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MED POL Survey of Organotins in the Mediterranean

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A pilot survey of tributyltin (TBT) and its derivatives in Mediterranean areas was undertaken in 1988 within the framework of the MED POL activities. The areas studied were the French Mediterranean coast, the Northern Tyrrhenian coast, the Southern coast of Turkey and the Alexandria (Egypt) coastal area. 113 water samples were analysed from the first three areas and 35 sediment samples from the fourth. Samples were collected at sites selected according to differing environmental conditions and potential inputs of TBT. Two shellfish-culture areas in the South of France located near marinas were included. The data reported represent the first coordinated survey of butyltin levels in seawater and sediments from Mediterranean areas. The concentrations generally exceed the no-observed effect level (NOEL) of 20 ng l^{-1} and are comparable to those previously reported for similar situations outside the Mediterranean area.

Of the organotins, the major concern for the marine environment is the use of TBT as an antifouling agent in boat paints and in power station cooling water systems. A number of countries have already taken action against the use of these compounds. So far in the Mediterranean, only France has restricted the use of organotin antifouling paints on hulls of boats less than 25 m long (UNEP/FAO/WHO/IAEA, 1989).

In 1988 the Co-ordinating Unit for the Mediterranean Action Plan in cooperation with FAO and IAEA organised a pilot survey in selected areas to generate data on existing levels of organotins in Mediterranean locations.

Materials and methods

Water sampling

Water samples were collected from the areas shown in Fig. 1.

French Mediterranean coast: Marseille harbour and the adjacent marina in 'Vieux Port', Bandol marina (near Toulon), Cap d'Agde marina, Etang de Thau and Gruissan bay were sampled. Etang de Thau is the largest shellfish (mussels and oysters) culture area on the French Mediterranean coast and the mouth of Gruissan Bay is used by the shellfish farmers for storing oysters and mussels during the winter season.

Northern Tyrrhenian coast: Leghorn harbour (Livorno) and Fossi Medicei, Piombino (harbour and marina), Foce Cecina marina, San Vincenzo marina and Punta Ala marina were sampled.

Southern coast of Turkey: Iskenderun harbour, Mersin harbour, Antalya marina and Marmaris marina were sampled. An offshore station was also sampled.

The number of stations in each area varied from 1 to 12 and each station was sampled 1–4 times during the period April–September 1988. All water samples (113 in total) were collected in pre-cleaned glass or Teflon® bottles which were immersed prior to opening and retrieved closed to prevent potential contamination from the surface micro-layer. Samples for AAS analysis were preserved using glacial acetic acid while those for cGC-FPD were stored in darkness at $<4°C$ and were analysed within two days of collection.

Sediment sampling

Surface sediment samples were collected using a Van Veen grab from 35 stations in the Alexandria coastal area. The sampling area extended more than 45 km along the coast from Agamy (west of Alexandria) to Abukir bay (east of Alexandria) and in a seaward direction to a depth of 35 m. The sediment samples were stored frozen in pre-cleaned polypropylene bags. After air drying each sample was ground and sieved (63 μm stainless steel mesh) prior to organotin analysis.

Analytical techniques

The following two techniques were used to determine butyltins in sea water:

