LIFE Stop CyanoBloom **LIFE12 ENV/SI/000783**

Innovative technology for cyanobacterial bloom control

FINAL Report
Publishable abstract

Project Data

Project location	Slovenia
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Project end date:	31/12/2016
Total Project Duration	42 months
Total budget	1,300,963 €
Total eligible budget	1,298,363 €
EU contribution:	648,792 €
(%) of total costs	49.87 %
(%) of eligible costs	49.97%

Beneficiary Data

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- *Arhel, projektiranje in inženiring d.o.o*., Slovenia – Marko Gerl, Gorazd Lakovič, Maja Zupančič Justin, Tinkara Rozina, Mario Marinović, Luka Teslič, Jošt Grum, Neža Finžgar, Maša Čater, Andrej Meglič, Andrey Yakuntsov, Lovro Pokorn, Dorjan Dolanc, Maja Čič, Tomaž Varlec, and Branko Hamiti.
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Marko Gerl, Arhel d.o.o.
Stop CyanoBloom project manager
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2 Executive Summary

2.1 Project background

Despite the efforts invested into measures to prevent water eutrophication, like prevention and treatment of point and dispersed discharges, the eutrophication still occurs. One of the unwanted consequences of eutrophic water state is the occurrence of excessive cyanobacterial blooms. Mass occurrence of cyanobacteria is a significant health risk related to surface waters in EU and worldwide since most of the cyanobacterial genera produce cyanotoxins. Besides toxicity, cyanobacterial blooms cause an increase in the turbidity of water and create taste and odour problems. All mentioned represents substantial economic losses in sectors like aquaculture, tourism, drinking water facilities and indirect losses due to increased healthcare expenditures and environmental degradation. It is, therefore, necessary to find appropriate solutions for rapid detection and also in-lake prevention of bloom occurrence despite, for example, existing high eutrophic conditions in the water body. The proposed project represents such a solution.

In the frame of the LIFE Stop CyanoBloom project, we have designed two solar powered robotic vessels for the in-lake detection and control of cyanobacterial proliferation. Each vessel is capable of three-dimensional localisation of cyanobacteria by measuring fluorescence of pigments involved in the process of photosynthesis. The vessels are additionally equipped with electrochemical cells using boron doped diamond electrodes fixed on board, producing short-lived hydroxyl radicals, which prevent cyanobacterial proliferation as well as cyanotoxins inactivation. Hydroxyl radicals cause different levels of damages to cyanobacterial cells as well as stress, that may also result in phage induced cyanobacterial lysis. Natural control of cyanobacterial density by lytic cyanophages is known for several years. The mixing of water during the electrolytic cell operation also increases the potential contacts of cyanobacteria with cyanophages, disturbs cyanobacterial buoyancy and generates additional nutrients for nontoxic phytoplankton population. Additional newly developed equipment of the vessel allows advanced automated navigation (automatic docking, avoiding obstacles, maintaining the position in windy conditions, etc.), sampling from different depths, real-time data transfer, statistical analysis and graphical presentation using the adequate software.

2.2 LIFE Stop CyanoBloom project objectives

The primary goal of the project was to demonstrate the operation of two robotic vessels with accompanied boathouses and docking stations in two freshwater bodies with a different pattern of cyanobacteria occurrence. The aim was to enable early on-line detection and differentiation of cyanobacteria from the rest of the phytoplankton and to prevent proliferation of harmful cyanobacteria directly in the lake without the addition of chemicals. For this purpose, electrolytic cell was used, producing hydroxyl radicals. The goal of the application of this innovative technology was to demonstrate its potential in the increase of bathing and drinking water quality. The aim of the implementation of the project in pilot scale was also to demonstrate the efficiency of the new technology, which is applicable to all EU water bodies. The objective was also to introduce a simple and efficient method for determination of certain physical and chemical parameters and concentration of cyanobacteria in the entire water body using innovative devices constructed for the proposed project. The plan was also to introduce the project and innovative technology on international events and convince the potential end-users of the applicability of the new technology for their water bodies.

2.3 Expected project results with key deliverables and outputs achieved

The main project results can be summarised as followed:

- ❑ Established two boathouses with automatic docking stations and robotic vessels with accompanying equipment for monitoring of phytoplankton, various chemical and physical water quality parameters and cyanobacterial control.
- ❑ Two-year demonstration and optimisation of the navigation and information system for on-line measurements and remote control. Software development for data transfer analysis and their presentation.
- ❑ Proof of appropriateness of simultaneous use of submersible phycocyanin and chlorophyll fluorescence sensors in continuous flow monitoring chamber for on-site and online detection and quantification of cyanobacteria and phytoplankton (*sensu stricto*) occurring simultaneously.
- ❑ Demonstration of a selective efficiency of electrochemical oxidation on cyanobacteria, preventing their further proliferation and enabling controlled release and inactivation of cyanotoxins.

According to the expected results presented in the project proposal, the following outputs can be presented:

Expected Result 1: The absence of cyanobacterial blooms during the time of the implementation of technology on a water body. After triggering the lysis of cyanobacteria, the concentration will decrease to approximately 90% of the initial concentration (it will never be entirely removed). We expect a 50% lower concentration of toxic cyanobacteria in the period, which is usual for the development cyanobacterial blooms. We also expect the absence of microcystins in the water body during the implementation of the technology on water bodies.

Output achieved:
The effect of electrochemical oxidation using electrolytic cells equipped with boron-doped electrodes on cyanobacteria was proved by 16 different laboratory and pilot-scale experiments. The control system in the robotic vessel was integrated with the electrolytic cell, enabling its switch-on when the measuring system detects defined concentration of cyanobacteria (e.g. >10.000 cells/mL). The results allowed the calculation of the operation range of the vessel with evaluation of investment and operation and maintenance costs for the case of a fishpond. From the methodological point of view, a longer project duration would be needed to test the treatment with electrolytic cells on the vessel directly in the natural environment. The rapid implementation of the tests was limited by the seasonal occurrence of cyanobacteria and needed previous insight on the behaviour of cyanotoxins during the treatment. Monitoring of the released cyanotoxins concentration in the water after electrochemical oxidation has confirmed the decrease of extracellular microcystins after the

treatment. Later on, the concentration of the released toxins did not exceed the concentration of the controls.
With the performed experiments, we confirmed that electrochemical oxidation induces a large variety of damages to cyanobacterial cells, like direct lysis, induction of various types of apoptosis and senescence and induction of the lytic cycle in lysogen cells.

Expected result 2: Improved ecological status of chosen water bodies: increased biodiversity - more diverse species of phytoplankton; turbidity of the water body during the implementation of the new technology for cyanobacterial bloom control will be significantly reduced in comparison with the previous state.

Output achieved: In a short period of the project duration it is hard to prove the increase of biodiversity on the ecosystem level. Several seasons are sometimes needed for restoring the natural balance and increase the biodiversity. Besides, external factors also affect the capacity for the restoration of the aquatic ecosystem. One of such is the intended use of the water body. The biodiversity of shallow fish ponds and dynamics of changes in them are entirely different from, for example, deep natural lakes. Therefore, we have established a few controlled trials to study the impacts on biodiversity, which is caused by direct control of the growth of cyanobacteria in the lake by electrochemical oxidation. Using the electrolytic cells, we observed, whether there is a different effect on algae and cyanobacteria at a selected intensity and duration of the electrochemical oxidation. Two on-site experiments for testing the proposed method for cyanobacterial population reduction by electrochemical oxidation were conducted at Koseze Pond. In the third experiment, the effect and toxicity of the products from treating cyanobacteria *Microcystis aeruginosa* on algae *Pseudokirchneriella subcapitata* has been evaluated. The results have shown that the treatment in set conditions had no adverse impact on the algal population in the pond. Ecotoxicological tests have shown that the growth of algae is mostly affected by high concentration of cyanobacteria in water and released cyanotoxins. The growth rate of green algae *Pseudokirchneriella subcapitata* was higher in the case of exposure to the products of electrochemical oxidation than in the event of a presence of cyanobacteria or cyanotoxins. Based on the results, we can conclude, that we can provide a selective effect on cyanobacteria at selected operating conditions, not harming the green algae in the water and therefore positively affecting the biodiversity of the water body.

Expected result 3: Daily reporting of measured parameters and daily updating of the presentation of results on the website of the project from the time of installation of technology until the end of the project. Technology is expected to continue operating even after the end of the project.

Output achieved:
We have carried out a sufficient number of monitoring with the two robotic vessels for a horizontal and vertical profiling of two water bodies to analyse the spatial and temporal dynamics of phytoplankton distribution and cyanobacteria occurrence during the growing season. Real-time presentation of the monitored data was established, visible on the operator server as well as website. Both vessels are in operational state and ready to be also used in the future.

Expected Result 4: At the end of the project we expect a serious interest in the new technology to implement it abroad.

Output achieved: Aquacultures were evaluated as an important market for the technology. Facilities in Serbia were visited. An expression of interest for the application of the technology by aquaculture owner and a statement about the technology received from cyanobacteria expert is added to the ANNEX 25.

2.4 Summary of each chapter of the Final Report

The report starts with the Introduction and general description of the project background, repeated at the beginning of the summary. Further on, the administrative part of the project is presented with project management phases, their implementation and deliverables produced. New organogram template of the project consortium has been added to the presentation of all project members during the project. In the description of the Technical part of the report, each project Activity has been presented, and comments have been given in the case of a delay or change of the project plan. A short description of the undertaken activities of each Action is presented with reference to ANNEXES (30 technical Annexes altogether) of Deliverables and Technical Reports, where these activities are presented in details. The technical part of the project report ends with an extensive table summarising Evaluation of the Project Implementation and Analysis of long-term project benefits. Comments on the financial report are given with a summary of costs occurred.

3 Introduction

The unwanted mass occurrence of cyanobacterial blooms and potential release of their toxins is a result of eutrophication of water bodies. The prevention measures to stop the eutrophication processes are not always efficient. Numerous lakes and surface drinking water accumulations are therefore subject of seasonal cyanobacteria bloom occurrence. The unpleasant consequences are the closure of bathing water area, loss of tourist income, a ban on drinking water use, losses in commercial aquaculture or in the worst scenarios occurrence of poisoning and illnesses due to cyanobacterial toxins. The traditional monitoring approaches of water quality represent a substantial cost and the results on phytoplankton composition, an important indicator of water quality, are obtained with a time lag from only limited locations in the lake. It is, therefore, necessary to find a solution for the prevention of harmful algal bloom occurrence despite of, for example, existing high eutrophic conditions in the water body.

The goal of the project was therefore to introduce an innovative solution for simultaneous detection of cyanobacteria and in-lake bloom control in natural environmental conditions. For the demonstration purpose of the project, two fully equipped robotic vessels have been constructed and put into operation in Koseze Pond in Ljubljana and Lake Bled. The selected water bodies differ in their size and occurrence of cyanobacteria (population structure, time and water column level of occurrence). The objectives of the project were to upgrade the already proven concept of cyanobacteria detection using phycocyanin and chlorophyll sensors and provide controlled lysis of cyanobacteria using advanced oxidation methods of water treatment in an electrolytic cell equipped with BDD electrodes.

Water resources are increasingly under pressure and innovating in the water sector is one of the important objectives of European Union. The expected outcome of the displayed technology in the project was to contribute to the improvement of the state of water resources, an important goal of Water Framework Directive and consequently contribute to substantial economic benefits on other EU sectors dependent on clean freshwater resources. Phytoplankton monitoring is becoming one of the key elements in water quality assessment in the Water Framework Directive. With the implementation of the Bathing water directive, cyanobacterial monitoring becomes compulsory in the EU. The presented *in-situ* automated detection is at present not a part of the official monitoring methodology but coupled with existing taxonomic approach it can clearly improve phytoplankton monitoring goals. Methods combining simultaneous monitoring and in-lake harmful cyanobacteria bloom control, without the addition of chemicals or sediment forming materials, are missing. The technology presented within the project offers such a solution.

The solution has a potential on the market such as freshwater monitoring, water management for tourism, fishing and aquaculture, drinking water abstraction and protection of endangered freshwater habitats. During the project deployment, inland aquaculture was identified as an important market sector looking for the solutions demonstrated in the project. There may be substantial losses in this sector due to the harmful cyanobacterial blooms. In the report, we pointed out that a legislation controlling the levels of cyanotoxins in consumer products from inland aquaculture as it is established for seafood is missing.

The developed technology has a transferability potential in other sectors demanding high-tech measuring equipment and advanced water treatment techniques.

