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Occurrence of Butyltin Compounds in Water and Mussel Samples Collected in an Oil Port

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The aim of the present study was to follow the seasonal variations of butyltin compounds in water and mussels under natural conditions in the Genoa oil port. To this purpose, water and organisms were collected in the same location within the port, at two-monthly intervals, between 1997 and 1998. The extraction of organotin compounds was performed both on unfiltered and filtered seawater to determine partitioning between the dissolved and the particulate phases. Moreover the extraction was carried out on the whole body, gills and digestive glands of mussels to analyze the distribution in marine organism tissues. The determinations were carried out using a HPLC–hydride generation–ICP/AES system. The results revealed the presence of tributyltin, dibutyltin and monobutyltin in all matrices, showing variations during the sampling period. In particular, in oil port waters, butyltin compounds were predominantly in the dissolved phase. Moreover, comparing butyltin trends in water and in mussel tissues, the best correlation was found in gill distributions, in this tissue a higher tributyltin bioaccumulation factor was observed.

Keywords: butyltin; concentrations; analysis; water; tissues; port

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INTRODUCTION

It is well known that tributyltin (TBT) is used in a number of commercial applications, including biocide additives in antifouling ship-paint formulations. In spite of efforts to reduce their use, levels in the marine environment are still high. The use of TBT in antifouling paints is still important for its applications on large sea-going vessels, resulting in environmentally significant TBT water concentrations in the open sea;[1] in fact about 69% of shipping vessels are still being painted with paints containing tributyltin as antifouling agent.[2]

In the marine environment, dibutyltin (DBT) and monobutyltin (MBT), which is less toxic than TBT to aquatic organisms, are also present as a result of TBT degradation processes, together with triphenyltin (TPT), employed as a co-toxicant with TBT in some long-performance antifouling paints. The fate of TBT in the water column is a result of different processes: input rate, mixing and dilution, biodegradation, photodegradation, sorption onto particulate suspended matter etc.[3] Several studies show that biodegradation due to microbial and phytoplanktonic activities is the main TBT degradation processes in inshore waters.[4–6] Experiments on TBT degradation in a yacht basin showed that the TBT half-life ranged from six to seven days and that DBT was the principal degradation product.[5]

The partitioning of organotin compounds between dissolved and particulate phases is considered one of the most important processes responsible for the reduction of concentrations and toxicity of organotin in water.[1] Sorption depends on the nature and on the concentration of the suspended matter present in the studied ecosystem. Several studies on distribution of organotin compounds in the water column reported the highest percentage of TBT in the dissolved phase[3] or in the particulate phase.[7,8]

Mussels are often used as biomonitors in monitoring programmes assessing the quality of the marine environment, because they can provide a time-integrated estimate of the contaminants in water.[9] A growing number of studies has evaluated the levels of alkyltins in mussel tissues from a variety of locations worldwide.[10–12] Nevertheless the input of organotins from large vessels, such as

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oil tankers, in coastal waters has seldom been assessed,[13] because these vessels spend the majority of the time offshore, where dilution and dispersion processes reduce the release of TBT into the water.[14]

The aim of the present study is to assess the presence, the levels, the partitioning between dissolved and particulate phases of organotin compounds in the water of the Genoa oil port and in tissues of mussel sampled in the same location, in order to evaluate their variation in concentration and speciation during the monitoring time.

METHODS

Study area and sampling procedures

The Genoa oil port is on the west side of the town; there are four wharves, 250–500 m long and 11–14 m deep. The terminal handles 500–600 oil tankers each year, which remain in the port for one to two days. Seawater and mussels of a local naturally occurring bivalve population of *Mytilus galloprovincialis, Lam*, 5–6 cm in length, were sampled at two-monthly intervals between July 1997 and May 1998. At each sampling, 10 litres of seawater were collected in a low-density polyethylene (LDPE) container, opened and filled at 1 m below the surface to avoid contamination from the surface microlayer, which can exhibit high concentrations of butyltins. The samples were stored at 4 °C in the dark, until organotin extraction; the storage time was never longer than three days. At the same time, 25 mussels were collected; the whole soft tissues of eight individuals and the digestive glands and the gills of the remainder were dissected, homogenized and frozen at −20 °C until analysis.

