ORGANOTIN COMPOUNDS IN MARINE MUSSELS COLLECTED FROM ITALIAN COASTS

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Abstract - Organotin compounds were determined in mussel samples collected in several Italian harbours and coastal environments. Interesting data were obtained from La Spezia and Taranto harbours (organotin levels in mussels were found to be relatively high), where some of the most representative Italian mussel farms are located. Preliminary results are here summarized and discussed.

Key words mussels, organotin compounds, GC analysis, Italian harbours

Environmental contamination by organotin compounds has been well documented starting from '70s, when these compounds replaced copper salts as active ingredients in antifouling paints. In particular, tributyltin (TBT) and, in less extent, triphenyltin (TPHT) [1] are widely employed. Due to the release of TBT and TPHT from antifouling paints and to the high toxicity to aquatic organisms [2-4], several studies have been carried out in the last years in order to determine concentration levels in the environment, accumulation rate in several organisms and effects on the health and productivity. In the early '80s many authors found that TBT in antifouling paints also had deleterious effects on non-target organisms, some of which are economically important [2,5]. Alzieu et al. [6] reported shell deformities (chambering) of the oyster *Crassostea gigas* in the Arcachon Bay (France) and the same type of thickening was later observed by Waldock and Thain [7] along the British East coast. Such compounds were suspected to be responsible for similar anomalous shell thickening in oysters from Tejo estuarine [8]. Other filter-feeding organisms, like the mussel *Mytilus edulis*, display growth inhibition of larvae and veliger stages [9]. In a recent work Quevauviller et al. [10] have suspected TBT inputs, originated from a shipyard, to be responsible for the death of oysters in the Sado estuary (Portugal). Laboratory experiments on TBT bioaccumulation showed high bioconcentration factors for oysters [7] and for blue mussels [11-12]. The above exposed considerations clearly show the necessity to regulate the use of these compounds on the legislative point of view and to improve the knowledge of environmental data. In the last years, our laboratory carried out monitoring programmes in several Italian harbours and coastal environments collecting water, sediment and mussel samples. The relatively high organotin levels found in mussel samples from La Spezia and Taranto and the fact that important mussel farms are located there, suggested a deeper investigation. The preliminary results are presented and discussed in this paper.

EXPERIMENTAL

Apparatus Gas chromatography was carried out on a Varian Vista 6000 chromatograph equipped with FPD detector operated without filter. With respect to the standard configuration gas flows were modified as following: air (1) 200 cc/min; Hydrogen (1) 120 cc/min; Hydrogen (2) 80 cc/min; other chromatographic conditions were: carrier gas flow Helium 9 cc/min; column Megabore DB1 (methylsilicone) 0.53 i.d., 1.5 μ m film thickness, 30 m. length; J&W Scientific; temperature programme: 80°C x 1 min., then 10°C/min to 280°C; injector: hot on-column, 240°C; detector temperature: 240°C.

Reagents Organic solvents were "RS-Pesticide Grade" from Carlo Erba (Milan, Italy) - Tropolone (2-hydroxycycloheptatrienone) from Lancaster Synthesys (Morecambe, U.K.) - n-pentylmagnesium bromide from Aldrich (Steinheim, FRG) - Sulphuric acid from BDH (Poole, Dorset, U.K.) - Florisil 100-200 mesh from BDH, activated overnight at 180°C - Anhydrous sodium sulfate RPE-ACS from Carlo Erba, treated at 550°C for 6 hours before use - Tributyltin chloride (TBT) from BDH - Dibutyltin chloride (DBT), Monobutyltin chloride (MBT), Diphenyltin chloride (DPHT), Monophenyltin chloride (MPHT) from Aldrich - Tripropyltin chloride and Triphenyltin chloride from Merck (Hohenbrunn, FRG).

