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Methylation of Inorganic Tin by Decaying *Spartina alterniflora* in Estuarine Water and by Estuarine Water

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Methyltin compounds (MeSn) which do not originate from man-made pollution are common in estuaries and particularly in salt marshes containing the marsh grass *Spartina alterniflora*. This study reports the results of experiments in which estuarine water containing *S. alterniflora* leaves is spiked with inorganic tin, and estuarine water alone is spiked with inorganic tin and MeSn. When decaying leaves are present, inorganic tin concentrations in the water decrease and there is a 10-fold increase in inorganic tin concentration in the leaves. This biosorption follows pseudo-first-order kinetics. $MeSn^{3+}$ and Me_2Sn^{2+} occur occasionally in the water. The Me_2Sn^{2+} concentration decreases with time and the Me_3Sn^{2+} concentration increases with time in *S. alterniflora* leaves. The results of estuarine water amended with inorganic tin and MeSn in the absence of leaves are quite different. The overall inorganic tin concentration decreases significantly during the experiment, the $MeSn^{3+}$ concentration is approximately constant, and concentrations of Me_2Sn^{2+} and Me_3Sn^{+} increase. This means that net methylation of inorganic tin has occurred. We conclude that decaying *S. alterniflora* is likely to be important in the cycling of tin in salt marshes.

Keywords: Tin, methylation, *Spartina alterniflora*

INTRODUCTION

Methyltin compounds (MeSn) are found throughout estuarine environments, including sediment,[1] shellfish,[2] macroalgae[3] and marsh grass.[4] In fact MeSn may constitute up to 80% of the total tin in water and more than 90% of the total tin in biota.[5] The only major industrial use for MeSn, as a minor component in PVC piping,[6] is not enough to account for their concentrations in the aquatic environment. For this reason we believe that the major source of MeSn in estuaries is environmental methylation of inorganic tin. This is of concern because MeSn are much more toxic to marine life than their inorganic tin precursors, in part because of their ability to bioconcentrate in certain tissues.

Donard *et al.*[3] studied the effect of the algae *Enteromorpha* on the formation and stability of methyltin. They found that all three methyltin compounds were produced as the algae decomposed. They also found that several seaweeds are capable of concentrating MeSn relative to the surrounding estuarine water. Wright and Weber[7] investigated the rates of accumulation of inorganic tin and MeSn by healthy *Fucus vesiculosus* and a mixed community of *Enteromorpha* incubated in modified estuarine water. They found that all four tin compounds were biosorbed by both species of algae, but that no methylation or demethylation occurred. Accumulation broke down into three distinct phases: (1) a rapid initial phase associated with surface adsorption; (2) a moderately fast second phase attributed to an extracellular process; and (3) a slow final phase of cellular accumulation.

There are *ca* 4.1 km^2 of salt marsh encompassed in the Great Bay Estuary, New Hampshire, USA, Making this the third most abundant habitat within the estuary.[8] Marsh grass ecosystems provide homes, feeding grounds and breeding grounds for many species of marine organisms and birds. Several species residing within the Great Bay Estuary salt marshes are classified as rare or endangered by the state of New Hampshire.[8] The low marsh (the region between the low and high tide levels) is dominated by stands of *Spartina alterniflora* which constitute a large portion of the organic detritus that fluxes into the estuary each year.[9]

Weber and Alberts[10] previously demonstrated that hydroponically grown *S. alterniflora* plants

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accumulate Me_3Sn^+ from Sn(IV)-amended media, but several unpublished studies in this laboratory on the ability of decaying *S. alterniflora* to methylate and demethylate tin have been inconclusive. For example, Proulx-Curry (personal communication) studied decaying *S. alterniflora* leaves, roots and rhizomes in estuarine water that had been amended with low (5 ng ml^{-1}) and high (150 ng ml^{-1}) concentrations of inorganic tin. In some of the plant parts incubated, she found high MeSn concentrations. However, the plants spiked with the higher amounts of inorganic tin did not exhibit higher MeSn concentrations. The high inorganic tin concentrations probably significantly altered the chemistry of the samples and/or killed all but the tin-resistant bacteria, thus eliminating some of the potential methylating bacteria. A possible conclusion is that methylation during the decay process resulted directly from a bacterial enzymic process or from chemical(s) released or formed during the decay process.

No published study has investigated the role of decaying *S. alterniflora* in the cycling of tin compounds. In this study, we examined the role of decaying *S. alterniflora* leaves in the uptake of inorganic tin and the subsequent formation of MeSn. Decaying *S. alterniflora* leaves concentrate inorganic tin from estuarine waters. A significant decrease in Me_2Sn^{2+} concentrations accompanied by a significant increase in Me_3Sn^+ concentrations indicates MeSn rearrangement in conjunction with the decay process. Because of the importance of *S. alterniflora* in estuaries, formation of MeSn during the decay of this grass may be a significant contributor to MeSn found in it.

