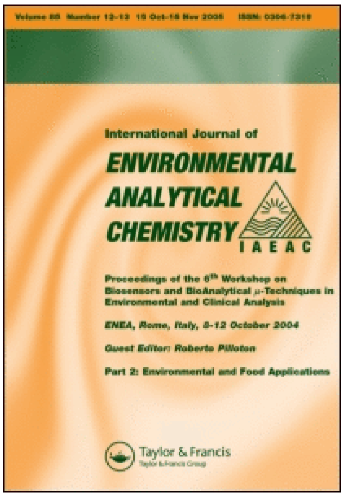

International Journal of Environmental Analytical Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713640455

Spatial and seasonal behaviour of organotin compounds in protected subtropical estuarine ecosystems in Okinawa, Japan

Mohammed Ali Sheikh[a]; Keiko Tsuha[a]; Xiaochun Wang[a]; Kenzaburo Sawano[a]; Sia Taema Imo[a]; Tamotsu Oomori[b]
[a] Marine and Environmental Sciences, Graduate School of Engineering and Science, University of the Ryukyus, Okinawa 903-0213, Japan [b] Faculty of Science, Department of Chemistry, Biology and Marine Science, University of the Ryukyus, Okinawa 903-0213, Japan

To cite this Article Sheikh, Mohammed Ali , Tsuha, Keiko , Wang, Xiaochun , Sawano, Kenzaburo , Imo, Sia Taema and Oomori, Tamotsu(2007) 'Spatial and seasonal behaviour of organotin compounds in protected subtropical estuarine ecosystems in Okinawa, Japan', International Journal of Environmental Analytical Chemistry, 87: 12, 847 — 861
To link to this Article: DOI: 10.1080/03067310701415629
URL: http://dx.doi.org/10.1080/03067310701415629

Intern. J. Environ. Anal. Chem.
Vol. 87, No. 12, 15 October 2007, 847–861

Taylor & Francis
Taylor & Francis Group

Spatial and seasonal behaviour of organotin compounds in protected subtropical estuarine ecosystems in Okinawa, Japan

MOHAMMED ALI SHEIKH*†, KEIKO TSUHA†, XIAOCHUN WANG†,
KENZABURO SAWANO†, SIA TAEMA IMO† and TAMOTSU OOMORI‡

†Marine and Environmental Sciences, Graduate School of Engineering and Science,
University of the Ryukyus, 1 Senbaru, Nishihara-Town, Okinawa 903-0213, Japan
‡Faculty of Science, Department of Chemistry, Biology and Marine Science,
University of the Ryukyus, 1 Senbaru, Nishihara-Town, Okinawa 903-0213, Japan

(*Received 30 November 2006; in final form 23 April 2007*)

The spatial and temporal behaviours of the organotin compounds (OTCs) (butyl- and phenyltin) were investigated in the Manko and Okukubi protected estuarine ecosystems on Okinawa Island, Japan from February to October 2006. Butyltin compounds (BTCs) were frequently detected in all seasons, while phenyltin (PhTs) were found in winter and early spring. In Manko estuary, the total mean concentrations of BTCs and PhTs were 22.78 ± 30.85, (mean ± SD, $n = 53$) and $0.08 \pm 0.27\,\mathrm{ng(Sn)\,L^{-1}}$, respectively. In Okukubi estuary, BTCs and PhTs were 12.58 ± 23.96 and 0.47 ± 1.67 ($n = 55$) $\mathrm{ng(Sn)\,L^{-1}}$, respectively. The Manko sediments can be classified as lightly contaminated, while the Okukubi sediments were uncontaminated with tributyltin (TBT). The mean levels of TBT shown in Manko estuary exceeded the threshold level and represent an ecotoxicological risk to sensitive aquatic life. Generally, the present study reports the occurrence and continuous input of OTCs in the protected estuaries, even 16 years after legal restriction of TBT usage in coastal waters was implemented by the Japanese Environmental Authorities.

Keywords: OTCs; Manko; TBT; Estuarine; Okukubi; Sources

1. Introduction

The Manko and Okukubi estuaries are protected wetlands located in a subtropical climate on Okinawa Island. These estuaries are recognized as an important visiting and wintering area between southeast Asia and Japan for migratory birds. They are also used for local people and tourists for recreation. Furthermore, they preserve diverse species of organisms. However, they are vastly exposed to wastewater discharge from domestic, agricultural, shipping, and livestock farming [1, 2].

Organotin compounds (OTCs) have grown in importance since the 1940s and have been used for various industrial applications [3]. To date, about 70% of OTCs are still used as mono/dialkyltin in polyvinylchloride (PVC) industry as a derivative

---

*Corresponding author. Fax: +81-98-895-8565. Email: sheikhmali2003@yahoo.com

International Journal of Environmental and Analytical Chemistry
ISSN 0306-7319 print/ISSN 1029-0397 online © 2007 Taylor & Francis
DOI: 10.1080/03067310701415629

stabilizer [4]. Triphenyl (TPhT) tin compounds have been widely used in agriculture as fungicides and insecticides. TBT was used mostly as biocides in marine antifouling agents [5]. The agricultural and biocidal use of organotin probably increases the load of organotin compounds in the environment [6]. Sewage disposal could be a potential source of OTCs in urban coastal areas [7].

Triorganotin compounds such as tributyltin (TBT) and triphenyltin (TPhT) are the most toxic forms of OTCs that have caused deleterious effects on some aquatic organisms [8–10]. It has been revealed that TBT concentrations of around $1\,\mathrm{ng\,L^{-1}}$ may affect calcification in adult oysters, mortality of larvae, and growth of phyto- and zooplankton [11, 12]. Owing to their high toxicity, butyl- and phenyltin compounds have been listed among the most pollutant substances in European countries [13]. As a consequence, many countries have imposed regulations on the usage of TBT containing paints [14, 15].

Japanese environmental authorities have controlled the use and sale of organotin compounds under the 'Law Concerning the Examination and Regulation of Manufacture etc. of Chemical substances' since 1990 [16]. Despite the legal restrictions, organotin compound residues continue to be widely reported in coastal areas around Japan [10, 16–22].

Contamination levels of organotin compounds (OTCs) are higher in port areas, estuaries, or semi-closed areas [23]. In general, most organotin compounds studies are focused in ports, marinas, and coastal areas. However, very little is known about the behaviour of OTCs in freshwater and estuarine ecosystems of subtropical areas. The main objective of this article is to provide detailed information on distribution and behaviour of butyl- and phenyltin compounds in protected subtropical estuaries in the Okinawa Islands, Japan.

2. Experimental

2.1 *Sampling*

Details of the sampling points are shown in table 1 and figure 1. Water samples were collected bimonthly in four seasons (winter – February, early spring – April, late spring – June, summer – August, and autumn – October, 2006). Sediment samples were taken in the winter.

