

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 (TBC), and TPT tolerant bacteria. Concentrations of MPT, DPT and TPT varied between 1- 26 ng Sn g⁻¹ dry wt, 3 – 28 ng Sn g⁻¹ dry wt, 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.

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Until recently, investigations concerning environmental pollution by organotins have primarily focused on TBT which is used as biocide in antifouling paints. Contamination of water and sediments by 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. 1994). 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 tributyltins (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. 1994), 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. 2005). 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).

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 hectars, has 24 berths and has largest shipbuilding 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 1M 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 0.1M 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 (6-8g). Phenyltins were then eluted using 8- 10 ml of hexane: toluene (1:1) mixture and concentrated to 1ml 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 et al. 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 $13 \mu\text{M}$ of TPT. All the bacterial analysis was done in triplicates.

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 $2 \text{ ng Sn g}^{-1} \text{ dw}$ (station 15, GCB-S) to $200 \text{ ng Sn g}^{-1} \text{ dw}$ (station 9, WRF). The content of MPT, DPT and TPT ranged from $0.84 \text{ ng Sn g}^{-1} \text{ dw}$ (station 15) to $26 \text{ ng Sn g}^{-1} \text{ dw}$ (station 22), $1.45 \text{ ng Sn g}^{-1} \text{ dw}$ (station 21) to $28.25 \text{ ng Sn g}^{-1} \text{ dw}$ (station 1), and $0.31 \text{ ng Sn g}^{-1} \text{ dw}$ (station 2, 18 and 21) to $145 \text{ ng Sn g}^{-1} \text{ dw}$ (station 19), respectively. In the inner harbour Fertilizer warf (station 9, $200 \pm 14 \text{ ng Sn g}^{-1} \text{ dw}$), H & Y jetty (station 10, $72 \pm 6 \text{ ng Sn g}^{-1} \text{ dw}$), East quay berth 9 (Station 4, $57 \pm 3 \text{ ng Sn g}^{-1} \text{ dw}$) and Boat basin berth (station 12, $54 \pm 8.4 \text{ ng Sn g}^{-1} \text{ dw}$) 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 ($188 \pm 10 \text{ ng Sn g}^{-1} \text{ dw}$) 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, $50 \pm 1.5 \text{ ng Sn g}^{-1} \text{ dw}$) and Oil berth (station 22, $89 \pm 10 \text{ ng Sn g}^{-1} \text{ dw}$) also showed higher contamination due their extensive usage, in 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 were 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 – 3663 ng Sn g⁻¹, Beselli et al. 2000), Spain (18120 ng Sn g⁻¹ Tolosso et al. 1992), western Mediterranean enclosures, (45 – 945 ng g⁻¹, Diez et al. 2002), Otuchi bay, Japan (<1- 9390 ng Sn g⁻¹, Harino et al. 2006) 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 x 10⁶ to 9.20 x 10⁹ 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 x 10³ to 1.67 x 10⁵ cfu/g dw, while in the outer harbour sediments it varied from 4.43 x 10³ to 4.17 X 10⁵ 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 (TBTCI) in the oil fields.

Suehiro et al (2007) reported up to 34 % of natural population resistant to TBTCI in Mekong River wherein occurrence of TBT and TBT tolerant bacteria was unrelated. They hypothesized that the abundance of TBTCI resistant bacteria towards TBT was not related with TBTCI 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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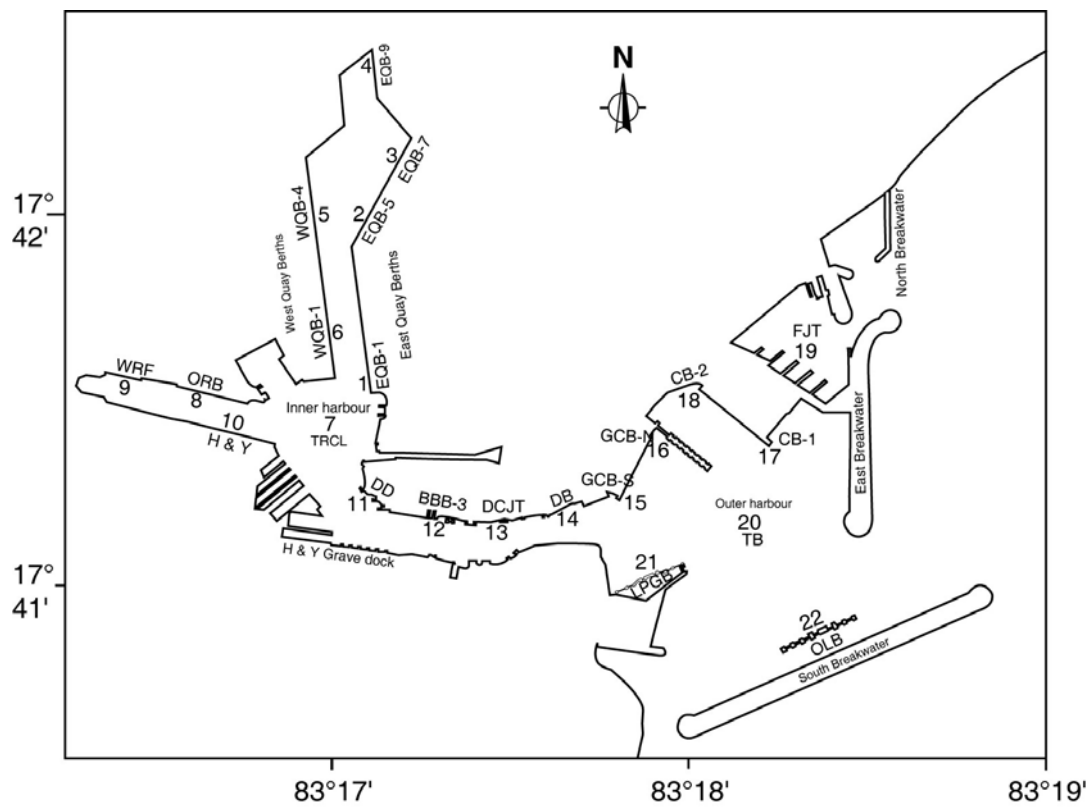


