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Organotin Species in Fish and Bivalves Samples Collected from the Egyptian Mediterranean Coast of Alexandria, Egypt

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The environmental monitoring and toxicological studies dealing with mussels and fish (Chengzuolian and Arnejensen 1989; Jantzen and Prange 1995; Alzieu 2000; Svavarsson et al. 2001) indicated that organotin compounds continue to pose a major excotoxicological threat in the aquatic environment. Several years after regulation, organotin compounds are still detected in marine organisms world-wide, and their effect on gastropods is widely reported. A Tributyltin (TBT) level of about 0.8 ng/L inhibits calcification in *Crassotrea gigas* and causes disease symptoms in the dog whelk *Nucella lapillus* (Gomez Ariza *et al.* 2000). The harmful effects of coastal environment contamination by TBT were first observed in the Bay of Arcachon oyster. In addition, significant and repeated disturbances were observed to occur in the *Crassostrea giges* oyster farms of the Arcachon Bay along the French Atlantic coastline (Quevuviller et al. 1994). They proved that organotin compounds are extremely toxic to aquatic organisms in general and to bivalves, molluscs and gastropods in particular, for which lower effective levels were below 1 ng/L.

Alexandria region was one of the most important marine fishery ground in Egypt. Many fish of economic importance migrate to coastal waters for breeding, penetrate into brackish water lakes connected to the sea to feed and then they attain sexual maturity and migrate back to the sea. Nevertheless, the presence of areas with high ship and pleasure boating activities, incorporating numerous harbors and marines in Alexandria, where the available data on organotin levels are scarce.

The present investigation was carried out to determine the residual levels of organotin compounds in different length groups of demersal fish (*Triglaparus lastovizo*), pelagic fish (*Diplodus sargaus*) and bivalves (*donex trunculus*) collected from the Egyptian Mediterranean coast of Alexandria.

MATERIALS AND METHODS

The sampling area extended along the Mediterranean coast of Alexandria from El-Max Bay (west of Alexandria) to Abu Qir Bay (East of Alexandria) and in a seaward direction to a depth of 25 m (Fig. 1).

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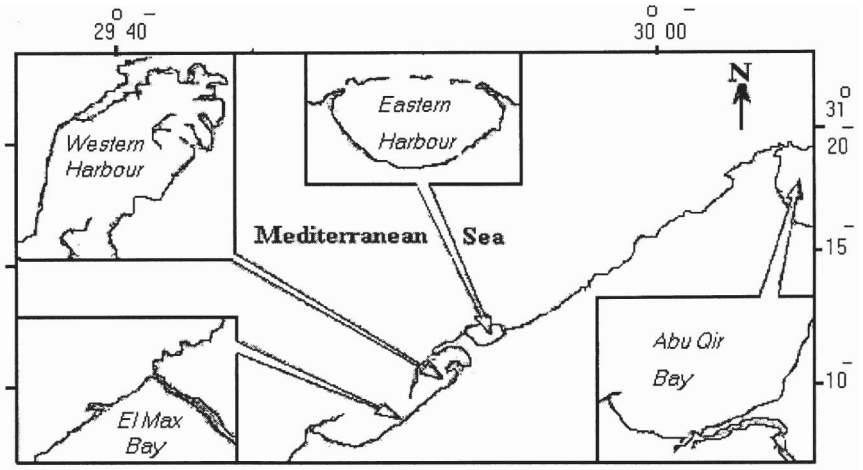

Figure 1. Area of investigation along the Mediterranean coast of Alexandria

Three length groups of demersal fish (*Triglaparus lastovizo*), pelagic fish (*Diplodus sargaus*) and bivalves (*donex trunculus*) were collected and measured for total tin and organotin species.

