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RESEARCH ARTICLE

In situ experiments for element species-specific environmental reactivity of tin and mercury compounds using isotopic tracers and multiple linear regression

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Abstract The fate of mercury (Hg) and tin (Sn) compounds in ecosystems is strongly determined by their alkylation/dealkylation pathways. However, the experimental determination of those transformations is still not straightforward and methodologies need to be refined. The purpose of this work is the development of a comprehensive and adaptable tool for an accurate experimental assessment of specific formation/degradation yields and half-lives of elemental species in different aquatic environments. The methodology combines field incubations of coastal waters and surface sediments with the addition of species-specific isotopically enriched tracers and a mathematical approach based on the deconvolution of isotopic patterns. The method has been applied to the study of the environmental reactivity of Hg and Sn compounds in coastal water and surface sediment samples collected in two different coastal ecosystems of the South French Atlantic Coast (Arcachon Bay and Adour Estuary). Both the level of isotopically enriched species and the spiking solution composition were found to alter dibutyltin and monomethylmercury degradation yields, while no significant changes were measurable for tributyltin and Hg(II). For butyltin species, the presence of light was found to be the main source of degradation and removal of these contaminants from surface coastal environments. In contrast, photomediated processes do not significantly influence either the methylation of mercury or the demethylation of methylmercury. The proposed method constitutes an advancement from the previous element-specific isotopic tracers' approaches, which allows for instance to discriminate the extent of net and oxidative Hg demethylation and to identify which debutylation step is controlling the environmental persistence of butyltin compounds.

Keywords Species-specific stable isotope tracers · Biogeochemical cycling of organometallic pollutants · Coastal field experiments

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Introduction

The environmental impact of an element depends on its chemical form (Ure and Davidson 1995). The importance and relevance of chemical speciation is reflected by the increasing number of regulations in which the total element content in the samples has been replaced by elemental species-specific levels (Proust et al. 2005). Among all the environmentally relevant elements, Sn and Hg have been widely studied due to the presence and persistence of toxic alkyltin and alkylmercury compounds in the aquatic environment.

In the case of Hg, the amount of monomethylmercury (MMHg) results from concomitant inorganic mercury (Hg (II)) methylation and MMHg demethylation. Also, photochemically induced redox reactions between elemental Hg (Hg°) and Hg(II) determine its long range transport through

the atmospheric pathway (Lin and Pehkonen 1999). Hg methylation is typically a biotic process mediated by sulfur-reducing bacteria (Compeau and Bartha 1985). The formation of MMHg in sediments is of special importance because it is a highly toxic species, readily bioaccumulated and biomagnified in the trophic network (Fitzgerald et al. 2007), and also the main species involved in human Hg exposure via fish consumption (Dabeka et al. 2004).

In the case of Sn, organotin compounds have been extensively used as pesticides, as toxic additives in antifouling paints, and during the processing of plastic materials. The use of trisubstituted organotin species such as tributyltin (TBT) in antifouling paints is being restricted worldwide; however, municipal solid wastes are still important pools of organotin compounds (Pinel-Raffaitin et al. 2008). TBT can be rapidly degraded in the water column by abiotic and/or biotic dealkylation reactions to form less toxic species such as monobutyltin (MBT) or dibutyltin (DBT) but is more persistent in sediments (Dowson et al. 1996).

New environmental regulations such as EU Registration, Evaluation and Authorization of Chemicals require an improved assessment of the contaminants impact on ecosystems (Lahl and Hawxwell 2006). Thus, besides the concentration of contaminants, it is also important to study their thermal and chemical stabilities, biodegradability, and transformation pathways that alter their toxicity. Field or in-lab incubations with real environmental samples are useful tools to study compound-specific environmental reactivity. However, the extractable information using these strategies is very limited particularly when complex reaction pathways and parallel formation and degradation processes need to be separated for different species of the same element. Experiments using radiotracers were developed in the past to study the biogeochemical cycle of some elements (McCubbin and Leonard 1995). Besides the radioactivity, the main drawback of the use of radiotracers is the concentration level required to specifically follow those reactions which are usually far from real environmental conditions (Ramlal et al. 1986). The continuous development of mass spectrometric techniques has boosted the use of stable isotopes as a better alternative to radiotracers. Stable isotopes can be added in closed systems at natural background concentrations without perturbing the system under study (Hintelmann et al. 2000). Also, if the element under study presents several isotopes, multi-isotopic labeling experiments open the possibility of following species-specific reaction routes (Björn et al. 2007). Usually, stable isotopes are added to the sample in aqueous (Lambertsson et al. 2001; Drott et al. 2008) or acidified (Eckley and Hintelmann 2006; Whalin and Mason 2006) solutions or after an equilibration process with organic ligands or pore water (Hintelmann et al. 2000; Hammerschmidt and Fitzgerald 2004; Heyes et al. 2004). The concentration level employed is normally similar (Drott et al. 2008) or lower (Hammerschmidt and Fitzgerald 2004) than the natural background concentration of the elements under study but higher concentrations have been also reported (Eckley and Hintelmann 2006; Lehnherr et al. 2011).

The validity of field incubations of samples spiked with several isotopically enriched species always relies on the assumption that the chemical behavior of the added enriched tracers during the incubation process is the same as that of the endogenous species. Therefore, all the obtained data are estimations of the chemical behavior of the ambient compounds. Two different stages can be considered in this type of experiments: (1) the addition of the environmental tracers to follow specific reaction pathways and (2) the addition of analytical tracers to correct for incomplete recoveries and undesired degradation reactions during the analysis. Such approaches require complementary mathematical approaches for the calculation of the initial and final endogenous and exogenous concentrations (Rodríguez-González et al. 2007). However, it has been recently demonstrated that previous mathematical procedures employed in the second stage could lead to erroneous results when opposite and simultaneous reactions are occurring in a significant extent (Meija et al. 2009). Thus, previous data on kinetic constants and degradation rates or half-lives might be significantly biased (Hintelmann and Evans 1997; Qvarnström and Frech 2002; Rodríguez-González et al. 2004). In contrast, the deconvolution of isotopic patterns by multiple linear regression has been found to provide accurate results because it obeys the conservation of the amount law. An alternative multi-spiking strategy to study mercury species transformations during field incubation experiments was proposed by Lambertsson and coworkers (2001; Drott et al. 2008; Björn et al. 2007). However, this procedure requires that the isotopic enrichment of both environmental and analytical tracers must be higher than 99 % in order not to significantly contribute to the other isotopic sources of the system.

