Arch. Environ. Contam. Toxicol. 51, 608–614 (2006)
DOI: 10.1007/s00244-005-0252-z

ARCHIVES OF
Environmental
Contamination
and Toxicology

Assessment of Butyltin and Phenyltin Pollution in the Sea Mullet, *Mugil cephalus*, Along the Moroccan and Spanish Coasts (Mediterranean Sea)

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Received: 20 October 2005 /Accepted: 2 March 2006

Abstract. Levels of three butyltin (BTs) compounds, monobutyltin (MBT), dibutyltin (DBT) and tributyltin (TBT), and three phenyltin (PTs) compounds, monophenyltin (MPhT), diphenyltin (DPhT), and triphenyltin (TPhT), were determined in the sea mullet, *Mugil cephalus*, collected along the northern Mediterranean coast of Morocco, and the south Mediterranean coast of Spain. Also, sediment samples from the Guadalquivir River (Spain) near to its mouth in the Mediterranean Sea were analysed. TBT and TPhT were the predominant compounds in the analyzed samples, and TBT concentrations were higher in liver than in muscle. In general, the total content of BTs in these samples was higher than PT levels. In the Moroccan coast, the highest value of BTs (18.00 mg/Kg wet weight) was found in the west harbour of the Tangier site, while the lowest concentration (0.01 mg/Kg wet weight) was detected in the Oued Laoue site. Concerning PTs compounds, the highest value (4.00 mg/Kg wet weight) was found in the harbour of the M'diq Site and the lowest value (0.01 mg/Kg wet weight) was detected in the Oued Laoue site. On the other hand, organotin (OT) residues were not detected in the water samples of the Spanish coast although TBT was identified in four of the nine sediment samples from the Guadalquivir River at concentrations between 15.45 and 245.07 mg/Kg wet weight.

The need for effective chemical compounds that prevent the growth of marine organisms on submerged structures such as fish cages and on immersed equipments such as fishing nets is widely recognised. Organotin (OTs) compounds have been used worldwide as biocides in antifouling paints for this aim. Specifically, tributyltin (TBT) and triphenyltin (TPhT) compounds have been shown to have very good fungicidal and bactericidal properties. So, during the past several decades they have been widely used as antifouling agents in marine paints (Fent, 1996), and from this use derives the larger input of OTs into marine aquatic systems. In addition, OTs are hydrophobic compounds that present a long half-life and lipophilic properties, which facilitate their accumulation in adipose tissues of organisms. These compounds have been found in a variety of marine organisms, often at concentrations exceeding the acute and chronic toxicity levels (Le Thi *et al.* 1999).

A reduction of contamination levels occurred after restrictive regulations prohibiting the use of TBT for boats of less than 25 m were gradually adopted in Western countries, and paints were developed that ensured a better control of the solve out (Fent and Meier 1992; Michel *et al.* 2001). Despite optimistic forecasts by various scientists after these regulatory measures taken in the 1980s, coastal TBT contamination is still a major problem. Nowadays their use has been restricted in many countries due to their high toxicological relevance, but contamination of harbour sediments persists due to the low degradation of these compounds in sediments (Fent and Hunn 1991).

Several monitoring programmes or survey studies, conducted in Atlantic and North sea areas, have shown that in most of these European coastal waters TBT levels were greater than ng/dm^3. The information available for Mediterranean sea also reveals contamination by OTs compounds in waters (Alzieu *et al.* 1990; Kubilay *et al.* 1996; Gómez Ariza *et al.* 1998; Gómez Ariza *et al.* 2001), sediments and marine organisms (Tolosa *et al.* 1992; Gómez Ariza *et al.* 1995; Kubilay *et al.* 1996; Gómez Ariza *et al.* 1998; Amouroux *et al.* 2000; Gómez Ariza *et al.* 2001; Borghi *et al.* 2002; Chiavarini *et al.* 2003; Díez *et al.* 2002). On the other hand, in Morocco, an important maritime country situated between the Atlantic and Mediterranean waters, no legislation is currently in place to control the importation or use of TBT-based paints despite its interdiction in the nearest European country. The waters of the Mediterranean are very important transportation corridors between European countries and Morocco, and towns in northern Morocco and the South of Spain (Andalusia region) are especially known to have heavy shipping traffic.

Chemical analysis for OTs, both parent compounds and metabolites, is complicated, expensive, and requires the application of effective extraction and sample purification techniques, followed by a final chromatographic determina-