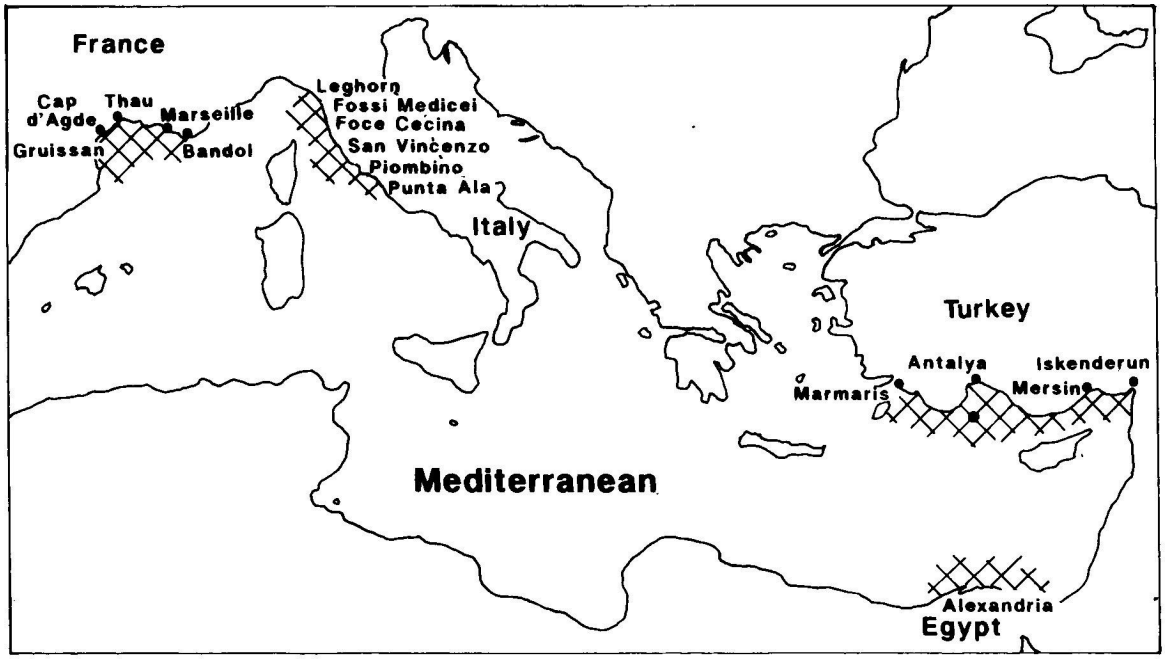

Fig. 1 Mediterranean areas investigated by the organotin pilot survey. Coastlines sampled are shaded by cross-hatching and individual sites are named.

1. Hydride generation—AAS: This technique was derived from the methods desribed by Braman and Thompkins (1979) and Hodge *et al.* (1979). It was used to quantify butyltins in samples from the Southern coast of Turkey and the French Mediterranean coast, the latter using the automated analytical protocol described by Michel (1987). Detection limits were approximately 1 ng l^{-1} for DBT and MBT and 2 ng l^{-1} for TBT using the automated technique (10 ng l^{-1} for TBT using the manual technique).

2. Capillary gas chromatography—flame photometric detection (cGC-FPD): The samples from the North Tyrrhenian coast were analysed by cGC-FPD using an application of the simultaneous hydridization and extraction procedure introduced by Matthias *et al.* (1986) with minor modifications including those according to Waldock *et al.* (1987). The detection limit was approximately 20 ng l^{-1} for tri- and di-butyltins and 100 ng l^{-1} for monobutyltins.

The various techniques employed in the different laboratories to quantify individual butyltins in seawater were intercalibrated through replicate analyses of a water sample spiked (at 100 ng l^{-1}) with TBT. Results gave a mean concentration of 139 ng l^{-1} with a standard deviation of ±18 ng l^{-1} (n = 15).

The sediment samples were extracted as described by Weber *et al.* (1986) by shaking with $CaCl_2$ solution and hydrochloric acid for 15 h. They were then filtered through a 0.4 µm Nucleopore polycarbonate filter apparatus and the filtrate after dilution was analysed using the HG-AAS technique described above. Detection limits for individual alkyltins were approximately 1 ng Sn g^{-1} dry sediment while for inorganic tin it was 2 ng g^{-1}. The recovery ranged from 79 to 105% for all alkyltin species. Triethyltin was used as an internal standard.

Results and discussion

Seawater samples

Results from this pilot survey can be considered as a first approximation, representative of the various contaminated sites occurring in the Mediterranean region. Typical TBT contaminated sites can be grouped as follows:

- those receiving industrial discharges, mainly related to the use of TBT as an antifoulant in cooling pipes;
- harbours with commercial shipping activities and ship maintenance operations. These often receive large quantities of industrial and other effluents. Marinas are also frequently located within these areas;
- marinas, occupied by pleasure boats;
- mariculture areas.

Concentrations of organotins recorded in samples from the Mediterranean are summarized in Table 1.

