Papers

Butyltins and phenyltins in biota and sediments from the Lagoon of Venice*

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Abstract

Sediments and organisms were sampled to determine organotin contents – butyltins (BTs) and phenyltins (PhTs) – at 12 locations in an estuarine ecosystem, the Lagoon of Venice, characterised by varying contamination impacts. The results showed that organotin contamination in sediments is at lower levels, ranging from 2.5 ± 0.1 to 84 ± 1 ng g^{-1} (d.w.) for ΣBTs and from 0.8 ± 0.2 to 7 ± 1 ng g^{-1} (d.w.),

for ΣPHTs, than in organisms, where the highest concentrations were found in filter feeders like mussels – from 60 ± 3 to 7632 ± 148 ng g^{-1} (d.w.) for ΣBTs and from 0.80 ± 0.01 to 4005 ± 121 ng g^{-1} (d.w.) for ΣPHTs. The possible risk to human health was assessed on the basis of the consumption of edible species sampled in some areas of the lagoon.

1. Introduction

Organotin compounds have been used since the 1940s in different industrial applications. Initially, they were used in the plastics industries as PVC stabilisers and as catalysts for polyurethane foams and silicones, which still represent their main uses. Once their biocide properties were discovered (1950s), they were also used as wood preservatives, pesticides in agriculture and as the active compound in antifouling paints, applied on aquaculture cages, ship hulls and harbour facilities to prevent settling of barnacles, seaweeds or tubeworms (Hoch 2001).

The relatively high toxicity of organotin compounds released into the environment from the applications cited above, in the beginning mostly underestimated, has been extensively investigated since the 1980s, when negative effects on non-target organisms were discovered. Alzieu et al. (1986) found that organotin compounds were responsible for the appearance of symptoms like shell calcification anomalies and the absence of spatfall, with consequent losses in oyster culture farms in Arcachon Bay. Bryan et al. (1986) observed that tributyltin was able to superimpose male sexual characters in female gastropods (*Imposex*), causing sterility and the consequent decline of entire populations, even at very low water concentrations (< 1 ng dm^{-3}). The effects on humans have not yet been studied in detail. Despite TBT restrictions in antifouling paints since 1982 in many European countries, organotin compounds are still consistently being introduced into the environment. To tackle the problem, the International Maritime Organisation has proposed the complete prohibition of these compounds in antifouling paint formulations after 2008. Nevertheless, owing to their hydrophobicity and persistence in sediments, organotins will probably cause problems long after they have been banned, remaining a matter of major concern and requiring constant monitoring for many years to come.

So far, a lot of studies have reported the monitoring of organotin compounds world-wide (Morcillo & Porte 1998, Shawky & Emmons 1998, Elgethun et al. 2000, Yang et al. 2001), but few have taken Italy into consideration. Di Cintio (1988), Bacci & Gaggi (1989), Gabrielides et al. (1990), Caricchia et al. (1992) and Amodio Cocchieri et al. (1993) have focused their studies on water samples, Caricchia et al. (1991) and Rivaro

et al. (1997) on mussel tissues, Terlizzi et al. (1998) on *Imposex*. Gallina et al. (2000) surveyed exclusively mussel tissues from the southern part of the Lagoon of Venice. No other information is available for other species and parts of the lagoon, although organotin pollution seems to have reached significant levels there, as indicated by the appearance of imposex induction on *Hexaplex trunculus* (S. Wilson, personal communication).

The present study was focused on the Lagoon of Venice (Northern Adriatic Sea, Italy), a shallow transitional environment, 550 km^2 in area, characterised by a limited water exchange with the sea. Entering from three inlets – the Ports of Lido, Malamocco and Chioggia – tidal seawater periodically floods the inner areas. This water flows into progressively smaller channels that divide the lagoon into marshes, islands and shelves (Cossu & de Fraja Frangipane 1985). The industrial district of Porto Marghera adjacent to the lagoon has until recently been the source of broad spectrum contamination and most probably still is, owing to the large amounts of contaminated sediments resting in the canals of the industrial port (Pavoni et al. 1987, Pavoni et al. 1992, Frignani et al. 1998). Freshwater enters the lagoon from a number of tributaries conveying agricultural run-off from the drainage basin. The use of TBT-based antifouling paints has been regulated since 1982, with application on vessels shorter than 25 metres being prohibited.

