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# Atomic absorption spectrometric screening and gas chromatographic-mass spectrometric determination of organotin compounds in marine mussels: an application in samples from the Venetian Lagoon

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**Abstract** A new analytical approach for the determination of organotin compounds (OTC) in mussel samples has been developed and evaluated. A preliminary step, performed by graphite furnace atomic absorption spectrometry (GFAAS) for the total tin determination may be followed by gas chromatographic-mass spectrometric (GC/MS) speciation only for the characterization of those samples exhibiting total tin concentration higher than 30 ng/g wet weight (GFAAS limit of detection). The GFAAS method was optimized using Pd nitrate under reducing conditions as matrix modifier to minimize NaCl interferences. Organotins were derivatized with a Grignard reagent for GC/MS analysis (TBT limit of detection = 80 ng/g). An application of this strategy was performed on mussel samples collected from the Venetian Lagoon.

## Introduction

The importance of tin and organotin compounds (OTC) as exogenous presence in environment and biota has become increasingly remarkable. Owing to their wide use both in industrial processes (as catalysts or stabilizing agents) and as antifouling additives in ship paint formulations, traces of their presence are detectable in environmental and biological samples. Recently, the use of OTC has been restricted or banned in many countries [1], however, their presence in ecosystems is still significant and should be monitored. As a consequence, many analytical procedures have been developed.

The negative impact of triorganotin compounds on the worldwide aquatic environment, mainly tributyltin (TBT) and triphenyltin (TPT) released from the paint of ships and boats, is of great environmental and ecotoxicological concern because of the proven toxicity of these compounds towards non target organisms like mussels and gastropods [2, 3]. A wide range of adverse effects on aquatic biota are well documented, and appeared even at OTC concentration levels of few ng/L in water. Furthermore, results obtained from in vitro experiments noticed the potential genotoxicity of these compounds towards human cells as well [4].

Owing to their high bioconcentration factors, mussels are usually chosen as sentinel organisms for the detection of marine pollutants [5]; therefore, in order to assess the quality of marine ecosystems, the development of alternative and easily applicable analytical methods to detect OTC compounds on mussel samples are of strong interest.

Several methods have already been proposed to analyze OTC in mussels. Analyses were performed mainly by gas chromatography – GC, coupled with suitable detection systems (such as electron capture detector – ECD; flame photometric detector – FPD; mass spectrometric detector – MSD; atomic emission detector – AED; electrothermal atomic absorption spectrometric detector – ETAASD) [6–9]. In spite of the good separation properties typical of GC techniques, the required derivatization step is usually time consuming.

This work was aimed at the optimization of a new analytical strategy consisting of a preliminary screening of mussel samples by graphite furnace atomic absorption spectrometry (GFAAS) for the determination of *total tin* amount, followed by speciation by GC/MS for samples exhibiting tin concentrations higher than the estimated level of contamination [5]. In this way a high sample throughput per day is achieved, reducing the laborious, time and reagent consuming step of speciation, which needs only be performed when suspect of polluted samples occurs.

Finally, an application of this approach was performed on samples of mussels (*Mytilus galloprovincialis*) collected from some areas of the Venetian Lagoon, which was chosen considering: – the wide density of mussel colonies throughout the lagoon and their closeness to docks and shipyards; – the geographical circular conformation of this lagoon; – the presence of river mouths carrying industrial pollutants.

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## Experimental

### Instruments

*Atomic absorption instrumentation.* A Varian (Mulgrave, Victoria, Australia) SpectrAA 250 Plus atomic absorption spectrometer equipped with a GTA-97 graphite furnace atomizer, a PSD-56 autosampler and deuterium background corrector was used. A Varian Sn hollow cathode lamp served as the line source. Operating condition: *calibration mode*: concentration; *measurement mode*: peak height; *slit width*: 0.5 nm; *slit height*: normal; *wavelength*: 235.5 nm; *time constant*: 0.05; *measurement time*: 1.0 s; *replicates*: 1. The graphite furnace temperature program is given in Table 1.

*Digestion equipment.* The digestion of mussel samples was performed in a CEM (Matthews, NC, USA) model MDS-2100 Microwave Sample Preparation System 950 W oven, using CEM ACV advance composite digestion vessels.

