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Acid Extraction Treatment of Sediment Samples for Organotin Speciation; Occurrence of Butyltin and Phenyltin Compounds on the Cadiz Coast, South-West Spain

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A method is described for leaching of nanogram amounts of mono-, di and tri-butyltin compounds and mono-, di- and tri-phenyltin compounds from sediments. The procedure is based on soaking the sediments in a water–hydrogen bromide mixture (2:3) with magnetic stirring for 1 h followed by extraction with 0.02% (w/v) tropolone solution in pentane for 2 h. Organotins are determined by GF FPD after clean-up through a Florisil column and derivatization by Grignard pentylation. The method has been applied to the study of water and sediments in different areas of south-west Spain. Predominant species are butyltins, especially tributyltin (TBT), which has high values in waters and sediments of Puerto de Santa Maria and Cadiz Bay, as well as in sediments of the Sancti Petri Channel, which suggests a harmful action on biota. A direct relation has been found beween organotin levels and distance of potential focus determined by boating activities. In addition, the relative occurrence of dibutyltin (DBT) and monobutyltin (MBT) together with TBT has been noted, possibly as a result of a degradation process, and the influence of grain size of sediment and presence of organic matter on organotin accumulation has been studied.

Keywords: tributyltin; dibutyltin; monobutyltin; triphenyltin; diphenyltin; monophenyltin; speciation; sediments; gas chromatography; flame photometry; south-west Spain

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INTRODUCTION

Tributyltin (TBT), triphenyltin (TPT), and other organotin species generally formed by their environmental degradation, are now subject to environmental analysis, due to the important environmental impact of these compounds which are widely used as biocides and algicides in antifouling paints and agriculture. The toxic effect of TBT on nontarget organisms makes necessary the quantification of organotin species in environmental samples to assess pollution incidents.

Analytical speciation techniques for organotin compounds in sedimentary samples are more complex than those for water,[1] owing to the complexity of environmental matrices. This makes it necessary to pay special attention to sample preparation, as well as to species extraction and preconcentration.

Organotin extraction from sediments generally involves leaching of the species with acidic solutions. Tugrul *et al.*[2] sonicated 0.5–1 g of dry ground polluted sediment for 1 h in 40 ml of 0.1 M hydrochloric acid, Seidel *et al.*[3] used a *mild* 0.3 M

Table 1 Detection limits of organotin compounds in water and sediment

	TBT	DBT	MBT	MPT	DPT	TPT
Water (ng l^{-1})	4.6	5.2	5.3	12	12	11
Sediment (ng g^{-1})	0.61	0.75	0.76	2.8	3.2	2.7

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hydrochloric acid leach to extract tin and organotin compounds from sediments and Randall *et al.*[4] used a 2.5 M calcium chloride and 2.5 M HCl extraction and analyzed the tin with hydride generation atomic absorption spectrometry (HG AA). In addition, Astruc *et al.*[5] and Quevauviller and Donard[6,7] extracted organotins with acetic acid in two successive steps: stirring overnight and ultrasonic extraction. Other authors combined acid leaching with the extraction with a nonaqueous solvent. Tsuda *et al.*[8] proposed the extraction with hexane after acid leaching with HCl, Muller[9] used a HCl and diethyl ether extraction followed by methylation with methyl-

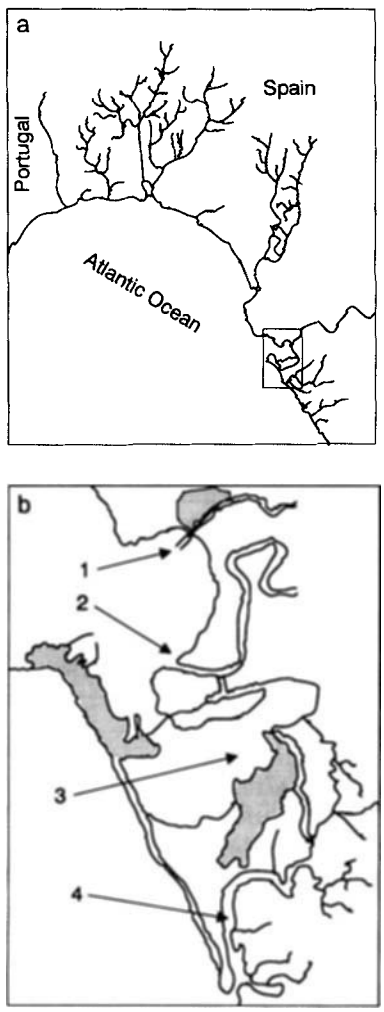

Figure 1 (a) Area of study on the Cadiz coast, south-west Spain. (b) Location of the different zones under study: 1, Guadalete river; 2, San Pedro river; 3, Cadiz Bay; 4, Sancti Petri river.

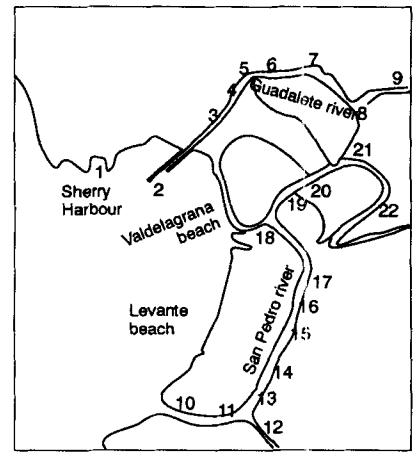

Figure 2 Sampling sites in Guadalete and San Pedro rivers.

magnesium chloride and analysis with flame photometric gas chromatography and GC MS. Hattori *et al.*[10] used methanolic hydrochloric acid and benzene extraction and analysis with ECGC. Other authors proposed the use of a chelating agent, generally tropolone, to enhance the organotin extraction. Maguire *et al.*[11] used HCl, tropolone and benzene, Muller[12] and Tolosa *et al.*[13] proposed acid treatment with HCl to pH 2–3 and extraction of the resulting slurry with an ethereal tropolone solution, and Batley *et al.*[14] used sonication of the sediments with concentrated HCl and methanol followed by extraction with tropolone in dichloromethane. Ashby and Craig,[15] also proposed a similar procedure based on the use of HCl for leaching of tin species and extraction with tropolone in dichloromethane. Finally, Martin-Landa *et al.*[16] recommended treatment with concentrated hydrobromic acid and extraction with tropolone in pentane.