The aim of the present investigation was to establish the levels of contamination by organotin compounds in sediments and edible organisms belonging to different trophic levels in the Lagoon of Venice, to identify the most contaminated areas and species, and to estimate the possible risk to human health resulting from seafood consumption.

2. Material and methods

2.1. Sampling

In the autumn and winter of 1999–2000, sediments and organisms from different trophic levels (*Mytilus galloprovincialis*, *Tapes* sp., *Carcinus maenas*, *Atherina boyeri*, *Anguilla anguilla*, *Zoosterisessor ophiocephalus*) were collected at 12 sampling sites in the Lagoon of Venice variously affected by contamination: 10 sites were located close to shipyards and the other 2 (Stations 7 and 9) were in areas with few shipping activities, to serve as 'references' (Fig. 1). Every sampling area corresponded to 200 m^2, in which 500 g of sediments and individuals of each species of organisms sampled. All samples were collected from a boat free of antifouling paints; superficial sediments were sampled with a grab.
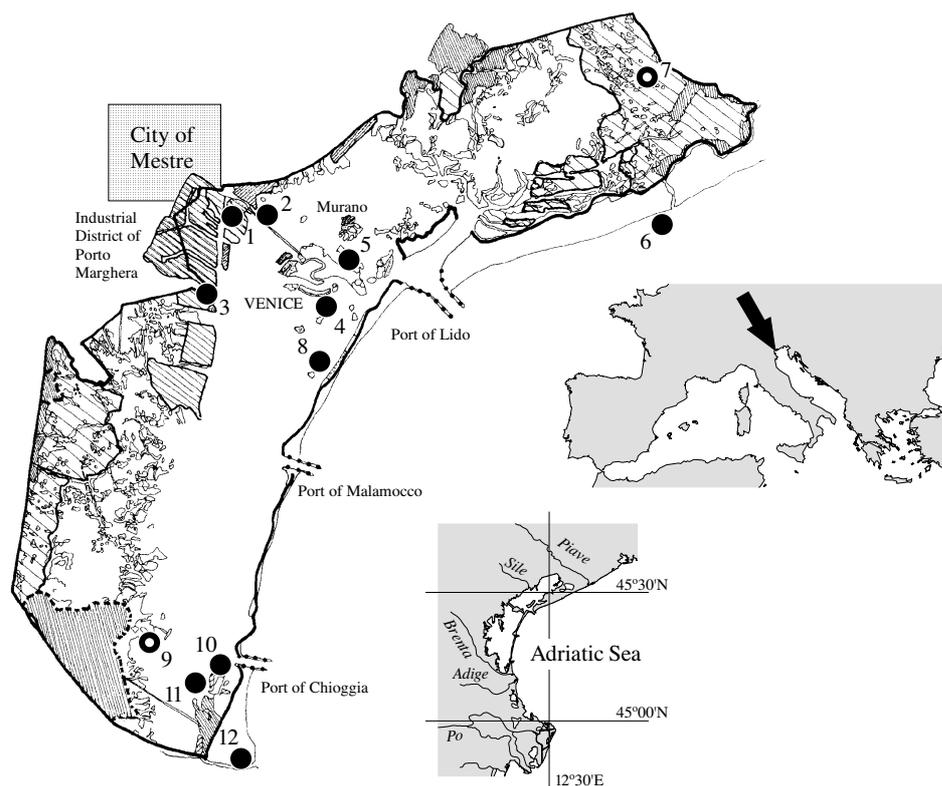
Fig. 1. Locations of the sampling stations in the Lagoon of Venice

Immediately after collection, the organisms were carried to the labora-
tory, shelled or dissected, pooled, homogenised, divided into 5 sub-samples,
freeze-dried and maintained at $-20°C$ in the dark till analysis in order
to preserve their organotin content and speciation from degradation, as
suggested by Caricchia et al. (1994) and Gomez-Ariza et al. (1999).

2.2. Organotin analyses

The following procedure was used to quantify tributyltin (TBT),
triphenyltin (TPhT), dibutyltin (DBT), diphenyltin (DPhT), monobutyltin
(MBT) and monophenyltin (MPhT).