Subsurface water samples were taken using 2-L polycarbonate amber bottles. The bottles were washed with soapy water and then decontaminated with 10% HNO_3 solution and kept overnight. They were rinsed with Milli-Q water again and dried in a clean room before sampling. In addition, the bottles were always rinsed thoroughly with waters before sample acquisition. Water samples were transported in a cooler box and filtered immediately after arrival in the laboratory using $0.45\,\mu\mathrm{m}$ GF/F. All water samples were stored at 4°C in the dark and extracted within 5 days. Sediment samples were collected using a grab sampler, kept in aluminium foil, and then sealed in clean polyethylene bags. Samples were brought to the laboratory under 4°C and then kept in a deep freeze at −20°C. All sediment samples were dried by a freeze-dryer (FRD-51, Iwaki, Japan) before extraction.

Table 1. Details of sampling areas.

Estuary	Transect	Station	Location	Activities
Manko (26° 13′ N, 127° 41′ E)	TK 1	MK 1a	Naha port	Commercial, passengers, Cargo, US navy, Japanese Coast guard shipping activities
		MK 1b		
		MK 1c		
		MK 1d		
	TK 2	MK 2a	River mouth (Tsubogawa port)	Fishing and leisure Boats, residential
		MK 2b		
		MK 2c		
		MK 2d		
	TK 3	MK 3a	Middle of river	Residential, commercial and public park (Koen)
		MK 3b		
		MK 3c		
		MK 3d		
	TK 4	MK 4a	Upstream	Residential, commercial and public park (Koen)
		MK 4b		
Okukubi (26° N, 128° E)	TO 1	OT 1a	Coastal area	Local fishing
		OT 1b		
		OT 1c		
		OT 1d		
	TO 2	OT 2a	River mouth	Agriculture
		OT 2b		
		OT 2c		
		OT 2d		
	TO 3	OT 3a	Middle of river	Agriculture
		OT 3b		
		OT 3c		
		OT 3d		
	TO 4	OT 4a	Upstream	Agriculture
		OT 4b		
		OT 4c		

2.2 *Chemicals*

Organotin standards, tripropyltin chloride (TPrT, 98%), monobutyltin trichloride (MBT, 95%), dibutyltin dichloride (DBT, 96%), and tributyltin chloride (TBT, 96%), were purchased from Merck. Stock solutions of $1000\,\mathrm{mg(Sn)\,L^{-1}}$ of organotin compounds were prepared in methanol (pesticide analysis grade) and then stored at 4°C. Working solutions were prepared weekly by diluting the stock solutions. Sodium tetraethylborate ($NaBEt_4$) 98%, hydrochloric acid ($\mathrm{Sn} < 0.1\,\mathrm{pg\,mL^{-1}}$), acetic acid (ultrapure grade), anhydrous sodium sulfate, tetramethyl ammonium hydroxide (TMHA, 25%, $\mathrm{Sn} < 20\,\mathrm{pg\,mL^{-1}}$) and ammonium solution (ultrapure grade) were obtained from Kanto Chemicals (Tokyo).

2.3 *Analysis*

2.3.1 Sediments. The extraction of organotin compounds from sediments was performed as follows [24]: to 0.5 g of freeze-dried sediment in a centrifugal tube, 100 µL of TPrT ($50\,\mathrm{\mu g\,L^{-1}}$) was added as an internal standard. After 10 min, 2 g of NaCl, 12 mL of toluene containing 0.1% tropolone, and 10 mL of $1\,\mathrm{mol\,L^{-1}}$ of HCl methanol were added. The tubes were capped and mixed for 60 min afterwards, and

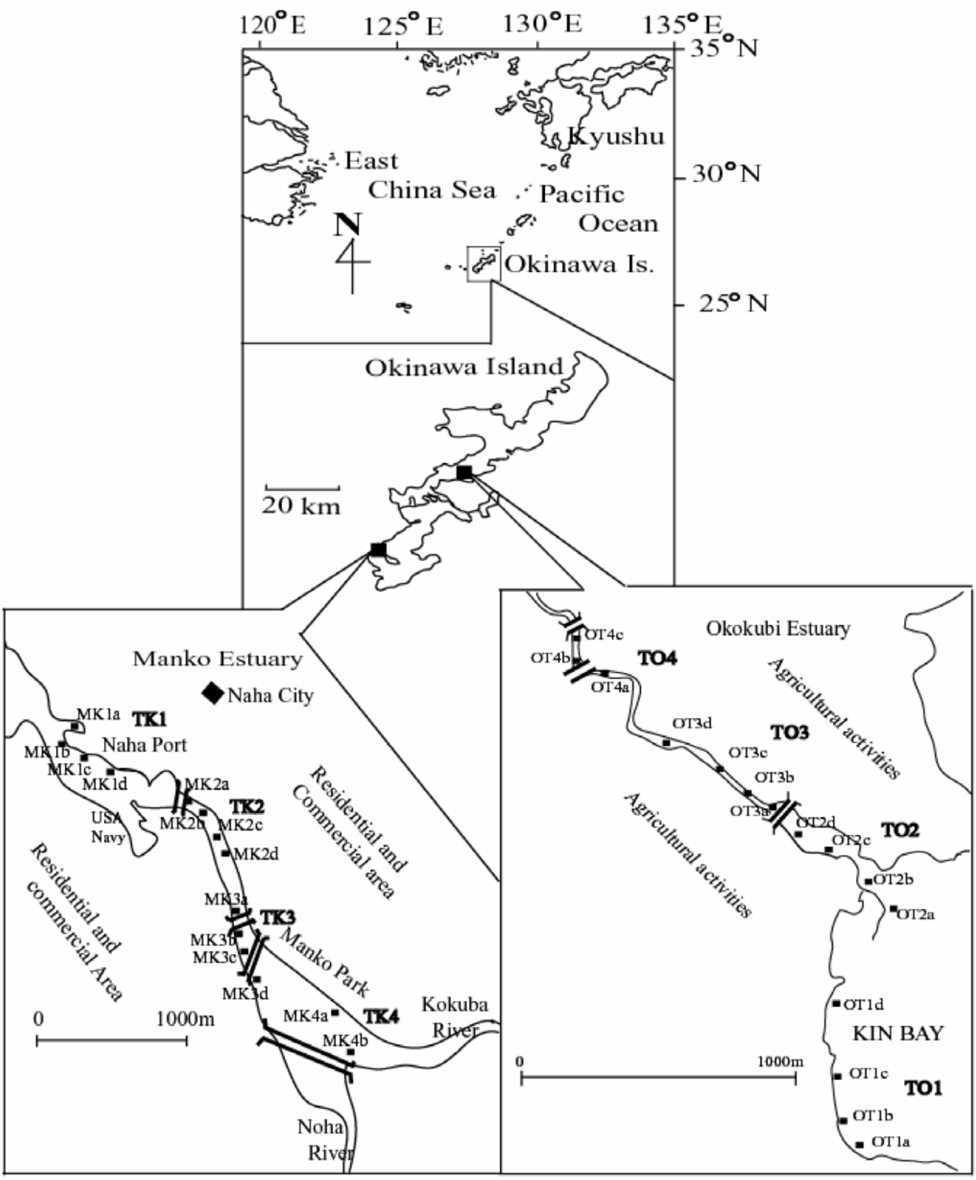

Figure 1. Map showing transects and sampling points.

