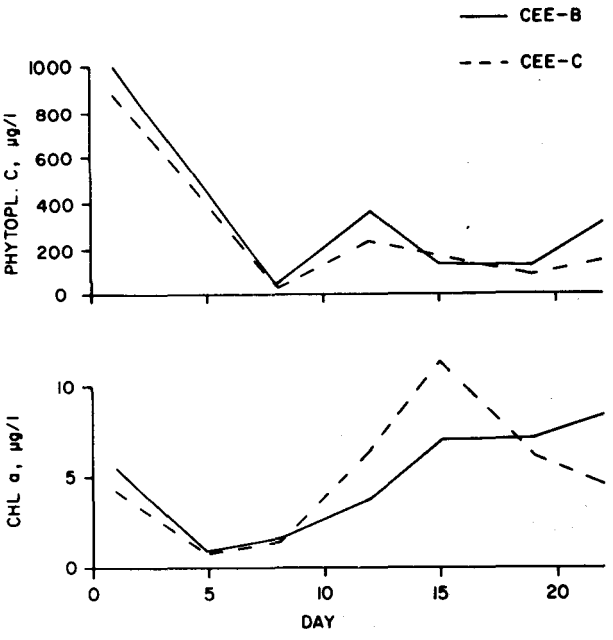

Fig. 2—Phytoplankton carbon and chl *a* in enclosures

Total As concentrations—Total As within enclosures CEE-B and CEE-C remained constant at 6.49 ± 0.39 μg/litre for the entire experiment. There was no measureable decrease due to uptake, sedimendation, or adsorption in either enclosure. Total As in the surrounding water of Saanich Inlet did not vary averaging 1.41 μg/litre. As speciation in Saanich Inlet also did not vary significantly; As(V) comprised about 82%, As(III) about 4%, and DMA 13%.

