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Determination of tributyltin oxide in coastal marine sediments and mussels by electrothermal atomic absorption spectrometry

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We have devised a new method for bis(tributyltin)oxide (TBTO) determination in marine sediments and mussels. This technique involves an n-hexane/methylene chloride mixture extraction and extract purification with a sodium hydroxide wash in order to eliminate interfering compounds. TBTO is then extracted again by nitric acid and converted into an inorganic tin species; the analysis has been effected using Zeeman graphite furnace–atomic absorption spectrophotometry. The method detection limit for the matrices examined is 0.004 μg TBTO g^{-1} (wet weight) and is sufficient for the analysis in real samples. The percentage recovery of TBTO from sediments and mussels samples is higher than 85% and 95% respectively. This method has been applied to TBTO level determination in sediments and mussels (*Mytilus galloprovincialis*) sampled in the harbour area in Taranto, where mussel culture activities are much developed; the TBTO levels obtained in sediments and mussels were in the range 15–47 ng g^{-1} (wet weight) and 11–30 ng g^{-1} (wet weight) respectively. Such values are comparable with those found in other harbour areas in the Mediterranean Sea.

Keywords: Butyltin, analysis, graphite furnace, atomic absorption, sediments, mussels

INTRODUCTION

Starting from about 1970, organotin compound world production has increased step-by-step up to $(35–40) \times 10^3$ t $year^{-1}$. Such compounds are used in industry as, for example, stabilizers for PVC, catalysts in polyurethane foam synthesis and silicon rubbers additives.[1] In particular, tributyltin derivatives are used as antifouling agents, both in industrial cooling-water treatment and in marine paints, but they have high toxicity to non-target organisms (bacteria, algae, fungi and invertebrates). Among tributyltin derivatives, bis(tributyltin) oxide is one of the most toxic compounds: 0.1 μg TBTO dm^{-3} concentrations are sufficient in fact to cause death to 50% of *Mytilus edulis* larvae, within 15 days (15 days LC_{50});[2] the rest of the larvae appeared to be dying or showed a sensible decrease in speed of growth. Reduction of growth speed has been observed not only in larvae, but also in *Mytilus edulis* juveniles (size 5–8 mm; age 5 months) with 0.4 μg TBTO dm^{-3} concentrations;[3] similarly in Pacific and European oysters[4,5] and in many species of marine microalgae[6] growth reduction is observed at TBTO concentrations between 0.02 and 0.3 μg dm^{-3}. Correlation between TBTO concentration and growth decrease is approximately hyperbolic. Shell malformations have been observed in commercial oysters (*Crassostrea gigas*) at such levels.[7]

Such results show TBTO level determinations in environmental matrices are fundamental, mostly for harbour coastal areas used for mariculture, in relation to the phenomenon of compound release from hulls treated with antifouling paints and to TBTO accumulation, both into sediments and marine filter-feeders.

In order to evaluate effectively the environmental risks associated with tributyltin biocide usage and to develop monitoring strategies, different analytical methods have been developed in recent years for trialkyltin compounds and, in particular, TBTO determination in sediments and marine biological materials. They usually involve a preliminary TBTO extraction by an organic solvent; interfering substances can be eliminated by washing with sodium hydroxide,[8,9] by cation exchange resin column[10] or by purification on a Florisil column.[11]

Analysis is sometimes by Zeeman graphite furnace–atomic absorption spectrophotometry

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(GF AA)[12] or hydride generation AA.[13] As detection techniques we have used fluorimetry and electron capture–gas chromatography (ECD GC) or flame photometric detector–gas chromatography (FPD GC). Wade *et al.*[14] also used, besides extraction, a Grignard derivatization procedure with hexylmagnesium bromide and determination of hexylated organotin compounds by FPD GC or GC MS.

