Distribution of antifouling biocides in coastal seawater of Egadi Islands

The pollution level due to antifouling biocides in the Marine Protected Area of Egadi Islands (MPA) has been evaluated by both grab and passive sampling. Analyses of tributyltin (TBT), diuron, irgarol, chlorothalonil and dichlofluanid have been carried out on seawater and sediments. The results indicate a good condition of the coastline, but further studies with passive sampling for TBT are required to help the MPA administrators to control the status of the seawater with a methodology suitable to reach the Environmental Quality Standard values established by the Water Framework Directive

DOI 10.12910/EAI2015-068

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Introduction

Colonization by fouling organisms is a problem for any structure placed in the aquatic environment and can be controlled through both chemical biocides and non-biocidal technologies. In spite of the progress made on diverse non-biocidal technologies and an increase in the commercial use of fouling-release coatings, the majority of vessels are still protected by antifouling (AF) paints containing biocides.
Formulations containing tributyltin (TBT) were the most successful against biofouling but they were banned in 2008, due to the detrimental impact on sealife. Currently, most antifouling paints contain copper or zinc as an active ingredient and a "booster" biocide to strengthen the effectiveness of the formulation [1].
In particular, the herbicides irgarol 1051 (2-methylthio-4-tert-butylamino-6-cyclopropilamino-s-triazine) and diuron (3-(3,4-dichlorophenyl)1,1-dimethylurea) and the fungicides chlorothalonil (2,4,5,6-tetrachloro iso-phthalo nitrile) and dichlofluanid (N-dichlorofluoromethylthio-N′, N′-dimethyl-N-phenylsulfamide) are extensively used worldwide in AF paints [1].
The widespread use of biocides in AF paints has resulted in high levels of contamination in the environment and has raised concerns about their effects on marine communities (shell malformation in oysters, mortality of mussel larvae and imposex in gasteropods), leading to policy actions to regulate their utilization and to set environmental quality standards (EQSs).
The Water Framework Directive (WFD) of the European Commission (EC 2000/60/EC) describes the monitoring of priority substances and other pollutants in surface waters, including coastal waters. The daughter directive 2008/105/EF of the European Parliament and the Council of the European Union has defined EQSs for priority substances in water and sediment, with the aim to protect the aquatic environment from adverse effects of these substances. Amongst the priority substances, specific compounds have been classified as priority hazardous substances, with the aim to cease or phase out their discharges, emissions and losses.
The list of priority substances include the biocides TBT (priority hazardous substances) and diuron and, after a recent revision (Directive 2013/39/EU), irgarol.

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Passive sampling (PS) has proven to be a reliable alternative to grab water sampling as it allows high volume sampling systems to ensure significant results while maintaining low detection limits [2]. Semi-permeable membrane devices (SPMDs) have been used extensively for the screening and the source identification of a variety of non-polar organic contaminants. SPMDs consist of a thin layer of a neutral lipid (usually trioleine) enclosed within a thin-walled, flat-lying, low-density polyethylene (LDPE) tubing. In the aquatic environment, SPMDs allow to measure not only the presence but also the bioavailability and potential bioconcentration of organic lipophilic compounds with octanol/water partition coefficients $\log K_{OW} > 3$.

In the last few years, the assessment of anthropogenic pressure on marine environment has led to stronger protection efforts of marine ecosystems. Marine protected areas (MPAs), in particular, have become an extensively advocated form of marine conservation and their number is constantly increasing worldwide. It is generally recognized that MPAs are essential for conservation as they can provide unique protection for critical areas and spatial escape for overexploited species. MPAs safeguard populations or assemblages within their boundaries, but they are less effective for protection from some major threats to marine environments [3]. These threats include coastal modifications and subsequent changes in local hydrodynamic and sedimentary regimes, the spreading of exotic species, disease epidemics and, above all, contamination by chemicals that is not possible to control directly.

Limited data and information are available on the environmental occurrence, fate, toxicity, and persistence of antifouling biocides. In particular, to our knowledge, no direct evaluation of these compounds is available for the Marine Protected Area of Egadi Islands.