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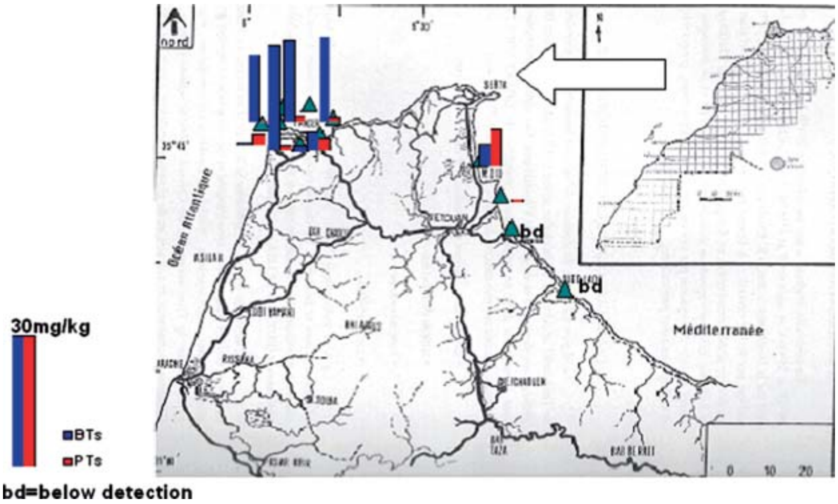

Fig. 1. Map of the Northern Mediterranean coast of Morocco showing sampling locations and concentrations of OT compounds in fishes of each sampling location

tion. We are interested in evaluating the extent of OT contamination in the fish specie Sea mullet, *Mugil cephalus*. It was selected because it can be fished for in the harbours, it presents a good adaptation with the hostile surroundings, and it is a popular fish in the Moroccan diet. For this reason, there is great concern about the OT content of this species because of its toxicological implications for public health. This study is considered the first one that was carried out to evaluate the level of OT contamination in Morocco.

Hence, the main objective of this study was to determine the occurrence of OT compounds, including TBT and its metabolites monobutyltin (MBT) and dibutyltin (DBT), and TPhT and its metabolites monophenyltin (MPhT) and diphenyltin (DPhT), in harbours and open areas in the north of Morocco and to compare the results with those obtained in harbours and open areas of the South of Spain (Andalusia coast). For this aim, the sea mullet, *Mugil cephalus*, was used as sentinel organism. In addition, sediment samples from the Guadalquivir River (Andalusia) were also analysed. Monitoring of target analytes was carried out by gas chromatography (GC) using the tandem mass spectrometry (MS/MS) operation mode, which is particularly useful for the analysis of organic compounds at trace levels in complex samples, because it enables the separation and certaintly of identification with each detected compound.

Materials and Methods

Chemicals

OTs standards were obtained from Riedel-de-Haën (Seelze-Hannover, Germany); purity always >99%. OT chloride stock standards were prepared at 300 mg/l in acetone, except TBT stock standard that was prepared in n-hexane. All standards were stored at 4°C and working solutions were prepared weekly by diluting the stock solutions with acetone. Pesticide-quality solvents (n-hexane, toluene, acetone) and acid chloride were supplied by Panreac (Barcelona, Spain). Sodium diethyldithiocarbamate (DDTC) was obtained from Fluka (Madrid, Spain), purity >99%. Sodium chloride and ammonium chloride were obtained from Panreac with a purity >99%. Grignard reagent (n-pentylmagnesium bromide, PeMgBr, 2.0 M in diethyl ether) was obtained from Aldrich (Barcelona, Spain). Alumina (type N) in syringe barrel format was supplied by Waters Corporation (Milford, MA).

Site Description and Sampling

Sampling sites for fish samples were distributed in the Northern Moroccan Mediterranean coast (Fig. 1) and in the South Spanish coast (Fig. 2), both in the Mediterranean Sea. Also, sediment samples were taken from the Gualdalquivir River (Fig. 2).

Fish samples captured were stored immediately in a deep freezer and transferred to the laboratory within 4 h of collection. At the laboratory, samples were pooled to 10 or 12 individuals with the same size. Then, liver and muscle were separated and mixed in the pool, and stored frozen at −20°C in the dark until analysis. Sediment samples were collected in glass containers. Samples were cooled to 4°C or below in the field and kept refrigerated at 4°C away from light prior to extraction, which was done within 48 h. The extracts were analyzed within two weeks of collection. Handling avoided the contamination of the samples.

Analytical Procedure

OT analysis was based on the method described by Martínez Vidal *et al.* (2003) with some modifications. Briefly, to 5 g of fish sample weighted into a beaker were added tripropyltin chloride (TPrTCl) (10 μg/L) as internal standard (IS), and 10 mL HCl 10%. The mixture was sonicated for 30 min. Afterward, 10 mL of toluene and 0.02 g of DDTC were added and the mixture was sonicated for 15 min, and centrifuged at 3000 r.p.m. for 10 min. The organic extract was filtered through a conic Buchner with anhydrous sodium sulfate to a glass conical vessel for the derivatization step cooled by ice. TPrTCl (10 μg/L) was added as IS to 2 g of sediment samples weighted into a beaker, as well as 5 mL of toluene, and 0.02 g of DDTC. The mixture was sonicated for 5 min, and then filtered in a conic Buchner containing anhydrous sodium sulfate. The process was repeated 3 times and the organic extracts were combined in a glass conical vessel for the derivatization step. This was performed by adding 0.5 ml of Grignard reagent (n-pentymagnesium bromide), then by shaking for 1 min and standing for 20 min at ambient temperature. The addition of 2 mL of NH_4Cl 20% eliminated the excess of Grignard reactive. The derivatized extracts were centrifuged at 3000 r.p.m. for 10 min and