Determination of total tin in biota was carried out according to the method of Saeki *et al.*, (1999). About 0.5 g of the dried sample were weighed in 30mL Teflon containers with screw caps, and 5mL aliquots of concentrated HNO_3 (69%) were added, predigestion step was carried out at room temperature for 6 hrs. The digestion was carried out in a microwave oven (LG-convection) for 6 min at 200 watt (high level). After cooling 0.5 mL of HNO_3 (69%) and 1mL of deionized distilled water were added into the sample container and again heated in the microwave oven for 40 sec. When digestion is completed, the sample was transferred into a measuring flask (10 mL) and diluted with deionized distilled water up to 10mL, then the concentration of total tin was measured by atomic absorption spectrometer (Perkin Elmer 2380).

Tributyltin (TBT), diphenyltin (DPhT) and dibutyltin (DBT) were determined according to the method of Tsuda *et al.,* (1987). Thirty g of homogenized sample were placed into 300-mL reparatory funnel, and extracted for 30 min with 50mL ethyl acetate after adding 100 mL water, 15 g NaCl and 10 mL HCl. The mixture was centrifuged (2500 rpm; 5min) and 30 mL of the organic layer was transferred into 50 mL round-bottom flask. The organic layer was evaporated nearly to dryness (0.1mL) under rotary-vacuum at 40°C. The residue was dissolved in 1mL ethanol after adding 2 mL hydrogenation reagent ($NaBH_4$) with shaking and left for standing 10min at room temperature. Five mL water was added to the reaction mixture, shaken slightly, and transferred to 50 mL separatory funnel. The flask was rinsed with 5 mL portions of water and transferred to the funnel. Then extracted for 5 min with 5mL hexane after adding 5g NaCl on Silica gel column. Hexane layer was poured onto silica gel column, and passed through the column.

The first 20mL was collected in round-bottom flask, evaporated to 2 mL under rotary-vacuum at 40°C. The concentrate was transferred to 5mL gradual test tube with the rinsed flask with hexane. The final volume was adjusted to 1mL under nitrogen stream in 40°C dry bath. The sample was injected into gas chromatograph-electron capture detector (GC/ECD HP 5890 II). Method validation and quality control samples were done using standard solutions and applying the computerized 4.3 quality system program provided by DANIDA from VKI. Two natural samples were analyzed in duplicate in each of six batches of samples after spiking by a known concentration from the standard solution. The same two natural samples were analyzed without spiking. The highest and lowest percentages of recovery for spiked samples were used to determine the accuracy which ranged between 90 and 105%, while precision was agreed to be within 10%.

RESULTS AND DISCUSSION

The concentrations of DBT, TBT, DPhT and total tin species were measured in three length groups of pelagic fish (*Diplodus sargaus*) from Egyptian Mediterranean coastal water of Alexandria Table (1). These concentrations fluctuated in the ranges from undetectable to 16 ng/g; wet wt for DBT; 44– 82 ng/g; wet wt for TBT; 10 – 43 ng/g; wet wt for DPhT; and 131- 240 ng/g; wet wt for total tin. The highest concentration of organotin and total tin species were found in the length group 17-19 cm, while the lowest concentration of organotin and total tin species were found in length group 11-13 cm suggesting the bioaccumulation of these pollutants in pelagic fish. *Diplodus sargus* is considered as one of most important species in family *Sparidae* which are living in tropical and temperate littoral or inshore waters, sometimes brackish waters, young and small species gregarious in shallow waters on rocky bottoms entering brackish water and lagoons in spring returning to sea at end of autumn they feed on algae and warms, small molloscs, crustaceans echinoderms (Abdallah 1995).

Table 1. Concentrations of organotin species (ng/g; wet wt) and total tin (ng/g; dry wt) in biota samples collected from Alexandria coasts during 2004.