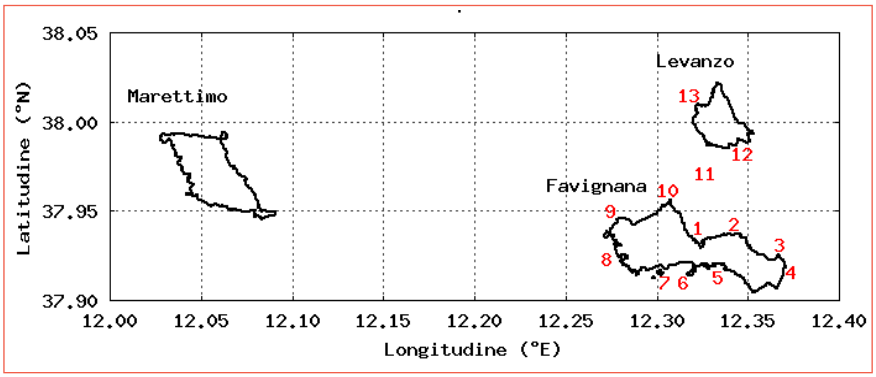

FIGURE 1 Location of sampling points for 2012 monitoring campaign

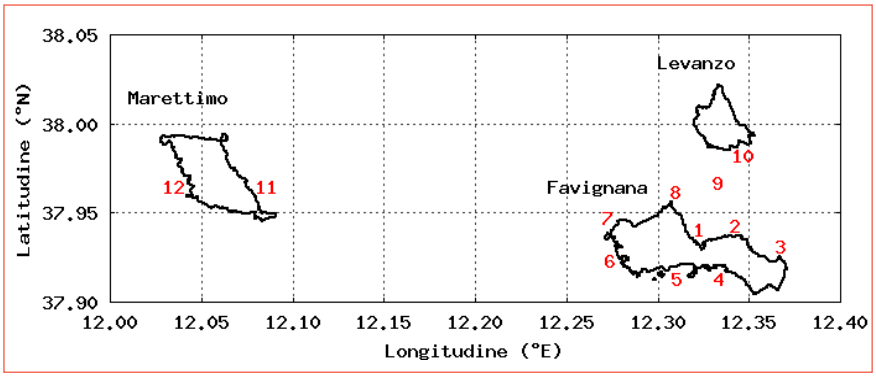

FIGURE 2 Location of sampling points for 2013 monitoring campaign

The goal of this research is therefore to estimate the pollution level by TBT and other antifouling biocides in the 4 zones of the MPA through spot sampling for seawater. Organotin-compound sediments analysis and TBT seawater passive sampling have been carried out, due to TBT particular persistence in sediment and possible presence at very low concentration (<1 ng/l as Sn).

Materials and methods

Study areas

The Egadi Islands' Marine Protected Area includes the islands of Favignana, Levanzo, Marettimo and the islets of Formica and Maraone. Figures 1 and 2 show sites selected foryears 2012 and 2013 monitoring,

respectively. All the points were in zone C, with the exclusion of points 10 and 13 in the first campaign (Faraglioni and Levanzo, B zone) and 8 and 12 in the second campaign (Faraglioni, B zone, and Marettimo, A zone, respectively).

Sampling

Water and sediment samples were collected in September 2012 and September 2013, before the end of summer, when boating activity is still intense and the contamination from AF paints is expected to be significant. Seawater was collected in all 25 sites; in 7 sites it was not possible to collect any sediments due to the rocky bottom (4 in 2012 and 3 in 2013).

A Glass-Sampler Probe (International PBI, Milan, Italy) was submerged to a depth of 0.5 m below the sea surface and seawater samples were collected in pre-cleaned 1 l glass bottles. All the containers were additionally rinsed with seawater before sample collection. The aqueous samples for analysis of Monobutyltin (MBT), Dibutyltin (DBT) and Tributyltin (TBT) were acidified *in situ* with 0.8 ml 37% HCl per liter. The aqueous samples for analysis of biocides compounds were added *in situ* with 2 ml of dichloromethane per liter. All water samples were stored in a fridge at 4 °C until the analysis.