Extremely high and relatively constant levels of TBT were found in water samples collected at the Leghorn power plant outlet (selected as an example of TBT load from antifouling treatments in cooling pipes). In the same samples, DBT and MBT appear as minor components. The TBT contamination in the Fossi Medicei (which originates from the plant outlet), is 4–10 times less owing to dilution and to a lesser extent to degradation processes (as indicated by some relatively high concentations of DBT). TBT contamination of the Leghorn harbour and of Fossi Medicei appears substantial owing to the contribution (about 7 kg.d^{-1}) from the power plant outlet (Bacci & Gaggi, 1989).

The harbours of Leghorn (810 ng l^{-1}) and Mersin (936 ng l^{-1}) appear to be the most contaminated sites; others ranged between 83 ng l^{-1} (Iskenderun) to 210 ng l^{-1} of TBT (Marseille). At Iskenderun the high concentration of MBT (2774 ng l^{-1}) might be explained by

increased degradation of TBT. Further research is in progress.

Of the marinas, the highest level of TBT was recorded at Cecina (3930 ng l^{-1}) but the majority of values range between 100 and 1000 ng l^{-1} of TBT. In some marinas with low capacity for boats or well exchanged water, the level can be less than 100 ng l^{-1}. DBT level was generally lower than 200 ng l^{-1}, with the exception of Cecina. The particularly high TBT and DBT concentrations found at the Foce Cecina marina, probably relate to the high density of boats, the shallow depth (1–2 m), and restricted water exchange.

The mariculture areas, sampled on the French coast, exhibited less contamination by TBT, with concentrations ranging from <2 to 17 ng l^{-1}. With respect to TBT effects on mollusc larval development the highest concentration approached the 'No Observed Effect Level' (NOEL) of 20 ng l^{-1}.

A few water samples were also collected away from directly impacted areas: approximately 1 km away from the Leghorn harbour and also offshore Turkish waters. Concentrations in both samples were below the detection limits of the adopted analytical techniques (<10 ng l^{-1}). Although concentrations within the range 1–10 ng l^{-1} can be harmful to highly sensitive marine organisms (Bryan *et al.*, 1986), it is clear that the areas of significant environmental impact will be confined to inshore waters.

The data presented in Table 1 show that for each type of area sampled, the concentrations of butyltins vary within a wide range. The variations encountered of TBT concentrations in harbours and marinas are summarized in Figs 2 and 3, respectively. Such variations can be caused in part by the location of specific sampling sites, the density of boats present, and the water turnover. It is therefore inappropriate, to sum the data and provide average values for each type of site.

The concentrations of butyltins reported are, however, comparable to those reported for similar situations outside the Mediterranean area, and therefore there is no evidence that the areas covered by this pilot survey present a uniquely different problem to that encountered elsewhere (UNEP/FAO/WHO/IAEA, 1989).

TABLE 1
Range* of butyltins (ng l^{-1}) in seawater samples from the survey areas.

Site	TBT	DBT	MBT
Industrial discharges			
Leghorn Power Plant outlet	11 930; 12 150	70; 95	<100
Fossi Medicei	1125–3180	65–385	<100
Harbours			
Marseille	41–201	38–141	20–98
Leghorn	<20–810	<20–340	<100
Piombino	<20	<20	<100
Iskenderun	<10; 83	56; 484	8; 2774
Mersin	936	266	30
Marinas			
Gruissan	16; 102	17; 26	4; 9
Thau	54; 59	23; 25	8; 38
Cap d'Agde	34–536	16–36	16–56
Bandol	10–390	<1–161	<1–83
Vieux Port de Marseille	410; 736	119; 190	78; 98
Cecina	440–3930	90–750	<100
San Vincenzo	260–570	45–170	<100
Punta Ala	505–960	100–190	<100
Marmaris	11–353	121–742	<0.5
Antalya	154; 184	121; 677	<0.5
Mariculture			
Gruissan	13; 17	16; 16	10; 10.5
Thau	<2–16	<1–38	<1–26

*A single number represents the result from a single sample. Two numbers separated by a semicolon represent the results from two samples. Two numbers separated by a dash represent a range of results of more than 2 samples taken from one or more stations.

Sediment samples

Concentrations of tin species in sediments from the Alexandria coastal area are reported in Table 2 [as Sn · g^{-1} dry sediment (Aboul Dahab, 1988)].

