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Arsenic and phosphorus in seagrass leaves from the Gulf of Mexico

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Abstract

Arsenic is a common contaminant in the marine environment, but little is known about arsenic in seagrasses. Arsenate is taken up by the phosphate uptake systems of plants, and there is often a relationship between P availability and As uptake. We sampled green leaves of the seagrass Thalassia testudinum from six estuaries in the Gulf of Mexico to document the As content of seagrasses and to determine the relationships between P availability and As. Arsenic content of seagrasses was generally lower than literature values of other marine primary producers. Arsenic content varied from 0.90 to 3.36 ppm and phosphorus content varied from 544 to 6294 ppm, and there were significant differences among the estuaries studied. Nutrient stoichiometry (N:P) indicated that there were differences in P availability among estuaries: Charlotte Harbor had high P availability, while St. Joseph Bay and Florida Bay were P-limited. The Homosassa River, the Anclotte Estuary and Tampa Bay had intermediate P availability. When data from all estuaries were pooled, there was a significant, negative relationship between P and As content, as predicted by the model of competitive uptake of As and P. However, more extensive sampling within one estuary (Florida Bay) showed a significant positive relationship between P and As, suggesting that factors other than strict competitive uptake kinetics influence the relative content of As and P. We suggest that the P and As availability ratio in estuaries, and therefore in the seagrasses that inhabit them, is largely controlled by the relative importance of freshwater and marine inputs of the two elements. © 2001 Elsevier Science B.V. All rights reserved.

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1. Introduction

Arsenic is a common contaminant in marine and estuarine environments. Its potent biocidal properties make it ideal for use in many preservatives and anti-fouling agents, and As is an important active ingredient in wood preservatives used in marine pilings, piers and bulkheads (Weis et al., 1993). Arsenic enters marine food chains through primary producers, and As contamination has the potential of severely altering the marine food webs (reviewed in Sanders et al., 1994).

Arsenate (As(V), the dominant form of As in marine and estuarine surface waters) is taken up by the phosphate transport systems of plants because of the chemical similarity of arsenate and phosphate (Dixon, 1997). Inorganic arsenate is toxic because it uncouples phosphorylation and inhibits phosphate uptake. Much of the arsenate that is taken up by marine algae is rendered non-toxic to the plant by the processes of reduction to As(III) and methylation (Andreae and Klumpp, 1979; Sanders and Windom, 1980; Howard et al., 1995). This less-toxic form of As is stored within the plants as relatively nontoxic arsenosugars (Francesconi and Edmonds, 1997). Macroalgae can concentrate As in their tissues to levels 3-4 orders of magnitude higher than found in their environment (Klumpp and Peterson, 1979). There is an apparent phylogenetic difference in the concentration of As in macroalgae, with Chlorophytes and Rhodophytes having lower As concentrations than Phaeophytes (Sanders, 1979; Francesconi and Edmonds, 1997). Many terrestrial plants have a limited ability to detoxify As, probably an outcome of evolution in a relatively low As environment (Nissen and Benson, 1982). Little is known about the ability of seagrasses to detoxify and accumulate As. These marine angiosperms are thought to be evolutionarily derived from terrestrial angiosperm ancestors (Den Hartog, 1970).

Because of the chemical similarity between arsenate and phosphate, there is a first-order positive correlation between total As and total P contents of broad taxonomic groups of marine macroalgae (Sanders, 1979); but there are reasons to suspect that this positive relationship may not always hold within a taxon. Arsenate inhibits phosphate uptake in yeast (Rothstein and Donovan, 1963) and phytoplankton (Blum, 1966). Similarly, arsenate uptake is suppressed by high concentrations of phosphate in both phytoplankton (Planas and Healey, 1978) and terrestrial angiosperms (Meharg and MacNair, 1992). Therefore, there should be a negative correlation between the phosphorus availability to marine plants and the arsenic content of the plants, even when the P and As availability ratio is constant. Few, if any, data exist on the relationships between phosphorus availability and arsenic uptake by seagrasses.