Fig. 1 Surface sediments sampling sites in the Visakhapatnam harbour, east coast of India.

Table 1 Concentrations of phenyltins in surface sediments of Visakhapatnam harbour

S. No.	Station name	MPT	DPT	TPT	Σ 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 berth1 (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

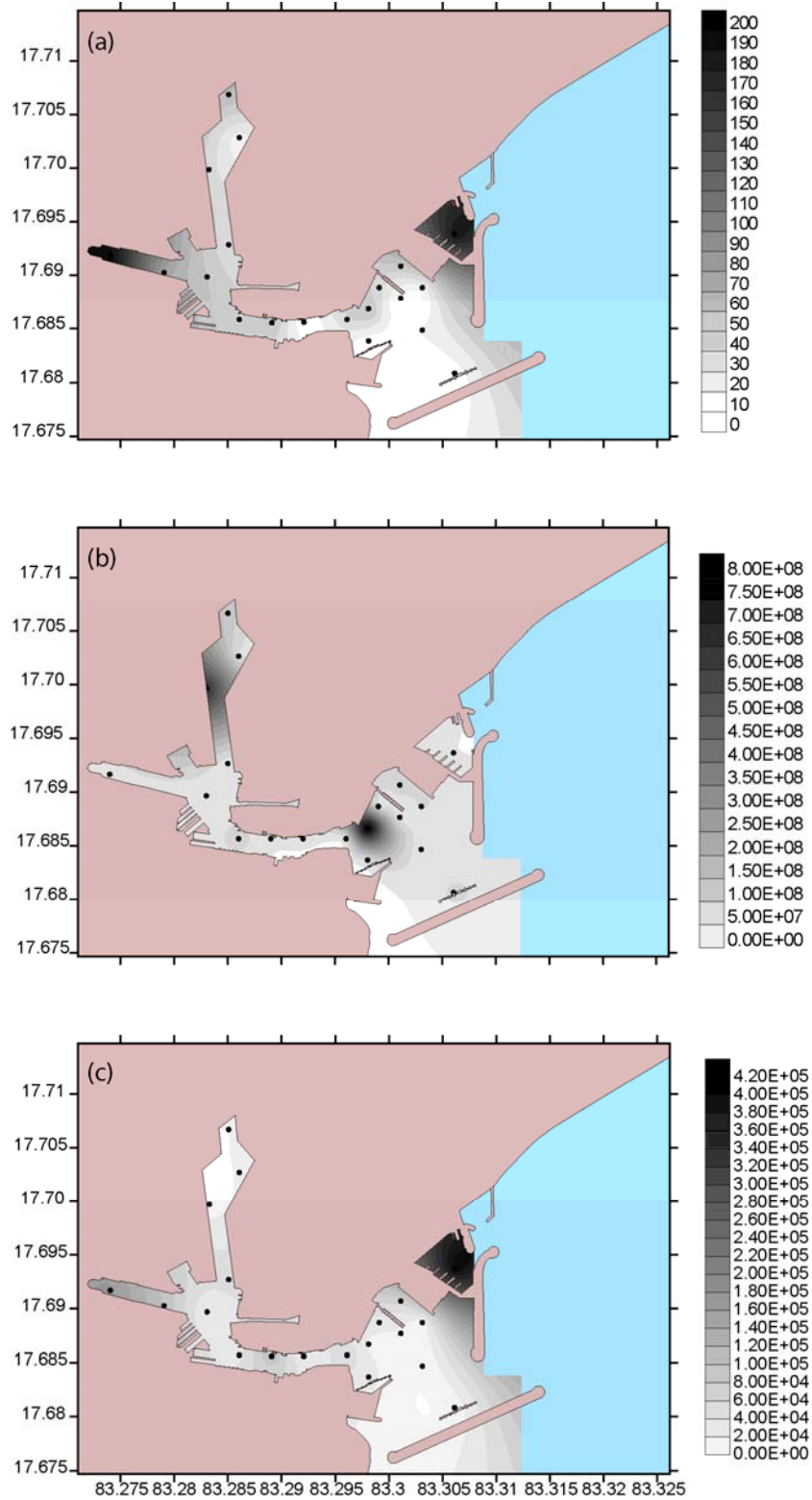


Fig. 2 Distribution of (a) Total phenyltin (ng Sn g⁻¹ dw), (b) TBC (cells. g⁻¹ dw), and (c) TPT tolerant bacteria (CFU g⁻¹ dw) in Visakhapatnam harbour