

SURVEY OF CYANOBACTERIA AND RELATED TOXIN GENES ON PORTUGUESE COASTAL WATERS: DO FRESHWATER TOXINS THREATEN MARINE ENVIRONMENTS?

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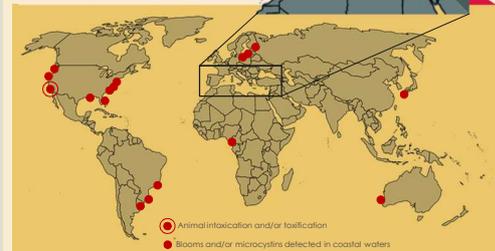
01 INTRODUCTION

The reports of **Freshwater Cyanobacterial Blooms (FCHABs)** exposure incidents of acute illness in humans have been reported for the La Plata estuary^[6]. Some countries, like Australia and USA, have defined microcystins guidelines for fish, prawns, and molluscs to apply in some states^[7]. However, in general, food standards regulations and legal guidelines for seafood safety do not yet include regulatory limits for freshwater cyanobacteria toxins^[8].

Furthermore, recreational exposure incidents of acute illness in humans have been reported for the La Plata estuary^[6]. Some countries, like Australia and USA, have defined microcystins guidelines for fish, prawns, and molluscs to apply in some states^[7]. However, in general, food standards regulations and legal guidelines for seafood safety do not yet include regulatory limits for freshwater cyanobacteria toxins^[8].



Fig. 1 : Worldwide occurrence of FCHABs and their toxins with emphasis in the MED area. Adapted from Preece et al.^[6]



In Italy discharges from Lake Occhito were reported to reach the sea, contaminating mussel farms with microcystins^[9]. In Portugal 42% of the water reservoirs are eutrophic^[10] and Cyanobacteria blooms have also been observed in river estuaries^[11,12] and recently in coastal waters^[13].



Fig. 2 : Cyanobacterial blooms in Portuguese Reservoirs

With a constant and persistent eutrophication of freshwater reservoirs, concerns exist whether toxic freshwater cyanobacteria are reaching marine shellfish production areas, posing a potential risk for consumers.

For this purpose, a screening was made crossing information from microscopical observations from monitoring water samples with cyanobacterial toxin gene presence.

References
 [1] Gopal et al. 2014 doi: 10.1016/j.mbs.2014.07.004. [2] Preece et al. 2015 doi: 10.1016/j.mbs.2015.07.013. [3] Gopal et al. 2014 doi: 10.1016/j.mbs.2014.07.004. [4] Preece et al. 2015 doi: 10.1016/j.mbs.2015.07.013. [5] Preece et al. 2015 doi: 10.1016/j.mbs.2015.07.013. [6] Preece et al. 2015 doi: 10.1016/j.mbs.2015.07.013. [7] Preece et al. 2015 doi: 10.1016/j.mbs.2015.07.013. [8] Preece et al. 2015 doi: 10.1016/j.mbs.2015.07.013. [9] Preece et al. 2015 doi: 10.1016/j.mbs.2015.07.013. [10] Preece et al. 2015 doi: 10.1016/j.mbs.2015.07.013. [11] Preece et al. 2015 doi: 10.1016/j.mbs.2015.07.013. [12] Preece et al. 2015 doi: 10.1016/j.mbs.2015.07.013. [13] Preece et al. 2015 doi: 10.1016/j.mbs.2015.07.013.

02 MATERIALS AND METHODS

NATIONAL SHELLFISH MONITORING PROGRAM

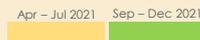
Weekly monitoring of parameters in water and shellfish:



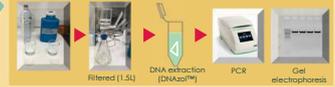
SAMPLING

- ▶ 13 coastal and estuarine-lagoon production areas
- ▶ 50 sampling stations
- ▶ Weekly alert levels
- ▶ National communication and outreach

In this work 9 sampling stations were analyzed in a total of 72 water samples across two periods of time



LIVE WATER SAMPLES :



PRESERVED WATER SAMPLES :

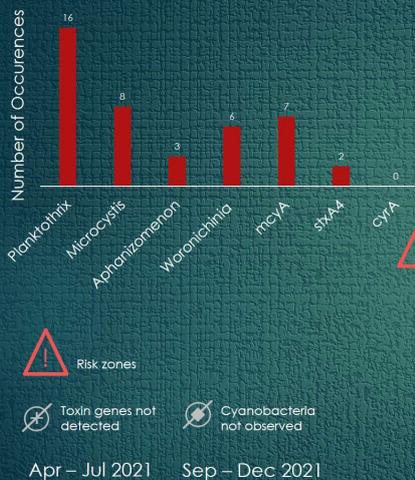


TOXIN GENES :

- ▶ Microcystins - *mcyA* [Sobregado et al. 2009]
- ▶ Saxitoxins - *stxA4* [Sobregado et al. 2013]
- ▶ Cylindrospermopsin - *cyrA* [Estroff et al. 2006]

CYANOBACTERIA AND GENE DETECTION

03 RESULTS AND DISCUSSION



Risk zones

Toxin genes not detected

Cyanobacteria not observed



04 CONCLUSION

The transfer of cyanobacteria and cyanotoxins from inland to marine waters in Portugal and other European countries is still neglected.

This work confirms the presence of potential toxic freshwater cyanobacteria in coastal and transitional waters and highlights the importance in increasing the knowledge and understanding on this subject.

Freshwater Cyanobacterial Blooms and Cyanotoxins in marine environment is an emergent issue that needs specific research.

- ▶ The most frequent cyanobacteria observed in the samples were the microcystin producers **Planktothrix** and **Microcystis**
- ▶ Likewise, the ***mcyA*** gene was the most frequent detected
- ▶ Saxitoxin related gene ***stxA4*** was detected in two locations, but no saxitoxin **phytoplankton** producer was observed
- ▶ Cylindrospermopsin related gene ***CyrA*** was not detected

- ▶ **Risk locations** of microcystins occurrence and shellfish contamination are Peniche at center north and Mira Estuary in the center South.

FUTURE RESEARCH...

- Species composition
 - ▶ Who is contributing to the presence of the *stx* gene?
- Bloom occurrence
 - ▶ Which season is more prone to favor cyanobacteria appearance?
- Toxin profile in water and wildlife
 - ▶ Are the shellfish and marine life accumulating freshwater toxins?

Acknowledgements

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