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Phenyltins in Surface Sediments of the Visakhapatnam Harbour, India

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Abstract Twenty-two surface sediment samples were collected from Visakhapatnam harbour, east coast of India, and analysed for monophenyltin (MPT), diphenyltin (DPT), triphenyltin (TPT), total bacteria, and TPT tolerant bacteria. Concentrations of MPT, DPT and TPT varied between 1–26, 3–28, and 0.31–145 ng Sn g^{-1} dry wt, respectively. Phenyltin concentrations were influenced by ship related activities, agricultural waste and sewage. These phenyltin concentrations indicate sediments are contaminated. Abundance of TPT tolerant bacteria was strongly influenced by the levels of phenyltins.

Keywords Phenyltins · Phenyltin degradation index · Total bacterial count · Culturable bacteria · TPT tolerant bacteria

Until recently, investigations concerning environmental pollution by organotins have primarily focused on tributyltins (TBT) which is used as biocide in antifouling paints. Contamination of water and sediments by triphenyltin (TPT) as biocide has received relatively less attention. However, the agricultural and biocidal application of TPT compounds probably gives rise to significant amount of pollution in aquatic environment (Kannan et al. 1995). TPT enters ecosystem via leaching from painted ship hull, land runoff from agricultural waste, industrial usage and recreational activities. Phenyltins are often found in harbours, estuaries and sites where shipping and recreational activities take place (Jadhav et al. 2009). The use of TPT has not been as strictly regulated as TBT, but it is acknowledged to produce similar levels of toxicity (Hoch 2001). Phenyltins are equally toxic and has higher bioaccumulation potential than TBT (Kannan et al. 1995), and is subjected to lower rate of metabolism than TBT. Like TBT, they are endocrine disruptors and cause imposex in gastropods (Horiguchi et al. 1997). The effects of phenyltins on invertebrates are well documented (Ishaaya et al. 1980).

It is a well known fact that microbes play significant role in nutrient cycling which is necessary to maintain ecosystem health (Grandlic et al. 2006). The presence of pollutants in the sediments may cause reduction in bacterial biomass due to inhibition of growth and loss of biochemical activities. If the pollutants are persistent in the environment, there is a gradual change in microbial community structure due to natural selection, gene exchange and immigration (Joynt et al. 2006). Effects of phenyltins on indigenous bacteria in the sediments are hardly known. Changes in carbohydrate—lipid complex were observed when bacterial cell encountered organotin molecules (Ph. Daniel et al. 2008). Boopathy and Daniels (1991) demonstrated inhibition of methanogenic bacteria by MPT at concentrations <0.05 mM. In another study, TPT was scarcely degraded by estuarine bacteria in 60 days of incubation and culturable bacterial counts reduced initially on addition of 0.025 mM of MPT (Harino et al. 1997). Microscopic staining technique and plate count technique are used to estimate total and culturable bacterial count, respectively. Culturable fraction of sediment bacterial communities are particularly useful and sensitive while studying sediments contaminated with relatively low levels of pollutants (Grandlic et al. 2006).

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The aims of present research were to assess distribution of phenyltins in the harbour sediments and evaluate their effect on the abundance of total and TPT tolerant bacteria.

Materials and Method

Visakhapatnam harbour is one of the busiest and largest natural harbours on the east coast of India. Spread over area of 300 hectors, has 24 berths and has largest ship-building yard in India. Due to extensive ship related activities, this site is selected for the study. Twenty-two sediment samples were collected from different locations of the Visakhapatnam harbour using Van Veen grab in January 2008 (Fig. 1). All samples were stored in ice and transported to the laboratory and stored at −20°C.