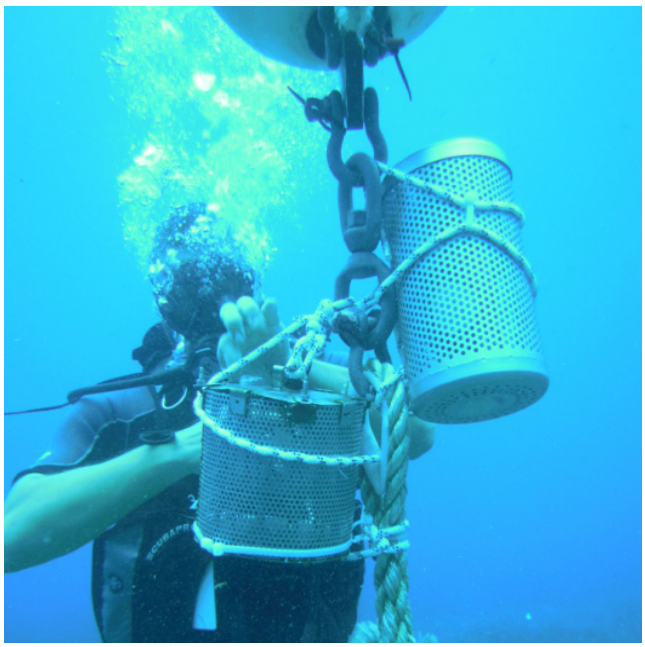

FIGURE 3 Passive sampler placement by scuba diver in Preveto site

Surface sediment samples were collected in 18 sites (9 in 2012 and 9 in 2013) by a stainless steel Van Veen grab sampler. After collection, sediments were stored at -20 °C and then transported to ENEA laboratories, where the sediments were sieved at 2 mm: the fraction <2 mm was considered for analysis. After sieving, sediments were freeze-dried.

Standard SPMDs (length 91.4 cm; width 2.5 cm; LDPE wall thickness: 70–95 µm) with 1 ml of 99% purity triolein) were purchased from ExposMeter AB, Tavelsjö, Sweden. Before use, all the support devices were washed with tap and distilled water before being rinsed with acetone and hexane. The SPMDs were transported in sealed clean metal cans and refrigerated at 4 °C. The SPMDs were assembled with proper supports and inserted into stainless steel cages (canisters, ExposMeter AB, Tavelsjö, Sweden) on the boat just before the positioning. The canisters were deployed between 1 to 6 meters below the water surface and properly fixed to a buoy or to a quay by a scuba diver (Figure 3). After retrieval, the SPMD strips were immediately roughly cleaned with acidic (HCl) water and stored at 4 °C in the metal can.

Analysis

Biocides

500 ml of water were placed into a 1 l separatory funnel, 50 ml of dichloromethane were added, and the mixture was shaken for 2 min. The layers were allowed to separate and the organic layer was filtered through anhydrous sodium sulphate and collected in a round bottom flask. Another 20 ml of dichloromethane were added and the sample was extracted as above. The extracts were reduced to approximately 0.5 ml on a rotary evaporator and then transferred to a vial for GC-MS analysis. [4].

To evaluate the recovery, samples of synthetic seawater were spiked with biocides at two different levels: 10 and 100 ng/l. The samples were analyzed according to the procedure described above. Four replicates of each level were analyzed. The recovery values were greater than 86% for both levels with a standard deviation of 9% in the most unfavorable case. LODs for this method were 1 ng/l for all the biocides.

OT compounds

Spot samples

1 L water samples (pH adjusted to 2 at the moment of sampling) were added with an appropriate amount of a solution of ^{119}Sn -enriched butyltin compounds (an isotopically enriched solution of MBT, DBT and TBT) as procedure/quantification standard, and allowed to equilibrate for 15 min with occasional agitation.