Seagrasses most often occur in clear, low nutrient waters, since they have a high light requirement and in high nutrient environments they are outcompeted by microalgae and epiphytes for light (Duarte, 1995). Seagrasses from tropical habitats are often limited by the availability of phosphorus (Atkinson, 1987; Short et al., 1990; Fourqurean et al., 1992a; Agawin et al., 1996; but see Terrados et al., 1999 for an exception) instead of nitrogen which is the norm in temperate marine ecosystems (Smith, 1984). In these P-limited environments, water column concentrations of phosphate can be below detection ($0.02 \mu M$) (Fourqurean et al., 1993), roughly the same concentration as arsenate (Andreae, 1978). The neotropical seagrass *Thalassia testudinum* has a broad distribution in the Gulf of Mexico, from warm temperate environments along the northern shore of the Gulf to subtropical

environments in extreme South Florida. Florida Bay, at the southern end of this continuum, is a seagrass-dominated, P-limited embayment (Fourqurean et al., 1992a, 1993). The availability of phosphorus relative to nitrogen generally increases to the north, so that more temperate embayments in the Gulf of Mexico tend to be N-limited (Pennock et al., 1999). Unfortunately, data on As concentrations in the water column and sediments are generally not available for these estuaries of Gulf of Mexico (but see Froelich et al., 1985 for an exception).

There is a general dearth of information relating to the effects of As on seagrasses or the ecosystems supported by seagrass primary production. Only recently has the concentration of As in seagrass leaf tissues been reported (Cai et al., 2000). In this paper, we document the variability of As content of seagrasses across estuaries in the eastern Gulf of Mexico. The gradient in relative P availability for seagrasses in the Gulf of Mexico, combined with the inhibitory effect of phosphate availability on arsenate uptake, led us to suspect that there would be differences in the arsenic content of *T. testudinum* leaves among estuaries in the gulf. Previous work has shown P content of seagrasses to be a good indicator of P availability (Atkinson and Smith, 1983; Duarte, 1990; Fourqurean et al., 1992a,b); hence we hypothesized that arsenic content of the seagrass would decrease as P content increased.

2. Methods

For comparisons among estuaries, five sampling sites were randomly chosen within each of six estuaries along the west coast of Florida (Fig. 1), from St. Joseph Bay in the north $(30^{\circ}00'N, 85^{\circ}30'W)$ to Florida Bay in the south $(25^{\circ}00'N, 81^{\circ}00'W)$ during the summer of 1997. For the analysis of the spatial pattern in elemental content within Florida Bay, an additional 31 sites were sampled. At each sampling site, five intact shoots of *T. testudinum* were haphazardly collected from a 10 m^2 area. These were returned to the lab, where all attached green leaves were cleaned of adhering epiphytes by gently scraping with a razor blade. All leaves from a site were pooled and dried at 80° C. Dried leaves were ground to a fine powder using a ceramic mortar and pestle.

Arsenic was determined by a HNO₃/H₂O₂ digestion followed by inductively coupled plasma mass spectrometry, with standard additions to correct for matrix effects. This method has been shown to accurately determine the As content of standard reference materials (citrus leaves, SRM 1572) and seagrass leaves (Cai et al., 2000). Because of the documented importance of the availability of P relative to other nutrients in determining As content of other marine plants, we analyzed our leaf samples for P content. To help us determine whether P was the most limiting nutrient for seagrasses at a location, we also analyzed for nitrogen content. Powdered samples were analyzed in duplicate for N content using a CHN analyzer (Fisons NA1500). Phosphorus content was determined by a dry-oxidation, acid hydrolysis extraction followed by a colorimetric analysis of phosphate concentration of the extract (Fourqurean et al., 1992a). Elemental content was calculated on a dry weight basis; elemental ratios were calculated on a mole:mole basis.

Differences in elemental content among estuaries were assessed using ANOVA with Tukey post hoc tests. Relationships between elements were examined using linear regression. Spatial patterns in elemental content were examined visually by mapping and contouring the elemental content data using a kriging interpolation algorithm.