Total phenyltins were extracted from the sediment samples by using Grignard's reagent (Jadhav et al. 2009). Briefly, a known quantity of the homogenized sediment sample (1–2 g) was transferred to a clean test tube to which 15 mL of 0.03% tropolone in methanol, 1 mL of conc HCl, 100 ng of tripropyltin chloride (TPrT, internal standard) were added and then vortexed for 10 min. After the mixture settles, the supernatant was taken in a separating funnel and 100 mL of 5% NaCl was added. Liquid–liquid partitioning was performed twice with 15 mL of dichloromethane. Like wise procedural blanks and standards (100 ng of each MPT, DPT, and TPT added with internal standard) were prepared with every set. Dichloromethane extracts were pooled and dried over anhydrous sodium sulphate. One mL of iso-octane was added as keeper; extract was then evaporated almost to dryness and reacted with 1 M pentyl magnesium bromide (Grignard's reagent) for a minute. Grignard's reagent was destroyed by drop wise addition of water (2 mL) and 5 mL of 0.1 M sulphuric acid. Phenyltin derivatives were then extracted using n-hexane (3 mL, thrice). The derivatives were pooled and concentrated using a rotary evaporator (1 mL), and then purified on an activated florisil column (6–8 g). Phenyltins were then eluted using 8–10 mL of hexane: toluene (1:1) mixture and concentrated to 1 mL using gentle stream of nitrogen. 1 μL of sample were injected into GC–MS (Shimadzu QP 2010) equipped with electron impact ionization (70 eV). The minimum detection concentration for phenyltins was 0.2 ng Sn g^{-1} dw. Recovery for MPT, DPT and TPT were 80 ± 10%, 79 ± 8%, 94 ± 12%, respectively.

Total bacterial counts (TBC) for sediment samples were analysed by using 0.05% DAPI (Aistegui and Montero 2005). The enumeration of TPT tolerant bacteria was done by spread plate technique. Wet sediment sample was serially diluted (tenfold) and 100 μL of the dilution was spread plated on ZoBell marine agar plate containing 50 μg of TPT (13 μM). TPT stock solution was prepared in methanol (10 mg mL^{-1}). Control agar plate contained equal quantity of methanol (50 μL). Plates were incubated for 4 days at 28 ± 2°C. TPT tolerant bacteria were defined as those growing on agar plates containing 13 μM of TPT. All the bacterial analysis was done in triplicates.