We propose here a comprehensive and accurate approach based on the deconvolution of isotopic patterns by multiple linear regression applied for the first time to the study of the environmental reactivity of different species of the same element. As a proof of concept, the combination of stable isotope tracers, isotope pattern deconvolution, and field incubations has been applied to study the species-specific environmental reactivity of Sn and Hg in coastal sediment and waters from the Arcachon Bay and the Adour River estuary (SW French Atlantic Coast). We demonstrate in this work the potential of such methodology and its application to complex natural media and reaction scheme. Also this work presents, to the best of our knowledge, the first data

on the environmental reactivity of alkyltin compounds obtained using enriched stable isotopes.

Experimental

Field incubation experiments

Seawater incubations

Surface water samples were collected from two different sampling sites in the south of France: the Adour Estuary and the Arcachon Bay. The surface waters from the Adour Estuary were collected in the estuarine plume whereas those from the Arcachon Bay were collected from a subtidal station. Coastal surface waters were collected at 0.5 m depth using 5 L Teflon-coated GoFlo sampler (General Oceanic, Miami). The samples were transferred directly to previously acid-cleaned PFA bottles (Nalgene, USA) and submitted to incubation experiments. A more detail description and maps (Figs. S1 and S2) of the sampling sites are included in the Electronic supplementary information (ESI).

Incubation experiments were performed within 2 h after sampling. The experimental design for water incubations has been described in a previous work (Monperrus et al. 2007) and is detailed in Fig. S3 of the ESI. Briefly, known amounts of the isotopically enriched tracers were added to the bulk samples in order to obtain concentrations similar than those of the ambient species. For Hg experiments, the added enriched species were ^{199}Hg-enriched Hg^{2+} and ^{201}Hg-enriched $MMHg^{+}$ solutions whereas for Sn experiments ^{116}Sn-enriched Sn^{4+}, ^{118}Sn-enriched DBT, and ^{117}Sn-enriched TBT were added. ^{201}HgO was obtained from Oak Ridge National Laboratory (TN, USA). The ^{201}MMHg synthesis is described in a previous work (Rodriguez Martin-Doimeadios et al. 2002). $^{199}HgCl_2$ was prepared by dissolving ^{199}HgO (Oak Ridge National Laboratory) in HCl. A spike solution of ^{117}Sn-enriched TBT was supplied by the Laboratory of the Government Chemist (Teddington, UK) during the course of an intercomparison exercise, and the synthesis of the ^{118}Sn-enriched DBT employed in this work has been described in a previous publication (Ruiz Encinar et al. 2000). A mixture of MBT, DBT, and TBT enriched in ^{119}Sn was purchased to ISC-Science (Oviedo, Spain). The resulting concentrations of the isotopic tracers in the water samples were 0.2 ng L^{-1} for MMHg and 2 ng L^{-1} for tin species and Hg^{2+}. As reported in Tables S4 and S6, the concentration of enriched isotope in comparison with the endogenous compounds in the water samples was always lower than 14 times for $MMHg^{+}$ and TBT and lower than 10 times for Hg^{2+} and DBT. All incubations were performed in triplicate and incubated directly in the field or in temperature-controlled seawater baths performed on board by continuous pumping of surface seawater. Thus, the uncertainties reported in this work represent in all cases the standard deviation from three independent incubations. The samples were incubated during a complete diurnal cycle (24 h, dawn to dawn), either exposed to sunlight or in the dark. The dark incubations were employed as a control for the investigation of photochemically induced transformation processes. To discriminate the abiotic processes from those mediated by plankton biomass, most of the suspended matter/plankton were removed by filtration through a 0.45-μm PVDF membrane (Durapore, Millipore). Then, the filtered water samples were spiked with the same amount of enriched stable isotope species. All incubations were stopped by adding high purity HCl (1 %*v*/*v*) and stored at +4 °C in the dark until analysis. The analysis of dissolved species in the water samples was carried out within less than 3 weeks after field sampling and incubation as explained in the ESI. The analysis for dissolved gaseous mercury was performed directly on site at the end of the incubation period as explained in the ESI. Two independent sets of incubations (one for Sn and one for Hg) were always performed to avoid any influence between Hg and Sn compounds.

Superficial sediment incubations

Surface (0–3 cm) sediments were collected from the intertidal mud flat of the Arcachon Bay on a seasonal basis during three different campaigns from 2006 to 2008 (May 2006, October 2007, and January 2008). Approximately 5 kg of sediment sample was collected with the help of an acid-cleaned plastic spatula and transported in an acid-cleaned plastic container. Also, a 5-L volume of the interstitial percolating water at low tide of the sampling station was collected from the adjacent small channels surrounding the sampling point. The slurries were prepared in the field by mixing 5 g of sediment with 5 mL of percolating water in an acid-precleaned 30 mL headspace glass vial. Then, the isotopically enriched tracers ^{199}Hg-enriched Hg^{2+} and ^{201}Hg-enriched $MMHg^{+}$ or ^{116}Sn-enriched Sn^{4+}, ^{118}Sn-enriched DBT, and ^{117}Sn-enriched TBT were added to the slurry and the vials were sealed with a silicone/PTFE septum using an aluminum crimp cap. All incubations were performed in triplicate and incubated directly in the field or in temperature-controlled seawater. Thus, the uncertainties reported in this work represent in all cases the standard deviation from three independent incubations. The concentrations of the isotopically enriched tracers in the incubated

samples were 2 ng g^{-1} for Sn compounds, 40 ng g^{-1} for Hg(II), and 0.2 ng g^{-1} for MMHg. As reported in Tables S3 and S5, the concentration of enriched isotope in comparison with the endogenous compounds in the sediment samples was always lower than 2 times for $MMHg^{+}$ and Hg^{2+} and between 2–10 times for TBT and DBT. The samples were manually shaken for 5 min to facilitate homogenization of the slurry and the added isotopically enriched tracers. At this point, a set of three independent control incubations was stopped by freezing the samples at −18 °C. The rest of the samples were divided into two groups and incubated in the field using temperature-controlled water baths. One group of samples was exposed to a diurnal cycle of light and the rest were kept in the same conditions but in the dark during 24 h. All incubations were stopped by freezing the samples at −18 °C and stored in these conditions until lyophilization in the laboratory. Two independent sets of incubations (one for Sn and one for Hg) were always performed to avoid any influence between Hg and Sn compounds.