Analytical procedures

Organotin extraction

Seawater

An aliquot of the sampled seawater was filtered through 0.45 μm PC filters, in order to separate the particulate suspended matter.

The extraction of organotins was carried out on both filtered and unfiltered samples, to obtain their partitioning between dissolved and particulate phases. The procedure, optimized in our laboratories for this study, consisted of a liquid–liquid extraction. Seawater (800 ml) was extracted twice with 80 ml methylene dichloride (CH_2Cl) containing 0.05% tropolone by shaking vigorously for 5 min in a separating funnel. The methylene dichloride phases, containing the organotins, were collected and evaporated to dryness by a vacuum rotary evaporator. These were no losses of TBT in this procedure. The samples were redissolved in 1 ml methanol and 200 μl was injected into the HPLC column. Calibration curves were prepared for each compound using seawater which was spiked with standard compounds. Recoveries of TBT averaged 80%; reproducibility, assessed by triplicate extractions of spiked seawater, was within $\pm$10% (standard deviations of mean values of triplicate extractions of spiked samples) for all compounds.

Mussel tissues

The extraction from the tissues, based on the method employed by Caricchia *et al.*,[15] consisted of a liquid–liquid extraction. Tissue 2 g, (wet mass) was homogenized in a methanol/tropolone (0.05%) mixture and the organotins were extracted with 30 ml methylene dichloride by shaking vigorously for 5 min in a separating funnel. The methylene dichloride phase, containing the organotins, was collected and evaporated to dryness by a vacuum rotary evaporator. The samples were redissolved in methanol and 200 μl was injected into the HPLC column. The accuracy of the method was tested by using CRM 477, produced by BCR of European Commision. Recoveries of TBT, DBT and MBT averaged 108%, 79% and 102% respectively.

Organotin compound determination

To separate and quantify organotin compounds, an HPLC–hydride generation–ICP–AES system was used as previously described.[16] $NaBH_4$ and HCl were added on-line.

The HPLC instrument used in the experiments was a Varian LC system 5000 equipped with a 200 μl Rheodyne injector. Separations were performed on a Partisil SCX 10 μ analytical column (10 μm particle size, 25 cm $\times$ 4.6 mm i.d.) (Whatman, Englewood Cliffs, NJ, USA). The flow rate was 1 ml min^{-1} and no gradient elution devices were used. The mobile phase was 0.1 M ammonium acetate in 80% methanol/water containing 0.1% tropolone. The inductively coupled plasma atomic emission spectrometer used as the detector was a Jobin Yvon JY24 (Jobin Yvon, Lonjumeau, Paris, France). The ICP–AES parameters were: power, 1.1 kW; argon flow rate (1 min^{-1}), intermediate 16, coolant 0.5, nebulizer 0.5. The Jobin Yvon hydride

Table 1 Concentrations of butyltin compounds[a] in seawater samples from the Genoa oil port

	July	September	October>	December	February	May
Unfiltered						
Total butyltin	1308	1843	1237	314	1906	680
TBT	528 ± 15	1200 ± 21	734 ± 28	225 ± 18	629 ± 14	297 ± 4
DBT	571 ± 12	518 ± 29	290 ± 7	41 ± 4	1028 ± 19	210 ± 8
MBT	209 ± 21	124 ± 14	213 ± 11	48 ± 3	249 ± 2	173 ± 15
Filtered						
Total butyltin	603	1541	1175	288	1471	580
TBT	161 ± 20	1041 ± 95	724 ± 3	200 ± 9	394 ± 37	222 ± 15
DBT	296 ± 30	384 ± 38	238 ± 16	40 ± 2	872 ± 1	185 ± 10
MBT	146 ± 15	116 ± 10	213 ± 11	48 ± 4	205 ± 6	173 ± 15

[a] Values are in ng l^{-1} for whole molecule and represent the mean ± standard deviation of three replicates.

generator was used as a gas–liquid separator. The best sensitivity was obtained with 0.3 M HCl and 0.25 M $NaBH_4$ in 1 M NaOH with a 0.7 ml min^{-1} flow for both reagents.