Many of these methods are quite lengthy and not conveniently usable in routine analyses; extraction procedures are often not very quantitative. In this paper we show a simple procedure for TBTO extraction from environmental matrices and its determination by Zeeman GF AA. We discuss percentage recoveries from different mtrices and detection limits of the technique. At the end we show the results of a TBTO contamination survey into marine sediments and mussels sampled from four stations in the Taranto harbour area (Ionian Sea), where such contamination seems to be important. Filter-feeder organisms such as mussels are indeed particularly important both for toxic metal compound bioaccumulation and as important parts of a food chain which involves man.

EXPERIMENTAL

Apparatus

A Perkin–Elmer Zeeman 3030 atomic absorption spectrometer with an HGA 600 graphite furnace, AS 60 autosampler, was used.

Reagents

Reagent-grade methylene chloride, n-hexane and sodium hydroxide and Ultrex-grade acetic acid, hydrochloric acid and nitric acid were purchased from J. T. Baker Chemical Co.; a 1000 μg cm^{-3} tin(II) chloride solution (BDH) was used for preparing tin standard solutions. Bis(tributyltin) oxide was obtained from Fluka Chemie AG; a 1000 μg Sn cm^{-3} solution was prepared by dissolving TBTO in anhydrous acetic acid. More dilute solutions were prepared from this by dilution with 2% acetic acid.

Procedure

Hydrochloric acid (10 cm^3 of 6 mol dm^{-3}) were added to 10 g of wet homogenized mussel tissue or sediments. After mixing by a vortex mixer, the sample was treated with ultrasound for 10 min; 15 cm^3 of methylene chloride and 15 cm^3 of n-hexane were then added; the sample was put on the shaker set for 60 min. After centrifugation at 4000 rpm for 15 min, the supernatant was poured off into a 100 cm^3 Erlenmeyer flask. The rest of the tissue or sediment was extracted again with a methylene chloride/n-hexane mixture; after putting all the extracts together, these were concentrated to 5 cm^3, under a nitrogen flow and washed with 4 cm^3 NaOH solution (3% by weight) in order to eliminate interference of mono- and dibutyltins. NaOH treatment converts mono- and dibutyl species to solids removable by centrifugation. After transferring to a 10 cm^3 centrifuge and to remove organic species which would cause matrix interference in the GF AA method tube, the sample was centrifuged at 4000 rpm for 10 min, to separate the aqueous phase. We then added 2 cm^3 of n-hexane and 1 cm^3 of concentrated nitric acid to the organic extract, to convert organic tin to inorganic tin and to remove organic species which would cause matrix interference in the GF AA method. Solvent evaporation in the presence of nitric acid removed concern about tin losses through volatilization. Acid was then evaporated; 2 cm^3 of 3 mol dm^{-3} nitric acid was added to the residue which was then analysed. Reagent blanks were put through the same procedure and were extremely low. The analysis was effected by Zeeman GF AA, using pyrolytically coated graphite tubes, fitted with L'vov platforms. The analytical wavelength was 286.3 nm; 20 μl of sample and 10 μl of matrix modifier (20 g dm^{-3} of $NH_4H_2PO_4$ and 2 dm^{-3} of $MgNO_3$ in 1 % nitric acid) were automatically dispensed onto the platform, using an AS-60 autosampler. The conditions for the stabilized temperature platform furnace are summarized in Table 1. Peak area integration has been used for signal measurement.

Sediments and mussels samples to be analysed were taken from four stations in Mar Piccolo and Mar Grande in Taranto (see Fig. 1). Station Nos 1 and 2 are located in mussel culture plants, in the second and first inlets in Mar Piccolo, respectively; in the latter an Italian Navy base is located, as well as the shipyards. The other two stations are located in Mar Grande: station No. 3 in mussel culture plants and station No. 4 on natural mussel banks. In each station, five sediment samples were taken by a gravity core barrel. For mussels, we sampled two series of samples on the surface (S_1, S_2), two series at half-depth (M_1, M_2)

Table 1 Zeeman HGA 600 graphite furnace parameters used for analysis of bis(tributyltin) oxide in mussels and sediments samples

Step	Temp. (°C)	Ramp (s)	Hold (s)	Recorder	Int. gas ($cm^3 min^{-1}$)
1	90	1	30	–	300
2	110	50	60	–	300
3	200	30	60	–	300
4	800	30	60	–	300
5	2000	0	6	+	0
6	2500	1	7	–	300
7	20	1	20	–	300

and two series on the bottom (D_1, D_2). Each series was made up of about 30 mussels on average, having a shell length of 4–5 cm. Individuals for each series were homogenized and 10 g of homogenized matter was analysed.