The present paper considers the use of

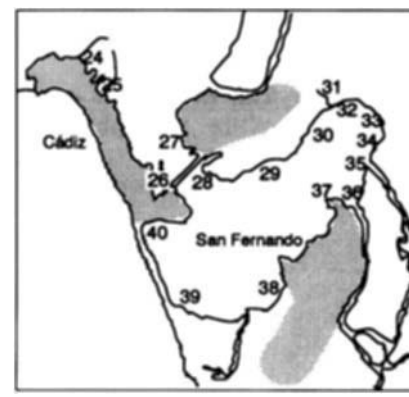

Figure 3 Details of sampling locations in the Cadiz Bay.

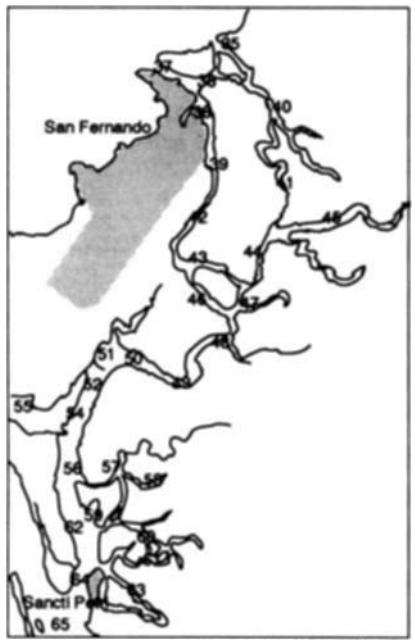

Figure 4 Sampling point locations in the Sancti Petri river.

HBr/pentane and HCl/pentane as leaching systems for butyltin and phenyltin species in sediment samples and the methods are applied to evaluate the occurrence of these species in waters and sediments of the Cadiz coast (south-west Spain).

EXPERIMENTAL

Water extraction procedure

A 1000 ml portion of the unfiltered water sample containing the organotin species, acidified with 10 ml of HBr (Merck), was extracted by shaking vigorously in darkness with 300 ml of a 0.07% (w/v) solution of tropolone (Aldrich) in pentane for 10 min; the tropolone concentration was optimized to prevent dismutation.[17] The organic extract was dried with anhydrous Na_2SO_4 and reduced in volume to 0.5 ml in the rotary evaporator. The extract was then derivatized by pentylation, for analysis by GC.

Table 2 Recoveries (%) of organotin species in spiked sediments using HBr as leaching agent

HBr (%)	TBT	DBT	MBT	MPT	DPT	TPT
2	85	41	0	0	39	41
10	90	85	0	0	60	86
16	90	85	0	0	100	102
30	95	86	24	0	95	98
40	102	94	53	0	95	100
50	99	96	75	20	96	102
60	100	100	90	60	101	99
70	101	99	97	58	140	91
80	98	100	100	91	207	56
90	50	55	62	54	145	0

Table 3 Recoveries (%) of organotin species in spiked sediments using HCl as leching agent

HCl (%)	TBT	DBT	MBT	MPT	DPT	TPT
40	40	50	0	0	60	62
50	60	60	0	0	64	92
60	98	84	0	0	80	95
70	97	100	0	0	151	90
80	98	96	0	0	160	31
90	95	94	0	0	165	0
100	97	96	0	0	123	0

Sediment chemical treatment/ extraction procedure

A suitable weight of wet homogenized sediment (about 20 g) was placed in a 250 ml Erlenmeyer flask with a PTFE screw closure and treated with 50 ml of a water hydrogen bromide mixture (2:3) with magnetic stirring for 1 h. The mixture was extracted with 100 ml of 0.02% (w/v) tropolone solution in pentane for 2 h. The phases were separated in 50 ml Teflon tubes by centrifugation at 10 000 rpm for 10 min. Both aqueous and organic phases were transferred to a separating funnel, in which the organic phase was separated and dried with anhydrous sodium sulfate. Reduction of the resulting extract in volume to about 0.5 ml by rotary evaporation was followed by the derivatization and determination steps.

Table 4 Recoveries (%) of organotin species in spiked sediments using HCl as leaching agent in the presence of 10 ml concentrated HBr

HCl (%)	TBT	DBT	MBT	MPT	DPT	TPT
40	95	97	25	0	82	95
50	99	98	85	68	98	100
60	98	99	93	89	106	94
70	99	100	98	75	127	87
80	100	98	99	54	61	36
90	98	97	98	0	0	0
100	99	99	100	0	0	0

Table 5 Recoveries (%) of organotin species in spiked sediments using different amounts of tropolone as chelating agent

Tropolone (g)	TBT	DBT	MBT	MPT	DPT	TPT
0	98	97	80	45	98	95
0.01	100	98	91	61	99	98
0.05	99	96	90	60	101	99
0.10	98	98	91	57	99	99

Table 7 Influence of leaching time on recoveries (%) of organotin species in spiked sediments

Time (min)	TBT	DBT	MBT	MPT	DPT	TPT
5	99	97	80	71	90	99
30	98	98	95	80	97	98
60	100	100	100	85	98	100
120	97	99	99	83	99	99

Analytical methods

Derivatization

The pentane extract was treated with 4 ml of 1 M pentylmagnesium bromide solution in ether for 1 h in a sealed vessel at room temperature.

Clean-up

The excess of reagent was removed with 0.5 M sulfuric acid and the organic extract reduced to 1 ml. Purification by chromatography on a 7 cm × 1 cm i.d. column of florisil (Merck) followed 10 ml of pentane was used as the eluent. This pentane extract was then reduced to 2 ml and transferred to a microevaporator together with an internal standard (dimethyldipentyltin) and concentrated to 0.5 or 0.2 ml under a stream of nitrogen.