Freeze-dried samples (0.5 g) were spiked with the internal standard
tripropyltin and extracted twice for 15 minutes with 15 cm^3 of a methanol
solution of tropolone (0.03%) and 1 cm^3 of HCl in a sonication bath. The
extract was then centrifuged for 10 minutes and liquid-liquid-extracted
twice in a separating funnel with 15 cm^3 of CH_2Cl_2 and 100 cm^3 of 5%
NaCl in deionised water. The organic phase (dewatered with activated

sodium sulphate, diluted with 1 cm^3 isooctane, then reduced to 1 cm^3 in a gentle stream of nitrogen) was treated with the derivatising agent pentylmagnesium chloride (1 cm^3, 2 M in diethylether) for 5 minutes at room temperature. The excess Grignard reactant was destroyed by adding carefully, drop by drop, 2 cm^3 of deionised water. The derivatised organotins were subsequently extracted twice with 2 cm^3 of n-hexane and 5 cm^3 of 1 M sulphuric acid. Then, as a clean-up step, the sample, reduced to 0.5 cm^3 under a stream of nitrogen, was eluted with 10 cm^3 of 1 : 1 hexane-toluene through a 3 g column of activated silica gel (sediment samples) or florisil (biota samples), previously soaked with the same solution. The eluate was finally evaporated to 1 cm^3 under nitrogen and analysed with an HRGC mass-spectrometer. The chromatographic conditions were as follows: capillary column HP–5 (5% phenyl methyl silicone, i. d. 0.32 mm, length 25 m, film thickness 0.25 μm); injector temperature, 240°C; temperature programme, 80°C for two minutes, then 10°C min^{-1} up to 280°C; transfer line temperature, 280°C; splitless injection; carrier gas helium at 120 kPa head pressure. The MS detection was performed using electron impact ionisation (70 eV) in the selected ion monitoring mode (SIM): the masses and retention times used for identification of organotin compounds are reported in Table 1. All samples were analysed in triplicate.

Table 1. Ion masses, precision and sensitivity (LOD: level of detection) of the analytical procedure

	Retention time	Ion mass	% recovery	Precision [%] n = 3	LOD [μg kg^{-1} d.w.]
TPrT (i. s.)	19.58	277, 275, 273			
TBT	22.18	305, 303, 301	89	7	0.2
DBT	23.07	319, 317, 315	92	8	0.2
MBT	23.92	319, 317, 315	92	4	0.3
MPhT	26.60	339, 337, 335	75	6	0.6
DPhT	29.02	345, 343, 341	76	5	0.6
TPhT	32.63	351, 349, 347	71	9	0.4

Organotin compounds are known to cause toxic effects even at very low concentrations. For this reason, the analytical determination and speciation of this class of compounds have to be performed by a technique with good precision and sensitivity, even with the most complex matrix, targets which can be achieved by Grignard derivatisation followed by GC/MS (Stäb et al. 1994).

The analytical procedure used in this work was adapted from Morabito et al. (1995). The accuracy of the analytical methods was measured by

participating in an interlaboratory exercise and using a reference material (mussel tissue CRM 477), certified only for butyltins (certified values [mg kg^{-1}]: TBT: 2.20 ± 0.19; DBT: 1.54 ± 0.12; MBT: 1.50 ± 0.27; obtained values: TBT: 1.89 ± 0.26; DBT: 1.45 ± 0.11; MBT: 1.50 ± 0.20). Recovery values were determined by means of a spiking experiment: yields were about 90% for butyltins and more than 70% for phenyltins (Table 1). Detection limits of the entire procedure, expressed as 3σ of the blanks, are less than 0.3 μg kg^{-1} (d.w.) for butyltins and less than 0.6 μg kg^{-1} (d.w.) for phenyltins, while the precision of the determination, expressed as CV, is $\pm$ 10%.

3. Results and discussion

Among the heterogeneous class of organotin compounds, tributyltin (TBT), triphenyltin (TPhT), and their breakdown products containing one (mono-) or two (di-) organic groups, DBT, MBT, DPhT and MPhT were considered of interest for the Lagoon of Venice in view of their actual presence in this area. In fact, TBT is used mainly as an active compound in antifouling paints, and is most likely still used in the vicinity of the stations under investigation, DBT is used as a catalyst in PVC polymerisation in the industrial zone of Porto Marghera, and TPhT is used as a pesticide in agriculture and a co-toxicant in some antifouling paints.