EXPERIMENTAL

Materials

All fresh water used was doubly deionized and distilled through a Corning Mega-pure still. Monomethyltin ($MeSn^{3+}$), dimethyltin (Me_2Sn^{2+}), trimethyltin (Me_3Sn^+) and inorganic tin(IV) chlorides (97 + % purity) were obtained from Alfa Chemicals. Stock solutions of *ca* 1000 mg ml^{-1} (all concentrations throughout this paper are as tin) were prepared in 1 M hydrochloric acid (HCl). Standards were made by diluting stock solutions to *ca* 5 μg ml^{-1} in 0.05 M nitric acid (HNO_3) for methyltin chlorides or 1 M HCl for $SnCl_4$. Sodium borohydride ($NaBH_4$) solution (6%) was prepared by dissolving 12 g $NaBH_4$ (Aldrich Chemicals, 98%) in 100 ml water and allowing the solution to stand overnight in a refrigerator. The solution was then filtered through a 0.2 μm polycarbonate filter (Nuclepore) to remove any inorganic tin colloids, and diluted to 200 ml with water. All other chemicals were of reagent grade. All glassware and plasticware was soaked overnight in a 10% HNO_3 solution. The containers used for the experiments were 1000 ml Florence flasks modified with a nozzle at the base and connected to an air source. A porous frit separated the interior of the flask from the air source to prevent flow of flask contents into the air lines.

Sample collection

Estuarine water was obtained in 2.5 l bottles off the dock at Jackson Marine Laboratory in the Great Bay Estuary, New Hampshire, USA. To avoid collecting the top microlayer of water, which tends to have higher concentrations of inorganic tin and methyltin compounds,[11] care was taken to uncover and cover the bottles at least 5 cm below the surface. The water was collected upstream from the dock to avoid any contamination from the dock. The inorganic tin concentration in the estuarine water was 2.4 ng ml^{-1}. *S. alterniflora* leaves were collected in early July 1993 at Chapman's Landing in the Squamscott River, New Hampshire, by cutting leaves from the stalk. Leaves were cut into 0.5 cm lengths and mixed together to homogenize them as much as possible.

Experiment 1

Three flasks were filled with 10 g homogenized leaf and 500 ml estuarine water. Each flask was spiked with 11.75 μg inorganic tin (from $SnCl_4$ stock solution). House compressed air was bubbled through each flask at a rate just great enough to create a steady stream of small bubbles to keep the sample aerated. All flasks were placed on a laboratory bench exposed to indirect sunlight for *ca* 16 h day^{-1}. Every 6 h for the first 30 h, *ca* 1 g leaf (weighed accurately) and 20.0 ml water were removed from each experiment. The water samples were immediately acidified to pH 0 through addition of 1.8 ml 12.1 M HCl. Unamended estuarine water (20 ml) was added to each sample to replace that removed. Fresh water was added

to replace any that had evaporated. Leaf samples were washed by shaking in a 10 ml aliquot of 0.05 M HNO_3 and the washings collected. Leaves were then rinsed with two 5 ml aliquots of water and dried by pressing lightly between two Kimwipes (Kimberly-Clark). Leaves were ground in liquid nitrogen and extracted in 1 M HCl–75% methanol (v/v).[4]

Experiment 2

Three flasks were each filled with 500 ml estuarine water. To each flask 36.7 μg of tin (as above) and $MeSn^{3+}$, Me_2Sn^{2+} and Me_3Sn^+ (12.5 μg of each from stock solutions) were added. House air was bubbled through the experiments as in Experiment 1. Samples were again exposed to indirect sunlight. Aliquots (20 ml) were removed immediately and again on days 2 and 4 from each flask and replaced with unamended estuarine water. Each aliquot was immediately acidified to pH 0 and stored in the freezer until analyzed.