4 Technical part

4.1 Technical progress per task

4.1.1 A1 Design of the working platform and collection of existing data

Description of undertaken activities	
The A1 action was composed of two phases: A1 Phase 1 Design of the working platform A1 Phase 2 Collection of existing monitoring data on both selected water bodies The action has been finished on time with the elaboration of the project documentation for the wharfs and the vessels and with the overview of existing data on selected water bodies (presented in Inception report). As explained in the Inception report, the location of the fishpond in Hotinja Vas has been changed with the location Koseze Pond in Ljubljana. The new location is closer to Arhel`s and NIB seat and therefore enabled easier daily testing of the equipment. It is also an important recreation point for Ljubljana`s population and therefore much more visited as a pond in Hotinja Vas, what was important from the promotional point of view. The cyanobacteria have also been detected in Koseze Pond in the previous year, and the pond was, therefore, a suitable selection. At Koseze Pond we, however, needed to follow the existing landscape architecture/design. Therefore, the designs of the boathouse have been worked out by the city architects. With the start of the activities at Lake Bled, we needed to change the location of planned wharf of the vessel. From the former location in "Grajska" boathouse, the wharf has moved to the boathouse of the Hotel Vila Bled. For this reason, some adaptations to the original designs have been made. For the performance of the project activities, we obtained all the needed permits (**ANNEX 5**) The final versions of the designs of the boathouses with docking stations (Koseze Pond and Lake Bled) and for the both working platforms – the robotic vessels are presented in **ANNEX 7**. A review of the existing monitoring data carried out in the past for the Koseze Pond and Lake Bled are presented in **ANNEX 6**.	
Expected outputs in quantifiable terms	Delivered outputs
• Elaborated outline plan • Elaborated detailed project • Collection of existing analysis on both water bodies with abstract	Completed
Implementer of activities	
Arhel and NIB have worked together at gathering the existing data on selected water bodies. Municipality Bled assisted in gathering the allowances and existing water quality data of Lake Bled. Arhel has prepared the outline designs for both wharfs. A detail architectural design for the Koseze wharf has been developed by architects: Miha Kajzelj and Rok Žnidaršič. The designs (bodies of the vessels, mechanical parts, and electronics) of both vessels have been done by Arhel. In certain parts of programming of the software for data	

transfer through a mobile network, communication among computer modules and measuring processor board, an external expert has been hired.	
Indicators of progress (as set in the project)	
Meetings with experts from different working fields	Accomplished
Defined location for installation of both wharf	Accomplished
Elaborated outline plan	Accomplished
Elaborated detailed project	Accomplished
Problems / drawbacks / delays and consequences	
The elaboration of the detailed designs of the boat house at Koseze Pond according to the architect's instructions took longer as planned. A correction in plans was needed with the change of the location of the boathouse at Lake Bled.	
Complementary actions outside LIFE	
None.	
Perspectives for continuing the action after the end of the project	
Foreseen are meetings with the Municipality of Ljubljana on how to keep and maintain the boathouse at Koseze Pond. The same is in plan with the Municipality Bled. We will also look for new interested end-users to test the vessels.	
Evaluation	
Changing the location of the fishpond in Hotinja Vas in the north-eastern part of the country with Koseze Pond in Ljubljana, enabled us enormous savings in time and travel costs on a daily basis during the establishment phase of the docking station and robotic vessel. The design of the boathouse at Koseze Pond took longer time and slightly higher costs since we need to follow the guidelines and plans of the architects. However, the design of the boathouse, mimicking with the natural environment, brought a lot of positive attention from the public and turned the service platform into an attractive demonstration and education point. Changing the boathouse at Lake Bled to a southern part of the lake enabled us much easier manipulation of the boat, avoiding possible incidents with rowers and tourist.	

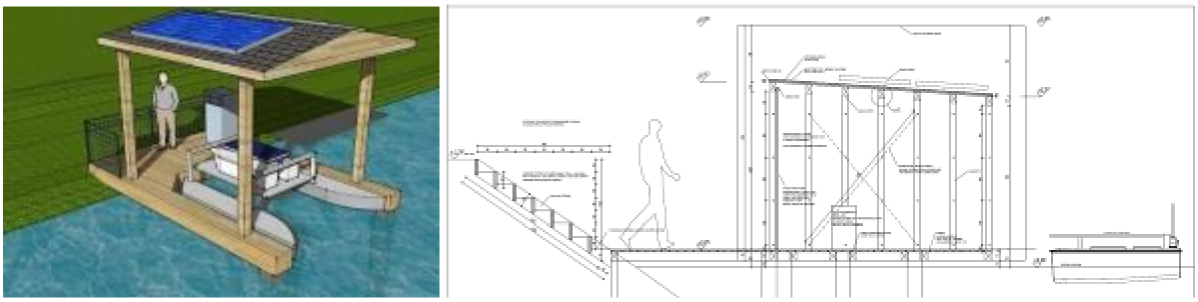

Figure 1: The first plan of the wharf at Koseze Pond, and the new one, which was changed according to the instruction of architects.

Figure 2: The agreement and overview of the location for setting up the wharf (docking station) at a new location below the Hotel Vila Bled.

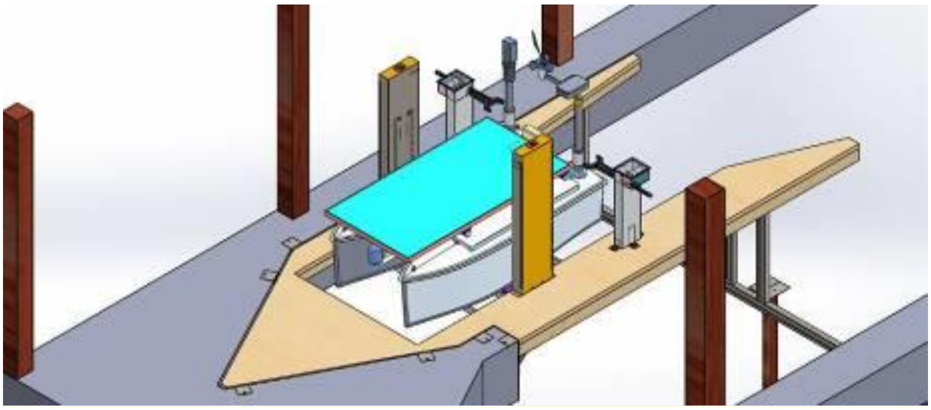

Figure 3: New design for the wharf at Lake Bled location

4.1.2 B1 Manufacture and test operation of service wharfs and mobile working platforms

Description of undertaken activities
B1 Phase 1 Purchase procedures B1 Phase 2 Manufacture of service wharfs and mobile measuring working platforms B1 Phase 3 Laboratory calibration of phycocyanin probe B1 Phase 4 Testing of the operation of the mobile measuring working platform B1 Phase 5 Elaboration of guidelines for operation and maintenance of devices Basic requirements of the action B1 have been carried out on time, and the set objectives have been achieved. The boathouses, their docking stations and the vessels were finished in several stages. First, the outer constructions and the mechanical parts were elaborated and separately the electrification, electronics and equipment with the software and information system. Certain parts have, however, not been completed into the details due to the complexity of the product, during the duration of this action. This were the details in remote navigation, automatic docking, coherent automatic operation and details in data transmission, analysis and presentation of the measured data. All these parts didn`t affect the continuation of the project and were be completed during the testing and monitoring activities (actions B2, B3, B4, B5, C1). Therefore, also the final version of the Deliverable Guidelines for the operation

and maintenance of the vessels and wharfs were completed by the end of the project (**ANNEX 8**). The tests within “Laboratory calibration of the phycocyanin probe” have been performed together by Arhel and NIB. This activity has taken longer time as originally planned. The procedures of cultivation of cyanobacteria in laboratory turned out to be more complicated as initially planned. Some infections of the cultures happened. To avoid delays, the cultures are now kept in two places, in Arhel` s and NIB laboratory. Additional time took the optimisation of the extraction of cyanobacterial pigment phycocyanin, for which no ISO standards exists. The quantification of phycocyanin by extraction was needed for the interpretation of the results measured by phycocyanin sensor. Besides extraction of pigments, the results obtained by sensors were compared with algal and cyanobacterial biomass. The determination of biomass by biovolume is also very time consuming (the determination of species composition is needed, counting of at least 500 cells in the sample and determination of the average volume of ca 100 cells of individual algal species). An internal report on the activities is gathered in the **ANNEX 10A**. The report from the testing of autonomous navigation is presented in **ANNEX 11**.

Expected outputs in quantifiable terms	Delivered outputs
Two service wharfs completed Two mobile measurement working platforms (vessels) completed with sensors and sampler The vessels will be equipped with software and connected to stationary computers to achieve autonomous navigation, measurements, data transfer and data interpretation Elaborated laboratory calibration curve to determine the number of cyanobacteria with the application of the sensors Successful test navigation of the vessel with minimal operation errors. Elaborated clear and useful guidelines for operation and maintenance	Completed

Implementer of activities
Some elements of Koseze wharf (like vessel lifting mechanism) have been worked out by external providers, but installed by Arhel and external support. The wooden parts of the wharf have been purchased from an external provider. Aviotech has done the manufacture and assemblage of the vessels. Arhel has developed and connected the electronic devices. Arhel has also performed testing of the equipment and its optimisation. Arhel has also finalised the guidelines for the operation and maintenance of the equipment. Arhel and NIB have performed laboratory calibration of sensor equipment for cyanobacteria sensing and quantification.

Indicators of progress (as set in the project)	
Completed laboratory testing of phycocyanin & chlorophyll probes. Elaborated statistically reliable calibration curve.	Accomplished
Elaborated software for autonomous operation, measurements and data transfer.	
Constructed wharf and vessel with all the measurement sets.	
Elaborated guidelines for operation and maintenance.	
Successful test navigation.	

Problems / drawbacks / delays and consequences

Although some Phases had some delays, the overall action was finished on time. Certain details were finished in the frame of other actions.
Complementary actions outside LIFE
A broader laboratory research on cyanobacteria has also been performed in the frame of the national research project Control of harmful cyanobacteria bloom in fresh-water bodies (applied research project), where NIB was a partner of this project.
Perspectives for continuing the action after the end of the project
Based on the results obtained in the framework of the project, the design of the vessel may be given certain changes (e.g., higher number electrolytic cells, increased photovoltaic panels).
Evaluation
Numerous test and demonstration routes performed by both vessels prove the successful manufacture of the vessel and docking station. The complete construction and assemblage took longer as planned, but the complexity of the work can be seen from the produced designs. Due to the phase construction and assemblage of the docking station and the vessels, and gradual improvements of individual elements on the vessel, this Action overlapped with actions B2, B3, B4 and B5.

Figure 4: Photos from the assemblage of the vessels.

Figure 5: Laboratory calibration of the phycocyanin and chlorophyll sensors in measuring chamber. In the course of the laboratory tastings, a transfer from double (left) to single measuring chamber equipped with two fluorescence sensors (middle) has been made. The right picture shows measuring chamber with electronics prepared for integration into the vessel.

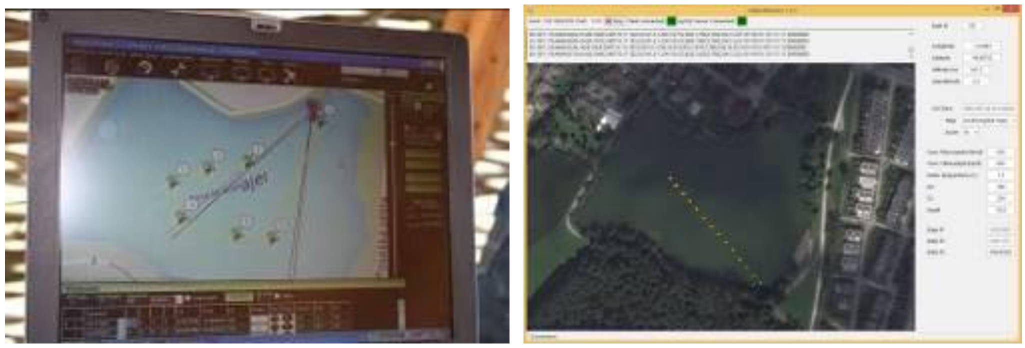

Figure 6: Testing of the navigation system of the vessel. Computer input of the route (left) and recording of the carried out route on the monitor screen (right).

Figure 7: Photo of both vessels (Stop CyanoBloom 1 and 2) – mobile working platforms

4.1.3 B2 Start-up of operation of the mobile measuring working platform on Koseze Pond

Description of undertaken activities
B2 Phase 1 Installation of service wharf B2 Phase 2 Providing the autonomy of the device on Koseze Pond B2 Phase 3 Calibration of phycocyanin probe in the natural environment B2 Phase 4 Determination of representative sampling line The boat house (wharf) at Koseze Pond has been constructed, although with a little delay. As it is an ecologically important area in Ljubljana, we had to follow the instructions of the Ljubljana city architects. Additional demands concerning the design, caused some delays as well as higher costs, as initially planned. The boat house has been however very good accepted from the local population and represents now an important promotional facility. The Internal report in **ANNEX 11** presents the work that has been carried out to achieve autonomous navigation of the vessels. The use of the software is described together with the possible modes of communicating with the vessel. The Internal report on the performed Calibration of the phycocyanin probe in the natural environment is presented in **ANNEX 10**. It gathers the activities performed on Koseze Pond and Lake Bled.

<table>
<tr><td colspan="2">According to the results of the first calibrations in the natural environment, the decision on the further on-line representative sampling paths of the monitoring has been accepted.</td></tr>
<tr><th>Expected outputs in quantifiable terms</th><th>Delivered outputs</th></tr>
<tr><td>Finished calibration curve of phycocyanin probe in the natural environment and adjusted software for interpretation of these results
Autonomous operation of mobile measuring working platform
Daily reporting on measured parameters and daily promptness in their presentation on the internet from the time of the installation of the equipment until the end of the project
Weekly sampling of the water samples on the representative line</td><td>Completed.

We have established a separate sub-page on the project website for the reporting of the measured data in the water body. The regular presentation of the data started later, with the start of the monitoring activities in Action C1. The actual data were visible only during the monitoring events.

Water samples were sampled on a weekly basis during the summer periods, with the start of the development of the phytoplankton.</td></tr>
<tr><th colspan="2">Implementer of activities</th></tr>
<tr><td colspan="2">The activities on the calibration of the phycocyanin probe in the natural environment and determination of representative sampling line have been carried out by Arhel and NIB.</td></tr>
<tr><th colspan="2">Indicators of progress (as set in the project)</th></tr>
<tr><td>Calibration curve for the given conditions on selected water body statistically reliable</td><td rowspan="3">Accomplished</td></tr>
<tr><td>The measuring working platform works autonomously</td></tr>
<tr><td>Representative sampling line defined</td></tr>
<tr><td>The internet page publishes the data about the state of the Koseze Pond once per day</td><td>The data were published in the days of the monitoring</td></tr>
<tr><td>At least 200 days of undisturbed autonomous data transfer in 2015</td><td>The activities to assure the autonomic navigation of the vessel provided its ability to transfer the data in real time when the vessel is in use, enabling undisturbed daily data transfer. However, due to some delays in the establishment of the system and a sophisticated data analysis work, which needs to be done after performed navigation, a total of 76 sailings were carried out with robotic vessels at Koseze Pond in 2015 and 2016 (activities of the Action C1). Additional sailing with the boat was carried out on fish Pond in Hotinja Vas.
We still evaluate, that we managed to obtain enough data for the assessment of the robotic vessel sensing and monitoring capacity.</td></tr>
<tr><th colspan="2">Problems / drawbacks / delays and consequences</th></tr>
<tr><td colspan="2">The boathouse with the docking station was installed with a slight delay as explained in action B1.</td></tr>
</table>

There have been some delays in the establishment of the autonomous operation of the vessel due to change of personnel and technical issues. Among the technical problems, we had to solve the problems with accumulation of bubbles in the measuring chamber, leakage of electromagnetic valves, the performance of automatic docking and data transfer. The regular monitoring with the vessel therefore begun with a delay and was substituted in this period with manual sampling and measurements with the equipment from the boat. After the completion of all the details, automatic monitoring was performed with autonomous data transfer, giving optimum results.