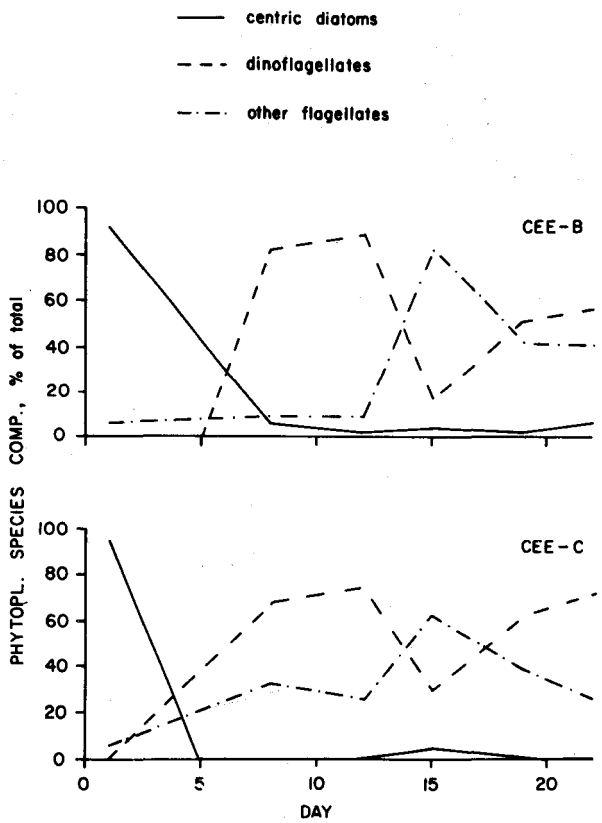

Fig. 3—Phytoplankton composition in enclosures

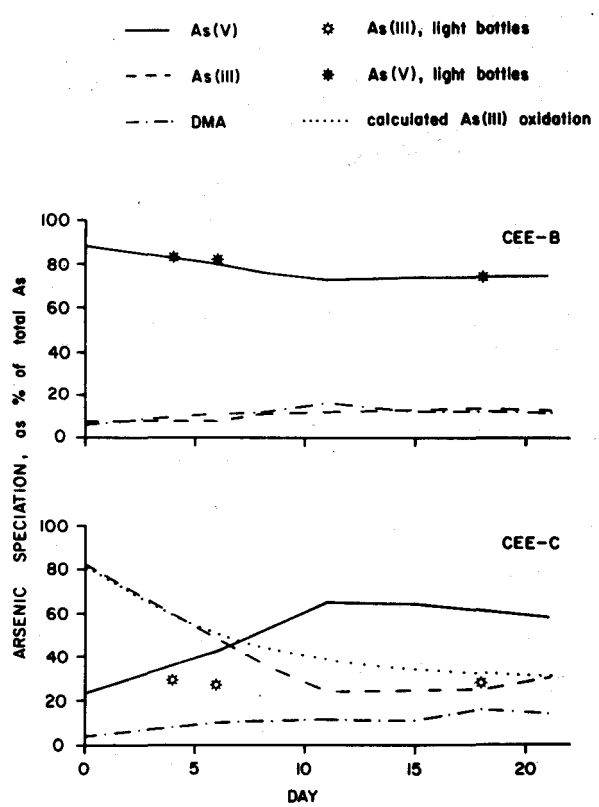

Fig. 4—Arsenic speciation [Lines represent concentrations of arsenic species in the integrated water column. The three discrete points indicate results of analysis of samples collected from bottles suspended in enclosures]

Although the enclosures were sampled at 3 depths (1,5, and 10 m) for analysis of As species, there was no significant difference between the depths, so data of all depths were pooled.

Changes in As speciation—Arsenate addition (CEE-B): Changes in the speciation of As throughout the experiment were not large. During the first 10 days, As(V) decreased from 88% to approximately 70% of the total As content. As(III) and DMA increased from approximately 6 to 14% of the total As (Fig. 4). After an initial decrease, As(V) did not change significantly, As(III) decreased slightly, and DMA continued to increase slowly, eventually doubling its original concentration. None of these changes may be significant given the precision of analysis.

Arsenite addition (CEE-C): Changes in speciation within the As(III) enclosure were more dramatic than in CEE-B, due largely to the unstable nature of As(III) under oxidizing conditions. Arsenite decreased rapidly from 82% of the total to 24% in 10 days, then remained essentially constant, increasing somewhat on day 20. Arsenate concentrations correspondingly increased rapidly, then leveled off and decreased slightly. Increases in DMA, from approximately 4 to 12% of total As (Fig. 4) are probably not significant.

Although thermodynamically unstable, As(III) does not oxidize immediately in the water column. Its rate of oxidation depends on the concentration present[16], and organic compounds present in the water may also influence oxidation rates[17]. Using an oxidation rate determined in the laboratory[18] we predicted that the As(III) concentration in CEE-C should be approximately 33% of the total As at the end of the experiment, a value close to that measured (Fig. 4).

Biological mediation of speciation—Light bottles treated with antibiotics and incubated within enclosure CEE-B [As(V) addition] showed shifts in As speciation similar to those already described (Fig. 4). The dark bottle treated with antibiotics to reduce both microbial and phytoplankton growth was also similar on day 18; however, it was not placed in the enclosure until day 5. In addition, both the dark and light bottles had similar *in vivo* fluorescence values on day 18 (Table 1), and were only approximately 50% as active as a sample taken directly from the enclosure. Microbial activity in the bottles was predictably low, about 3% of natural activity at most (Table 1).

As speciation within light bottles incubated in CEE-C [As(III) addition] was quite different from the enclosure itself (Fig. 4). Arsenite concentrations in bottles sampled on days 4 and 6 were much lower than predicted. Only the bottle sampled on day 18 had As speciation similar to that within the enclosure.

Phytoplankton activity within the light bottle sampled on day 18 was approximately 70% of that in the enclosure; microbial activity was < 1% (Table 1).

Arsenic in the sediment phase—As speciation in sedimented material removed from the bottom of the enclosures changed during the experiment. Sediment filtrates (interstitial water) had total As concentrations similar to the overlying water, and their speciation on day 8 was also similar (Table 2). On day 21, however, As speciation in filtrate from both enclosures was dominated by the reduced species, As(III). The proportion of DMA remained largely unchanged (Table 2).

Acid digests of the dried sediments indicated that sediments in both enclosures increased in total As concentration by 30 - 40% (Table 2). The small absolute amount of As tied-up in the sediments, however, is insufficient to produce significant As decreases in the water column. As speciation remained consistently around 50% inorganic As, and 50% methylated (Table 2).