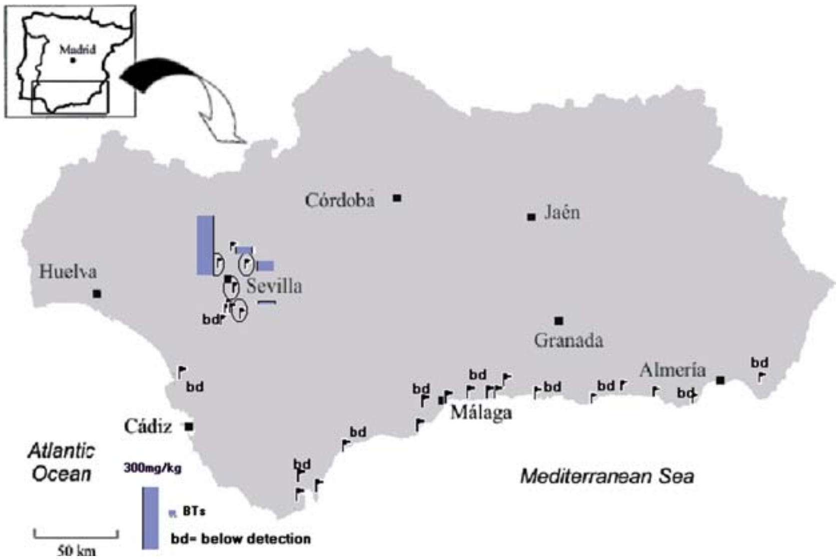

Fig. 2. Map of the South Mediterranean coast of Spain showing sampling locations and concentrations of OT compounds in samples

Table 1. LOD, LOQ, calibration date, average recoveries, and precision (n = 10) values of the target compounds by GC-MS-MS in liver samples

Compound	Precursor ion (m/z)	Quantification ion (m/z)	LOD (μg/Kg)	LOQ (μg/Kg)	Linear range (μg/Kg)	R^2	Recovery (%)[a]	RSD (%)[a]
TBT	179	121 + 135	1.25	3.80	20–100	0.996	74.6	9.7
MBT	193	133:135	5.49	18.6	20–100	0.998	82.6	12.0
DBT	319	191:193	0.98	3.41	20–100	0.992	81.7	12.4
MPhT	269	197	5.10	16.3	20–100	0.997	74.9	14.8
DPhT	275	195:197	0.15	0.31	20–100	0.998	78.6	10.4
TPhT	351	195:197	0.12	0.40	20–100	0.999	78.8	11.9

[a] 50 μg/kg.

cleaned up by passing the extracts through alumina cartridges used as received. OT compounds were eluted with 5 mL of n-hexane, and the eluate was gently vented with nitrogen down to a final volume of 0.5 mL; 10-μL aliquots of the last solution were injected into the gas chromatograph operating at a syringe injection flow rate of 1 μL/s.

Instrumental Analysis

OTs were determined by low-pressure gas chromatography (LP-GC) using a Varian 3800 gas chromatograph with Electronic Flow Control (EFC) coupled with a Saturn 2000 ion-trap mass spectrometer (Varian Instruments, Sunnyvale, CA). The chromatograph was equipped with an 8200 autosampler and an SPI/ 1079 split/splitless programmed-temperature injector operated in the large volume injection technique. The glass liner contained a plug of carbofrit (Resteck Corp., Bellefonte, PA). A fused silica capillary column 2 m × 0.25 mm i.d. from Supelco (Bellefonte, PA) was used as guard column. Separations were carried out on a Rapid-MS (0.6 m × 0.10 mm i.d. restriction coupled to WCOT fused silica CP-Sil 8 CB low bleed of 10 m × 0.53 mm i.d. × 0.25 μm film thickness) analytical column. The initial injector temperature, 70°C, was held for 0.5 min and then increased at 100°C min^{-1} to 310°C, which was held for 10 min. After injection, the column temperature, initially 70°C, was held for 3.5 min, and then increased at 20°C min^{-1} to 300°C, which was held for 3 min

The ion-trap mass spectrometer was operated in EI-MS/MS mode. The transfer line, manifold, and trap temperatures were 280°C, 50°C, and 200°C, respectively. The analysis was performed with a filament-multiplier delay of 6 min in order to prevent instrument damage. The Automatic Gain Control (AGC) was activated with an AGC-target of 5000 counts. The specific precursor and quantification ions are shown in Table 1. The computer that controlled the system also held an EI-MS/MS library specially created for the target analytes under our experimental conditions. Other EI-MS libraries were also available. The mass spectrometer was calibrated weekly with perfluorotributyamine. Helium (99.999%) at a flow rate of 1 mL/ min was used as carrier and collision gas.

Results and Discussion

Evaluation of Analytical Method

In order to check the feasibility of the LP-GC method for the analysis of OTs in fish and sediment samples, it was validated in both matrices. All experiments were carried out using wet samples. In consequence, all the results (mg/kg) are referred to wet matrices.