Measurement of organotin concentrations

Organotin species were determined using a GC FPD system, following a procedure reported previously.[18,19] A Perkin–Elmer 8140 gas chromatograph fitted with a split/splitless injector, glass capillary column (Supelco SPB-1 15 m in length, 0.53 mm i.d., film thickness 1.5 μm) and a flame photometric detector was used. The detector was operated with a 610-nm cut-off interference filter, at a temperature of 250 °C, using hydrogen and air flow rates of 46.5 and 88.0 ml min^{-1}, respectively. The injector temperature was set at 250 °C and helium (9.5 ml min^{-1}) served as carrier gas, using a split ratio of 3.8. Sample aliquots of 5–10 μl were injected and the compounds of interest were eluted with the following temperature programme: initial column temperature 50 °C, heating to 250 °C at 10 °C min^{-1} and isothermal at this temperature for 7 min.

Table 6 Influence of pentane volume on recoveries (%) of organotin species in spiked sediments

Pentane (ml)	TBT	DBT	MBT	MPT	DPT	TPT
25	100	99	72	0	100	99
50	100	98	90	58	101	99
75	99	99	92	67	99	100
100	100	100	100	85	98	100
200	100	100	98	81	99	99

Calibration and analytical quality control

Organotin concentrations were deduced from calibration curves derived from derivatized standard solutions using peak heights. Calibration experiments were not carried out directly on natural sediments or waters. Calibration curves were linear for tin amounts less than 40 ng; this limit increased to 55 ng and 60 ng for diphenyltin DPT and TPT, respectively. The determinations were carried out using Me_2SnPe_2 as internal standard, which improved the precision.

Quality control of results was monitored by preparing a calibration graph each week and injecting a derivatized standard with all tin species each day to test the instrument signal. The absolute limit of tin able to be detected for the instrument evaluated as three times the standard deviation of blank is in the range of about 0.3 ng for butyltins and 0.5 ng for phenyltin species. The detection limits in water and sediment analysis (including the extraction step) are presented in Table 1.

Both water and sediment samples were analyzed at least five times with relative standard deviations (RSDs) in the range 4–10% when peak height was used. The precision of the detector response using the area peak decreases; for this reason peak height was used throughout.

Table 8 Influence of extraction time on recoveries (%) of organotin species in spiked sediments

Time (h)	TBT	DBT	MBT	MPT	DPT	TPT
0.5	89	85	75	68	89	92
1	100	98	97	86	100	99
2	100	100	100	85	98	100
3	99	99	98	84	100	98

Table 9 Influence of percentage of HBr as leaching agent on organotins recovery from polluted sediments from south-west Spain

		Recovery of organotin (ng Sn g^{-1})			
Sample	Organotin	40% HBr	60% HBr	80% HBr	60%HCl + 10% HBr
1	TBT	73	77	78	75
	DBT	272	270	296	276
	MBT	64	97	93	100
	MPT	<DL[a]	12.7	17.3	<DL
	DPT	11.9	13.5	13.7	<DL
	TPT	7.4	7.4	<DL	<DL
2	TBT	149	156	159	148
	DBT	51	49	47	50
	MBT	18.6	26	30	27
	MPT	<DL	12.5	13.1	<DL
	DPT	13.2	12.9	18.3	<DL
	TPT	23.8	22.5	13.4	<DL
3	TBT	1.63×10^4	1.73×10^4	1.70×10^4	1.69×10^4
	DBT	1.20×10^3	1.23×10^3	1.28×10^3	1.19×10^3
	MBT	442	560	537	549
	MPT	92	323	399	<DL
	DPT	181	193	240	<DL
	TPT	331	329	225	<DL
Blank	TBT	<DL	<DL	<DL	<DL
	DBT	<DL	<DL	<DL	<DL
	MBT	<DL	<DL	<DL	<DL
	MPT	<DL	<DL	<DL	<DL
	DPT	<DL	<DL	<DL	<DL
	TPT	<DL	<DL	<DL	<DL

[a] DL, detection limit.

Area of study

The presence of organotin compounds has been noted in waters and sediments of the Cadiz coast in south-west Spain [Figs. 1(a) and 1(b)]. High levels of TBT were previously found in waters nearby.[7] This area has a remarkable ecological importance because it is a feeding ground of nearby importance for migrating birds. Sampling sites were selected in relation to the suspected presence of organotins caused by boating activity in wharves, docks, shipyards and so on.

Four locations with the following characteristics have been studied.

Guadalete River (Fig. 2)
This flows into Puerto de Santa Maria, a small town with fishing and shell-fishing activities, and also a harbour that could be a source for organotins. In addition the neighbouring location Puerto Sherry is a big international marina with numerous recreational vessels which could increase the inputs of organotin species in this area.

San Pedro River (Fig. 2)
This flows by Cadiz Bay, being connected with it through a channel (Caño de la Cortadura). It is a marsh area without potential organotin sources such as harbours or dockyards, and a number of fish-farms are present.

Cadiz Bay (Fig. 3)
It is a more complex area with both commercial and fishing basins (in Cadiz city) and a shipyard (Puerto Real village) on the opposite side of the bay. The depth of the bay is variable but it is possible for large vessels to reach La Carraca, a military port situated at the end of the bay. In addition the bay is connected with the San Pedro River and the Santi Petri Channel through a system of channels.

Sancti Petri Channel (Fig. 4)
This is a marsh area between San Fernando and Sancti Petri villages, with numerous saltworks and fishfarms, which undergoes influence from both a recreational (Sancti Petri) and fish port (La Gallinera, next to San Fernando). The river is connected with the Cadiz Bay by two channels with the mouth next to La Carraca being important as a source of organotins.

Sample collection

All the samples were collected during January and February 1992, and analyzed within seven days after sampling.