Complementary actions outside LIFE

No

Perspectives for continuing the action after the end of the project

Discussions are planned with the Municipality Ljubljana, Bled and fish pond managers in Hotinja Vas for a potential continuation of the activities. The discussions were also held with aquaculture managers in Serbia.

Evaluation

The regular activities on the lake started with a delay, due to technical issues to be solved on the vessel and construction of the boathouse as well as security issues on how to prevent eventual vandalism and damage to the boat.

To obtain the data on water quality, manual sampling was performed (in connection with the Action C1) and the parameters measured with the equipment from the vessel. Dedicated work, however, resulted in finalised and operative vessel enabling water measurements on computer defined route, data transfer and their real-time presentation.

Although not as many demonstrative routes with the vessel were performed as planned in the proposal, the number of performed ones demonstrate the full operability of the robotic vessels.




Figure 8: Construction steps of the wharf at Koseze Pond

Figure 9: Completed wharf at Koseze Pond

Figure 10: Test navigation of the Stop CyanoBloom 1 at Koseze Pond

4.1.4 B3 Upgrade of the mobile measuring working platform with the system for triggering of cyanobacteria lysis and monitoring of its performance in Koseze Pond

Description of undertaken activities
B3 Phase 1 Laboratory optimisation for the triggering of cyanobacteria lytic cycle B3 Phase 2 Production of the systems for triggering of the lysis B3 Phase 3 Installation of the system for triggering cell lysis on Koseze Pond In **ANNEX 12,** we have gathered all the tests performed on the Laboratory optimisation for the triggering of cyanobacteria lytic cycle. The results describe the achieved delayed growth, decreased autofluorescence, loss of buoyancy and cell lysis of cyanobacteria due to the electro-oxidation treatment with an electrolytic cell. The laboratory experiments have been performed with a use of laboratory scale electrolytic cells (boron doped diamond electrode, 8 cm^2 electrode surface, 1.6 mL electrolytic cell volume, current density ten mA/cm^2, ten mL/min flow rate). The laboratory part of the experiments was repeated also using fluorescence sensors as a measuring tool for considering the cyanobacterial physiological state. Following the obtained laboratory results, electrolytic cells were constructed, which were installed in the vessels, used in a fixed position in the ponds, as well as in the pilot scale experiments with canisters and aquariums. Beside electrolytic cells, the accompanying electronics and software have been developed for the integration of the components in the robotic vessel.

In **ANNEX 15** (Pilot-scale optimisation of in-lake cyanobacterial control with electrochemical oxidation) altogether 16 experiments are presented where we tested the efficiency of the electrolytic cell with different treatment settings of the electrolytic cell and at various environmental conditions. We have performed three experiments with a pure culture of *Microcystis aeruginosa* and one with several species of pure cultures of cyanobacteria and green algae. Experiments with different natural water samples has been performed, two experiments with the water from Koseze Pond (with prevalence of green algae population), three experiments with the water from Lake Bled (containing cyanobacteria *Plantktothrix rubescens* and phytoplankton) and seven experiments with the water from fish pond in Hotinja Vas, which contained high concentrations of *Microcystis aeruginosa* and green algae. The tests were performed at the beginning on the laboratory scale and were further continued in bigger pilot scale - in the canisters and aquariums, simulating the natural conditions. The results of the tests performed directly in the lake environment (evaluation effect on biodiversity) are presented in **ANNEX 16**. We managed to define the application mode at which we achieve selective control of cyanobacteria growth and undisturbed phytoplankton population. These data were further used in the economic justification of the project.	
Expected outputs in quantifiable terms	Delivered outputs
• Produced optimised (maximum efficiency at minimal power use) systems (electrolytic cell and heat collectors) for the control of cyanobacterial populations, able of autonomous triggering of lytic cycle to such an extent that the concentration of cyanobacteria will reduce on approximately 90% of its original concentration • Autonomous operation of upgraded mobile working platform on Koseze Pond • Expecting absence of cyanobacterial blooms on Koseze Pond • Expecting higher biodiversity due to reduced cyanobacteria population with proliferation of different green algae and consequently algae consumers	• In actually all of the performed experiments, we managed to demonstrate higher efficiency than the reduction to the approximately 90% of the original concentration of cyanobacteria (**ANNEX 15**). • The experiments with the electrolytic cell were placed in fixed position in the Koseze and Hotinje pond, and not fixed onto the vessel, to assure controlled environment. • Cyanobacteria were not blooming in Koseze Pond during the project. The reduction capacity on Microcystis in pilot scale conditions was demonstrated for the fish pond in Hotinja Vas (**ANNEX 15**). • Positive effect on biodiversity has been demonstrated in pilot scale experiments (**ANNEX 16**).
Implementer of activities	
The activities on laboratory optimisation of direct lysis and triggering lytic cycle of cyanobacteria were carried out by Arhel and NIB. The design of the sampler and electrolytic cell has been done by Arhel.	
Indicators of progress (as set in the project)	
Achieving complete autonomous start-up of the electrolytic cell and triggering of the heat shock at detected border concentration of cyanobacteria 10,000	Accomplished After the performed experiments, the decision was made to use the electrolytic cell

cells/mL, before the beginning of increased growth of cyanobacteria population	only and not also the heat shock, to optimise the use of energy.
Absence of cyanobacteria blooming during the operation of new technology on Koseze Pond	Accomplished
The concentration of cyanobacteria above 10,000 cells/mL will not be detected during the monitoring	
The phytoplankton species composition found in the analyses of monthly monitoring will be more diverse as in the former year	Demonstrated on a pilot level by experiments (**ANNEX 16**).
Problems / drawbacks / delays and consequences	
A delay in this action started already with the performance of laboratory tests since we had difficulties in the cultivation of sufficient concentration of cyanobacteria and it was also not always possible to obtain the sample from the natural environment. An additional problem we confronted was a fungal infection of the laboratory cyanobacterial culture, which caused early cell decomposition or autolysis. We managed to solve the problems and laboratory experiments were successfully finished. The analysis after each performed test with the electrolytic cell was also very comprehensive. Time-consuming were especially the tests determining cyanobacterial and the rest of the phytoplankton biovolume, determining damages on the cells and their DNA material and measuring cyanotoxins. To evaluate the potential lysogeny of the cyanobacterial cells (proliferation of cyanophages) turned to be out of the feasibility of this project. Due to a longer time needed to finish all the works with the monitoring part of the prototype, the part of the project, which dealt with the triggering of the cyanobacteria lysis with an electrolytic cell, was also having some delay. In order not to miss the entire 2015 season with electrolytic cell activities in the natural environment, we proceeded with experiments in the natural environment with the installation of a fixed electrolytic cells in Koseze Pond and fish pond in Hotinja Vas. This enabled us to follow the effects in the natural environment under more controlled experimental conditions and to evaluate the effect of the electrolytic cell on the phytoplankton as well as on other water organisms. The second concern was eventual release of cyanotoxins from cyanobacteria after their lysis caused by electrolytic cell and their effect on other water organisms. This was, therefore, a second reason justifying the establishment of controlled pilot scale experiments. The 16 experiments with different volumes and sources of water provided us with sufficient results to evaluate the capacity of the electrolytic cell, boundaries of its selectivity to algae and cyanobacteria and therefore conclusions on potential positive effects on biodiversity. The seasonality of cyanobacterial blooms and the time frame of the project, however, didn`t allow us to perform the demonstration of the electrolytic cell activity directly from the robotics vessel on an open water body.	
Complementary actions outside LIFE	
/	
Perspectives for continuing the action after the end of the project	

The discussions were held with aquaculture managers in Serbia on how to reduce their problems with cyanobacterial blooms.

Evaluation

We evaluate that the general goals of the Action were achieved. The effect of electrochemical oxidation using electrolytic cells equipped with boron-doped electrodes on cyanobacteria was proved by 16 different laboratory and pilot-scale experiments. The control system in the robotic vessel was integrated with the electrolytic cell, enabling its switch-on when the measuring system detects defined concentration of cyanobacteria (e.g. >10.000 cells/mL). The results enabled the calculation of the operation range of the vessel with evaluation of investment and operation costs for the case of a fishpond.

From the methodological point of view, a longer project duration would be needed to test the treatment with electrolytic cells on the vessel in the natural environment. The rapid execution of the tests is limited by the seasonal occurrence of cyanobacteria and needed previous insight on the behaviour of cyanotoxins during treatment. Detecting induction of lysogeny of cyanobacteria would also need comprehensive laboratory trials first, with their continued monitoring in the environment with advanced equipment enabling analysis of viruses (cyanophages).

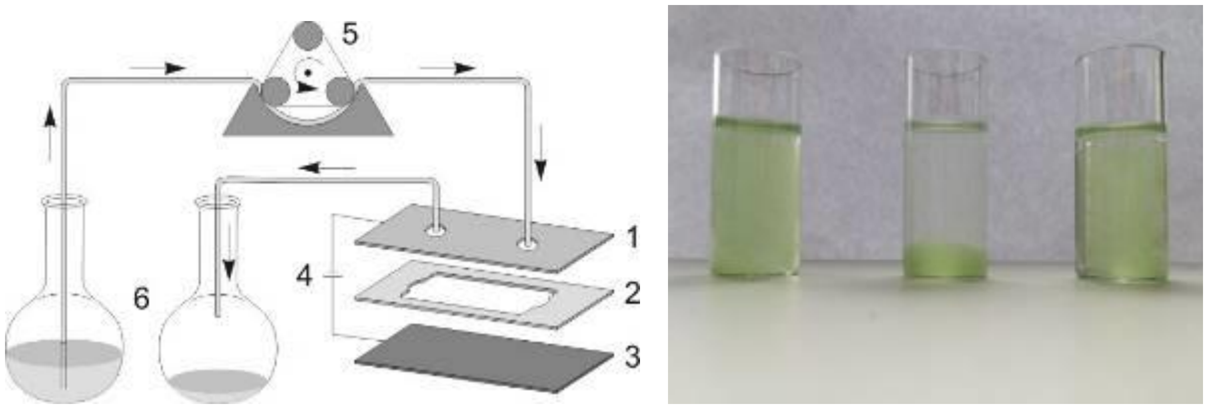

Figure 11: Laboratory tests on cyanobacteria treated with an electrolytic cell. Left: Scheme of experiment set-up. Right: jars with cyanobacteria from left to right: control (uniform distribution of cells), treated in the electrolytic cell (cells precipitate on the bottom), flow through switch off electrolytic cell (uniform distribution of cells).

Figure 12: Left: Set-up of the laboratory experiment. Right: images of the cyanobacterial cultures after different replications of treatment in the electrolytic cell.

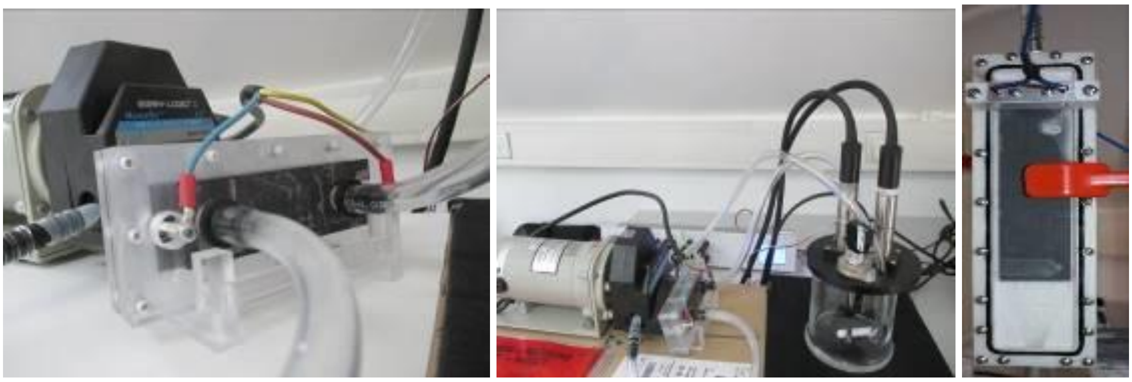

Figure 13: Different versions of electrolytic cells used for laboratory testing. Left: Small lab size electrolytic cell, middle: electrolytic cell connected to the measuring system with phycocyanin and chlorophyll sensor, right: bigger laboratory cell.

Slika 1: Set-up of the electro-oxidation cell for water treatment and pump along the aquariums with the water taken from a fish pond in Hotinja Vas.

Figure 14: Fixed electrolytic cell in the inner side of the service wharf and location of the experimental canisters

Figure 15: Set-up of the second pilot test experiment on Koseze Pond with electrolytic cell: a system with power supply, pump and an electrolytic cell.

Figure 16: Set-up of the pilot experiment in a fishpond in Hotinja Vas.