Determination of inorganic tin and methyltin

Inorganic tin and MeSn in each sample were determined as tin atoms by on-line hydride generation followed by cryogenic trapping, chromatographic separation and detection by atomic absorption spectrometry.[12] Samples of 0.1–2 ml are placed in the hydride generation flask with 40 ml water and acidified through the addition of 0.5 ml acetic acid. Larger volumes of strongly acidic samples are neutralized by addition of 10 M sodium hydroxide (NaOH) to ensure constant initial pH. After the flask is connected to the column, 2.5 ml of a 6% (w/v) $NaBH_4$ solution is added. This has the dual purpose of generating the hydrides of the tin compounds and creating a large volume of hydrogen gas which serves to purge SnH_4 and MeSn hydrides from the flask. From there they are trapped in a liquid-nitrogen-cooled U-tube (40 cm × 5 mm i.d.) packed with 3% SP-2100 on Chromosorb G AW-DMCS 45/60 which has been silanized with dimethyldichlorosilane [5% (v/v) in toluene].[12] Prepurified helium at a flow rate of 120 m min^{-1} is the carrier gas. Upon removal of the liquid nitrogen from the trap, the tin compounds elute according to their boiling points and are detected in an electrothermally heated (750 °C) quartz furnace with a Perkin–Elmer 503 atomic absorption spectrophotometer. A hydrogen-rich oxygen–hydrogen flame (hydrogen flow rate, 830 m min^{-1}; oxygen flow rate, 21 m min^{-1}) improves the atomization efficiency of the hydrides. Retention times are 0.7 min for SnH_4, 1.5 min for $MeSnH_3$, 2.1 min for Me_2SnH_2, and 2.7 min for Me_3SnH.

The absolute limit of detection for each tin compound is 0.25 ng Sn. Quantitation is based on calibration curves of the analytes. Actual detection limits depend on dilution factors and the volume of aliquot used and can be found at the foot of Tables 1 and 2. The maximum sample volume used depends on the amount of inorganic tin present, because a large inorganic tin peak can obscure the $MeSn^{3+}$ peak. Thus a large inorganic tin spike decreases the limit of detection. Each determination was performed at least in duplicate. If the relative deviation was greater than 10%, a third determination was carried out.

RESULTS

Experiment 1

Concentrations of inorganic tin in the estuarine water containing decomposing *S. alterniflora* leaves (Table 1) decrease from 28 ng ml^{-1} (mean at 6 h) to 13 ng ml^{-1} (mean at 120 h) during the 120 h study. Analysis of variance (ANOVA) at the 95% confidence level indicates that this decrease is significant ($F = 16.97$, degrees of freedom (df) = 6, probability (P) > $F = 0.0000$). Table 1 also shows that $MeSn^{3+}$ and Me_2Sn^{2+} occur occasionally in water samples, but with no temporal trend.

The initial concentration of inorganic tin in leaves (Table 2) is *ca* 28 ng g^{-1}. There is approximately a 10-fold increase in inorganic tin concentration in decaying leaves during the five days studied. This represents a statistically significant increase ($F = 8.56$, df = 7, $P > F = 0.0002$).

Prior to adding inorganic tin ($t = 0$) the $MeSn^{3+}$ concentration in the leaf samples was *ca* 2 ng g^{-1} and that of Me_2Sn^{2+} was *ca* 9 ng g^{-1}. No Me_3Sn^+ was detected in the leaf samples [limit of detection (LOD) = 0.44 ng g^{-1}]. After addition of inorganic tin, $MeSn^{3+}$ (Table 2 and Fig. 1) occurs occasionally with no apparent trend. There is no statistically significant difference between the initial $MeSn^{3+}$ concentrations and later ones. Me_2Sn^{2+} occurs quite regularly during the first 12 h of sampling and then concentrations drop below the limit of detection. Me_3Sn^+, in contrast,

Table 1 Variation in concentrations of inorganic tin and methyltin compounds with time, in estuarine water containing decomposing *S. alterniflora* leaves and supplemented with inorganic tin (32.5 ng ml^{-1})[a, b, c]

Time (h)	Concentration (ng ml^{-1} ± 1 standard deviation) Sn	MeSn	Me_2Sn
0[d]	2.08 ± 0.26	0.16 ± 0.004	1.4 ± 0.003
0[d]	1.43 ± 0.13	0.08 ± 0.01	0.49 ± 0.03
6	23.9 ± 0.88	D/ND	D/ND
6	32.0 ± 1.6	ND	ND
6	27.2 ± 3.2	0.594 ± 0.019	0.835 ± 0.094
12	22.6 ± 0.2	ND	ND
12	27.1 ± 2.7	ND	ND
18	32.2 ± 2.6	ND	ND
18	32.9 ± 4.2	ND	ND
18	33.2 ± 6.4	ND	ND
24	22.1 ± 1.2	ND	ND
24	26.5 ± 0.6	ND	0.506 ± 0.038
24	28.7 ± 1.2	ND	D/ND
30	18.2 ± 2.8	ND	ND
30	16.7 ± 1.3	0.61 ± 0.14	0.74 ± 0.16
30	13.3 ± 0.9	ND	ND
48	20.6 ± 2.4	2.04 ± 0.33	1.01 ± 0.25
48	15.9 ± 0.6	ND	D/ND
48	14.3 ± 1.8	ND	ND
120	15.3 ± 0.6	ND	ND
120	14.3 ± 0.5	ND	ND
120	9.8 ± 1.1	D/ND	0.70 ± 0.22

[a] ND, compound is not detected. Limit of detection (baseline + 3σ) at $t = 0$ (sample volume = 5.0 ml) is 0.044 ng ml^{-1} and is 0.44 ng ml^{-1} for all other times (sample volume = 0.5 ml).