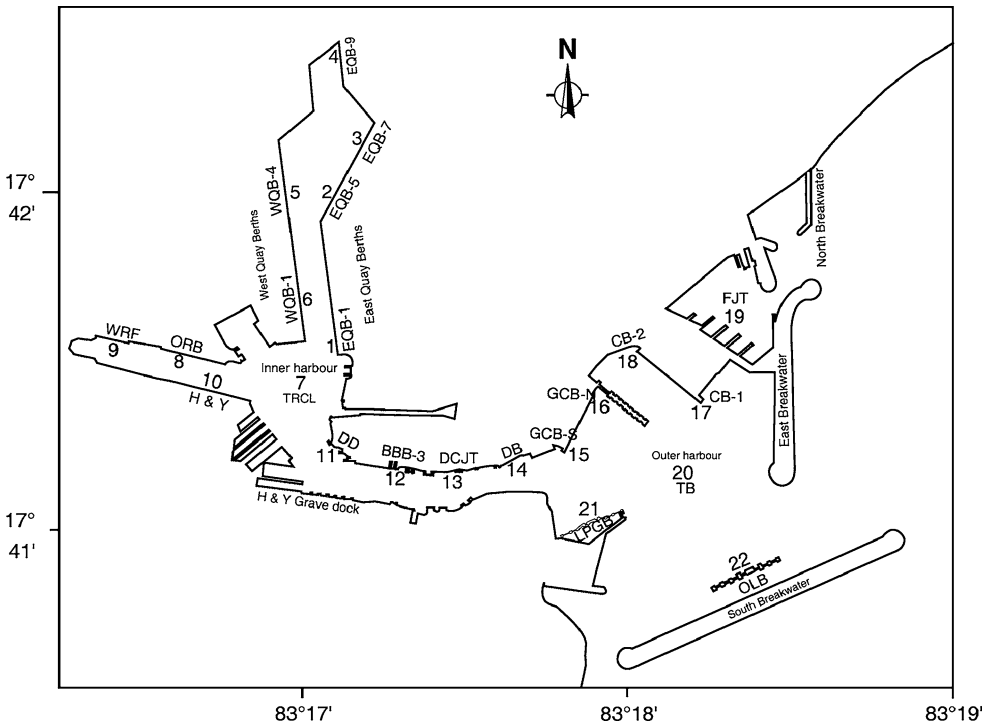

Fig. 1 Surface sediments sampling sites in the Visakhapatnam harbour, east coast of India

Shapiro's test was used to check the normality of the data. TPT content and microbial abundance data failed the requirements for parametric analysis. Therefore non parametric Kruskal–Wallis test was performed to evaluate differences across the stations. *P* value less than or equal to 0.05 was considered to be significant. Relationship between TPT concentration and microbial abundance (i.e., TBC and TPT tolerant bacteria) was assessed using correlation analysis.

Result and Discussion

Twenty-two sediment samples were analysed for the MPT, DPT and TPT in the Visakhapatnam harbour (Table 1). Out of these, 13 samples were from inner harbour while 9 samples were from the outer harbour. Total phenyltin (MPT + DPT + TPT) content in these samples ranged from 2 ng Sn g^{-1} dw (station 15, GCB-S) to 200 ng Sn g^{-1} dw (station 9, WRF). The content of MPT, DPT and TPT ranged from 0.84 ng Sn g^{-1} dw (station 15) to 26 ng Sn g^{-1} dw (station 22), 1.45 ng Sn g^{-1} dw (station 21) to 28.25 ng Sn g^{-1} dw (station 1), and 0.31 ng Sn g^{-1} dw (station 2, 18 and 21) to 145 ng Sn g^{-1} dw (station 19), respectively. In the inner harbour Fertilizer warf (station 9, 200 ± 14 ng Sn g^{-1} dw), H & Y jetty (station 10, 72 ± 6 ng Sn g^{-1} dw), East quay berth 9 (Station 4, 57 ± 3 ng Sn g^{-1} dw) and Boat basin berth (station 12, 54 ± 8.4 ng Sn g^{-1} dw) were found to have high levels of TPT contamination. This was probably due to extensive shipping activities and lack of water movement, as these stations are situated in enclosed area of the port. Surprisingly, phenyltin concentrations were less at stations experiencing dry docking activities (Station 10 and 11). This was perhaps due to dredging activities in the harbour or effect of current. The current pattern can affect spatial distribution of organotins (Bhosle et al. 2004). In the outer harbour, Station 19 (Fishing harbour) was found to be most contaminated (188 ± 10 ng Sn g^{-1} dw) probably due to extensive fishing activities at this station. Moreover, this station is land locked from all sides with the narrow outlet. As a result, there is lack of water movement which may account for greater concentration of phenyltins at this station. Turning basin (station 20, 50 ± 1.5 ng Sn g^{-1} dw) and Oil berth (station 22, 89 ± 10 ng Sn g^{-1} dw) also showed higher contamination due their extensive usage, in

Table 1 Concentrations of phenyltins in surface sediments of Visakhapatnam harbour