10 mL of Milli Q water was added. The tubes were shaken for 10 min and then centrifuged at 2000 rpm for 3 min. The toluene part was concentrated to 5 mL for further analysis. $NaBEt_4$ was applied as a derivatization agent for organotin compounds in sediments as follows: 5 mL of $1\,mol\,L^{-1}$ acetate buffer of pH 5 was added to the acetone part. Fifteen millilitres of milli Q water and 1 mL of 5% $NaBEt_4$ were added. The tubes were shaken for 10 min for ethylation and extraction, then centrifuged. The hexane layer was collected from the centrifugal tube using a pasteur pipette. Water traces were removed from the sample by applying 2 g of anhydrous

Na_2SO_4. Finally, the hexane extract was evaporated to 100 µL using a stream of nitrogen gas. 1 µL was injected into the GC for OTCs analysis.

2.3.2 Water. Five millilitres of 1 M acetate buffer was added to 1 L of a filtered water sample, and then the pH of water was adjusted to 5. One hundred microlitres of 50 ng (Sn) L^{-1} of TPrT was then added as an internal standard. The derivatization of OTCs was performed by adding 1 mL of 5% $NaBEt_4$, and then 30 mL of hexane was added for liquid/liquid extraction. The hexane layer was dried by Na_2SO_4. Finally, the extract was concentrated to 100 µL for OTCs analysis.

Temperature and pH were measured *in situ* using a pH meter (Thermo orion 290+, Japan).The pH meter was calibrated with NBS-scaled buffer solutions (TOA Golden buffer) of pH (6.863 at 25°C) and pH 4 (4.006 at 25°C). Water salinity was determined in laboratory by a portable salinometer (Model 8410A, Canada). Salinity values were obtained as the conductivity of seawater relative to reference standard seawater (UK-Ocean Scientific International IAPASO ($K_{15} = 0.99987$, $S = 34.995$). DOC was determined by a total organic carbon analyser (TOC-V_{CSH}, Shimadzu, Japan).

2.4 *Instrumental and quantification*

Organotin compounds were analysed by a gas chromatography (GC) (HP 6890 Series) equipped with a HP-1 methyl siloxane column (30 m length, 320 µm i.d., 0.25 µm film thickness) and a flame photo-detector (FPD) fitted with a 610-nm bandpass optical filter. The carrier gas was He (2.0 mL min^{-1}), in splitless mode, and a sample of 1 µL was automatically injected to the GC. The temperature was programmed as (1) oven parameters, initial temperature 55°C, final temperature 300°C, ramp rate 15°C (from 55 to 100°C), 30°C (from 100 to 300°C); hold time 55°C (0 min), 100°C (0 min) and 300°C (1.33 min). (2) FPD temperature 240°C, He flow rate 150 mL min^{-1}, and air flow rate 90 mL min^{-1}. The peak areas of the individual butyl- and phenyltin were used for quantification. Peaks of butyl- and phenyltin in the chromatograms were assigned to individual organotin species on the basis of retention times (MBT 5.54 min, TPrT 5.87 min, DBT 6.32 min, TBT 6.95 min, MPhT 6.69 min, DPhT 8.25 min, TPhT 9.52 min) (see figure 2).

Quality assurance of the results was monitored by preparing calibration curves every week and injecting a derivatized standard with all butyl- and phenyltin species each time to test the instrument signal. The accuracy of the analytical method for butyl tin compounds in sediments was validated by analysing certified reference material (CRM 7301-a) for butyltins in marine sediments, obtained from the National Meteorological Institute of Japan. Recoveries of TBT, DBT, and MBT were 74 ± 12%, 106 ± 9%, and 57 ± 15 ($n = 3$), respectively. The accuracy of the analytical procedure for water samples was tested by spiking the OTCs (MBT, DBT, TBT, MPhT, DPhT, and TPhT) in Milli Q water. They were then extracted following the same procedure as that used for the water samples. Satisfactory recovery results (>70%) were obtained.

The quantification limits were obtained by a FPD signal greater than three times the standard deviation of the procedural blank values ($n = 8$) of each OTC species (MBT, DBT, TBT, MPhT, DPhT, and TPhT); they were <0.01 µg L^{-1} and <0.3 ng g^{-1} (dw) for both butyl- and phenyl tin compounds in water and sediment samples, respectively.

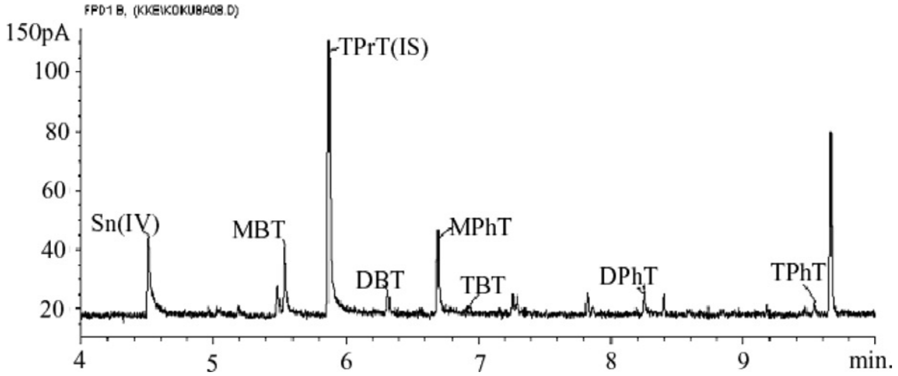

Figure 2. Typical FPD chromatogram for water from station MK 3d (February 2006).

All organotin concentrations reported in this article are expressed as the mass of Sn per mass or volume unit ng (Sn) g^{-1} dry weight for sediment or ng (Sn) L^{-1} for water samples.

3. Results

3.1 *Spatial variations of OTCs in water*

Table 2 presents the mean concentrations of all OTCs in waters from the Manko and Okukubi estuaries.