[b] N/ND: value is above the limit of determination (baseline + 1.5σ) but below the limit of detection

[c] At each time three different samples were analyzed. Each number represents the average of at least two determinations.

[d] Represents the concentration prior to the addition of an inorganic tin spike.

is rarely seen during the first 18 h and is regularly detected after that. While both Me_2Sn^{2+} and Me_3Sn^+ exhibit apparent trends in concentration with time, the actual data are insufficient to support this observation statistically. Based on the assumption that MeSn are present in most of the samples at concentrations below the limit of detection (LOD = 5 ng g^{-1}) and that detection errors will be similar to those in the actual data, we fabricated 'dummy' values for the undetected concentrations.[13] That is, we replaced undetected (zero) concentrations of Me_2Sn^{2+} and $MeSn^{3+}$ with concentrations below the LOD that have a 20% relative deviation. By this method, ANOVA

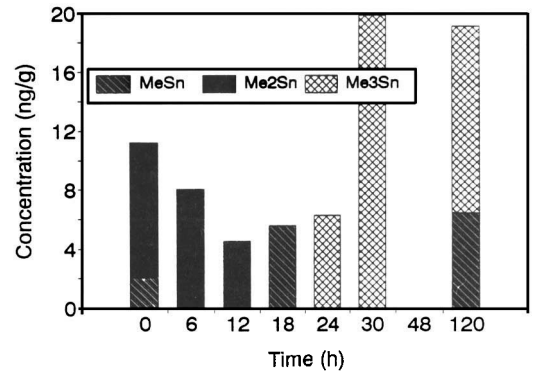

Figure 1 Variation of averaged MeSn concentrations in decaying leaves of *S. alterniflora* in estuarine water with time after spiking with 1.17 μg g^{-1} inorganic tin.

shows that both Me_2Sn^{2+} and Me_3Sn^+ exhibit a statistically significant trend with time (Me_2Sn^{2+}: $F = 9.10$, df = 7, $P > F = 0.0003$; Me_3Sn^+: $F = 10.29$, df = 7, $P > F = 0.0001$).

Inorganic tin, $MeSn^{3+}$ and Me_2Sn^{2+} appear regularly in leaf washings (Table 2) with no apparent temporal trend. The mass of inorganic tin found in the wash accounts for 2.2% of the total inorganic tin associated with the leaves, but the percentages of $MeSn^{3+}$ (14%) and Me_2Sn^{2+} (38%) are more significant. The washings contained a significantly greater concentration of Me_2Sn^{2+} than of $MeSn^{3+}$ (t-statistic = 4.14, df = 33, $P > t = 0.0005$).

Since there are two methyl groups for every Me_2Sn^{2+} ion, and three methyl groups for every Me_3Sn^+ ion, the total concentration of methyl groups attached to tin atoms ($[Me]_{TOT}$) (Table 2) in leaves plus washings is shown in Eqn [1].

$$[Me]_{TOT} = [MeSn^{3+}] + 2[Me_2Sn^{2+}] + 3[Me_3Sn^+] \quad [1]$$

$[Me]_{TOT}$ associated with leaves does not vary with time ($F = 1.11$, df = 6, $P > F = 0.4063$).

Experiment 2

Table 3 and Fig. 2 show concentrations of tin compounds in estuarine water samples (no leaves) that have been spiked with inorganic tin (75 ng ml^{-1}) and MeSn (25 ng ml^{-1} of each). The inorganic tin concentration (Table 3 only) decreases substantially ($F = 36.43$, df = 2, $P > F = 0.0000$) during the four days of study. The $MeSn^{3+}$ concentration (Fig. 2) decreases on day 2 to *ca* 16 ng ml^{-1}, then returns to *ca* 19 ng ml^{-1}.

$MeSn^{3+}$ concentrations on days 0 and 4 are significantly different from those of day 2 ($F=7.36$, df = 2, $P>F=0.0050$). Me_2Sn^{2+} concentrations increase significantly ($F=17.24$, df = 2, $P>F=0.0001$) from 24 ng ml^{-1} (day 0) to 29 ng ml^{-1} (day 4). Me_3Sn^+ concentrations, which follow the same pattern as Me_2Sn^{2+}, increase significantly ($F=6.13$, df = 2, $P>F=0.0099$) during the four-day experiment. $[Me]_{TOT}$ increases significantly with time ($F=8.27$, df = 2, $P>F=0.0189$).

