Session: Toxins

DETERMINATION OF PALYTOXINS IN SAMPLES FROM *OSTREOPSIS* OUTBREAKS IN LLAVANERES (NW MEDITERRANEAN COAST)

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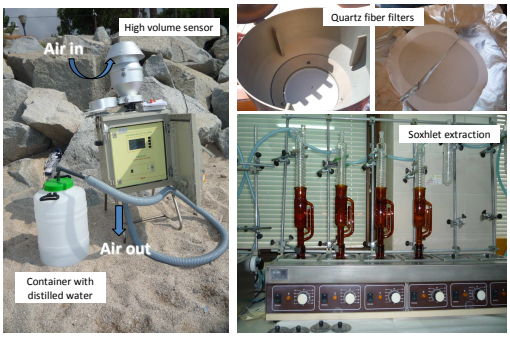
BACKGROUND

Dinoflagellates of the genus *Ostreopsis* have been related to harmful episodes in many Mediterranean coastal areas since 1998. In August 2004 one important event occurred in Llavaneres beach (Catalan coast, Spain) affecting 74 people with rhinitis and breathing problems. Since then, many Mediterranean heavy blooms of *Ostreopsis* have coincided with respiratory problems in people staying near the beach, suggesting a possible link between them.
To test this hypothesis, samples of seawater, macroalgae, benthic marine invertebrates and aerosols were collected from this location during 2009 and 2010 in the framework of the EBITOX project to determination of palytoxin (see poster Vila et al.; Pop Dyn. Session).

OBJECTIVE: TO LINK *OSTREOPSIS* BLOOMS WITH RESPIRATORY PROBLEMS

AEROSOL SAMPLING



A high volume air pump was installed on the beach during *Ostreopsis* outbreak. It was working continuously for 3 days. The air volume provided by the sensor was 30 m^3/h. Filters were replaced every 6 or 7 hours. A total air volume of 1326 m^3 was filtered and bubbled into a container with 6L of distilled water.

- Extraction from filters was performed with MeOH in a Soxhlet extractor with 10-12 hours cycles.
- Distilled water was evaporated to dryness and then dissolved in 30 mL of MeOH for toxin analyses.

RESULTS

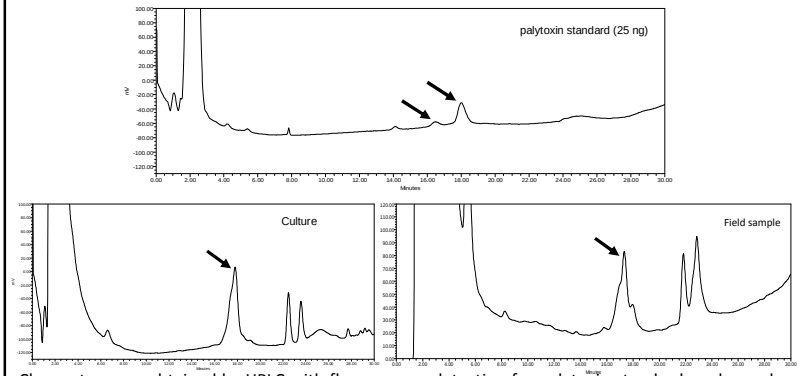
No Palytoxin was detected by LC-FLD in filters neither in distilled water.

Filters and distilled water were found no toxic by haemolytic assay.

CULTURES

Ostreopsis cells from the study area were isolated and cultured in laboratory (see poster Bravo et al.; Pop. Dyn. Session). Morphologic and genetic analysis revealed that all the cultured strains were *Ostreopsis ovata*. Toxins extraction was performed with MeOH.

Presence of palytoxin was confirmed by hemolytic assay (Riobó et al 2008), LC-FLD and LC-MS (Riobó et al. 2006). A palytoxin analog with a molecular weight of 2647 Da was found and the content of toxin in cells from cultures has been estimated to be 0.3 pg/cell.



Chromatograms obtained by HPLC with fluorescence detection for palytoxin standard and samples derivatized with ACCQ reagent following the method described by Riobó et al (2006) with slight modifications.

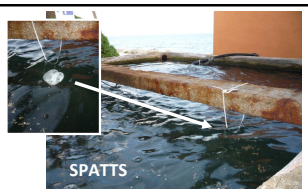
SEAWATER SAMPLING

Respiratory poisoning could be due to:

- Presence in the aerosol drops of *Ostreopsis* cells. Then two explanations are possible for breathing problems:
  - allergic
  - produced by palytoxin
- Presence in the aerosol drops of palytoxin. Then toxin must be dissolved in sea water.

In order to extract palytoxins from sea water during *Ostreopsis* blooms, different procedures have been tried:

1. SPE with Sep pak C18 cartridges
2. Partition with Butanol (Ciminiello et al.2008)
3. Adsorption on Diaion Column.
4. C18 EMPORE Disks (De la Iglesia et al., 2009)
5. SPATTS (Mackenzie et al., 2004)



RESULTS

•Palytoxin was no detected with procedures 1 to 4.

•Seawater extracts obtained following SPATTS procedure described by Mackenzie (2004) showed typical haemolytic activity due to palytoxin.

•Confirmation by analytical methods is required.

SEA URCHINS

Six pieces were collected in the study area. Extraction of the whole flesh was made with 100% MeOH.

Samples were analyzed by haemolytic assay and LC-FLD.

No palytoxin was detected.

REFERENCES:

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ACKNOWLEDGMENTS:

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