Sediments

The organotin concentrations at all the sampling stations are reported in Fig. 2. It seems that the degree of pollution of the sediments is quite homogeneous in the Lagoon of Venice: concentrations of these contaminants are similar at all the stations. In each area butyltins were found at higher concentrations than phenyltins, which are below or just above the detection limit of the analytical procedure. Station 9, though considered to be a clean reference area, surprisingly presents concentrations comparable to the other zones. By contrast, at the second clean reference site, station number 7, butyltins and phenyltins were near the detection limits. By inspecting the relationships between TBT and its degradation products, it appears that TBT generally prevails over DBT and MBT. In fact, the relative percentage of the parent compound towards the entire butyltin concentrations ranges from 35% to 81%, with an average value of 60%, while the relative percentages of DBT and MBT range from 2.8 to 29.1% and from 9.9 to 37% respectively. These values suggest a fresh input of TBT into the environment or a slow degradation process occurring in this matrix.
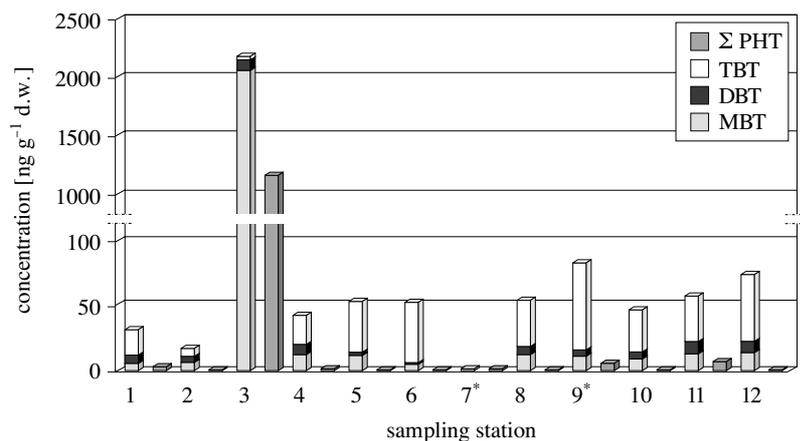
Fig. 2. Concentrations of organotin compounds (ng g^{-1} d.w.) in sediments at the sampling sites in the Lagoon of Venice

By contrast, the concentrations detected at station 3, which is quite close to four dockyards and to the industrial zone of Porto Marghera, indicate the occurrence of old butyltin pollution, since the levels of TBT degradation products is considerable (MBT: 2053 ng g^{-1} d.w.), while the parent compound remains at a relatively low level (TBT: 32 ng g^{-1} d.w.). Here, TPhT reaches concentrations three orders of magnitude higher than at the other stations (TPhT: 840 ng g^{-1} d.w.), revealing a possible recent input of this pollutant into the environment. The concentrations found in the Lagoon of Venice are consistent with those reported by Gomez-Ariza et al. (1998) in south-west Spain (TBT: 1.1–130 ng-Sn g^{-1} d.w.), by Quevauviller et al. (1989) in the Sado Estuarine System (TBT: 12–520 ng g^{-1} d.w.), by Gabrielides et al. (1990) in the Mediterranean region (TBT: 35–975 ng-Sn g^{-1} d.w.), but lower than those found by Tolosa et al. (1992) in Western Mediterranean coastal enclosures (TBT: 9260 ng g^{-1} d.w.; TPhT: 4160 ng g^{-1} d.w.), by Diez et al. (2002) on the north-western and south-western coasts of Spain (TBT: 124–18722 ng g^{-1} d.w.; TPhT: 15–271 ng g^{-1} d.w.), by Barakat et al. (2001) in the commercial harbour of Alexandria City (TBT: 1–2067 ng-Sn g^{-1} d.w.) and by Page et al. (1996) (TBT: 24–3900 ng g^{-1} d.w.) in intertidal locations in Maine (USA). Comparison of butyltin and phenyltin levels in the sediments of the Lagoon of Venice with other ecosystems suggests a relatively low organotin contamination. However, it is worth noting that the half-life of organotin compounds has been estimated at a few weeks in the water and from 1.3 to 4.4 years in sediments (De Mora et al. 1995, Sarradin et al. 1995). Therefore, this matrix can be regarded as the ultimate sink for

organotins, but also as a possible future source for resuspension in water and bioavailability to organisms (Page et al. 1996, Berg et al. 2001).

Biota

Six species of organisms were chosen, featuring different mobility, habitat and behaviour: two sessile species, *M. galloprovincialis* and *Tapes* sp., indicators of water columns of different depths; one benthic species *C. maenas*, and three species of fish (*Z. ophiocefalus*, *A. boyeri* and *A. anguilla*) with different ecological behaviours.