Analytical procedures

The measurement of the isotopic composition of the Sn and Hg compounds in the samples was carried out by gas chromatography combined with inductively coupled plasma mass spectrometry (GC-ICP-MS). Also, the determination of the elemental species concentration was carried out by species-specific isotope dilution mass spectrometry. More information on the applied analytical procedures can be found in the ESI. All sample preparation procedures applied in this work have been developed and validated previously for water (Amouroux et al. 1998, Rodríguez-González et al. 2002, Monperrus et al. 2005; Bouchet et al. 2011a) and sediments (Rodríguez-González et al. 2007; Rodríguez Martín-Doimeadiós et al. 2003).

Mathematical approach

A sample (such as seawater or sediment slurry) is contained in a closed system in which a certain number of species-specific isotopically enriched tracers have been previously added and homogenized with the matrix. Thus, the total amount of a given elemental species in the system is the sum of all its analogues with a different isotopic composition. Then, we can define the concept of molar fraction as the relative amount of an analogue containing a specific isotope pattern from the total amount of such species in the sample. For example, if a species a is present in the sample as a mixture of the species with natural isotope abundances and a number n of isotopic tracers with different isotopic composition, we can define the following molar fractions as follows:

$$ {}^{a}x_{\mathrm{nat}} = \frac{{}^{a}N_{\mathrm{nat}}}{{}^{a}N_{\mathrm{nat}} + {}^{a}N_{t,1} + {}^{a}N_{t,2} + \cdots + {}^{a}N_{t,n}} = \frac{{}^{a}N_{\mathrm{nat}}}{{}^{a}N_{T}} \quad (1) $$

$$ {}^{a}x_{t,1} = \frac{{}^{a}N_{t,1}}{{}^{a}N_{\mathrm{nat}} + {}^{a}N_{t,1} + {}^{a}N_{t,2} + \cdots + {}^{a}N_{t,n}} = \frac{{}^{a}N_{t,1}}{{}^{a}N_{T}} \quad (2) $$

$$ {}^{a}x_{t,2} = \frac{{}^{a}N_{t,2}}{{}^{a}N_{\mathrm{nat}} + {}^{a}N_{t,1} + {}^{a}N_{t,2} + \cdots + {}^{a}N_{t,n}} = \frac{{}^{a}N_{t,2}}{{}^{a}N_{T}} \quad (3) $$

$$ {}^{a}x_{t,n} = \frac{{}^{a}N_{t,n}}{{}^{a}N_{\mathrm{nat}} + {}^{a}N_{t,1} + {}^{a}N_{t,2} + \cdots + {}^{a}N_{t,n}} = \frac{{}^{a}N_{t,n}}{{}^{a}N_{T}} \quad (4) $$

The subscript nat refers to the species of natural isotopic composition and the subscript t refers to the different isotopically enriched added tracers whereas x refers to the molar fractions and N to the number of moles.

Once the environmental processes under study have taken place, the calculation of the species-specific isotope abundances of all the species in the sample involved is carried out by using a chromatographic technique coupled to a mass spectrometer. In this way we will obtain a chromatographic peak not only for each species but also for each of the isotopes monitored. The isotope abundances of the species can be easily calculated from the species-specific isotope intensities. It can be assumed that the abundances in the sample are linear combinations of the individual isotopic sources present in the system. This can be consider as a case of multivariate linear regression in which our observations (species-specific isotope intensities) depend on a set of $n+1$ independent variables (the isotope abundances of the different isotopic sources present in the system). If we assume that the response is first order in all the independent variables, we can write the general linear function for the isotope *1* of the species a as follows:

$$ {}^{a}A^{1}_{\mathrm{sample}} = {}^{a}A^{1}_{\mathrm{nat}} \cdot {}^{a}x_{\mathrm{nat}} + {}^{a}A^{1}_{t,1}\,{}^{a}x_{t,1} + {}^{a}A^{1}_{t,2}\,{}^{a}x_{t,2} + \ldots + {}^{a}A^{1}_{t,i}\,{}^{a}x_{t,i} + \ldots + {}^{a}A^{1}_{t,n}\,{}^{a}x_{t,n} \quad (5) $$

Considering all the available isotopes of the element, this set of equations can be rewritten in shorthand matrix notation as: $y = A \cdot x + e$, where y is the measurement vector (isotope abundances of the sample), e is the error vector, x

the parameter vector, and A the independent variable matrix. Or in detail matrix notation as:

$$\begin{pmatrix} {}^{a}A^{1}_{\text{sample}} \\ {}^{a}A^{2}_{\text{sample}} \\ {}^{a}A^{3}_{\text{sample}} \\ \vdots \\ {}^{a}A^{m}_{\text{sample}} \end{pmatrix} = \begin{pmatrix} {}^{a}A^{1}_{\text{nat}} & {}^{a}A^{1}_{t,1} & {}^{a}A^{1}_{t,2} & \cdots & {}^{a}A^{1}_{t,i} & \cdots & {}^{a}A^{1}_{t,n} \\ {}^{a}A^{2}_{\text{nat}} & {}^{a}A^{2}_{t,1} & {}^{a}A^{2}_{t,2} & \cdots & {}^{a}A^{2}_{t,i} & \cdots & {}^{a}A^{2}_{t,n} \\ {}^{a}A^{3}_{\text{nat}} & {}^{a}A^{3}_{t,1} & {}^{a}A^{3}_{t,2} & \cdots & {}^{a}A^{3}_{t,i} & \cdots & {}^{a}A^{3}_{t,n} \\ \vdots & \vdots & \vdots & \cdots & \vdots & \vdots & \vdots \\ {}^{a}A^{m}_{\text{nat}} & {}^{a}A^{m}_{t,1} & {}^{a}A^{m}_{t,2} & \cdots & {}^{a}A^{m}_{t,i} & \cdots & {}^{a}A^{m}_{t,n} \end{pmatrix} \cdot \begin{pmatrix} {}^{a}x_{\text{nat}} \\ {}^{a}x_{t,1} \\ {}^{a}x_{t,2} \\ \vdots \\ {}^{a}x_{t,i} \\ \vdots \\ {}^{a}x_{t,n} \end{pmatrix} + \begin{pmatrix} {}^{a}e^{1} \\ {}^{a}e^{2} \\ {}^{a}e^{3} \\ \vdots \\ {}^{a}e^{m} \end{pmatrix} \tag{6}$$

The estimates of the unknown parameters can be determined with the m observations which are the measured isotopes of the element under study. Providing that the system comprises more measured isotopes than isotopic sources ($m > n+1$), the best values of the molar fractions can be calculated by least squares minimization of the error vector "e" given by: $x = (A' \cdot A)^{-1} \cdot A' \cdot y$, being A' the transpose of A and -1 the inverse matrix. Then, we can calculate the concentrations of the different analogues from their molar fractions and the total number of moles of the sample ${}^{a}N_T$.

At this point, two different ways of calculating the total number of moles ${}^{a}N_T$ can be proposed. If an additional isotopically enriched tracer is available (e.g., tracer n), a known amount ${}^{a}N_{t,n}$ can be initially added before analysis and introduced as an additional isotopic source into the matrix equation. Then, the total number of moles can be directly calculated from the number of moles added (known) and the molar fraction of the analytical spike n ${}^{a}x_{t,n}$ and ${}^{a}N_{t,n}$, respectively using Eq. (7):

$$N_{t,n} = {}^{a}x_{t,n} \cdot {}^{a}N_T \tag{7}$$

However, if the availability of enriched isotope tracers is limited, the total number of moles in the sample must be calculated by conventional isotope dilution analysis using natural abundance standards as analytical spikes (providing that the natural abundance species in the sample have been isotopically modified by the addition of the isotopic tracers). Although less expensive, this mode is more time-consuming as it requires the additional analysis of the re-spiked sample with natural abundance standards. Once ${}^{a}N_T$ is calculated, the individual concentrations of ambient and isotopically added species can be deconvoluted after the environmental process has taken place by rearranging Eqs. (1) to (4).

Calculation of alkylation/dealkylation yields and half-lives of the elemental species

Species-specific alkylation and dealkylation yields and half-lives were calculated from the values obtained from single incubation experiments. The purpose for this study was the evaluation of the method to provide reliable, precise, and quick estimations of these parameters in coastal environments under different environmental conditions. Those parameters were calculated always at a fix time (typically 24 h or 1 week) but performing several independent replicates in the field aimed at providing reliable information with a satisfactory experimental uncertainty. In all cases, the data presented in this work correspond to the average half-lives from three independent incubation experiments.

The alkylation/dealkylation yields of the considered elemental species (i.e., MMHg, Hg(II), TBT, DBT, and DBT) were calculated in two different ways: (1) measuring the increase of the newly formed species concentrations (formation yields) and (2) measuring the decrease of the initial added species concentrations (degradation yields). In all cases, the reported yields presented in this work correspond to the average yield from three independent incubation experiments.

If we consider the simplest case of two endogenous natural abundance species of the same element (a_{nat} and b_{nat}) contained in a system in which two analogues enriched in a different isotope are added (a_{t1} and b_{t2}), The final concentration of the enriched analogues will provide the potential formation and degradation yields after the process under study has taken place. The potential formation of a from b (F_a (in percent)) was calculated from the amount of the newly formed a_{t2} found after the incubation whereas the potential formation of b from a (F_b (in percent)) was calculated from the amount of b_{t1} found after the incubation. Such calculations are given in Eqs. (8) and (9):

$$F_a(\%) = \frac{a_{t2}}{b_{t2} + a_{t2}} \times 100 \tag{8}$$

$$F_b(\%) = \frac{b_{t1}}{a_{t1} + b_{t1}} \times 100 \tag{9}$$

In a similar way, degradation yields (D_a (in percent) and D_b (in percent)) can be calculated from Eqs. (10) and (11)

$$D_a(\%) = 100 - \frac{a_{t1}}{(a_{t1})_0} \times 100 \tag{10}$$

$$D_b(\%) = 100 - \frac{b_{t2}}{(b_{t2})_0} \times 100 \tag{11}$$

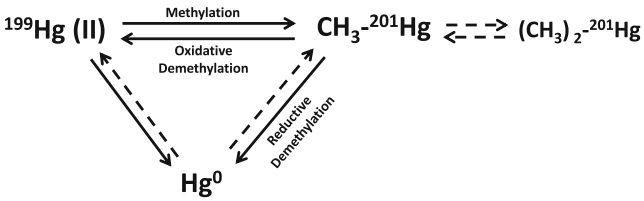
where the subscripted 0 corresponds to the amount of species determined for a non-incubated sample. Finally the half-life of the contaminants was evaluated from the yields using Eq. (12), where K is the resulting constant calculated from the yield and the incubation time.

$$t_{1/2} = \frac{\ln 2}{K} \tag{12}$$

For more complicated cases involving more than two species, the same strategy was adopted. In this way, it is possible to obtain species-specific potential formation or degradation yields and half-lives and to follow individually any degradation route.