Analytical procedure

The eluent coming from the HPLC column was mixed in a PTFE (polytetrafluoroethylene) T-piece with hydrochloric acid, and then the organotin compounds were converted into their hydrides by addition of $NaBH_4$ in another PTFE T-piece.

Evolved hydrides were drained to the gas–liquid separator, where an argon flow carried the tin vapours into the ICP–AES torch, while the organic eluent was sent to waste.

The tin emission signal was detected leaving the monochromator fixed at the analytical wavelength for tin 189,989 nm (the scanning step around this wavelength was 0 nm), recording the signal every 300 ms. The detection limit was 7ng for Sn.

Reagents analytical grade and the methanol was HPLC grade. The water was de-ionized from a Milli-Q system.

RESULTS

Seawater

Butyltins, TBT, DBT and MBT were found in every sample throughout the months examined, in both the dissolved and the particulate phases, while no phenyltin compounds were detected. In Table 1 TBT, DBT and MBT concentrations are reported; the data represent the mean ± standard deviation of three determinations.

The total butyltins varied during the months, showing a maximum in September (1842 ng l^{-1}) and in February (1906 ng l^{-1}) and a minimum in December (314 ng l^{-1}). TBT was the predominant species during the summer and autumn, showing the highest concentration in September, in both unfiltered and filtered samples (1200 and 1041 ng l^{-1}, respectively), and reaching minimal values in December and May.

Regarding to the degradation products, DBT showed its maximum in February in both unfiltered and filtered samples (1029 and 872 ng l^{-1}, respectively) and lowest concentrations in December (about 40 ng l^{-1} in both unfiltered and filtered samples). MBT did not show any significant changes in the months examined except in December, when the lowest concentration (48 ng l^{-1}) was found. MBT concentrations were generally lower than DBT values. Butyltins were predominantly in the dissolved phase. In fact 62–98% of the total TBT, 74–97% of the total DBT and 82–99% of the MBT were present in the samples

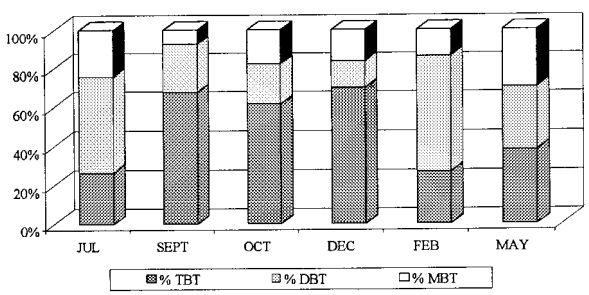

Figure 1 Percentage distribution of butyltin compounds in filtered seawater samples.

