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Venezuelan Caribbean Sea under the threat of TBT

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HIGHLIGHTS

- Imposex and TBT levels were detected along of the Venezuelan Caribbean coast shore.
- The most contaminated sites were under direct influence of marinas (pleasure boats).
- Marinas are likely to be the new source of fresh TBT to the region.
- The fresh input of TBT could be happening to other areas of the Caribbean Sea.

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ABSTRACT

Although environmental tributyltin (TBT) contamination is considered a solved problem, imposex occurrence in *Plicopurpura patula* as well as butyltins (BTs) contamination in sediments and tissues were detected along 700 km of the Caribbean coastal shore. Areas under the influence of five main ports of Venezuela were covered, as well as large marinas and sites located away from expected sources. Marinas were the most contaminated areas, whilst imposex incidence and TBT levels were relatively low in areas nearby commercial harbors. Thus, it is evident that marinas have become the main source of fresh TBT to the region. This might explain why imposex incidence seems to be widely distributed along the Venezuelan coast, since leisure boats are circulating along the whole coastal region. In fact, this could be the pattern for other areas of the Caribbean Sea.

1. Introduction

Tributyltin (TBT) is a very toxic synthetic organic compound that was released into the environment during the last 5 decades mainly through the use of antifouling paints (Gibbs and Bryan, 1987). Nowadays, TBT has been reported as one of the most concerning pollutants in aquatic coastal systems since the levels detected in the environment are in the same order of magnitude of those causing deleterious effects in the biota (Meador et al., 2011). Malformation in oysters (Alzieu, 2000), hormonal imbalance in dolphins (Tanabe, 1999), and imposex in gastropods (Smith, 1981) are among the most reported environmental damages related to the use of TBT-based antifouling paints. Imposex incidence (the most well-known TBT biological effect) has been widely used as a biomarker of exposure to TBT and is directly associated to coastal areas under the influence of intense maritime activities such as ports, harbors, marinas and shipyards (Lee et al., 2006).

Hence, several countries have restricted the use of TBT-based antifouling paints (Champ, 2000). In addition, the Antifouling Systems (AFS) Convection was adopted in 2001 by the International Maritime Organization which restrained the global use of TBT-based protective coatings in 2003 and totally banned them from September 2008 (IMO, 2008). Consequently, TBT concentrations and imposex levels started to decline in environments worldwide (Ruiz et al., 2010) and there are many reports about recoveries of previously contaminated coastal areas after restrictive regulations towards the use of TBT. For example, imposex reduction was verified for populations of *Nucella lapillus* (Morton, 2009; Galante-Oliveira et al., 2009) and *Nassarius reticulatus* (Sousa et al., 2009) after the banning of TBT-based antifouling paints by the European legislation (EC Regulation 782/2003). *Hexaplex trunculus* from the Tunisian coast (Lahbib et al., 2009) and *Stramonita haemastoma* from southern Brazil (Castro et al., 2012d) have also showed reductions in imposex levels after global and local

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restrictions. Similarly, many studies have also detected a reduction of butyltins (BTs) concentration in sediment and biota samples from Asia (Choi et al., 2010), Europe (Ruiz et al., 2008) and North America (Wade et al., 2008).

Based on these international restrictions along with evidences of its reduction, the environmental issues related to TBT-based antifouling paints have been considered as solved by many governmental and academic organizations (Morton, 2009). However, this decrease in imposex and TBT levels might not be the case for every coastal area as high TBT and imposex levels have recently been detected in several South American countries, such as Peru (Castro and Fillmann, 2012), Ecuador (Castro et al., 2012a), Brazil (Azevedo et al., 2012;Borges et al., 2013), and Chile (Pinochet et al., 2009). These data pointed out that TBT-based antifouling paints are still being used in the region, where evidences of production and retailer of TBT oxide and chloride were recently found (Castro et al., 2012b). Although Peru, Ecuador and Chile have no specific legislation against TBT-based antifouling paints and are not signatories of the Antifouling convention by IMO (IMO, 2014), this is a clear indication that the illegal use (in the case of Brazil), uncontrolled commercialization, and high environmental persistence turns TBT into an environmental problem that is still far from being solved, at least in South American coastal areas.

Most of the studies aiming to verify the effectiveness of the TBT ban were focused in areas under the influence of major commercial harbors (i.e. Castro et al., 2012b). However, it is relevant to study other coastal areas affected by potential anthropogenic sources, such as marinas, small fishing harbors, and shipyards. In addition to commercial ports, the Caribbean Sea is well known by its touristic activities and is, consequently, crowded with marinas mostly harboring leisure boats. In the Caribbean, imposex and BT contamination had already been reported in Virgin Islands (Strand et al., 2009) and Margarita Island (Venezuela) (Miloslavich et al., 2007;Peralta et al., 2014). However, the possible sources of BTs were not identified nor its relationship with different ship activities (i.e. commercial harbors, marinas). Thus, the present study comprehensively appraised imposex occurrence and BTs contamination along 700 km of the Venezuelan Caribbean coastal shore covering areas under the influence of five of the main ports of Venezuela, but also large marinas and sites located further away from expected sources.

