AN IN-SITU MARINE POLLUTION MONITORING DEVICE, BASED ON NEW BIOSENSORS, DESIGNED FOR LONG-TERM UNSUPERVISED AUTONOMY: SYSTEM REQUIREMENTS AND DESIGN APPROACH

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ABSTRACT

In the context of an ongoing European Commission project (MariaBox – FP7 Grant Agreement 614088), a novel, fully autonomous device is being developed, based on the use of biosensors for the detection of selected pollutants and algal toxins in seawater. The device will be capable of long-term unattended operation and wireless data transmission under various conditions. It is being designed as a device suitable for installation on buoys or similar platforms, on ships or to be used as a portable instrument. The first step towards this development has been to collect and analyse the system requirements, in order to be able to present a first design approach

Keywords: biosensors, water pollution, autonomous monitoring device, algal toxins

1. Introduction

Due to growing concerns about the health of the oceans and their capacity to continue to provide resources, as well as associated risks to the human health, there is an increasing demand for real-time monitoring of the environmental status of marine water quality and the provision of early warning systems. Real-time in situ monitoring of marine contaminants (including man-made chemical pollutants and algal toxins) is of utmost importance for the sustainable management and exploitation of the sea.

Monitoring the seawater quality is an essential part of the European marine strategy and agenda, with several projects currently ongoing on the development of novel autonomous sensing devices for marine water quality [1]. Following this strategy, the European Union has established several directives for the protection of the marine environment:

- Directive 2008/56/EC – Marine Strategy Framework Directive
- Article 16 of the Water Framework Directive (2000/60/EC) [2] sets out the "Strategies against pollution of water" (outlining the steps to be taken),
- Decision 2455/2001/EC [3] establishes the first list of priority substances
- The Commission Directive 2009/90/EC [4] lays down the technical specifications for chemical analysis and monitoring of water status.

All EC directives, as well as national regulations and research programs have the common necessity of monitoring several pollutants and toxins in the seawater. This is though currently not possible to be done in an autonomous way for a long period of time. Thus, analysis are usually performed either taking samples in the lab or through the use of expensive and voluminous devices that have to be carried on the field [5, 6].

The MariaBox project is developing a wireless, portable marine environment analysis device, based on novel biosensors of high-sensitivity, capable of repeating measurements over a long time for monitoring chemical and biological pollutants. It is being designed as an instrument to support the European strategy for the sea and ocean protection and exploitation. There is clearly an advantage also for the aquaculture industry to be able to monitor toxins in real-time.

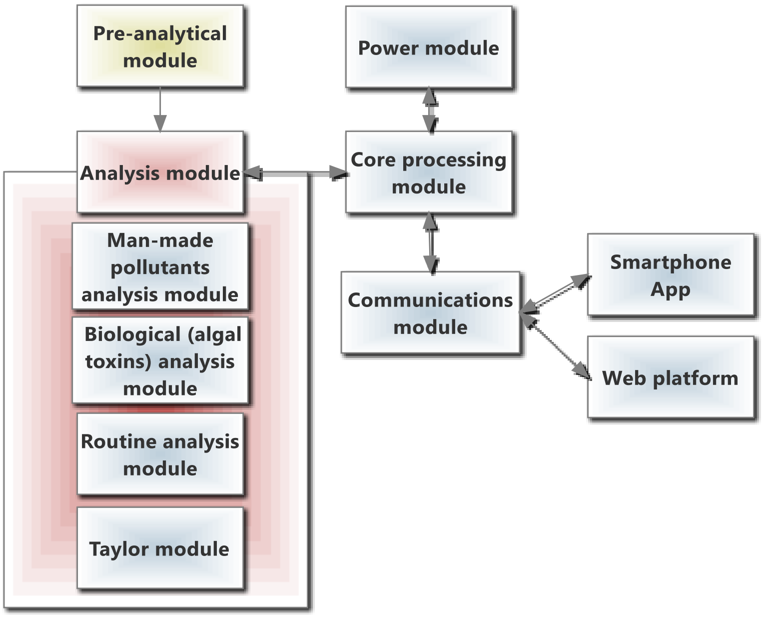

Figure 1: MariaBox modular approach

The approach includes: a) a sensing and analysis device, b) a modular communication system, c) a flexible power module, d) a software platform with open data services, and e) a cell phone application. The main device includes the pre-analytical module, which is responsible for acquiring and preparing the water sample for the analysis, and the analysis module. The later contains four sub-modules, namely the man-made pollutants analysis module (Chemical-POD), the algal toxins analysis module (Biological-POD), the routine environmental parameters analysis module (Routine-POD) and a custom module (Taylor-POD), which offers additional inputs for system customization and expansion.