Mercury species transformation model

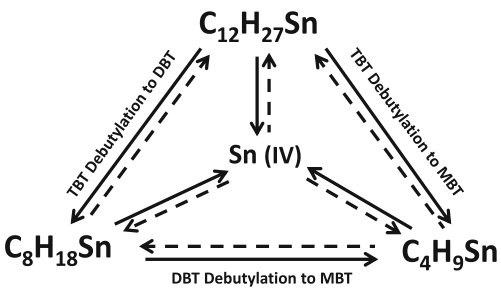
Equation (13) shows the reactivity model of Hg compounds. The solid arrows correspond to the reaction pathways that can be calculated with the methodology employed in this work and the dotted arrows show the pathways that cannot be quantified due to the absence of compound-specific isotopically enriched tracers. In water samples, gaseous mercury was also measured thus, using two isotopic tracers, the method allows not only the determination of the global degradation yield of MMHg but also the simultaneous determination of the reductive demethylation and the oxidative demethylation.



(13)

Butyltin species transformation model

Equation (14) shows the reactivity model of Sn compounds. Unidirectional degradation or formation models are assumed so that yields can be calculated from the individual concentrations of the isotopically enriched tracers.



(14)

Advantages of this methodology in comparison with previous works

Mathematical approach

The approach proposed by Lambertsson and coworkers (2001; Drott et al. 2008; Lambertsson et al. 2007) is based on the use of four different enriched Hg isotopes. The endogenous MMHg concentration is calculated from a ^{200}Hg-enriched MMHg spike added as analytical standard after the incubation period. The methylation of the previously added ^{201}Hg(II) environmental tracer and the demethylation of the previously added ^{198}Hg-enriched MMHg tracer are both calculated from the endogenous concentration of MMHg. This procedure requires that the isotopic enrichment of both environmental and analytical tracers must be higher than 99 % in order not to significantly contribute to the other isotopic sources of the system. In addition, this methodology, not allowing to determine produced and remaining Hg(II) from both isotopic tracers, quantifies the global demethylation of MMHg and cannot discriminate between the oxidative or reductive demethylation.

The mathematical approach proposed here uses the whole isotope abundances of every tracer to calculate the resulting concentrations after the incubation and sample preparation. Thus, it is not required to use tracers with a high isotopic enrichment and less expensive enriched isotopes can be employed. An important specific advantage of this method is the possible correction of isobaric spectral interferences due to the presence of co-eluting compounds (such as the interference of organolead species at m/z 204 during mercury measurements). To do so, the isotopes of the interfering element must be introduced in the matrix to calculate its corresponding molar fraction (Rodríguez-Castrillón et al. 2009). Also, as we have more isotopes than isotopic sources, we can calculate the standard uncertainty of the molar fractions using the variance covariance matrix. In this way, the correction of the mass bias is possible without the additional measurement of reference isotope ratios reducing both measurement time and data treatment (Rodríguez-González et al. 2007). The method proposed here can also be extended to other polyisotopic elements providing that the element presents a higher number of isotopes than species and that enriched species can be synthesized or are commercially available.

Quantification of both remaining and formed species

In cases in which the number of isotopes available for the element under study is limited (e.g., less than four isotopes) or when there is a lack of commercial or in-house-synthesized enriched isotope tracers, the proposed procedure offers a

specific advantage for quantification in comparison with previously published approaches. A less expensive but more time-consuming strategy can be adopted as explained in the "Experimental" section. The first analysis is employed for the calculation of the modified isotope abundances in the sample and the second analysis for the quantification of the species using natural abundances standards. The only requirement of this double approach is that both analyses must be performed under previously optimized conditions in the absence of any analytical degradation route.

Another important advantage of the proposed methodology is the simultaneous determination of formation and degradation yields by the quantification of all the formed and remaining species derived from each isotope. It is thus possible to assess yields for coupled and simultaneous processes. For example, in the case of Hg species, it is possible to quantify the oxidative demethylation (degradation of MMHg) and global demethylation obtaining the reductive demethylation by the substraction of both values.

Determination of the source of newly formed species

The last important advantage of the proposed methodology in comparison with previous approaches is the possibility of obtaining qualitative information of any formed species of the element under study. For unknown compounds, the calculation of the molar fractions in the chromatographic indicates their origin. In addition, performing the double analysis of the sample, the concentration of newly formed species can be obtained provided that the identity of the species is known and that the natural abundance standard is available. This could be applied here to the formation of methylated tin species or dimethyl mercury. However such species were not observed in any of the samples analyzed in this work.

Results and discussion

Preliminary considerations

As reported in previous studies (Bouchet et al. 2011b), the presence of dimethylmercury in the sediment samples from the same station in the Arcachon Bay was found to be negligible. In addition, the presence of dimethylmercury in the water samples from the Arcachon Bay and Adour Estuary was also studied. However, in all cases, the concentration was below the detection limit of 2 fmol L^{-1} of the cryotrapping GC-ICP-MS method (Amouroux et al. 1998). Two representative GC-ICP-MS chromatograms of the water samples of the Arcachon Bay and Adour Estuary are given in Figs. S5 and S6 of the ESI, respectively. As can be observed in both figures, dimethylmercury was below the detection limit of the method (the retention time of dimethylmercury was 130 s).

The standard deviation of the results presented in this work comes mostly from the environmental variability rather than that originated from the mathematical and analytical methodologies. In fact, all samples were injected in the GC-ICP-MS system in triplicate and the results obtained were always lower than a 3 % relative standard deviation (RSD) (analytical uncertainty). As reported by Meija et al. (2009), multiple spiking approaches increase the uncertainty of the analytical results (typically 3 % RSD) in comparison with single spiking (typically 1–2 %) but such increase does not justify the standard deviation of our results.