Species	no. of samples	length, cm	wt, g	DBT	TBT	DPhT	Total tin
Donex trunculus	75	1.5	15	11	161	41	370
Donex trunculus	65	2	20	20	191	53	498
Donex trunculus	50	2.5	20	28	250	66	580
Trigloporus lastoviza	10	9	99	11	155	32	371
Trigloporus lastoviza	10	9 12	104	15	192	44	404
Trigloporus lastoviza	10	>12	121	20	212	61	530
Diplodus sargus	3	11 13	91	10	44	10	131
Diplodus sargus	3	14-16	191	13	56	22	230
Diplodus sargus	3	17-19	261	16	82	43	240

TBT species exhibited the highest concentration of organotin species in all length groups of *Diplidus sargaus*. This is due to the fact that TBT compounds have been

used most extensively as the main biocide in marine antifouling and TBT accumulates in a variety of marine organisms.

The residual concentration levels of of DBT, TBT, DPhT and total tin species in three length groups of demersal fish (*Triglaparus lastovizo*) from Egyptian Mediterranean coastal water of Alexandria are summarized in Table (1). The concentrations of these species of organotin compounds are fluctuated in the ranges from (11 - 20 ng/g; wet wt); (155 – 212 ng/g; wet wt); (32 - 61 ng/g; wet wt) and (371 - 530 ng/g; wet wt), respectively. The highest concentration of organotin and total tin species were found in the length group >12 cm.; while the lowest concentration of organotin and total tin species were found in length group <9 cm, revealing the bioaccumulation of these compounds in fish. TBT exhibited also the highest concentration than other organotin species reflecting their extensive use and application. *Trigluparus lastoviza* is considered as one of most important species in demersal fish living on rough rocky grounds or sand and muddy sand near rocks from the shoreline to about 150m depth. They mainly fed on small-sized crustaceans with shrimp and crab which were the most important food items. These two items made up more than 75% of the total food weight consumed and had a relative importance indices of 77 and 15%, respectively (Faltes 1996). In demersal fish, organotin compounds enter via food or it is adsorbed through gills in contact with sea water and it is rapidly metabolized in the liver (Borghiand and Porte 2002).

Table 1 indicates that the concentration of DBT, TBT, DPhT and total tin species in bivalves (*donex trunculus*) were fluctuated in the ranges (11 - 28 ng/g; wet wt), (161-250 ng/g; wet wt), (41-66 ng/g; wet wt) and (370-580 ng/g; dry wt), respectively. The highest concentrations of organotin and total tin compounds were found in the length group 2.5 cm, indicating a bioaccumulation of those pollutants in bivalves, on contrast the lowest concentrations of organotin compounds and total tin were found in length group 1.5 cm regarding organo tin species, the highest concentration of organotin species in three length groups of bivalves was TBT species. This is due to the worldwide use of TBT species as biocide in antifouling paints and agriculture. TBT was the most predominant butyl tin component in almost all bivalve specimens surveyed, suggesting a low rate of TBT metabolism (Horiguchi *et al.* 2003). Pollutants are usually accumulated by aquatic organisms providing a time integrated measure of the pollutant bioavailability by the analysis of their tissues, bivalve being the most employed organism as biomonitor accumulating TBT in direct proportion to their environmental levels (Gomez Ariza et al., 2000).

A comparison was made between the concentrations of TBT and DBT recorded in fish samples of the present study with that recorded in other areas (Table 2). It is obvious that the present levels were comparable to other studies recorded in Italian coast, Baltic Sea and North Sea.

The direct comparison between the concentration of organotin and total tin compounds in the bivalves and the other two fish species indicated high concentration in bivalve (Fig. 2).