Concentration levels in biota (Figs. 3a–f) are higher than in sediments, therefore organotin compounds can be considered persistent and bioaccumulative pollutants in the same way as other organic compounds such as PAHs and PCBs. For most species, the highest concentrations were found at stations 1, 2, 3 and 4, whereas at the other stations the organisms were less contaminated. As the former stations were selected very close to dockyards, the extensive use of antifouling paints is a reasonable explanation for these higher levels of contamination.

The levels of concentrations in the different organisms, averaged over all the stations (Figs. 4a–b), indicate that butyltins are much more important contaminants than phenyltins. In fact, whereas the latter were found at concentrations just above the detection limit in most of the organisms, the former accumulate mainly in filter-feeding organisms (*M. galloprovincialis* and *Tapes* sp.). The concentrations detected in the whole soft body of *M. galloprovincialis* were similar to those found in specimens of the same organisms in other areas by Quevauviller et al. (1989) (TBT: 16–114 ng g^{-1} d.w.), Rivaro et al. (1997) (TBT: 250–1060 ng g^{-1} w.w.), Morcillo et al. (1997) (TBT: 3516 ng-Sn g^{-1} d.w.) and Tselentis et al. (1999) (TBT: $\sim$800 ng-Sn g^{-1} d.w.). The concentrations found in this species at stations 10 and 11, located in the southern part of the lagoon, are comparable with those reported by Gallina et al. (2000) for sampling stations located nearby.

The relationships between the different congeners in the two molluscs considered in this study show that in general TBT>DBT>MBT. This is in agreement with other studies on bivalves that have described the limited capability of these organisms to metabolise TBT and their ability to accumulate it (Laughlin et al. 1986, Wade et al. 1990, Regoli et al. 2001, Roper et al. 2001). The high values of TBT concentrations in these species and its slow metabolism can also be deduced from the TBT : total butyltin

Fig. 3. Organotin concentrations (ng g^{-1} d.w.): in *Mytilus galloprovincialis* (a), in *Tapes* sp. (b), in *Carcinus maenas* (c), in *Zoosterisessor ophiocephalus* (d), in *Atherina boyeri* (e), in *Anguilla anguilla* (f)

a
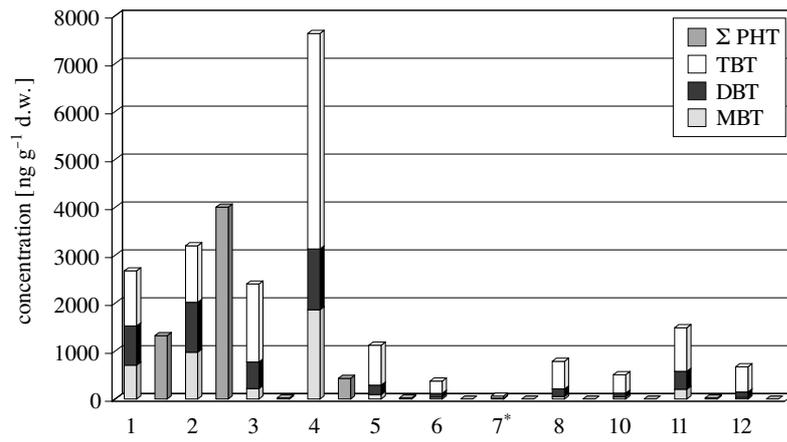
b
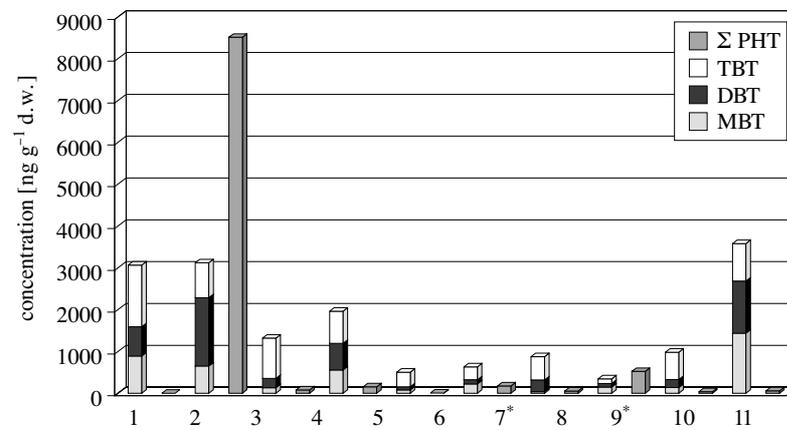
c
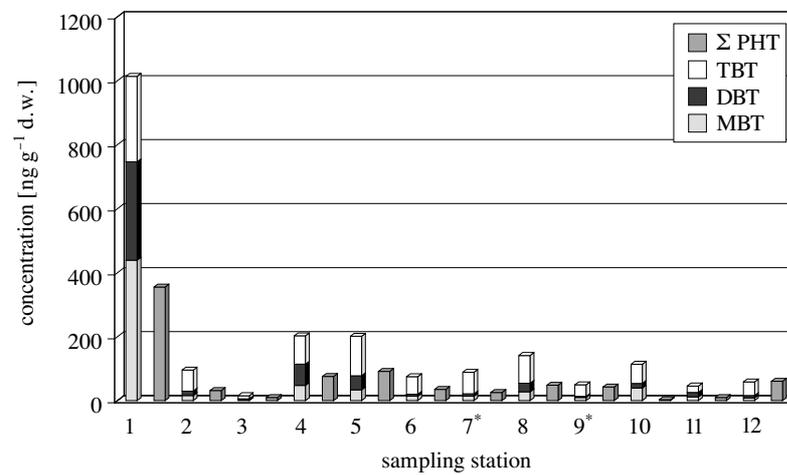
d

