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Contamination of natural and cultured mussels (*Mytilus galloprovincialis*) from the northern Adriatic Sea by tributyltin and dibutyltin compounds†

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The levels of tributyltin (TBT) and dibutyltin (DBT) compounds were measured by gas chromatography ion-trap mass spectrometry (GC–MS/MS) in natural and cultured mussels (*Mytilus galloprovincialis*) from the northern Adriatic Sea in spring 2003. The highest (725 ng g^{-1} dry weight) and lowest levels (198 ng g^{-1} dry weight) of butyltins (TBT + DBT) were found in native mussels from the Lagoon of Venice, with the higher values being detected in specimens collected next to a dockyard and the lower values in specimens from a location characterized by strong water exchanges between the lagoon and the Adriatic Sea. The two cultured samples exhibited intermediate concentrations, with marine harvested samples being more contaminated than those from the lagoon.

Concentrations of TBT were higher than those of DBT for several reasons: the limited capability of bivalves to metabolize TBT and their ability to accumulate it (with recent input of TBT into the environment as TBT-based anti-fouling paints being the most important contributor of organotin contamination in our study area); and higher lipophilicity of TBT than DBT. In order to find correlations between organotin pollution and the state of nourishment of the mussels, the condition index was also evaluated as the mass ratio of meat dry weight to shell dry weight. There was no correlation between condition index and butyltin concentrations. These results indicate that condition index could not give information about stress conditions, as the contamination level is probably too low to cause significant reductions in mussel growth.

KEYWORDS: tributyltin; dibutyltin; GC–MS; mussel; bioconcentration factor; condition index; northern Adriatic Sea

INTRODUCTION

Mussels are generally recognized as good bioindicators of water-column purity, being resistant; and tolerant, but not insensitive, to low contaminant levels of many toxic compounds, such as organotins.[1–3] They are easy to find close to sources of butyltin pollution, such as dockyards, ports and other sites of human activity.

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The state of nourishment of bivalve molluscs at the seasonal scale or in long-term studies, both in natural[4–6] and farmed[7,8] populations, can be evaluated by the condition index, a ratio of different body parameters of the molluscs. This index is influenced by many factors, such as salinity and concentration of metals in the water,[9] the nutritional state of the individual[10] and its reproductive cycle.[11] The condition index has been applied in environmental monitoring, to correlate with the degree of pollution of the marine environment.[12–14]

The concentrations of tributyltin (TBT) and dibutyltin (DBT) compounds have been measured by gas chromatography ion-trap mass spectrometry (GC–MS/MS) in natural and cultured mussels (*Mytilus galloprovincialis*) from the northern Adriatic Sea. The contaminant concentrations were then compared with values of the condition index, assuming that

butyltin exposure causes lower levels of this parameter in mussels reflecting stress conditions.

MATERIALS AND METHODS

Sampling

M. galloprovincialis samples were collected from cultured and natural populations in spring 2003.

Marine Harvesting (MH) is a long-line offshore mussel farm placed 9.3 km off Porto Caleri (Rovigo, Italy), and Lagoon Harvesting (LH) is a mussel farm situated along channel edges in the southern basin of the Lagoon of Venice. Native mussels were gathered in two lagoon sites next to Chioggia (Venice, Italy): the NA site was located near Chioggia outlet and NB was a relatively internal location, close to a marina (Fig. 1). Water samples (1 l) were collected from the same sites, except for the marine site (MH), filtered through 0.7 μm glass-fibre filters, and adjusted to pH 2 and then stored at −20 °C. Soft bodies of 40 specimens (length range: 5–6 cm) per sample were homogenized and freeze-dried.