*GC/MS instrumentation.* The GC/MS speciation was carried out by a Hewlett Packard (Avondale, PA, USA) 5890 gas chromatograph equipped with a 5970 Mass Detector. Chromatographic conditions: column, J&W Scientific (Rancho Cordova, CA, USA) 5% phenyl methyl polysiloxane DB-5 (30 m × 0.25 mm id × 0.25 μm ft); carrier gas, helium, 1 mL/min; injection port temperature, 300 °C; splitless injection, 1.5 μL injection volume, purge time 0.5 min; transfer line temperature, 270 °C; oven temperature program, 60 °C held for 1 min, increased at 15 °C $min^{-1}$ to 270 °C and maintained for 10 min.

### Reagents

*GFAAS.* Concentrated nitric acid (37%, *pro analysi*), L (+) ascorbic acid, palladium modifier (Pd = 10.0 g/L as $Pd(NO_3)_2$ in $HNO_3$, 15%) were all of analytical grade and obtained from Merck (Darmstadt, Germany). Water used for preparing solutions was distilled and deionized using a Millipore (Milford, MA, USA) MilliQ Water Purification System. Sn stock solution (Sn = 1000 mg/L as $SnCl_4$ in HCl 5 M) was purchased from Merck. Calibration standards (2.5, 5.0, 10.0 ng/mL) were prepared daily.

*GC/MS.* Mono- (MBT), di- (DBT), tri- (TBT) and tetrabutyltin (TTBT) chlorides (95, 96, 96 and 93% purity, respectively), methylmagnesium bromide (3 M in diethyl ether) and tropolone (98% purity) were all of analytical grade and obtained from Aldrich (Milwaukee, WI, USA). Dichloromethane, n-hexane, methanol, all RPE reagent grade, were purchased from Carlo Erba (Milano, Italy).

*Sample preparation.* The whole animals were removed from their shells, about 20 g were homogenized with an Ultra-Turrax Ika Labortechnik (Janke & Kunkel).

*Digestion procedure for GFAAS.* Homogenized sample (0.5 g) was mixed with $HNO_3$ (10 mL) in the Teflon liner of Advance Composite Vessel, and digested in a microwave oven, according to the conditions listed in Table 2. After cooling at room temperature, the digests were quantitatively transferred into 50 mL class A volumetric flasks and adjusted to volume with distilled deionized water.

*Extraction procedure for GC/MS.* To 2 g of homogenized sample, placed in 50 mL glass screw tubes, 15 mL of 0.05% w/v tropolone solution in methanol were added. After 2 min stirring by Vortex, the sample was treated in a Branson 3200 ultrasonic bath (Dansbury, Connecticut, USA) for 15 min and then centrifuged at 4000 rpm for 10 min: the supernatant was collected with a Pasteur pipette. This extraction procedure was repeated three times. Combined extracts were placed into 100 mL polypropylene centrifuge screw tubes, mixed with distilled deionized water (50 mL) and extracted with dichloromethane (2 × 30 mL). The separation was improved by centrifugation at 6000 rpm for 5 min. The combined dichloromethane extracts were dried on anhydrous sodium sulfate and the solvent was evaporated by a Büchi (Flawil, Schweiz) RE111 vacuum rotary evaporator. The samples were solved in n-hexane (1 mL), and quantitatively transferred into 5 mL screw vials with PTFE septum closure for the derivatization step.

*Derivatization for GC/MS.* To the hexane extracts, a solution of methyl magnesium bromide (500 μL, 3 M in anhydrous diethyl ether) was added dropwise (reaction time: 15 min at room temperature). The reaction was slowly quenched with aqueous ammonium chloride 20% (2 mL) at 0 °C (cautions must be observed according to the manufacturer indications). After 3 min stirring by Vortex, 1.5 μL of the upper organic layer were injected in GC/MS for the analysis.

**Table 1** GFAAS temperature program for Sn determination

| Step n°. | Temp. (°C) | Time (s) | Ar flow rate (L/min) |
|---|---|---|---|
| 1 | 85 | 5.0 | 3.0 |
| 2 | 95 | 40.0 | 3.0 |
| 3 | 120 | 10.0 | 3.0 |
| 4 | 600 | 10.0 | 3.0 |
| 5 | 600 | 1.0 | 3.0 |
| 6 | 600 | 2.0 | 0.0 |
| 7 | 2600 | 1.2 | 0.0 |
| 8 | 2600 | 2.0 | 0.0 |
| 9 | 2600 | 2.0 | 3.0 |