DISCUSSION

Experiment 1

In a preliminary study we examined the effect of light and air on the methylation of inorganic tin by decaying *S. alterniflora* leaves in estuarine water. Inorganic tin spikes ranged from 0 to 100 ng ml^{-1}. Plant samples maintained in a light/dark cycle and in an aerobic environment seemed to produce higher concentrations of MeSn; how-

Table 2 Variation of inorganic tin and methyltin concentrations in decomposing *S. alterniflora* leaves and leaf washings in estuarine water supplemented with 1.18 μg Sn per g of leaf material[a,b,c]

	Concentration (ng g^{-1} ± 1 standard deviation)							
	Within leaf				Leaf washings[d]			
Time (h)	Sn	$MeSn^{3+}$	Me_2Sn^{2+}	Me_3Sn^+	Sn	$MeSn^{3+}$	Me_2Sn^{2+}	$[Me]_{tot}$[e] (ng g^{-1})
0[f]	34.2 ± 2.8	2.32 ± 0.08	10.6 ± 0.2	ND	—	—	—	23.5
0[f]	24.8 ± 3.2	1.72 ± 0.18	7.69 ± 0.93	ND	—	—	—	17.1
0[f]	29.7 ± 3.8	ND	ND	ND	—	—	—	ND
0[f]	24.7 ± 5.8	ND	9.18 ± 0.51	ND	—	—	—	18.4
6	125 ± 16	ND	ND	ND	2.66 ± 0.005	1.49 ± 0.004	4.59 ± 0.54	10.7
6	69.7 ± 8.7	ND	5.09 ± 0.43	ND	7.68	2.42 ± 0.16	7.42 ± 0.17	27.4
6	119 ± 17	ND	8.06 ± 0.95	ND	8.7 ± 1.6	31.1 ± 2.7	50.2 ± 0.7	148
12	193 ± 21	N/ND	12.0 ± 2.9	N/ND	7.69 ± 0.48	D/ND	D/ND	27.2
12	106 ± 20	N/ND	N/ND	N/ND	2.50 ± 0.08	D/ND	1.42 ± 0.06	3.99
12	114 ± 13	ND	ND	ND	2.34 ± 0.09	ND	1.46 ± 0.06	2.92
18	90.2 ± 0.1	ND	ND	ND	10.6	1.31 ± 0.15	1.60 ± 0.13	4.51
18	174 ± 11	12.8 ± 1.1	ND	ND	1.87 ± 0.26	1.71 ± 0.26	7.02 ± 0.54	28.6
18	144 ± 3	5.56	ND	ND	3.36 ± 0.58	21.6 ± 8.5	5.4 ± 1.6	37.9
24	148 ± 10	N/ND	ND	6.27 ± 0.54	3.05	D/ND	5.48 ± 0.72	30.3
24	182 ± 7	ND	ND	ND	6.93	1.29 ± 0.14	8.83 ± 0.50	19.0
24	215 ± 16	ND	ND	ND	2.94 ± 0.01	30.7 ± 2.2	29.8 ± 1.7	90.3
30	147 ± 20	N/ND	ND	11.4 ± 2.6	5.44	D/ND	4.70 ± 0.16	44.5
30	188 ± 23	N/ND	N/ND	N/ND	1.74 ± 0.75	D/ND	6.09 ± 0.29	13.1
30	226 ± 37	ND	ND	19.9 ± 0.4	1.41 ± 0.004	2.79 ± 0.60	10.2 ± 0.1	82.9
48	167 ± 4	ND	ND	ND	1.68 ± 0.08	D/ND	4.57 ± 0.58	10.3
48	364 ± 1	ND	N/ND	N/ND	1.70 ± 0.22	1.65 ± 0.36	9.99 ± 0.36	21.6
48	229 ± 19	ND	ND	ND	2.05	D/ND	6.70 ± 0.15	14.6
120	317 ± 35	6.45 ± 0.16	ND	12.68	6.96 ± 0.26	ND	D/ND	46.1
120	293 ± 34	ND	N/ND	N/ND	7.42	D/ND	D/ND	2.66
120	542 ± 80	N/ND	ND	N/ND	4.65 ± 0.22	ND	D/ND	1.28

[a] ND compound is not detectable in that particular sample. The limit of detection (baseline = 3σ) at $t=0$ (sample volume = 1.0 ml) in the leaf washings is 1.25 ng g^{-1} and in the remainder of the samples (sample volume = 0.25 ml) is 5.0 ng g^{-1}.

