Suitability of a hemolytic assay and a cell-based ELISA to quantify palytoxins in marine microalgae

V. Brovedani[1], M. Pelin[1], S. Sosa[1], L. Tartaglione[2], Z. Ninčevi? Gladan[3], C. Dell'Aversano[2], A. Tubaro[1]

[1] Department of Life Sciences, University of Trieste, Via A. Valerio 6, 34127 Trieste, Italy
[2] Department of Pharmacy, University of Napoli 'Federico II', 80131 Napoli, Italy
[3] Institute of Oceanography and Fisheries, Šetalište Ivana Meštrovi?a 63, 21000 Split, Croatia

Palytoxin (PLTX) and its analogs are highly toxic marine polyethers detected in *Palythoa* corals, *Ostreopsis* dinoflagellates and *Thricodesmium* cyanobacteria. The main concern for human health associated to these toxins is their accumulation in marine edible organisms and the possible entrance in the human food chain. In tropical areas, human cases of even fatal foodborne poisonings, characterized by gastrointestinal, respiratory, skeletomuscular and cardiac problems, had been ascribed to consumption of PLTXs contaminated seafood. In the Mediterranean Sea human poisonings ascribed to PLTXs were often associated with inhalation of marine aerosol and/or cutaneous exposure to seawater during *Ostreopsis* blooms. Given the growing cases of adverse effects during *Ostreopsis* blooms in this area and PLTXs detection both in *Ostreopsis* and marine aerosols, the development of specific and rapid methods for PLTXs detection in microalgae suitable within monitoring programs is recommended. Thus, two methods for PLTXs quantitation were developed and characterized: a hemolytic assay, based on the PLTXs ability to convert the Na^+/K^+ ATPase into an unspecific cationic channel leading to erythrocytes lysis, and a cell-based ELISA, measuring the binding of PLTXs to Na^+/K^+ ATPase of skin HaCaT keratinocytes by a mouse monoclonal anti-PLTX antibody targeted by an enzyme-conjugated anti-mouse detection antibody. The limits of PLTX detection (LOD) and quantitation (LOQ) by hemolytic assay were $1.4x10^{-10}$ M and $3.4x10^{-10}$ M, respectively, while those by the cell-based ELISA were $1.2x10^{-11}$ M and $2.8x10^{-11}$ M, respectively. Studies are in progress to assess the suitability of these methods for PLTXs quantitation in microalgae (matrix effect, PLTX recovery from matrix-matched samples and PLTXs quantitation in field *Ostreopsis* cf. *ovata* samples) in comparison to an indirect sandwich ELISA and high resolution liquid chromatography/mass spectrometry.