The extraction was performed in a separatory funnel with at least 2 aliquots (30 ml) of a 0.03% tropolone solution in dichloromethane (to improve the extraction efficiency of the monosubstituted species), and the organic phase was collected through anhydrous sodium sulphate. The organic phase was evaporated on a rotary evaporator down to a final volume of 1 ml at the temperature of 30 °C. The organic extract was transferred into a vial, added with 2 ml of hexane and 1 ml of isooctane (as keeper solvent) and then evaporated almost to dryness under a gentle stream of nitrogen. The organotin compounds were pentylated by Grignard reagent and then were extracted twice with 1 ml of hexane. The extract was concentrated and purified on a silica gel column. After concentration down to 0.5 ml, 1 µl of the final solution was injected for GC-MS-SIM analysis and organotin quantitative determination was based on isotope dilution method [5].

For sediments, approximately 1.0 g of freeze-dried material was taken as sample and extracted with 15 ml of 0.03% (w/v) tropolone in methanolic solution and 1 ml of concentrated hydrochloric acid. The supernatant was transferred to a separatory funnel and the extraction procedure was repeated. After the addition of the 0.03% tropolone solution in dichloromethane, the subsequent steps were the same as in the seawater extraction.

Method limits of detection for seawater (as cations) were 2.5 ng/l for TBT and 2.0 ng/l for DBT and 1.5 ng/l MBT. All the analyses were carried out by the same operator. Method limits of detection for sediment (as cations) were 1.2 µg/kg for TBT and 1.0 µg/kg for DBT and 0.8 µg/kg for MBT.

A certified reference material, a coastal sediment (IRMM BCR 462) and fortified blank seawater samples (TBT, DBT and MBT spiked at 10 ng/l and 100 ng/l as cation) were used for validation of the procedures. The analysis of the reference material (n = 3) showed a good performance, results overlapped the certified values ± their uncertainty and recoveries from fortified blank seawater samples (n = 5) were: TBT 92 ± 21%, DBT 87 ± 23%, MBT 82 ± 24% (10 ng/l) and TBT 96 ± 10% DBT 92 ± 13% MBT 86 ± 20% (100 ng/l). The GC-MS determination was done in a single run for all the samples including blanks and BCR 462.

Passive samples

After an exposure time of 21 days the collected SPMD samples strips were washed with acidic (HCl 1%) water and then stored at -20 C°.

The samples were dialyzed with 150 ml of hexane (two cycles of 24 hours). The organic phase was evaporated on a rotary evaporator down to a final volume of 1 ml at the temperature of 30 °C. The organic extract was transferred into a vial, added with 1 ml of isooctane (as keeper solvent) and then evaporated almost to dryness under a gentle stream of nitrogen. After that, the samples were treated as described above for organotin determination, whereas quantitative determination was based on the isotope dilution method.

As quality control, SPMD blanks (for the procedure and for the campaign) were analysed in parallel with the samples. No TBT was found in the blanks analysed.

The concentration in the seawater has been calculated with the method and constants described by Harman et al [6] , where the sampling rate of TBT for the SMPD was 2.67 l/d.

Results and discussion

The major input of TBT, diuron, irgarol, chlorothalonil and dichlofluanid into marine systems derives from anti-fouling paints, but occurrences have been related also with their use in other human activities (farming and conservation)[7]. For TBT, diuron and recently irgarol, EQS values for annual averages (AA-EQS) and maximum allowable concentrations (MAC-EQS) have been established in the WFD. Table 1 reports the EQS values. For chlorothalonil and dichlofluanid EQS standards are not present but values around 5 -10 ng/l are in discussion for proposed EQS [8-10].

	AA EQS µg/l seawater	MAC EQS µg/l seawater	AA EQS µg/kg sediment
TBT as cation	0.0002	0.0015	5
Diuron	0.2	1.8	N.A.
Irgarol	0.0025	0.016	N.A.