Detection (LOD) and quantitation (LOQ) limits were calculated through the definition based on the standard deviation of the signal of blank (in our study blank fish or sediment extracts) injections following IUPAC recommendation. In liver samples, LOD values ranging from 0.12–5.49 μg/Kg and LOQ values ranging from 0.31–18.6μg/kg. In sediment samples LOD values ranging from 0.12–0.67 μg/Kg and LOQ ranging from 0.38–2.12 μg/kg were obtained (Tables 1 and 2). Linear calibration graphs were constructed by least-squares regression of concentration versus peak area ratio (analyte /IS)

Table 2. LOD, LOQ, calibration date, average recoveries, and precision (n = 10) values of the target compounds by GC-MS-MS in sediment samples

Compound	LOD (μg/Kg)	LOQ (μg/Kg)	Linear range (μg/Kg)	R^2	Recovery (%)[a]	RSD (%)[a]
TBT	0.50	1.63	10–100	0.999	94.6	10.4
MBT	0.67	2.12	10–100	0.992	92.6	9.0
DBT	0.12	0.46	10–100	0.990	87.0	7.9
MPhT	0.49	1.82	10–100	0.998	79.0	11.5
DPhT	0.14	0.38	10–100	0998	82.9	12.4
TPhT	0.17	0.50	10–100	0.999	88.5	9.9

[a] 100 μg/Kg.

Table 3. Concentrations of TBT and DBT and their sum (BT) and TPhT and DPhT and their sum (PT) in fish (in mg/Kg weight wet) from Morocco

Location	N	Organ	TBT	DBT	ϵBTs	TPhT	DPhT	ϵPTs
Tangier								
1. East harbour 1	10	Liver	6.83	4.20	11.03	0.26	0.07	0.33
	10	Muscle	3.02	nd	3.02	0.05	nd	0.05
2. East harbour 2	11	Liver	8.57	3.20	11.77	1.01	0.34	1.35
	11	Muscle	5.54	Traces	5.54	nd	nd	-
3. West harbour 1	10	Liver	18.00	nd	18.00	0.58	0.13	0.71
	10	Muscle	nd	nd	-	0.36	nd	0.36
4. West harbour 2	10	Liver	10.17	6.54	16.71	0.77	0.05	0.82
	10	Muscle	8.11	nd	8.11	0.04	nd	0.04
5. Marina	9	Liver	4.15	nd	4.15	2.82	0.01	2.83
	9	Muscle	nd	nd	-	0.04	nd	0.04
6. Open area 1	10	Liver	0.21	nd	0.21	1.12	0.04	1.16
	10	Muscle	0.09	nd	0.09	0.19	nd	0.19
7. Open area 2	12	Liver	0.13	0.34	0.47	nd	nd	-
	12	Muscle	0.07	0.09	0.16	nd	nd	-
M-diq (Telouan)								
8. Harbour	8	Liver	1.48	2.80	4.28	1.30	2.4	3.70
	8	Muscle	0.53	nd	0.53	0.76	4.00	4.76
9. Open area	10	Liver	0.09	0.01	0.09	0.26	0.33	0.59
	10	Muscle	nd	0.06	0.06	0.11	nd	0.11
Oued Laou (Tetouan)								
10. Open sea	7	Liver	0.01	nd	0.01	nd	nd	0.01
	7	Muscle	nd	nd	nd	0.004	nd	0.04
Amsa (Tetouan)								
11. Open sea	9	Liver/muscle	nd	nd	nd	nd	nd	nd

N: Number of samples pooled; nd; not detected.

of the calibration standards. Good linearity was found in the concentration range studied, with correlation coefficients between 0.990 and 0.999 (Tables 1 and 2).

Recovery efficiency data were obtained analysing uncontaminated liver and sediment extracts (n = 10) spiked at 100 μg/Kg of the target compounds (Tables 1 and 2). Recoveries higher 74.0% were obtained for all the target compounds. The precision (repeatability, n = 10) of the overall method was also evaluated at the same concentration level (100 μg/Kg) and expressed as a relative standard deviation (RSD). Tables 1 and 2 show the results, with RSD values lower than 15% for compounds.

Organotin Analysis on the Northern Coast of Morocco

TBT remains an important pollutant in areas with high traffic, because its use continues on commercial and navy vessels of more than 25 m length in many countries, such as Morocco. The harbours of Tangier and Tetouan are by far the largest local users of TBT-based paints. Various sources of TBT marine contamination related to ship-repairing activities have been identified, including: losses during paint spraying, blasting residues, high pressure washings, and scraping of ship hulls. Since OT levels in harbours were higher in areas dedicated to some special activities, for instance sites of boat maintenance, it can be concluded that these activities are about equally important as sources of OT contamination of the local marine environment.

