# Sensitive biosensing of potentially toxic cyanobacteria for risk application in freshwater environments

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# Sensitive biosensing of potentially toxic cyanobacteria for risk application in freshwater environments


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## INTRODUCTION

Harmful cyanobacterial blooms are a major threat to freshwater ecosystems globally weighing down the Good Environmental Status of waters and posing risk for human health. The system established by current French regulation to detect and monitor these blooms is based on microscopic observations with a 24 to 48 hours delay, while requiring trained taxonomists. To improve and reduce analysis times, biosensors are a highly promising technology that can produce near real-time measurements that could be used by early warning systems safeguarding human and freshwater ecosystem health. To this end, we develop **a rapid, sensitive and economical test** to identify and daily monitor the blooms of potentially toxic cyanobacteria in aquatic environments based on their genetic identity. Nevertheless, to validate this innovative solution, we still have to compare our data with microscopic traditional cell counting and biovolume estimation present in the samples.

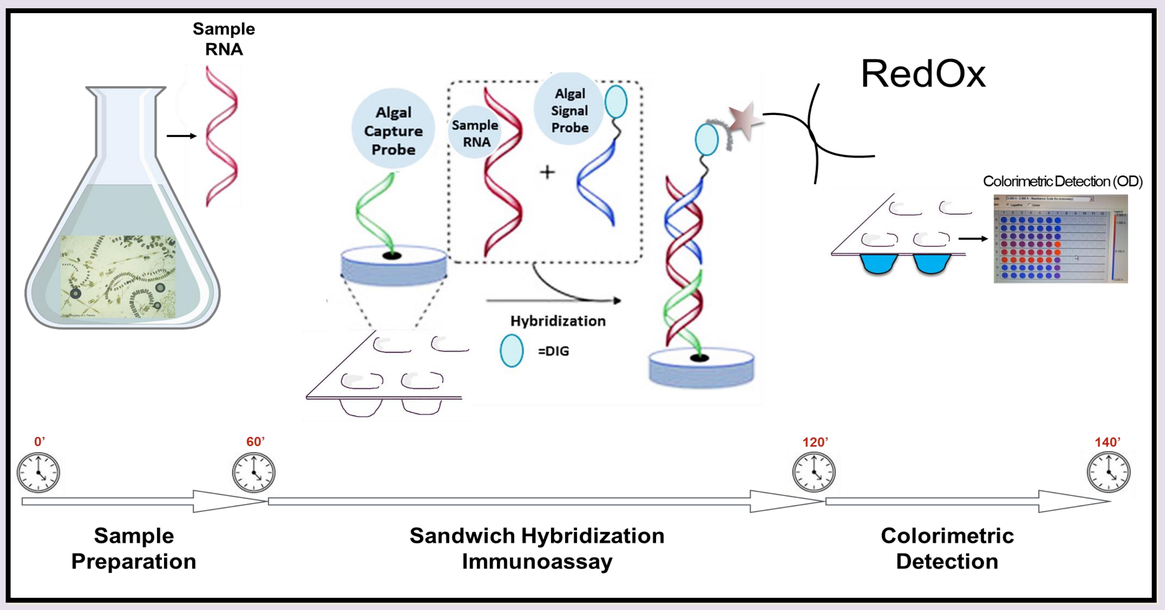
## MATERIAL & METHODS

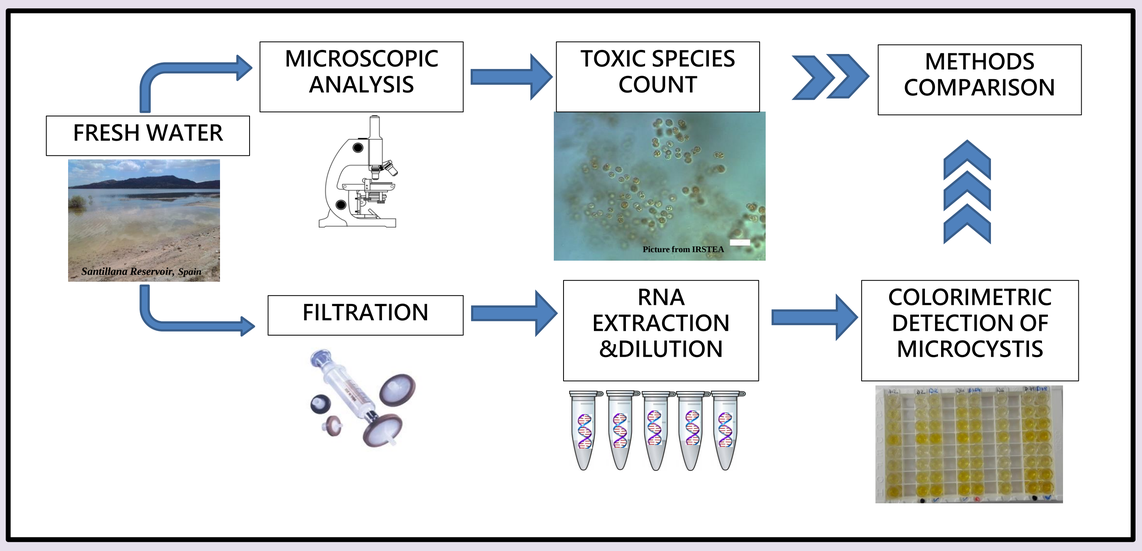
### BIOSENSOR PRINCIPLE

The biosensor is a genus-specific ribosomal RNA/DNA probe-based assay for potentially toxic genera and is calibrated to equivalent cell biovolume.