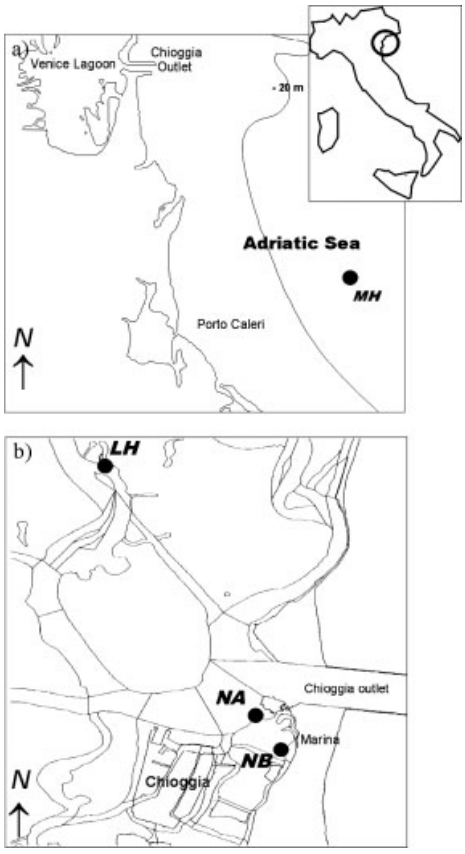


Figure 1. Maps of sampling sites: (a) northern Adriatic Sea; (b) southern Lagoon of Venice.

Analytical procedure

Butyltins (TBT and DBT) were determined in mussel and water according to Binato *et al*.[15] and Caricchia *et al*.[16] respectively. Tributyltin chloride, dibutyltin chloride and tetra-*n*-butyltin (TTBT) standards (96%, 97% and 96% purity respectively) were purchased from Sigma Aldrich.

Extraction

100 mg of each freeze-dried sample were spiked with 50 μl of a TTBT solution (10 mg l^{-1}) as internal standard and extracted with a methanol solution (15 ml) of tropolone (0.05%) and HCl (1 ml) in an ultrasonic bath for 15 min. The methanol solution was then centrifuged at 4000 rpm for 10 min. The supernatant was mixed with MilliQ water (15 ml) and extracted with dichloromethane (10 ml); the procedure was repeated three times. The combined dichloromethane extracts were dried on anhydrous sodium sulfate and after centrifugation the supernatant was separated and evaporated. Finally, the sample was dissolved in *n*-hexane (1 ml).

50 μl of a TTBT solution (10 mg l^{-1}) as internal standard was added to 1 l of water sample, placed into 1.5 l glass separation funnel and the solution was allowed to equilibrate for 10 min. The sample was extracted with a dichloromethane solution (10 ml) of tropolone (0.05%) three times. The combined dichloromethane extracts were dried on anhydrous sodium sulfate and after centrifugation the supernatant was separated and evaporated. Finally, the sample was dissolved in *n*-hexane (1 ml).

Derivatization and clean up

A solution of methyl magnesium bromide (500 μl, 3 mol l^{-1} in anhydrous diethyl ether) was added dropwise (reaction time: 15 min at room temperature) to the mussel and water hexane extracts. The reaction was slowly quenched with 20% aqueous ammonium chloride (2 ml) at 0 °C. 1.5 ml of the upper organic layer of the water sample was directly injected for GC–MS/MS analysis (Trace GC Polaris Q Thermo Finningam). In order to clean up the derivatization product of mussel sample, 1.5 ml of the upper organic layer was purified and eluted with hexane through a 3 g column of activated florisil and anhydrous sodium sulfate (1 g). The eluate was finally evaporated to 1 ml and injected for GC–MS/MS analysis.

GC–MS analysis

The chromatographic conditions were as follows: capillary column Rtx-5MS (i.d. 0.25 mm, length 30 m, film thickness 0.25 μm), carrier gas, helium, 1 ml min^{-1}; injection temperature 270 °C; split-less injection, 1.5 μl injection volume; 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. The fragmentation was obtained by an ion trap in EI (electronic impact) with MS/MS. The parameters used for chromatographic quantitative analysis in the GC–MS/MS analysis are described in Table 1.