4.1.5 B4 Start-up of the operation of the mobile measuring working platform of Lake Bled

Description of undertaken activities
B4 Phase 1 Installation of a service wharf B4 Phase 2 Providing the autonomy of the device on Lake Bled

<table>
<tr><td colspan="2">B4 Phase 3 Adjustment of the interpretation of the measurements of the phycocyanin probe to the conditions in the Lake Bled
B4 Phase 4 Determination of the representative sampling line – Lake Bled
A docking station (service wharf) in the boathouse of the Vila Bled at Lake Bled has been established with a slight delay. The selection of this new location was more suitable for the vessel operation since we didn`t have to cross the sport rowing trails on the lake and deal with the dense tourist boat traffic to access the so-called West depression of the lake. The West depression of the lake, on the western side of the lake island, is the deepest point of the lake and is the point of the highest cyanobacteria occurrence.
The work carried out on the providing the autonomy of the vessel operation, adjustment of the
interpretation of the measurements of the phycocyanin probe to the conditions in the Lake Bled and determination of the representative sampling line is presented in the ANNEXES 10A, 10 and 11. The monitoring activities with the vessel differed from the monitoring at Koseze Pond. Here, vertical profiles of the water were monitored. For each monitoring event, we decided to take samples at two locations with usually different cyanobacteria occurrence pattern, east and west from the lake island.</td></tr>
<tr><td>Expected outputs in quantifiable terms</td><td>Delivered outputs</td></tr>
<tr><td>• Phycocyanin probe adapted to the Lake Bled conditions
• Autonomous operation of the mobile working platform, daily reporting of the measured parameters and daily promptness of the data presentation on the internet page from the time of vessel installation to the end of the project
• Weekly gathering of the samples on the representative sampling line</td><td>• The calibration of the sensors in the natural environment has been accomplished
• The robotic vessel exhibits the autonomous operation, and the reporting of the measured data on the operator`s server and the web page is established immediately after recording.<br>• During the top season weekly gathering of the samples has been provided by manual sampling and measured with the vessel` measuring equipment</td></tr>
<tr><td colspan="2">Implementer of activities</td></tr>
<tr><td colspan="2">Občina Bled has organised a new location for the boat house with the docking station of the vessel in Bled, as the initially selected location was occupied. Arhel has prepared the updated designs for the new location of the wharf on the Lake Bled, and the installation works (additional wooden elements, a lifting mechanism for the vessel, and electronics for automatic docking) were performed by the same supplier as for the Koseze Pond. The outer infrastructure of the boathouse was arranged by Municipality Bled and their external service. The first testing of the vessel in the Lake Bled has been done by Arhel. The activities on the adjustment of the interpretation of the measurements with the phycocyanin (as well as chlorophyll) probe to the conditions in the Lake Bled were carried out together with NIB.</td></tr>
<tr><td colspan="2">Indicators of progress (as set in the project)</td></tr>
<tr><td>Calibration curve for the given conditions on selected water body statistically reliable</td><td rowspan="3">Accomplished</td></tr>
<tr><td>The measuring working platform works autonomously</td></tr>
<tr><td>Representative sampling line defined</td></tr>
</table>

<table>
<tr><td>The internet page publishes the data about the state of the Lake Bled once per day</td><td>The data were posted in the days of the monitoring.</td></tr>
<tr><td>At least 200 days of undisturbed autonomous data transfer in 2016</td><td>The activities to assure the autonomic navigation of the vessel provided its ability to transfer the data in real time when the vessel is in use, enabling undisturbed daily data transfer. However, due to some delays as explained in the action B2, a total of 19 sailings were performed with the robotic vessels at Lake Bled in 2015 and 2016 (activities of the Action C1). Despite the fact that we made a much smaller number of monitoring, the monitoring has been carried out in two key periods, during the absence and presence of cyanobacteria. We have performed a monitoring of vertical profiles as well as horizontal recording on two depths.</td></tr>
<tr><td colspan="2">Problems / drawbacks / delays and consequences</td></tr>
<tr><td colspan="2">The measurements of the water quality through the water vertical profile took longer time as expected. Therefore, only two points with the depth profile during each monitoring event with the vessel were taken. After the start of the testing period, some technical also problems emerged, which had to be solved:
1. Formation of the bubbles in the monitoring chamber: they were disturbing the ridings on the fluorescence sensors; the installed whisk turned out not to be sufficient. To eliminate the problem, the frequency of the whisk`s shifts have been increased and shorter period of the recordings at one depth was taken into the analysis; all the parts were tested for water tightness. Additional glass measuring chamber has been elaborated to observe and follow the changes inside the chamber.
2. Some connectors turned out not to be waterproof, and the vessel stops operating. The connectors were changed.
3. Damage on the surface of the chlorophyll and phycocyanin sensors have been detected, which caused false results during the monitoring; measures have been undertaken to repair the sensors` surface.
Due to confronted problems, less monitoring events have been successfully performed. Additional problems in data presentation and prediction of blooming events represented the specific winter and early spring occurrence of cyanobacterial blooms in Lake Bled and very clear water conditions with low phytoplankton presence during the project.
In spite of a lower number of the monitoring events with the vessel, we managed to present automatic monitoring with the vessel, vertical and horizontal profiling of the water, data transfer, their (statistical) analysis and graphical presentation.</td></tr>
<tr><td colspan="2">Complementary actions outside LIFE</td></tr>
<tr><td colspan="2">/</td></tr>
<tr><td colspan="2">Perspectives for continuing the action after the end of the project</td></tr>
<tr><td colspan="2">We will try to find new financial resources to perform additional monitoring in Lake Bled or similar lakes with the occurrence of cyanobacteria in metalimnion.</td></tr>
</table>

Evaluation
Regular operation of the vessel in Lake Bled with the performance of vertical water profiling started with a delay due to several technical issues explained in paragraph 5.1.5. To obtain the data on water quality, manual vertical sampling was performed (in connection with the Action C1), and the parameters were measured with the equipment from the vessel. After we have resolved the problems, we carried out a sufficient amount of monitoring with a vertical profiling at two Lake locations and with horizontal routes, with which are presented the spatial and temporal comparison of the locations and times of occurrence of cyanobacteria. The results prove that the vessels equipment for the vertical water samples abstraction and the vessel`s software for the maintenance of the vessel position, also enable monitoring of deep-water lakes with meta-limnetic cyanobacteria occurrence.

Figure 17: Warf at Lake Bled and test operation of the vessel Life Stop CyanoBloom 2.

Figure 18: Installation of the service wharf at boat house of Vila Bled (timber elements with electricity).

Figure 19: Damages on the epoxy surface of the fluorescence sensors and emerging of the bubbles in the measuring chamber, both causing a false reading of the produced fluorescence signals.

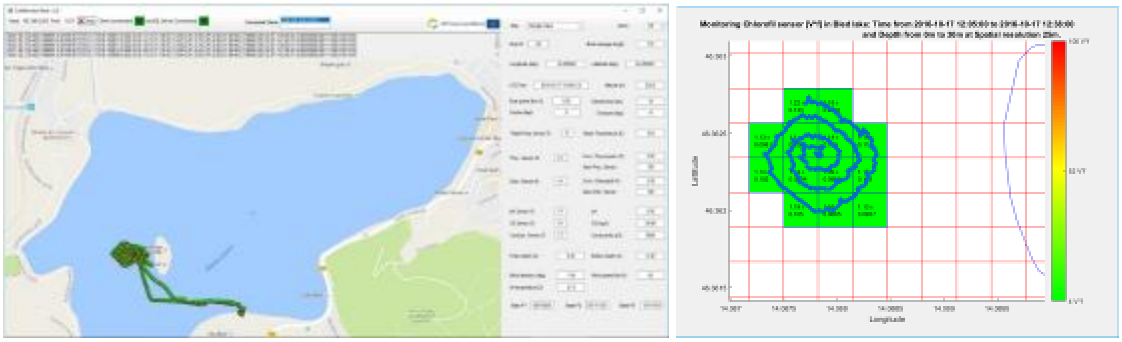

Figure 20: Presentation of the electronic record of the performed paths of the vessel on the Lake Bled during monitoring event measuring horizontal profile on the west side of the lake island and the analysis of measured data with the chlorophyll sensor.

4.1.6 B5 Upgrade of the mobile measuring working platform with the system for triggering of cyanobacteria lysis and performance monitoring on Lake Bled

<table>
<tr><td colspan="2">Description of undertaken activities</td></tr>
<tr><td colspan="2">The electrolytic cell developed in the Action B3 has also been used for the system tested in the Lake Bled. As explained under Action B3, control tests with electrolytic cell have been undertaken and not directly treating the lake water from the robotic vessel. The activities with Lake Bled water started later as planned, and were postponed to March 2016. In ANNEX 12 and 15, all the tests performed on the Laboratory optimisation for the triggering of cyanobacteria lytic cycle and the Pilot scale optimisation of in-lake cyanobacteria control are gathered, and three experiments among them were dedicated to water and cyanobacteria from Lake Bled. The predominant cyanobacteria in Lake Bled are filamentous Planktothrix rubescens. The tests were performed on the effect of electrochemical oxidation on cyanobacteria and its cyanotoxin reduction.</td></tr>
<tr><td>Expected outputs in quantifiable terms</td><td>Delivered outputs</td></tr>
<tr><td>Autonomous operation of the mobile working platform on Lake Bled
Prevented emerging of cyanobacterial bloom on Lake Bled. In the case of detected increase of cyanobacteria, we expect a reduction of cyanobacteria on 90% of the beginning concentration (before treatment) after triggering of lytic cycle on the part of the cyanobacterial population in electrolytic cell or with heat shock
With the decrease of cyanobacteria population, we expect visible increase of biodiversity with immediate proliferation of different green algal species and consequently different consumers of green algae, what will be evident from regular monitoring from other institutions on the lake</td><td>Autonomous operation of the vessel has been demonstrated.
In all of the performed experiments, we managed to demonstrate a reduction of the cyanobacteria proliferation, which was much higher than only reducing to the 90% of the beginning concentration
The potential of increasing the biodiversity was demonstrated in the experiments presented in ANNEX 16.</td></tr>
<tr><td colspan="2">Implementer of activities</td></tr>
</table>

The experiments and the analysis of the obtained data were carried out together by Arhel and NIB.	
Indicators of progress (as set in the project)	
Achieving complete autonomous start-up of the electrolytic cell and triggering of the heat shock at detected border concentration of cyanobacteria 10,000 cells/mL, before the beginning of increased growth of cyanobacteria population	Accomplished After the performed experiments, the decision was made to use the electrolytic cell only and not also the heat shock, to optimise the use of energy.
Absence of cyanobacteria blooming during the operation of new technology on Lake Bled	There was a noticeable occurrence of *Planktothrix rubescens* in the winter, and early spring in Lake Bled during the project, but not in a severe form of blooming. The vessel was not operating during the winter period.
The concentration of cyanobacteria above 10,000 cells/mL will not be detected during the monitoring	During the performed monitoring events, we managed to detect also this concentration of *Planktothrix rubescens* in the depths of about 10 to 15 m.
The phytoplankton species composition found in the analyses of monthly monitoring will be more diverse as in the former year	Such an influence on biodiversity was not possible to prove in the natural environment is such short period of time. A prof with performed experiment was provided presented in **ANNEX 16.**
Problems / drawbacks / delays and consequences	
During the project, we found out that the cyanobacteria *Planktothrix rubescens*, which blooms are significant for Lake Bled, emerges in upper water layers only during the winter season. Due to the project plan, which envisaged the start of the activities on Lake Bled first in 2015 and difficulties at the development of the equipment, we have lost two potential winter season periods when the cyanobacterial blooms are most expressed. The next possible occasion to detect expressed *P. rubescens* occurrence was from December 2016 to March 2017, which was from the point of view of the project duration already too late. For this reason, we performed the experiments with collected water samples from the lake containing the cyanobacteria. We managed to prove the efficiency of the electrochemical stimulation on *P. rubescens* in the controlled environment.	
Complementary actions outside LIFE	
/	
Perspectives for continuing the action after the end of the project	
We will try to find interested end-users having problems with filamentous cyanobacteria *P. rubescens* or new financial resources to test and demonstrate the technology directly in the lake.	
Evaluation	
As for the action B3, we evaluate that the general goals of the Action B5 were achieved. The effect of electrochemical oxidation using electrolytic cells equipped with boron-doped electrodes on cyanobacteria was confirmed. The control system in the robotic vessel switches on when the measuring system detects defined concentration of cyanobacteria.	

From the methodological point of view, also in the case of deep water lakes, a longer project duration would be needed to test the treatment with electrolytic cells on the vessel in the natural environment.

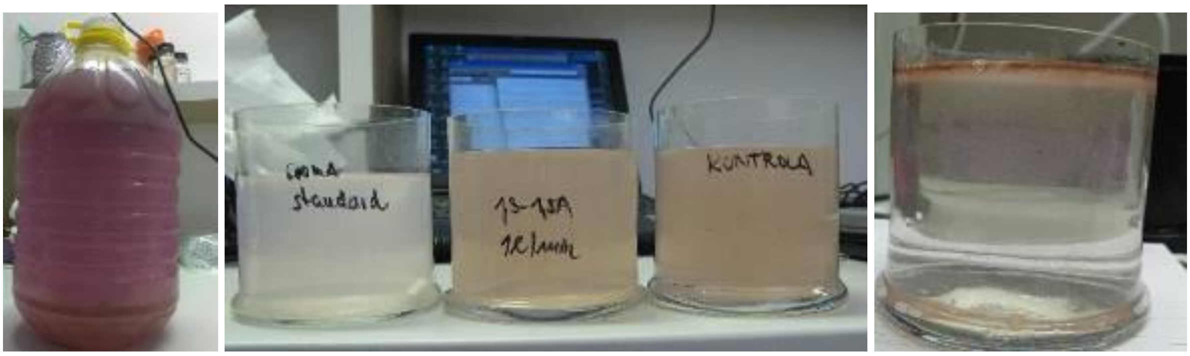

Figure 21: Samples used in the experiment.