Water samples were collected in 2.5 l polycarbonate bottles. Where possible, samples were collected from land, or else they were collected by boat. All of the samples were manually collected from just beneath the surface; to avoid microlayer contamination the bottle cap was carefully removed under the water surface. The samples were then stored at 4 °C in a refrigerator.

Sediment samples were collected at the surface taking aliquots of about 10 cm depth, and these were carefully homogenized. The aliquots from each sampling point were mixed to produce composite samples using four sites spaced out 2 m, then homogenized and placed in polyethylene bottles for freezing and storage.

Grain size of the sediments

Bulk samples of the sediments were subjected to grain size analysis by a combination of wet sieving (for fractions coarser than 63 μm) and sedimentation analysis by Sedigraph 5000 procedures, the latter after elimination of carbonates by treatment with acetic acid.

Organic matter in the sediments

The content of organic matter (as percentage of carbon) in sediments was characterized by oxidation with an excess of potassium dichromate and back-titration with a standardized solution of iron(II) (oxidizable organic matter).

RESULTS AND DISCUSSION

Accumulation of organotin species in sediments in the environment is caused by their dynamic interaction with water, which mobilizes organo-

Table 10 Influence of leaching and extraction times on organotins recovery from polluted sediments from south-west Spain, using pentane as extracting agent

		Recovery of organotin (ng Sn g^{-1})			
		Leaching time		Extraction time	
Sample	Organotin	1 h	3 h	2 h	3 h
1	TBT	75	73	76	76
	DBT	291	273	269	286
	MBT	95	91	92	97
	MPT	12.8	13.1	13.5	12.8
	DPT	14.1	13.8	14.0	12.9
	TPT	6.7	6.4	6.9	7.2
2	TBT	157	144	149	151
	DBT	52	48	47	49
	MBT	30.2	30.2	27.9	29.6
	MPT	10.8	11.4	13.1	12.8
	DPT	12.0	13.1	12.7	12.9
	TPT	24.1	25.0	23.9	22.1
3	TBT	1.60×10^4	1.60×10^4	1.69×10^4	1.71×10^4
	DBT	1.17×10^3	1.21×10^3	1.27×10^3	1.28×10^3
	MBT	555	529	568	572
	MPT	233	249	251	224
	DPT	171	195	188	191
	TPT	315	327	331	320

Table 11 Influence of percentage of HBr and leaching and extraction times on the recovery of organotins from polluted sediments from south-west Spain, using dichloromethane as extractant

		Recovery of organotin (ng Sn g^{-1})						
		HBr			Leaching time		Extraction time	
Sample	Organotin	40%	60%	80%	1 h	3 h	2 h	3 h
1	TBT	75	78	75	76	78	76	74
	DBT	298	275	282	271	272	293	283
	MBT	100	94	91	97	98	93	95
	MPT	14.9	16.2	15.8	16.3	15.9	14.8	15.0
	DPT	13.0	14.2	13.4	12.8	14.1	13.8	12.9
	TPT	7.1	6.8	6.3	7.2	6.8	6.9	7.1
2	TBT	156	149	150	155	145	147	150
	DBT	48	51	48	46	48	51	51
	MBT	28	29	30	30	27	28	30
	MPT	14.3	15.9	16.3	14.8	15.6	16.0	15.9
	DPT	13.2	13.3	11.8	12.1	11.7	13.0	12.7
	TPT	23	24	22	23	24	24	24
3	TBT	1.66×10^4	1.60×10^4	1.65×10^4	1.71×10^4	1.66×10^4	1.64×10^4	1.60×10^4
	DBT	1.21×10^3	1.29×10^3	1.26×10^3	1.20×10^3	1.21×10^3	1.26×10^3	1.28×10^3
	MBT	533	566	532	521	574	578	561
	MPT	372	395	401	388	374	363	367
	DPT	180	196	186	174	185	188	189
	TPT	330	331	309	311	317	322	332
Blank	TBT	<DL[a]	<DL	<DL	<DL	<DL	<DL	<DL
	DBT	<DL	<DL	<DL	<DL	<DL	<DL	<DL
	MBT	<DL	<DL	<DL	<DL	<DL	<DL	<DL
	MPT	<DL	<DL	<DL	<DL	<DL	<DL	<DL
	DPT	<DL	<DL	<DL	<DL	<DL	<DL	<DL
	TPT	<DL	<DL	<DL	<DL	<DL	<DL	<DL

[a] DL, detection limit.

Table 12 Concentrations of butyl- and phenyl-tin compounds in waters of Guadalete River

	Organotin concentration (ng Sn l^{-1})					
Sample[a]	TBT	DBT	MBT	MPT	DPT	TPT
W1	116	32.3	25	<DL	<DL	7.4
W2	<DL[b]	<DL	<DL	<DL	<DL	<DL
W3	25.3	12.1	10.7	<DL	<DL	<DL
W5[c]	73	19.7	15.1	<DL	<DL	<DL
W7	11.4	9.8	10.7	<DL	<DL	<DL
W9	9.9	5.7	<DL	<DL	<DL	<DL
W5[d]	98	39.5	9.8	<DL	<DL	<DL

[a] W, water. [b] DL, detection limit. [c] W5, sampled in the riverside opposite Puerto de Santa Maria harbor. [d] W5, sampled in the Puerto de Santa Maria harbor.

tins from the contaminated focus in solution or adsorbed on particulate matter. Organotin species adsorbed on sediments can suffer degradation and desorption processes; the latter are strongly influenced by the physicochemical characteristics of the sediment, such as grain size or organic matter content.

Extraction of the different organotins from the sediments is a decisive step for their analytical characterization. For this purpose a double treatment is usually necessary: acid treatment (leaching) to break the bonding between the species and sediments, followed by extraction of organotin in a nonaqueous solvent for later derivatization and analysis.

In order to set up the optimum extraction procedure for total recovery of organotin species from sediments, tests have been carried out to determine the more important experimental variables affecting the separation step.