In the Manko estuary, the mean concentration of ΣBTCs was 22.78 ± 30.85, (mean ± SD). The highest mean concentrations of ΣBTCs were observed as TK1 > TK2 > TK4 > TK3. TBT mean concentrations ranged from 0.05 to 8.68 ng(Sn) L^{-1} (2.45 ± 7.12). Our results revealed that TK2 and TK1 are more contaminated with TBT than TK3 and TK4 (see figure 3). The total mean concentrations of MBT and DBT were 14.9 ± 29.86 and 5.42 ± 5.03 ng (Sn) L^{-1}, respectively. The highest mean concentrations of MBT and DBT were observed in TK1 and TK2, respectively. PhTs were found in few samples, and the total mean value was 0.08 ± 0.27. Maximum mean concentrations of PhTs species; TPhT, DPhT, and MPhT were 0.04 ± 0.11, 0.03 ± 0.1, and 0.15 ± 0.32 ng(Sn) L^{-1}, respectively. MPhT was the dominant degradation product detected among the PhTs. The highest mean concentration of TPhT was observed in TK4. No significant correlation was found between ΣBTCs and ΣPhTs (Pearson correlation coefficient, $r = -0.182$, $p > 0.05$) (see table 3).

In the Okukubi estuary, the mean concentration of BTCs was 12.58 ± 23.96 ng(Sn) L^{-1}. The maximum mean concentration of BTCs was observed at TO3 at 21.24 ± 32.78 ng(Sn) L^{-1}. The total mean values of TBT, DBT, and MBT were 0.19 ± 1.4, 0.19 ± 1.1, and 12.2 ± 23.82 ng(Sn) L^{-1}, respectively. The maximum mean concentration of TBT was detected in TO1. MBT was the dominant BTCs degradation product detected in the estuary. The overall mean value of ΣPhTs was 0.47 ± 1.67 ng (Sn) L^{-1}. Maximum mean concentrations of TPhT, DPhT and MPhT were 0.39 ± 1.13, 0.79 ± 2.59, and 0.01 ± 0.02. All maximum values of PhTs degradation

Table 2. Main basic data for OTCs in Manko and Okukubi estuaries.

		Concentrations (ng(Sn) L^{-1})								
	Transect	MBT	DBT	TBT	ΣBTs	MPhT	DPhT	TPhT	ΣPhTs	
Manko	TK1	Mean	27.00	3.07	0.31	30.38	0.04	nd	0.02	0.06
		±SD	48.06	6.68	0.70	47.05	0.16	–	0.06	0.21
	TK2	Mean	9.23	9.35	8.68	27.25	0.01	nd	nd	0.01
		±SD	10.42	12.65	12.06	21.17	0.04	–	–	0.04
	TK3	Mean	7.19	2.13	0.21	9.52	0.08	nd	nd	0.08
		±SD	9.23	9.35	8.68	27.25	0.01	–	–	0.01
	TK4	Mean	12.02	8.52	0.05	20.59	0.15	0.03	0.04	0.22
		±SD	21.33	14.19	0.15	22.30	0.32	0.10	0.11	0.536
	Total[a]	Mean ($n = 53$)	14.90	5.42	2.45	22.78[b]	0.06	0.01	0.01	0.08
		±SD	29.86	10.03	7.12	30.85	0.20	0.04	0.06	0.27
Okukubi	TO1	Mean	5.96	0.58	0.61	7.15	nd	nd	0.08	0.08
		±SD	8.91	1.95	2.51	10.78	–	–	0.18	0.20
	TO2	Mean	11.64	nd	nd	11.64	0.01	0.25	0.23	0.49
		±SD	21.74	–	–	21.73	0.03	0.99	0.93	1.92
	TO3	Mean	20.23	0.01	nd	21.24	0.01	0.79	0.39	1.18
		±SD	32.79	0.03	–	32.78	0.02	2.59	1.13	2.71
	TO4	Mean	14.95	0.02	nd	14.97	nd	nd	0.30	0.30
		±SD	32.65	0.07	–	32.65	–	–	0.93	0.94
	Total[a]	Mean ($n = 55$)	12.20	0.19	0.19	12.58[b]	0.01	0.24	0.23	0.47
		±SD	23.82	1.10	1.40	23.96	0.02	1.28	0.82	1.67

[a] All sampling stations.
[b] Significant different (paired t test, $p < 0.05$).

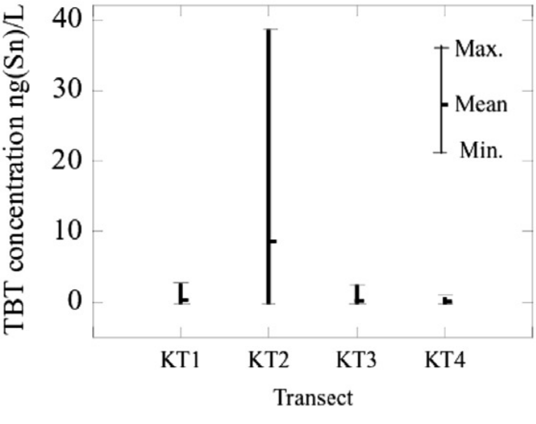

Figure 3. Range of TBT in waters from Manko estuary.

products were observed at TO3. No significant correlation was found between ΣBTCs and ΣPhTs (Pearson correlation coefficient, $r = -0.10$, $p > 0.05$).

3.2 *Spatial variations of OTCs in sediments*

Organotin compounds contents of sediments are summarized in figure 4. In Manko estuary, MBT was detected in all sediment samples analysed. The maximum TBT value was 38.66 ng(Sn) g^{-1} dwt. detected at TK2. The second highest mean concentration was

Table 3. Pearson correlation coefficients of OTCs and physico-chemical factors in waters.[a]

	MBT	DBT	TBT	ΣBTs	MPhT	DPhT	TPhTs	ΣPhTs	Salinity	Temperature	pH	DOC
MBT	1	0.00	0.01	0.99	−0.09	−0.06	−0.09	−0.10	0.01	0.35	−0.18	−0.04
DBT	**−0.13**	1	0.99	0.10	−0.02	−0.03	−0.03	−0.04	0.15	0.01	0.19	0.23
TBT	**−0.08**	**0.18**	1	0.11	−0.03	−0.03	−0.04	−0.04	0.13	0.02	0.17	0.25
ΣBTs	**0.90**	**0.23**	**0.21**	1	−0.10	−0.07	−0.10	−0.10	0.02	0.35	−0.16	−0.02
MPhT	**−0.11**	**−0.15**	**−0.09**	**−0.18**	1	−0.03	0.06	0.02	0.17	−0.34	0.16	−0.16
DPhT	**−0.06**	**−0.07**	**−0.04**	**−0.10**	**0.61**	1	0.23	0.88	−0.11	−0.08	−0.56	−0.03
TPhT	**−0.09**	**−0.12**	**−0.07**	**−0.14**	**0.67**	**0.80**	1	0.67	−0.13	−0.17	−0.06	−0.04
ΣPhTs	**−0.11**	**−0.15**	**−0.09**	**−0.18**	**0.96**	**0.77**	**0.82**	1	−0.15	−0.15	−0.45	−0.05
Salinity	**0.18**	**−0.44**	**0.43**	**0.04**	**0.07**	**−0.02**	**0.12**	**0.08**	1	−0.07	0.61	−0.48
Temp.	**0.09**	**0.06**	**0.15**	**0.14**	**−0.39**	**−0.23**	**−0.31**	**−0.39**	**0.22**	1	−0.15	0.10
pH	**0.03**	**−0.20**	**0.04**	**−0.03**	**0.05**	**0.01**	**0.04**	**0.05**	**0.26**	**0.61**	1	−0.12
DOC	**0.15**	**−0.13**	**−0.05**	**0.13**	**−0.30**	**0.11**	**−0.02**	**−0.21**	**−0.05**	**0.43**	**−0.47**	1

[a] Values in bold are for the Manko estuary. Values in normal font are for the Okukubi estuary.

