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Cyanobacterial blooms and toxins in water resources:
Occurrence, impacts and management.

Short Term Scientific Mission (STSM)

LCMS detection methods for cyanotoxins in tissue and environmental samples from brackish and freshwater water bodies

Researcher

Ekaterina Chernova

Dpt. of Eco-chemical studies, SRCES, Russian Academy of Sciences, St-Petersburg, Russia

Group of Dr.Zoya Zhakovskaya

Host Organization

Dr. Hanna Mazur-Marzec,

Dpt. Marine Biotechnology, Institute of Oceanography University of Gdansk, Poland

Objectives

Sample preparation, detection and identification of different kinds of cyanotoxins in biomass and tissue samples.

Methodology

Saxitoxins.

Sample preparation.

biomass samples : The procedure started with sonication with 1 ml of complex solvent mixture consisted of (A) 4mM ammonium formate buffer, pH 3.5 and (B) acetonitrile with buffer (95:5, v/v) in ratio 40:60 (A:B), followed by treatment in ultrasonic bath and centrifugation.

tissue samples: Tissue samples were freeze-dried prior extraction.

Two extraction methods were compared: i) boiling at 100C during 5 min in 0,1 M HCl and ii) extraction in a mixture of acetonitrile / water (80:20, v/v) with 0,1% formic acid. Both extraction were followed by LLE using hexane for removing lipids and SPE (Sep-Pack C18, Waters) for decreasing matrix effects. The passed through the cartridge fraction was analysed.

LCMS-analysis.

Separation was performed on TSKgel Amide-80 column 2.0x250 mm, particle size 5 µm (Tosoh Bioscience, Germany) in isocrate regime. Mass-spectrometry analysis started with screening in MRM-mode followed by collection of full MS/MS spectra for confirmation purposes. Data dependant regime was used for detection of other saxitoxin variants

Microcystin, anabaenopeptins, cyanopeptolins and anatoxin-a analysis

Sample preparation tissue samples

Three extraction procedures were tested i) boiling at 100 degrees during 5 min in 0.1 M HCl; ii) solvent mixture Methanol:Butanol:Water (20:5:75); iii) 5% acetic acid. All extraction were followed by LLE using hexane for removing lipids, dilution extracts for adjusting the content of organic phase to <20% and conducting of SPE (Sep-Pack C18, Waters) for decreasing matrix effects and concentrating. Eluted with 90% methanol.

LCMS-analysis

Separation was performed using reverse-phase column Zorbax Eclipse XDB-C18 column (4.6x150 mm; 5 µm, Agilent Technologies, Santa Clara, California, USA) in gradient regime. Mass-spectrometry analysis was run in MRM and data dependant regimes.

Results

We detected saxitoxins in 6 among12 biomass samples from reservoirs of Central part of Russia, which is in agreement with phytoplankton composition and previously obtained data of PCR analysis.

8 microcystins varints, anatoxin-a, cylindrospermopsin? and cyanopeptolins, anabaenopeptins were identified in biomass samples.

No cyanotoxins were detected in analyzed tissue samples from Curonian Lagoon, northwestern part of Russia.

Highlights

I consider this Short Term Scientific Mission (STSM) as an important step in my research, because it provided the methods and background necessary to carry out investigation in the field of detection and identification of cyanotoxins in different matrixes.

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