# ALARMTOX: assays and biosensors for the detection of biotoxins from aquatic media

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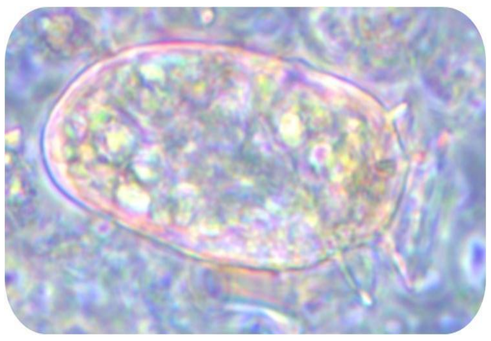
## INTRODUCTION

Several microalgae species produce toxins with detrimental consequences: food safety is compromised by contamination of shellfish, drinkable water and nutritional algae; direct exposure to biotoxins may cause diseases in humans and animals; closure of shellfish production areas and contamination of aquatic recreational areas have negative economical implications.



## THE PROJECT

The ALARMTOX project is focused on the development and validation of assays and biosensors for the detection of aquatic biotoxins, with the aim of establishing new technologies to guarantee the quality of continental waters and aquaculture products. These technologies should be more specific, more sensitive, faster and less expensive than the currently used biological and analytical methods.

## STEP 1: Production of protein phosphatases

Protein phosphatases (PPs) have been genetically engineered to improve their sensitivity to microcystins (MCs), okadaic acid (OA) and derivatives. They should be more stable than the commercial enzymes. Lyophilisation will allow their storage at room temperature. The produced PPs have been purified and will be used in the subsequent steps of the project.

## STEP 2: Development of colorimetric assays and electrochemical biosensors

The extent of PP activity inhibition by MCs and OA is proportional to the amount of toxin present in the sample. This is the basis for both the colorimetric assay and the electrochemical biosensor. At present, the colorimetric approach provides lower limits of detection than the electrochemical one with the enzyme in solution or immobilised into polymers. It is expected to improve the sensitivity by immobilising the enzyme through magnetic particles.

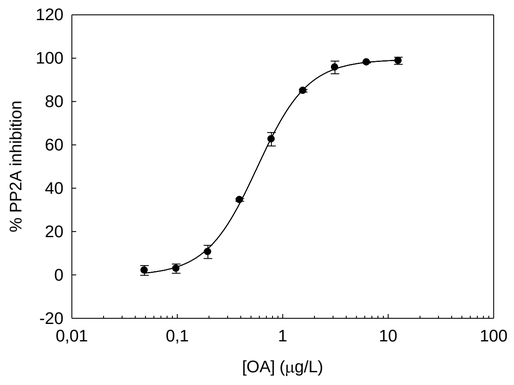
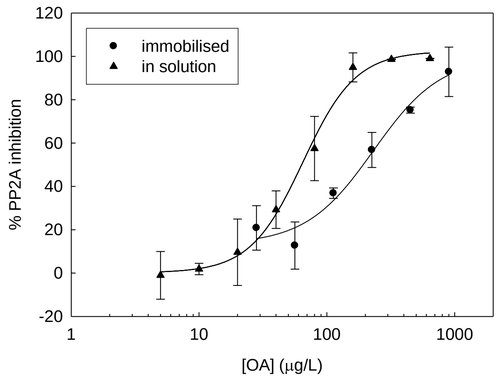



*Figure 1 (left): OA calibration curve obtained with the colorimetric assay. p-Nitrophenyl phosphate is hydrolysed by PP and its coloured product is detected at 405 nm.*

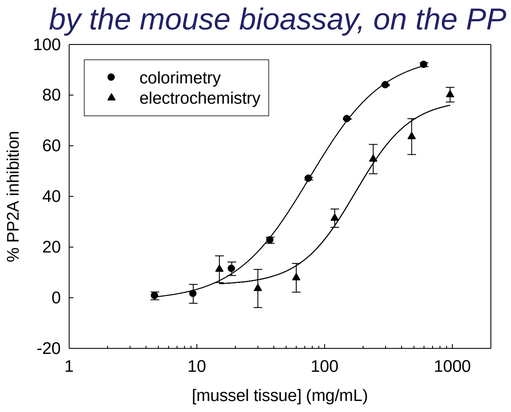
*Figure 2 (right): OA calibration curve obtained with the electrochemical assay (PP in solution) and biosensor (PP immobilised by entrapment). α-Naphthyl phosphate is hydrolysed by PP and its electroactive product is detected by Differential Pulse Voltammetry.*




## STEP 3: Validation of assays and biosensors

Several public institutions and private enterprises will provide water, microalgae and shellfish samples from a great variety of ecosystems, which will be tested with the developed assays and biosensors. Their performance and validity will be checked by comparison with LC-MS/MS analysis. Matrix effects will be also evaluated.

*Figure 3: Effect of a mussel, negative by the mouse bioassay, on the PP activity.*




ALARMTOX is a project co-financed by the European Union through the Interreg IV B SUDOE (South-West Europe) Territorial Cooperation Programme. It is integrated into the first priority of the Programme: “Promotion of innovation and constitution of stable cooperation networks in technological matters”. The presented opinions only compromise the partners and, consequently, in no way represent the opinion of the Territorial Cooperation Programme’s management bodies.