Fig. 1. Map showing location of estuaries sampled for arsenic, phosphorus and nitrogen content of green leaves of the seagrass *T. testudinum*. Inset shows distribution of sampling stations for the analysis of regional spatial pattern in the elemental content of green leaves of *T. testudinum* in the Florida Bay region.

3. Results

3.1. Inter-estuary comparisons

Total arsenic content of the 30 inter-estuary comparison samples ranged from 0.90 to 3.00 ppm, with a mean of 1.84 ± 0.55 (± 1 S.D., Fig. 2). Phosphorus content of the samples was ca. three orders of magnitude higher and more variable, with a range of 700–6300 ppm, with a mean of 2300 ± 1500 ppm (Fig. 2). Nitrogen was a larger component of the leaf samples; the mean N content was 22, 700 ± 2200 ppm with a range of 19,000–26,800 ppm (Fig. 2).

Arsenic content of the samples varied significantly among estuaries (ANOVA, F = 15.71, P < 0.001; Fig. 2). Samples from St. Joseph Bay had the highest As content, with a mean of 2.68 ppm. Charlotte Harbor samples had the lowest As content, with a mean of 1.18 ppm. The other estuaries fell between these two end points: Tampa Bay, 2.12 ppm; Homosassa River, 1.92 ppm; Florida Bay, 1.66 ppm; and Anclotte Key, 1.51 ppm. Note that the five Florida Bay samples collected for the inter-estuary comparison had a similar mean



Fig. 2. Elemental content of green leaves of *T. testudinum* in six estuaries on the eastern shore of the Gulf of Mexico. StJ: St. Joseph Bay; HO: Homosassa River; AN: Anclotte Key; TB: Tampa Bay; CH: Charlotte Harbor; FB: Florida Bay (five stations for comparison to other estuaries); FBr: regional mean from 31 sites across Florida Bay, exclusive of the five sites used for the cross-estuary comparisons. Lower case letters represent membership in a homogenous group for an element (ANOVA with Tukey post-hoc test, $P \le 0.05$). FBr samples were not analyzed in the ANOVA.

to the larger Florida Bay data set collected for analysis of spatial pattern (1.66 ppm versus 1.77 ppm).

There were also significant differences in the mean P content of the samples among estuaries (ANOVA, F = 58.39, P < 0.001; Fig. 2). The estuaries were segregated into three groups: Charlotte Harbor had the highest P content ($\bar{x} = 5200$ ppm); St. Joseph Bay and Florida Bay had the lowest P content ($\bar{x} = 1100$ ppm for St. Joseph Bay and $\bar{x} = 1000$ ppm for Florida Bay). Homosassa River, Anclotte Key and Tampa Bay had intermediate P content (means of 1700, 2300 and 2400 ppm, respectively). The P content of the inter-estuary comparison samples from Florida Bay and the Florida Bay spatial pattern samples was similar (1000 and 1100 ppm, respectively).

Nitrogen content of the samples varied among estuaries, but the magnitude of the differences was much less for As and P (Fig. 2). The significant differences in N content among the estuaries (ANOVA, F = 6.82, P < 0.001) were characterized by three groups: a low



Fig. 3. Elemental ratios (mol:mol) of green leaves of *T. testudinum* in six estuaries on the eastern shore of the Gulf of Mexico. StJ: St. Joseph Bay; HO: Homosassa River; AN: Anclotte Key; TB: Tampa Bay; CH: Charlotte Harbor; FB: Florida Bay (five stations for comparison to other estuaries); FBr: regional mean from 31 sites across Florida Bay, exclusive of the 5 sites used for the cross-estuary comparisons. FBr samples were not analyzed in the ANOVA. Lower case letters represent membership in a homogenous group (ANOVA with Tukey post-hoc test, $P \le 0.05$). The horizontal band on the N:P ratio panel represents the range of values associated with N:P balance ratio in the plant tissues, i.e. a seagrass "Redfield" ratio (Atkinson and Smith, 1983; Duarte, 1990; Fourqurean et al., 1992a). N:P ratio above this band indicates P limitation and below indicates N limitation.