**Table 2** Microwave oven digestion conditions

| Step n°. | Power (%) | Pressure (kPa) | Temp. (°C) | Time (min) |
|---|---|---|---|---|
| 1 | 20 | 140 | 120 | 5 |
| 2 | 40 | 520 | 150 | 5 |
| 3 | 50 | 980 | 170 | 10 |
| 4 | 55 | 1260 | 200 | 10 |
| 5 | 55 | 1330 | 200 | 10 |

## Results and discussion

The analytical results from several studies concerning the OTC amounts in mussel tissues have been summarized worldwide [5, 10]. The authors determined TBT concentration ranges corresponding to standardized degrees of contamination, i.e. 100 ng of TBT per gram of wet tissue was chosen as the critical threshold level between non or low contaminated mussels and mid or high contaminated ones. In order to respect these indications, the screening

**Table 3** GFAAS screening: total tin recovery (performed on five spiked samples, $n = 5$, at each concentration level)

| Spiking level (ng/g) | Mean recovery (%) | SD | CV% |
|---|---|---|---|
| 50 | 87.4 | 4.72 | 10.8 |
| 100 | 89.0 | 13.4 | 15.1 |
| 200 | 91.2 | 17.2 | 9.4 |
| 400 | 84.6 | 47.4 | 13.9 |

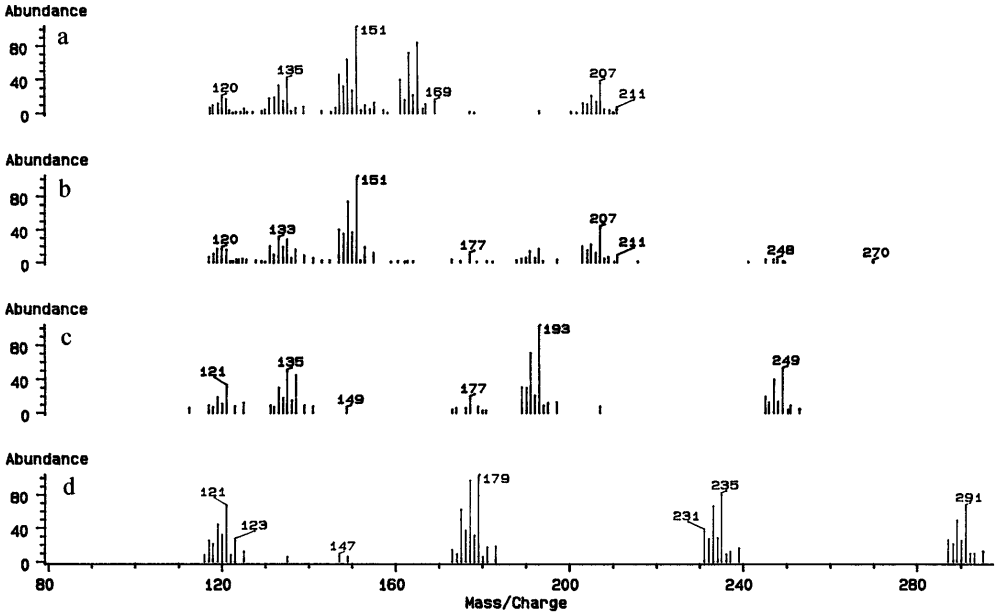


**Fig. 1** **a**) MBT, **b**) DBT, **c**) TBT and **d**) TTBT full scan spectra

method was optimized in order to have a detection limit of 30 ng/g of total tin (for the GFAAS screening) which corresponds to about 82 ng/g in TBT. For this reason the GC/MS detection limit of TBT has to be equal to or lower than this value.

*GFAAS screening.* Owing to the characteristics of the matrix, rich in sodium chloride, $Pd(NO_3)_2$ was used under reducing conditions as the best matrix modifier [11]. Recoveries of total tin by GFAAS were estimated on different levels of concentration, each of them performed on five spiked samples, and are listed in Table 3.

*GC/MS speciation.* Several procedures [8, 13, 14] have been proposed for leaching OTC from biological samples; we have chosen the extraction with methanolic solution of tropolone (2-hydroxy-2,4,6-cycloheptatrienone) [4]. The extracted cationic OTCs were then derivatized to the non-polar tetra-alkyl compounds with a Grignard reagent in order to make them suitable for GC analysis.