Table 1. Parameters used in the GC–MS/MS analysis

Ion	Retention time (min)	Precursor ion (m/z)	Fragmentation ion (m/z)
TBT	7.73	249	193
DBT	6.14	151	135
TTBT	9.35	291	291/235/179

The extraction efficiency was evaluated by analysing spiked uncontaminated filtered sea water (20 ng l^{-1}) and using a certified reference material (BCR-CRM 477) for mussel tissue. The quantification limits of butyltin (TBT and DBT) compounds in mussel tissues and in water were 9 ng g^{-1} and 9 ng l^{-1} respectively. All mussel samples were analysed in duplicate. The recoveries of TBT and DBT in the BCR-CRM 477 were 81 ± 16% and 82 ± 15% respectively of the certified values. The recoveries of TBT and DBT in spiked seawater samples were 97 ± 9% and 101 ± 12% respectively.

The concentrations of TBT and DBT are expressed as cation mass: $Sn(C_4H_9)_3^+$ and $Sn(C_4H_9)_2^{2+}$ respectively.

Bioconcentration factor and condition index

TBT concentrations in mussel tissues were related to water TBT concentrations detected during a concurrent sampling survey (no available data from MH site), to estimate the bioconcentration factor (BCF), defined as the ratio between the TBT concentrations in tissue and in water.

Condition index (DW/SW: DW is dry weight of flesh, oven-dried for 48 h at 104 °C; SW is dry weight of shell, oven-dried for 24 h at 104 °C) was measured on samples of 40 specimens.

Statistical analysis

Results were compared by Tukey tests, as one-way analysis of variance tests showed significant differences between mean values. STATISTICA software (StatSoft Inc., Tulsa, OK, USA) was used for statistical processing.

RESULTS

TBT and DBT concentrations and total amount of butyltins (TBT + DBT) in mussel tissue and in water samples detected by GC–MS/MS are reported in Tables 2 and 3 and in Figs 2 and 3. TBT and DBT concentrations were highest in NB native mussels and lowest for the NA native mussel and MH. At all sites, TBT prevails over DBT.

Water samples from the NB and NA sites show the highest values for TBT and DBT respectively. Also, in water samples, TBT is the prevailing form of the two organotin compounds analysed.

The BCF for TBT at the LH site is the highest (Table 3).

Table 4 and Fig. 4 show the condition index values and levels of significance of Tukey's test. MH had the highest condition index, whereas LH exhibited the lowest index, with significant differences between values ($p < 0.001$). The two native mussel samples had intermediate and not significantly different values ($p < 0.05$).

Table 4. Mean values and standard deviation of condition index (DW/SW) of mussels at different sites

Site	DW/SW (ng g^{-1})
Marine Harvesting	0.171 ± 0.022
Lagoon Harvesting	0.124 ± 0.022
Native A	0.164 ± 0.050
Native B	0.149 ± 0.042

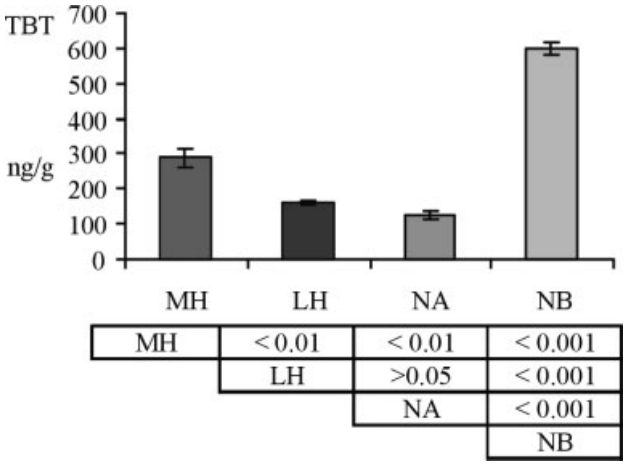
Table 2. Mean values and standard deviations of butyltin concentrations in mussel tissues