Figure 1 shows the concentration and dispersion pattern of BT and PT compounds in fishes from the northern Mediterranean sea of Morocco. The levels of BT and PT compounds in the sea mullet, *Mugil cephalus*, are shown in Table 3. The results show that the contents of BTs and PTs in fish varied with both location and target organ. The total content of BTs (sum of TBT, DBT, and MBT) was higher than the total content of PTs (sum of TPhT, DPhT, and MPhT). Neither MBT nor MPhT was detected in any of the analysed samples. The highest value of BTs (18.00 mg/Kg) was found in the west harbour of the Tangier site, while the lowest concentration (0.01 mg/Kg) was detected in Oued Laoue site. Concerning PT compounds, the highest value (4.00 mg/Kg) was found in the harbour of the M'diq Site

Table 4. Concentration (ng g^{-1} dry wt) of organotin compounds in marine organisms and sediments from different Mediterranean areas

Sample	Location	Sampling year	MBT	DBT	TBT	MPhT	DPhT	TPhT	Reference
Bivalves	Southwest Spain	1992	27–139	40–110	110–281	n.d.	n.d.	n.d.	Gómez-Ariza *et al.* (1997)
Mollusca	Southwest Spain	1992–1994	1.1–170	12–390	23–1500	n.d.	n.d.	n.d	Gómez-Ariza *et al.* (2001)
Deep sea fish	Northwestern Mediterranean	1996	<1–54	<1–67	<l–52	<1–152	<l–85	<1–1430	Borghi and Porte (2002)
Snails	Sicilian coast (Italy)	1999–2000	n.d.–167	n.d.–316	n.d.–9l	n.a.	n.a.	n.a.	Chiavarini *et al.* (2003)
Sediments	Southwest Spain	1992–1994	0.9–90	<0.6–120	<0.6–140	n.d.	n.d.	n.d.	Gómez-Ariza *et al.* (2001)
Sediments	Sicilian coast (Italy)	1999–2000	<1	<1	3–27	n.a.	n.a.	n.a.	Chiavarini *et al.* (2003)
Sediments	Southwest Spain (Huelva coast)	1998	2.1–95	2.1–284	1.2–130	n.d.	n.d.	n.d.	Gómez-Ariza *et al.* (1998)
Sediments	Southwest Spain (Cadiz coast)	1992	27–350	73–1200	250–14150	33–630	34–430	15–940	Gómez-Ariza *et al.* (1995)
Sediments	French coast (Arcachon harbour)	1996–1997	4025	3980	8150	n.d.	n.d.	n.d.	Amouroux *et al.* (2000)
Sediments	Mediterranean northeastern coast (Spain)	1998	430	1800	9260	8	5840	12272	Tolosa *et al.* (1992)
Sediments	Mediterranean southeastern and northeastern coast (Spain)	1995–2001	7–1674	92–6860	124–l8,722	8–5160	11–419	15–236	Diez *et al.* (2002)

n.d.: not detected; n.a.: not analyzed.

and the lowest value (0.01 mg/Kg) was detected in the Oued Laoue site. No BTs or PTs were detected in Amsa, an uncontaminated site that was taken as control.

In our study, TBT was found in fish samples at higher concentrations than other BTs, and much of the detected PT compound was TPhT. These data are in agreement with that reported by Gómez Ariza *et al.* (1997, 1998) in different marine organisms (Table 4). In all samples of harbours and open sea from Tangier, TBT was the predominant compound with levels always higher than TPhT values, except in samples of muscle of West harbour 1 and Marina, and in samples from open area 1, all these exceptions located in Tangier. These results can be attributed to the continuous high inputs of TBT into the marine environment.

Analysis of the results of monitoring reported in Table 3 shows a large sample-to-sample variation in contaminant concentrations, the highest levels can be caused by proximity, certainly caused by proximity to effluents discharges and the site of boat maintenance. Generally, sites of samples situated near or in sites where ships are repaired and maintained were subject to high contamination levels. Among the harbours included in this study, Tangier harbour, the main commercial and fishing harbour, shows the greatest contamination, followed by the M'diq harbour. These differences can be explained partially by factors like the density of the boats and the currents. Considering the importance of the Tangier harbour, we chose 7 points of samplings. Points corresponding to the place of careenage, and maintenance of boats (East harbour 1 and 2, and West harbour 1) have shown a very high contamination. This can be explained by the fact that the majority of the boats remain in water all year. The OTs come from paint antifouling but also from other sources such as pesticides in wood and PVC stabilizers. Site 4, which is located near the receptacle of domestic and industrial wastes waters, also shows a high contamination. So, domestic primary sewage sludge effluents are also a source of OT contamination. The sites of reference such as Oued laou and Amsa are located far from all known sources of OTs, and this explains the absence of contamination or the decrease in contamination that was found.

Concentrations of BTs in fish samples ranged from 0.01–18.00 mg/Kg wet weight in liver and 0.06–8.11 mg/Kg wet weight in muscle. The results are comparable to those reported for the muscle of Tuna from Italian coastal waters (Kannan *et al.*1996) or fish samples from Taiwan coastal waters (Tsu-Chang *et al.*1998).