4.1.7 C1 Monitoring of the impact of the project actions

Description of undertaken activities

C1 Phase 1 Monitoring of the socio-economic impacts of the project implementation on the local economy and population

Action 1 Monitoring of the population awareness of cyanobacteria bloom problematic
Action 2 Monitoring of tourist development and number of employments in tourism and improvement of tourist offer
Action 3 Monitoring of local public and bathers (swimmers) health
Action 4 Monitoring of fish quality in water body

C1 Phase 2 Monitoring of environmental impacts of the project

The deliverable with the Report on the socio-economic effects of the project in presented in **ANNEX 19.** Monitoring of public awareness on the problem of cyanobacterial blooms was implemented with two questionnaires. Socio-economic effects of the project were also researched through a literature survey of the effects of cyanobacterial blooms on tourism and human health. Additionally, delivery with a Report on the composition and quality of the fish community was prepared and presented in **ANNEX 18**. Interviews with the representatives of the fish pond managers and representatives of the fishing societies.

Monitoring has been carried out according to the plan. Besides monitoring on the Koseze Pond and Lake Bled, the additional visit has been made to Hotinje pond with pronounced blooming of the cyanobacteria. The reports on lake monitoring activities and data analysis are gathered in two extensive reports:
ANNEX 13: Monitoring of the ecological status of lakes (Koseze Pond, Lake Bled, Fish Pond in Hotinje) during the LIFE Stop CyanoBloom project
ANNEX 14: Performed on-line lake monitoring with the robotic vessels Stop CyanoBloom 1 and Stop CyanoBloom 2

After evaluating all the monitoring and experimental data on electrochemical oxidation, an economic justification of the technology has been performed and presented in the Deliverable Report on the economic justification of the project in **ANNEX 17.**

<table>
<tr><td>Expected outputs in quantifiable terms</td><td colspan="2">Delivered outputs</td></tr>
<tr><td>Elaborated graphs and schemes are showing proofs of equipment efficiency in controlling toxic cyanobacterial bloom. All collected data will be gathered in a special report.</td><td colspan="2">All collected data are collected in reports presented in ANNEXES: 13, 14, 15, 16, 17, 18 and 19.</td></tr>
<tr><td colspan="3">Implementer of activities</td></tr>
<tr><td colspan="3">The activity is carried out by Arhel and NIB.</td></tr>
<tr><td colspan="3">Indicators of progress (as set in the project)</td></tr>
<tr><td colspan="2">Water turbidity reduced by half.</td><td rowspan="3">Proved with the pilot scale experiments</td></tr>
<tr><td colspan="2">Increased biodiversity.</td></tr>
<tr><td colspan="2">50% lower concentration of toxic cyanobacteria.</td></tr>
<tr><td colspan="2">Improved tourist offer and visitors well-being due to a safer environment.</td><td>Could not be directly assessed, since the place where the vessel was operating were not affected severely by cyanobacteria.</td></tr>
<tr><td colspan="2">No income loss due to the prohibition of fishing as a consequence of harmful algal bloom.</td><td>Fishing was not prohibited during the project.</td></tr>
<tr><td colspan="2">No illnesses of the visitors connected to the cyanobacterial poisoning.</td><td>No illnesses reported due to cyanobacteria.</td></tr>
<tr><td colspan="3">Problems / drawbacks / delays and consequences</td></tr>
<tr><td colspan="3">The potential drawbacks were presented already in the description of the actions B2 do B5.</td></tr>
<tr><td colspan="3">Complementary actions outside LIFE</td></tr>
<tr><td colspan="3">/</td></tr>
<tr><td colspan="3">Perspectives for continuing the action after the end of the project</td></tr>
<tr><td colspan="3">After the planned meetings with the municipalities and managers of the water bodies, we will see whether they are interested in the continuation of the monitoring and treatment activities.</td></tr>
<tr><td colspan="3">Evaluation</td></tr>
<tr><td colspan="3">The activities proceeded according to the plan. In addition to regular monitoring on two lakes, occasional visits of the fishpond in Hotinja Vas were made with cyanobacteria blooms. Parallel monitoring at all three locations represents a very extensive engagement the project team.
To enable better social and economic assessment of the effect of cyanobacterial blooms, two big carp breeding aquaculture institutions in Serbia were visited and two shallow lakes are suffering from cyanobacterial blooms in the surroundings. Interviews with the fishpond managers were held to assess the severity of the problems and the concrete needs they have to solve their problems.
Due to the changed activities and cyanobacterial presence on the lake, certain societal and economic effects were evaluated by the literature review.
All performed activities enabled us the evaluation of the economic justification of the technology and prospect markets for the replicability and transfer of the technology.</td></tr>
</table>

Figure 22: Images from monitoring on Koseze Pond

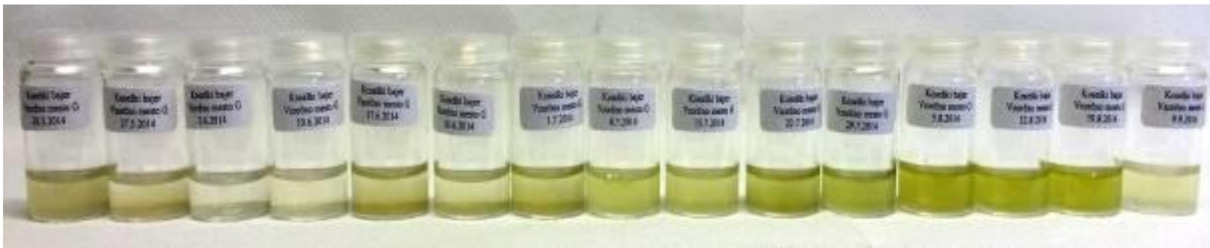

Figure 23: Fixed phytoplankton samples from monthly monitoring on Koseze Pond

Figure 24: Images from monitoring on Lake Bled

Figure 25: Images from the monitoring event on Hotinje pond

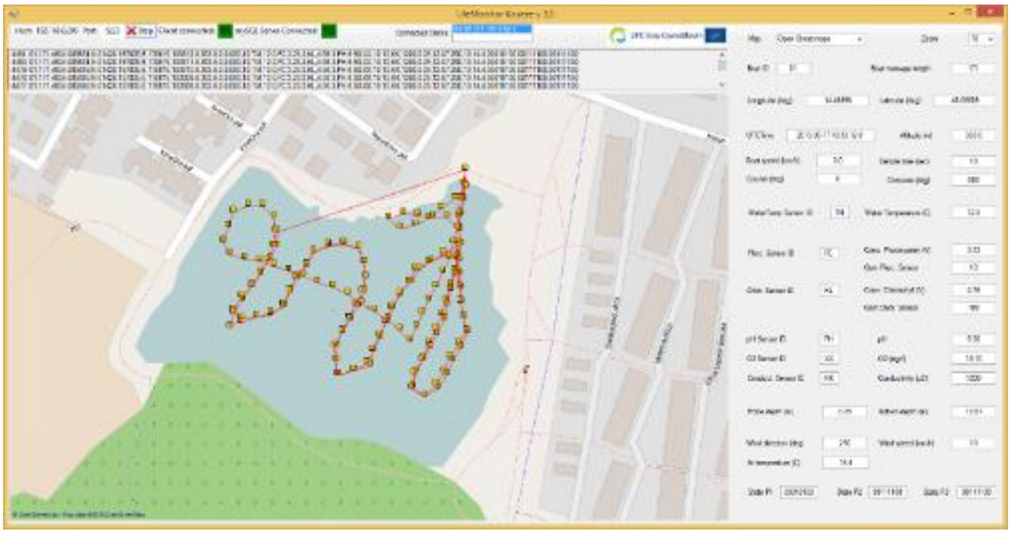

Figure 26: Presentation of the electronic record of the performed paths of the vessel on the Koseze Pond during the monitoring event. The path of the vessel is followed from a remote computer. The pictures of completed paths are presented.

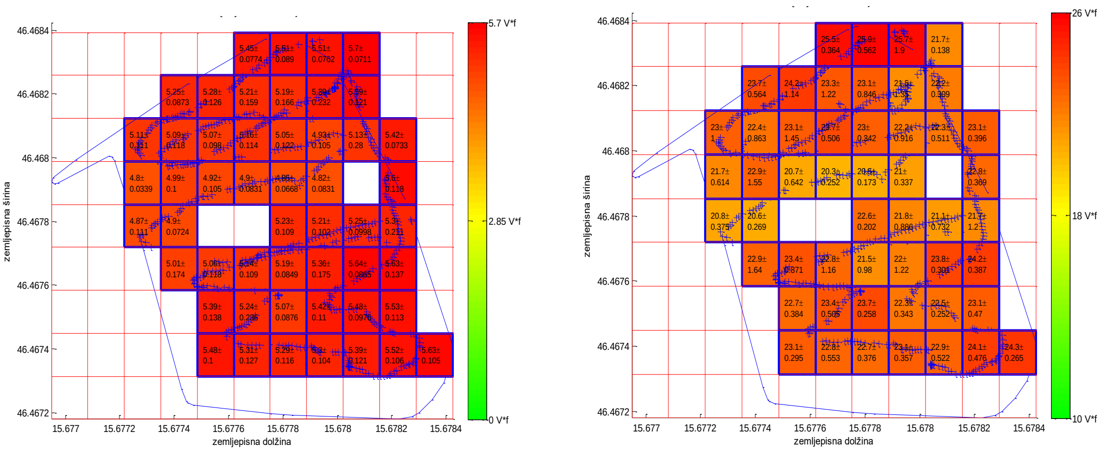

Figure 27: Presentation of the analysed data received from the phycocyanin and chlorophyll fluorescence sensors during the additional monitoring performed on the pond in Hotinja village. Average values are presented with standard errors. Left phycocyanin sensor, right chlorophyll sensor.

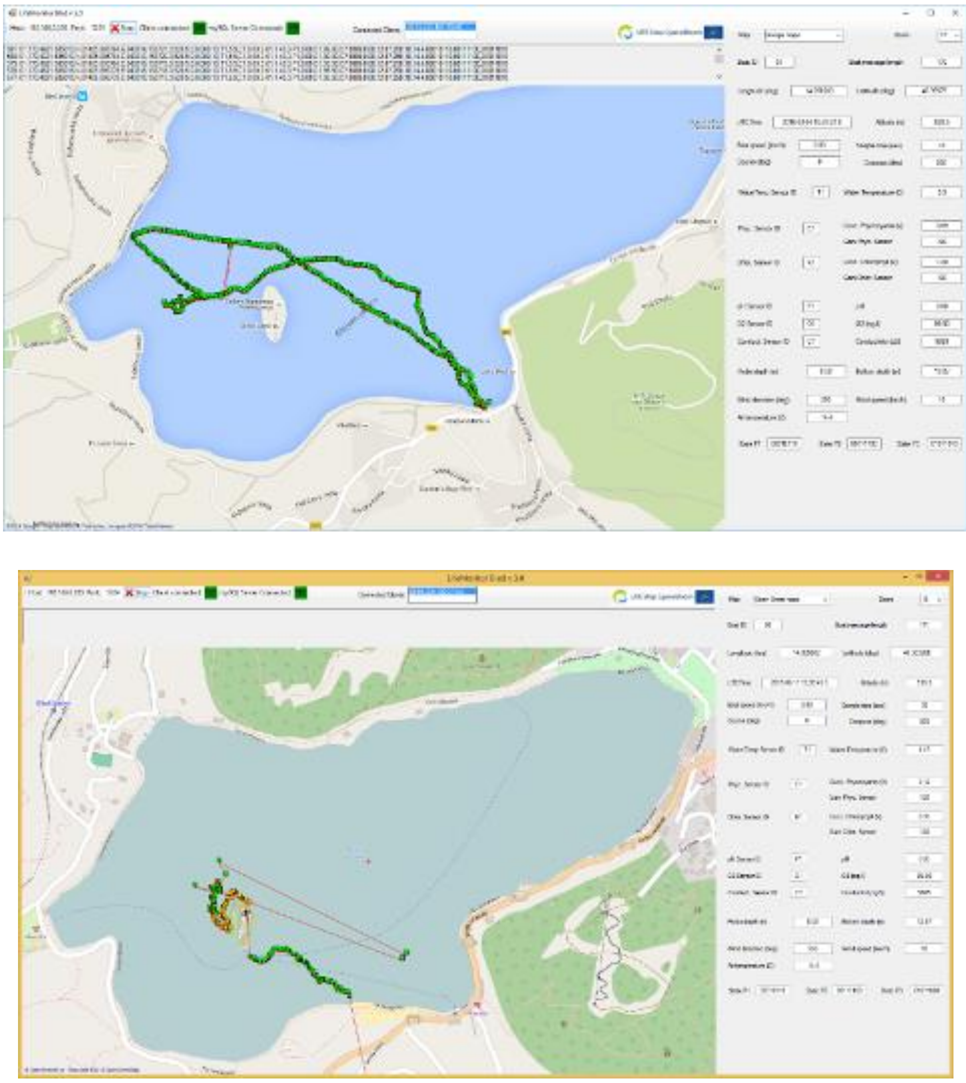

Figure 28: Presentation of the electronic record of the performed paths of the vessel on the Lake Bled during two monitoring events. The path of the vessel is followed from a remote computer. The pictures of completed paths are presented.

Figure 29: Operators are preparing the vessel for the route.

4.2 Dissemination actions

A list of published papers in scientific and professional journals, poster and other communication material is presented in After-LIFE Communication Plan document.

A lot of material can also be downloaded from Home/Documents and News sections of the project webpage http://lifestopcyanobloom.arhel.si. A separate sub-page is dedicated to the closure project conference where all the project presentations and Conference Proceeding can be downloaded.

From the feedback received on our dissemination and communication activities, we evaluate our work as very successful.
We were hosting a visit from two experienced scientists working on cyanobacteria and engaged in CYANOCOST action from Finland and Serbia. Their statement on Stop CyanoBloom technology is attached in **ANNEX 25.** They presented their lectures also at the Project closure conferences (23 presentations altogether).