Site	TBT (ng g^{-1})	DBT (ng g^{-1})	TBT + DBT (ng g^{-1})	TBT (%)	DBT (%)
Marine Harvesting	288 ± 26	80 ± 2	368	78	22
Lagoon Harvesting	161 ± 5	53 ± 1	214	75	25
Native A	124 ± 12	74 ± 12	198	63	37
Native B	597 ± 17	128 ± 1	725	82	18

Table 3. Values of butyltin concentrations in water samples and BCF

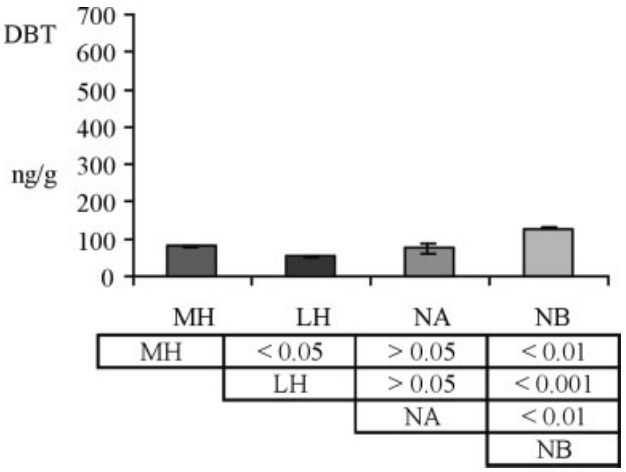
Site	TBT (ng g^{-1})	DBT (ng g^{-1})	TBT + DBT (ng g^{-1})	TBT (%)	DBT (%)	BCF TBT
Marine Harvesting[a]	–	–	–	–	–	–
Lagoon Harvesting	10	<9	<19	53	<47	16 087
Native A	119	67	186	64	36	1044
Native B	667	32	699	95	5	895

[a] Not measured.



MH	< 0.01	< 0.01	< 0.001
	LH	>0.05	< 0.001
		NA	< 0.001
			NB

Figure 2. TBT concentrations at different sites and levels of significance of Tukey's test. MH: Marine Harvesting; LH: Lagoon Harvesting; NA: Native A; NB: Native B.



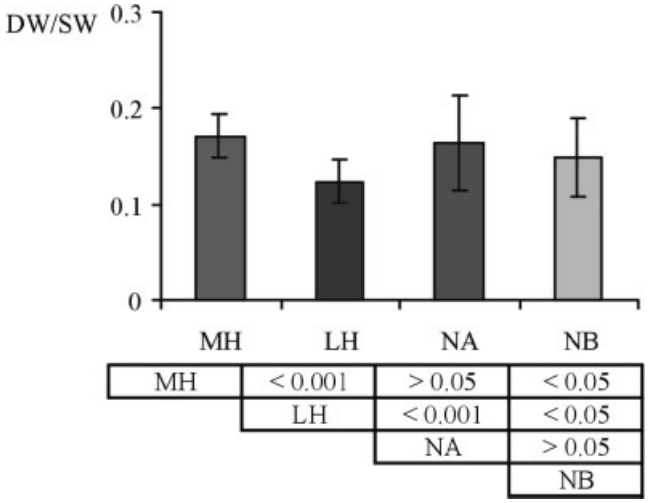
MH	< 0.05	> 0.05	< 0.01
	LH	> 0.05	< 0.001
		NA	< 0.01
			NB

Figure 3. DBT concentrations at different sites and levels of significance of Tukey's test. MH: Marine Harvesting; LH: Lagoon Harvesting; NA: Native A; NB: Native B.

DISCUSSION

Butyltin results for the mussel tissue of MH samples show higher concentrations than expected at the 9 km offshore site. This could be due to the influence of the Po river plume, as well as to the proximity of a big shipyard. The native mussels of the NB lagoon site, close to the marina and with low circulation, exhibited the highest levels of contamination, with mussels being directly exposed to the principal sources of butyltin pollution, such as marina and shipping activities. Lower butyltin concentrations at LH and in native mussels of the NA lagoon site are probably a consequence of the strong hydrodynamic conditions characterizing these areas.