RESULTS AND DISCUSSION

The TBTO extraction method described above allows us to reduce analysing times, but to obtain quantitative recoveries. In previous papers,[8,9] sample treatment with hydrochloric acid lasted a few hours or a whole night; moreover, the extraction procedure lasted 4–16 h. In some sediment samples,[8] however, non-quantitative recoveries equal to 72–82% were obtained. On the other hand, in this paper matrix dissolution, after acidification with hydrochloric acid was effected by ultrasound, whereas our extraction has been made with an n-hexane/methylene chloride mixture (1:1) and solvent evaporation by nitrogen flow on the surface. Such devices have allowed us to increase the extraction yield, to reduce TBTO losses caused by volatilization, and to shorten analysis times.

Extract washing by NaOH allows us to eliminate the interference of other co-extracted alkyltin compounds, whereas the digestion process by nitric acid and the consequent TBTO transformation into inorganic tin is necessary in order to eliminate the organic phase, which would cause serious interference problems in AA analysis.

A mixture of n-hexane/methylene chloride (1:1) has been found to be the most effective for TBTO extraction from mussels and marine sediments samples; n-hexane presence together with methylene chloride allows us to recover the latter quantitatively, when it would otherwise remain partly solubilized in the acid aqueous phase. Two extractions are necessary successively, made with the above solvent mixture, putting together the organic extracts at the end. Before treating with NaOH, it is necessary to concentrate the extract

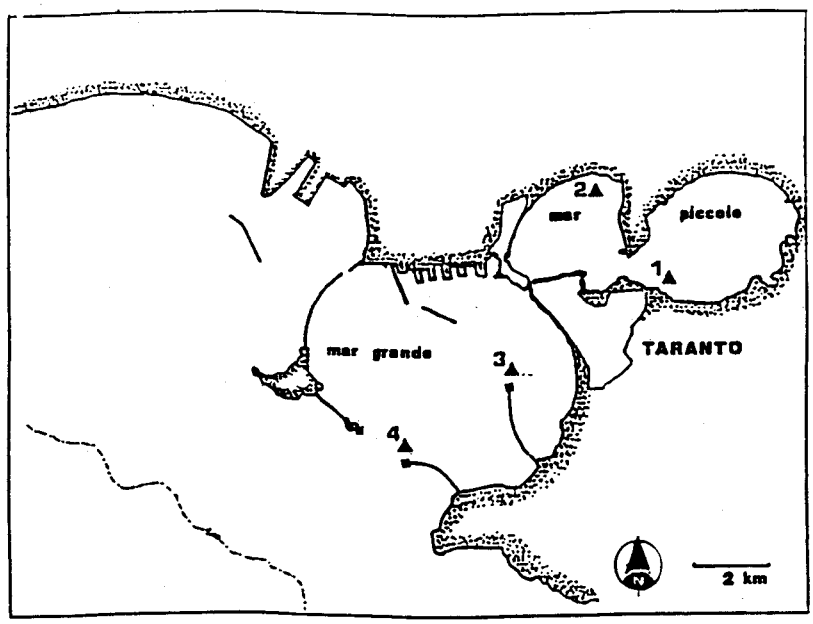

Figure 1 Mussel and sediment sampling stations.