The technology was also awarded two first prizes at the 10th Slovenian Innovation Forum Nova Gorica, in 2015. We won the first price in the category of micro-enterprises, and we have received a special award for the innovation with a societal impact.

Additionally, we received an award for the photography of the vessel at Europe in my Region competition.

With the numerous presentations, we evaluate that we have exceeded the envisaged dissemination activities.

4.2.1.1 A list of attended conferences with the title of the presentations

1. 6th Croatian Water Conference with International Participation, Opatija 20. – 23 May 2015. Poster presentation: Real-time phytoplankton quantification using phytoplankton chlorophyll a and phycocyanin fluorescence sensors.
2. Conference 4th Conference with International Participation. Conference VIVUS – on Agriculture, Environmentalism, Horticulture, and Floristics, Food Production and Processing and nutrition. 20th and 21st April 2016, Biotechnical Centre Naklo, Strahinj 99, Slovenia: Tinkara Rozina et al.: Seasonal succession of phytoplankton and water quality of Koseze Pond«
3. (XI international scientific conference "Ecology for better environment", 31. 3. – 1.4. 2016, Rakičan: Test operation of robotic vessel for monitoring and control of phytoplankton in the water body: simultaneous application of fluorescence sensors and electro-oxidation method
4. European robotic form 2016 on the entrepreneurship competition with a pitch, 21 – 23. March, 2016.
5. Konference of the Society of the Biology Students, BIOSFERA, Ljubljana, September 2016: 2 poster presentations: Čater et al., Robotic Floating platform and Rozina et al., The effect of electrochemical oxidation on Microcystis aeruginosa.
6. Power of microbes in industry and environment, 28. 9 – 1.10. 2016, Krk, Croatia: Poster and lecture presentation, Čater et al.: Reduction of extracellular microcystin concentration by boron-doped diamond electrodes to control cyanobacterial blooms.
7. 10th International Conference on Toxic Cyanobacteria, Wuhan, China, 23. – 28. October, 2016. Poster presentation: Rozina et al.: PILOT SCALE DEMONSTRATION PROJECT LIFE Stop CyanoBloom
8. 5th International Conference of Mechanical Engineers, 18th October 2016. Poster presentation: Marinović et al.: Daljinsko vodeno plovilo z on-line sistemom razločevanja in kvantifikacije fitoplanktona in lokalizirano kontrolo cianobakterij v vodnem telesu.

9. LIFE Stop CyanoBloom Closure project conference, 1. 2. December 2016, Ljubljana. 7 presentations with lectures of the project team members:
   1. Maja Zupančič Justin: Introduction
   2. Mario Marinović, Jošt Grum, Luka Teslić, Gorazd Lakovič, Marko Gerl: Robotsko plovilo Stop CyanoBloom: tehnične lastnosti, navigacija, upravljanje sistema za analizo, tretiranje in vzorčenje vode / *Robotic vessel StopCyanoBloom: technical properties, navigation, operation control of the systems for water analysis, treatment and sampling*
   3. Luka Teslić, Mario Marinović, Jošt Grum, Gorazd Lakovič, Marko Gerl: Robotsko plovilo Stop CyanoBloom: informacijski sistem - prenos, shranjevanje, obdelava in prikaz podatkov / *Robotic vessel StopCyanoBloom: information system - data transfer, storage, analysis and presentation*
   4. Bojan Sedmak: Potreba po sodobnejših pristopih pri monitoringu fitoplanktona / *The need of advanced approaches for phytoplankton monitoring*
   5. Tinkara Rozina, Maja Zupančič Justin, Tina Eleršek, Maša Čater, Bojan Sedmak: Rezultati aktivnosti monitoringa vodnih teles v okviru projekta LIFE Stop CyanBloom: primerjava on-line meritev s standardnimi metodami vrednotenja fitoplanktona / *Results of monitoring activities in the frame of the LIFE Stop CyanoBloom project: comparison of online monitoring with standard methods of phytoplankton assessment*
   6. Tina Eleršek, Maja Zupančič Justin, Tinkara Rozina: Vrednotenje učinka elektrokemijske oksidacije cianobakterij s standardiziranimi testi na zelenih algah / *Evaluation of the effect of the electrochemical oxidation of cyanobacteria with a standardized test on green algae*
   7. Maša Čater, Tinkara Rozina, Maja Zupančič Justin : Results of testing the application of an anodic oxidation with the electrolytic cell for the control of cyanobacteria and degradation of cyanotoxins within the LIFE Stop CyanBloom project / *Rezultati testiranj uporabe anodne oksidacije z elektrolitsko celico za izvajanje kontrole nad cianobakterijami in razgradnjo cianotoksinov v okviru projekta LIFE Stop CyanBloom*

4.2.1.2 Published papers in professional and scientific journals

1. MEGLIČ, Andrej, PECMAN, Anja, ROZINA, Tinkara, LEŠTAN, Domen, SEDMAK, Bojan. Electrochemical inactivation of cyanobacteria and microcystin 2 degradation using a boron-doped diamond anode : a potential tool for cyanobacterial bloom control. *Journal of Environmental Sciences(China)*, ISSN 1001-0742, 2016, vol. , iss. , 14 str., [in press], ilustr., doi: 10.1016/j.jes.2016.02.016.
2. Tinkara Rozina, Cyanobacteria small giants, GEA July 2014. Slovene language Printed in http://www.mladinska.com/gea/tekoca_stevilka/clanek?aid=4104
3. ZUPANČIČ JUSTIN, Maja, ROZINA, Tinkara, MARINOVIĆ, Mario, TESLIĆ, Luka, GRUM, Jošt, YAKUNTSOV, Andrey, FINŽGAR, Neža, LEŠTAN, Domen, SEDMAK, Bojan, LAKOVIČ, Gorazd, GERL, Marko. [Demonstracija robotskega plovila s funkcijo zaznavanja in nadzora rasti cianobakterij v stoječih vodah]. *Ekolist*, ISSN 1854-3758, dec. 2015, [Št.] 12, str. 22-27, ilustr.
4. ROZINA, Tinkara, MEGLIČ, Andrej, LAKOVIČ, Gorazd, GERL, Marko, LEŠTAN, Domen, ZUPANČIČ JUSTIN, Maja, FINŽGAR, Neža, SEDMAK, Bojan. Real-time phytoplankton quantification using chlorophyll a and phycocyanin fluorescence sensors = Kvantificiranje fitoplanktona u realnom vremenu uz korištenje fluorescentnih senzora za klorofil a i fitocijanin. V: BIONDIĆ, Danko (ur.), HOLJEVIĆ, Danko (ur.), VIZNER, Marija (ur.). *Hrvatske vode na investicijskom valu : zbornik radova = Croatian waters on the investment wave : proceedings*, 6th Hrvatska konferencija o vodama s međunarodnim sudjelovanjem, Opatija 20. - 23. svibnja (May) 2015. Zagreb: Hrvatske vode, 2015, str. 1345-1354
5. Web-page publication:Tinkara Rozina, Maja Zupančič Justin: "Nad vodne bakterije s solarnim plovilom" published in the portal: energetika.net, 16. 10. 2016: http://www.energijadoma.si/novice/nad-vodne-bakterije-s-solarnim-plovilom#.VvoctEckTVI
6. MARINOVIĆ, Mario, ROZINA, Tinkara, TESLIĆ, Luka, ČATER, Maša, GRUM, Jošt, GERL, Marko, LAKOVIČ, Gorazd. Daljinsko vodeno plovilo z on-line sistemom razločevanja in kvantifikacije fitoplanktona in lokalizirano kontrolo cianobakterij v vodnem telesu. V: *Akademija strojništva 2016 : inženirstvo - sodelovanje industrijskega in akademskega okolja za kakovostnejše življenje*, 5. mednarodna konferenca strojnih inženirjev 2016, Ljubljana, Cankarjev dom 18. oktober 2016
7. ČATER, Maša, ROZINA, Tinkara, ZUPANČIČ JUSTIN, Maja. Effect of short exposure to electro-oxidation treatment on Planktothrix rubescens = Učinek kratke izpostavitve elektro-oksidaciji na Planktothrix rubescens. *Acta biologica slovenica*, ISSN 1408-3671. [Tiskana izd.], 2016, vol. 59, št. 1, str. 77-80.
8. ATER, Maša, ROZINA, Tinkara, ZUPANČIČ JUSTIN, Maja. Environmentally non-aggresive reduction of cyanobacterial populations in lakes by electro-oxidation with baron-doped diamond electrode. V:

ANTOLOVIĆ, Roberto (ur.). *Programme and abstracts*, 6th Croatian Congress of Microbiology with International Participation, June 15-18, 2016, Sveti Martin na Muri, Croatia. Zagreb: Croatian Microbiological Society, 2016, str. 75.

9. ROZINA, Tinkara, ZUPANČIČ JUSTIN, Maja, LEŠTAN, Domen, SEDMAK, Bojan. Pilot scale demonstration project LIFE Stop CyanoBloom. V: *Cyanobacterial and cyanotoxins : from research to risk management*, 10th International Conference on Toxic Cyanobacteria, Wuhan, China, 23. 10.-28. 10. 2016. [S. l.: s. n., 2016], str. 53-54
10. ČATER, Maša, ROZINA, Tinkara, ZUPANČIČ JUSTIN, Maja. Reduction of extracellular micrcystin concentration by an advanced oxidation technique : potential of boron-doped electrodes to control cyanobacterial blooms. V: MRŠA, Vladimir (ur.), TEPARIĆ, Renata (ur.), KIFER, Domagoj (ur.). *Programme and abstracts*, Simposium Power of Microbes in Industry and Environment September 28 - October 1, 2016 Krk, Croatia. Zagreb: Croatian Microbiological Society, [2016], str. 48.
11. ROZINA, Tinkara, ZUPANČIČ JUSTIN, Maja, ELERŠEK, Tina, SEDMAK, Bojan, LEŠTAN, Domen. Sezonska sukcesija fitoplanktona in kvaliteta vode Koseškega bajerja. V: MAČEK JERALA, Milena (ur.), MAČEK, Melita Ana (ur.), KOLENC ARTIČEK, Majda (ur.). *Z znanjem in izkušnjami v nove podjetniške priložnosti : zbornik referatov = With knowledge and experience to new entrepreneurial opportunities : collection of papers*, 4. konferenca z mednarodno udeležbo - konferenca VIVUS s področja kmetijstva, naravovarstva, hortikulture in floristike ter živilstva in prehrane, 20.-21. april 2016, Strahinj, Naklo, Slovenija. Strahinj: Biotehniški center Naklo: = Biotechnical Centre Naklo, 2016, str. 352-360, ilustr.
12. ROZINA, Tinkara, TESLIĆ, Luka, ZUPANČIČ JUSTIN, Maja. Testiranje robotskega plovila za spremljanje in nadzor fitoplanktona v vodnem telesu : sočasna uporaba fluorescenčnih senzorjev in metode elektro-oksidacije. V: DUH, Matjaž (ur.), et al. *Ekologija v konceptu širših družbenih sprememb*. Maribor: Pedagoška fakulteta; Rakičan: RIS Dvorec, 2016, str. 225-238, ilustr
13. ROZINA, Tinkara, ZUPANČIČ JUSTIN, Maja, ELERŠEK, Tina, SEDMAK, Bojan. Učinek elektrokemijske oksidacije na Microcystis aeruginosa = The effect of electrochemical oxidation on Microcystis aeruginosa. V: PREMATE, Ester (ur.), et al. *Zbornik konference*, Biosfera - konferenca študentov bioloških znanosti, Ljubljana, 23. 9. 2016. Ljubljana: Društvo študentov biologije, 2016, str. 11-12. u http://biosfera.si/files/2016/09/Biosfera_zbornik.pdf?e9270b.
14. ROZINA, Tinkara, ZUPANČIČ JUSTIN, Maja (avtor, fotograf). Za cianobakterije in proti njihovemu cvetenju. *Delo*, ISSN 0350-7521, 2. jun. 2016, leto 58, št. 127, str. 14, ilustr.

4.2.1.3 Invited lectures and presentations to end-users

- Lecture held for the students of Environmental College Velenje (Visoka šola za varstvo okolja): Sensors – senses of machines, Gorazd Lakovič; Methods for detection of phytoplancton, Dr. Andrej Meglič, 2014.

Figure 30: Gorazd Lakovič and Andrej Meglič lecturing at Visoka šola za Varstvo okolja.

- Lecture: "Electrochemical wastewater treatment and remote sensing of cyanobacteria" presented at the International Training Programme on Wastewater Management and Solar Energy, organised by ICPE, 11 February 2014, Ljubljana, Slovenia.

- Presentation of the project in Belgrade, Serbia, 22-23 December 2014: The project was presented to the Serbian Ministry of the Interior Affairs, Department for the management of emergency situations as well as the National Directorate for Water of the Ministry of Agriculture and the Environment. The purpose of the presentation was to demonstrate the technology and the product which is the subject of a demonstration project LIFE Stop CyanoBloom in cases of solving the problems of Serbian water reservoirs, such as accumulation Vrutci in Užice and Grlište in Zaječar. Visit on 22 and 23 December 2014. The power-point presentation has been prepared and leaflets distributed.
- Presentation of the project and technology in Užice, Serbia, 29-30. April 2015: Presentation of the technology in Užice, Serbia: The possibility of monitoring and control of cyanobacteria in the accumulation Vrutci, which has been constructed to supply drinking water to the town Užice. Due to cyanobacteria presence, the accumulation is currently not in use for drinking water supply. Visit on: 29, 30 April. The power-point presentation has been prepared and leaflets distributed.
- On July 2015 we hosted a visit from Serbian and Finish professionals and demonstrated the robotic vessel operating at Koseze Pond.

Figure 31: Demonstration for guests from Serbia and Finland.