S. no.	Station name	MPT	DPT	TPT	∑PT	PDI
	Inner harbour					
1	East quay berth 1 (EQB-1)	1 ± 0.2	28 ± 0.7	2 ± 0.5	31 ± 1	13.5
2	East quay berth 5 (EQB-5)	1 ± 0.3	6 ± 0.6	1 ± 0. 2	8 ± 1	5.8
3	East quay berth 7 (EQB-7)	6 ± 0.5	5 ± 0.5	2 ± 0.3	13 ± 1	4.7
4	East quay berth 9 (EBQ-9)	10 ± 0.2	26 ± 0.7	21 ± 2.1	58 ± 3	1.7
5	West quay berth 4 (WQB-4)	14 ± 0.5	13 ± 0.5	11 ± 1	37 ± 2	2.4
6	West quay berth 1 (WQB-1)	8 ± 1	5 ± 1	16 ± 1	29 ± 3	0.6
7	Turning circle (TRCL)	12 ± 0.5	9 ± 0.5	12 ± 0.5	33 ± 2	1.7
8	Ore berth (ORB)	17 ± 1	12 ± 1	25 ± 1	54 ± 3	1.2
9	Fertilizer wharf (WRF)	23 ± 2	35 ± 3	142 ± 9	200 ± 14	0.4
10	H & Y Jetty (H & Y)	26 ± 2	16 ± 1.5	30 ± 2.5	72 ± 6	1.4
11	Dry dock (DD)	9 ± 2.5	4 ± 0.5	30 ± 5	42 ± 8	0.4
12	Boat basin berth (BBB-3)	16 ± 1	18 ± 1	20 ± 6.4	54 ± 8	1.7
13	DC jetty (DCJT)	3 ± 0.2	4 ± 0.4	2 ± 0.4	9 ± 1	3.7
14	Dredger berth (DB)	1 ± 0.1	22 ± 0.5	15 ± 2.5	38 ± 3	1.5
	Outer harbour					
15	General cargo berth S (GCB-S)	0.84 ± 0.1	2 ± 0.5	0.3 ± 0.5	2 ± 1	5.5
16	General cargo berth N (GCB-N)	12 ± 1	9 ± 1	12 ± 0.8	38 ± 3	1.7
17	Container berth 1 (CB-1)	2 ± 0.2	6 ± 1	2 ± 0.1	10 ± 1	3.6
18	Container berth 2 (CB-2)	8 ± 0.5	6 ± 0.4	1 ± 0.2	15 ± 1	16.1
19	Fishing harbour (FJT)	20 ± 1	22 ± 1	145 ± 10	188 ± 10	0.3
20	Turning basin (TB)	11 ± .5	27 ± 0.5	13 ± 0.5	50 ± 2	2.9
21	LPG berth (LPGB)	7 ± 0.5	3 ± 0.5	1 ± 0.5	11 ± 2	6.7
22	Oil berth (OLB)	25 ± 2.5	19 ± 2.5	45 ± 5	89 ± 10	1.0