[b] N/ND: value is above the level of determination (baseline + 1.5σ) but below the limit of detection.

[c] At each time three different samples were analyzed. Each number represents the average of at least two determinations.

[d] Leaves were not washed prior to immersion in tin supplemented water ($t=0$).

[e] $[Me]_{TOT}=[MeSn^{3+}]+2[Me_2Sn^{2+}]+3[Me_3Sn^+]$ and includes both the leaves and washings.

[f] Represents concentration prior to the addition of an inorganic tin spike.

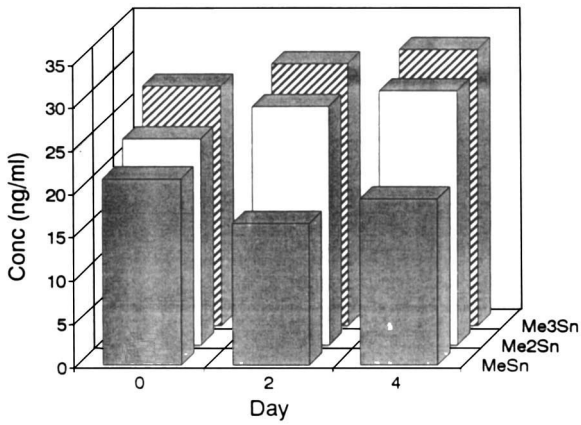

Figure 2 Variation of averaged MeSn concentrations in estuarine water with time after spiking with 75 ng ml^{-1} inorganic tin and 25 ng ml^{-1} $MeSn^{3+}$, Me_2Sn^{2+} and Me_3Sn^{+}.

ever, the difference was not statistically significant. As a result, we eliminated light and air as possible factors and ran all experiments in an aerobic environment with a natural light/dark cycle. An additional experiment confirmed that bubbling of air caused no loss of MeSn from solutions due to volatilization during our experiments. Thus, any loss of MeSn must result from methylation and/or demethylation processes.

The concentration of the inorganic tin spike in this experiment was chosen as 23.5 ng per ml estuarine water (or 1.18 μg Sn per g leaf) for two reasons. Typical tin concentrations in the waters of Great Bay Estuary are in the low nanograms-per-milliliter range,[11] and the estuarine water used in this experiment had an inorganic tin concentration of 2.4 ng ml^{-1}. The detectable concentration in environmental samples typically has about 20% error associated with it. We wanted to be able to detect changes in the spiked concentration at better than 90% accuracy but still to be within realistic environmental concentrations. A higher concentration would allow for more accurate detection. However, that would then place the spiked concentration higher than that possibly found in estuarine water samples, and also decrease the LOD for $MeSn^{3+}$ because of peak overlap.

It is not surprising that the inorganic tin concentration in the estuarine water above decomposing *S. alterniflora* leaves decreases with time. The pH of estuarine water is about 7 and inorganic tin is known to adsorb strongly to container walls at that pH. At the end of Experiment 1 about 30% of added inorganic tin was associated with the plant leaves (Table 2), and *ca* 50% remained in solution (Table 1). This left about 20% of added inorganic tin that adsorbed to walls of the flask or became otherwise undetectable.

The increase in inorganic tin concentration in the leaves was also predictable. Huang *et al.*[14] found that *S. alterniflora* concentrates metals from the surrounding water column. Additionally, Alberts *et al.*[15] found that the elemental concentration of many metals increases in decaying *S. alterniflora* leaves. Algae also bioconcentrate inorganic tin from the surrounding water column. For example, Wright and Weber[7] determined the uptake of inorganic tin onto the marine algae *Fucus vesiculosus* and a mixed community of *Enteromorpha* and found a three-phase biosorption process. The first phase was too fast to

Table 3 Variation in concentrations of inorganic tin and MeSn compounds with time in estuarine water supplemented with inorganic tin (75 ng ml^{-1}) and MeSn compounds (25 ng ml^{-1})

	Concentration (ng Sn ml^{-1} ± 1 standard deviation)				
Time (d)	Sn	MeSn	Me_2Sn	Me_3Sn	$[Me]_{TOT}$[a] (ng ml^{-1})
0	86.4 ± 0.6	23.1 ± 0.8	25.1 ± 0.7	27.3 ± 3.6	155
0	73.5 ± 0.7	22.6 ± 0.2	22.6 ± 1.5	26.6 ± 0.2	148
0	69.2 ± 0.5	18.6 ± 1.6	23.9 ± 0.9	29.3 ± 0.8	154
2	49.4 ± 0.7	17.0 ± 0.6	25.9 ± 2.6	28.8 ± 2.5	155
2	26.1 ± 0.5	15.5 ± 0.4	28.1 ± 0.3	30.8 ± 1.1	164
2	33.1 ± 5.9	16.5 ± 2.6	28.7 ± 0.8	31.4 ± 1.2	168
4	39.3 ± 0.1	22.1 ± 0.2	28.8 ± 1.1	32.0 ± 0.7	175
4	31.0 ± 2.9	19.5 ± 1.0	30.7 ± 0.2	33.3 ± 1.2	181
4	34.0 ± 3.4	15.8 ± 1.2	28.6 ± 2.6	30.7 ± 3.6	165