Table 13 Concentrations of butyl- and phenyl-tin compounds in sediments of Guadalete River

Sample[a]	Moisture content (%)	Organotin concentration (ng Sn l^{-1})					
		TBT	DBT	MBT	MPT	DPT	TPT
S3	25.9	150	36	16.1	4.3	2.9	22
S6	54.6	100	58	26.7	8.4	5.9	12.6
S7	33.9	601	510	129	21.5	14.5	19.0
S8	30.7	580	338	115	36.6	<DL[b]	11
S9	42.3	26.5	20.5	20.5	18.2	<DL	<DL

[a] S, sediment. [b] DL, detection limit.

Table 14 Grain type and organic matter content (%) in the sediments of Guadalete River

Sample[a]	Organic carbon	Coarse sand	Fine sand	Silt	Clay
S3	0.39	2.8	71.8	13.4	12
S6	0.54	1.8	58.8	23.6	15.8
S7	2.3	1.3	13.6	37.8	47.2
S8	2.14	2.2	12.5	31.6	53.7
S9	2.22	1.4	12	36.6	50

[a] S, sediment.

Table 15 Concentrations of butyl- and phenyl-tin compounds in the waters of San Pedro River

Sample[a]	Organotin concentration (ng Sn l^{-1})					
	TBT	DBT	MBT	MPT	DPT	TPT
W11	11.2	5.5	6.9	<DL[a]	<DL	<DL
W16	9.3	<DL	7.0	<DL	<DL	<DL
W18	16.3	13.0	14.4	<DL	<DL	<DL
W21	12.6	14.3	11.7	<DL	<DL	<DL
W22	11.4	<DL	8.6	<DL	<DL	<DL
W23	10.4	7.7	10.2	<DL	<DL	<DL

[a] W, water. [b] DL, detection limit.

Recoveries from spiked sediments

Influence of acid concentration

The action of HBr and HCl as leaching agents has been tested on sediments spiked at the appropriate levels with TBT, DBT, MBT, TPT, DPT and MPT. These doped sediments have been prepared by stirring overnight an organotin-free sediment (obtained from sites remote from organotin sources, e.g. harbors, wharves, dockyards, etc.) with an aqueous solution of these compounds of known concentration. The ratio water/sediment

Table 16 Concentrations of butyl- and phenyl-tin compounds in the sediments of San Pedro River

Sample[a]	Moisture content (%)	Organotin concentration (ng Sn g^{-1})					
		TBT	DBT	MBT	MPT	DPT	TPT
S10	29.3	6.8	4.8	2.9	<DL[b]	0.7	<DL
S12	26.5	4.7	4.3	3.2	<DL	<DL	<DL
S13	23.9	11.1	4.1	1.9	<DL	<DL	<DL
S11	35.5	7.9	5.5	3.4	1.1	0.8	0.6
S14	37.3	8.6	6.8	3.5	0.9	0.8	<DL
S15	55.0	11.1	8.7	5.6	<DL	0.9	0.9
S16	50.8	7.4	7.7	3.9	<DL	0.9	<DL
S17	39.2	3.3	4.9	4.3	<DL	<DL	<DL
S19	42.7	4.1	3.2	<DL	<DL	<DL	<DL
S20	35.4	7.8	4.6	<DL	<DL	<DL	<DL
S21	49.3	1.3	1.9	3.7	<DL	<DL	<DL
S22	54.1	1.5	2.8	6.1	<DL	<DL	<DL
S23	29.4	0.70	1.5	2.0	<DL	<DL	<DL

[a] S, sediment. [b] DL, detection limit.

Table 17 Grain type and organic matter content (%) in the sediments of San Pedro River

Sample[a]	Organic carbon	Coarse sand	Fine Silt	Silt	Clay
S10	0.85	2.3	51.7	20.8	25.2
S12	0.71	0.6	49.6	24.6	25.2
S13	0.67	0.9	41.6	20.6	36.9
S11	1.03	<DL[b]	30.3	19.4	50.3
S14	0.86	1.6	33.4	23.6	41.4
S15	0.63	2.6	43.8	19.5	34.1
S16	0.68	3.1	41.4	22.7	32.8
S17	0.57	2.6	39.5	19.6	38.3
S19	0.61	3.6	36.2	24.7	35.5
S20	0.65	2.6	39.9	23.7	33.8
S21	0.53	1.3	41.2	18.6	38.9
S22	0.47	0.8	41.4	17.3	40.5
S23	0.38	1.6	49.5	12.7	36.3

[a] S, sediment. [b] DL, detection limit.

for these experiences was maintained at 1:1, which was generally observed in the real samples. Tables 2–4 show the results obtained using different concentrations of acids for extraction of these doped samples; the presence of a high concentration of HBr or HCl is critical for total recovery of organotin species, specially di- and mono-derivatives, possibly because these are more strongly adsorbed on the sediment owing to their higher electrical charge. Butyltin species require an HBr concentration between 70 to 80% for complete recovery and above 90% for degradation. For phenyltin species, extraction is more difficult because recoveries decrease above 70% HBr due to degradation; therefore 60% HBr is proposed as optimal, although recoveries for MBT and MPT are lower: 90% and 60%, respectively. With HCl (Table 3) it is not possible to leach the mono-derivatives (MBT and MPT) and the quantitative extraction of TBT and DBT requires a higher concentration of acid than when using HBr, since degradation is not observed. HCl is not suitable for extraction of TPT and DPT owing to degradation.

Table 18 Concentrations of butyl- and phenyl-tin compounds in the waters of Cadiz Bay

	Organotin concentration (ng Sn l^{-1})					
Sample[a]	TBT	DBT	MBT	MPT	DPT	TPT
W28	41	27	13.2	<DL[b]	<DL	<DL
W33	50	34	29	42	<DL	<DL
W26	25.6	21.9	8.9	<DL	<DL	<DL
W25	488	68	41	<DL	<DL	<DL
W37	16.0	8.3	7.7	<DL	<DL	<DL
W38	40.0	8.5	<DL	<DL	<DL	<DL
W39	8.3	<DL	<DL	<DL	<DL	<DL
W30	11.8	10.3	13.5	<DL	<DL	<DL
W36	54	13.1	9.8	<DL	<DL	<DL
W24	<DL	<DL	<DL	<DL	<DL	<DL
W27	99	25.0	21.0	<DL	<DL	<DL

[a] W, water. [b] DL, detection limit.