Discussion

As speciation in CEE-B, to which arsenate was added, was similar to that in other productive marine systems such as the Georgia Bight[10,19], and the pattern of change [continuous reduction of As(V), increased as As(III), DMA] in many ways was similar to that seen in algal cultures spiked with As(V)[10]. This reduction of As(V) to As(III) and subsequent methylation to DMA is biologically mediated[10,19] and was largely borne out by experiments described here. Dark bottles were placed into the enclosures in an attempt to discover to what extent speciation changes take place in the absence of biota. Apparently, these bottles received enough light around the stoppers to permit some phytoplankton growth (Table 1). We were, however, able to effectively remove the influence of the microbial assemblage by using antibiotics (Table 1). The results suggest that microbes were not a major factor in determining the rate of As(V) reduction.

Rate of As(V) reduction in CEE-B was approximately 0.1 μg/litre/day over the first 10 days. Phytoplankton carbon averaged approximately 450 μg C/litre during the same period (Fig. 2), yielding a reduction rate of about 220 ng As(V)/mg phytoplankton C/day. This rate compares remarkably well with a rate of 190 ng As(V)/mg C/day obtained in a diatom culture[10] enriched with the same level of As(V). This reduction rate when compared to primary productivity within CEE-B, also compares well with culture data, yielding an average of 1.2 ng As(V) reduced/μg C produced in the enclosures and 0.7 ng As(V)/μg C in cultures[10,18]. Omitting the very low measurement of productivity during the diatom crash, the rate in CEE-B was about 0.8 ng As(V)/μg C, which compares very well with cultures.

Arsenate reduction in CEE-B virtually stopped after day 11. The uptake of As(V) by phytoplankton depends on the concentration of available PO_4; as PO_4 increases, As(V) uptake is reduced greatly[10]. Therefore the steady increase in PO_4 concentration in CEE-B may have caused a large reduction in the uptake of As(V) by phytoplankton and a subsequent slowing of the rate of As(V) reduction. In addition, the chemical oxidation of As(III) (discussed below) produces new As(V). This would also lessen the apparent As(V) reduction rate.

Since little As is incorporated in biological material[10,18], total As concentrations within the water column do not change measurably. Algae, however, incorporate small amounts of As[10]. Incorporation followed by death and sinking of the plants led to increases in As in sedimented materials. Increases of about 40% were also very similar to increases of As in

Table 1—Phytoplankton and Microbial Activity in Light and Dark Bottles

[Activity in relative units. Sample collected on day 18]

Sample	Phytopl. activity	%	Microb. activity	%
CEE-B	72	100	54,000	100
CEE-B, light bottle	39	54	400	1
CEE-B, dark bottle	38	53	2,400	3
CEE-C	87	100	53,000	100
CEE-C light bottle	63	72	400	1
CEE-C, dark bottle	21	24	600	1

Table 2—As Concentration and Speciation in Sediment and Filtrate Removed from the Bottom of CEE-B and C

[As conc: in filtrate, μg/litre, in total sediment, μg/g]

Sample	Filtrate							Total sediment				
	As(V)	(%)	As(III)	(%)	DMA	(%)	Total As	Inorg. As	(%)	Methyl As	(%)	Total As
CEE-B, Day 08	5.19	(77)	0.68	(10)	0.91	(13)	6.78	1.92	(57)	1.45	(43)	3.37
CEE-B, Day 21	0.68	(10)	4.75	(74)	1.00	(16)	6.43	2.72	(57)	2.04	(43)	4.76
CEE-C, Day 08	4.55	(70)	1.02	(16)	0.96	(14)	6.53	2.24	(41)	3.51	(59)	5.93
CEE-C, Day 21	0.37	(6)	5.50	(83)	0.73	(11)	6.60	4.15	(54)	3.53	(46)	7.68

cultured algae[10]. As speciation within the sediment, about 50% inorganic arsenic and 50% methylated, is also similar to that found in marine algae[10].