2. Materials and methods

2.1. Study area and sampling

The present study was carried out along the Caribbean coast of Venezuela, covering areas under the influence of marinas and 5 of the main commercial ports. The study area was divided in 4 regions: West (Paraguana Port), Central-West (Cabello Port), Central (La Guaira Port) and East (Guanta and Sucre Ports) (Fig. 1). Up to 30 adult specimens of *Plicopurpura patula* were collected in the intertidal zone (either manually or by snorkeling) at 29 sites distributed along the study area. Strand et al. (2009) also reported *P. patula* as a species affected by imposex in the Virgin Islands (Caribbean Sea). Surface sediments (upper 2 cm) were collected using a stainless steel "Van-veen" dredge at 10 sites only (G3, G4, G9, C2, C3, C4, C5, C6, C7 and C10) since areas dominated by coarse sediments (>90% sand) were not sampled. Sediments were frozen (−20 °C) and freeze-dried before analysis.

2.2. Imposex determination

The organisms were previously narcotized with a 3.5% $MgCl_2$ solution (1:1 seawater/distilled water) for 2 h. Shells lengths (SL) were measured with a digital caliper (0.01 mm) from the apex to the top of the siphonal canal. Soft bodies were removed from the shell and sex determination was based on the presence or absence of sexual accessory glands (albumen, capsule and seminal receptacle). The penis length (PL) and the presence of vas deferens in females and males were also registered.

Imposex levels were assessed using the following parameters:% of imposex in females (I%), Female Penis Length (FPL = mean penis length of all females in the population, including the zero values), Relative Penis Length Index (RPLI=[mean penis length in females/ mean penis length in males] × 100) (Bryan et al., 1987). The vas deferens sequence index (VDSI), based on the development of male sexual characters (particularly the vas deferens) by females, was evaluated according to Gibbs and Bryan (1987). In brief, the VDSI stages were determined as following: 0 – normal female, I – beginning of penis or vas deferens formation, II – penis developed [length < 2 mm], III – penis developed [length > 2 mm], IV – completely developed vas deferens, V – vulva blocked by vas deferens growth and VI – Dark mass of aborted eggs in capsule gland. After imposex determinations, the gastropod tissues (visceral coils) were frozen (−20 °C) and freeze-dried before analysis. Visceral tissue was chosen due to its lipid content (higher BT levels) and to allow for comparison with other studies.

2.3. Sample preparation and Butyltin analysis

Butyltin (TBT, DBT and MBT) levels were analyzed according to Castro et al. (2012a). Briefly, 5 g of freeze-dried sediment or 1 g of freeze-dried visceral tissue were placed into 40 mL vials. These samples were spiked with 100 ng of tripropyltin (TPrT) as surrogate standard and left for equilibration (30 min.). Afterwards, 15 mL of tropolone solution (0.05% w/v) in methanol and 1 mL of concentrated HCl (37%) were added. The vials were ultra-sonicated for 15 min and centrifuged at 3000 rpm for 10 min. This procedure was repeated three-times. The supernatants were transferred to a 250 mL separatory funnel filled with 150 mL of a 10% NaCl solution and extracted with 3 × 20 mL of dichloromethane. The extracts were collected through anhydrous sodium sulphate, added to 5 mL of hexane and blown down to approximately 2 mL using a Syncore system. Extracts were then derivatized by adding 2 mL of n-pentyl magnesium bromide in diethyl ether solution (2 M). After derivatization, the pentylated butyltins were extracted with 3 × 5 mL of hexane and evaporated down to 0.5 mL with N_2 flow. A cleanup was then performed using a silica-gel column (3.5 g). Analytes were eluted with 15 mL of hexane/ toluene (1:1). Finally, the solution was concentrated down to 0.9 mL (N_2 flow) and 100 μL of tetrabutyltin solution (1000 ng Sn mL^{-1}) was added as internal standard. Extracts were analyzed by gas chromatography using a Perkin Elmer Clarus 500MS equipped with mass spectrometer detector, split/splitless injector and auto sampler. An Elite-5MS (5% diphenyl/95% dimethyl polysiloxane) capillary column (30 m × 0.25 mm I.D., 0.25 μm film thickness) was used.

The analytical curves were made using matrix addiction technique to avoid matrix effects. The quality assurance and quality control was based on regular analyses of blanks, spiked matrices and certified reference material (PACS-2/National Research Council of Canada, Ottawa, Canada). Results obtained for the PACS-2 (TBT – 798 ± 23 ng Sn g^{-1}; DBT – 1104 ± 12 ng Sn g^{-1} and MBT – 670 ± 20 ng Sn g^{-1}) were in good agreement with the certified (TBT – 890 ± 105 ng Sn g^{-1} and DBT – 1047 ± 64 ng Sn g^{-1}) and reported values (MBT – 600 ng Sn g^{-1}). The samples recoveries were between 88.5% and 109% and RSD (relative standard deviation) below 20% (IUPAC, 2002). All concentrations were reported as ng Sn g^{-1} (dry weight).