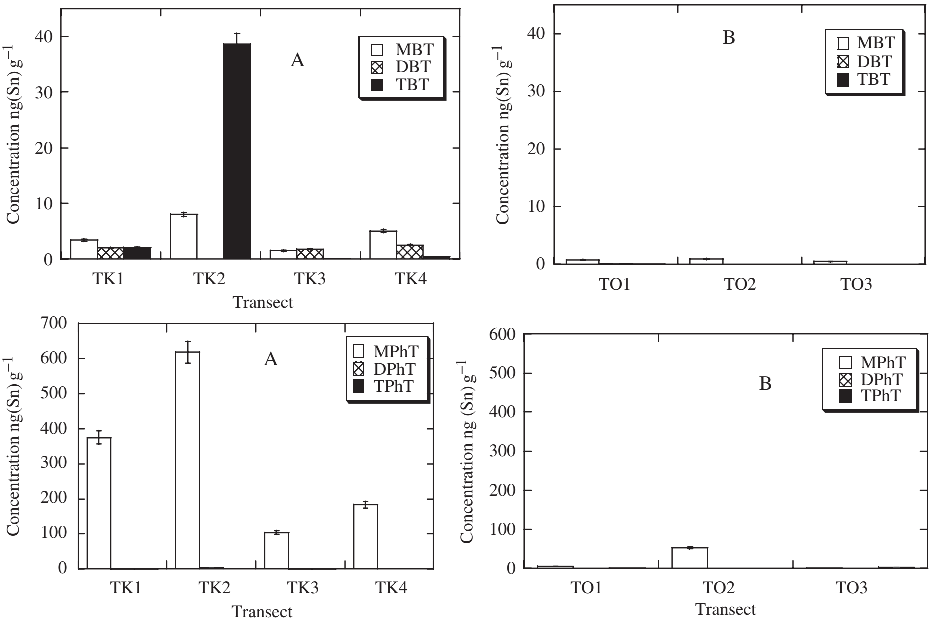

Figure 4. Distribution of BTs and PhTs in sediments from (A) the Manko estuary and (B) the Okukubi estuary.

2.08 ng(Sn) g^{-1} of TBT observed at TK1. Minimum concentration of the TBT was 0.1 ng(Sn) g^{-1} found at TK3. The gradient of TBT contamination pattern shown in sediment is TK2 > TK1 > TK4 > TK3.

Phenyltin compounds were also found in Manko sediments. The maximum mean values of TPhT, DPhT, and MPhT were 1.65, 2.08, and 5.42, and 619.8 ng (Sn) g^{-1} at TK2. TPhT was under the detection limit at TK4. The ratio of MPhT in PhTs was higher in all transects in the Manko estuary (see figure 5).

A significant positive correlation was found between ΣBTCs and ΣPhTs (Pearson correlation coefficient, $r = 0.812$, $p < 0.05$) as well as TBT and TPhT (Pearson correlation coefficient, $r = 0.64$, $p < 0.05$).

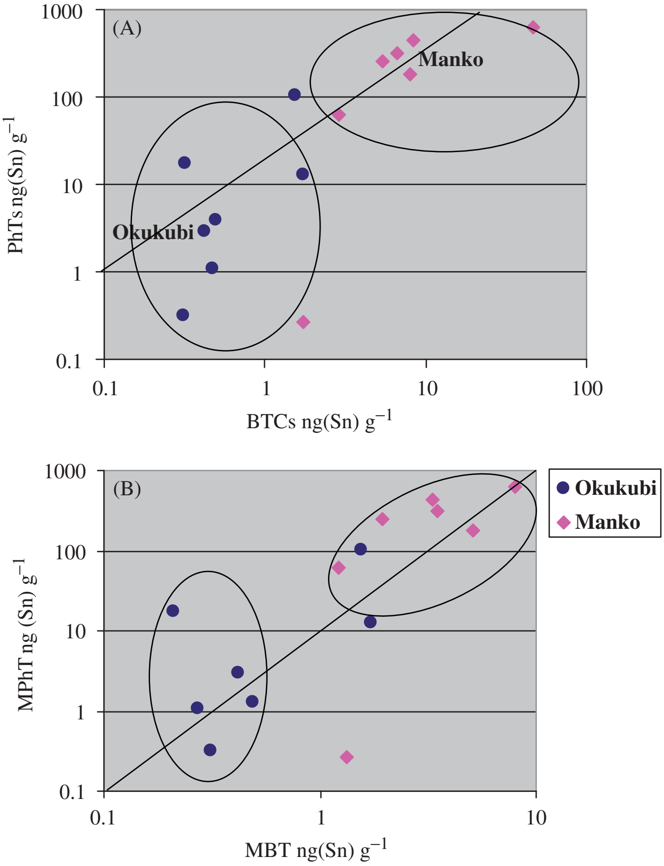

Figure 5. OTCs in sediments from the Manko and Okokubi estuaries: (A) total OTCs; (B) mono-substituted OTCs.

In the Okukubi estuary, TBT was only detected at TO1 with the mean concentration of 0.025 ng(Sn) g^{-1}. MBT was a predominant degradation product among BTCs found in the estuary. PhTs were frequently detected at TO2 and TO3. Contamination patterns in the Okukubi sediments were MBT > DBT > TBT and MPhT > TPhT for BTCs and PhTs, respectively. DPhT was not detected in all transects. In comparison, Manko estuary sediments were contaminated more than 100 times with OTCs than Okukubi estuary sediments (see figure 5).

Correlations were not significant between total organic carbon (TOC) and OTCs (ΣBTCs and ΣPhTs, Pearson correlation coefficient, $r = 0.69$ and -0.03 respectively, $p > 0.05$) for Okukubi and (ΣBTCs and ΣPhTs, Pearson correlation coefficient, $r = -0.8$ and -0.9 respectively, $p > 0.05$) for the Manko estuary.

3.3 *Seasonal variation of OTCs*

Seasonal variations of OTCs are shown in figure 6. BTCs have been detected frequently in all seasons in both estuaries; however, MBT was predominant.