TABLE 1 Environmental Quality Standards (EQS), Annual Average value (AA-EQS) and Maximum Allowable Concentration (MAC-EQS) defined by the Directive on Environmental Quality Standards (2008/105/EC) for TBT, Diuron and Irgarol in seawater coastline and sediment. N.A Not Available

	TBT ng/l
Favignana Port	2.8±0.5
Preveto	1.0±0.3
Faraglioni (B zone)	0.3±0.1

TABLE 2 Concentration of TBT in SPMD samples from 2012 monitoring campaign. All results are expressed as ng/l cations

In all the seawater samples collected by spot sampling, the concentration of TBT and biocides were below the limit of detection, i.e. 2.5 ng/l for TBT, 2.0 ng/l for DBT, 1.5 ng/l for MBT and 1 ng/l for all the other biocides.
For diuron, irgarol, chlorothalonil and dichlofluanid the absence of detectable traces in seawater samples and the LOD of the analytical method are such to state that the presence of these biocides are well below the EQS defined by WFD or internationally recognized (Table 1).
Other studies have shown the absence of diuron, irgarol, chlorothalonil and dichlofluanid in areas of the Mediterranean sea [7] including other MPAs.
Since the marine environment is not affected by these biocides, at the moment, their utilization as antifoulants and in farming activities as herbicides and/or fungicides seems to have a limited or not relevant impact on the seawater.
Despite the total ban of TBT-based paints [11], TBT was still a commonly encountered contaminant in the seawater, and the presence of organotin compounds is yet recorded in the coastline sites. Many studies have involved surveys on TBT distribution in the water column, sediments, and biota [12-14]. Measurements taken prior to restrictions on TBT have shown levels higher than 500 ng l^{-1} in North American and European marinas. In recent investigations, it has been reported that TBT concentrations have generally declined, rarely exceeding 100 ng l^{-1}, even if hot spots have been reported especially in those countries where IMO restrictions have not been applied. When concentration in the seawater is at least 3-5 ng/l, the conventional methods of spot sampling, coupled with classical methods of analysis, are sufficient to detect the presence of TBT and its degradation products. In this study, TBT concentration in seawater samples collected by spot sampling was always lower than the method detection limit (2.5 ng/l); consequently it was impossible to verify water contamination and quality standards, considering that EQS values for TBT are 0.2 and 1.5 ng/l, for AA and MAC, respectively (Table 1), that is well below the LOD. Passive sampling devices are therefore necessary for levels below 2 ng/l. Passive samplers have been validated and provide high sampling rates (liters/day) for various contaminants, thus allowing to quantify extremely low pollution levels in water, using similar methods of analysis as for grab sampling. In particular, SPMD can be used for the analysis of TBT [15, 16] thanks to its octanol/water partition coefficient $\log K_{OW}$ >3. The TBT data of passive samples are in agreement with the results obtained with samples collected with classical procedures (Table 2). Indeed, all the samples collected by passive devices have showed a concentration around or lower than 2.5 ng/l, which is the LOD of TBT with grab sampling. The lowest concentration of 0.3 ng/l was found in the B zone of the protected area (Faraglioni), 1.0 ng/l in the Preveto samples and, finally,

	TBT µg/kg	DBT µg/kg	MBT µg/kg
1) Favignana Port	4.0±0.5	3.0±0.4	3±0.5
2) Punta San Nicola	n.a	n.a	n.a
3) Cala Rossa	n.d.	n.d.	n.d.
4) Punta Marsala	n.d.	n.d.	n.d.
5) Cala Monaci	n.a	n.a	n.a
6) Punta Longa	n.d.	n.d.	n.d.
7) Preveto	n.d.	n.d.	n.d.
8) Cala Rotonda	n.d.	n.d.	n.d.
9) Punta ferro	n.a	n.a	n.a
10) Faraglioni (B zone)	n.d.	n.d.	n.d.
11) Canal Favignana Levanzo	n.d.	n.d.	n.d.
12) Levanzo Port	2±0.3	8 ±0.6	2±0.3
13) Levanzo (B zone)	n.a	n.a	n.a

TABLE 3 Concentration of butyltin compounds in sediment from 2012 monitoring campaign. All results are expressed as cations µg/kg d.w. (n.d. not detectable: LOD TBT 1.2 µg/kg d.w., DBT 1.0 µg/kg d.w., MBT 0.8 µg/kg d.w; n.a, not available)