[a] Represents the sum of $[MeSn^{3+}] + 2[Me_2Sn^{2+}] + 3[Me_3Sn^{+}]$.

determine rate constants. Phase 2 was slower with pseudo-first-order rate constants of 0.86 h^{-1} for *F. vesiculosus* and 0.65 h^{-1} for *Enteromorpha*. Phase 3, which was associated with cellular accumulation, was the slowest phase in the biosorption process with rates of 350 ng g^{-1} h^{-1} for *F. vesiculosus* and 4 ng g^{-1} h^{-1} (not significantly greater than zero; $P=0.05$) for *Enteromorpha*.

Biosorption of inorganic tin by *S. alterniflora* (Table 2) does not continue until all the tin has been adsorbed, but by 120 h the rate of absorption begins to level off. Because inorganic tin remains in the water, the process must be in equilibrium (Eqn [2]).

$$[\mathrm{Sn}]_{\mathrm{water}} \underset{k-1}{\overset{k_1}{\rightleftharpoons}} [\mathrm{Sn}]_{\mathrm{leaves}} \qquad [2]$$

Equation [2] can be treated as opposing pseudo-first-order reactions. The integrated rate equation is analogous to that used for unidirectional reactions, but the derived first-order rate constant, k, is the sum of forward and reverse first-order rate constants (k_1+k_{-1}).[16] The integrated rate equation for first-order or pseudo-first-order kinetics is shown in Eqn [3].

$$\ln([\mathrm{Sn}]_e-[\mathrm{Sn}]_t)=-kt+\ln([\mathrm{Sn}]_e-[\mathrm{Sn}]_0) \qquad [3]$$

The inorganic tin concentration at $t=120$ h was used as the equilibrium concentration ($[\mathrm{Sn}]_e$). We determined the pseudo-first-order rate constant by plotting $\ln([\mathrm{Sn}]_e-[\mathrm{Sn}]_t)$ against time (Fig. 3), where $[\mathrm{Sn}]_t$ is the inorganic tin concentration at time t. Biosorption of inorganic tin appears to be

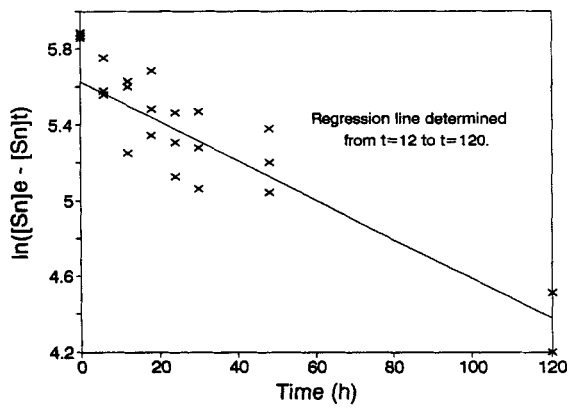

Figure 3 Pseudo-first-order kinetic plot for biosorption of inorganic tin by decaying *S. alterniflora* leaves from surrounding estuarine water. Regression line represents biosorption from 12 h to 120 h.

at least a two-phase process with slow and fast steps. The range of the second phase was determined by deleting the earliest data points and performing linear regressions until the correlation coefficient (R^2) was optimized.

The second phase began at *ca* $t=12$ h and continued until the completion of the experiment. Prior to 6 h there was rapid uptake of inorganic tin which was not measured. The pseudo-first-order rate constant for uptake of tin from estuarine water ($t=12$–120 h) is 0.010 h^{-1}. Thus *F. vesiculosus* (0.86 h^{-1}) and *Enteromorpha* (0.65 h^{-1}) biosorb inorganic tin much faster than *S. alterniflora*. This is probably due to increased surface area on the algae as compared with marsh grass, and to differences in cell wall structure.