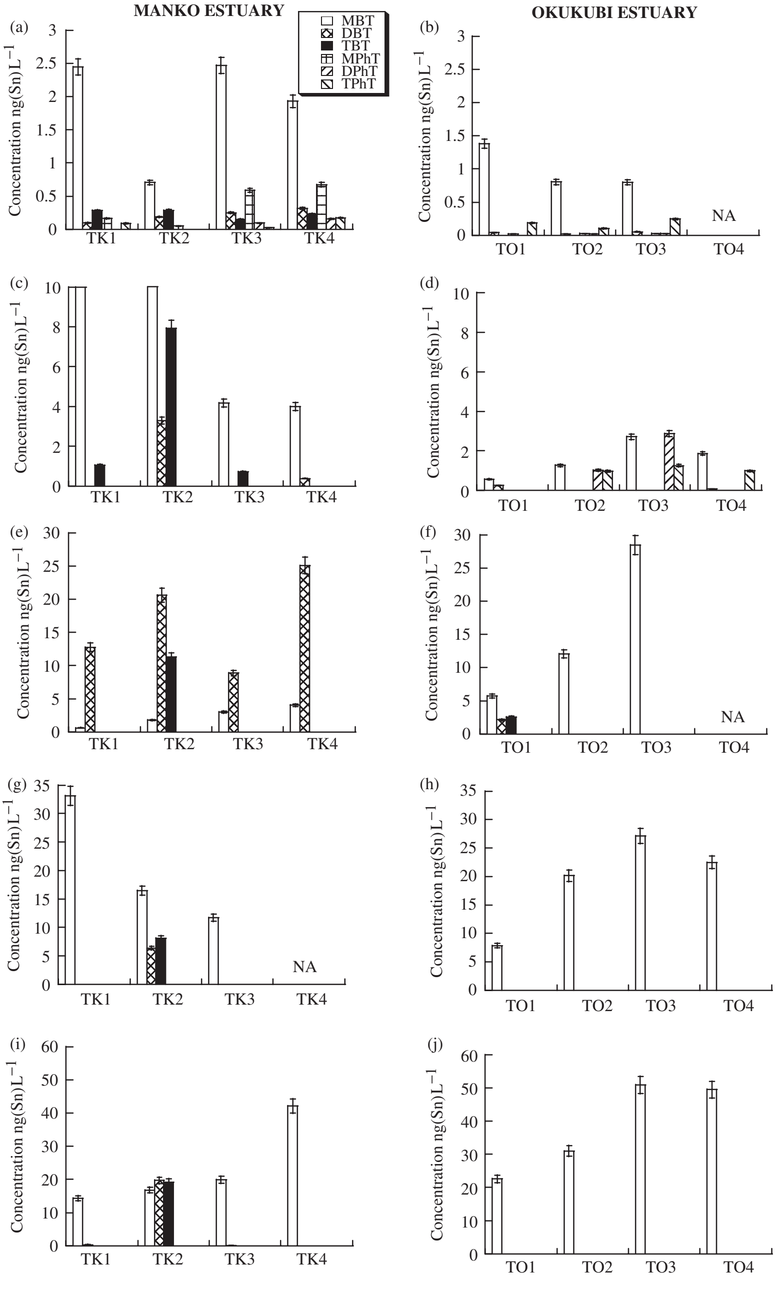

Figure 6. Seasonal variations of OTCs in waters from the Manko estuary (left) and Okukubi estuary (right). (a and b: February; c and d: April; e and f: June; g and h: August; i and j: October). NA: sample not available.

In the Manko estuary, high values of TBT were detected in the summer and autumn, and the lowest values were found during the winter. The maximum concentrations of MBT and DBT were found in early and late spring, respectively. PhTs were detected in the winter only. BTCs showed a significant seasonal difference (paired *t*-test, $p<0.05$)

Table 4. Concentrations of TBT in water (ng(Sn) L^{-1}) and in sediment (ng(Sn) g^{-1}) reported in the literature.

Location (sampling year)	TBT range	Reference
Water		
Port of Osaka (1992)	2000–33,000	[45]
Coast of west Japan (2001)	nd–40	[17]
Coastal of Peninsular, Malaysia (1991–1992)	<3.4–281.8	[25]
French coast (1997)	1.7–87.7	[11]
Ganga Plain, India (1995)	8.7–32.6	[43]
Coos Bay estuary, USA (1986–1997)	nd–1,400,000	[46]
Huelva Coast, Spain (1993)	nd–101	[42]
Taiwan coastal areas(2001–2004)	nd–229.4	[44]
China (1998–1999)	nd–976.9	[47]
Tamar estuary, UK	0.3–3.8	[34]
Portugal (1999–2000)	3–30	[15]
Manko estuary	nd–38.4	This study
Okukubi estuary	nd–10.36	This study
Sediments		
Coastal of Peninsular Malaysia (1991–1992)	<0.7–216.5	[25]
Taiwan coastal areas (2001–2004)	nd–8547.6	[44]
Port of Osaka (1992)	2–966	[45]
Barcelona habor, Spain (2002)	98–4702	[48]
Coastal areas, Japan (1994)	nd–440	[22]
Otsuchi Bay, Japan (1995–1996)	10–640	[18]
Tamar estuary, UK (1991)	10–44	[34]
Gipuzkoa estuary, Spain(2000)	50–5480	[49]
Dar es salaam Port, Tanzania (2004)	nd–3670	[50]
Manko estuary	nd–39.2	This study
Okukubi estuary	nd–0.09	This study

between winter and late spring, winter and summer, and winter and autumn. PhTs showed a significant difference between winter and the remaining seasons.

In the Okukubi estuary, TBT was detected during the spring while in other seasons MBT was predominant. Phenyltin compounds were detected in winter and early spring with a maximum value of TPhT detected in early spring. BTCs showed a significant seasonal difference (paired *t*-test, $p < 0.05$) between winter and summer, and early spring and summer. For PhTs, a significant seasonal difference ($p < 0.05$) was observed between winter and late spring, and winter and summer.

4. Discussion

This study shows that the OTCs are widely spread in the protected estuaries of Okinawa. Spatial distribution patterns OTCs appeared to reflect the type of activities in these sampling areas. The Manko and Okukubi waters contained lower concentrations of TBT than those found in other studies as shown in table 4. However, 17% of the of TBT measurements in Manko estuary waters have exceeded some international permissible targets such as the Canadian estuarine waters quality guideline for TBT (1 ng L^{-1}) for the protection of aquatic life [25], the UK Environmental Quality Target (2 ng L^{-1}), [26] as well as 'no observed effect levels' (NOEL) (1 ng L^{-1} cause gastropod imposex and phyto- and zooplankton growth and 2 ng L^{-1} cause *C. gigas* calcification anomalities) proposed by Alzieu [23].