Table 2 Concentrations of butyltin compounds[a] in mussel tissues

	July 1997	September 1997	October 1997	December 1997	February 1998	May 1998
Whole tissues						
Total butyltin	4.61	11.06	8.46	3.62	6.85	2.18
TBT	2.50 ± 0.25	5.79 ± 0.28	3.29 ± 0.33	0.73 ± 0.07	3.36 ± 0.02	0.89 ± 0.08
DBT	1.57 ± 0.14	3.08 ± 0.01	3.72 ± 0.07	1.04 ± 0.11	2.84 ± 0.12	0.69 ± 0.06
MBT	0.54 ± 0.01	2.19 ± 0.12	1.45 ± 0.03	1.85 ± 0.18	0.65 ± 0.01	0.60 ± 0.01
Gills						
Total butyltin	13.73	16.03	11.40	7.07	12.46	3.63
TBT	6.52 ± 0.13	7.59 ± 0.56	6.02 ± 0.22	2.54 ± 0.11	5.94 ± 0.03	1.21 ± 0.06
DBT	3.70 ± 0.03	5.84 ± 0.37	3.38 ± 0.18	2.32 ± 0.24	5.40 ± 0.03	0.84 ± 0.04
MBT	3.51 ± 0.03	2.60 ± 0.01	2.00 ± 0.06	2.21 ± 0.01	1.12 ± 0.02	1.58 ± 0.08
Digestive glands						
Total butyltin	6.47	4.46	3.82	3.70	6.68	2.50
TBT	2.76 ± 0.11	2.46 ± 0.08	2.03 ± 0.02	1.66 ± 0.17	2.75 ± 0.02	1.20 ± 0.10
DBT	2.05 ± 0.16	1.15 ± 0.07	0.83 ± 0.04	0.19 ± 0.04	3.34 ± 0.04	0.52 ± 0.06
MBT	1.66 ± 0.10	0.85 ± 0.01	0.95 ± 0.01	1.85 ± 0.02	0.69 ± 0.03	0.78 ± 0.03

[a] Values are in $\mu g\ g^{-I}$ (whole molecule) dry weight and represent the mean ± standard deviation of three replicates.

after filtration in all the months except July, when they were equally distributed between dissolved and particulate. Butyltin partitioning was not dependent on particulate matter concentration ($r^2 = 0.43$).

In Fig. 1 the butyltin species distribution in the dissolved phase is shown. As can be seen, TBT represents about 70% of the total organic tin in autumn and winter and ranged from 25 to 40% in spring and summer. However, DBT showed the highest percentage among butyltins, in samples collected in spring and summer, varying from 49 to 72%. MBT represented less than 25% of butyltin, every month.

Mussel tissues

As regards the distribution of butyltin compounds in mussel tissues, in Table 2 the organotin concentration found in the examined tissues are reported; the data represent the mean ± standard deviation of at least three replicates.

In detail, in all the tissues, total butyltin varied during the months, ranging from 2.18 to 11.07 $\mu g\ g^{-1}$ in whole tissues, from 3.63 to 16.03 $\mu g\ g^{-1}$ in gills and from 2.50 to 6.68 $\mu g\ g^{-1}$ in digestive glands, showing in all cases a minimum in May. Total tissues and gills presented the same TBT trends with a maximum in September and a

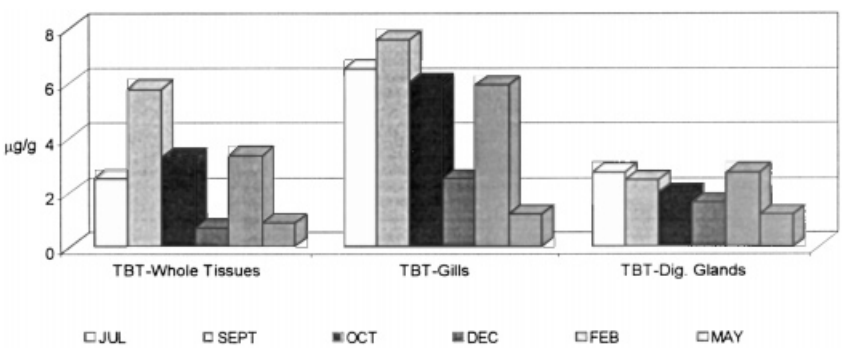

Figure 2 Tributyltin trends in whole tissues, gills and digestive glands of mussels.