In their study of the cycling of methyltin compounds in the presence of *Enteromorpha*, Donard *et al.*[3] found that high concentrations of Me_3Sn^+ were concurrent with low concentrations of $MeSn^{3+}$ and Me_2Sn^{2+}, and vice versa. Initially they found Me_2Sn^{2+}, but no Me_3Sn^+, in the algae samples. However, by day 4 the majority of MeSn were Me_3Sn^+. They suggested a detoxification mechanism which ultimately converts the toxic MeSn to volatile Me_4Sn via redistribution processes (Eqns [4]–[6]).

$$4\,Me_3Sn^+ \rightarrow 2\,Me_2Sn^{2+} + 2\,Me_4Sn \qquad [4]$$

$$2\,Me_2Sn^{2+} \rightarrow MeSn^{3+} + Me_3Sn^+ \qquad [5]$$

$$3\,Me_3Sn^+ \rightarrow MeSn^{3+} + 2\,Me_4Sn \qquad [6]$$

We find the same pattern with the Me_2Sn^{2+} and Me_3Sn^+ in the *S. alterniflora* leaves up to 120 h (Fig. 1). Me_3Sn^+ concentrations increase significantly while Me_2Sn^{2+} concentrations decrease. The increase in Me_3Sn^+ concentration is probably not due to absorption from the surrounding water as there was never any detectable Me_3Sn^+ in the estuarine water (Table 1). This indicates cycling of the MeSn. Our study did not continue beyond day 5, so it remains undetermined whether the pattern found by Donard *et al.*[3] would be repeated in its entirety. There was no overall change in $[\mathrm{Me}]_{\mathrm{TOT}}$ (Table 2) ($F=1.26$, df = 8, $P>F=0.3319$), so no net methylation of inorganic tin occurred during the five-day experiment.

Experiment 2

The $[\mathrm{Me}]_{\mathrm{TOT}}$ (Eqn [1]) is an important consideration for the understanding of Experiment 2. ANOVA of the variation of $[\mathrm{Me}]_{\mathrm{TOT}}$ (Table 3)

with time indicates a significant increase in concentration ($F=8.27$, df$=2$, $P>F=0.0189$). There were also significant changes in concentrations of each MeSn compound from day to day.

The methylation/demethylation of MeSn in estuarine water alone (Fig. 2) was different from that when *S. alterniflora* leaves were present (Fig. 1). In the absence of leaves, concentrations of both Me_2Sn^{2+} and Me_3Sn^+ increased throughout the experiment. In addition, the concentration of $MeSn^{3+}$ was approximately constant because it decreased on day 2 and then returned to the original concentration on day 4. These data are consistent with the following methylation and redistribution reactions (Eqns [7]–[10]).

$$5\,Sn + 5\,Me \rightarrow 5\,MeSn^{3+} \quad [7]$$

$$4\,MeSn^{3+} \rightarrow 2\,Me_2Sn^{2+} + 2\,Sn(IV)^{4+} \quad [8]$$

$$MeSn^{3+} + Me_2Sn^{2+} \rightarrow Me_3Sn^+ + Sn(IV)^{4+} \quad [9]$$

$$5\,Sn + 5\,Me \rightarrow Me_2Sn^{2+} + Me_3Sn^+ + 3\,Sn(IV)^{4+} \quad [10]$$

In Eqn [7] inorganic tin is expressed as Sn without an oxidation state because its oxidation state is generally unknown in environmental media, and the unidentified methyl donor is designated by Me. In contrast, inorganic tin resulting from redistribution reactions is Sn(IV) (Eqns [8]–[10]). Methylation may occur by oxidative addition of a methyl cation to Sn(II), as observed in model systems with for example, methyl iodide as methyl donor[17] (Eqn [11]).

$$Sn(II)^{2+} + MeI \rightarrow MeSn^{3+} + I^- \quad [11]$$

Further methylation to Me_2Sn^{2+} or Me_3Sn^+ via a carbocation donor is unlikely since it would first require a reduction step to unknown methyltin(II). Thus, the above rearrangement reactions are more likely.

CONCLUSIONS

Apparently two separate processes account for changes in MeSn concentrations in *S. alterniflora* leaves surrounded by estuarine water (Experiment 1) and in estuarine water alone (Experiment 2), since there are two distinctly different trends. There is no net methylation in the leaves (Experiment 1) but there is a noticeable rearrangement of MeSn from Me_2Sn^{2+} to Me_3Sn^+. In Experiment 2 (water alone) we see an increase in both Me_2Sn^{2+} and Me_3Sn^+ concentrations accompanied by overal net methylation. The task remains to elucidate the methylators and potential methylation mechanism in the estuarine water. *S. alterniflora* leaves absorb inorganic tin (and probably MeSn) from the surrounding water. Then MeSn are rearranged in the decaying *S. alterniflora* leaves, predominantly forming Me_3Sn^+. Decaying *S. alterniflora* leaves make up a large portion of the organic litter in the estuary each year. This addition to the organic matter at the surface of the sediment may contribute to MeSn found regularly in healthy *S. alterniflora*.

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