Corso di Dottorato di Ricerca in Scienze della Vita e dell'Ambiente - Ciclo XXXVI

Emerging Biotoxins in marine organisms: chemical methods of analysis, bioaccumulation and eco-toxicological studies.

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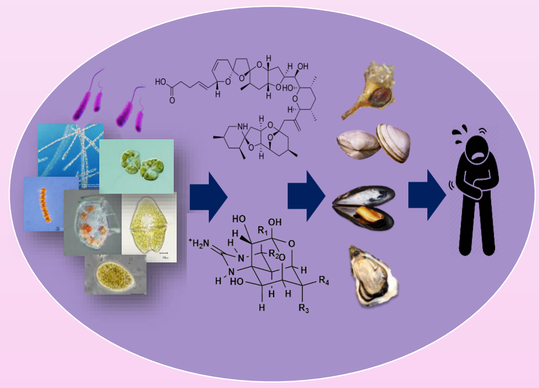
OVERVIEW

Emerging Marine Biotoxins (EMBs) are toxins of microalgal and/or bacterial origin including palytoxins (PLTX-analogues), cyclic imines (CIs), azaspiracids (AZAs) and tetrodotoxins (TTXs) for which toxicity data are limited. Algae and toxins are detected in seawater and seafood representing a threat for the environment and human health [1-3]. This PhD work aims to elucidate EMBs toxicological impact through a multidisciplinary approach including chemical, microbiological and ecotoxicological studies on EMBs trophic transfer in the aquatic ecosystems.

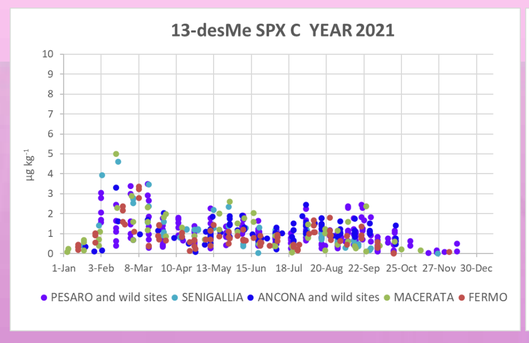


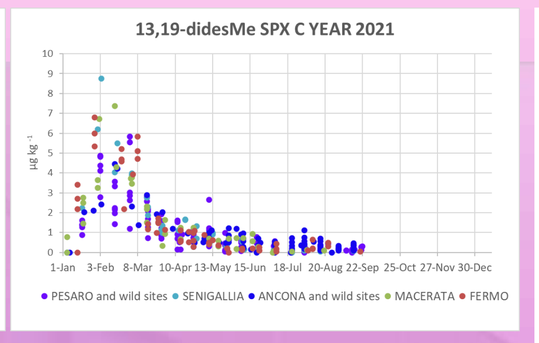
METHODOLOGIES

LC-MS/MS methods were implemented and validated for AZAs, CIs, PLTX-analogues and TTXs. PCR protocols were set up for: species-specific detection of *V. alginolyticus* and identification of PKS and NRPS genes (TTX biosynthesis genes). Mesocosm experiments, to study the PLTX-analogues trophic transfer, were performed feeding sea breams with *Ostreopsis cf. ovata* contaminated mussels.

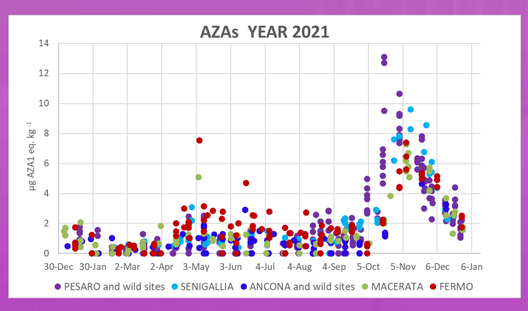
RESULTS

AZAs and CIs





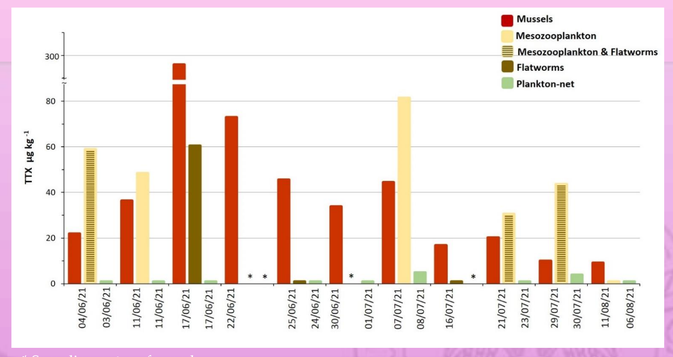
Spirolides (SPXs) were the most abundant CIs detected, with a maximum contamination of 12-14 µg Kg^{-1} in the first part of the year. Concerning the analogues: the 13-desMe SPX C was present all the year although at low levels. The 13,19-didesMe SPX C showed a maximum of contamination in the first part of the year but became undetectable at the end of 2021. Traces of GYM A reached higher concentration in August and December. Its presence was reported in Italian coastal waters, for the first time, in mussels during 2014-2015 [1].



AZAs were reported in mussels at levels of 10-13 µg AZA1 eq. Kg^{-1}, with the predominance of AZA2 (65-100%) and maximum levels recorded in October-December, in the Marche North coast.

AZAs and CIs still seem not to represent a risk for human health; in fact, levels are very far from legal limit (160 µg AZA1 eq. Kg^{-1}, Reg. EC 853/2004) for AZAs, and guidance level (400 µg sum of SPXs Kg^{-1}, EFSA opinion) for CIs.