The device will be able to transmit the collected data through different channels according to local needs and geographical location. Power to the system comes from an external module, which may include various renewable sources, depending on the location. A web platform and a smartphone application complete the system and deliver the acquired data to the interested stakeholders. The heart of the system are the novel biosensors that are being developed for 5 man-made chemicals and for 4 categories of micro-algal toxins.

2. System requirements

As a first step, the project underwent a phase of user and pilot-site specific requirements collection. Four pilots are foreseen in locations of different characteristics: (a) Alfacs Bay in Spain and (b) Vassilikos Port in Cyprus, both using a new buoy, (c) Galway Bay in Ireland using an existing buoy and (d) the Skagerrak in Norway, using a ferryboat. These requirements included both the necessities of the end-users in terms of substances that will have to be monitored, and the necessities in terms of measurements' acquisition frequency, data communication channels availability and measurement required resolution. EU directives were also examined in detail, in order to specify the biosensors within the needed constraints of sensitivity and selectivity.

The second project step and second iteration of collaborative review of end-user requirements was focused on the mechanical design, the power consumption, space and geometrical limitations, electronics and communication systems, and the water sampling system. This step had to take into account the biosensors functional and operational constraints, such as their temperature working range, storage conditions (temperature, humidity) for long measurement campaigns and the choice of biosensor electronics.

The third iterative step was focused on the communications module, focusing also on developing a device that could easily be connected to an existing communication infrastructure, whenever this will be available. This step included also significant effort in the area of interoperability and data exchange standardization.

Identifying user requirements has been a very significant task, which allows the development of a system with high value and immediate applicability. Initially, the possible MariaBox end-user categories were identified and then, selected end-users were asked to provide their requirements for the system, through a questionnaire. The following have been identified as the main MariaBox end-user categories:

- Governmental agencies, responsible for marine water quality monitoring. This is the end-users major category, since these agencies are responsible for legislation in relation to water quality issues as well as for the implementation of related EU Directives. Currently those agencies rely mostly on samples analysed in the lab.
- Research institutes. Marine research institutes, as well as environmental research institutes. Those include mainly Universities and Public research institutes.
- Oil/gas companies. This is related to the fact that those industries present significant risk of environmental damage and have to follow very strict regulations.
- Additionally, the aquaculture industry and NGOs have been identified as possible users of the MariaBox measurements and open data.

Following user and EC regulatory requirements, eight different biosensors will be developed: (a) four for man-made chemicals, and (b) four for micro-algal toxins. Table 1 summarises the target analytes.

Table 1: List of target analytes

Chemical pollutants for which biosensors will be developed		**Algal toxins for which biosensors will be developed**	
Category	**Analyte**	**Algae**	**Analyte**
1. PAHs	Naphthalene	5. Dinoflagellate, *Alexandrium*	Saxitoxin and derivatives
2. Fluorinated surfactants	PFOS	6. Cyanobacteria: *Microcystis, Nodularia, Anabaena, Oscillatoria*, Nostoc	Microcystin and structurally related variants
3. Heavy metals	All heavy metals	7. *Azadinium spinosum*	Azaspiracid
4. Pesticides	Camphechlor	8. *Pseudo nitzschia* sp.	Domoic acid

3. Biosensors and complete system design approach

For the above analytes, novel biosensors are being developed. Once the target analytes were specified, the feasibility of all eight biosensors was examined and confirmed. Antibodies for these analytes (except for heavy metals) are currently being developed. In order to achieve the best results, the strategy of chemically modifying their structure with a bi-functional linker in order to obtain an amino reactive derivative was followed. A retro-synthetic analysis for each compound was performed. The presence of the amino reactive group in the structure of the derivative compound allows to covalently attaching the molecule to an immunological protein carrier (such as Bovine Serum Albumin and/or Glutamine Binding Protein). The obtained conjugates were used to produce high affinity polyclonal antibodies using a standard immunization procedure. Currently, the conjugates (analyte-carrier) for each analyte have been produced and injected in the host animals (two rabbits for each analyte) for the immunization procedure. At the end of this process, mono-specific antibodies will be purified and the specificity against the analytes will be evaluated in Surface Plasmon Resonance and ELISA experiments under different operating conditions.

A different, bioinformatics, approach is being followed for the development of heavy metal detection biosensors. From the amino acid sequence of protein domains that are able to bind heavy metals, several peptides have been designed. In the peptides design, additional requirements are considered, such as peptide stability and presence of amino acid residues useful for the heavy metal detection.