Concentrations are expressed in ng Sn g^{-1} dw

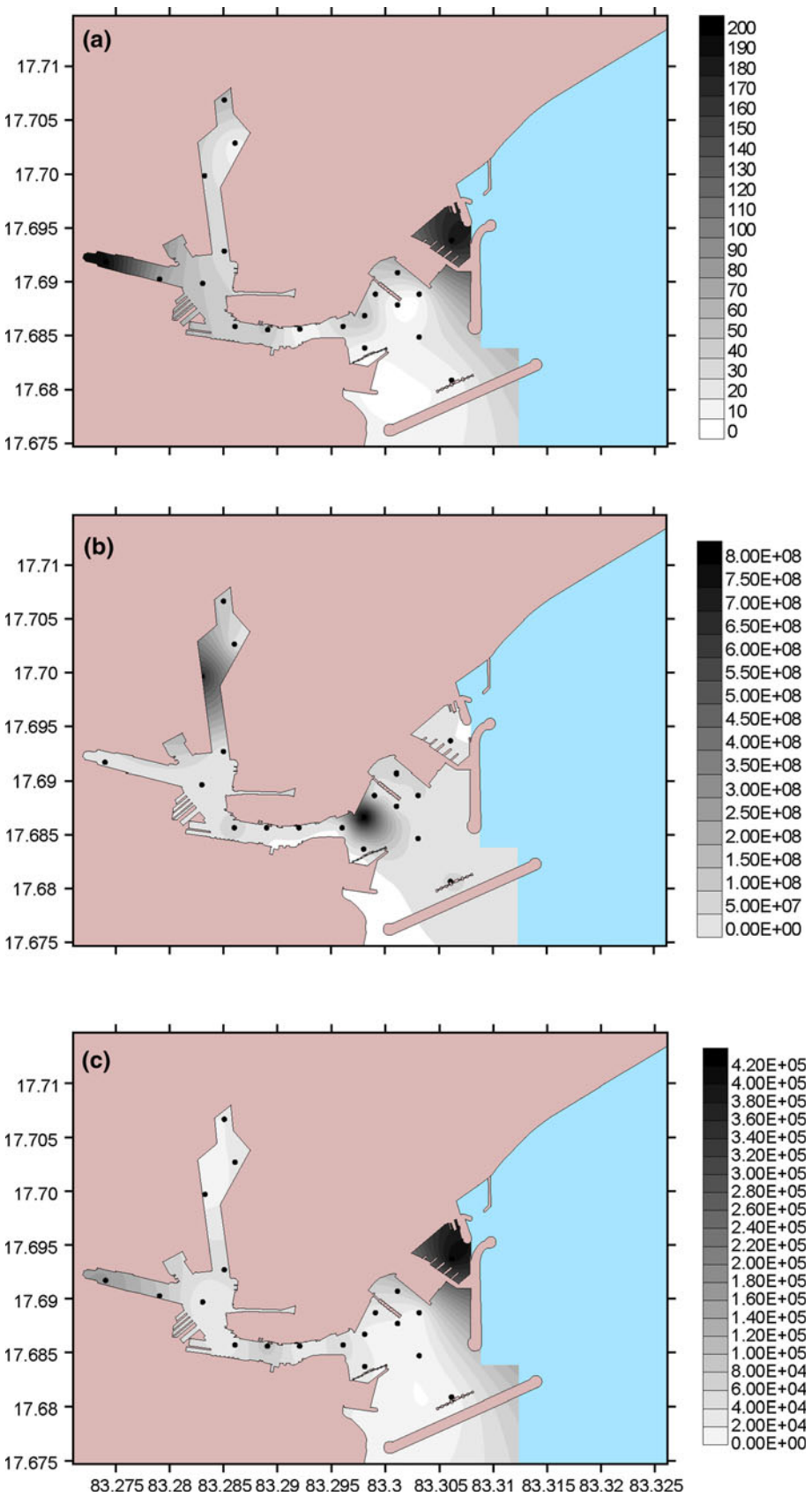

Fig. 2 Distribution of **a** Total phenyltin (ng Sn g^{-1} dw), **b** TBC (cells g^{-1} dw), and **c** TPT tolerant bacteria (CFU g^{-1} dw) in Visakhapatnam harbour

otherwise moderately contaminated outer harbour. The distribution of phenyltins across the stations varied significantly (Kruskal–Wallis test, $P = 0.003$). The presence of degradation products such as MPT and DPT indicates biotic and/or microbial degradation of TPT compounds, respectively. In order to know, the status of degradation at these stations, phenyltin degradation index (PDI) was calculated (Table 1). PDI was calculated following equation given below, assuming similar degradation rates between both the compounds (Diez et al. 2002)