Influence of the tracer solution medium and concentration in sediment incubations

First, we studied the influence of the media in which the isotopically enriched tracers are added to sediments collected from the intertidal station of the Arcachon Bay. To do so, the tracers were added at the beginning of the incubation either diluted in an acidic media (1 % HCl for Hg and 1 % acetic acid for Sn) or diluted in the sediment pore water to reach similar concentrations than ambient compounds. Also, the concentration level of the isotopically enriched tracers was evaluated by performing incubations at a 10-fold higher concentration dissolving the enriched tracers in acidic media. The potential yields and half-lives obtained from those experiments are listed in Table 1. The full data set of those experiments, including the concentration of the endogenous and exogenous species, is given in Tables S1 and S2 of the ESI.

Hg species transformations

Methylation of Hg(II) was not observed at the beginning of the incubations (t=0) in any of the different conditions tested. However, when the Hg isotope tracers were diluted and added in 1 % HCl, we observed an important oxidative demethylation (71.6±15.2 %) of the isotopic tracer just after the addition to the sample (t=0). Such instantaneous degradation was decreased to 48.8±14.3 % when increasing 10 times the tracer concentration and it was significantly lower when the tracer is added to the sample diluted in the sediment pore water (12.2±3.1 %). The accurate detection of Hg species transformations is limited by these initial transformations. Thus, a proper choice of the MMHg spiking solution medium is critical. Although it is possible to stabilize MMHg with chemicals, such as methanol or acetic acid, they probably modify the sample biogeochemistry and especially the microbial activities by acting as fresh and labile substrates. The preparation of the tracer solutions in sediment pore waters might be a suitable strategy considering the initial degradation of MMHg (which is probably

Table 1 Methylation, demethylation and debutylation yields and half-lives of Hg and Sn compounds in sediments under different spiking conditions

	Tracer in 1 % HCl (Hg) or acetic acid (Sn)			Tracer diluted in pore water			10 fold concentrated Tracer in 1 % HCl (Hg) or acetic acid (Sn)		
	t=0	*t*=1 day	*t*=7 days	*t*=0	*t*=1 day	*t*=7 days	*t*=0	*t*=1 day	*t*=7 days
Net methylation yield (%)	0.1±0.0	0.9±0.5	0.5±0.1	0.1±0.0	1.4±0.4	0.9±0.2	0.1±0.0	1.4±0.6	2.8±0.6
Net demethylation yield (%)	–	81.4±6.5	97.3±0.7	–	85.0±4.3	96.4±0.1	–	55.1±19.1	83.8±7.3
Oxidative demethylation (%)	71.6±15.2	93.8±1.1	99.3±0.3	12.2±3.1	92.5±2.3	98.9±0.1	48.8±14.3	74.3±7.7	90.7±4.1
Half-lives (days)									
Hg (II)	–	99±62	1058±183	–	55±18	682±231	–	57±25	194±53
MMHg	–	0.4±0.1	1.3±0.1	–	0.4±0.1	1.5±0.0	–	1.0±0.6	2.7±0.7
Debutylation yields (%)									
TBT to DBT	1.1±0.3	0.7±0.3	2.6±0.5	0.9±0.2	1.0±0.6	4.7±1.8	1.0±0.2	0.6±0.1	3.1±0.1
TBT to MBT	0.1±0.8	1.0±0.1	0.7±0.4	1.2±0.1	1.0±0.3	1.5±1.2	1.0±0.4	0.8±0.3	1.1±0.6
TBT to Sn (IV)							2.9±2.7	3.1±0.5	1.7±1.5
DBT to MBT	1.8±1.0	1.7±0.2	4.0±0.4	5.8±0.6	6.2±0.1	8.1±0.3	6.4±1.3	5.5±0.8	6.8±0.5
DBT to Sn (IV)							2.3±2.4	0.2±0.4	1.3±3.9
Half-lives (days)									
TBT to DBT		111±47	195±46		87±53	113±40		126±29	155±3
TBT to MBT		73±5	912±423		78±29	251±105		97±46	576±409
TBT to Sn (IV)								23±4	203±63
DBT to MBT		42±4	121±11		11±0	60±2		13±2	72±5
DBT to Sn (IV)								99	85

The uncertainty values correspond to 1 s standard deviation of three independent incubation experiments

efficiently stabilized by dissolved organic matter) and the pre-equilibration of Hg species with natural ligands (Hintelmann et al. 2000; Rodríguez Martín-Doimeadiós et al. 2003).

The methylation yield observed after 24 h under all conditions ranged from 0.9 to 1.4 %. Only after 1 week that we could observe differences in the methylation yields between the different conditions employed. At a 10-fold higher concentration, the methylation yield was 2.8±0.6 % but when the incubations were prepared in 1 % HCl or diluted in pore waters the yield decreased to 0.5±0.1 and 0.9±0.2 %, respectively. This demonstrates that both the level of concentration and the duration of the experiment are critical parameters in the evaluation of methylation yields. The methylation yields obtained after 1 week at high concentrations cannot be extrapolated to natural MMHg because the bioavailability of added IHg is higher than that of ambient IHg. The methylation yields obtained at lower concentrations are more representative but decreased between 1 and 7 days, which indicated that a back reaction of demethylation occurred. It therefore hinders the calculation of a true gross methylation rate.

Net demethylation and oxidative demethylation values under all conditions are not statistically different indicating that reductive demethylation is mostly negligible. After 24 h, demethylation yields are close to 90 %, and after 1 week, the demethylation is almost 100 % for the low level incubation regardless of the medium in which the tracer is diluted. This values decrease when incubating at a 10-fold higher concentration level reaching values close to 90 % after 1 week. When incubated at low concentration, there is a strong probability to bias the assessment of the demethylation rate. At high concentrations, this problem does not appear and the time evolution is consistent with demethylation being often described as a first-order reaction (Hammerschmidt and Fitzgerald 2004). According to these data the half-lives obtained for MMHg in sediments ranges from 0.4 (24 h in acidic medium) to 2.7 days (1 week).