BT accumulation in the liver of *Sea mullet* is similar to several trace metals, which prominently concentrate in the liver (Honda *et al.* 1982). Also, several analyses of BTs in Dolphin, Tuna, and Blue shark collected from the Italian coast of the Mediterranean Sea showed the highest concentrations in liver with low concentrations in kidney and muscle (Kannan *et al.* 1995; Kannan *et al.* 1996). Generally, the higher accumulation of BTs in liver than in muscle may result from binding of TBT to a specific protein that is abundant in the liver (Kannan *et al.* 1995). The occurrence of TBT as the predominant compound in fish livers suggests a slow metabolism of TBT, which could be possibly due to the inhibition of microsomal enzymes by TBT at high exposure concentrations (Fent and Stigman 1993).

TBT was present at higher concentration levels than its metabolite DBT, except for samples from an open area 2 from Tangier and two samples from M-Dip. MBT metabolites were not detected, which indicates that fresh inputs of the antifouling agents dominate over the degradation products.

As for the PTs (TPhT, DPhT and MPhT) levels in liver and muscle varied from non detected- 3.7 mg/Kg wet weight and non detected-4.76 mg/Kg wet weight respectively. The highest concentrations were recorded in M'diq harbour (PTs = 4.76 mg/Kg wet weight), and in Tangier harbour in the Marina. With respect to high concentrations of TBT in liver of most samples analysed, TPhT was also higher in liver than in muscle. This is an agreement with Watanabe *et al.* (1998) and also De Mora *et al.* (2003). Abalat *et al.* (2002) reported the presence of TPhT in flatfish samples from the Polish coast (Baltic Sea), the highest levels were observed in the liver and decreased respectively in digestive tube and gills. TPhT and their metabolites (MPhT and DPhT) were also found in deep sea fish from Nortwestern Mediterranean Coast (Borghi *et al.* 2002). The tendency of TPhT to accumulate in marine organisms was first noticed by Takami *et al.* (1988).

