

## IMPOSEX AS A BIOMONITORING TOOL OF CONTAMINATION BY TBT IN THE MEDITERRANEAN

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Environmental monitoring within the Mediterranean is essentially based on chemical analysis of a range of suspected pollutants. The limitations and inadequacies of the present chemical monitoring programme have been identified by JOANNY (1990). Analytical problems and difficulties of interpretation of data are further compounded when dealing with certain pollutants which exert their biological effects at extremely low environmental levels. These include a specific organotin (Tributyltin, TBT) which has been used as an antifouling biocide on marine craft.

Elevated levels of TBT and its degradation products have been recently reported in the Mediterranean (GABRIELIDES *et al.*, 1990). Such pollutants may cause harm to inshore species (including economically important organisms such as oysters) at very low concentrations. The present author believes that a biomonitoring programme based on the use of imposex as a bioassay, is the only monitoring strategy which at present may provide data which is sufficiently reliable and which may cover the whole Mediterranean region at minimal costs.

Imposex is the imposition of male genitalia (penis and/or vas deferens) on females in certain marine prosobranchs. FIORONI *et al.* (1991) list 69 species of 46 genera of prosobranchs in which this phenomenon has been identified. At present there is a near-general consensus that no xenobiotic other than TBT is known to cause imposex in such species. A number of TBT-biomonitoring programmes based on the use of imposex have been successfully conducted in a number of regions, such as in U.K. using *Nucella lapillus*, and in North America using *Ilyanassa obsoleta*. More recently, this bioassay has been used in field biomonitoring of TBT contamination in Malta, using *Hexaplex* (*Murex*) *trunculus* (AXIAK *et al.*, in press). This species has proved to be one of the most sensitive in its imposex response to TBT, of all species investigated so far.

The degree of imposex may be quantified by various indices, including: the Relative Penis Size Index (RPS) which is the ratio between the cubed mean penis length in imposexed females to that in males for a given population; and the Vas Deferens Sequence (VDS) Index, whereby imposex development is divided into various stages of vas deferens development, with each stage being given a score. The vas deferens sequence may vary according to species (FIORONI *et al.*, 1991).

While imposex indices are always strongly correlated with proximity to marinas or harbours, in several reported cases the mean TBT body burdens for exposed population are found to be correlated with imposex indices, only at extremely low levels of TBT. This is because imposex induction occurs at levels as low as 1 ng Sn l<sup>-1</sup>. Imposex is generally induced over a period of 6-12 weeks (eg. GIBBS *et al.*, 1991). Furthermore it is apparently an irreversible phenomenon, and may be found in populations where TBT contamination is no longer present. For example, when *H. reticulata* affected by imposex was kept for 18 months under TBT-free conditions in the laboratory, no evidence for imposex remission was found (STROBEN *et al.*, 1992).

Imposex may lead to sterility and preferential female mortalities in some species such as *Ocenebra aciculata* and *Nucella lapillus* (GIBBS *et al.*, 1988). In *Nucella* normal egg capsule production occurred only in females held at a TBT concentration of 1-2 ng Sn/l; at higher TBT levels females were sterilised by imposex. Oogenesis was suppressed at TBT levels above 3-5 ng Sn/l to be supplanted by spermatogenesis leading to sperm production. The decline (and in some cases, complete eradication) of populations of *Nucella*, in U.K., France and Norway, during the past decade, has been well documented. More recently, as a result of the enforcement of TBT-controlling regulations, recovery of some of such populations has also been successfully monitored through imposex biomonitoring (eg. EVANS *et al.*, 1994).

Apparently, imposex is related to an increase in testosterone titre in exposed females in response to exposure to TBT (SPOONER *et al.*, 1991). LEE (1991) has suggested that the cytochrome P-450 dependent mono-oxygenase system is mainly responsible for the elimination of TBT from the body of marine organisms. Moreover, MFO activity is important in steroid metabolism. The susceptibility of molluscs to TBT (including imposex in prosobranchs) may be related to their relatively low MFO activities. In any case, the exact molecular basis of imposex has still to be determined. It is concluded that if properly calibrated, imposex in a limited number of species, may be successfully used for TBT biomonitoring over the whole Mediterranean due to the high sensitivity and specificity of this response, as well as the low-costs involved.

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(Note: A full bibliography list will be made available during the presentation.)

## BIOREMEDIATION OF AN OIL POLLUTED BEACH

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Bioremediation of oil pollution in open systems presents several problems (BARTHA, 1990; GLASER, 1991; ROSENBERG, 1991). These include:

1. The long period required for biodegradation
2. Difficulties in making available a supply of nutrients, mainly nitrogen and phosphorus compounds, which dilute rapidly and become inaccessible.
3. The biological solutions have not been suitable for immediate emergency response.

We have been developing a novel technology for treating oil pollution in open systems - at sea, fresh water (lakes, ponds and rivers) and on beaches. The basis for this procedure is the combined use of specific bacterial strains that adhere to hydrocarbons (ROSENBERG & ROSENBERG, 1985; ROSENBERG, 1991) and a unique oleophilic, controlled-release, nitrogen and phosphorous source.

This technology was used for the bioremediation of the north beach of Haifa (30,000 m<sup>2</sup>) following an oil spill of several hundred tons of heavy crude oil. The rate of oil degradation was 0.13 mg per gram sand per day in the summer (25°C), and half this rate in the winter (less than 10°C). The major treatment took place in the winter and was completed in four months. It should be noted that the winter was unusually hard, and temperatures were around 5-10°C for a couple of months. At the end of the treatment about 90% of the oil has been degraded, and this included the heavy (up to C40) as well as the aromatic fractions of the oil.

Visual examination of the beach sand following the treatment, in addition to the analytical data described above, indicated that this technology was applicable for bioremediation of the sand, that also became light in color.

Biodegradation of hydrocarbon-contaminated sand at Haifa beach during summer and winter

% Biodegradation	August		January	
	Natural	Treated	Natural	Treated
Day				
0	0	0	0	0
4	0	30		
9	18	50	11	25
14	26	77		
25	15	85	25	50
38			5	66
87			0	80
123			5	89

The initial concentration of hydrocarbon-contamination in the in the upper 10 cm of sand of the control plot was 2.3 mg/g sand and 3.8 mg/g sand in the experiment. The average standard deviation was 0.15 mg/g sand.

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