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Determination of Organotin Compounds in Coastal Sediments by Propylation and GC-MS analysis.

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ABSTRACT

In this study, an attempt was made to determine concentrations of six species of organotins in sediment samples collected from 17 sites in the Eastern Province of Saudi Arabia. These samples were extracted using solid-liquid extraction and then derivatized by propylation using Grignard reagent. Analytes were separated and quantitatively determined on gas chromatography – mass spectrometric system. Recoveries for 0.1 µg g^{-1} spiked samples were between 70 and 84%, and limits of detection of 6.6-13 ng/g were estimated at S/N=3. Total organotin concentration found at each location was averaged at 2.31 µg $g^{-1.}$ Butylated organotin species were found as tributyltin (0.441-0.695 µg g^{-1}), dibutyltin (0.580-0.820 µg g^{-1}), and monobutyltin (0.150-0.190 µg g^{-1}) while phenylated tins were determined as triphenyltin (0.248-0.327µg g^{-1}), diphenyltin (0.256-0.303µg g^{-1}), and monophenyltin (0.203-0.350 µg g^{-1}). Precision as %RSD for three determinations ranged from 11 to 14%. We used the ratios of tributyltin and triphenyltin to their respective degradation products to infer fresh input of these micro-contaminants into the marine environment.
Keywords: Antifouling paint, coastal sediment, derivatization, GC-MS, organotin species, solid-liquid extraction.

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INTRODUCTION

Organotins are organometallic compounds of tin that have been employed in various spheres of life; thus they are found to contaminate different environmental media. High butyltin concentrations, 0.05-5.48 mg Sn/Kg, in Gipuzkoa sediments of North Spain, were found with gas chromatography flame ionization detector (GC-FID) determination, reflecting pollution related to the area's historical industrial as well as fishing activities [1]. In the same vein, GC-FID analysis of butyltins and phenyltins in sediments, plankton and mussels at Port of Osaka, Japan, has revealed higher concentrations of tributyltin (TBT) than triphenyltin (TPhT) in all matrices. The levels of TBT were also high in marinas and mooring areas of small and medium-hull vessels [2]. Organotin determination using GC-MS under retention time locked conditions has availed easy peak location based on mass spectra and retention time of target analytes: concentrations ranging between 15µg/kg and 43 mg/kg were recorded at port of Antwerp, Belgium and near ship repair station respectively, in water and sediments samples [3]. In a study by Harino *et al*. [4], organotin compounds were detected in surface sediments and mussels *Mytilus edulis* from two major estuaries of the UK, the Mersey and the Thames, approximately one decade after legislation banning the use of TBT on small boats. The study showed that TBT concentration can be correlated to shipping activity in the Manchester Ship Canal (MSC). Results also showed that TBT was the predominant BT species in sediments (approx. 50%) and that only 4% of the total tin in sediments was made up of BTs; concentration of BTs in mussels can be correlated to the total extractable tin in sediment, though in contrast to sediments, 85% of the total tin in mussels was made up of BTs, the most predominant of which was TBT.

First effects of organotin compounds on higher marine organisms were observed in the form of imposex in early 1970s in female dogwhelks (*Nucella lapillus*) [5] in the United Kingdom and in American mud-snails [6] in the United States. In the latter case a much larger prevalence of the deformity was observed close to the harbor than further away. Despite the severity of the phenomenon the connection to shipping was not established until analytical capabilities improved towards late 70s to early 80s.

Exposed humans have also demonstrated some signs of toxicity. Workers handling dibutyl- and tributyltin have reported eye irritation and skin lesions [7, 8] and mucus irritation after exposure to interior paints containing tin [9].

While photochemical degradation of trialkyltin compounds in the water column converts them to less toxic di- and monoalkyltin compounds within days, once they are adsorbed in sediments or bioaccumulated in marine organisms they can be active for months if not years. Owing to the adverse effects of organotin compounds on marine life, revealed by the cases cited above and their potential presence in the global marine environment due to shipping, much attention has been focused on the determination of organotin compounds in the environment.

