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Automated *In-Situ* Cyanotoxin Assessment Toolbox for Real-Time Surface Water Monitoring (CYANOBOX)

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Abstract. Automated *In-Situ* Cyanotoxin Assessment Toolbox for Real-Time Surface Water Monitoring, CYanoBox, is a 3-year project, coordinated by the innovation and technology company CyRIC, for the development of novel biosensors, and reliable at-source detection of toxic metabolites. The project aims to deliver an autonomous, affordable, and easy to operate water monitoring system as an early-warning tool for surface waters affected by cyano-HABs. CYanoBox comprises of an innovative water processing system that can remotely filter and lyse the cyanobacterial cells *in-situ* so that an accurate measurement is taken for both the extracellular and intracellular concentration of one of the most important groups of cyanotoxins, the hepatotoxic microcystins. The focus is on optimizing the method to lyse the cells in a way that maximizes the recovery of targeted cyanotoxins, followed by the development of biosensors for cyanotoxins identification and quantification. Being able to detect accurately and remotely the concentration of cyanotoxins in surface water without the physical presence of humans, it will be beneficial to the actual waterbodies, administrators, local communities, and researchers. This system will remotely evaluate the severity of a bloom based on its toxicity and track changes in water quality that traditional discrete monitoring activities usually miss.

Keywords: cyanobacteria, monitoring, sensors, surface water

1. Introduction

Cyanobacteria (blue-green algae) are fresh-water photosynthetic bacteria, and an essential organism for maintaining a balanced aquatic ecosystem. Excess load of nutrients into the waterbodies favor their rapid and extensive growth leading to the formation of cyanobacteria harmful algal blooms (cyano-HABs) which deplete surface water quality due to their potentially high toxicity. Their toxicity derives from their ability to produce bioactive metabolites, known as cyanotoxins. Cyanotoxins are categorized based on their structure into cyclic peptides, alkaloids, and lipopolysaccharides and their bioactivity in neurotoxins, hepatotoxins, cytotoxins, and endotoxins. In addition to their toxicity, cyanobacterial blooms are making the water have undesirable odor, color and taste, while at the same time they negatively impact the environment, cause financial loses to the tourist industry and burden financially the health sector.

Over the past years, different approaches, methodologies, and strategies were developed to monitor the surface water quality and record changes into a waterbody. Most of the monitoring strategies are based on recording common physicochemical water parameters such as pH, conductivity, salinity, total dissolved and suspended solids, dissolved oxygen, and nutrient levels, whose change indicates possible water contamination. Additional to these parameters, cyanotoxins levels and water characteristics related to cyano-HABs blooming (i.e., pigmentation levels), found to be a necessary addition to the existing monitoring tools. The most known and analyzed cyanotoxin group is the hepatotoxic microcystins which have been detected in waterbodies all over the globe. The structure of microcystins comprises of comprised by seven amino acids in a cyclic configuration with the amino acids in positions 1 and 4 varying, giving so far 249 different variants. Microcystin-LR (Figure 1) is known for its high toxicity to humans and animals, having a lethal dose- LD_{50} 10 times lower than the venom of cobra. Its bioactivity found to be associated with hepatotoxicity as MC-LR inhibits protein phosphatase type 1 and 2A through interactions and bonds formation, promoting tumors in the hepatic cells.

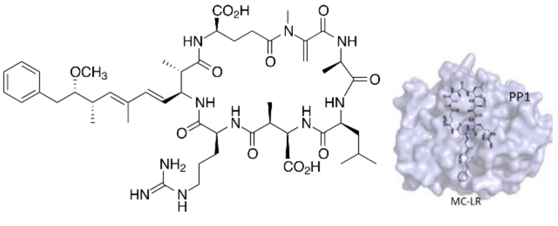


Figure 1. Left: Structure of Microcystin-LR; right: protein phosphatase inhibition

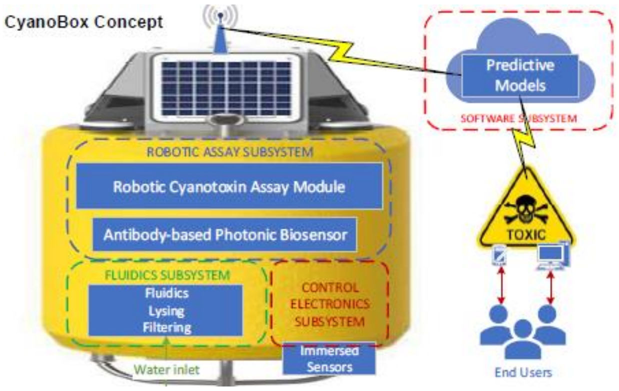
The first organization that officially provided guidelines for cyanotoxins in drinking water supplies was the World Health Organization (WHO) in 1984 and 1993, while in 2021 an updated version of those guidelines was published. Toxic cyanobacteria present in drinking water reservoirs pose a severe public health, recently prompting the EU to include safety levels for certain toxins in the revised Drinking Water Directive (EU) 2020/21842. More specifically, the revised directive suggests that by 2026 all Member States shall take the measures necessary to ensure that water intended for human consumption complies with the parametric values given by the Commission (1 μg/L for microcystin-LR).