Table 2 TBTO recovery percentage in mussels and sediment samples

Sample (1 g)	μg TBTO added	% recovery
Mussel tissue	1	98 ± 6.0
	2	99 ± 8.5
Sediments	1	89 ± 6.5
	2	90 ± 9.1

at 5 ml by flowing nitrogen upon the surface of the liquid. Solvent evaporation lowers extract temperature, reducing TBTO losses. The extraction procedure validity has been tested, calculating TBTO recovery percentages from mussels and sediment samples artificially contaminated. One sample of mussels and one of sediments, taken from Station 4 and containing respectively 10 and 15 ng TBTO/g (w:w), has been divided, after being homogenized, in various sub-samples. To each of these, known quantities of TBTO have been added (1 or 2 μg of TBTO/g). Samples have then been analysed. Each recovery test has been repeated 5 times, so calculating the average value of percentual recoveries. The results obtained are shown in Table 2. As we notice, percentual recoveries vary according to matrices: for mussels recoveries are quantitative, for sediments recoveries are of about 90%. The variation coefficient, obtained analysing mussels and sediments samples for 5 times, is respectively 7% and 8%.

Concerning tin determination in atomic absorption spectrophotometry it is to be underlined that tin chemistry and spectroscopy have proven to be very complex: using a matrix modifier (a mixture of dibasic ammonium phosphate and magnesium nitrate in 1% nitric acid) in combination with a pyrolytically coated graphite tube, fitted with L'vov platform, Zeeman background correction and a rapid temperature ramp, during atomization, is necessary to remove matrix interferences mainly due to the presence of chlorides and sulphates.[12] The matrix modifier raised the temperature of tin volatilization higher than 1000 °C. Most interfering agents are removed in such a way, in the ashing phase, with a background decrease. It comes out that calibration straight lines obtained with TBTO and inorganic Sn standard solutions have the same slope. Within experimental error, the results obtained by standard addition and by external calibration are coincident. Calibration results to be linear up to 150 μg Sn l^{-1}. This allows a remarkable saving of time for routine analysis. At 286.3 nm wavelength, measuring the signal in a peak-height mode, 10 pg of tin produced 0.005 absorbance. The method detection limit for biological samples (twice the standard deviation of the blank sample) is 0.004 μg TBTO/g (wet weight) and is sufficient for the analysis in real matrices. There are not any peculiar problems due to tin contamination, deriving from glassware, pipette tips and cups of the autosampler. The main source of tin contamination is due to the reagents used and in particular to the matrix modifier. For this reason the blank signal corresponds to a concentration of 2.3 ng Sn/cm^{-3} and it is negligible in the analysis of mussels and sediment samples, taken in harbour areas. Using the reagents actually sold, it is not possible to reduce in a remarkable way the blank signal. The tin level in the matrix modifier can be further reduced instead, through electrolytic purification.

This method has been applied for TBTO determinations in sediments and mussels (*Mytilus galloprovincialis*) sampled from four stations in the Taranto basin. The results obtained are shown in Table 3. Average TBTO concentration values in the sediments and mussels analysed vary in the four stations in the range 15–47 ng g^{-1} (wet weight) and 11–30 ng g^{-1} (wet weight) respectively. For each station, remarkable differences among TBTO levels are not observed in the various samples of sediments analysed; the CV ranged between 4 and 19%.

For mussels, too, there is no significant difference in TBTO levels of samples taken on the

Table 3 TBTO concentrations (ng g^{-1} wet wt) in mussels and sediments from the four sampling sites in Taranto basins

Mussels	Station				Sediments	Station			
Sample	1	2	3	4	Sample	1	2	3	4
S_1	11	34	11	10	1	21	46	19	13
S_2	16	23	13	11	2	29	49	14	15
M_1	13	23	18	11	3	16	45	22	15
M_2	11	34	24	11	4	21	45	17	15
D_1	11	34	21	13	5	19	51	14	19
D_2	10	31	17	12					
$\bar{x}$	12	30	17	11	$\bar{x}$	21	47	17	15
SD	2	5	5	1	SD	4	2	3	2
CV (%)	17	17	29	9	CV (%)	19	4	18	13