Dealkylation of butyltin compounds

Butylation of butyltin compounds has never been reported under environmental conditions. Indeed, such processes require conditions of high temperature and pressure which cannot be found in the environment. Thus, butyltin reactivity was assumed as a unidirectional model. For TBT degradation, we have not found any significant difference at the beginning of the experiments when the tracer solutions were added diluted in

acidic media, in pore water, or at a 10-fold higher concentration level. However, this was not the case for the DBT debutylation to MBT as reflected in the approximately 6 % degradation observed when diluting the tracer solution in pore water or when increasing its concentration. The most significant degradation values both for TBT and DBT were obtained when diluting the tracer solution in the pore water reaching values of 4.7 ±1.8 and 8.1±0.3 %%, respectively, after 1 week. In order to have a better estimation of debutylation yields, pore waters could be employed for the preparation of the TBT tracer solutions, but limitations may occur due to the DBT liability. According to these data, different values of TBT half-life can be obtained depending on the specific dealkylation route being 576 days, the highest value obtained for the debutylation of TBT to MBT (obtained after 1 week of incubation at a 10-fold higher concentration level). DBT half-lives were, as expected, much lower in the range of 11 to 72 days.

Incubation time

According to these results, the selection of the duration of the incubation experiment is not a simple task and should be established considering the ability of the method to detect transformations. In our case, as demethylation occurred to a rather large extent, 24 h incubations appear as the best compromise to fulfill the requirements of Hg transformation studies. Given unidirectional and low degradation extents of butyltins in sediments, longer incubation duration could be more suitable. As such, it might not be appropriated to systematically perform simultaneous Hg and Sn incubations.

Application of the method to a preliminary assessment of Hg and Sn reactivity in coastal sediments and waters

As a proof of concept, the proposed methodology involving species-specific stable isotope tracers, multiple linear regression, and field incubations was applied to perform a study of the species-specific environmental reactivity of Sn and Hg in coastal sediment and waters from the Arcachon Bay and the Adour River estuary (SW French Atlantic Coast).

Seasonal variations in intertidal sediments from the Arcachon Bay

The seasonal variability of the reactivity of Sn and Hg in sediments was studied by performing diurnal cycle incubations and incubations in the dark during 24 h at the intertidal station of the Arcachon Bay during three different campaigns: May 2006 or September 2007, October 2007, and January 2008. The results obtained for the potential transformation yields of Sn and Hg species are presented in Fig. 1.

Figure 1 shows that Hg methylation is not significantly influenced by the presence of light and exhibits rather low potential methylation yields (between 0.12±0.07 and 0.69± 0.15 %) but consistent with previous coastal investigations (Hammerschmidt and Fitzgerald 2004; Lambertsson and Nilsson 2006; Bouchet et al. 2011b). The highest potential methylation yield was found for late summer or fall. No significant differences were found for the potential demethylation yields between dark and light conditions. The highest demethylation was observed for both conditions in October 2007 (28.9±7.3 and 34.1±7.1 % for light and dark conditions, respectively), while the lowest occurred in

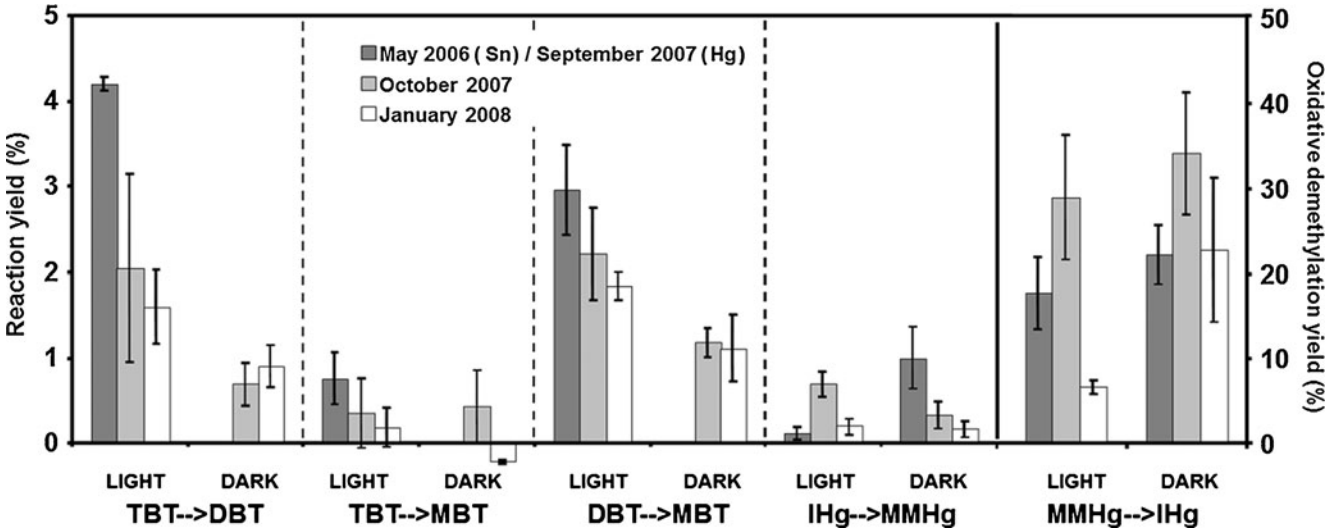


Fig. 1 Seasonal variability on the formation and degradation yields (in percent) of butyltins and Hg species in sediments of the Arcachon Bay. The uncertainty values correspond to 1 s standard deviation of three independent incubation experiments

January 2008 (6.7±0.8 %) under light conditions. According to these results, demethylation process in the sediment is not directly influenced by light but it is by the seasonal and/or sample heterogeneity variability.

Concerning butyltin compounds, Fig. 1 exhibits that sunlight radiations influence the degradation pattern of the butyltin species. Thus, photochemical- or photobiological-mediated reactions seem to be the main degradation pathway in sediment samples. This is also in agreement with the fact that higher debutylation is systematically observed in spring than October or January in the same sampling station of the Arcachon Bay. In this particular case, the main degradation route was found to be that of TBT to DBT under light conditions (4.2±0.1 %) followed by the degradation of DBT to MBT (2.9±0.5 %) in May 2006. The lowest degradation yields were obtained in winter under dark conditions, confirming the significant influence of seasonal light and temperature variations.