All samples contained inorganic Sn with concentrations ranging from 310 to 5200 ng g^{-1}. Monomethyltin (MMT) was detected in 31 out of 35 samples and the concentrations ranged from undetectable to 1200 ng g^{-1}. Dimethyltin (DMT) was detected in 29 of 35 samples with concentrations ranging from undetectable to 135 ng g^{-1}. Trimethyltin (TMT) was present in 6 of 35 samples, ranging from undetectable to 80 ng g^{-1}. (MBT) was detected in all samples and ranged from undetectable to 450 ng g^{-1}. DBT was absent from 6 samples and ranged between undetectable and 425 ng g^{-1} in the remainder. All samples contained TBT with concentrations ranging from 30 to 1375 ng g^{-1}.

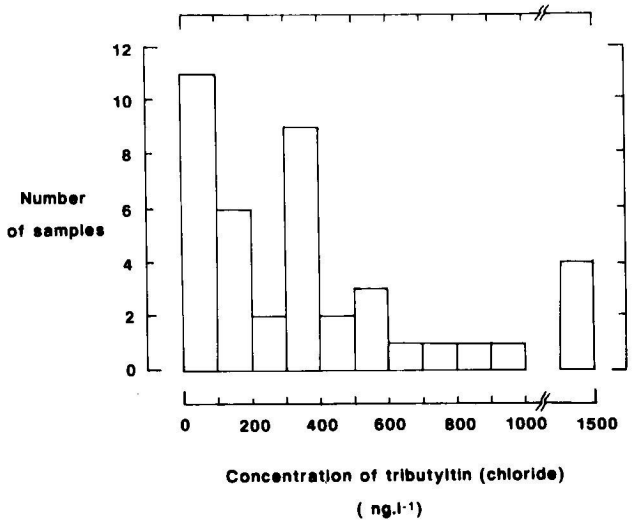

Fig. 2 Frequency histogram of TBT concentrations in water samples from marinas.

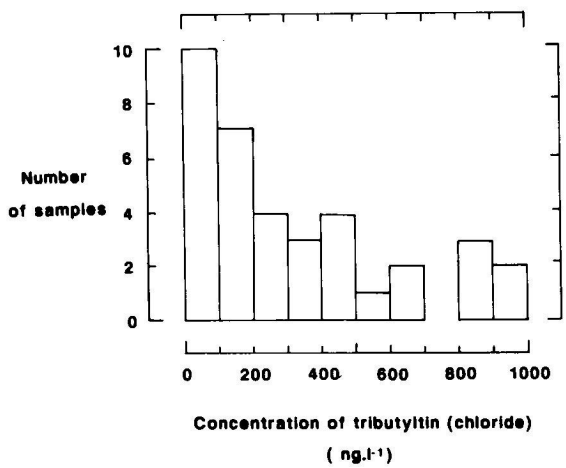

Fig. 3 Frequency histogram of TBT concentrations in water samples from harbours.

TABLE 2
Spatial concentrations of tin species in the Alexandria coastal belt sediments (ng (as Sn) g^{-1} dw) (from O. Aboul Dahab, 1988).

	I Ref. St	II Agamy	III Mex Bay	IV Western Harbour	V Off Sewage	VI Off Eastern outfall	VII Eastern Harbour	VIII Eastern coast	IX Abu Kir
Station No.	4	1–3	5–11	12–14	15	16	17–18	19–26	27–35
No. of samples	1	3	7	3	1	1	2	8	9
Inorg. Sn	310	488	2147	4720	2430	1080	3645	1075	2252
MMT	0	20	69	1000	85	0	500	41	127
DMT	15	12	35	77	70	45	23	23	43
TMT	0	0	0	60	0	0	23	0	3
Total MT	15	32	104	1136	155	45	545	64	173
MBT	0	41	57	330	45	55	138	60	83
DBT	10	17	49	305	65	60	120	63	67
TBT	35	40	187	975	185	160	260	63	252
Total BT	45	97	294	1610	295	275	518	186	401

The highest levels for all Sn species is reported for a sample taken from the Western Harbour, Alexandria, the main commercial harbour of Egypt. Next highest concentrations are reported for the Eastern harbour, Alexandria (a small fishing harbour). Low levels are reported for the reference station and adjacent 'Agamy' stations. The trend reveals a gradual increase in Sn species concentrations in sediments from the Eastern Alexandria coast to the area off the Eastern harbour to Mex Bay. Abu Kir Bay sediments showed rather higher concentrations of tin species than those of Mex Bay.