e

f

Fig. 3. *(continued)*

a

b
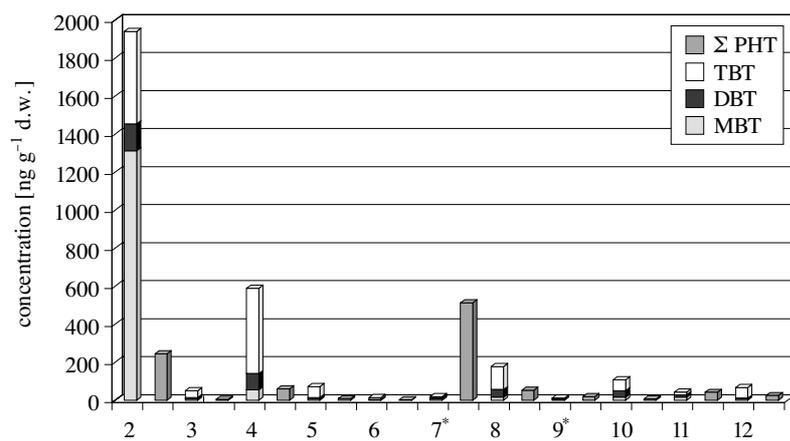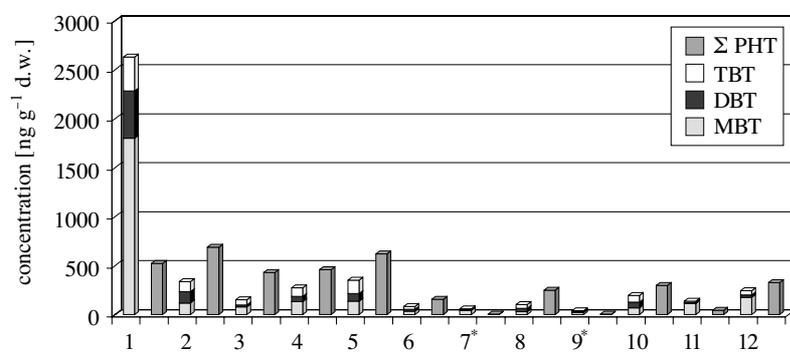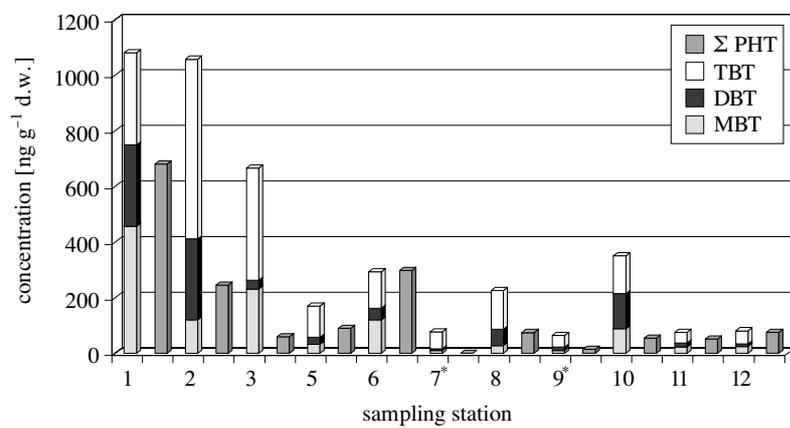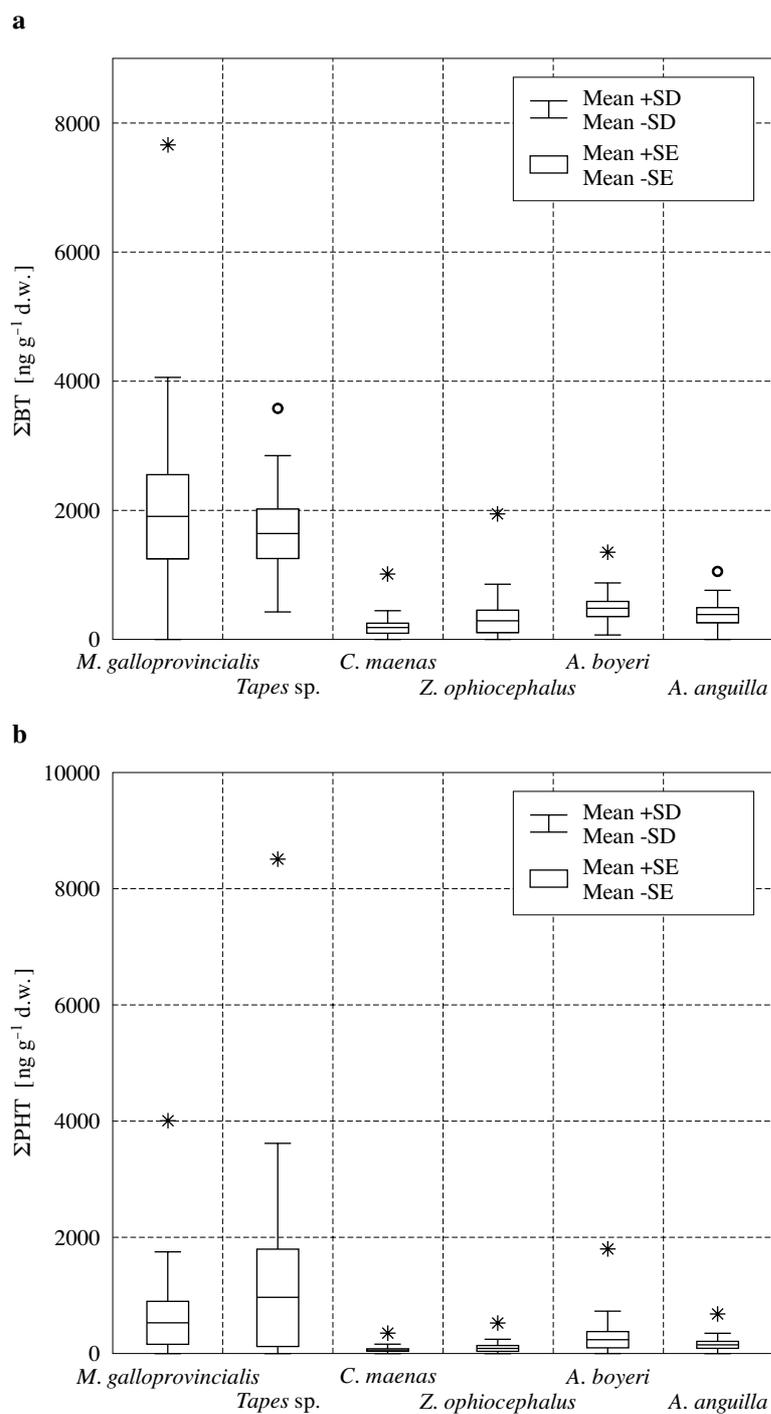
Fig. 4. Box plots of average concentrations (ng g^{-1} d.w.) for butyltins (a), for phenyltins (b)

ratio, which is in the 0.4–0.8 range for *M. galloprovincialis* and 0.3–0.7 for *Tapes* sp., figures that are in agreement with the values of 0.5–0.8 reported by Morcillo et al. (1999).