The biosensor device for detecting toxic cyanobacteria is based on a sandwich hybridization assay, which utilizes a capture probe to capture target RNA from a complex environmental sample. The captured RNA is then detected by a signal probe that is coupled to a colorimetric detection.

Basically, the signal intensity obtained from the assay is proportional to the amount of RNA applied to the biosensor, which is a proxy of the biovolume of a target cyanobacteria genus.




### SAMPLING CAMPAIGN

The sampling campaigns took place in August 2018 at the Santillana reservoir in Spain. Twice a week, 1,2 L were filtered through 1 µm polycarbonate filters, which were kept in 2 mL *RNA lysis buffer* (Zymo Research) and immediately send to Microbia Environnement for cyanobacteria detection. All the samples were extracted and then diluted in order to have an equivalent volume of 100, 50, 25, 10 and 5mL. This step is very important to find the linear phase and then eliminate outliers or points in the stationary phase. This prevents the under-estimation of the sample biovolume and elucidates the biosensor saturation.

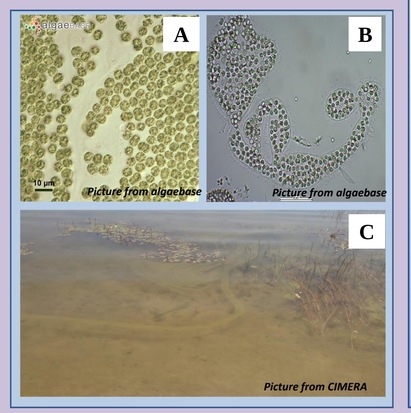
During this campaign, **14 samples** were fixed with Lugol for taxonomic analysis of cyanobacteria species by CIMERA and they were all compared to colorimetric detection.

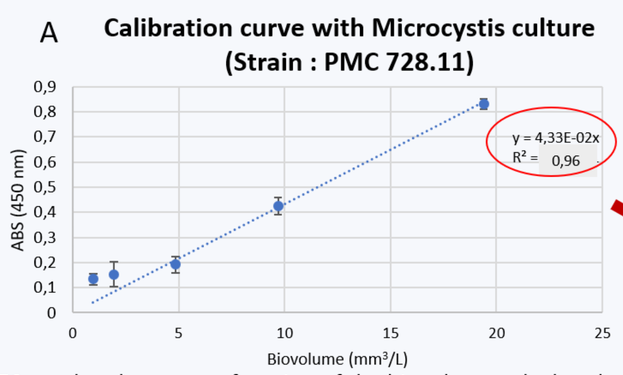
## RESULTS

Here the field validation results, performed in Santillana reservoir concerning the detection of *Microcystis* genera during the summer 2018 campaign, are presented. The samples were tested for the presence of *Microcystis* genera while the different cyanobacteria species were counted by microscopic analysis (CIMERA). Results were qualitatively and quantitatively compared with microscope counting.