The above legislations arose the need for the development of accurate and reliable monitoring tools and biosensors for the remote and *in-situ* analysis of cyanotoxins. Those tools will work as an early detection and warning systems to prevent blooming and toxicity into the waterbodies. CYanoBox, is a 3-year project, for the development of novel biosensors, dedicated to the reliable at-source detection of toxic metabolites. The project aims to deliver an autonomous, affordable, and easy to operate water monitoring system as an early-warning tool for surface waters affected by cyano-HABs. The present paper outlines the steps taken to design, optimize, and build the system from the beginning of the project until the present stage.

2. Device development plan

2.1. Concept definition

The first design of the device is illustrated on Figure 2, showing the four subsystems and the apparatus of the device. The system has an inlet for sampling water and transferring it to the fluidics subsystem where the sample is filtered to collect the scum and lysed mechanically for the intracellular cyanotoxins analysis. The supernatant is transferred to the robotic cyanotoxin assay module comprised of a novel anti-body biosensor and the results are uploaded to the software subsystem where they are processed and send to the end user as a signal. Immersed sensors monitoring the common water quality characteristics are part of the electronics system which is sending data to the software system also for building predictive models based on the water quality characteristics and cyano-HABs blooming relationships.



2.2. Collection of user requirements

For ensuring a high-quality device that corresponds to the needs of the market, we have conducted a three-month survey collecting user requirements. The survey was sent to stakeholders such as universities, research institutes, public authorities, and private companies related to water monitoring and in total 57 questionnaires were completed. Based on the results, the most analyzed cyanotoxins is microcystin group followed by anatoxin and nodularin groups. Liquid chromatography coupled with mass spectroscopy (LC-MS/MS) is the most used technique for analyzing cyanotoxins among the survey participants. They all mentioned that LC-MS/MS and other chromatographic methods require time and experienced personnel to run the analysis while there is no option of *in-situ* reliable detection which costs in terms of money, time, and response to the emergency.

2.3. Development of the fluidics, the robotic assay, and the software subsystem

Based on the user requirements, collection and the on-going directives and regulations in the USA and EU, we have decided to develop the system to sample, process, and analyze for MC-LR. An auto-sampler is taking samples from a certain depth below the surface and filter it through nylon membrane filters which collect the water scum. The scum is collected mechanically and lysed in an extraction solvent, transferred to the robotic assay subsystem for determine the MC-LR levels. If the concentration is above 1 μg/L a red signal will be sent to the end user, while in cases of below the limit, signal will be an orange or green light depending on the corresponding levels of MC-LR in combination with the other physicochemical characteristics.

2.4. From lab calibration and validation to field validation of the device

Biosensor-prototype lab calibration

- Calibration of the biosensors on the developed photonics module with reference microcystin-LR solutions at different known concentrations

Biosensor-prototype lab validation

Comparison between the following analyses:

- Lab analysis of reference microcystin-LR solution or equivalent using the CYANOBOX biosensor prototype.
- Lab analysis using commercially available cyanotoxin-specific ELISA immunoassays kits for microcystins.
- Advanced analytical techniques: liquid chromatography, mass spectroscopy

CYANOBOX-prototype field validation

The field validation of the device will be performed most probably in St. George Lake, at the National Park of Athalassas (Nicosia, Cyprus). The location was decided based on our 12-month monitoring study conducted in the lake, which showed that MC-LR producing species are present in the water and have a blooming cycle of 4-months during the summer-autumn period. Its surface water will be used earlier for pre-testing experiments and troubleshooting of the developing system.

3. Conclusions

Easy, reliable, and fast *in-situ* monitoring tools are of a high-need not only as tools to constantly test the water quality but also as an early warning tools that will be used to predict cyano-HABs and act fast to prevent blooming events. Being able to detect accurately and remotely the concentration of cyanotoxins in the water without the physical presence of humans, it will be beneficial to water bodies, administrators, local communities, and researchers. This system will remotely evaluate the severity of a bloom based on its toxicity and track changes in water that traditional discrete monitoring activities usually miss.

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