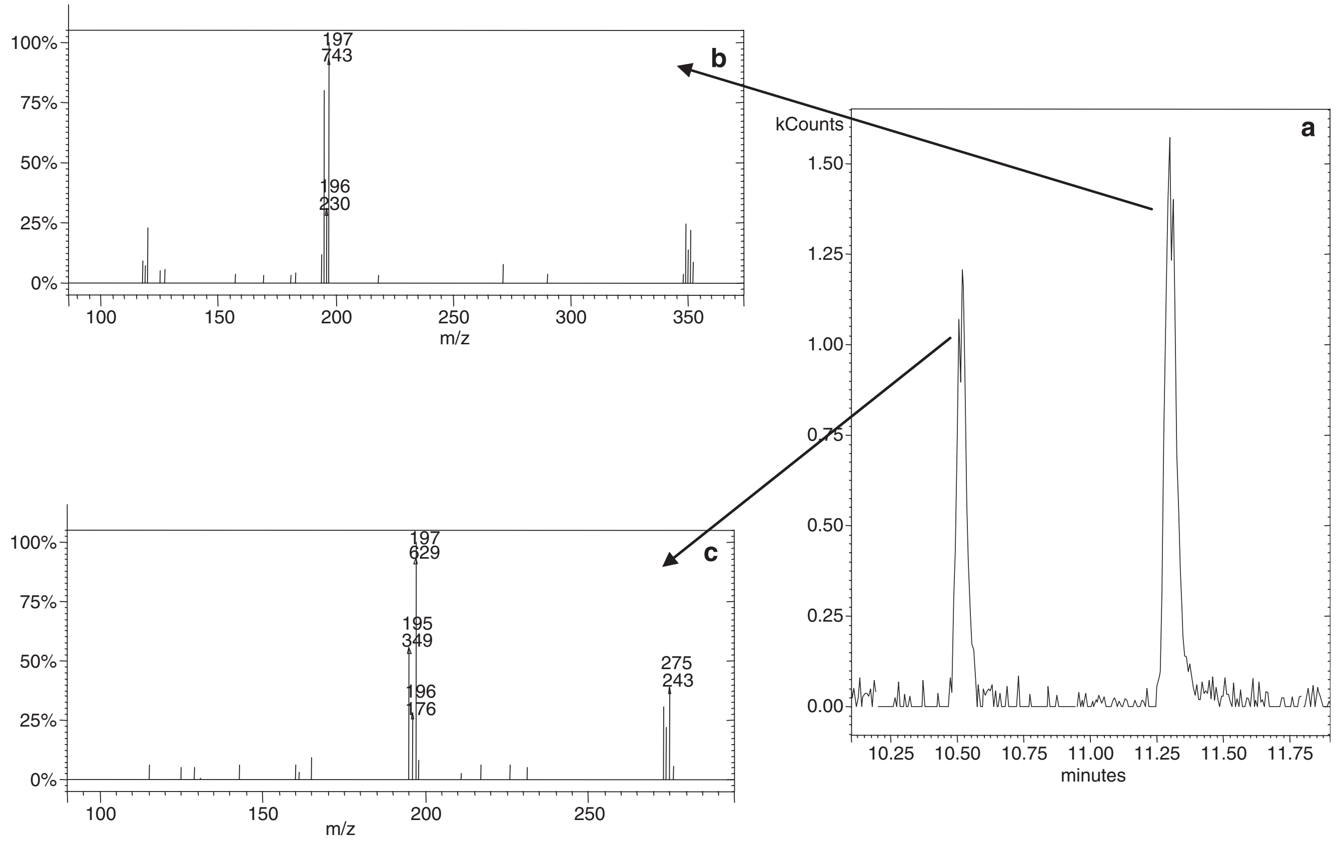

Fig. 3. Analysis of a real fish liver sample from M-diq (open area). **a:** Gas chromatogram obtained for TPhT and DPhT; **b:** MS/MS spectrum obtained for TPhT; **c:** MS/MS spectrum obtained for DPhT in the sample

The distribution of PT residues in fish shown here, differs from that of BTs species. The origin of TPhT in our fish samples may be marine antifoulants or local marina traffic. In the literature, TPhT in fish from the Mediterranean (Morcillo and Porte 2000) and Baltic (Abalat *et al.* 2002) seas was deemed to originate from fungicide usage.

Generally, the total OT (OTs = BTs+PTs) concentration in most liver samples was higher than in muscle suggesting preferential accumulation of these compounds. The presence of OT compounds in fish samples collected from the harbours of Tangier and Tetouan suggests that these compounds are widely distributed in the studied area and bioaccumulated in fish. Figure 3 shows, as an example, the GC-MS/MS chromatogram of TPhT and DPhT in a real fish sample.

Organotin Analysis in the South Coast of Spain

TBT was identified in four of the eight sediment samples from the Guadalquivir River at concentrations of 15.45, 45.03, 48.78, and 245.07 mg/Kg, respectively. However, PT compound was not detected in the sediment or the fish samples analysed. Figure 2 shows the concentration and dispersion pattern of BT and PT compounds in samples from Spanish coasts. These data are in agreement with the recent study carried out by Díez *et al.* (2005) in a nearby area (Portugal). However, the fact that PT compounds were not detected in sediment or fish samples does not mean that they are not nowadays used in the area, for instance in agriculture. PTs are unstable compounds that are easily degraded (Quevauviller and Ariese 2001; Morabito *et al.* 1999). The presence of TBT in some sediment samples indicates the high persistence of this compound in the environment. These levels are related to heavy boat traffic and shipyard activities.

Conclusions

In summary, all harbour sites from Morocco showed high levels of contamination, correlating well with berthing capacity and traffic and also with high maritime activity. BT and PT contamination decreased significantly from the West to the open area in the Harbour of Tangier. The Harbour of Tangier is considered the most contaminated site. It has many ship reparing/modelling factories and more than a thousand ships passing daily through the Strait of Gibraltar. Compared to Tangier Harbour, M'diq harbour situated in Tetouan due is a small fishing harbour, but it also can be considered as a contaminated site to the use of nets, boats, and others utilities treated with antifouling biocides.

Contamination by BTs in fish from the two harbours (Tangier and M-diq) investigated here must to be taken in consideration because we do not have any data on the dangers to public health. A strict legislation banning the use of these compounds must be introduced here to prevent a major risk to human health.

Oued Laou and Amsa sites can be considered uncontaminated sites because they are situated far from the sources of contamination by OTs and there are few fishing boats there. In addition, this study demonstrates the absence of pollution in fish from the Spanish coast, and only the presence of TBT in some sediment samples owing to its long persistence and probable indiscriminate use in the past.

Acknowledgments. The authors are very grateful to the Framework Agreement between the Consejería de Medio Ambiente de la Junta de Andalucía and the University of Almería (Analytical Chemistry Department), and to the Consejería de la Presidencia Junta de Andalucía (project AM58/04) for financial support.

References

Abalat A, Potrykus J, Pempkowiak J, Porte C (2002) Assessment of organotin pollution along the Polish coast (Baltic sea) by using mussels and fish as sentinel organisms. Chemosphere 47:165–171

Alzieu C, Michel P, Sanjuan J, Averty B (1990) Tributyltin levels in French Mediterranean coastal Waters. Appl Organometal Chem 4:55–61

Amouroux D, Tessier E, Donard OFX (2000) Volatilization of organotin compounds from estuarine and coastal environments. Environ Sci Technol 34:988–995

Borghi V, Porte C (2002) Organotin pollution in deep-sea fish from the Northwestern Mediterranean. Environ Sci Technol 36:4224–4228

Chiavarini S, Massaniso P, Nicolai P, Nobili C, Morabito R (2003) Butyltins concentration levels and imposex occurrence in snails from the Sicilian coasts (Italy). Chemosphere 50:311–319

De Mora SJ, Fowler SW, Cassi R, Tolosa I (2003) Assessment of organotin contamination in marine sediments and biota from the Gulf and adjacent region. Mar Pollut Bull 4:401–409

Díez S, Ábalos S, Bayona JM (2002) Organotin contamination in sediments from the Western Mediterranean enclosures following 10 years of TBT regularion. Water Res 36:905–918