Significant variations in TBT levels were observed between stations. TBT concentrations in sediments of the Western Harbour were 28 times higher than that of the offshore station and were approximately 7 times greater than 'background' in Eastern Harbour and in Abu Kir Bay samples (Table 2). This could indicate that TBT release to the coastal area of Alexandria is mainly from the Western Harbour followed, to a limited extent, by the two fishing harbours, Eastern Harbour and Abu Kir Bay. This is consistent with its use as an antifouling agent for ships.

Conclusions

This pilot survey has shown conclusively that high and potentially toxic concentrations of TBT occur in the vicinity of harbours and marinas where there are high densities of ships and pleasure craft. The range of concentrations found (from below detection limit to 12 000 ng l^{-1}) are comparable with those known to be present in similar situations outside the Mediterranean region.

In addition to the well-known source of TBT from antifouling paints, its use in industrial cooling water systems has been shown to result in very high concentrations in the locality of the discharge (up to 12 000 ng l^{-1}).

This survey has also shown that the ratios of TBT to the breakdown products di- and monobutyltin found in the Mediterranean are not dissimilar to those found elsewhere (see Table 1). There is no evidence, therefore, that the breakdown of TBT occurs at a significantly different rate in the Mediterranean.

The use of TBT in the Mediterranean is likely to produce similar environmental effects to those identified elsewhere. Although debatable, general consensus of opinion is that concentrations as low as 20 ng l^{-1} are harmful to a number of sensitive marine organisms some of which appear to be affected by concentrations as low as 1 ng l^{-1}. It is beyond doubt that the results of this pilot survey (Table 1) reinforce the need for regulatory action.

In contrast to the large amount of information on harmful effects of TBT in water, much less is known about contaminated sediments. Such sinks may by recycling form a continuing source of pollution even after the use of TBT has been regulated (especially as the degradation rates in sediments may be very slow). Also, in areas close to shipyards, the TBT may be attached to persistent paint fragments. There is a need for continuing research to identify the importance of this potential problem, including studies on the mechanisms of adsorption and desorption, alkylation and de-alkylation and the bio-availability of TBT to sediment dwelling organisms, so that appropriate predictive models can be constructed.

Aboul Dahab, O. (1988). Speciation of tin compounds in sediments of the Alexandria coastal belt. *Water, Air & Soil Pollut.* **40**, 433–441.

Bacci, E. & Gaggi, C. (1989). Organotin compounds in harbour and marina waters from the Northern Tyrrhenian Sea. *Mar. Pollut. Bull.* **20**, 290–292.

Braman, R. S. & Thompkins, M. A. (1979). Separation and determination of nanogram amounts of inorganic tin and methyltin compounds in the environment. *Anal. Chem.* **51**, 12–19.

Bryan, G. W., Gibbs, P. E., Hummerstone, L. G. & Burt, G. R. (1986). The decline of the gastropod *Nucella lapillus* around south-west England: evidence for the effect of tributyltin from antifouling paints. *J. Mar. Biol. Assoc. U.K.* **66**, 611–640.

Cardwell, R. D. & Sheldon, A. W. (1986). A risk assessment concerning the fate and effects of tributyltins in the aquatic environment. In *Oceans '86 proceedings*, vol. 4, Organotin symposium, pp. 1117–1119. IEEE. New York.

Hodge, V. F., Seidel, S. L. & Goldberg, E. D. (1979). Determination of tin (VI) and organotin compounds in natural waters, coastal sediments and macro algae by atomic absorption spectrometry. *Anal. Chem.* **51**, 1256–1259.

Matthias, C. L., Bellama, J. M., Olson, G. J. & Brinkman, F. E. (1986). Comprehensive method for the determination of aquatic butyltin and butylmethyltin species at ultratrace levels using simultaneous hydridization/extraction with gas chromatography–flame photometric detection. *Environ. Sci. Technol.* **20**, 609–615.