Different methods have been employed for the separation and quantitative determination of species of these compounds in various matrices. Using gas chromatography atomic emission spectrometry, GC-AES, a fast and accurate method was developed for the determination of butyltins in several sea foods. The limit of detection was reported as 3-6 ng/g [10]. As previous studies have failed to obtain baseline resolution between dibutyltin (DBT) and triphenyltin (TPT), Ace C-18 stationary phase with decreased particle size was used to achieve this resolution in mussel and oyster matrices. The concentration of the analytes could be determined down to 40 pg/g with HPLC-ICP-MS set up [11].

For the determination of eight organotin compounds in water and sediments, gas chromatography with pulsed flame photometric detector, GC-PFPD, was used. In this method, tripropyltin and diheptyltin were applied as internal standards for volatile and semi volatile compounds respectively [12]. Based on commercially available spike solution containing mixture of mono-, di- and tributyltin (MBT, DBT and TBT) enriched with ^{119}Sn, isotope dilution method was used in conjunction with gas chromatography electron impact ionization mass spectrometry, GC-EII-MS, for the identification of MBT, DBT and TBT in water. This method limit of detection was calculated as 0.18-0.25 ng/L [13]. Also in water matrix, good resolution was obtained with methanol: water: acetic acid (80:19:1) mixture as mobile phase for ion-pair reversed phase chromatography with hydride generation quartz furnace atomic absorption spectrometry detection, IP-RPC/HG-QFASS. Ion pairs for the organotin compounds were generated by reaction with decane sulfonate [14].

In many instances, derivatization of the analytes has been performed in order to improve recoveries and detectability. Grignard reagents are commonly used for derivatization of the organotin compounds. Ethylation using sodium tetraethyl borate, STEB, is a type of derivatization employed in the gas chromatographic determination of organotin compounds [10, 15]. However, the reagent is highly flammable and toxic and not applicable for routine environmental applications. When liquid chromatography coupled with atmospheric pressure chemical ionization mass spectrometry, LC-APCI-MS, was harnessed for the separation and quantitation of TBT and its hydroxylated intermediate in seawater, tropolone was used as complexing agent and recoveries of 72-96% were obtained [16].

In this study, we employed an efficient method that utilized derivatization by propylation in combination with GC-MS to determine the amounts of OTs in sediment samples from three coastal locations within Saudi Arabia.

MATERIALS AND METHODS

Chemicals and Reagents

Analytical grade standards of monobutyltin (MBT) as monobutyltin trichloride, dibutyltin (DBT) as dibutyltin dichloride, monophenyltin (MPhT) as monophenyltin trichloride and diphenyltin (DPhT) as diphenyltin dichloride were supplied by Sigma-Aldrich (St. Louis, MO), while standards of both TBT and triphenyltin (TPhT) as tributyltin chloride and triphenyltin chloride respectively were purchased from Fluka (Buchs, Swizerland). 1000 mg/ml solutions were prepared in acetone and stored as stock from which necessary dilutions were made as needed. Organotins were derivatized using the Grignard reagent, isopropyl magnesium chloride (Sigma-Aldrich, St. Louis, MO). Sodium sulfate anhydrous was supplied by Riedel-de-Haen, AG, Switzerland, and dichloromethane (DCM) by Sigma-Aldrich (St. Louis, MO). N-hexane was purchased from J.T. Baker Chemical Co, USA. Ultra pure water was prepared using Nanopure water purification system (Barnstead, Dubuque, IA, USA).

Sampling area and sample collection

The study area is located in southern coast of the Gulf which is situated in the Eastern province of Saudi Arabia, with Jubail at the northern end and Dammam at the southern end of the study area. The area is home to commercial fishing activities with small to medium, as well as big boats birthing the sea. Seventeen stations were sampled from three locations within the area: King Abdulaziz Port, Inside (KPI) (Sampling IDs 7-10), King Abdulaziz Port, Outskirts (KPO) (Sampling IDs 11-17) and Near Dammam Corniche (NDC) (Sampling IDs 1-6). King AbdulAziz Port is the main gateway through which cargoes from all over the world enter the Eastern and Central Provinces of the Kingdom. The Port has fully functional, self-sufficient mechanical and marine workshops, water treatment plants and about 39 berths. It is one of the busiest ports in the Kingdom of Saudi Arabia. Dammam Corniche area is considered to be highly polluted due to inputs from nearby industrial plants and agricultural and runoff waters.