Furthermore, Horiguchi *et al.* [10] also found that TBT 1 ng L^{-1} would produce imposex for adult females rock shells *T. clavigera* during 3-month flow-through exposure experiments. Based on the *in vivo* findings shown above, it is clear that the mean concentration of 2.45 ng L^{-1} of TBT reported in Manko estuary seawater is sufficient to cause imposex in gastropods, phyto- and zooplankton growth anomalities, and inhibition of oyster calcification. In fact, 100% imposex occurrence for *T. clavigera* and *T. bronni* (Muricidae: Neogastropa) in Seto inland and the Senriku region in Japan has already been reported [10]. Future studies on TBT in the Manko estuary need to take into account the toxicological effects of TBT on biota.

Higher levels of TBT relative to those reported in the present study have been found in sediments from different aquatic environments, as shown in table 4. These findings suggest that the Manko and Okukubi basins contain a relatively low TBT contamination level in comparison with those reported from other regions. The sediments in Manko estuary sediments can be considered lightly contaminated (0–20 ng g^{-1} dw), and Okukubi as uncontaminated (below 3 ng g^{-1} dw) according to the criteria of Dowson *et al.* [27]. Nevertheless, 71% of the sediment samples from Manko estuary showed TBT levels exceeding the Australian guidelines of acceptable TBT levels in estuarine sediments 0.5–7 ng g^{-1} [28]. Furthermore, the mean concentration of 6.21 ng g^{-1} TBT found in the sediments in this area is more than 100 times greater than the high trigger value of the OSPAR sediments guideline for TBT (0.05 ng g^{-1}) [29]. This indicates that the sediments may pose a threat to the benthic biota.

The overall mean concentrations of TBT followed the order TK2 > TK1 > TK3 > TK4 (see figure 3). TK2 showed high concentrations of TBT throughout all seasons (figure 6), indicating that this is a key point source of TBT in the Manko estuary. It could be supposed that the illegal use of TBT in small fisheries and recreational boating activity close by contribute a notable amount of TBT contamination in TK2 more than the contribution of leached TBT from TK1. Similar results were observed by Dowson *et al.* [27] and Ko *et al.* [30], who found that smaller boats, rather than large ships, remain a potential source of TBT, although TBT use was banned for aquaculture and coastal boating activities in Japan more than a decade ago [17]. Illegal usage of TBT in aquaculture facilities remains a potential source of TBT contamination in the northern part of Japan [17, 31–32].

The seasonal concentration pattern of TBT at the Manko estuary was autumn > spring > summer > winter (figure 6). This corresponds with fishing and leisure boat usage patterns, which are the minimum in winter and maximum in summer. Similar observations have been reported by other studies [20, 27, 33]. Additionally, the effects of precipitation and typhoons may also be considered in the seasonal variation of TBT in the Manko estuary. These natural phenomena may cause the instability of surface sediments in the estuaries due to high tides, high speed winds, freshwater–seawater mixing, and turbulence [28]. Eventually, TBT will be introduced in the water column as a resuspension of surface sediments [28, 34–36]. In this study, maximum concentrations of TBT were detected in the spring and autumn (see figure 6). This coincides with the peak precipitation and typhoon occurrences in Okinawa. This suggests that TBT that settled on the Manko surface sediments from previous months may be freed in the water column during the turbulent spring and autumn months. Buggy and Tobin [28] have concluded that SPM bound TBT were deposited in surface sediments during the summer and released in the water column by turbulence during the winter months in Tolka estuary, Ireland.

No significant correlations were found between TBT and its degradation products (MBT and DBT) (see table 3) in the Manko estuary. This implies that DBT and MBT have more than one source apart from TBT degradation. It is suggested that municipal wastewater input may be another important source of DBT and MBT in the estuaries. The detection frequency of MBT and DBT upstream from Manko estuary (TK4) shows the importance of terrestrial inputs of these compounds to the estuary. Numerous studies have reported the occurrence of MBT and DBT in raw municipal wastewater and agricultural activities [6, 13, 15, 37]. MBT and DBT may enter the waters via leaching from organotin-stabilized PVC [13].

TPhT showed a significant correlation with TBT in sediments, thus indicating that they are coming from the same source in the Manko estuary. MPhT was the dominant PhT degradation product observed and significantly correlated with TPhT in the estuary. This demonstrates the importance of TPhT degradation in the estuary surface sediments. TPhT has been commonly used in antifouling paints in Japan [10], and its residues have been widely observed in the marine environment around Japan [16, 18–20].

TPhT was detected in the middle (TO2 and TO3) and upstream (TO4) of the Okukubi estuary. In the Manko estuary, however, TPhT was detected upstream (TK4) (see figure 6). The spatial distribution of TPhT in the waters shows that the main possible source of TPhT could be agricultural activities. It seems that TPhT is leached from farm soils and transported with wastewater directly to the estuaries via pipes located on estuary embankments [2]. The occurrence of PhTs during the winter in Okukubi and during winter and early spring is not surprising, since it is well known that PhTs are unstable in environment and CRMs, even under recommended sampling and storage procedures [38–40]. We suggest that PhTs are relatively stable during winter and early spring in the Manko and Okukubi estuaries compared with other seasons. In the Adour-Garonne basin, France, phenyltin compounds were also detected in water, particularly in spring and at the end of summer [13].

A laboratory-based study by Inaba *et al.* [41] showed that physico-chemical parameters such as salinity, pH, and temperature influence the solubility of OTCs. Furthermore, it was revealed that these parameters play an important role on the behaviour of OTCs in the estuarine environment [28, 34]. In contrast, we found that physico-chemical parameters (salinity, temperature, pH, DOC, TOC) have little influence on the distribution of OTCs in the Manko and Okukubi estuarine ecosystems (see table 3). The high concentrations of OTCs at certain transects are not influenced by physicochemical factors; rather, they may be influenced by point-source influx of discharge containing high OTCs from shipping and agricultural activities around the estuaries. Similar findings have been revealed in the southwest of Spain [42] and India [43]. It has also been noted that the important physico-chemical parameters influencing OTCs behaviour are related to the characteristics of the specific area of study [15, 34, 42].

5. Conclusions

The present work reveals the presence of organotin compounds (butyl and phenyltin) in water and sediments of the Manko and Okukubi protected estuaries. At some locations in the Manko estuary, the concentrations of TBT reported may pose a risk to marine life.

The distribution of OTCs clearly reflects activities occurring in those areas; notable levels of TBT and TPhT were found in the area of high boating and agricultural activities, respectively. Furthermore, the results showed a significant seasonal variations indicating that more monitoring studies of OTCs should remain a priority in the preserved estuarine ecosystems. As such, a better understanding of the risk posed by OTC pollution in subtropical estuarine ecosystems around Okinawa Island can be achieved.

Acknowledgements

The authors wish to thank their colleagues of the analytical chemistry group of the University of the Ryukyus for their cooperation during this study. We are also grateful to the Instrumental Research Center of the University of the Ryukyus for the use of their research facilities. The constructive comments of three anonymous referees also greatly contributed to the present form. This study was partially supported by a grant from the 21st century COE programme of the University of the Ryukyus and a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology, Japan (No. 17201006).