	TBT µg/kg	DBT µg/kg	MBT µg/kg
1) Favignana Port	3±0.3	2±0.2	2±0.2
2) Punta San Nicola	n.a	n.a	n.a
3) Punta Calarossa	n.d.	n.d.	n.d.
4) Cala monaci	n.a	n.a.	n.a.
5) Preveto	n.d.	n.d.	n.d.
6) Cala Rotonda	n.d.	n.d.	n.d.
7) Punta ferro	n.a	n.a	n.a
8) Faraglioni (B zone)	n.d.	n.d.	n.d.
9) Canal Favignana Levanzo	4±0.5	2±0.2	4±0.4
10) Levanzo Port	2±0.2	5±0.5	3±0.3
11) Marettimo Port	2±0.2	1±0.1	1±0.1
12) Marettimo (A Zone)	n.d.	n.d.	n.d.

TABLE 4 Concentration of butyltin compounds in sediment from 2013 monitoring campaign. All results are expressed as cations µg/kg d.w. (n.d. not detectable: LOD TBT 1.2 µg/kg d.w., DBT 1.0 µg/kg d.w., MBT 0.8 µg/kg d.w; n.a, not available)

the highest concentration, 2.4 ng/l, was found in the Port of Favignana.

Clearly, only a passive sampling strategy can deal with these concentrations, which are below the range of routine methods of analysis.

Marine sediments still represent a problem in the long term because they are a source of biocides for the water column and biota even after the source of contamination has been removed. The ability of marine sediments to accumulate these compounds varies geographically and geologically, according to the physicochemical characteristics of the sediment (e.g., particle size and organic carbon content). In particular, the restriction on the use of TBT [11] has not yet led to TBT disappearance in port areas, owing to its hydrophobicity and long persistence (years) in sediments [12-14]. The TBT contaminated sediment may continue to act as input sources of TBT to overlying water by desorption or resuspension of sediment-bound TBT in areas where maritime traffic is intense.

The sediment samples collected in the MPA of Egadi Islands clearly showed that butyltin compounds are consistently detected only in port areas, even if at low concentrations, in agreement with published work for sediment collected in others Sicilian MPAs [17]. Favignana, Levanzo and Marettimo ports showed TBT concentrations ranging from 2 µg/kg to 4 µg/kg (Tables 3 and 4) in both monitoring campaigns. Such concentrations can be classified as the lowest among the Italian port areas [18], always below the EQS of WFD (5 µg/kg).

Conclusions

The analysis of seawater samples from different location of Egadi Islands' coastline indicates the absence of diuron, irgarol, dichlofluanid and chlorothalonil in all the MPA locations studied.

The LOD of the analytical method is sufficient to state that the presence of these biocides in the seawater is well below the EQS defined by the WFD for diuron and irgarol and the accepted international quality standards for dichlofluanid and chlorothalonil.

TBT presence was not detected by grab sampling but only using passive sampling, at concentration levels higher than the extremely low EQS defined by the WFD (<1 ng/l).

The results confirm that only passive sampling by SPMD allows to measure TBT levels in the investigated areas reaching quantification limits similar to the requested EQS for this contaminant. TBT in sediments is only detected in portual samples, with concentrations below

the EQS of 5 µg/kg. This presence could explain the detection in passive sampling due to the remobilization of sediment with consequent resuspension of TBT.
TBT will probably cause problems long after it has been banned, remaining a matter of concern and requiring monitoring for the years to come.
These findings confirm that pollution threats to marine systems (like the dispersion of pollutants, in our case) should be addressed by MPAs also through the development and implementation of smarter monitoring systems [19]. Passive sampling devices proved to be useful to cope with this kind of pollution, particularly if extremely low levels have to be detected [20], in compliance with the EQS (annual average in particular) defined by the WFD for priority substances.

Acknowledgements
The authors would like to acknowledge the MPA Egadi Islands' Director, Dr. Stefano Donati, for his assistance, Dr. Daniela Sammartano and Dr. Monica Russo for the coordination and logistic help on site, and the divers, Dr. Niccolò Ponzè and Dr. Pietro Patti Genovese, for the crucial work of positioning passive sampling devices.

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