N group consisting of St. Joseph Bay, Florida Bay and Homosassa River (means of 21,000, 20,200 and 22,200 ppm, respectively); an intermediate group consisting of Homosassa River and Anclotte Key (22,200 and 23,800 ppm, respectively); and a high N group consisting of, Anclotte Key, Tampa Bay and Charlotte Harbor (means of 23,800, 24,500 and 24,700 ppm, respectively). Note that the samples collected from Florida Bay for the spatial pattern study had significantly higher N content than the Florida Bay inter-estuary comparison samples (25,900 ppm versus 20,200 ppm), this was a consequence of the marked spatial pattern in N content of seagrass leaves from within Florida Bay (Fourqurean et al., 1992a).

There were significant differences in the ratio of N to P in *T. testudinum* leaves among estuaries (ANOVA, F = 32.03, P < 0.001; Fig. 3). St. Joseph Bay and Florida Bay had similar mean N:P ratio, both substantially higher than the seagrass "Redfield" ratio of 25 to 30 (Atkinson and Smith, 1983; Duarte, 1990; Fourqurean et al., 1992a), indicating P limitation. Charlotte Harbor *T. testudinum* had a very low N:P ratio, indicating strong N limitation. There were no significant differences in the mean N:P ratio from Homosassa River, Anclotte Key and Tampa Bay; these estuaries had N:P ratio lower than the "Redfield" range for seagrasses, indicating potential N limitation, but the signal of N limitation was not nearly as strong for these estuaries as for Charlotte Harbor.

Phosphorus:arsenic ratios also differed among estuaries (ANOVA, F = 24.73, P < 0.001; Fig. 3), but the post-hoc test indicated that Charlotte Harbor was the only



Fig. 4. Relationship between phosphorus and arsenic content of green leaves of *T. testudinum* from six estuaries in the eastern Gulf of Mexico. Regression and 95% confidence interval for all data is shown.

estuary significantly different from the others (Tukey, P = 0.05). In Charlotte Harbor, the P:As ratio was more than three times higher than the other estuaries.

Arsenic content of the inter-estuary comparison samples was related to phosphorus availability, as measured by P content of the *T. testudinum* leaves (Fig. 4). As P content increased, there was a decrease in As content. Using the data from all the six estuaries, there was a significant linear regression using P content as a predictor of As content (As = $2.37-2.28 \times 10^{-4}$ (P), $R^2 = 0.37$, P < 0.001). Florida Bay samples, which had relatively low P and As content, seemed to deviate from the general relationship exhibited by the other estuaries; when Florida Bay data were removed, the predictive ability of the regression improved (As = $2.65-3.01 \times 10^{-4}$ (P), $R^2 = 0.56$, P < 0.001).

3.2. Within Florida Bay comparisons

The range of As concentrations of *T. testudinum* leaves was greater in the 31 samples collected within Florida Bay than encountered in the inter-estuary comparison. In Florida Bay, As content was in the range of 0.96–3.36 ppm, with a mean of 1.76 ± 0.62 ppm. Phosphorus content of samples from within Florida Bay was between 500 and 1900 ppm, with a mean of 1100 ± 400 ppm. Nitrogen content averaged $25,100 \pm 5700$ ppm, with a range of 15,200–39,600 ppm. The N:P ratio of samples from within Florida Bay was highly variable, with a mean of 59.4 ± 25.3 and a range of 22.6–107.1.

Phosphorus availability, as indicated by the N:P ratio of leaves of *T. testudinum*, decreased from the open western boundary of Florida Bay to the enclosed eastern end of the bay (Fig. 5). This is the same pattern documented previously (Fourqurean et al., 1992a; Frankovich and Fourqurean, 1997). There was also a strong gradient in the As content of the seagrass leaves (Fig. 5), with the highest As content (ca. 3 ppm) in the western portion of the bay, and low (ca. 1 ppm) concentrations in the eastern bay. There was a strong, significant positive relationship between As and P content within Florida Bay (Fig. 6), in contrast to the negative relationship observed in the inter-estuary comparison samples (Fig. 4).