Tetrabutyltin (TTBT) was chosen as the internal standard: 625 ng were added to each sample. Characteristic ions were chosen among those typical of the tin clusters. The fragmentation produced in EI was satisfactory for all the compounds studied, allowing to choose up to four ions for each analyte among a wide number of abundant ones. Mass spectrometric data were acquired according to the following selective ion monitoring: MBT: 147; 149; 151; 163; 165. DBT: 147; 149; 151; 193; 205. TBT: 189; 191; 193; 247; 249. TTBT: 177; 179; 233; 235. In this way, a good signal-to-noise ratio was maintained without losing specificity. Full scan spectra of the three molecules and of the internal standard are shown in Fig. 1. Quantitative analyses were performed on a single ion: 165 for MBT, 149 for DBT, 193 for TBT and 179 for TTBT (int. std.).

In agreement with Page et al. the linearity of recoveries in the range 100–400 ng/g wet sample was tested only for TBT, the original compound used in paint formulations [5]. Recoveries for MBT and DBT (products of TBT decomposition) were tested only at 100 ng/g. In Table 4 the results of recoveries obtained at all levels of concentration, each of them performed on five spiked samples ($n$ = 5), are presented. The regression equation is the following: $y = 0.0022x + 0.5515$, $r^2 = 0.992$, where y is the ratio of peak areas of recovered TBT and the area of the internal standard, and x is the concentration of TBT spiked samples; $r^2$ is the correlation coefficient of the equation. The detection limit was found equal to 80 ng/g wet weight. Owing to the non satisfactory recovery results obtained at the spiking level of 400 ng/g, it was impossible to consider this linearity totally suitable for quantitative determinations on the whole range. However, considering the target level of 100 ng/g as the critical threshold level between non or low contaminated mussels and mid or high contaminated ones, the speciation of OTC can still be performed in the range 80–200 ng/g wet weight. In Fig. 2 the chromatograms of the standard solution of TBT (200 ng/g), of a mussel sample spiked with TBT at 200 ng/g and of the corresponding blank sample are shown.

**Table 4** GC/MS speciation: OTCs recoveries (performed on five spiked samples, $n$ = 5, at each concentration level)

| Spiking level (ng/g) | Mean recovery (%) | SD | CV% |
|---|---|---|---|
| TBT 100 | 89.8 | 13.6 | 15.2 |
| TBT 200 | 60.7 | 8.7 | 7.2 |
| TBT 400 | 53.8 | 14.6 | 6.8 |
| MBT 100 | 76.9 | 10.4 | 13.6 |
| DBT 100 | 90.1 | 10.9 | 12.1 |

*Analyses of real samples.* An application of the proposed analytical strategy was performed on samples collected from the Venetian Lagoon. This area was chosen considering the presence of mussel colonies spontaneously grow-

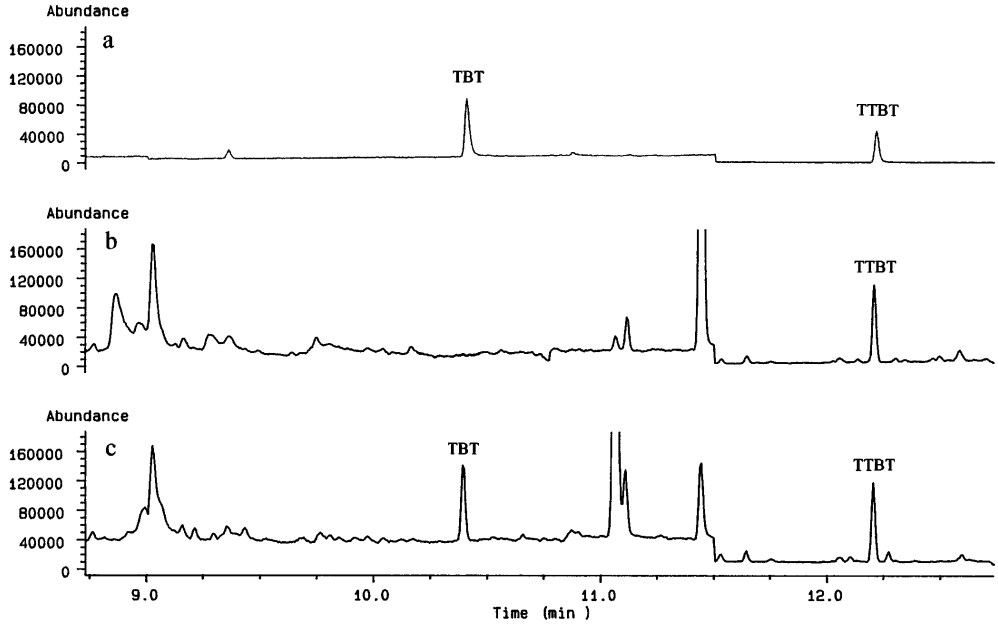


**Fig. 2 a–c** Chromatograms of: **a**) TBT Standard solution (200 ng/g), **b**) Blank sample, **c**) Sample spiked at 200 ng/g