- Presentation to a delegation from Kosovo, 1. February 2016 at Arhel premises, to the representatives of the Kosovo Landfill Management Company
- During 18. – 21. 12 2016 we visited two Serbian aquaculture companies, Ribnjačarstvo Kenđel in Mužlja at Zrenjanin and Kapetanski Rit at Subotica. Additionally, we visited other lakes suffering from cyanobacterial blooms, Paličko Lake and Lake Ludaš at Subotica. Both companies expressed their interest in finding a solution for cyanobacterial blooms in their fish ponds. Ribnjačarstvo Kenđes send us a letter of interest for further common cooperation (attached to **ANNEX 25**).

4.2.1.4 Awards

Awarded photography of Tomaž Varlec on the competition “Europe in my Region”, 12 – 14 October 2015

Figure 32: Europe in my Region Regioawards ceremony. Award is given to Tomaž Varlec.

The technology was presented on the 10th Slovenian Innovation Forum Nova Gorica, 17. – 18. November 2015. **Our innovation has won two awards. We won the first price in the category of micro-enterprises, and we have received a special award for the innovation with a societal impact**

Figure 33: Exhibition and award ceremony of the 10th Slovenian Innovation Forum

4.3 Analysis of long-term benefits

4.3.1 Environmental benefits

a. Direct/quantitative environmental benefits

For the demonstration and optimisation of the technology, two different types of lakes have been selected:

- Fish pond in Hotinja Vas, a shallow fishpond with high organic and nutrient loads (hypereutrophic) and dispersed cyanobacteria occurrence across the water columns (predominance of chroococcal *Microcysts aeruginosa*) and
- Lake Bled, a deep-water lake with seasonal stratification and lower organic and nutrient load (oligotrophic to eutrophic) and metalimnetic cyanobacteria occurrence with a predominance of filamentous *Planktohtrix rubescens*.

In the past, both lakes suffered occasional excessive cyanobacterial blooms. In the case of Hotinja Vas, the survival of fish population during the summer months was endangered. In the case of Lake Bled, the rise of cyanobacteria to the surface in the late winter and early spring months represented an unattractive appearance of the very important lake from the point of view of the tourist visits. In the case of severe blooms, cyanotoxins also posed a threat to the environment in the past. For the project purposes, also a third water body was selected, a fishpond Koseze in Ljubljana, close to Arhel`s and NIB premises. In previous years, cyanobacteria occurrence was also detected in this water body and alarming low concentrations of oxygen during the peak summer months, endangering the survival of the fish population. The pond was also selected with the reason to optimise the costs of the frequent visits of the pond during the period of testing and fine-tuning the equipment of the robotic vessel.

During the project, neither of the selected water bodies showed excessive cyanobacterial bloom, which would reflect in fish die off, poisoning of the population or other adverse environmental or economic consequences. The occurrence of cyanobacteria (or their absence in the case of Koseze Pond) was, however, sufficient to perform monitoring activities with the robotic vessel and tests of different scales with electrochemical stimulation. Immediate direct electrochemical stimulation in the lakes was, however, avoided, to prevent potential toxic effects of released cyanotoxins, before we checked what is happening to them in controlled conditions. The aim of the tests was to define the needed intensity of the treatment to achieve selective control over cyanobacteria (and not over the rest of the phytoplankton population) and to evaluate the treatment range.

Obtained detailed monitoring data on phytoplankton development:
During the monitoring activities performed in the action C1, we obtained a long-term data on the seasonal change in concentration and species composition of the phytoplankton in the fishpond Koseze and Lake Bled, and several measurements for the fishpond in Hotinja Vas, which would otherwise not be performed. With the automated on-line monitoring approach demonstrated in the project, we showed that it has a potential to substantially reduce the need for laboratory determination of phytoplankton and related costs (to approximately one tenth of actual costs related to laboratory measurements), while significantly increase the number of data about the status of the water body. The detailed information and report are presented in the ANNEXes 6, 10A, 10, 13 and 15.

Proved selective effect of hydroxyl radicals on cyanobacteria:
With more than sixteen experiments and in-lake pilot tests with the electrolytic cell, we demonstrated that we could achieve different levels of cyanobacteria cell damages, resulting in their in-lake growth control. With the performed experiments, we proved that electrochemical oxidation induces a large variety of damages to cyanobacterial cells, like direct lysis, induction of various types of apoptosis and senescence and induction of the lytic cycle in lysogenic cells.
The detailed results are presented in the ANNEXes 12, 15 and 16. Further calculations, based on the obtained experimental and demonstration results showed that the current boat size and the capacity of the photovoltaic panels (constructed within the project) would allow the installation of three electrolytic cells, each with the $60cm^2$ electrode surface. These cells would

enable treatment of 9 and 2,8 m^3 of water infested with cyanobacteria in one week or two days, respectively, with a goal of treating the entire affected water volume up to 80% cyanobacteria reduction efficiency. This would allow controlling one and 0.27 hectares of water surface if we presume that 1% of the water surface is affected and that we would like to achieve 80% reduction of bacterial cells in one week and two days, respectively. Since the goal is to start with electrochemical stimulation as soon as possible after the detection of cyanobacterial cloud, the need for the cell reduction can be as little as 10% efficiency. This means that the reach of the vessel could be up to 8 hectares. The effect range and the economic evaluation is further discussed in the ANNEX 17 of the Final Report.

Potential energy and costs savings:
From the point of view of energy savings, the solar powered robotic vessel can save up to 400 litres of gasoline in 100 days per year, which would be otherwise used for the motorised operation of a manned vessel.

The solar powered robotic vessel, used as a water body monitoring device, can eliminate at least one man-year costs in comparison with a manual monitoring by a manned vessel with a crew of two people, considering the operation of 100 days/year.

Potential savings of resources:
The operation of the three electrolytic cells, each with 60 cm^2 surface and applied current density 60 mA/cm^2 in 100 days/year would produce (in reactivity equivalents) the same amount of hydroxyl radicals as entering at least 6 m^3 of 30% H_2O_2 into the lake, which represents an enormous saving of the resources (Isarain-Chavez et a., 2013[1]). In case that only 10% of the lake is affected by cyanobacteria, the spraying with H_2O_2 would need to be done to the whole lake surface, amounting in at least 60 m^3 of 30% H_2O_2, while detection and production of hydroxyl radicals with an electrochemical cell on board would allow target operation.

The proposed technology (mobile working platform equipped with fluorescence sensors and an electrolytic cell for in-lake cyanobacteria control) could, therefore, provide the following environmental benefits:

- Early warning system for on-line monitoring on harmful algal bloom
- Localised growth control of cyanobacteria population with no chemicals addition into water and therefore resource savings
- Reduced toxicity risk (health and environmental) due to prevention of cyanotoxin release into water
- Reduced adverse effect on other water environmental components (reduced fish, amphibian and mollusc death)
- Improved biodiversity with prevention of cyanobacteria overgrowth
- Energy savings with renewable energy use from photovoltaics producing no emissions during the operation of the robotic vessel for cyanobacteria detection and control

b. <u>Relevance for environmentally significant issues or policy areas</u>

[1] Isarain –Chavez et al.,. On-site Hydrogen Peroxide Production at Pilot Flow Plant: Application to Electro-fenton Process. Int.J.Electrochem Sci., 8(2013) 3084-3094.

Inland waters are used for different purposes, such as recreational activities (swimming, rowing, water-skiing, sports fishing), abstraction of drinking and irrigation water and commercial aquaculture. Here, several local and governmental administrative bodies and authorities are responsible. For example, Ministries for Agriculture are usually responsible for the safety of aquaculture products for human consumption and quality of water used for irrigation. The Ministries for the Environment and Physical Planning are usually responsible for environmental issues and ecological water quality. By the new Water Directive, they are obliged to monitor phytoplankton in waterbodies, but only those greater than 10 hectares with a minimum frequency four times per year. Agency of the Republic of Slovenia for the Environment under the Ministry for the Environment also monitors the bathing water quality according to bathing water Directive. The drinking water quality is a responsibility of Ministries of health. The quality of the reservoirs for the hydroelectric power plants is, for example, the responsibility of the hydroelectric power plant companies. Commercial aquacultures and ponds for procreative fishing are managed by aquaculture companies and fishing/angling clubs. By different purposes of use of water bodies, there may be conflicts of interest between the various authorities and managers.

The following European Directives can be considered as relevant for the hazards due to cyanobacterial blooms and cyanotoxins exposure via water:

Water Framework Directive (WFD, Directive 2000/60/EC)
The aim of the **WFD** is that all European waters meet criteria for good ecological status by 2015. Criteria are of hydro-morphological, physical, chemical and biological nature, and this is to be achieved by management plans for the specific River Basin Districts, i.e. natural geographical and hydrological units. Eutrophication control in these River Basin Districts is a necessary means to achieve the goals of the WFD, to which all EU member states are committed.
Phytoplankton monitoring is becoming one of the key elements in water quality assessment. Presented *in-vivo* automated detection is so far not a part of the official monitoring methodology, but coupled with existing taxonomic approach can improve phytoplankton monitoring goals. We see the potential to upgrade the traditional monitoring techniques and no problem in market acceptance.

EU Bathing Water Directive (BWD, *Revised bathing water directive: 2006/7/EC, 15.2. 2006 concerning the management of bathing water quality and repealing Directive 76/160/EEC and Guidelines for safe recreational water environments, World Health Organization, 2003)*
The BWD lays down provisions for developing a bathing water profile. The potential sources of contamination of the bathing site should be assessed to define the classification of bathing water quality, its management and the provision of information to the public. By 2015 all bathing waters were required to have an acceptable minimum water quality. With respect to cyanobacteria, the BWD stipulates that if a bathing water profile for an individual site indicates the potential for cyanobacterial proliferation, appropriate monitoring shall be carried out to enable timely identification of health risks. If proliferation does occur and risks have been identified, adequate measures shall be taken immediately to prevent exposure, including information to the public.

EU Drinking Water Directive (DWD, *98/83/EC, 3.11. 1998 on the quality of water intended for human consumption, and WHO Guidelines on Drinking water (2003)*

The DWD provides a general degree of protection, without mentioning cyanobacteria specifically: it is intended to protect human health by ensuring that drinking water does not contain microorganisms, parasites or substances in concentrations, which constitute a potential health risk. The Member States shall take any action that is required to guarantee the safety and purity of water intended for human consumption.
A provisional guideline value for microcystins in drinking water is **one µg/L**; for drinking water, the treatment shall be optimised to remove cyanotoxins from the water.

There are far fewer regulations for exposure via food as compared to exposure via water. National guidelines concerning cyanotoxins in freshwater fish and mussels are in general missing, although they are prone to bioaccumulation. This applies to fish caught in the framework of sports fishing as for the fish commercially produced in aquacultures. An important document of the DG Health and Food Safety is the Overview Report – Implementation of the Rules in Finfish Aquaculture (https://ec.europa.eu/fisheries/cfp/aquaculture_en and http://ec.europa.eu/food/audits-analysis/news_detail.cfm?id=74). The report does not mention any threats connected to harmful cyanobacterial blooms in aquaculture nor fish food safety concerning the cyanotoxins. The Council Directive 96/23 EC, 1996 on measures to monitor certain substances and residues thereof in live animals and animal products and repealing Directives 85/358/EEC and 86/469/EEC and Decisions 89/187/EEC and 91/664/EEC also do not consider any of the representative of cyanotoxins as a substance of food contamination. The are also not the subject of the REGULATION (EC) No 853/2004 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 29 April 2004 laying down specific hygiene rules for food of animal origin. The COMMISSION REGULATION (EC) No 2074/2005 of 5 December 2005 laying down implementing measures for certain products under Regulation (EC) No 853/2004 …. considered saxitoxins to be measured and controlled. The new orders are still targeted specifically at monitoring algae and their toxins for sites where breeding and harvesting of mussels takes place, hence aimed at marine environment (*Mytilus edulis* and marine toxins). At present this specific EU regulation does not appear to be used for protection against cyanotoxins in freshwater seafood.

4.3.2 Long-term benefits and sustainability

a) Long-term / Qualitative environmental benefits

The determination of the presence of phytoplankton using sensors in the specially designed LIFE Stop CyanoBloom continuous-flow monitoring chamber would be an effective complementary method to conventional monitoring and laboratory analysis because it enables:

- Obtaining information on the state of water in the natural environment in real time.
- Better accessibility to all parts of the water body and collection of samples of interest at a selected location. The device excludes arbitrariness in determining the sampling points with its mobility.
- Higher spatial and temporal resolution - a three-dimensional snapshot of the water body.
- Optimising sampling strategies. Real-time obtained information allows a reasoned decision on the further time and location of sampling

- Quantitative and qualitative detection of phytoplankton with combined use of chlorophyll and phycocyanin sensors
- Assessment of the physiological state of the phytoplankton
- Determining the presence of stress and stressors in the aquatic environment based on changes the size and the ratio of single signals
- The establishment of early warning
- Detecting and tracking the position and movement of cyanobacterial bloom
- Possibility of immediate simultaneous treatment with electrolytic cells
- Local effect (treatment) only in selected areas with an established presence of dangerous cyanobacteria, bringing energy, resources and costs savings.

The operation of the electrolytic cell also causes (besides the production of reactive oxygen species, which are hindering cyanobacteria proliferation) mixing of the water in the local area of operation. This activity provides positive secondary effects on the rest of the phytoplankton population by enabling them easier access to nutrients, disrupts cyanobacterial buoyancy pattern, as well increase potential contacts of cyanobacteria with cyanophages. In general, 30% of cyanobacterial blooms collapse because of cyanobacterial infection. Electrochemical stimulations causing different levels of stress to cyanobacterial cells makes them more prone to infection or conversion to lytic cycle, and the water circulation increases the potential interactions with the cyanophages.