In mussel tissue, TBT concentrations were higher than those of DBT. This could be due to the following reasons:



MH	< 0.001	> 0.05	< 0.05
	LH	< 0.001	< 0.05
		NA	> 0.05
			NB

Figure 4. Condition index (DW/SW) values at different sites and levels of significance of Tukey's test. MH: Marine Harvesting; LH: Lagoon Harvesting; NA: Native A; NB: Native B.

(1) The limited capability of bivalves to metabolize TBT and their ability to accumulate it.[17–20] In fact the molluscs' higher susceptibility to organotin compounds seem to be related to a very low cytochrome P-450 and mixed-function oxygenase activity in the digestive gland.[21]
(2) A recent input of TBT into the environment. TBT can be degraded rapidly in the marine water column to DBT and to lesser amounts of monobutyltin (MBT) with a half-life of several days. Rates of TBT degradation may be influenced by several biotic and abiotic factors, such as the nature and density of microbial populations,[22–24] TBT solubility, dissolved and suspended organic matter, pH, salinity, temperature and light.[25–29]
(3) The concentration of TBT, DBT and MBT in the molluscs decreases with decreasing degree of alkylation[30] because of higher lipophilicity of TBT compared with DBT and MBT.[31]
(4) TBT-based anti-fouling paints are the most important contributors of organotin contamination in our study area, as a consequence of dry-docks and shipyards localized in neighbourhood.

On the basis of TBT concentrations in water, LH, NA and NB can be categorized as lightly, moderately and grossly contaminated sites respectively, in accordance with the criteria reported by Dowson *et al*.[32] Generally, the water TBT levels detected at these sites are due to late spring–early summer boating activities, and the elevated TBT concentration at the NB site is due to its location near to a marina. The highest value at the NB site is consistent with findings reported for other marinas.[33–36] Also, in water samples the TBT concentrations are higher than those of DBT, which is a typical degradation product of TBT in water.[26,37]

The significant positive linear relationship found between TBT accumulation in mussel tissues and water TBT concentrations ($y = 0.0007x + 0.1008$, $R^2 = 0.95$) is comparable to the results of Salazar and Salazar.[38] Our BCFs for NB and NA are in agreement with the BCFs < 9000, for water TBT concentrations >105 ng l^{-1}, reported by Salazar and Salazar;[38] the highest values, estimated for LH, also fall in the range from 5000 to 100 000, at water TBT concentrations <105 ng l^{-1} reported by them.[38] Our results confirm that the lowest concentrations in water correspond to the highest bioaccumulation in mussels. The increase in TBT concentration in molluscs is not proportional to the exposure time of the organisms to the toxic substance, as a consequence of a stationary state in which there is equilibrium between the kinetics of accumulation and degradation–detoxification.[39,40]

In the lagoon, mussels are generally exposed on a chronic basis to a complex mixture of contaminants, with the result being an acclimatization of the organism to severe environmental conditions through the activation of compensatory mechanisms.[13] Moreover, the biological responses to the presence of xenobiotics could be affected both by genetic and physiology variability of the organism (size, age, growth rate).[14] In this study, the hypothesis that the condition index may give information about the stress condition of mussels caused by butyltins has not been supported experimentally, as the butyltin concentrations found in sampled mussels were lower than the threshold value (1500 ng g^{-1} TBT for tissues) reported to cause significant reductions in mussel growth.[38] Furthermore, the condition index does not only depend on contaminant levels, but also on other biotic and abiotic factors.[11,41] Here, in fact, MH showed the highest mean value of DW/SW, probably because marine mussels are subjected less to variations of environmental parameters than lagoon mussels.[42]

In conclusion, the TBT levels in mussel tissues detected in this study are relatively low when compared with those reported in other geographical areas.[15,38,43,44] Considering that the tolerable daily intake for butyltins (TBT + DBT) is estimated as 250 ng per kilogram body weight for humans,[45] a person of 75 kg could tolerate no more than 50 g dry mussels tissue daily (about 90 g of fresh mussels) coming from the MH site.

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