STABILITY OF OKADAIC ACID, 13-DESMETHYL SPXC AND GYMNODIMINE IN GALICIAN COAST WATERS

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Summary: Nowadays, long records of cyclic imines are not available in Galicia. Sediment profiles could be an useful tool to study the incidence of harmful events in the past if toxins did persist during long periods in this environment. Last year, passive samplers in Galician seawaters have often shown cyclic amines presence such as 13 desmethyl SPX C. To elucidate if toxins levels found are due to constant toxin production or high toxin stability a stability toxin work was carried out.

Introduction: OA is one of the most common toxins in Galician coast related to *Dinophysis* dinoflagellates (Arévalo et al. 1995; Blanco et al. 2005) and reaching high concentrations. During last years, 13-desmethyl SPX C has been found but its origin remains uncertain in Galician waters nowadays. A study of persistence in different environments would be desirable to elucidate if toxins origin is steady production or high stabilities, and would be helpful to assess the risk of exposure to these substances and monitoring strategies (MacKenzie et al. 2011). The main aim of this work is to further the knowlegde about persistence of these biotoxins in Galician Rías in seawater column, interstitial seawater and sediments. In addition, another infrequent cyclic imine in Galician waters was studied (GYM).

Materials and methods: Artificial seawater was prepared following the formula of Lyman and Fleming (1940). Na₂S, Na₂S₂O₄ and NH₄Cl were added to pretend interstitial water conditions (Grossi & Raphel 2000). Both treatments were sterilized with 0.22 µm filter before OA and 13desmethyl SPX C were added, mixed and distributed in vials that were kept in darkness (21°) about a month. All the used sediments were obtained with corer. Superficial (2-4 cm) and deep (18-20 cm) subsamples (0.5 g of sediment) from two different locations were incubated with a toxic solution (OA, 13-desmethyl SPX C and GYM) prepared in methanol while more samples were used as control without toxin spiked. Samples were kept in darkness at 15° C and three vials were taken every sample time. Methanol extracts (1:4 p/v) were sonicated and centrifugated (914 g, 15 min). To assure reduction conditions the handling process was carried out at nitrogen atmosphere and vials were wrapped in Para film. OA was purchased from WAKO and 13 desmethyl SPX C and GYM from IMB-NRC. To elucidate the fraction where 13-desmethyl SPX C is located, surficial sediment samples (0.5 g of sediment, 0-2 cm in depth) were collected from different Galician Rías. Some of them were analysed by LC/MS to know the total toxin concentration, the other samples were diluted with 25 mL filtered seawater (GF/F Whatman) and sonicated before has been passed through a 10 µm mesh. Retained material was extracted with methanol (1/4 p:v), homogenised and centrifugated (48000 g, 15 min). All the samples obtained were preserved at -20° C before 0.2 µm filtering and LC-MS analysis. Toxins standards employed were purchased from WAKO (OA,500 µg) and IMB-NRC (13-desmethyl SPX C and GYM).

Results and discussion

OA was the most stable of the toxins studied in all environments without significant concentration changes (Figure 1) so it could pose an indicator of harmful events in past. 13-desmethyl SPX C is highly degraded in seawater than expected by the found levels in natural samples (Figure 2). GYM showed a fast degradation rate in sediments, values at the end of the experiment were below to the detection limit (LOD) at Esteiro station (Figure 3). 13-desmethyl SPX C and gymnodimine declined



speedily according to sediments location and depth. The 13-desmethyl SPX C persistence in sediments of Galician coast seems to be related to resistance forms of dinoflagellates (Figure 4).

References

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