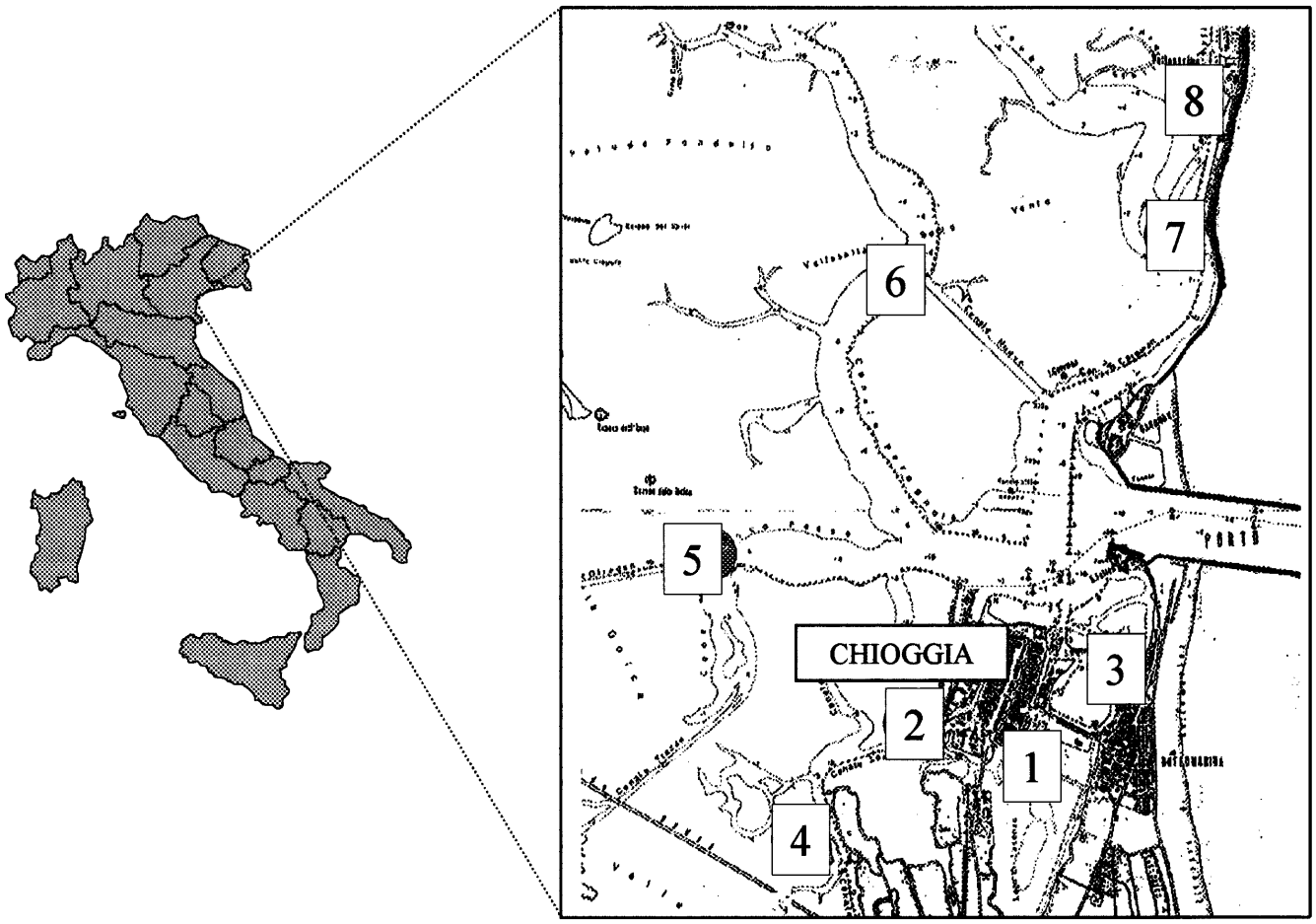


**Fig. 3** Map of the sampling area: 1) Canale S. Domenico; 2) Canale Lombardo int.; 3) Isola Buon Castello; 4) Aleghero; 5) Canale Poco Pesce; 6) Canale Perognola; 7) Bombae; 8) Pellestrina

ing throughout the whole region often near to docks and shipyards. Moreover, no data concerning organotin levels are available at the moment regarding this region. Specimen of collected mussel samples (*Mytilus galloprovincialis*) were both of adult and juvenile organisms (4–6 and 2–3 cm in length, respectively). In Fig. 3 the map