Sediments were sampled following the USEPA procedure for soil, water and solid waste sampling [17]. 300 g grab sediment samples were collected in Teflon bottles from each sampled location. Site I.Ds and coordinates of sampling stations are given in Table 1, while site quality indicators including temperature (^{o}C), dissolved oxygen (DO, mg/L), salinity (sal, ppt), total dissolved solids (TDS, ppt), turbidity (tur, NTU), pH and chlorophyll-a (chl-a, µg/L) are reported in Table 2. All the samples were returned in ice to the laboratory and analyzed immediately.

Extraction and derivatization of organotins

All glassware used for the extraction and derivatization were first washed with hot detergent water and rinsed with ultrapure water. These were then immersed in a pool of 12 M hydrochloric acid and left for about 24 hrs, removed and rinsed with ultrapure water after methanol rinsing and subsequently dried in oven at 50^{o}C. C_{18} SPE discs with 47mm Nu-phase fibers (CPI International, USA) were conditioned with deionized water and employed for the extraction of water samples. 50 g of each sediment sample was taken in Erlenmeyer flask and 50 mL of DCM added and stoppered. This was agitated for 30 min at 150 rpm on a Lab Companion Shaker (model SK-600, GEOL Tech, Korea) to effect extraction. After the extraction, traces of

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moisture were removed by addition of anhydrous sodium sulfate. Extracts were further pre-concentrated to 1 mL by a combination of Buchi Rotavapor R-200 equipped with heating bath B-490, and by slow stream of dry liquid nitrogen. Derivatization of OTs was performed using the Grignard reagent, isopropyl magnesium chloride. From the pre-concentrated extract, 500 µL was taken in a 10 mL vial and 1 mL n-hexane added. This was shaken for about 1 min, followed by the addition of 500 µL of the derivatization reagent. The mixture was vortexed for further 1min and left to stand at room temperature for 15 min. The reaction was then quenched by the addition of 0.05 M sulfuric acid. The vial was centrifuged, and the upper layer was analyzed in GC-MS.

Table 1: Sampling stations and coordinates of sampling IDs

Site ID	Station	Coordinates	
		X	Y
1	Near Dammam Corniche (NDC)	50.08182	26.46707
2		50.07827	26.47526
3		50.24008	26.45690
4		50.22756	26.47632
5		50.08036	26.48379
6		50.07976	26.49575
7	King AbdulAziz Port, Inside (KPI)	50.21303	26.50175
8		50.22078	26.50185
9		50.19113	26.51172
10		50.19837	26.52295
11	King AbdulAziz Port, Outskirts (KPO)	50.17426	26.47031
12		50.17113	26.50161
13		50.22474	26.53596
14		50.12070	26.55055
15		50.26665	26.57264
16		50.13410	26.58127
17		50.11090	26.61474

Table 2: Physico-chemical parameters of the sampling areas

Site ID	Physico-Chemical Parameters						
	Temp (^{o}C)	DO (mg/L)	TDS (ppt)	Salinity (ppt)	pH	Turbidity (NTU)	Chl-a (µg/L)
1	17.80	7.17	41.05	39.68	8.56	0.5	1.6
2	18.39	9.67	31.01	30.91	8.62	8.1	19.7
3	17.95	7.37	41.23	39.87	8.38	0.7	1.6
4	18.39	7.22	41.31	39.90	8.55	1.3	1.2
5	16.79	9.78	38.35	37.37	8.76	3.3	19.6
6	16.46	8.91	39.45	38.33	8.73	2.3	13.6
7	17.80	7.39	41.37	39.96	8.54	0.6	7.0
8	17.54	7.29	41.37	39.95	8.54	1.8	1.8
9	17.71	7.40	41.30	39.90	8.54	2.3	0.8
10	17.93	7.27	41.22	39.83	8.48	0.4	1.0
11	17.88	8.28	40.74	39.41	8.63	0.3	1.8
12	17.41	7.20	41.25	39.85	8.53	1.2	2.4
13	17.41	7.20	41.25	39.85	8.53	1.2	2.4
14	19.61	5.53	40.28	39.01	8.60	2.8	5.8
15	17.75	7.31	41.24	39.84	8.57	1.7	1.1
16	20.75	7.59	39.24	38.08	8.67	1.7	12.5
17	19.63	6.23	40.14	38.08	8.62	1.3	6.0