It is possible to achieve high recoveries of the six organotins under study using 60% HCl as leaching agent in the presence of 10 ml of concentrated HBr which contributes to mono-derivative leaching (Table 4).

Influence of the tropolone and volume of pentane

To avoid the problems arising from the extraction of more polar organotin compounds such as MBT and MPT, the use of tropolone has been tested. This reagent is frequently recommended for organotin species, and was used in previous studies on waters.[6,7] The results in Table 5 show that tropolone is not critical for the extraction of organotins from sediments. Experiments carried out on 60% HBr acidified samples did not give quantitative recoveries for MBT and MPT, although tropolone improved the extraction.

Pentane has been used as a nonpolar solvent for separation of leached organotin species from the resulting polar acid extract. It can be seen in Table 6 that optimizing the volume is an important factor for good recoveries of mono-derivatives, it being necessary to use at least 100 ml of solvent for suitable results.

Optimization of both leaching and extraction times

The successive application of leaching and extraction steps for the recovery of organotins from soils makes necessary the combined optimization of time involved in both stages of the procedure. For leaching optimization the extraction time was fixed at 3 h, (Table 7) at least 1 h of leaching being necessary for quantitative results. On the other hand, extraction time was varied, with leaching time at 1 h. It was verified that 1 h is necessary for satisfactory recoveries, although more reliable results were obtained after an extraction of 2 h (see Table 8).

Recoveries from polluted sediments

Procedures developed for the extraction of organotin species from doped sediments have to be checked in other sediments undergoing organotin

Table 19 Concentrations of butyl- and phenyl-tin compounds in the sediments of Cadiz Bay

Sample[a]	Moisture content (%)	Organotin concentration (ng Sn g^{-1})					
		TBT	DBT	MBT	MPT	DPT	TPT
S28	47.3	23.5	23.2	6.3	<DL[b]	<DL	<DL
S29	47.3	13.3	12.8	3.6	2.9	4.4	<DL
S30	48.6	1.6	1.1	1.7	<DL	2.4	<DL
S31	36.3	28.8	14.9	10.3	2.1	1.8	1.5
S32	43.0	34.1	23.7	11.6	4.8	2.2	<DL
S33	26.6	225	52	31	35	3.1	3.3
S34	25.0	40	4.9	3.1	1.0	<DL	<DL
S36	61.7	42.0	21.7	9.5	21.6	26.8	2.2
S35	42.42	11.2	7.2	7.3	12.3	18.0	<DL
S37	51.98	20.9	16.9	9.2	20.7	20.1	<DL
S38	58.2	42.4	38.3	12.6	<DL	3.7	2.9
S39	23.4	7.2	3.5	1.5	<DL	<DL	<DL
S40	21.1	6.0	4.3	1.2	<DL	<DL	<DL

[a] S, sediment. [b] DL, detection limit.

contamination in the environment, because adsorption of these species in real samples can follow different mechanisms from those in doped samples. For this purpose three sediments from the Andalusian coast (south-west Spain) which receive inputs from some boating activity (harbors, recreational boatyards, commercial wharves, fishing activities, etc.) have been tested. Sediment samples were mechanically homogenized for 7 h and stored in the refrigerator until use, and were then manually homogenized for 5 min.

In Tables 9 and 10 can be seen the influence of acid concentration (HBr) and leaching and extraction times on recoveries of organotins from the environmental samples. Results are similar to those from doped samples. High concentrations of HBr do not affect separation of butyltin derivatives but cause degradation of phenyltins. Therefore, a HBr/water (3:2) mixture has been selected for extraction. It is also possible to use dichloromethane as extraction agent with similar results (Table 11). The influence of digestion (leaching) and extraction steps are negligible.

Table 20 Grain type and organic matter content (%) in the sediments of Cadiz Bay

Sample[a]	Organic carbon	Coarse sand	Fine sand	Silt	Clay
S28	1.64	1.8	12.4	22.5	63.2
S29	1.27	9.9	4.9	15.1	70.1
S30	1.16	5.3	11.6	17.3	65.8
S31	0.71	0.8	51.4	31.9	15.9
S32	0.66	0.8	48.3	26.2	24.7
S33	0.36	12.9	50.4	25.6	11.1
S34	0.29	14.7	57.9	19.9	7.5
S36	1.86	1.6	1.1	18	79.3
S35	1.75	4.3	6.2	17.8	71.6
S37	1.69	3.8	6.1	22.8	67.3
S38	2.14	0.1	1.3	19.8	78.8
S39	0.12	17.2	53.3	14.1	15.4
S40	0.1	23.4	61.3	6.3	8.9

[a] S, sediment.

Table 21 Concentrations of butyl- and phenyl-tin compounds in the waters of Caño de Sancti Petri

Sample[a]	Organotin concentration (ng Sn l^{-1})					
	TBT	DBT	MBT	MPT	DPT	TPT
W64	15.8	6.6	6.7	<DL[b]	<DL	<DL
W63	<DL	6.8	6.1	<DL	<DL	<DL
W58	5.3	<DL	8.6	<DL	<DL	<DL
W56	8.3	37	19.7	<DL	<DL	<DL
W51	50	34	29	<DL	<DL	<DL
W48	8.4	12.4	13.6	<DL	<DL	<DL
W47	5.6	9.4	8.1	<DL	<DL	<DL
W43	6.3	6.8	6.5	<DL	<DL	<DL
W45	4.9	<DL	5.6	<DL	<DL	<DL
W38	56	18.6	20.1	<DL	<DL	<DL
W41	9.1	8.6	8.1	<DL	<DL	<DL
W65	<DL	<DL	<DL	<DL	<DL	<DL

[a] W, water. [b] DL, detection limit.