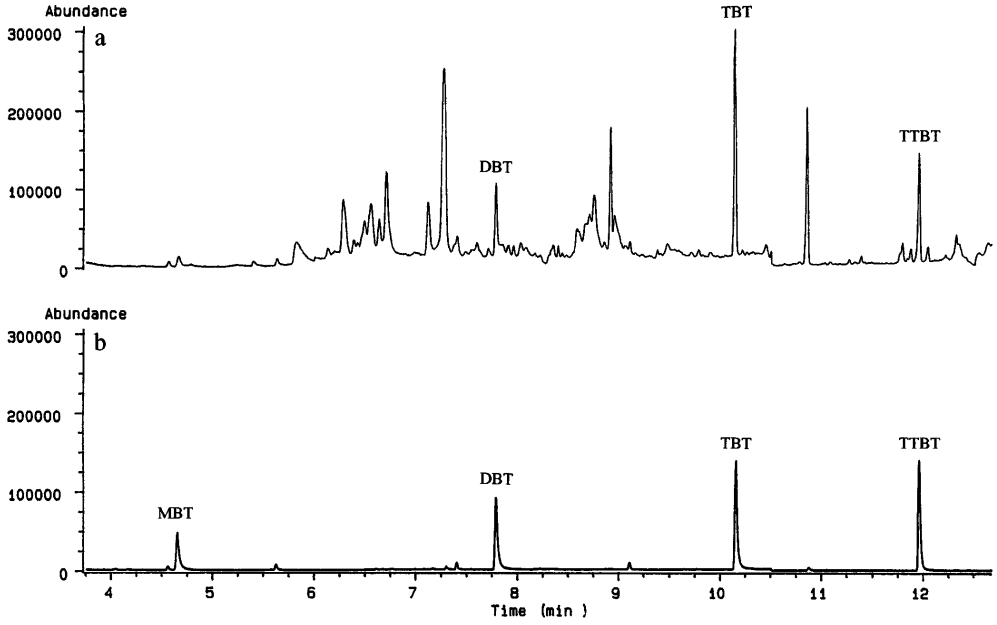


**Fig. 4 a, b** Chromatograms of: **a**) a real sample (collected from site 1) and **b**) MBT, DBT, TBT standard solution (200 ng/g)

of the sampling area is shown. Sampling sites were chosen either foreseeing their possible contamination due to the predictable pollution of the surrounding environment, or far from any possible source of contamination. Total tin concentrations were determined on both adult and juvenile specimens, and were found to be lower or equal to the GFAAS detection limit in sites 5 and 6, and varied between 169 and 568 ng/g in the other six locations.

No significant difference in total tin amount was noticed between adult and juvenile mussels collected in each site. Therefore speciation analysis was performed only on adult samples collected from sites 1, 2, 3, 4, 7, 8. Samples exhibiting TBT concentration higher than the upper limit of 200 ng per gram of wet sample were diluted in order to perform analyses in the acceptable linear range. GC/MS analysis revealed that TBT represents the main contribution; DBT and MBT were present in minor amounts confirming that they arise from TBT degradation. TBT concentrations varied between 390 and 1350 ng/g. An example of a real sample chromatogram compared with the standard mixture one is given in Fig. 4.

In conclusion, particularly relevant organotin levels were found in sampling positions 1, 2, 3 and 4 in accordance with their closeness to docks, shipyards, and commercial ship traffic.

## Conclusions

The proposed analytical strategy results to be sensitive, reproducible and allows a high sample throughput per day. In fact, the screening proved to be easily applicable by any routine analytical lab, avoiding the laborious and time consuming analysis necessary for the indiscriminate speciation of all samples. This step can be performed only in the case of suspect of heavy polluted samples, enabling to recognize the compound of interest. For all these reasons the method may be useful in monitoring programs for the assessment of quality of marine environments.

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