Table 2. Comparison between the concentrations of organo tin compounds (ng/g) in fish samples collected from the investigated area with that recorded in the surroundings

Investigated area	TBT	DBT	References
Alexandria coast, Egypt	44-82	0 -16	Prestnt study
Eastern Harbour, Egypt	97	39	Abdallah 1995
Western Harbour, Egypt	420	120	Abdallah 1995
Suruga Bay, Japan	72	-	Takahashi et al. 1997
Badaiya, Bahrain	8.8-20	-	Stephen et al. 2003
Italian coasts	16-230	-	Kannan et al. 1996
North Sea	293	-	Kannan and Falandysz 1997
Baltic Sea	14-455		Shawky and Emons 1998

This trend might be explained by different metabolic rates in biota according to the environment pollution levels that induce the activity of microsomal monoxygenase enzymes, leading to the cleavage of the tin - carbon bonds (Kimmel *et al.* 1977) and bivalve being the most employed organism as biomonitor accumulating organotins in direct proportion to their environmental levels. In addition, it is worth to mention that the organotin and total tin concentrations in the *triglaparus lastoviza* are higher than *Diplodus sargaus* (Fig. 2). This can be related to several factors such as habitat, dietary uptake, pollutant bioavailability and biotransformation in addition to the main factor for uptake of organotin compounds in demersal fish via food which is mainly feed through sediments leading to high uptake of pollutions. The fact that many demersal fish species are long-lived and tend to feed at higher trophic levels than their pelagic fish counterparts may lead to a potentially higher level of accumulation of persistent pollutants particularly those are not easily metabolized or degraded.

Table 3. Comparison between the concentrations of organotin compounds (ng/g) in worldwide bivalve samples

Investigated area	TBT	DBT	References
Alexandria coast, Egypt		161-250	Prestnt study
Eastern Harbour, Egypt		21	Abdallah 1995
Wetern Harbour, Egypt	320	51	Abdallah 1995
UK estuaries	8-3100	170-16100	Prestnt study
US Coasts	260	708	Takahashi et al. 1997
Barcelona, Spain	2500	2450	Stephen et al. 2003
Alfacs Bay, Spain	220	12	Kannan et al. 1996
Abu-Dabi, UAE	196	229	Kannan and Falandysz 1997
Sydney, Australia	1-90	-	Shawky and Emons 1998
Taiwan	130-620	-	Abdallah 1995
Chinhae Bay, Korea	100-1800	-	Abdallah 1995

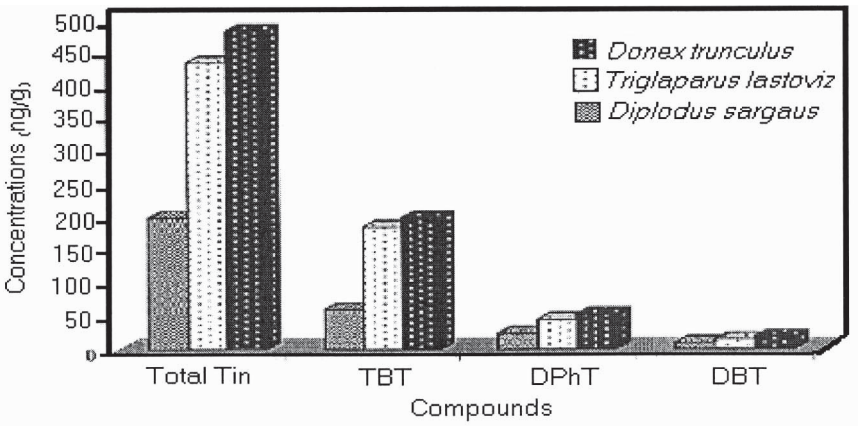

Figure 2. The average concentrations of organotin species and total tin in biota samples collected from the investigated area during 2004

A comparison recorded in Table 3 represented that the levels of both of TBT and DBT measured in bivalves of Alexandria coast were lower than that recorded in other areas of Spain, Australia, Taiwan, Korea, USA and UAE.

In general, the concentrations of TBT species showed the highest contents at all species of biota samples compared to DBT and DPhT. This trend might be due to the worldwide use of TBT not only as biocides in antifouling paints but also as preserving agents for wood, fungicides in agricultural activities, heat and UV stabilizers of PVC. The application of TBT results in direct release into the water body with the consequent accumulation in aquatic fauna and decrease the degradation rate into its derivatives.

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