Furthermore, Lee (1991) has observed the molluscs' higher susceptibility to organotin compounds and has hypothesised that this must be related to a very low cytochrome P–450 content and mixed-function oxygenase activity in the digestive gland. On the other hand, the higher concentrations of these enzymes in fishes induce a greater metabolic degradation of organotin compounds, thus explaining the lower contamination in these tissues with respect to inferior species in the food-web. This evidence makes molluscs, and in particular mussels, which were found to be better 'concentraters' than clams, the most suitable indicators of organotin pollution, sentinel organisms which accumulate and retain these compounds for a significant period (Laughlin et al. 1986).

Significantly lower concentrations, lower even by one order of magnitude, were found in the other species considered, which have not been studied in any report before the present one.

Concentrations in all the species of biota (6 variables and 67 observations) were processed by multivariate statistical methods to synthesise the information content by means of Statistica 5.1 software. Significant correlations ($r > 0.6$) were found between each of the butyl congeners and the others (TBT, DBT and MBT) and, similarly, among the phenyltin congeners (TPhT, DPhT and MPhT). As expected, no significant correlation was observed between butyltin and phenyltin compounds, thus confirming the different origins and use patterns of these classes of congeners.

Potential risk to human health

From the organotin concentrations in organisms the potential risk to human health was assessed in relation to the average consumption rate of local population; in fact, the species analysed are commonly available in fish markets.

The Tolerable Daily Intakes (TDI) of TBT and DBT were estimated at 0.25 μg kg$_{\text{body weight}}^{-1}$ per day by Penninks (1993), who extrapolated TBT toxicity from rats to humans, applying the most precautionary factors. Belfroid et al. (2000), starting from Penninks' TDI, introduced a new tool to establish when organotin concentrations are unsafe for human consumption: TARL (Tolerable Average Residue Level), which is defined as the level of TBT in seafood that is tolerable for the average consumer with an average weight of 60 kg. In view of a daily seafood consumption rate for the population of Venice of about 70 g day^{-1}, TARL for TBT in this region is equal to 857 μg kg^{-1} (d.w.). This threshold limit is slightly more restrictive

than the tissue screening level reported by Elgethun et al. (2000) for the USA (i.e. 1120 μg TBT kg^{-1} tissue), obtained from EPA's oral reference dose for TBTO (Tributyltinoxide) of 0.28 μg kg$_{\text{body weight}}{}^{-1}$ per day and a daily consumption of 15 g of seafood for a 60 kg adult. Above these values a potential risk exists for human health.

A comparison between the modified values of TARL and of TBT concentrations obtained in the lagoon biota shows that all samples are below this level, except in mussels and clams, whose TBT values averaged over all the stations exceed TARL. This observation has already been made by Belfroid et al. (2000) on marine mussels from the Italian coast. With respect to each sampling station, TBT levels in specimens of *M. galloprovincialis* exceed TARL at stations 1, 2, 3, 11 and especially 4 (4500 ng g^{-1} d.w.), while DBT levels exceed this value only at stations 2 and 4. In the case of *Tapes* sp., TBT concentrations are higher than TARL at stations 1, 3 and 11, and those of DBT at stations 2 and 11. Finally, if we consider the sum of TBT and DBT concentrations, more samples are above the tolerable level. As a matter of fact, at stations 1, 2, 3 and 4 fishing was declared illegal by the local authorities. At these stations, organotin pollution is only one aspect of the overall contamination, which includes a large spectrum of pollutants, namely heavy metals, PAHs and PCBs (Frignani et al. 2001, Bellucci et al. in press). More alarming is the contamination level found at station 11, where fishing is not prohibited at all.

TARL can be regarded as a useful tool for evaluating whether there is a potential risk in the human diet due to the consumption of seafood products. However, it is excessively simplistic in that it only considers the effects on the average person, and neglects diversity of diet between and within populations and possible synergic effects between different compounds. There is thus an urgent need for further investigations in this direction.

4. Conclusions

This study discusses the contamination level of organotin compounds at twelve sites in the Lagoon of Venice (Northern Adriatic, Italy), both for specimens of organisms at different trophic levels and for sediments. The two classes of compounds, butyltins and phenyltins, were detected at significantly different concentrations and showed no correlation to one another. The more contaminated stations were those located in lagoon areas affected by higher boat traffic or boat maintenance activities. Filter-feeding organisms in the Lagoon of Venice were found to accumulate TBT at levels comparable to those found in other areas. At some sampling stations

the levels of TBT contamination in organisms exceeded the tolerable level in seafood, demonstrating a possible risk to human health and the need for further investigations.

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