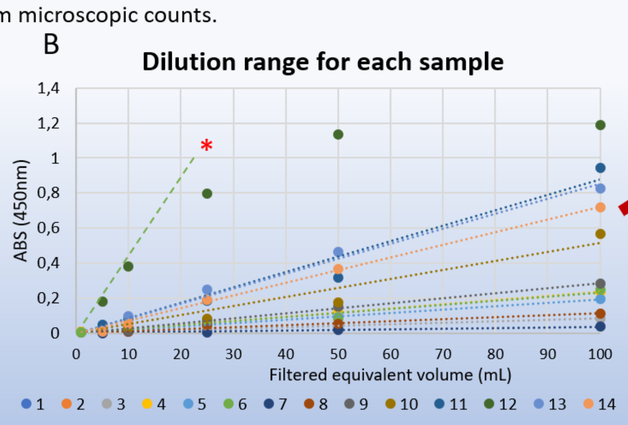
Data show that results are qualitatively comparable where the increase of biovolumes during the sampling period are correlated to the increase of biosensor absorbance signal.

*Pictures of Microcystis aeruginosa (A), M.wesenbergii (B) and bloom of Microcystis in Santillana reservoir (C)*

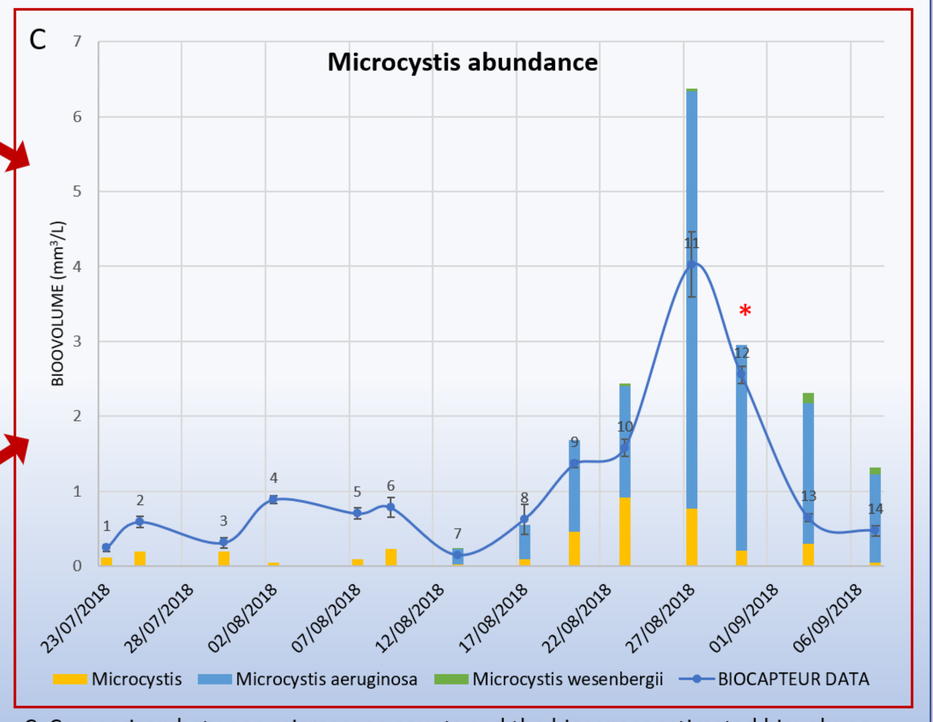



A. 450nm absorbance as a function of the biovolume calculated from microscopic counts.




B. Absorbance at 450nm as a function of dilutions of filtered water. Points on the linear slope are used to calculate the biovolume of the corresponding target genus.




C. Comparison between microscope counts and the biosensor estimated biovolume of Microcystis with the colorimetric detection assay in environmental samples from Santillana reservoir. These data were calculated using a proxy of Microcystis biovolume (A) and the linear phase of the dilution range (B)

## CONCLUSIONS

The preliminary data showed a good agreement between biovolumes estimated via microscopy and colorimetric biosensor results. Fourteen samples have been tested in this study, more data are needed to statistically validate the biosensor. Currently, two other sampling campaigns are in progress (nautical base of Champs-sur-Marne ; lakes in Aquitaine Region). These data will help us to tune sensitivity, reproducibility and robustness of the biosensor.

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