All produced biomolecules (antibodies and peptides) will be labelled with commercial dyes with spectral characteristics in the visible region of the light spectrum (Biotium CF488 nm). They will be characterized by advanced biophysical methods using circular dichroism, Fourier-transform infrared spectroscopy and steady-state and time-resolved fluorescence spectroscopy with regards to their stability and function under different operating conditions. Advanced nanostructured surfaces will be produced for the covalent immobilization of the conjugate carrier. An optical-based method will be used for the activation of surface groups of silicon wafers that will react towards the residues present in the biomolecules. The biosensor electronics will be based on LEDs as excitation source with spectral characteristic matching the optical properties of the fluorescent dye (488 nm excitation wavelength).

Regarding the MariaBox autonomous device design, from a mechanical point of view, one of the most critical challenges is corrosion, due to the marine environment. Thus, the system will be encapsulated in a custom-designed enclosure that ensures complete isolation of the electronics and other components susceptible to corrosion. This isolation is also necessary to guarantee stable operational conditions during the analysis process, especially in terms of temperature and darkness. Additionally, the unused biosensors need to be stored in controlled temperature conditions (4 ^{o}C) to ensure that they will remain active even after six-months. Another important challenge is the sampling and pre-analytical mechanism design. Salt, algae or other objects could obstruct system operation or could block the filters. For this reason, the device will include cleaning anti-fouling mechanisms, as well as a wastewater tank to avoid any contaminants leak to the environment.

The device electronics are also being designed in a modular way to allow taking advantage of the possibly existing resources in the field demonstration sites (for example, existing power supply and/or communication links). In any case, the independent MariaBox communication module integrates three different wireless technologies that can be enabled whenever required: Wi-Fi for short-range communication, 3G for medium/ long-range communication, and a satellite modem when there is no 3G coverage. The data collected are sent to a central server and application (MariaBox-NET) or to an authorized mobile device (MariaBox-MOB).

4. Data sharing

MariaBox-NET will make available all the information collected by the deployed MariaBox devices. The platform will be compatible with standards like INSPIRE directive, Copernicus and GOOS initiatives and SeaDataNet as well as technical standards such as the OGC Sensor Observation Service (SOS). Data availability will also facilitate the implementation of the Marine Strategy Framework Directive (MSFD).

The INSPIRE data standards implementation will enable copies of the datasets generated by MariaBox to be downloaded and accessed directly, according to the INSPIRE Directive. The metadata generated, which will adhere to INSPIRE standards, will be made available to users in conjunction with the datasets produced. Sensor Observation Service (SOS) will be used for implementation, as recommended by the INSPIRE Infrastructure for Spatial Information in Europe Guidelines.

The MyOcean online catalogue of marine data is managed by the MyOcean in situ Thematic Assembling Centre (TAC). TAC has been developed jointly with EuroGOOS. TAC collects and carries out quality control on data from outside MyOcean data providers, and is in charge of accumulating both near real-time quality controlled observations and historical data [7]. To add

MariaBox sensor data to TAC, the data needs to have sufficient meta-data to generate the standard NetCDF files. There is no requirement to transform the MariaBox data into NetCDF format as this activity is performed by the MyOcean TAC.
It is also anticipated that MariaBox data will be added to SeaDataNet via the Marine Institute of Ireland (partner in MariaBox project). SeaDataNet data follows the CDI Schema, which is fully INSPIRE Compliant.

By sharing the data in an Open way, as described, MariaBox will also facilitate the implementation of the MSFD. The implementation of the MSFD requires the establishment of a monitoring programme for the ongoing assessment and the regular update of targets. EU member states are required to provide data reports, initially for assessment purposes of their environmental status and then, periodically, every 6 years, for the re-assessment of targets, indicators and achievements [8]. MariaBox can be used to provide continuous data to cover the data reporting obligations of the Member States, initially focusing on the selected target parameters that have also been indicated by the MSFD working groups (such as PCBs, PAHs, pesticides, heavy metals) but, later-on (after the project period), also by measuring other analytes that may be required by future updates of the MSFD.

5. Conclusions

The MariaBox project is developing a wireless, portable marine environment analysis device, based on novel biosensors of high-sensitivity, capable of repeating measurements over a long time for monitoring chemical and biological pollutants. Following user and EC regulatory requirements, eight different biosensors will be developed: (a) four for man-made chemicals, and (b) four for micro-algal toxins.

The approach to develop the biosensors and some of the most crucial mechanical challenges have been presented. Data sharing has also been discussed. The MariaBox platform will be compatible with the INSPIRE directive, Copernicus and GOOS initiatives and SeaDataNet as well as technical standards such as the OGC Sensor Observation Service (SOS).

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