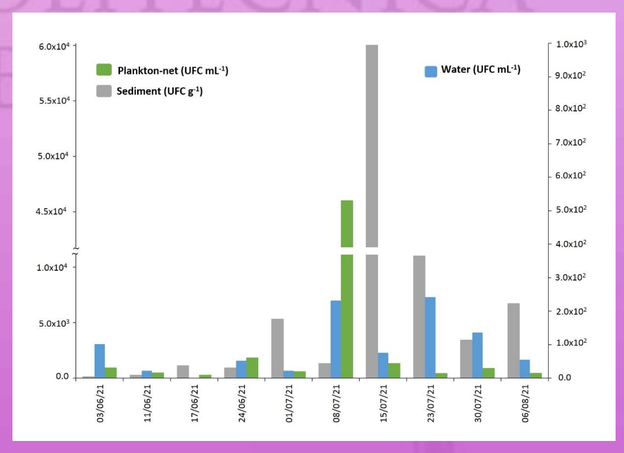
TTXs

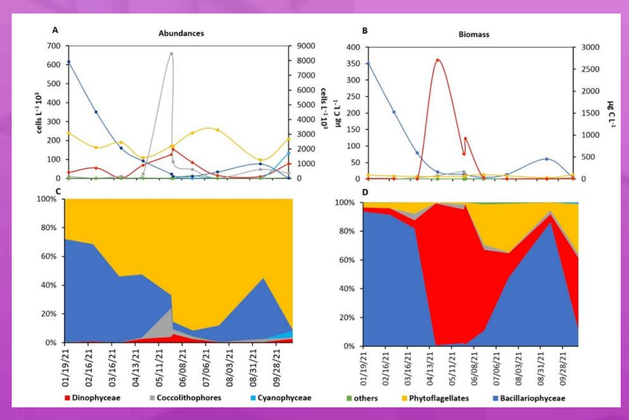


TTX was found in mussel samples from "Molo Portonovo" (MP) where it reached levels significantly higher in early June 2021. Contaminantion levels increased, probably as a result of the specific enviromental features of the study area. Other matrices such as mesozooplankton, flatworms and phytoplankton-net, were also contaminated by TTX [5].

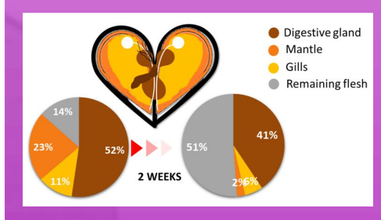
* Sampling not performed.

V. alginolyticus and NRPS and/or PKS genes were found in contaminated mussels. The *Vibrio* analyses of water, sediment and phytoplankton-net collected at MP site, showed similar trend with maxima recorded in July, in concomitance with TTX contaminated mussels [5].



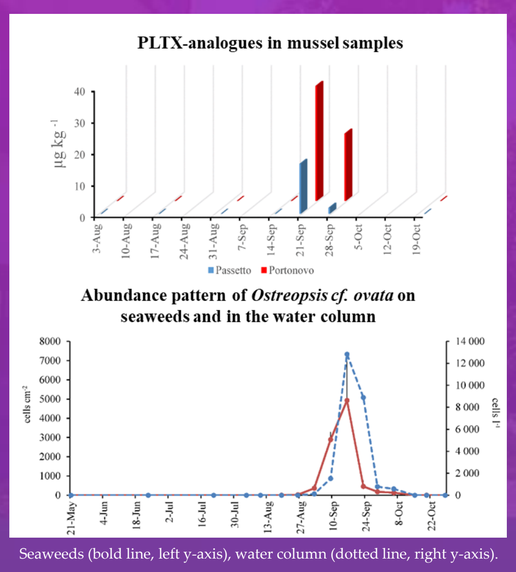


Significant higher abundance and biomass mean levels of dinoflagellates (the phytoplankton group most suspect as being involved in TTX contamination of mussels) were recorded during TTX mussel contamination, respect to the rest of the year [5].



The TTX distribution in mussel tissues showed a preferential accumulation in the digestive gland followed by mantle and gills [5].

PLTX-analogues

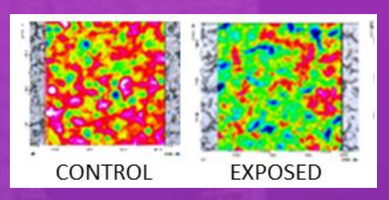


Seaweeds (bold line, left y-axis), water column (dotted line, right y-axis).

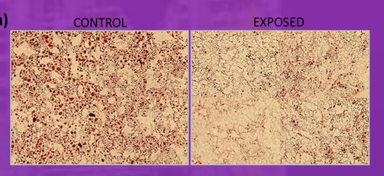
PLTX-analogues were measured at low contamination levels (16-36 µg kg^{-1}) in mussels collected in September 2021 in the Conero Riviera (EFSA guidance level is set at 250 µg kg^{-1}). Ovatoxin-a was the most abundant toxin (71–74%) in the contamination profile, followed by Ovatoxin–b (26–29%). Blooms of *Ostreopsis cf. ovata* were recorded in mid-September 2021 in the same sampling area. Mussel toxin profile fits well with profiles previously detected in field samples of *O. cf. ovata* collected in the same study site [4].

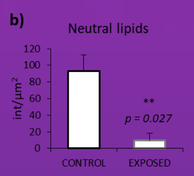
Trophic transfer: biological effects

DISTRIBUTION OF LIPIDS BY FTIRI



HISTOLOGICAL ANALYSIS





Sea breams fed with contaminated mussels did not show PLTX-analogues accumulation. Transcriptomic alterations of genes involved in lipid metabolism were observed in livers, suggesting further investigations. FTIRI and histological analysis showed a decrease of lipid content in exposed sea breams.

The diet contaminated by *O. cf. ovata* altered the hepatic metabolism of lipids, then the fish liver may be considerably affected by toxins even without an evident bioaccumulation.

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