$$\text{PDI} = [\text{MPT} + \text{DPT}]/[\text{TPT}]$$

where, MPT, DPT and TPT indicate concentration of these compounds. The PDI ranged from 0.3 (station 19) to 16.1 (station 18). The PDI for highly polluted stations (9, 10 and 19) was <1, suggesting continuous influx or usage of phenyltins at these stations (Diez et al. 2002), and there is hardly any degradation of TPT taking place at these stations. However, the stations with lower phenyltin concentration showed PDI >1 suggesting lesser input and higher degradation of phenyltins. The results show that, sediments in Visakhapatnam port were polluted with phenyltins (2–200 ng Sn g^{-1} dw, Fig. 2a). Ship trafficking, ship building/breaking docks, municipal sewage and agricultural wastes are sources of phenyltins in the harbour. The phenyltin concentrations were found to be higher than those recorded for Gdansk port, Poland, where MPT concentration ranged from 29–49 ng Sn/g dw, while DPT and TPT were not detected (Radke et al. 2008). In the Zuari estuary, west coast of India, the total phenyltins ranged from not detected to 46 ng Sn/g dw (Jadhav et al. 2009). But phenyltin concentrations where lower compared to Paranagua estuarine complex of Brazil (undetected to 800 ng Sn g^{-1}, Santos et al. 2009), German North and Baltic sea marinas (7–3,663 ng Sn g^{-1}, Biselli et al. 2000), Spain (18,120 ng Sn g^{-1} Tolosa et al. 1992), western Mediterranean enclosures, (45–945 ng g^{-1}, Diez et al. 2002), Otuchi bay, Japan (<1–9,390 ng Sn g^{-1}, Harino et al. 1997) and Taiwan harbours (445–946 ng Sn g^{-1}, Lee et al. 2006).

In the Visakhapatnam harbour, the total bacterial count varied from 5.72×10^6 to 9.20×10^9 cells/g dw (Fig. 2b). Statistically total bacterial count varied significantly across the stations (Kruskal–Wallis test, $P = 0.00001$). TPT concentrations showed significant negative relationship to the total bacterial count ($r = 0.719$, $P = 0.001$) indicating that bacterial abundance at different stations was affected by phenyltin concentrations. The TPT tolerant bacterial count varied significantly across the stations (Kruskal–Wallis test, $P = 0.00000$). For inner harbour sediments, the TBC varied from 1.00×10^3 to 1.67×10^5 cfu/g dw, while in the outer harbour sediments it varied from 4.43×10^3 to 4.17×10^5 cfu/g dw (Fig. 2c). Station 9, 10, 12, and 19 had highest % of TPT tolerant bacteria. Roy et al. (2004) reported the presence of 11% to 16% of total viable bacteria, resistant to tributyltin chloride (TBTCl) in the oil fields. Suehiro et al. (2007) reported up to 34% of natural population resistant to TBTCl in Mekong River wherein occurrence of TBT and TBT tolerant bacteria was unrelated. They hypothesized that the abundance of TBTCl resistant bacteria towards TBT was not related with TBTCl concentrations but with content of suspended solids containing other pollutants in the estuary. The present study also illustrated a fairly significant positive co-relation between TPT tolerant bacteria and phenyltin concentrations in the sediments of Visakhapatnam harbour ($r = 0.594$, $P = 0.01$). This indicates development of a community enriched with TPT tolerant bacteria which may have serious impacts on biogeochemical cycles and aquatic food web (Castle et al. 2006). For example many organic pollutants are known to alter the microbial communities and likely to influence microbial activities (Castle et al. 2006; Grandlic et al. 2006; Joynt et al. 2006).Visakhapatnam harbour sediments are contaminated with phenyltins and stringent legislative measures are required to curb their usage. Otherwise phenyltins would have detrimental effects on marine resources and ecosystem.

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References

Aistegui J, Montero M (2005) Temporal and spatial changes in plankton respiration and biomass in the canary Island region: effect of mesocale variability. J Mar system 54:65–82

Bhosle NB, Garg A, Jadhav S, Harji R, Sawant S, Venket K, Anil AC (2004) Butyltins in water, biofilm and sediments of the west coast of India. Chemosphere 57:897–907

Biselli S, Bester K, Hühnerfuss H, Fent K (2000) Concentrations of the antifouling compound Irgarol 1051 and of organotins in water and sediments of German North and Baltic Sea Marinas. Mar Pollut Bull 40:233–243