Díez S, Lacorte S, Viana P, Barcelò D, Bayona JM (2005) Survey of organotin compounds in rivers and coastal environments in Portugal 1999-2000. Environ Pollut 136:525–536

Fent K (1996) Ecotoxicology of organotin compounds. Crit Rev Toxicol 26:1–117

Fent K, Hunn J (1991) Phenyltins in water, sediment and Biota of freshwater marinas. Environ Sci Technol 25:956–963

Fent K, Meier W (1992) Tributyltin-induced effects on early life stages of minnows Phoxinus phoxinus. Arch Environ Contam Toxicol 22:428–438

Fent K, Stigman A (1993) Effects of TBT in vivo on hepatic cytochrome P450 Forms in Marine fish. Aquat Toxicol 24:219–240

Gómez Ariza JL, Beltrán R, Morales E, Giraldez I, Ruiz-Benitez M (1995) Acid extraction treatment of sediment samples for organotin speciation; occurrence of butyltin and phenltin compounds on the Cádiz coast, South-West Spain. Appl Organomet Chem 9:51–64

Gómez Ariza JL, Morales E, Giraldez I, Beltrán R, Pozas Escobar JA (1997) Acid/extraction treatment of bivalves for organotin speciation. Fresenius J Anal Chem 357:1007–1009

Gómez Ariza JL, Morales E, Giraldez I (1998) Spatial distribution of butyltin and phenyltin compounds on the Huelva coast (Southwest Spain). Chemosphere 37:937–950

Gómez Ariza JL, Giraldez I, Morales E (2001) Occurrence of organotin compounds in water, sediments and mollusca in estuarine systems in the Southwest of Spain. Water Air Soil Pollut 126:253–270

Honda K, Tatsukawa R, Fujiyama T (1982) Distribution characteristics of heavy metals in the organs and tissues of striped dolphin Stenella coeruleoalba. Agric Biol Chem 4:2959–2962

Kannan K, Tanabe S, Tatsukawa R (1995) Butyltin residues in fish from Australia, Papua New Guinea and the Solomon Islands. Int J Environ Anal Chem 61:263–273

Kannan K, Corsolini S, Focardi S, Tanabe S, Tatsukawa R (1996) Accumulation pattern of butyltin compounds in Dolphin, Tuna and Shark Collected from Italian Coastal Waters. Arch Environ Contam Toxicol 31:19–23

Kubilay N, Yemenicioglu S, Tugrul S, Salihoglu I (1996) The distribution of organotin compounds in the North-Eastern Mediterranean. Marine Pollut Bull 32:238–240

Le Thi HL, Shin T, Kazutoshi S, Nobutake N, Shinsuke T, Nobuyuki M, Yoshihiro F (1999) High percentage of butyltin residues in total tin in the livers of Cetaceans from Japanese Coastal Waters. Environ Sci Technol 33:11–19

Martínez Vidal JL, Belmonte Vega A, Arrebola FJ, González-Rodríguez MJ, Morales Sánchez MC, Garrido Frenich A (2003) Trace determination of organotin compounds in water, sediment and mussel samples by low-pressure gas chromatography coupled to tandem mass spectrometry. Rapid Commun Mass Spectrom 17:2099–2106

Michel P, Bernard A, Bruno A, Chiffoleau JF, Galgani F (2001) Tributyltin along the coasts of Corsica (Western Mediterranean): A persistent problem. Mar Pollut Bull 42:1128–1132

Morabito R, Muntau H, Cofino W, Quevauviler Ph (1999) A new mussel certified reference material (CMR 477) for the quality control of butyltin determination in the marine environment. J Environ Monitor 1(1):75–82

Morcillo Y, Porte C (2000) Interaction of tributy- and triphenyltin with the microsomal monooxygenase system of mollusks and fish from the western Mediterranean. Aqua Toxicol 38:35–46

Quevanviller PH, Ariese F (2001) New sediment reference material for the quality control of butyltin and phenyltin analysis. Trends Anal Chem 20:207–218

Takami K, Okumura H, Yamasaki H, Nakamoto M (1988) Determination of triphenyltin and tributyltin compounds in fish and shellfish by capillary GC. Bunseki Kagaku 37:449–455

Tolosa I, Merlín L, de Bertrand N, Bayona JM, Albaigés J (1992) Occurrence and fate of tributyl- and triphenyltin compounds in Western Mediterranean coastal enclosures. J Environ Toxicol Chem 11:145–155

Tsu- Chang H, Tung-Yi L, Tien-Fu L (1998) Determination of butyltins and phenyltins in oysters and fishes from Taiwan coastal waters. Environ Pollut 102:197–203

Watanabe M, Hashimoto S, Fujita K, Otsuki A (1998) Distribution of organotin compounds in fish and the ratio of phenyl-tin to total organic-tin in the ROPME sea area. In: Otsuki A (ed) Offshore environment of the ROPME sea area alter the war related Oil SIPI. Terra Scientific Publishing Company, Tokyo, pp 231–244