Depths: S = 1, M = 5, D = 9.

$$R_3—Sn—X \rightarrow R_2—Sn—X_2 \rightarrow RSnX_3 \rightarrow SnX_4$$

Scheme 1

surface, at half-depth and on the bottom. The average depth of various stations was about 10 m. TBTO levels in sediments were generally higher than in mussels, mainly in station No. 2 (first inlet of Mar Piccolo), where the highest concentrations were found, both in relation to the presence of the shipyards and the Navy base, and owing to a little water exchange. Results obtained show the main source of contamination is due to TBTO release by hull antifouling paints. After release, the compounds may be dispersed into water or adsorbed upon suspended particles which, by sedimentation, produce accumulation into sediments. TBTO degradation, caused both by abiotic and biological factors, occurs according to progressive dealkylation (Scheme 1). Such a degradation, which leads to less toxic compounds, has a slow kinetic pathway. According to various sources, it has come out that the main TBTO degradation processes are UV photodegradation and biological degradation. UV degradation will only be important near the surface; TBTO half-life, concerning UV degradation, is about 90 days.[15] In sediments, degradation is mainly of a biological kind, both in aerobic and anaerobic conditions, with a half-life time calculated around 1.5–5 months. TBTO losses, owing to volatilization, or to hydrolysis, are less significant. Accumulation into sediments is very significant, especially from the ecotoxicological point of view, mostly in benthic organisms such as crabs, oysters, etc.

Lee[16] has shown that in fish liver and in crab hepatopancreas, dibutyltin is the main TBTO metabolite; oysters (*Crassostrea virginica*) have a limited capacity for metabolizing the compound and so they accumulate it. Concerning mussels, Jensen[17] has shown that in *Mytilus edulis* organic tin bio-concentration factors range between 5000 and 60 000; depuration half-time was 40 days. Organotin compounds show degradation and bioaccumulation in mussels is a risk, particularly because of man's consumption of such products. As we note in Fig. 2, sediment contamination influences mussels; polluted sediment resuspension phenomena are, in fact, responsible for toxic compounds recycling along the water column. In closed basins like Mar Piccolo, with a low water exchange, accumulation phenomena can be increased.

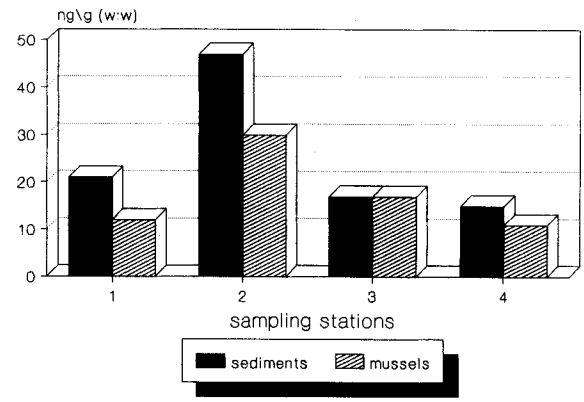

Figure 2 Distribution of TBTO average concentrations in mussels and sediments in the four sampling stations.

CONCLUSIONS

The TBTO extraction procedure described here has appeared to be a simple, quantitative and effective one for analysis of environmental matrices. TBTO concentration levels in the sediments of Taranto basins have the same order of magnitude as those found in some other harbour areas in the Mediterranean Sea.[18,19] TBTO concentration levels in mussels are difficult to compare, owing to the scarcity of literature concerning the Mediterranean Sea. However, the levels found, especially in Mar Piccolo, and the high toxicity of TBTO suggest that in harbour coastal areas this kind of contamination represents a risk, both for marine organisms and for man. Since 1982, it is fobidden in France to use organotin compound paints for ships less than 25 m long. Such a regulation, which is used in the USA too and also later in Italy, may be able to solve the problem for marinas, but it is certainly insufficient for harbour areas where mariculture activities live together with shipyard activities.

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