Occurrence of organotin species on the Cadiz Coast

Separation procedures have been applied to assess the presence of organotin species in southwest Spain, because few data have been reported on their possible environmental impact in this area (see Fig. 1). Sampling sites were selected where boating activity was high and where shellfish farms, fishery activity or migrating birds might be at risk.

Concentrations of organotin compounds in the Guadalete River (see Fig. 2) are shown in Tables 12 and 13. There are two potential foci for organotin inputs in the final river catchment: Puerto de Santa Maria and Sherry Marina. This is because TBT levels in water samples from points 1 and 5 reach appreciable values, decreasing with the distance from the focus. Results for sediment samples in Table 13 show very low levels of phenyltins, however concentrations of TBT are high, which suggests accumulation processes. This accumulation is specially marked points 7 and 8, possibly due to the low percentage of sand and the associated increase in organic matter content (Table 14). Sediment from point 9 has similar characteristics, but the increasing distance to the focus reduces accumulation. High DBT concentrations at points 7 and 8, which suggest a degradation process can be seen. The environmental impact caused by these high levels of TBT and DBT in the sediments of this area may be the reason for the appreciable decrease in shellfish production in recent years.

In the San Pedro River (Fig. 2) were detected only low concentrations of butyltins both in water and sediments (Tables 15 and 16) owing to the absence of harbors or dockyards. These sediments have similar percentages of sand and clay and low contents of organic matter (Table 17), which can reduce the accumulation of tin species.

As has been noted previously, Cadiz Bay (see Fig. 3) is a complex area, with numerous foci for organotin contamination and a complicated

Table 22 Concentrations of butyl- and phenyl-tin compounds in the sediments of Caño de Sancti Petri

Sample[a]	Moisture content (%)	Organotin concentration (ng Sn g^{-1})					
		TBT	DBT	MBT	MPT	DPT	TPT
S64	15.2	601	510	129.4	21.5	14.5	19.0
S60	40.63	8.6	1.2	8.0	7.1	12.3	<DL[b]
S61	41.79	3.8	1.9	4.6	11.7	15.3	<DL
S59	53.84	18.8	7.6	1.2	3.2	1.7	0.9
S62	34.5	1.6	2.4	1.6	<DL	<DL	<DL
S56	45.03	3.8	3.9	14.1	33	26	<DL
S57	40.18	1.6	3.9	14.0	20	28	2.0
S58	42.83	1.7	2.0	12.0	33	37	2.2
S54	48.37	2.3	4.9	15.3	8.8	21	<DL
S36	48.8	7.5	4.6	15.1	<DL	<DL	<DL
S52	52.09	82	9.3	10.9	25.2	15.9	61
S51	42.8	16.69×10^3	1.22×10^3	547.1	382	188	320
S53	47.2	10.0	18.9	22.3	<DL	<DL	<DL
S50	51.65	50	21	15.0	30	32	2.8
S49	50.74	1.7	2.4	14.3	21	21	<DL
S48	37.52	4.3	6.5	8.5	4.6	17.8	1.3
S47	46.76	3.7	6.8	19.9	31	22	<DL
S44	48.88	2.2	2.0	10.1	30	14	<DL
S45	54.7	2.7	3.8	2.7	2.2	<DL	0.8
S46	51.98	8.6	18.0	16.6	23	18	1.1
S43	49.38	7.6	15.0	18.1	20	20	<DL
S40	24.8	10.0	18.9	22.3	2.1	3.2	<DL
S42	59.7	57	61	20.4	4.8	4.6	3.4
S38	41.9	165	98	32	7.7	6.9	6.3
S39	57.4	675	26.0	13.8	2.5	2.3	3.6

[a] S, sediment. [b] DL, detection limit.

Table 23 Grain type and organic matter content (%) in the sediments of Sancti Petri Channel

Sample[a]	Organic carbon	Coarse sand	Fine sand	Silt	Clay
S64	0.52	4.2	46.5	25.7	23.7
S60	0.23	3.8	37.6	29.1	29.5
S61	0.18	2.3	29.4	22.7	45.6
S59	0.58	14.3	31.4	19.8	34.5
S62	0.12	<DL[b]	45.6	38.1	16.3
S56	1.58	16.5	25.4	16.6	41.4
S57	0.84	2.9	36.8	21.3	39
S58	0.24	0.8	31.0	39.7	28.5
S54	0.52	3.6	39.8	29.6	27
S36	0.80	8.1	34.1	29.3	28.5
S52	1.89	1.8	16.7	23.1	58.3
S51	1.77	21.7	9.2	20.4	48.7
S53	1.94	6.1	9.4	115.9	68.6
S50	1.79	0.6	9.6	25.7	64.1
S49	1.84	<DL	4.8	24.2	71.0
S48	1.63	0.9	7.8	27.1	64.2
S47	1.76	1.2	17.6	23.5	57.7
S44	1.59	0.6	9.4	17.3	72.7
S45	1.47	<DL	11.7	29.8	57.5
S46	1.94	<DL	6.8	25.0	68.2
S43	2.01	1.4	7.3	24.6	66.7
S40	1.14	11.4	23.0	17.1	48.5
s42	3.08	1.0	7.2	20.1	71.7
s38	2.21	27.4	8.7	12.4	51.5
s39	2.16	8.6	15.7	21.3	54.4

[a] S, sediment. [b] DL, detection limit.

hydrodynamic behavior which involves a number of interconnected channels flowing to the bay, which has a very variable depth. These facts make it difficult to establish a general picture of the presence of tin species in the area. The concentrations in Table 18 exhibit considerable differences, ranging from the highest levels in the waters of

Table 24 Concentration of butyltin species in harbors of the Cadiz coast

Site	Butyltin concentration (ng Sn l^{-1})			
	TBT	DBT	MBT	Total
Puerto Sherry	116	32	25	173
Puerto de Santa Maria	73	20	15	108
Puerto Real (dockyard)	99	25	21	145
Puerto Real (harbor)	45	34	29	108
Cadiz Fish Dock	489	68	41	598
La Carraca	56	19	20	95
Sancti Petri	16	7	7	30
La Gallinera	50	34	29	113