Boopathy R, Daniels L (1991) Pattern of organotin inhibition of methanogenic bacteria. Appl Environ Microbiol 57:1189–1193

Castle D, Montgomery M, Kirchman D (2006) Effects of naphthalene on microbial community composition in The Delaware estuary. FEMS Microbiol Ecol 56:55–63

Daniel Ph, Picart P, Bendriaa L, Sockalingum G, Adt I, Charrier Th, Durand M, Ergan F, Manfait M, Thouand G (2008) Effects of toxic organotin compounds on bacteria investigated by micro-raman spectroscopy. Spectrosc Lett 41:19–28

Diez S, Albalos M, Bayona J (2002) Organotin contamination in sediments from the Western Mediterranean enclosures following 10 years of TBT regulation. Water Res 36:905–91834

Grandlic J, Geib I, Pilon R, Sandrin T (2006) Lead pollution in a large, prairie-pothole lake (Rush Lake, WI, USA): effects on abundance and community structure of indigenous sediment bacteria. Environ Pollut 144:119–126

Harino H, Fukushima M, Kurokawa Y, Kawai S (1997) Susceptibility of bacterial populations to organotin compounds and microbial degradation of organotin compounds in environmental water. Environ Pollut 98:157–162

Hoch M (2001) Organotin compounds in the environmentdan overview. Appl Geochem 16:719–743

Horiguchi T, Shiraishi H, Shimizu M, Morita M (1997) Effects of triphenyltin chloride and five other compounds on development of imposex in the rock shell, *Thais clavigera*. Environ Pollut 95:85–91

Ishaaya I, Yablonsk S, Ascher K, Casida J (1980) Triphenyl and tetraphenyl derivatives of group V elements as inhibitors of growth and digestive enzymes of *Tribolium confusum* and *Tribolium castaneum* larvae. Pestic Biochem Physiol 13:164–168

Jadhav S, Bhosle N, Massanisso P, Morabito R (2009) Organotins in the sediments of the Zuari estuary, west coast of India. J Environ Manage 90:S4–S7

Joynt J, Bischoff M, Turco R, Konopka A, Nakatsu C (2006) Microbial community analysis of soils contaminated with lead, chromium and petroleum hydrocarbons. Microb Ecol 51:201–219

Kannan K, Tanabe S, Tatsukawa R (1995) Phenyltins residues in horse shoe crabs, *Tachylpleus tridentatus* from Japanese coastal waters. Chemosphere 30:925–932

Lee C, Hsieh C, Tien C (2006) Factors influencing organotin distribution in different marine environmental compartments, and their potential health risk. Chemosphere 65:547–559

Radke B, Leczynski L, Wasik A, Namiesnik J, Bolalek J (2008) The content of butyl- and phenyltin derivatives in the sediment from the Port of Gdansk. Chemosphere 73:407–414

Roy U, Dubey S, Bhosle S (2004) Tributyltin chloride-utilizing bacteria from marine ecosystem of west coast of India. Curr Sci 86:702–705

Santos D, Araújo I, Machado E, Carvalho-Filho M, Fernandez M, Marchi M, Godoi A (2009) Organotin compounds in the Paranaguá Estuarine Complex, Paraná, Brazil: evaluation of biological effects, surface sediment and suspended particulate matter. Mar Pollut Bull 58:1922–1952

Suehiro F, Mochizuki H, Nakamura S, Iwata H, Kobayashi T, Tanabe S, Fujimori Y, Nishimura F, Tuyen B, Tana T, Suzuki S (2007) Occurrence of tributyltin (TBT) - resistant bacteria is not related to TBT pollution in Mekong river and coastal sediment: with hypothesis of selective pressure from suspended solid. Chemosphere 68:1459–1464

Tolosa L, Merlini N, Bertrand M, Bayona J, Albaigés J (1992) Occurrence and fate of tributyl- and riphenyltin compounds in western Mediterranean coastal enclosures. Environ Toxicol Chem 11:145–155