Procedure The analytical procedure used for organotins determination in mussels is shown in Fig. 1. The sample is freeze-dried and homogenised before extraction. The procedure, after extraction in methanol/tropolone by sonication, include a liquid/liquid partitioning in water/dichloromethane. Solvent exchange from methanol to non-polar iso-octane was split into two steps because of easier handling of the water/dichloromethane system and complete recovery of the analytes (no significative influence of the evaporation step was observed). Derivatization is performed in a reaction vial (15 ml) for at least 15 minutes. Pentylmagnesium bromide often contains TBT impurities, so a careful blank evaluation is required. The excess of reagent is destroyed carefully adding 1 M sulphuric acid. The organic phase is collected and subjected to the clean up step after removal of ethyl ether. The clean up on Florisil is recommended for biological samples before gas chromatographic analysis. The organic layer (collected filtering on anhydrous sodium sulfate) was reduced to a 0.5-1 ml and transferred on the top of a column (1 g of Florisil in a Pasteur pipette) eluting with 2.5 ml of hexane/benzene 1:1. The solution was reduced to an exact volume (usually 1 ml) and 2 µl were injected in gas chromatography. Tripropyltin was used as internal standard for quantitative analysis, being the compound that closely matched with the compounds to be determined. Relative response factors are to be calculated (by injecting standard mixtures) before analytical determinations, due to the relatively small variability in flame.

RESULTS AND DISCUSSION

Figs. 2 and 3 show the location of the sampling stations. The selection of these two sites was suggested by the following considerations: a) the data (summarized in block diagram of Fig. 4) obtained on mussel samples in the preliminary general monitoring programme showed, as expected, higher organotin levels in samples from harbours (particularly for Genova, La Spezia and Taranto) than in samples from the other coastal environments; b) some of the most important Italian mussel farms are located in La Spezia and Taranto harbour areas. Exhaustive description of the La Spezia Gulf and analyses on water and sediments are reported in previous papers [13-14]. A mussel farm is located in point 1 just inside the La Spezia harbour and the sample from this point were directly furnished by the farm responsables. The other samples were collected on the bottom of the rocks using a stainless steel rake with a net. Mussel farms are located in each sampling point (1 to 4) inside the Taranto harbour and the samples were furnished by the farm responsables as well. The results obtained in the summer (August, Fig. 5) and winter (January, Fig. 6) sampling campaigns in the La Spezia Gulf are reported. As expected the organotins content is higher in point 1, located in the harbour area. As it can be seen from the diagrams, the organotins total content in each sampling point is practically the same in summer and winter. However, the organotins speciation is greatly different from summer to winter. In summer samples the degradation products are more than 50%; on the contrary, in winter samples the trisubstituted species are clearly prevalent. This fact is particularly true for phenylic species: in summer the prevalent form was MPHT, while in winter the prevalent one was TPhT and the MPHT concentration fall down to detection limit levels. The agreement between the summer and winter data expressed as total content seems to show that a steady state is achieved in both the cases. Analyses on sediment and water collected in the same area pointed out that probably the trisubstituted compounds are the only species accumulable at significative rate and that di- and monosubstituted ones are formed by degradation processes. Thus, the different speciation detected between summer and winter season, could be imputable to a different degradation rate occurring in mussels; in particular higher degradation rates seems to be effective in summer than in winter. As regards Taranto harbour, only the summer (June) sampling was carried out up to now and the obtained results are shown in Fig. 7. The concentrations found are ca. 5-fold

less than La Spezia ones and 8 to 10-fold less than concentration found in a sample collected in Taranto six months before the sampling campaign (sample from Fig. 4). Actually, the month choice for the sampling was not the most appropriate because of, in all sampling points, only juveniles animals were available. Due to this fact, the concentrations found could be affected by short exposure time. It will be interesting to verify if the second sampling campaigns will confirm this hypothesis.

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Fig. 1 Analytical procedure.

Fig. 2 Location of sampling stations in the La Spezia Gulf.

Fig. 3 Location of sampling stations in the Taranto harbour.

Fig. 4 Concentration levels of organotin compounds in mussels from 5 Italian harbours, Venice Lagoon (Venezia) and Tiber estuary (Rome).

Fig. 5 Concentration levels of organotin compounds in mussels from the La Spezia Gulf (summer sampling).

Fig. 6 Concentration levels of organotin compounds in mussels from the La Spezia Gulf (winter sampling).

Fig. 7 Concentration levels of organotin compounds in mussels from the Taranto harbour (summer sampling).