Fig. 5. Spatial pattern in N:P ratio (top) and arsenic content of green leaves of *T. testudinum* from 31 sites in Florida Bay plus the inter-estuarine comparison samples. Sample locations are indicated by + symbol. The five samples used for the inter-estuary comparison are enclosed in a dashed circle in the center of the bay.



Fig. 6. Relationship between phosphorus and arsenic content of green leaves of *T. testudinum* from 31 sites in Florida Bay plus the inter-estuarine comparison samples. Regression and 95% confidence interval for all data is shown.

4. Discussion

The As content of *T. testudinum* (0.9–3.4 ppm) from the six estuaries in the Gulf of Mexico was low compared to literature values for marine macroalgae and phytoplankton. Phytoplankton from the Great Barrier Reef contain around 9 ppm As (Benson and Summons, 1981). Worldwide, macroalgal As concentrations are quite variable, with Chlorophytes ranging from 0.1 to 23 ppm, Rhodophytes ranging from 0 to 45 ppm, and Phaeophytes ranging from 1 to 179 ppm (see Francesconi and Edmonds, 1997 for review). It has been argued that only plants that evolved in an environment with a low P:As availability ratio have had to evolve methods for detoxifying As, since only in such regions would As uptake be substantial (Benson et al., 1981; Nissen and Benson, 1982). Tropical seagrasses are often limited by the availability of P, hence they are likely to occur in environments with low P:As ratio; but the low As concentrations in *T. testudinum* compared to other primary producers may indicate the inability of this seagrass species to detoxify and accumulate As.

There were considerable differences among estuaries in the P availability (Fig. 3). *T. testudinum* from two of the estuaries, St. Joseph Bay and Florida Bay, was clearly P-limited. Phosphorus limitation of Florida Bay has been previously documented (Fourqurean et al., 1992a,b, 1993). The strong P-limitation signal for St. Joseph Bay is intriguing, because most P-limited marine ecosystems are subtropical–tropical in carbonate sediment environments [e.g. Florida Bay; Shark Bay, Australia (Smith and Atkinson, 1984); Bahamas seagrass beds (Short et al., 1990)] but St. Joseph Bay is a warm-temperate embayment with siliceous sediment. Seagrass from Charlotte Harbor was clearly N-limited, in agreement with what is known about nutrient biogeochemistry and phytoplankton limitation in this estuary (Froelich et al., 1985; McPherson et al., 1990). Seagrass N:P ratios from the Homosassa River, Anclotte Key and Tampa Bay suggest N limitation for these sites, although the deviation from the seagrass "Redfield" ratio is not so severe as for Charlotte Harbor. Few data exist on nutrient limitation for Homosassa River or Anclotte Key, but phytoplankton are probably limited by N availability in Tampa Bay (Pennock et al., 1999).

When comparing estuaries in the eastern Gulf of Mexico, the amount of As in seagrass leaves was inversely related to P availability (Fig. 4). The degree of discrimination between P and As by the seagrass, as indicated by the P:As ratio, increased as P availability increased (Fig. 3). Charlotte Harbor, where P availability was greatest, also had the highest P:As ratio. The two most P-limited estuaries, St. Joseph Bay and Florida Bay, had the lowest P:As ratio, suggesting less discrimination between As and P during uptake. These findings are in agreement with the kinetic competition model of As uptake described for phytoplankton (Planas and Healey, 1978) and terrestrial angiosperms (Meharg and MacNair, 1992).