Filtrates taken from the sediment slurry became more reduced during the experiment as evident from the large increase in As(III) over time (Table 2). Interestingly, the total As concentration in this interstitial fluid remained similar to that in the water above and did not increase with time. This suggests that As in particulate material was not desorbed from particles, or released due to microbial activity.

As speciation in CEE-C [As(III) addition] was largely controlled by the chemical oxidation of As(III) to As(V), but, in general, proceeded somewhat faster than predicted (Fig. 4). Considering that reduction of As(V) to As(III) occurs at rates similar to those measured in CEE-B, the As(III) within CEE-C should have oxidized at a slower rate than predicted because there was a constant production of new As(III) from biotic reduction. This was not the case. Microbes can oxidize As(III) and cause the discrepancy between measured and predicted oxidation rates[1,16]. In addition, since As(III) oxidation rates may be drastically affected by the concentration of dissolved organic matter present[17], an experimentally derived oxidation rate from Georgia coastal waters[18] should differ from that measured in Saanich Inlet.

As speciation in light bottles incubated in CEE-C was quite different than that measured in the enclosure itself (Fig. 4). Arsenite concentrations in the bottles were immediately much lower than those in the enclosure and remained lower until day 18. The reason for this discrepancy is not known because, if chemical oxidation is responsible for the majority of the disappearance of As(III), speciation in the bottles and enclosures should have been similar. Microbially mediated oxidation cannot account for the discrepancy since added antibiotics effectively eliminated 99% of the bacterial activity (Table 1). The only hypothesis we can advance is that organic compounds (antibiotics and dead microbes) in the bottles somehow catalyzed the rapid oxidation. Scudlark and Johnson[17], observed an order of magnitude increase in oxidation rates in the presence of dissolved organics.

Acknowledgement

The authors thank the CEPEX on site personnel and G.W. Wallace, the site manager at the time of this experiment, for their logistical support during this work. Thanks are also due to F. Azam for his help with aspects of the microbiological analyses. This work was supported by Grant OCE 76-222717 from the Office of the International Decade of Ocean Exploration of the US, National Science Foundation.

References

1 Ferguson J F & Garvis J, *Water Res*, **6** (1972) 1259.
2 Lowenthal D H, Pilson M E Q & Byrne R H, *J Mar Res*, **35** (1977) 653.
3 Braman R S & Foreback C C, *Science*, **182** (1973) 1247.
4 Braman R S, in *Arsenical pesticides*, edited by E A Woolson (American Chemical Society Symposium Series 7, Washington, DC) 1975, 108.
5 Andreae M O, *Deep-Sea Res*, **25** (1978) 391.
6 Waslenchuk D G, *Mar Chem*, **7** (1978) 39.
7 Johnson D L, *Nature Lond*, **240** (1972) 44.
8 Pilson M E Q, *Limnol Oceanogr*, **19** (1974) 339.
9 Andreae M O & Klumpp D, *Environ Sci Tech*, **13** (1979) 738.
10 Sanders J G & Windom H L, *Estuar Coast Mar Sci*, **10** (1980) 555.
11 Menzel D W & Case J, *Bull Mar Sci*, **27** (1977) 1.
12 Strickland J D H & Parsons T R, *A practical handbook of seawater analysis*, Bull 167 (Fish Res Bd, Canada) 1972.
13 Kovala P E & Laurence J D, *Computation of phytoplankton cell numbers, cell volume, cell surface and plasma volume per litre from microscopical counts* (Univ Wash, Dept Oceanogr, Spec Rept 38) 1966.
14 Strathmann R R, *Limnol Oceanogr*, **12** (1967) 411.
15 Braman R S, Johnson D L, Foreback C C, Ammons J M & Bricker J L, *Anal Chem*, **49** (1977) 621.
16 Johnson D L & Pilson M E Q, *Environ Lett*, **8** (1975) 157.
17 Scudlark J F & Johnson D L, *The role of dissolved organic matter in aquatic arsenite oxidation*. Paper presented at the 42nd annual meeting, ASLO, Stony Brook, New York, 1979.
18 Sanders J G, *Interactions between arsenic species and marine algae*, Ph D Thesis, Univ of North Carolina, 1978.
19 Sanders J G, *Mar Environ Res*, **6** (1980) 57.