Table 25 Averaged concentrations of butyltins in the waters of south-west Spain

Area	Butyltin concentration (ng Sn l^{-1})			
	TBT	DBT	MBT	Total
Guadalete River				
Harbor zone	95	30	17	142
Non-harbor zone	13	7	5	25
San Pedro River				
Harbor zone	—[a]	—	—	—
Non-harbor zone	12	8	10	30
Cadiz Bay				
Harbor zone	145	30	21	196
Non-harbor zone	15	9	8	32
Sancti Petri River				
Harbor zone	39	20	18	77
Non-harbor zone	6	10	9	24

[a] —, No harbor in this river.

the Fish Dock of Cadiz city, point 25, to the absence of tin species in the nearby Commercial Dock, point 24. Remarkable TBT levels have been detected in the fishing port of Puerto Real village, point 33, as well as at points 27 and 28 owing to the influence of Puerto Real dockyard. At point 36, a military harbor, the presence of organotins is also notable. However, phenyltins are not present in the Bay, with the exception of MPT at point 33 (Puerto Real). Analysis of sedi-

Table 26 Averaged concentrations of butyltins in sediments of south-west Spain

Area	Butyltin concentration (ng Sn g^{-1})			
	TBT	DBT	MBT	Total
Guadalete River[b]				
Harbor zone	125	47	21	193
Non-harbor zone	404	289	88	781
San Pedro River				
Harbor zone	—[a]	—	—	—
Non-harbor zone	6	5	3	14
Cadiz Bay				
Harbor zone	103	37	18	158
Non-harbor zone	19	11	7	37
Sanci Petri River				
Harbor zone	5800	611	236	6700
Non-harbor zone	16	10	13	39

[a] —, No harbor in this river. [b] Organotin concentrations are higher in non-harbor zone due to the high percentage of organic matter. Harbor sediments show a sandy, not adsorbent, character.

ments also reveals significant concentrations of organotins, especially around the Puerto Real harbor, point 33 in Table 19, with 224 ppb of TBT, in spite of the low concentration of organic matter in this sample (Table 20). The concentration of TBT decreases at other points such as 33 and 34, as distance from the harbor increases.

Finally, the study carried out in Sancti Petri Channel (Table 21) also indicates higher concentrations of TBT in waters near small harbors (points 38, 51 and 64), although levels in the last of these are low, possibly owing to the proximity of the coast and the diluting action of the tide. However, the enclosed character of point 51, La Gallinera harbor, in the middle of the channel with low water mobility, causes a higher concentration of TBT. Samples corresponding to intermediate points between harbors, such as points 60, 58 and 56, show relatively higher concentrations of DBT and MBT, which denotes the presence of degradation processes. In secondary channels the levels of all organotin species are very low as a consequence of the hydrodynamics of the system which mobilizes organotins through the main channel. In general, with the exceptions previously cited, waters in this zone exhibit little contamination by organotin compounds. Results for sediments in Table 22 show similar trends: high concentrations of butyltins, even phenyltins, in samples from harbor points 38, 51 and 64, specially at La Gallinera wharf (point 51), as well as at other points (such as 50 and 52) undergoing influence from those foci; and low concentrations of the other sites of the Sancti Petri catchment. Finally, the differences in organotin levels between points 51 and 64, La Gallinera and Sancti Petri harbors respectively, could be explained by the different contents of organic matter, clay and sand in them (Table 23), point 51 being richer in organotin-adsorbent components—organic matter and clay—which could explain the greater accumulation of butyl- and phenyl-tins observed in it.

CONCLUSIONS

The appraisal carried out on the Cadiz coast reveals that the presence of organotins in waters and sediments is important, with a number of points with high levels of butyltin species in waters and sediments, which could have important consequences for the biological quality of the ecosystem, depressing the growing of biota. Thain *et al.*[20] showed that shell thickening in *C. gigas* could be induced by TBT at levels of $20\,ng\,l^{-1}$. Even lower concentrations of TBT (approximately $2\,ng\,l^{-1}$) were shown to induce imposex in the dogwhelk *Nucella lapillus*,[21] and so impair reproduction.[22] As a consequence, an Environmental Quality Standard value (EQS) for TBT was set at $20\,ng\,l^{-1}$ in the UK In 1987 and latterly re-set at $2\,ng\,l^{-1}$ in order to achieve protection of marine life;[23] it has produced a clear reduction of TBT concentrations in UK estuaries.[23,24]

The presence of organotins in harbors and sites with high boating activity has been summarized in Table 24, which denotes the critical situation of the Fish Dock of Cadiz city, a site clearly under stress from tin species. Levels of organotins are also remarkable at Puerto Sherry marina, Puerto de Santa Maria harbor and Puerto Real dockyard. However the situation cannot be generalized for all sites on this coast, e.g. in Sancti Petri harbor the waters exhibit values low in organotins.

There is a strong connection between the presence of organotins in the environment and boating activity on the Cadiz coast. Tables 25 and 26 show the average organotin concentrations in waters and sediments, obtained from the data of this paper. In the different areas we establish two sub-areas—with high boating activity and with low boating activity—according to the relative presence of boats which could leach organotins from their hulls. However these figures have only a relative statistical value and they are not intended to provide a complete evaluation of organotins on this coast. From Tables 25 and 26 we can establish some trends: (1) TBT is the predominant species in waters, with accumulation at wharves, docks, shipyards, etc; (2) DBT exhibits higher concentrations than MBT in harbors, but the levels of the former species decrease with distance from these foci, a correlated increase in MBT concentration being verified which can be related to natural degradation processes;[25,26] (3) the predominance of TBT is not so marked in sediments, which show relatively high concentrations of DBT and MBT.

Finally, a general appraisal of data reveals the accumulation of organotin species in sediments in zones receiving direct inputs from boats; this effect decreases with distance, which makes it possible to explain the presence of productive fish farms at sites far from the focus. However, the

biological population at these foci and around them could be depressed.

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