References

[1] Y. Tashiro, A. Takemura, H. Fujii, K. Takahira, Y. Nakanishi. *Mar. Pollut. Bull.*, **47**, 143 (2003).
[2] T. Meziane, M. Tsuchiya. *J. Sea Res.*, **47**, 1 (2002).
[3] F. Pallizzato, E. Centanni, M.G. Marin, V. Moschino, B. Pavoni. *Sci. Total Environ.*, **332**, 89 (2004).
[4] M. Hoch. *Appl. Geochem.*, **16**, 719 (2001).
[5] S.J. de Mora, S.W. Fowler, R. Cassi, I. Tolosa. *Mar. Pollut. Bull.*, **46**, 401 (2003).
[6] K. Fent. *Sci. Total Environ.*, **185**, 151 (1996).
[7] S. Díez, M. Ábalos, J.M. Bayona. *Water Res.*, **36**, 905 (2002).
[8] N. Følsvik, J.A. Berge, E.M. Brevik, M. Walday. *Chemosphere*, **38**, 681 (1999).
[9] M.A. Fernandez, A. de Luca Rebello Wagener, A.M. Limaverde, A.L. Scofied, F.M. Pinheiro, E. Rodrigues. *Mar. Environ. Res.*, **59**, 435 (2005).
[10] T. Horiguchi, H. Shiraishi, M. Shimizu, S. Yamazaki, M. Morita. *Mar. Pollut. Bull.*, **31**, 402 (1995).
[11] P. Michel, B. Averty. *Mar. Pollut. Bull.*, **38**, 268 (1999).
[12] C. Alzieu. *Sci. Total Environ.*, **258**, 99 (2000).
[13] Ch. Bancon-Montigny, G. Lespes, M. Potin-Gautier. *Water Res.*, **38**, 933 (2004).
[14] S. Pierre-Marie, Y. Lapaquellerie, A. Astruc. *Sci. Total Environ.*, **170**, 59 (1995).
[15] S. Díez, S. Lacorte, P. Viana, P. Barcelo, J. Bayona. *Environ. Pollut.*, **136**, 525 (2005).
[16] H. Harino, M. Fukushima, S. Kawai. *Environ. Pollut.*, **105**, 1 (1999).
[17] R. Murai, S. Takahashi, S. Tanabe, I. Takeuchi. *Mar. Pollut. Bull.*, **51**, 940 (2005).
[18] H. Harino, M. Fukushima, Y. Yamamoto, S. Kawai, N. Miyazaki. *Environ. Pollut.*, **101**, 209 (1998).
[19] K. Sawano. MSc thesis, University of the Ryukyus (2003).
[20] X. Wang. MSc thesis, University of the Ryukyus (2004).
[21] K. Tsuha. MSc thesis, University of the Ryukyus (2006).

[22] Environmental Agency, Japan. *Summary of the Survey Results for Organotin Compounds, Chemicals in the Environment* (1996).
[23] C. Alzieu. *Ocean Coast. Manage.*, **40**, 23 (1998).
[24] B.R. Ramaswamy, H. Tao, T. Nakazato, A. Miyazaki. *Analyst*, **125**, 1757 (2000).
[25] S.L. Tong, F.Y. Pang, S.M. Phang, H.C. Lai. *Environ. Pollut.*, **91**, 209 (1996).
[26] UK Department of Environment. Water and Environment, Circular no. 7/89. 26, UK Department of the Environment, London (1989).
[27] P.H. Dowson, J.M. Bubb, J.N. Lester. *Mar. Pollut. Bull.*, **26**, 487 (1993).
[28] C.J. Buggy, J.M. Tobin. *Environ. Pollut.*, **143**, 294 (2006).
[29] OSPAR Commission for the Protection of Marine Environment of the North East Atlantic. *Quality Status Report* (2000). Available online at: http://www.ospar.org (accessed January 20, 2007).
[30] M.M.C. Ko, G.C. Bradley, A.H. Neller, M.J. Broom. *Mar. Pollut. Bull.*, **31**, 249 (1995).
[31] I. Takeuchi, S. Takahashi, S. Tanabe. *J. Mar. Biol. Assoc. UK*, **84**, 911 (2004).
[32] H. Sakai, R. Kasai, S. Takahashi and S. Tanabe. *Nippon Suisan Gakkaishi*, **69**, 10 (2003) (In Japanese with English abstract).
[33] J.L. Gómez-Ariza, I. Giráldez, E. Morales. *Environ. Pollut.*, **108**, 279 (2000).
[34] W.J. Langston, N.D. Pope. *Mar. Pollut. Bull.*, **31**, 32 (1995).
[35] R.A. Wheatcroft, C.A. Butman. *Cont. Shelf Res.*, **17**, 367 (1997).
[36] D.S. Page, C.C. Ozbal, M.E. Lanphear. *Environ. Pollut.*, **91**, 237 (1996).
[37] N. Mzoughi, G. Lespes, M. Bravo, M. Dachraoui, M. Potin-Gautier. *Sci. Total Environ.*, **349**, 211 (2005).
[38] R. Morabito, P. Soldati, M.B. De la Calle, P. Quevauviller. *Appl. Organomet. Chem.*, **12**, 621 (1998).
[39] R. Morabito, H. Muntau, W. Cofino, P. Quevauviller. *J. Environ. Mon.*, **1**, 75 (1999).
[40] P. Quevauviller, F. Ariese. *TrAC*, **20**, 207 (2001).
[41] K. Inaba, H. Shiraishi, Y. Soma. *Water Res.*, **29**, 1415 (1995).
[42] J.L. Gómez-Ariza, E. Morales, I. Giraldez. *Chemosphere*, **37**, 937 (1998).
[43] A.A. Ansari, I.B. Singh, H.J. Tobschall. *Sci. Total Environ.*, **223**, 157 (1998).
[44] C.-C. Lee, C.-Y. Hsieh, C.-J. Tien. *Chemosphere*, **65**, 547 (2006).
[45] H. Harino, M. Fukushima, Y. Kurokawa, S. Kawai. *Environ. Pollut.*, **98**, 163 (1997).
[46] K. Elgethun, C. Neumann, P. Blake. *Chemosphere*, **41**, 953 (2000).
[47] J. Gui-bin, Z. Qun-fang, L. Ji-yan, W. Di-Jing. *Environ. Pollut.*, **115**, 81 (2001).
[48] S. Díez, E. Jover, J. Albaigés, J.M. Bayona. *Environ. Int.*, **32**, 858 (2006).
[49] I. Arambari, R. Garcia, E. Millan. *Chemosphere*, **51**, 643 (2003).
[50] M.A. Sheikh, N.M. Noah, K. Tsuha, T. Oomori. *Int. J. Environ. Sci. Technol.*, **4**, 49 (2007).