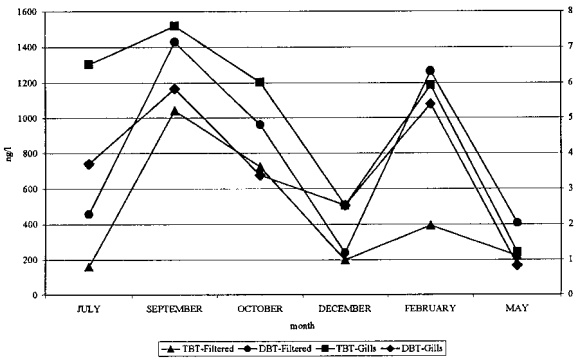

Figure 3 Comparison of tributyltin and dibutyltin trends in filtered seawater samples with tributyltin and dibutyltin trends found in mussel gills.

minimum in December and May, while digestive glands had a different pattern without showing maxima or minima. The bar diagram reported in Fig. 2 showing TBT trends found in whole tissues, gills and digestive glands points out the differences among the tissues.

Moreover, even when seasonal variation in concentrations were detected, the speciation was not greatly different from summer to winter, TBT representing 50% of butyltins in all seasons, both in gills and digestive glands.

Bioaccumulation Factors (BF) of TBT for the tissues considered were calculated (BF = TBT concentration in mussel tissue on dry weight basis relative to TBT water concentration). The results showed that the highest mean factor was reached for gills (9500), while for whole tissues and digestive glands the factors were 3700 and 4300 respectively. This result was not expected because the Bioconcentration Factor is usually higher for organs with a high lipid content such as digestive glands or mantle which are considered in this study as equivalent to whole tissue analysis with our specimens.

Comparing TBT and DBT trends in seawater and in mussel tissues, the best correlation was found between butyltins in the dissolved phase and gill levels, as can be seen in Fig. 3. The presence of degradation products in these tissues (gills) is due not only to metabolic processes but to direct take-up from seawater, therefore gills are the most suitable tissues to be considered as bioindicators of organotin seasonal variations. Nevertheless, it is interesting to point out that, even if gills reflect TBT and DBT seawater trends, as regards the speciation there were differences between organotin distributions in seawater samples and tissues: for instance the lowest TBT concentration value in seawater (225 ng l^{-1}) corresponded to its highest percentage (71%), while that in gills (2.5 μg g^{-1}) corresponded to its lowest (34%).

CONCLUSIONS

The results obtained by analysing seawater and mussel tissues sampled in the Genoa oil port show that butyltin compounds are present in this location. In particular, TBT levels determined in water were at high concentrations, owing to continuous input from vessels (despite a reduction in direct TBT use) and also due to poor water turnover in the basin. Data were compared with those available for Mediterranean waters, with particular reference to the Northern Tyrrhenian and Ligurian seas,[17,18] where there are similar or higher concentrations in commercial harbours with higher boat density and traffic compared with to the Genoa oil port. Therefore the release of TBT from oil tankers has to be considered relevant, even if these vessels spend few days in the basin.

DBT and MBT were also present because of degradation processes in the water column, while contributions from other applications were thought to be negligible. Butyltins were predominantly in the dissolved phase; however, little correlation was found between suspended solids and organotin compound concentrations. Regarding the speciation, differences were found in the distribution of species between summer and winter samples, the latter being the degradation products prevalent in spring and summer.

Mussel tissues, particularly gills, reflected the organotin variations found in the sampling period in the seawater. Comparing the results with other data on the speciation of organotin compounds in mussels sampled in different seasons, we emphasize that in our case no differences were found in the distribution of species between summer and winter samples. In fact, even if seasonal variations in concentrations are detected, TBT was prevalent in all seasons. Moreover, the total organotin compound content in mussel tissues was not different from the values found in our study carried out in the same location in 1995,[19] but the speciation was different, DBT being the prevalent species in 1995 monitoring, revealing change from year to year. We cannot confirm whether these differences are related to different butyltin inputs in

the basin or to different metabolic rates in organ tissues.

There are few reports on tetrabutyltin (TTBT) distribution along the water column, it being always below the detection limits. From an ecotoxicological point of view, tetraorganotins were found to exhibit a toxic effect because of their transformation to triorganotins, which can express their toxicity through different cellular mechanisms. Moreover, in our study we did not pay attention to this form, because it is well known that the maximum biological activity is related to the triorganotin compounds, which are used for this reason as antifouling agents.

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