Determination of organotins

The six species of OTs namely, MBT, DBT, TBT, MPhT, DPhT and TPhT were separated and detected using GC-MS 6890N system (Agilent) equipped with autosampler 7683B series and a 6890B injector. It was operated through a Chemstation with incorporated wiley7n.l and NIST 98.L libraries. Separation was carried

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out with the aid of an Agilent 19091Z-213 column of 30 m x 320 µm (i.d) x 1µm film thickness of HP-1 methyl siloxane stationary phase. High purity helium flowing at a rate of 2.0 ml min^{-1} was the carrier gas for 2 µL injected sample volume. Injection port temperature, MS detector temperature and interface temperature were set at 250°C each. The column temperature was initially set at 40°C which was held for 5 min, and then ramped to 300°C at the rate of 12°C/min. It was held at this final temperature for 4 min. Total ion current (TIC) in SCAN mode for ions of masses between 50 and 550 was used for acquisition and selected ion monitoring (SIM) mode was employed for quantitation using m/z of 246.8 (MBT), 277 (DBT), 291.1 (TBT), 283 (MPhT), 361(DPhT) and 351 (TPhT).

Five-point calibration curves were constructed and found linear between the concentration range of 0.05-10 µg g^{-1} (R^2, 0.9861-0.9998) (Table 3). Limit of detection (LOD) was calculated from signal-noise ratio of three (S/N = 3) and limit of quantitation (LOQ) from S/N = 10. Repeatability of analysis was investigated using percent relative standard deviation (%RSD) estimated from triplicate determinations.

Table 3: Analytical figures of merit for organotin determination in coastal sediment

Analytes	Linearity (R^2*)	LOD(ng g^{-1})	LOQ (ng g^{-1})	%R^1	%RSD (n=3)
MBT	0.9996	8.5	28.3	70	14
DBT	0.9861	12.2	40.7	74	13.7
TBT	0.9992	8.1	27	81	11.5
MPhT	0.9982	6.6	22	84	11
DPhT	0.9982	8.9	29.7	80	12.3
TPhT	0.9998	13	43.3	77	13.4

* For the concentration range of 0.05-10 µg g^{-1}
[1] Percent recovery for 0.1 µg/g spiked sediment sample

Table 4: Concentrations of organotin species in sediments from different locations

Analytes	Concentration, µg/g (Mean±SD) KPI	NDC	KPO
MBT	0.185±0.026	0.150±0.021	0.190±0.030
DBT	0.580±0.079	0.820±0.112	0.756±0.103
TBT	0.695±0.079	0.441±0.051	0.591±0.068
MPhT	0.283±0.031	0.350±0.039	0.203±0.022
DPhT	0.308±0.037	0.256±0.031	0.303±0.037
TPhT	0.248±0.033	0.256±0.034	0.327±0.044