Of note is the fact that seagrasses from Charlotte Harbor had very low As content compared to the other estuaries in this study (Fig. 2). This is striking, since bivalves collected from this estuary have high As concentrations relative to US national averages (Valette-Silver et al., 1999). The high As concentration in bivalves from Charlotte Harbor has been hypothesized to result from As in the Peace River originating from phosphate deposits of marine origin in the watershed (Valette-Silver et al., 1999). Since we found lower than average As content in seagrasses from this estuary, it is possible that elevated As concentration may be incorporated by filter-feeding bivalves consuming phytoplankton with a different As content than seagrass.

The relationship between As and P content observed over the Gulf of Mexico (Fig. 4) did not hold for the P availability gradient within Florida Bay, where As content increased as P content increased (Fig. 6). This observation suggests that the arguments about the kinetics of P and As uptake developed above do not explain all of the variability in As concentration of seagrasses. The source of P that maintains primary productivity in Florida Bay is Gulf of Mexico water brought into the western part of the bay by tidal action and by advection to replace water lost from the bay to evaporation (Fourgurean et al., 1992a, 1993; Rudnick et al., 1999). Little P is delivered to Florida Bay as stream flow (Rudnick et al., 1999), and we assume that As loading from the watershed is also very low. We further assume that the flux of dissolved As into Florida Bay follows the same pattern as the flux of P. The P availability to T. testudinum decreased from west to the east in Florida Bay, as indicated by the N:P ratio of seagrass leaves (Fig. 5). A similar pattern occurred for As content of T. testudinum (Fig. 5). The ratio of P to As in Florida Bay varied between 907 and 2047, with no coherent spatial pattern (data not shown). The resulting positive relationship between P and As (Fig. 6) indicated that As and P availability covaried in Florida Bay; further, there was no increase in As content as P content decreased. Interestingly, there was no apparent negative relationship between As content of seagrasses from Florida Bay and what is known about seagrass growth rates from Florida Bay (Zieman et al., 1989, 1999); both seagrass primary productivity and As content increase from a low in the northeast parts of the bay to a maximum in the western regions.

Clearly, factors other than the availability of P must influence the arsenic content of seagrasses. We suspect that riverine inputs of P to the five estuaries north of Florida Bay cause variability in the P:As ratio in the environment. Typical As concentrations in rivers in the southeastern US are low compared to typical marine water (Waslenchuk, 1978). Conversely, P concentrations tend to be much higher in rivers than marine waters. These conditions should therefore lead to higher P:As ratio near freshwater P sources and lower P:As ratio from marine sources. This pattern could explain the observed relationship between P availability and As content of the seagrasses among the estuaries (Fig. 4): those estuaries with high freshwater inflows should have higher P loads and elevated P:As ratio in the water column, and consequently higher P:As ratio in seagrass leaves. At one end of the spectrum of freshwater input for the six estuaries studied is Charlotte Harbor, where runoff from the Peace River delivers an average discharge of $70 \text{ m}^3 \text{ s}^{-1}$ of P-enriched runoff to the estuary (Miller and McPherson, 1991). Freshwater runoff leads to a P:As concentration ratio greater than 5000 in the water column near the head of the bay compared to less than 100 near the mouth of the bay (Froelich et al., 1985), despite the fact that the Peace River drains a watershed rich in phosphate deposits, which themselves are As sources (Valette-Silver et al., 1999). At the other end of the spectrum is Florida Bay, where freshwater runoff is a small part of the total water budget for the bay (Nuttle et al., 2000); consequently stream flow is a minor P source (and presumably a minor As source as well). Similar biogeochemical behavior of phosphate and arsenate (Sanders, 1980), then, would result in a constant relative availability of P and As across the estuary and produce a positive relationship between P and As concentration. This is the pattern in seagrass P:As concentration ratio that we observed from Florida Bay (Fig. 6). We interpret these data to indicate that variability in the P:As ratio in the source waters of the estuary must also play an important role in determining the As content of seagrasses, in addition to the hypothesized kinetic control of relative uptake of P and As that has been used to explain the accumulation of As by plants in low P environments. The critical experiments examining the effects of variable P:As availability ratio on P and As uptake by seagrasses remain to be done to fully address this hypothesis.

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