Comparison of two coastal water types

The study of the Hg and Sn reactivity in two different coastal seawater types is summarized in Fig. 2 and Tables S4 and S6. It was carried out with surface water collected in the coastal plume of the Adour River estuary, and in the inner lagoon of the Arcachon Bay, significantly influenced by the tidal cycles.

After 24 h incubation, we did not observed any significant methylation of inorganic Hg both for light and dark conditions (methylation extent was lower than the detection limit of newly formed $Me^{199}Hg$) indicating that Hg(II) may also exhibit negligible methylation extent in surface coastal waters (Monperrus et al. 2007). However, for both water types, a higher demethylation was significant and generally observed under light and dark conditions. We also observed a higher oxidative demethylation yield in the Adour Estuary than in the subtidal station of the Arcachon Bay under diurnal conditions (26.9±7.7 vs. 13.1±1.2 %, respectively), while net demethylation was similar for both waters (22.7±2.2 % and 20.8±6.6). These results also exhibit, for the first time in natural waters, a significant difference between net and oxidative MMHg demethylation in the case of the Arcachon Bay, although no significant difference can be found for the Adour Estuary (Table S4). Comparing with dark incubations, we can assume that mostly dark biotic oxidative demethylation was taking place in the Adour plume waters, while other pathways might be involved in MMHg demethylation in the Arcachon waters such as photo-reductive and oxidative demethylation. For instance, incubated filtered water (Fig. 2) also demonstrates that photochemical-induced demethylation is a major pathway for the Arcachon sample. Mercury global reduction yields indicate also distinct pathways between diurnal and dark conditions in the Arcachon water, whereas no difference can be distinguished in the Adour plume waters (Table S4). Overall, these data point out the importance to further investigate specific biotic and abiotic demethylation pathways in coastal waters leading either to inorganic ionic Hg or elemental gaseous Hg.

Similarly than in sediment samples, dealkylation of butyltin compounds was clearly influenced by the light. The degradation yields obtained in seawater incubations were significantly higher than those obtained with sediment incubations. The dealkylation of DBT to MBT under light conditions was the main route of degradation for both water types (18.7±1.3 and 17.7±3.3 for Adour and Arcachon waters, respectively) whereas the dealkylation of TBT to DBT was found to be 10.0±3.6 % under light conditions in the Arcachon Bay and close to 5.3±0.6 % in the Adour Estuary. Also, the dealkylation yields of bulk water from the Arcachon Bay were slightly higher than those obtained in filtered water indicating that photochemical-mediated

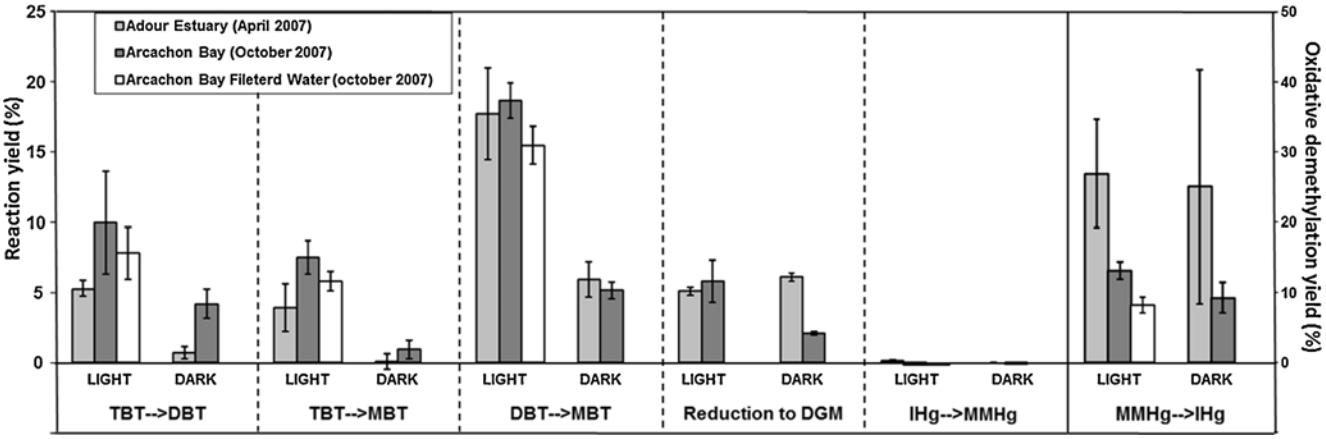


Fig. 2 Formation and degradation yields (in percent) of butyltins and Hg species measured in two different water types (Arcachon Bay and Adour Estuary). The uncertainty values correspond to 1 s standard deviation of three independent incubation experiments

degradations are more important than photomediated biological degradation reactions. These results confirm the lower stability of butyltin compounds in coastal waters. The half-life obtained for TBT to form DBT was always lower than 18 days under light conditions in both water types, whereas those for DBT to form MBT were lower than 5 days.

Conclusions

The methodology presented in this work provides an accurate experimental assessment of specific formation/degradation yields and half-lives of elemental species in different aquatic environments. Advantages of the proposed methodology include a simultaneous and quantitative determination of newly formed and remaining species derived from each isotope, identification of the origin of newly formed species, correction of isobaric interferences, and correction of instrumental mass bias. Also, the method can be applied to any polyisotopic element provided that the element presents a higher number of isotopes than species. Using this methodology, we have studied the environmental reactivity of Hg and Sn compounds in coastal water and surface sediment samples collected in two different aquatic environments of the South French Atlantic Coast (Arcachon Bay and Adour Estuary). The spike addition procedure and the incubation duration were found to be relevant parameters that must be specifically designed for each element before incubation. Both the level of isotopically enriched species and the spiking solution composition were found to alter DBT and MMHg degradation yields, while no significant changes were measurable for TBT and Hg(II). For butyltin species, the presence of light was found to be the main source of degradation and removal of these contaminants from surface coastal environments. In opposition, photomediated processes do not significantly influence either the methylation of mercury or the demethylation of methylmercury.

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