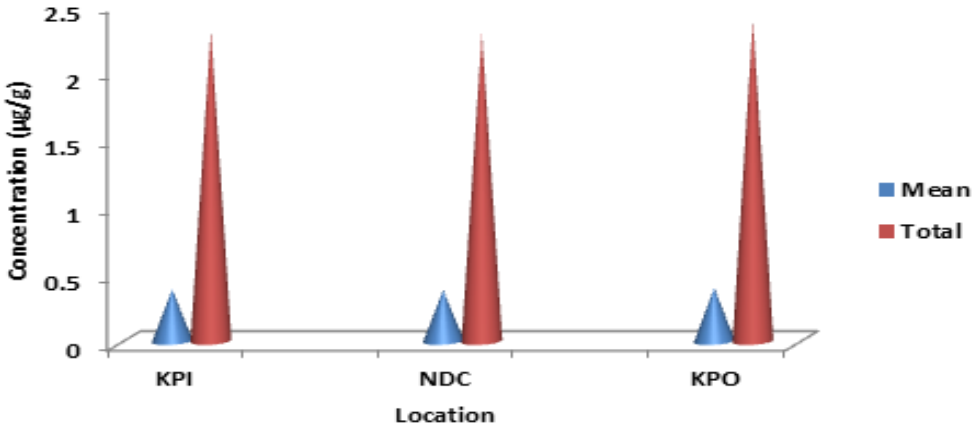

Figure 1: Mean and total concentrations (µg/g) of six organotin species in sediment samples from different locations

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Table 5: Ratios of TBT and TPhT to their respective degradation products in sediments

	TBT/DBT	TBT/MBT	TPhT/DPhT	TPhT/MPhT
NDC	0.539	2.944	1.000	2.857
KPI	1.198	3.757	0.805	0.876
KPO	0.783	3.113	1.011	1.613

RESULTS AND DISCUSSION

Recoveries for 0.1 ng g^{-1} spiked sediment samples were 70-84% as compared to 81-89% in water matrix. These slightly lower recoveries in sediments may be attributed to high amounts of organic matter as evidenced from the higher values of TDS over salinity (Table 2). Table 4 shows the concentrations of different species of OTs in all locations. Figure 1 shows the mean and total concentrations of organotin found at the respective locations. The mean concentration of each organotin species at KPI was 0.38 µg/g, NDC 0.37 µg/g and KPO 0.39 µg/g. The total of the six species averaged 2.31µg/g with KPO having the highest value of 2.37 µg/g and the lowest value of 2.28 µg/g was at NDC. Dammam Corniche area has inputs of pollutants from many industrial plants and agricultural run-off waters. Some agricultural and industrial activities may contribute large amounts of OTs into the maritime systems [18]. However, due to the effect of dispersion and mixing phenomena, the difference in concentrations of OTs was not significant between locations as confirmed from results of analysis of variance (ANOVA) at α=0.05 ($F_{cal} < F_{tab}$).

High ratios of TBT or TPhT to respective degradation species may suggest either long residence time where degradation occurs at slow pace or an evidence of new inputs from anthropogenic sources. The process and mechanism controlling the degradation of these analytes may determine which species is ultimately present and at what concentration. Where turbidity of the sea water is high, biodegradation is expected to be high [19] which would lead to low TBT/DBT ratios. In the study areas, sea water appeared to have high concentration of *chl-a* which might reach up to 19.7 µg L^{-1} at NDC. Although it was earlier reported that *chl-a* content of algae *Scenedesmus quadricauda* was impacted negatively by OTs [20], some bacterial and cyanobacterial communities known to carry out biodegradation of organic pollutants may be resistant to OTs and can facilitate their biodegradation [21, 22]. The relatively high ratios (1 and above) of the organotins to their respective degradation products (Table 5) may suggest slow rate of degradation of the parent organotin compounds or renewed inputs of these compounds from external sources.

According to according to the classification scheme of Dowson *et al.* [23], sediment with TBT level greater than 1.3 ng Sn/g is considered to be contaminated. From our study, sediment samples from the southern part of the Gulf were contaminated with OTs. With the heavy ship and boat traffic at the King AbdulAziz Port, in particular, as well as agricultural and industrial activities onshore, the level of OTs in our study locations was not surprising. High concentrations of OTs were earlier reported in the sediment samples of neighboring Bahrain (up to 1930 ng/g) [24] and Qatar (up to 60 ng Sn/g) [25]. Elsewhere, up to 43 mg/kg was recorded in samples of Antwerp, Belgium [3].

CONCLUSIONS

We have determined six different species of OTs in coastal sediment of the Eastern Province of Saudi Arabia using a simple method that involved derivatization by propylation in combination with solid-liquid extraction and GC-MS analysis. This method has analytical limits which were low enough for the determination and quantitation of the analytes. Concentrations determined indicate that the affected locations were contaminated with these organo-metallic species, possibly from ships and from agricultural and industrial inputs.

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