Michel, P. (1987). Automatization of a hydride generation/AAS system. An improvement for organotin analysis. In *Oceans '87 proceedings*, vol. 4, International Organotin symposium, pp. 1340–1343. IEEE. New York.

UNEP/FAO/WHO/IAEA (1989). Assessment of organotin compounds as marine pollutants in the Mediterranean. MAP Technical Reports Series no. 33, Athens, UNEP.

Waldock, M. J., Thain, J. E. & Waite, M. E. (1987). The distribution and potential toxic effects of TBT in UK estuaries during 1986.

Appl. Organomet. Chem. **1**, 287–301.
Weber, J. H., Donard, O. F. X., Randall, L. & Han, J. S. (1986). Speciation of methyl- and butyltin compounds in the Great Bay estuary (N.H.). In *Oceans '86 proceedings*, vol. 4, Organotin symposium, pp. 1280–1282, IEEE, New York.
Wong, P. T. S., Chan, Y. K., Kramar, O. & Bengert, G. A. (1982). Structure toxicity relationship of tin compounds on algae. *Can. J. Fish. Aquat. Sci.* **39**, 483–488.

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Metal Concentrations and Digestive Gland Lysosomal Stability in Mussels from Halifax Inlet, Canada

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Mussels (*Mytilus edulis*) from the Halifax Inlet contained copper, lead, and zinc in concentrations ranging from 13.7–154.3, 3.1–12.1 and 108.8–552.0 μg g^{-1} dry wt, respectively. Some of the variation in tissue concentrations could be attributed to the proximity of sampling sites to point sources such as sewage discharge points. In non-reproducing mussels, the stability of digestive gland lysosomal membranes was usually lower in samples containing high Zn concentrations.

For over 200 years, untreated wastes have been discharged into the Halifax Inlet. Increasing urbanization and industrialization in the Halifax/Dartmouth metropolitan area have resulted in a drastic increase in both the volume and diversity of waste categories being generated over the past 20 years. Recent estimates, involving a survey of 550 industries and institutions, indicate that about 22 800 t per annum of special (hazardous) wastes are generated around the Inlet (Porter Dillon, 1986). Of this, about 22% is discharged into the Inlet via sewer outfalls (Porter Dillon, 1986).

Several metal sources are included in these special wastes (Porter Dillon, 1986). Sewage discharges have been recognized as the most important source of metal loadings in the Inlet (Buckley & Hargrave, 1989). Other general sources which may also contribute to the total metal loadings into the Inlet include municipal storm water runoff, landfill operations, atmospheric fallout, and the dumping of sewage sludge. Dredging, dumping, and shore development activities involving infilling, are conducted periodically in and around the Inlet. These may play an important role in affecting the distribution and bio-availability of metals and other contaminants through the resuspension and redistribution of previously sedimented material.

Previous pollution studies in the Inlet have concentrated mainly on either the measurement of contaminant levels in abiotic components, or on assessments of the sanitary water quality through bacteriological surveys. The work described here focused on the uptake of metals by marine biota, and on their possible effects on health. The research strategy adopted was based on the 'Mussel Watch' bio-monitoring framework (NRC, 1980; Phillips, 1980; Bayne *et al.*, 1985; Goldberg, 1986), and the common mussel (*Mytilus edulis*) was employed as the indicator species. As well as determining tissue concentrations of metals in mussels residing in the Inlet, the stability of the lysosomal membrane in the digestive gland was measured as a possible index of their response to pollution (Bayne *et al.*, 1985; Moore & Stebbing, 1976; Moore, 1980; Moore & Viarengo, 1987; Moore, 1976, 1980; Harrison & Berger, 1982).

Methods

Samples were collected from nine stations in the Inlet (Fig. 1). These sites were chosen to reflect a range of possible metal concentrations, and on the basis of the availability of organisms of the size range 45–55 mm. Sampling, transportation, and the preliminary processing procedures used were in accordance with those described by the NRC (1980), or Phillips (1980). Approximately 100 individuals were collected from each site during the period mid-November to mid-December 1988.

Samples from some stations included organisms presumed to be reproductively active (RA) since they had a large ripe gonadal mass contributing > 50% to the total tissue weight. These were separated from non-active (NRA) organisms and analysed separately. NRA organisms were pooled in triplicate, according to sampling station, with each pool containing an identical