The activities would reflect in

- Balanced cyanobacteria presence with reduced cyanotoxins concentrations
- Higher bathing water quality and therefore reduced health hazards
- Improved fishpond water quality with improved fish growth, reduced fish die-off and higher fishes quality for consumption (without unpleasant odour and taste and cyanotoxins)
- Higher general fresh-water quality with greater biodiversity and higher ecosystem services

b) Long term / Qualitative economic benefits

Cyanobacterial blooms threaten vital socioeconomic infrastructure through the closure of important recreational areas and fishing grounds that sustain commercial and tourism industries, and other valuable cultural resources. Effects on the economy, livelihoods and cultural amenities can have long-term consequences for the local population well-being. Isolated blooms can cause short-term economic losses, but recurring blooms can have an adverse impact on the reputation of the area and therefore cause a long-term decline in tourism. The economic consequences of cyanobacterial blooms also depend on the season in which they appear. During the peak tourist season, the financial losses can be catastrophic, while during non-tourist season cyanobacterial blooms can occur without consequences.

The following long-term qualitative economic benefits can be exposed in case of use of the LIFE Stop CyanoBloom approach:

- Improved drinking water quality would reflect in the reduction of treatment costs for drinking water preparation.

- Higher bathing water quality would result in higher income from tourist visits and leisure activities.
- Higher fish-pond water quality would reflect in higher income from aquaculture and lower expenditure for in-lake cyanobacteria control measures.
- Higher general fresh-water quality with increased ecosystem services represents better prospects for general regional development.
- Applying fluorescence probes would reduce the expenditure for costly traditional algal monitoring approaches.
- The Early warning system would enable fast responses before the excessive algal blooms emerge and therefore reduce the expenditure for curative measures.

In ANNEX 17, we have presented a calculation of costs savings for the case of the fishpond. We estimated that with the purchase of the robotic vessel, the return of the investment for the 10 ha fishpond could be expected in 1.5 - 6 years. Here we took into account that 2-5 tonnes of fish are produced by 1 ha of fishpond with a market price two €/kg, and considered that fishpond owner would avoid the annual loss of 50% to 100 % of produced fish, which usually occurs due to harmful cyanobacterial blooms with the use of technology.

For the lakes needing regular monitoring, the adopted on-line monitoring, would substantially reduce the need for laboratory determination of phytoplankton and related costs (to approximately one tenth of existing costs related to laboratory measurements), while significantly increase the number of data about the status of the water body. With a frequent reduction of available budget for the implementation of lake monitoring activities and pressures on optimising the monitoring activities, on-line monitoring with unmanned equipment may offer a favourable solution.

c) Long term / Societal benefits

Consequences of cyanobacterial blooms on human health have social and economic impacts on the population. They cause deterioration in general well-being of people as well as the occurrence of costs for treatment of diseases. The biggest threat to human health is chronic exposure to cyanotoxins via drinking water. Evaluating health impacts caused by water pollution is very difficult. The first problem is actual identification and measurement of health impacts. The second issue is putting actual values to the illness and possible death.

Based on a comprehensive literature survey we concluded that reduction or prevention of health and economic consequences of cyanobacterial blooms would have a major impact on the local population and would improve the quality of life in the exposed area. These conclusions are made based on catastrophic examples from different countries.

As a general conclusion we can say:

- Improved water quality without toxic cyanobacterial toxins would have a positive effect on population health.
- Improved lake water quality in tourist areas would attract more tourist, offering higher employment possibilities.

d) Continuation of the project actions by the beneficiary or by other stakeholders

It is our strong belief that the research activity should not stop at achieving the goals of the existing LIFE project. We will put our efforts to continue with the project activities with the emphasis on optimising the technology operation in different geographical environments (e.g. cold Nordic climate and warm Mediterranean climate) and for water bodies with different trophic status (oligotrophic drinking water reservoirs versus eutrophic fishponds). Further development is expected in energy optimisation of anode oxidation and selection of electrode material adapted to low water environment with low conductivity. The potential of triggering the lytic cycle with induction of proliferation of phages in treated cyanobacterial cells is planned to be evaluated.

We will look for new financial opportunities for further research and development. After presenting the achieved results, we will start with the presentations of the technology and services at identified end-users. We evaluate that the approach is suitable among others for fish ponds and aquaculture, irrigation reservoirs, Lake water management for tourism purposes, drinking water abstraction and protection of endangered freshwater habitats. For the first market replication of the technology, freshwater aquaculture was planned.

Both robotic vessels will be offered into test operation to end-users looking for lake monitoring service and early direct in-lake cyanobacterial control. The vessels will be offered into use free of charge. The user will cover the operational and needed maintenance costs.

4.3.3 Replicability, demonstration, transferability, cooperation

4.3.3.1 Project replicability

In spite of the efforts to prevent nutrient loads into waters, which are the main drivers of cyanobacterial blooms, their worldwide incidence increase. While point sources of pollution are relatively well carried out in developed part of the world, less success is evident in controlling the dispersed sources of pollution. Very evidently, also the internal release of phosphorus from the sediment is triggering cyanobacterial blooms. With several million kilometres of running waters and more than a million lakes (European Environmental Agency), there are numerous needs for the use of technology.

For the further replication of the technology, we have considered markets such as:

- Bathing and recreational waters,
- Aquacultures,
- Wastewater stabilisation ponds,
- Irrigation reservoirs,
- drinking water abstraction and
- Protection of endangered freshwater habitats

A potential market represents ponds and accumulations for aquaculture. The transfer in the freshwater aquaculture from the non-fed to fed aquaculture resulted in a higher fish productivity, but also in increased water quality problems. Beside different infections, water anoxia and overgrowth of cyanobacteria are the main challenges confronted in intensive fish production.

A potential market also represents natural and artificial freshwater bodies with a high recreational potential. There are for example 1939 registered (big) lakes across Europe (The EEA European Union, 2014) with bathing waters. In 2013, there were 22 076 bathing waters identified in Europe (EEA Water bathing quality report, 2014). Here, high costs are spent for intensive water quality monitoring programmes as well as for rehabilitation and remediation measures to prolong the bathing season with removal or other cyanobacterial control measures.

Drinking water and irrigation reservoirs also represent a technology replication potential. In the EU, water supply is mainly fed by groundwater and by surface water, including artificial reservoirs. High water quality is an urgent precondition.

4.3.3.2 Project transferability

The sensors applied in the continuous flow monitoring chamber of the robotic vessel were dedicated to the direct detection of the cyanobacteria and green algae. These sensors could be exchanged, or additional sensors added to measure other water quality parameters of interest (e.g. nutrients, organic load, etc.), which are important indicators of eutrophication or also nutrient availability at hydroponics – cultivation of vegetables.

Besides sensors measuring water quality parameters, the vessel can be equipped with sonar equipment for identification of changes in water sediment and bottom, which are for example important data for hydropower plant reservoirs.

The continuous flow measuring chamber is designed in a manner to be used individually in for example laboratory environment, as portable equipment in a suitcase or used in affixed position in the water body.

The electrolytic cell with the production of hydroxyl radicals has a potential to be used in wastewater and drinking water treatment systems. The potential of its use as the fourth stage of wastewater treatment system for the removal of micro-contaminants like residues of pharmaceuticals has been presented in LIFE PharmDegrade project.

Green algae, as well as cyanobacteria, are widely used in biotechnology for production of algal biomass or various biomolecules produced by algae. Individual components designed in the project are applicable in processes of water sterilisation to be used in the process, sensing of different water parameters, as well as for treatment of outflows from such systems to prevent modified microorganisms enter into the environment.

The data transfer and the information system of the vessel have been used so far for the direct analysis and interpretation of the data by the vessel operator. The information system can be however upgraded for different bigger scale mapping applications (e.g. gathering data from aquacultures, bathing areas on larger geographical scale).

4.3.4 Best Practice lessons

4.3.4.1 Phytoplankton monitoring

The traditional approaches to phytoplankton monitoring include assessment of water transparency by Secchi disk, water sampling followed by analyses of chlorophyll a (Chl *a)* and phytoplankton biovolume, and determination of species composition. Laboratory standardisation of Chl *a* determination and the simplicity of the Secchi disk use has enabled the development of standards for general water trophic state. The microscopic counting and identification of phytoplankton species give very accurate data on species volume and composition in a given sample. However, there are several limitations of these approaches. Many samples are needed to follow trends; the laboratory analyses are time-consuming, as well as highly trained personnel is required for species determination. Such monitoring, therefore, results in high costs and the water examination reflects only the current situation of the sampled water spot. Because of specific migration patterns and specific occurrence of CB on a daily, seasonal and weather-induced basis, occasionally taken samples on a particular spot may bring misleading results or even do not show the problem. All this indicates the need for less costly methods of monitoring that can be carried out very often, with a large number of sample points and at a wide range of water body. Fluorometry, used in the project can greatly contribute to higher efficiency in phytoplankton monitoring.

4.3.4.2 In-lake cyanobacterial control

In **ANNEX 17** (Report on economic justification of the project) an extensive review of the existing in-lake cyanobacteria growth control options is given. As presented in the review, the existing in-lake rehabilitation and cyanobacteria control methods have efficiency, cost and manipulation limitations. The most appropriate method or their combination should be therefore selected by considering the following:

- environmental and geographically conditions of the water body in consideration,
- intended use of the water body (bathing, drinking, aquaculture, …),
- availability of funds (cost benefit),
- energy and resources use sustainability,
- selectivity to cyanobacteria,
- minimal toxicity to other organisms (avoiding disturbance of complete water ecosystem),
- avoiding the development of resistance,
- avoidance of additional sediment development.

Several advantages of the Sop CyanoBloom approach have been already mentioned. Among others, the technology uses combined automated on-line cyanobacterial monitoring and in-lake cyanobacteria control based on electrochemical oxidation. This is done without the use of chemicals or sediment-developing inputs, thus not changing the total capacity of the water body. The localised mixing of water with electrolytic cell operation facilitates the development of desirable phytoplankton in the water. Based on the positive effect of other in-lake cyanobacteria control methods, like the use of ultrasound, epilimnetic water circulation, floating wetlands and other ecological engineering approaches, etc., the Stop CyanoBloom approach could be used in combination with these approaches. Different water bodies would namely ask for different remediation approaches. The applied electrochemical oxidation induces a large variety of damages to cyanobacterial cells from direct lysis, induction of various types of apoptosis and senescence to the induction of the lytic cycle in lysogenic cells.

4.3.5 Innovation and demonstration value

The EU funding enabled the demonstration of the innovative technology on two Slovenian water bodies with different cyanobacterial species occurrence pattern. The first one was a representative of a shallow water body with the dispersed occurrence of globular *Microcystis aeruginosa*, the most common cyanobacterial species. The second one was a representative of deep water body with seasonal stratification and hypolimnetic occurrence of *Plaktothrix rubescens*, a filamentous cyanobacteria. Combining on-line monitoring equipment and cyanobacteria control tool on one mobile platform represent an innovation on a European level. To our knowledge, there haven`t been so far any demonstrations of the in-lake cyanobacteria control based on electrochemical oxidation.

Besides demonstrations performed in the laboratory and the natural environment, the EU funding enabling a development of numerous unique units composing the robotic vessel, like:

- Continuous flow chamber for measuring fluorometric and other water parameters
- Electrolytic cell for cyanobacteria growth control and cyanotoxin degradation
- Water flow regulation by smart valves
- Water auto sampler with motorised hose reel
- Automatic docking system and
- Integrated measuring, treating and sampling systems
- Information system for transfer and presentation of measured data

4.3.6 Long term indicators of the project success

Arhel will provide regular monitoring of the visits of the website. Maintaining at least the current number of visits to the website (more than 50 visits to the website each month; increase the visits from the other countries besides Slovenia) will be an indicator of the successful promotion of the technology through the website.

Partners will endeavour to further promote the technology with scientific and professional publications in journals and presentations at the conference, fairs and other events (>5 oral or poster presentations on different events in the next three years, at least one published scientific article). Arhel will cover the expenses from its budget.

Long-term indicators of the project success are seen in the commercialisation of the technology across Europe (orders of equipped vessels from different end-users). We expect at least one application in the next three years.

The following patents were filed for the technology or its parts:
LEŠTAN, Domen, SEDMAK, Bojan, LAKOVIČ, Gorazd. *Prevention of mass occurrence of harmful cyanobacteria (Preprečevanje masovnega pojavljanja škodljivih cianobakterij : patent) št. 23987 (A), 2013-08-30*. Ljubljana: Urad RS za intelektualno lastnino, 2013. 5 str., [ilustr.]. [COBISS.SI-ID 2885199]

LAKOVIČ, Gorazd, GERL, Marko, LEŠTAN, Domen, ZUPANČIČ JUSTIN, Maja, ROZINA, Tinkara, FINŽGAR, Neža. *Measuring chamber with circular laminar water flow (Merilna komora s krožnim laminarnim vodnim tokom) : patentna prijava P-201500301*. Ljubljana: Urad RS za intelektualno lastnino, 18. 12. 2015. [COBISS.SI-ID 8331897]

SEDMAK, Bojan, LAKOVIČ, Gorazd, LEŠTAN, Domen, MEGLIČ, Andrej, GERL, Marko. *Method and system for simultaneous detection of micro-particle concentration in suspension and their morphological and physiological traits : new European patent application no. EP 15161547.3, March 27 2015*. München: European Patent Office, 2015. 17 str., 15 str. pril., ilustr. [COBISS.SI-ID 3783759]

LAKOVIČ, Gorazd, GERL, Marko, LEŠTAN, Domen, ZUPANČIČ JUSTIN, Maja, MARINOVIČ, Mario, FINŽGAR, Neža. *Power supply for the electrolytic cell for the control of cyanobacteria bloom (Napajalnik elektrolitske celice za kontrolo cvetenja cianobakterij) : patentna prijava P-201500300*. Ljubljana: Urad RS za intelektualno lastnino, 6. maj 2015. [COBISS.SI-ID 8331641]